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Crop Stress and its Management: Perspectives and Strategies

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Preface

Abiotic and biotic stresses on crops are one of the most important limiting factors to crop growth, development and potential yield realization, production loss due to these stresses are enormous. Plants have complex system of responses to stress stimuli which change constantly with changes in the environmental stresses encountered by the plants. These changes in the structure and function of the plant systems are highly intricate and much more complex than found in animal systems notwithstanding the noted nonexistence of a well-defined immune system in plants. One can say that the simple reason for this is that plants do not possess the ability to simply move away from the region of stressful stimuli or in other words they are sessile. Multiple stresses or a stress and an external potential ameliorant can evoke very complex responses in plants systems, these responses may be of stress countering nature or simply an effect of the stimuli. The threat to productivity in global agriculture due to these stresses cannot be overstated, nor should it be overlooked especially in the light of the predicted climate change. Crops experience an assortment of environmental stresses which include abiotic viz., drought, water logging, salinity, and extremes of temperature, high variability in radiation, subtle but perceptible changes in atmospheric gases and biotic viz., insects, birds, rodents, nematodes and pathogens (viruses and other microbes). The ability to tolerate or adapt by effectively countering these abiotic stresses is a very multifaceted phenomenon; in addition the inability to do so rendering the crops susceptible, is again the result of various exogenous and endogenous interactions in the ecosystem. Both biotic and abiotic stresses occur at various stages of plant development and frequently more than one stress concurrently affects the crop. Stresses result in both universal and local effects on plant growth and development. One of the imposing tasks for the crop researchers globally is to distinguish and to diminish effects of both biotic and abiotic stress factors on the performance of crop plants, especially with respect to yield, quality of raw materials, and nutrient efficiency resources. This is of special significance in view of the impending climate changes with complex consequences for economically profitable and ecologically and environmentally sound global agriculture. The challenge at the hands of the crop scientist in such a scenario is to promote a competitive and multifunctional agriculture, leading to the production of highly nourishing, healthy and secure food and animal feed as well as raw materials for a wide variety of industrial applications. In order to successfully meet this challenge, researchers have to understand the various aspects of these stresses in view of the current development from molecules to ecosystems. The book will focus on the strategic aspects in addition to touching some mechanistic aspects. In addition, whole plant and crop community approach to rationally manipulate and optimize tolerance traits for improved crop productivity, evolve crop production packages with the aid of geoinformatics and precision agriculture to counter stress is highlighted in some of the chapters of the book. Of special significance in the book is the comprehensive state of the art of abiotic and biotic stress management in plantation crops and the chapter on socio economic and policy issues in abiotic stress management. The enormous pace at which advances and new discoveries that recently are taking place in the cutting edge areas of molecular biology and basic genetics, have strengthened and increased the efficiency of science outputs in dealing with crop stresses. We have entered a new phase in science, i.e. ‘post-genomics era’, where outcome in terms of translation of information generated on field performance of crops to increase productivity would be considered as the ultimate goal. With enormous body of knowledge available in the researchers’ domain, attempts are required to transfer this knowledge to the farm level for combating various crop stresses. The book also addresses the role of the novel information and communication technologies for technology transfer. This multi authored edited compilation will attempt to put forth a comprehensive picture in a systems approach wherein most aspects of stress will be dealt with. The chief objective of the book hence would be to deliver information for developing strategies to combat crop stress. We attempt here to present a judicious mixture of management as well as research outlooks so as to interest workers in all areas of crop stress. We trust that the information covered in this book would bridge the mechanistic aspects (what do we know) of stresses with the strategic aspects (what do we do).

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Chapter 1
Overview of Plant Stresses: Mechanisms, Adaptations and Research Pursuit

M. Maheswari, S.K. Yadav, Arun K. Shanker, M. Anil Kumar, and B. Venkateswarlu

Abstract Biotic and abiotic stresses in crops are a major hurdle in attaining potential yield worldwide. Finding an approach to sustain high yields of crop plants under biotic and abiotic stresses is an important goal of agriculture researchers and stakeholders alike. Among the abiotic stresses, drought, salinity, temperature and heavy metal accumulation are the major environmental stresses, which adversely affect plant growth and productivity. In addition, biotic stresses primarily, plant diseaseses are a significant constraint to the production of about 25 important food and fiber crops. Changing climate compounds these adverse effects of stresses on crops. To cope with biotic and abiotic stress it is of paramount significance to understand plant responses to these stresses that disturb the homeostatic equilibrium at cellular and molecular level in order to identify a common mechanism for multiple stress tolerance at least in the case of abiotic stresses. An integrated systems approach is essential in the study of complex quantitative traits governing tolerance to multiple biotic and abiotic stresses. A detailed account of specially abiotic stresses and combating strategies to effectively counter them are discussed in this chapter.

1.1 Introduction

Hostile biotic and abiotic environmental conditions, such as diseases and pests, drought, flood, heat and other stresses affect agricultural productivity greatly. Crop losses are caused by these abiotic and biotic environmental factors, leading to the

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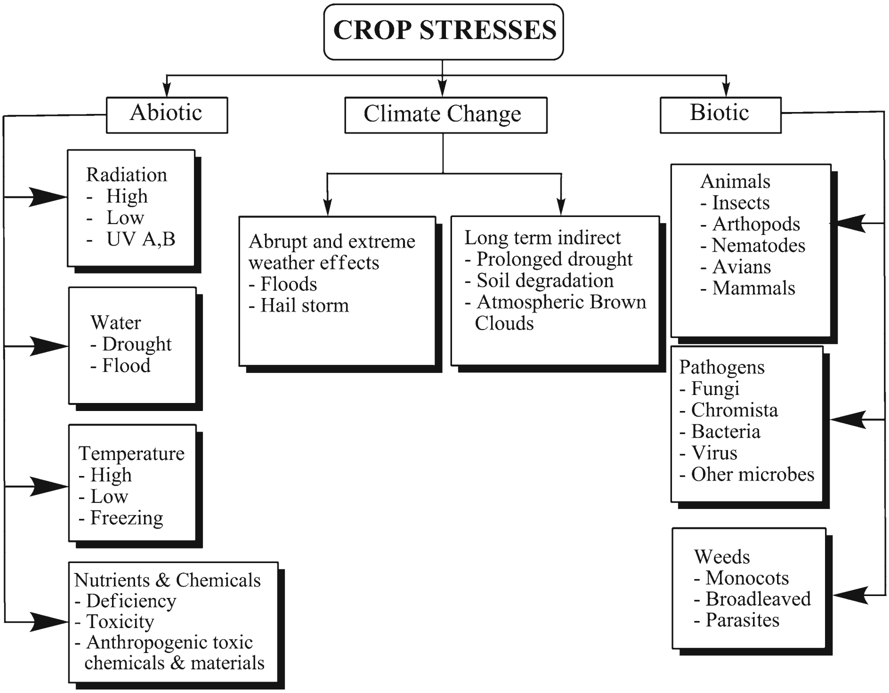

Fig. 1.1 An overview of biotic and abiotic factors that cause crop stress

significant decline of crop performance and subsequent lower actual yield than attainable yield (Fig. 1.1). Absolute losses vary between crops due to dissimilarities in their reaction to the effects of biotic and abiotic stresses. Finding an approach to sustain high yields of crop plants under stress is an important goal of agriculture researchers and stakeholders alike. Among the abiotic stresses, drought, salinity, temperature and heavy metal accumulation are among the major environmental stresses, which adversely affect plant growth and productivity. Although one third of the total land area is considered as potentially suitable for arable agriculture, only 10% of the world's 13 billion hectares is cultivated. By 2030, global cereal demand for food and animal feed alone is expected to be 2.8 billion tonnes per year, or 50% higher than in 2000 (Lobell et al. 2009). Various forms of abiotic stresses limit production on most of the world's 1.4 billion ha of cultivated land. In addition, biotic stresses primarily plant diseases are a significant constraint to the production of about 25 important food and fiber crops. Biotic interactions reduce crop productivity in various ways; examples being growth reducers (damping-off pathogens), photosynthetic rate reducers (fungi, bacteria, viruses), leaf senescence accelerators (pathogens), competition for light (weeds, some pathogens), assimilate sappers (nematodes, pathogens, sucking arthropods), and tissue consumers (chewing animals, necrotrophic pathogens). In general weeds affect crop productivity mainly due to the competition for inorganic nutrients (Oerke 2006). World-wide losses from diseases range from 9% to 16% in rice, wheat, barley, maize, potato, soybean, cotton (Chakraborty et al. 2000). Changing climate compounds adverse effects of stresses on crops. Potential effects of climate change on agriculture include reduced yields in warmer regions as a result of heat stress; damage to crops, soil erosion and inability to cultivate land caused by heavy precipitation events; and land degradation resulting from increasing drought. Crop simulation models, driven by future climate scenarios from global circulation models, suggest that the reduction in agricultural production would be more severe in tropical regions, where there is still a shortage of food production (Ghini et al. 2011). Pests cause extensive damage to crop production and contribute greatly to yield losses and this again is exasperated by variability in climate. The global loss potential caused by pests is particularly high in crops grown under high productivity environments and also in the tropics and sub-tropics where climatic conditions favour the damaging function of pests (Oerke 2006). In this chapter we present the major abiotic stresses and discuss the mechanism by which they affect crop growth and present some possible novel research strategies that can tackle this problem.

1.2 Significance of Stresses in Crop Plants

Abiotic stresses have a general mechanism of action although a degree of specificity can be attributed to the specific type of stress unlike biotic stresses with the exception of weeds wherein physical damage is one of the major result of infestations. In addition, in the case of abiotic stresses the degree of specificity is very high depending on the organism attacking the crop viz., insects, birds, viruses, microorganisms nematodes where each organism affects the crop in almost a distinctive way and furthermore the degree of complexity in the mechanism is higher due to specific interactions between the host and damage causing organism. Many pests and pathogens exhibit considerable capacity for generating, recombining, and selecting fit combinations of variants in key pathogenicity, fitness, and aggressiveness traits (Oerke 2006). A detailed discussion on this would be beyond the scope of this chapter and hence we confine ourselves to abiotic stresses and their mechanisms. Stress is defined as “any environmental variable, which can induce a potentially injurious strain in plants”. The concept of optimal growth conditions is a fundamental principle in biology. Since living organisms cannot control environmental conditions, they have evolved two major strategies for surviving adverse environmental conditions i.e. stress avoidance or stress tolerance. The avoidance mechanism is most obvious in warm blooded animals that simply move away from the region of stressful stimuli. Plants lack this response mechanism, which is mobility; hence they have evolved intricate mechanisms to avoid stress. For example, they alter life cycle in such a way that a stress sensitive growth period is before or after the advent of the stressful environmental condition. On the other hand, tolerance mechanisms mainly involve biochemical and metabolic means which are in turn regulated by genes.

1.2.1 Cold

Most crops of diverse origins – some tropical and subtropical are sensitive to chilling temperatures. Principal food crops like maize (*Zea mays*) and rice (*Oryza sativa*) are very sensitive to low temperatures. The growth of these crops are severely affected by temperatures below 10°C resulting in considerable yield loss or even crop failure. Among other crops, maximum economic loss is seen in fruit trees (Meirong and Yanli 2008). The temperature below which chilling injury can occur varies, ranging from 4°C for temperate fruits, 8°C for subtropical fruits, and 12°C for tropical fruits such as banana. Chilling during the seedling stage in cotton can reduce plant height, delay flowering and adversely affect yield and lint quality. Chilling injury is the physical and/or physiological changes that are induced by exposure to chilling temperatures. The physiological changes may be considered primary or secondary. The primary injury is the initial rapid response that causes a dysfunction in the plant, but is readily reversible if the temperature is raised to non-chilling conditions (Kratsch and Wise 2000). Secondary injuries are dysfunctions that occur as a consequence of the primary injury and that may not be reversible. The characteristic visual symptoms are the consequence of secondary chilling injuries. Enzymatic reactions, substrate diffusion rates, and membrane transport properties are amongst the main life processes affected by chilling wherein the entire internal environment of each cell and each molecule within the cells are affected. Physiological age, seedling development, and pre-harvest climate can also influence chilling sensitivity. The severity of injury to chill-sensitive tissues tends to increase with decreasing temperatures and with length of low-temperature exposure. Cellular autolysis and senescence is promoted by severe chilling stress. Leaf yellowing due to loss of chlorophyll, may occur in the light as a consequence of photo-oxidation. Loss of membrane integrity that allows the leakage of cellular fluids into the inter cellular (apoplastic) spaces gives chilled tissues a water-soaked appearance, failure to maintain cellular compartmentation and loss of turgor. Electron microscopy studies showed that the mitochondria of sensitive species to be swollen and distorted after chilling. The rates of CO_2 and C_2H_4 production usually increase; prior to the appearance of visual symptoms although C_2H_4 is not a causal agent of chilling injury symptoms (Limin and Fowler 2000). Chilling stress is perceived locally, probably by each individual cell. Chilling injury is not translocatable for example, this can be seen when a cucumber plant was divided so that one shoot was chilled, while the remainder of the plant remained at warm temperatures, the chilling injury was restricted to that one shoot. Individual cell injury is the main event which triggers the symptoms of chilling injury (Chang et al. 2006).

1.2.2 High Temperature

Heat stress often occurs when temperatures are hot enough for sufficient time to cause irreversible damage to plant function or development. In addition, high temperatures can increase the rate of reproductive development, which shortens the time for photosynthesis (Hall 2001). High temperature stress is also considered as a heat-stress effect even though it may not cause permanent irreversible damage to development of the plant because the acceleration does substantially reduce total yield. High day temperatures can directly increase tissue temperatures or indirectly cause plant-water-deficits due to high evaporative demands. Evaporative demand increases exponentially with increase in day-time temperatures and can result in high transpiration rates and low plant water potentials. High soil temperatures generally decrease rate of plant emergence or germination. The maximum threshold temperatures for germination and emergence are higher for warm-season than for cool-season annuals. For example, the threshold maximum seed zone temperature for emergence of cowpea is about 37°C compared with 25°C to 33°C for lettuce. During vegetative stage, high day temperatures cause damage to the photosynthetic machinery and decrease carbon assimilation as compared to optimal temperatures. Extreme temperatures can cause premature death of plants. Among the cool-season annuals, pea is very sensitive to high day temperatures with death of the plants occurring when air temperatures exceed about 35°C for sufficient duration, whereas barley is very heat tolerant. For warm season annuals, cowpea can produce substantial biomass when growing in one of the hottest crop production environments on earth (maximum day-time air temperatures in a weather station shelter of about 50°C), although its vegetative development may exhibit certain abnormalities. In monocotyledons, cool-season and warm-season annuals, high temperatures at daytime can cause leaf firing. Reproductive development in many crops is affected by heat due to which they produce no flowers or flowers do not progress to seed set. The heat tolerance limit of leaves of higher plants overlaps with the thermal sensitivity of primary photochemical reactions taking place in the thylakoid membrane system. Tolerance limits vary between genotypes of the same species and also among species; however, some effects are subject to acclimation to high temperature. Long-term acclimations can be overlaid upon fast adaptive modifications of the thermal stability, occurring in the time range of a few hours. Light causes an increase in tolerance to heat, and this stabilization is related to the light-induced proton gradient. In addition to irreversible effects, high temperature may also cause large reversible effects on the rate of photosynthesis. Energy dissipated by photorespiration can exceed that consumed by CO_2 assimilation, and a reversible, temperature-induced non-photochemical 'quenching' process, related to 'spillover' of excitation energy to photosystem I, decreases the efficiency of photosystem II with increasing temperature (Weis and Berry 1988). However, despite the general drop in the quantum efficiency, CO_2 assimilation may be inadequate, at high temperature by an imbalance in the regulation of the carbon metabolism, which is seen as an effect in down-regulation of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) activity (Leegood and Edwards 2004)

1.2.3 Salinity

The progress of saline stress is generally a three stage process. Firstly, high salt concentrations decrease the osmotic potential of soil solution creating water stress in plants. Secondly, they form the basis for severe ion toxicity; this is due to the fact that the Sodium ion is not readily sequestered into vacuoles as we see in halophytes. Thirdly, the exchange of salts with mineral nutrition results in major and micro nutrient imbalances and deficiencies. The consequence of this three stage process leads to plant death as a result of severe growth retardation and molecular damage. Salinity arises through natural or anthropogenic processes as a consequence of accumulation of dissolved salts in the soil water. Sodicity is a secondary result of salinity in clay soils, where leaching through either natural or anthropogenic processes has washed soluble salts into the subsoil, and left sodium bound to the negative charges of the clay Munns (2004). Salts in the soil water may inhibit plant growth for two reasons. First, the presence of salt in soil solution reduces plants ability to take up water, which is referred to as the osmotic or water-deficit effect of salinity (Munns 2009). On the hand if disproportionate amounts of salt enter the plant system through the transpiration stream, it causes physical injury to cells in the transpiring leaves, which in turn cause decrease in growth. The description of salt tolerance is generally described as the percent biomass production in saline soil relative to plants in non-saline soil, when grown for an lengthy period of time. Salinity imposes not only ionic stress but also osmotic stress. The ionic stress is primarily caused by sodium toxicity to plants. Some plant species are also sensitive to chloride toxicity. In certain saline soils, the ion toxicity is further aggravated by alkaline pH. The osmotic stress caused by high salt stress is often referred to as "physiological drought."

For halophytic plants that are tolerant of sodium toxicity, osmotic stress is the main cause of growth inhibition. However, most crop plants are glycophytes, and are sensitive to relatively low concentrations of salt (Munns 2002). Therefore, ionic toxicity is a significant and often predominant component of salt stress for crop plants. High salinity causes hyperosmotic stress and ion disequilibrium that produce secondary effects or pathologies. Fundamentally, plants cope by either avoiding or tolerating salt stress i.e. plants are either dormant during the salt episode or there must be cellular adjustment to tolerate the saline environment. Tolerance mechanisms can be categorized as those that function to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effects caused by these stresses (Yokoi et al. 2002).

The chemical potential of the saline solution principally creates a water potential difference between the apoplast and symplast that leads to turgor reduction. Growth cessation follows when turgor is reduced below the yield threshold of the cell wall. Cellular dehydration begins when water potential difference is greater than the difference that can be compensated for by turgor loss. The cellular response to turgor reduction is osmotic adjustment. Since plant cell growth occurs chiefly because of directional growth mediated by an escalation in vacuolar volume, compartmentalization of Na^+ and Cl^- enables osmotic adjustment which is vital for cellular development (Munns 2002). As of now there is no indication of adaptations in enzymes to the presence of salt, so mechanisms for salt tolerance at the cellular level involve keeping the salt out of the cytoplasm, and sequestering it in the vacuole. This happens in most species as evidenced by high concentrations found in leaves that are still functioning normally; concentrations well over 200 mM, which are known to completely repress enzyme activity in vitro. In general Na^+ starts to hinder most enzymes at a concentration above 100 mM. The concentration at which Cl^- becomes toxic is not very well defined, but is possible that it is in the same range as that for Na^+. Since Na^+ and Cl^- are sequestered in the vacuole K^+ and organic solutes should accumulate in the cytoplasm and organelles to balance the osmotic pressure of the ions in the vacuole. The organic solutes that accumulate most commonly under salinity are proline and glycinebetaine, although other molecules can accumulate to lesser degrees. Salt tolerant species have transport systems on the tonoplast that can sequester Na^+ and Cl^- at high concentrations within the vacuoles, while maintaining much lower concentrations in the cytoplasmic compartments (Munns and Tester 2008).

1.2.4 Water

1.2.4.1 Drought

Plant water deficit develops as the demand surpasses the supply of water. This is determined by the quantity of water held in the soil to the depth of the root system. The demand for water is dependent on plant transpiration rate or crop evapotranspiration, which includes both plant transpiration and soil evaporation. While some of this energy is important for photosynthesis, most of it is not utilized and it must be dissipated (Blum 2011). It is partly dissipated by radiation emitted from the plant in the form of heat, but most of it must be dissipated by transpiration. Transpiration causes leaves to cool relative to ambient temperature when the environmental energy load on the plant is high (Centritto et al. 2011). The rate of transpiration is also affected by vapour pressure deficit (or relative humidity) and wind. Water deficit has effect on flowering and may manifest as advanced or delayed flowering. Osmotic adjustment induces roots grow deeper under stress. Root distribution within the soil changes as stress develops, in a way that helps the plant to explore soil moisture from deeper layers. In cereals, dry top soil inhibits the formation and establishment of new roots in topsoil while assimilates partitioned to the root are used in furthering the growth of existing roots into deeper soil. In small grains and rice, tillering is associated with the development of new roots from tillers. Therefore, extensive tillering is generally associated with dense and shallow roots while limited tillering is associated with sparser and deeper roots. This is one of the reasons why most cereal crop cultivars developed in dry regions tend to have a limited tillering habit (Bray 1997). It is not known which are the primary mediators of cellular responses to water deficit and their order of importance, be it cellular water status, turgor, bound water, hormones (mainly ABA), cellular membrane functions or other agents. It is also not clear how cells perceive cellular water deficit and how cellular water deficit is transduced and transcribed into the various consequences of this stress (Bartels and Sunkar 2005).

1.2.4.2 Flooding

Crop plants require a free exchange of atmospheric gases for photosynthesis and respiration. The most common impediment to gas diffusion is water that saturates the root environment in poorly drained soils or that accumulates above soil capacity as a result of the overflow of rivers, excessive rainfall or excessive irrigation. Long-term flooding shifts the microbial flora in the soil in favour of anaerobic micro-organisms that use alternative electron acceptors to oxygen (Sairam et al. 2008). As a consequence, soil tends to accumulate more reduced and phytotoxic forms of mineral ions such as nitrite and ferrous ions. Few plants are adapted to grow in such soils. Short-term anaerobic stress to plants due to periodic flooding reduces oxygen levels around roots and influence root development directly, whereas changes in shoot development may follow as a result of metabolic alterations in the roots (Bramley et al. 2007). When the soil is waterlogged, gas exchange between soil and atmosphere becomes negligible. Initially, the flood water contains oxygen, but this is depleted within hours, depending on temperature and respiration rates. Therefore, in nature, the plant experiences hypoxia prior to anoxia, and this gradual depletion has two major effects. The first is a stimulation of ethylene synthesis in response to depression of internal oxygen concentration. Ethylene then initiates and regulates many adaptive responses that allow the plant to avoid anaerobiosis by increasing oxygen availability to the roots in a flooded or waterlogged soil. Furthermore, ethylene triggers other symptoms like epinasty, chlorosis and leaf senescence that enables the plant to cope with low amounts of gas exchange in the roots (Parlanti et al. 2011). In fields with temporarily water-saturated soils or a high water table, roots grow only in a small region near the surface and do not exploit large soil volume as they would under aerated conditions. This makes them more susceptible to subsequent droughts and increases their fertiliser requirements. Long-term flooding promotes senescence and leaf abscission as the consequence of numerous negative and positive signals that accumulate during flooding. The adaptive significance of this response is to reduce the shoot: root ratio as a final adjustment to an impaired root system.

1.2.5 Heavy Metals

Metal contamination issues are becoming increasingly common in cultivated areas. Metals are a natural part of terrestrial systems occurring in soil, rock, air, water and organisms. A few metals, including Cu, Mn and Zn, are however essential to plant metabolism in trace amounts. Heavy metals have become one of the main abiotic stress agents for living organisms mainly due to their increasing use in various anthropogenic activities which causes its high bioaccumulation and toxicity. The effect of their toxic influence on plants is basically inhibition of growth processes of the above and underground parts, in addition to decrease in the activity of the photosynthetic apparatus, which associated with early senescence. The presence of metals in bioavailable forms at disproportionate levels develop the potential to become toxic to plants. Plant responses to metals are by and large dose dependent. For essential metals, these responses cover the phases from deficiency – sufficiency/tolerance – toxicity. For non-essential metals, only the tolerance and toxicity phases occur. The concept of critical or threshold toxicity is frequently used to understand the point at which metals cause substantial growth decreases. Critical concentrations that can cause deleterious effects vary considerably across metals and plant species (Maksymiec 2007; Reichman 2002).

Plants have developed a range of mechanisms to obtain metals from the soil solution and transport these metals within the plant. Understanding of mechanisms of heavy metal toxicity in plants and crops has been at sufficiency and deficiency levels of most metals. Uptake of metals into plant roots is a complex process involving transfer of metals from the soil solution to the root surface and inside the root cells. Understanding of uptake processes is hampered by the complex nature of the rhizosphere which is in continual dynamic change interacted upon by plant roots, the soil solution composing it and microorganisms living within the rhizosphere. The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms which mainly comprise of uptake and accumulation of metals through binding to extracellular exudates and cell wall constituents, efflux of heavy metals from cytoplasm to extranuclear compartments including vacuoles, complexation of heavy metal ions inside the cell by various substances, for example, organic acids, amino acids, phytochelatins, and metallothioneins, accumulation of osmolytes and osmoprotectants and induction of antioxidative enzymes and activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures (John et al. 2009; Cho et al. 2003). Each metal has a different mode of action. However, in general, metal toxicity reduces photosynthesis, affect enzyme and protein production and utilisation, alter nutrient transport.

1.3 Improving Stress Tolerance – Conventional and Molecular Approaches

Although considerable progress was made during the twentieth century to improve crop yield and quality through conventional breeding progress in improving the tolerance of crops against abiotic stresses, is very modest. Nonetheless, the genetic variation of crops was exploited well at intra-specific, inter-specific and inter-generic levels so as to produce stress-tolerant lines cultivars (Ashraf and Akram 2009). As a result some tolerant genotypes of different crops were developed through conventional breeding and tested under natural field conditions. Availability of genetic variation in most of the crop species is one of another problem encountered by conventional breeders. The conventional approach as a whole is time-consuming and labor-intensive; undesirable genes are often transferred in combination with desirable ones; and reproductive barriers limit transfer of favorable alleles from inter-specific and inter-generic sources. Due to these reasons genetic engineering is being employed as a potential option worldwide for improving abiotic stress tolerance. For instance plant engineering strategies for abiotic stress tolerance have been focused largely on the expression of genes that are involved in osmolyte biosynthesis (glycine betaine, mannitol, proline, trehalose etc.); genes encoding enzymes for scavenging ROS (SOD, glutathione S- transferase, Glutathione reductase, glyoxylases etc.); genes encoding LEA proteins (LEA, HVA1, LE25, Dehydrin etc.); genes encoding heterologous enzymes with different temperature optima; genes for molecular chaperons (HSPs);genes encoding transcription factors (DREB 1A,CBF 1, Alfin 1); engineering of cell membranes; proteins involved in ion homeostasis (Trethowan et al. 2010; Valliyodan and Nguyen 2006). Development of transgenic plants has certainly opened a new possibility to enhance abiotic stress tolerance in crop plants. However, to fine-tune transgenic technology into a successful and practical approach, it is important to address issues like using tissue and stage specific and stress inducible promoters to avoid unnecessary biological costs; to target multiple gene regulation rather than single genes; developing near natural field stress evaluation schemes to critically assess the benefits of transgenics rather than at seedling stage and under controlled environments (Bhatnagar-Mathur et al. 2008). Another molecular technology which gained considerable importance in developing abiotic stress tolerance is marker assisted selection (MAS) it improves the efficiency of plant breeding through precise transfer of genomic region of interest (foreground selection) and accelerate recovery of the recurrent parent genome Considerable efforts were made in crops like maize and rice through MAS (Mehboob-ur-Rahman et al. 2011). With the advent of molecular biology techniques it was presumed that developing stress-tolerant cultivars would be convenient and relatively less time consuming. However, the progress so far does not seem to be as rapid as it was envisaged. An effective integration of transgenic, QTL, MAS and genomic approaches into conventional breeding program seems to be the most essential requirement in developing stress tolerant genotypes (Fig. 1.2).

1.3.1 Systems Biology Strategy

Omics is a science and engineering for analyzing the interactions of biological information objects. These include genomics, proteomics, metabolomics, expressomics and interactomics. The main focus is on (1) mapping information objects such as genes and proteins, (2) finding interactive relationships among the objects and (3) engineering the networks and objects to understand and manipulate the regulatory mechanisms (Gu 2008). Bioinformatics refers to the study of biological information using concepts and methods in computer science, statistics, and engineering. It can be divided into two categories: biological information management and computational biology. The potential of omics and informatics as a tool in biotechnology for development of multiple stress tolerant plants and crops is enormous as evident from the hypothetical experimental strategy described below (Shanker et al. 2009).

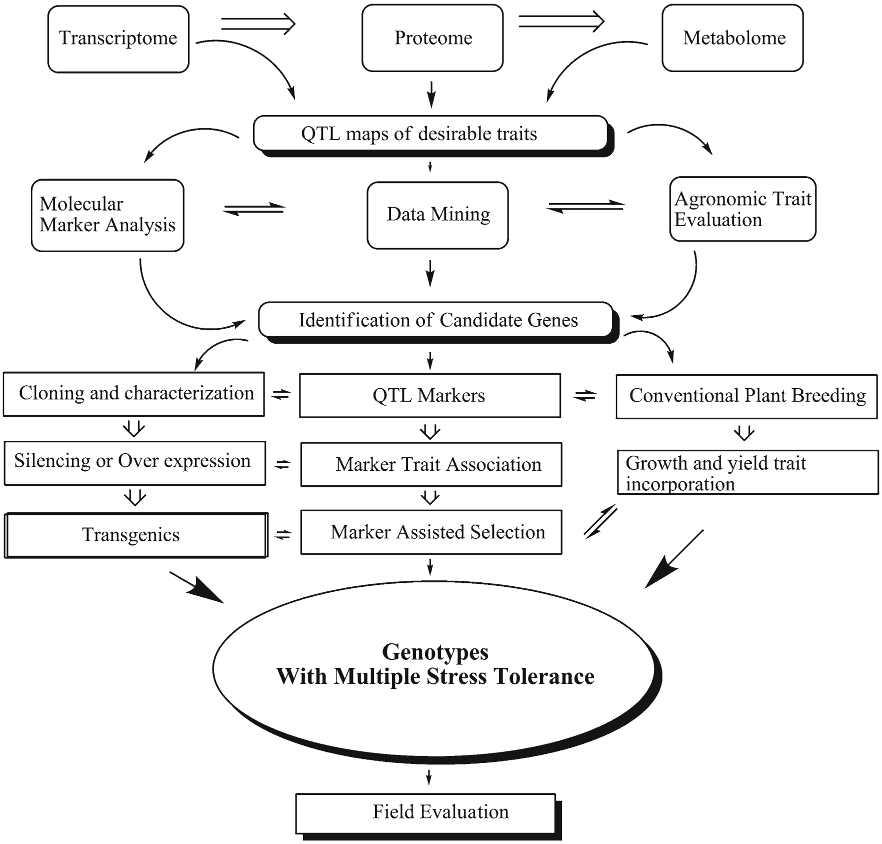

Fig. 1.2 Process network in development of stress tolerant cultivars

A continuing shift to advanced analytical methods and integration of biological experimentation and bioinformatics with these methods will throw up immense meaningful data towards understanding the complete interactome in various crop stresses. An all inclusive omics study of stresses would involve transcript profiling for gene expression, global DNA methylation detection, proteomic characterization by online coupling of electrophoretic techniques, chromatographic separation technique, targeted metabolite analysis and in the case of metal stress a high power sensitive and element-specific oxidation state detection system seamlessly integrated with algorithmic data analysis. The initial step in wholesome omics would be to start with the gene-driven approach – to ask the question as to what is the transcript profile of plants under different biotic and abiotic stresses. The application of microarrays for gene expression profiling has been demonstrated to be one of the most powerful and direct ways of using the sequence data for functional studies.

It represents an approach that is both comprehensive in its scope and high-throughput in its application and can be effectively applied for deciphering the transcriptome in case of stress affected crops and plants and in addition making it online with the other -omics and hyphenated analytical methods would be a worthy strategy.

1.3.1.1 Transcript Profiling

Transcript profiling can be done by taking the Whole Genome Array (WGA) as against cDNA arrays as it often misses very low abundance and non-polyadenylated transcripts and are often devoid of transcripts that are expressed in response to a specific physiological or environmental condition. WGA tiling arrays can also detect alternatively spliced forms which may not have been previously known or predicted. These arrays can be used for gene expression studies by hybridizing targets made from RNA samples of different tissues viz., flower, leaf, root, stem cultured cells, all exposed difference stresses singly or multiple simultaneous (Fig. 1.3). Total RNA is isolated from these samples and double stranded cDNA is synthesized, used as a template for transcription of complementary RNA (cRNA) which equally represents all expressed gene products in the total RNA, in addition to serving as amplification of targets in adequate magnitude for hybridization to WGAs. After that hybridization signal detection and data processing is carried. The normalized signal intensities devoid of noise of each target from repetitive hybridizations is averaged and changes under treatmental condition is calculated as the ratio of the average intensity in treated samples to that in the appropriate control sample. Simultaneously WGA can also be used to map sites of DNA methylation (also known as the methylome) within the specific crop or plant genome used for the study, this technique has been perfected in *Arabidopsis thaliana* and the simplified procedure is to use an antibody that recognizes methylated cytosine bases of genomic DNA of flower, leaf, root, stem and cultured cells all exposed to stresses, these regions are immunoprecipitated then these DNA fragments are super amplified to get higher DNA yield and later they are cut down to small DNA fragments (to increase hybridization efficiency) and hybridized with the WGA. A similar bioinformatics analysis of this microarray can be done to obtain expression patterns. Microarray data of these two processes should be superimposed to obtain a map which would include epigenetic aspects of the stress treatment. Alternatively, total DNA of the samples can be isolated and digested and a global DNA methylation pattern for quantification of 5-methyl-20-deoxycytidine (5-mdC) is arrived at by isocratic cation exchange high-performance liquid chromatography and this can be compared with the processed WGA data. This would be an important aspect in the stress interactome study as stress intensity increases in sequence alterations, extensive methylation changes in CCGG-sequences, and genome-wide hypermethylation leading to epigenetic silencing or reactivation of gene expression has been reported due to biotic and abiotic stresses. The transcriptome analysis is likely to show functionally undefined hypothetical genes and genes with annotated functions as affected by the imposed treatments.

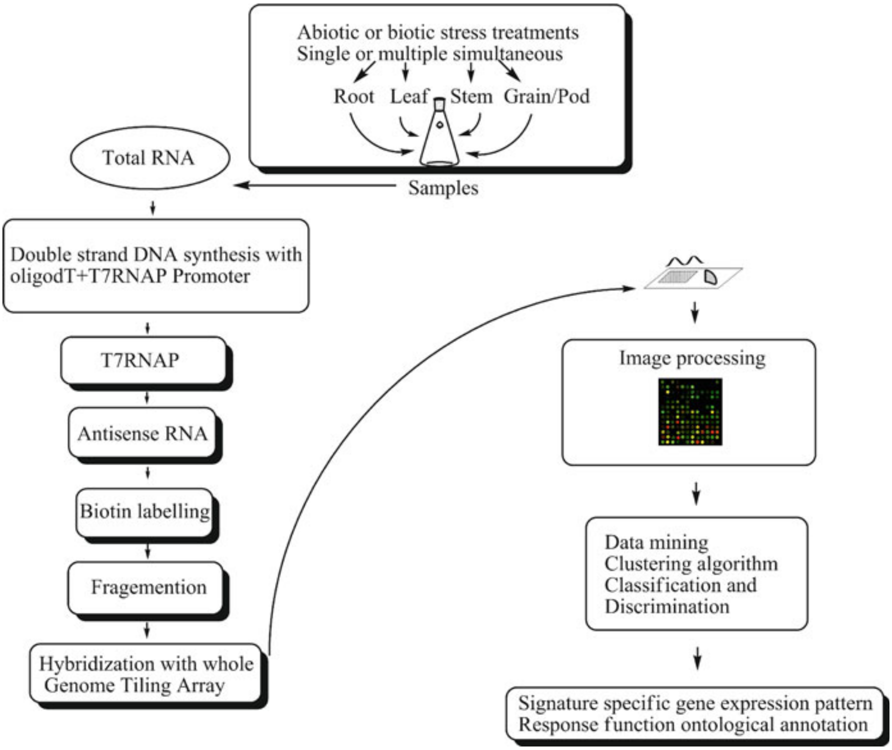

Fig. 1.3 Schematic representation of the transcriptome exemplified for analysis of expression of the whole genome as influenced by biotic and abiotic stress treatments imposed singly or multiple simultaneous

1.3.1.2 Proteome Analysis

The next step would be high-throughput proteomic method, based on LA-ICP-MS to detect stress-proteins in protein bands of 1D gel electrophoresis (1D-GE) or protein spots separated after 2D gel electrophoresis (2D-GE) and matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry (MALDI-TOFMS) analysis of tryptic 2D electrophoresis (2-DE) spot digest and peptide matching with crop/plant protein database. The transcriptome and the proteome data are superimposed to assess the parallelism between DNA transcription and protein expression (Fig. 1.4). The proportion of detectable proteins to that of the transcriptionally active genes will throw light on the physiological, biochemical and molecular mechanism of the stresses in plants in detail. In addition to protein profiling, protein-DNA interaction is of importance especially in the case of heavy metals and some other biotic stresses because stress induced metabolites complexes are known bind to DNA, causing lesions that can alter interactions with proteins and disrupt normal cellular function. An array of various proteins created on a nitrocellulose membrane and screened by using a labelled stress-modified DNA probes containing appropriate promoter region, this method has been used to discover DNA binding ability in proteins with other identified functions. This method offers a high-throughput means for recognizing proteins that bind to a particular DNA recognition sequence, an achievement that is hard to accomplish using other methods.

1.3.1.3 Metabolomic Studies

The next step in the strategy is to construct a complete metabolic profile. The components of the metabolome can be seen as the final products of gene expression and describe the biochemical phenotype of a cell or tissue in comparison with the molecular biological genotype. Quantitative and qualitative measurements of the entire cellular metabolites consequently provide a clear insight of the biochemical status of an organism, an extension of proteomic expression data in relation to pathway dynamics that can be used to monitor and assess gene function. The procedure would involve LCQ-Duo ion trap mass spectrometer fitted with an electrospray source, this hyphenated mass spectrometry method will offer good sensitivity and selectivity, but relatively longer analysis times (Fig. 1.4). The analysis of the metabolome would provide the most complete functional interaction of the stress in question and the crop/plant. On the other hand, transcriptome and proteome profile can effectively point to functionality, and consequently a judicious integrated approach can be adopted with available resources. The all-inclusive quantitative and qualitative scrutiny of all the metabolites contained by a cell, tissue or organism is an extremely difficult goal and is still in its infancy in a given system, even though considerable steps forward are being made.

1.3.1.4 Bioinformatics

Reduction of the dimensionality of the data set and to envisage the data from a metallomics perspective by separating noise from signal is imperative to arrive at a wholesome picture. This would involve apart from the algorithmic methods at every end stage of each component of the –omics study, unsupervised methods such as principal component analysis (PCA), hierarchical clustering (HCA) and K-means clustering and machine learning methods like Markov models, feature extraction and selection and network structure deduction. Although most of the above techniques would be beyond the scope of this chapter a small note on a batch-learning self-organizing map (BL-SOM) would be informative. BL-SOM is an alteration of the original SOM, which provides coloured attribute self-determining maps of data input. In short a matrix is constructed from the transcriptome and metabolome dataset in which signal intensities are ordered in various columns (experimental series) and multiple rows (gene and metabolite IDs). BL-SOM analyzes this integrated matrix of both transcriptome and metabolome data after suitable normalization of the data and initial calculations, this will give us a visual picture of the correlations

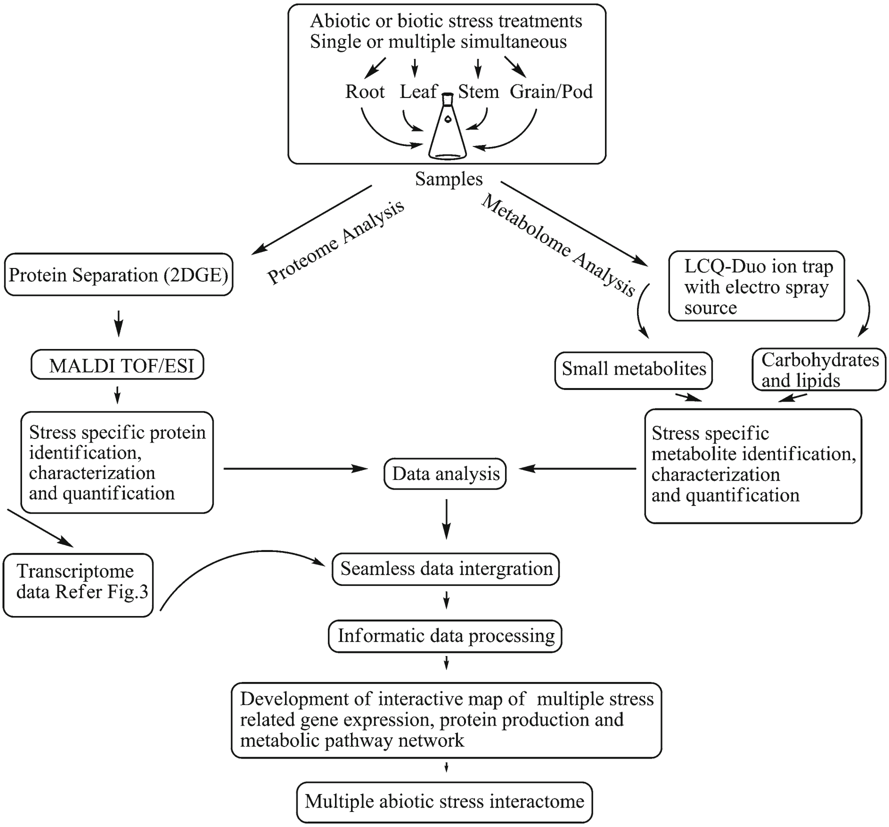

Fig. 1.4 Schematic representation of proteomic and metabolomic data acquisition and integration with transcriptomic data leading to stress interactome

between components. Genes and metabolites are classified into clusters in a two-dimensional "feature map" based on their expression and accumulation patterns. A fairly good picture of the stress interactome can be arrived at by using the above strategy.

1.4 Future Outlook

Crop stresses pose a serious challenge to growth and productivity. The problem may get further compounded by the changing climate scenario. Understanding plant responses to major stresses will help us devise strategies to evolve multiple stress tolerant crop plants. To cope with biotic and abiotic stresses it is of paramount significance to understand plant responses to these stresses that disturb the homeostatic equilibrium at cellular and molecular level in order to identify a common mechanism for multiple stress tolerance at least in the case of abiotic stresses. A very crucial and highly productive role is envisaged here for biotechnology in identifying metabolic alterations and stress signaling pathways, metabolites and the genes controlling these tolerance responses to stresses and in engineering and breeding more efficient and better adapted new crop cultivars. Future strategies should take into account several species combinations and the wealth of genetic diversity existing in the land races and wild relatives and should provide a way to harness the existing evolutionary adaptive diversity to develop multiple stress-tolerant crops. Yield stability should be the top priority in crop breeding programs and it should be kept in mind that increased stress tolerance would be beneficial in terms of only yield stability and not mere survival. An integrated systems approach is essential in the study of complex quantitative traits governing tolerance to multiple biotic and abiotic stresses. Selection for yield and stress tolerance per se necessitates a "top-down" approach, starting from the dissection of the complex traits to components. Marker-assisted selection (MAS) for stress related traits should preferably target 'major' QTLs characterized by a sizeable effect, consistent across germplasm and with a limited interaction with the environment. Analysis of both biotic and abiotic response must: (i) quantify on a field scale the genetic variation for the grain yield response of major crops to stress; (ii) consider both inter-and intra specific variation and classify traits that allow screening of a much wider range of germplasm; (iii) use existing genetic variation and new tools from high throughput omics, quantitative genetics, molecular breeding and bioinformatics to elucidate the mechanisms of crop yield response to stresses Evolving crops traits tolerant to multiple abiotic stresses is still in its infancy. An integrated systems approach is essential in the study of complex quantitative traits which govern tolerance to multiple abiotic stresses from the current work much clearer picture of abiotic stress signal transduction pathways is likely to emerge and more examples of genetic improvement for multiple tolerances by fine-tuning plant sensing and signaling systems. The research essentially must use the latest omics resources combining novel technologies in quantitative genetics, genomics and bioinformatics to come up with an ecophysiological understanding of the interactions between crop/plant genotypes and the changing environment. Most current research programmes lack this interdisciplinary approach. Such co-ordination of the various concepts and use of methods is particularly appropriate for the projects in Asian countries. The clustering of these projects should yield substantial added value (Gregory et al. 2008). Multiple abiotic stress signaling has mainly continued to be a mystery until lately. Although the molecular characteristics of major signaling elements have been recognized we are yet a great distance from having a perfect picture. The primary struggle in solving the puzzle is not having all the pieces of it. Consequently, the test in the near future remains to recognize and categorize all the signaling elements. Signaling has been a major area of research in the quest for stress tolerance mechanisms. Any signaling component has to be established by functional obligation and functional sufficiency. That is to say, plant phenotypes, be they molecular, biochemical, or physiological, are essential to establish precisely that a specific factor functions in stress signaling (Zhu 2002). The host of genomics tools has provided a wealth of data for enhanced understanding of the changes in cellular metabolism but fewer results have been useful with respect to the functioning of the whole plant. The conversion of many data points into understanding is still incomplete. Incorporation and sifting of data and validation by independent means in combination with cutting-edge bioinformatics tools will lessen this deficit. An understanding of plants as a system of interacting functions will emerge, but a more immediate problem seems to be finding applications for all this knowledge (Bohnert et al. 2006).

Probing for and recording quantitative traits has the advantage of a balanced method. Systems biology platform provides high value information on the molecular means of massive adaptive functional diversity in several characters involved in stress tolerance. Such strategies would deliver a way to harness the existing evolutionary adaptive diversity to develop stress-protected crops in which growth and yield are less compromised by both biotic and abiotic stresses. The practical value of any genes or pathways for stress tolerance in crop plants can only be useful if there is evidence of superior performance in the field especially in terms of yield. Eventually, the functional determination of all genes that contribute to biotic or abiotic stress adaptation is likely to provide an integrated understanding of the biochemical and physiological basis of stress responses in plants. Armed with such evidence from conventional and cutting edge models, it will be possible to sensibly deploy and augment tolerance traits for improved crop productivity well into the future (Cushman and Bohnert 2000). To achieve such a goal, an interdisciplinary and inter institutional approach would be needed with well-defined targets on crops and problems prioritized at the national and international level.

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Chapter 2
Dryland Agriculture: Bringing Resilience to Crop Production Under Changing Climate

B. Venkateswarlu and Arun K. Shanker

Abstract Drylands of the world are affected in addition to the impending climate change by various other inherent biotic and abiotic limitations like water availability, declining soil quality and pest and disease infestations. The challenges facing dryland agriculture, global food security and the sustainable management of natural resources are many and are interrelated. Productivity of dryland crops can be increased only if the problems are understood well and in turn combated effectively. Major dryland agro ecological regions of the world and their problems are outlined in this chapter. Sustainable Natural Resource Management (SNRM) is stressed here as an important way to addresses the problems faced by these regions of the world. Resilience to predicted climate change will depend on increasing agricultural productivity with available water resources; refining technologies and timely deployment of affordable strategies to accomplish potential levels of arable land and water productivity. An account into the adaptation strategies to increase resilience to combat climate change related effects by management of water, soil and biodiversity are detailed here. We propose here that research on adaptive capability of crops by increasing their resilience to abiotic stresses, pests and diseases will have to expand to new horizons with a systems biology perspective.

2.1 Introduction

The challenges facing agriculture, global food security and the sustainable management of natural resources are many and are interrelated. Global climate change poses greatest danger to world food security and sustainable development. This is evident

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in the drylands of the world which are affected in addition to the impending climate change by various other inherent biotic and abiotic limitations like water availability, declining soil quality and pest and disease infestations. In addition to the subtle variation in climate that affect normal crop growth and productivity, drought, extreme weather events such as heat waves, cyclones, intense rainfall, frost and hailstorms and dust storms also cause adverse effects on agriculture across the arid and semiarid regions of the world (Holmgren et al. 2006). The growing world population together with the lack of expansion in arable lands underscores the importance of raising crop yields and adaptation to climate. There is an urgent need to assess the impact of climate change on dryland agricultural production systems, and food security in specific regions of the world (Fedoroff et al. 2010). Design and implementation of effective coping mechanisms against biotic and abiotic stresses that threaten crop productivity in these regions requires a clear and scientific understanding of the effects and impacts so that appropriate adaptation and mitigation measures can be formulated. Cereal food grain production systems in the drylands should be considered as one of the main targets for improvement in order to achieve food security. Globally, 69% of all cereal area is rainfed, including 40% of rice, 66% of wheat, 82% of maize and 86% of other coarse grains. Worldwide, rainfed cereal yield is about 2.2 metric tons per hectare, which is about 65% of the irrigated yield (3.5 metric tons per hectare). Rainfed areas currently account for 58% of world cereal production (Rosegrant et al. 2002). Although expansion of production into marginal areas is one of the options available to increase rainfed food grain yield this has several negative environmental and social impacts such as soil erosion and increasing poverty, leading to increased malnutrition and poor health. Growth in crop yield in rainfed farming systems is the only sustainable option. For this to be achieved various improved technologies should be strategically deployed along with suitable policy measures.

2.2 Major Dryland Agricultural Regions of the World

Drylands are found in climate regimes that are not very favorable to crop production. They are typically characterized by low annual rainfall (300–500 mm per annum or less) and its high variability presenting tough challenges for growing crops successfully with minimum crop need for water in excess of rainfall (Fig. 2.1). Arid and semiarid regions where rainfed farming is practiced comprise almost 40% of the world's land area and are populated by some 700 million people. Roughly two-thirds of these drylands are in developing countries where population growth is high. Many dryland regions are characterized by extremely fragile natural resource base (Stewart et al. 2006). Soils are often coarse textured, characteristically low in fertility, organic matter and water-holding capacity and highly vulnerable to wind and water erosion. Africa includes about one-third of the world's total semi-arid lands. Semi-arid lands occur also in Eastern Europe, Middle East and Western Asia. In Eastern Asia, semi-arid lands occur within summer precipitation zones (Russia, China, Mongolia,

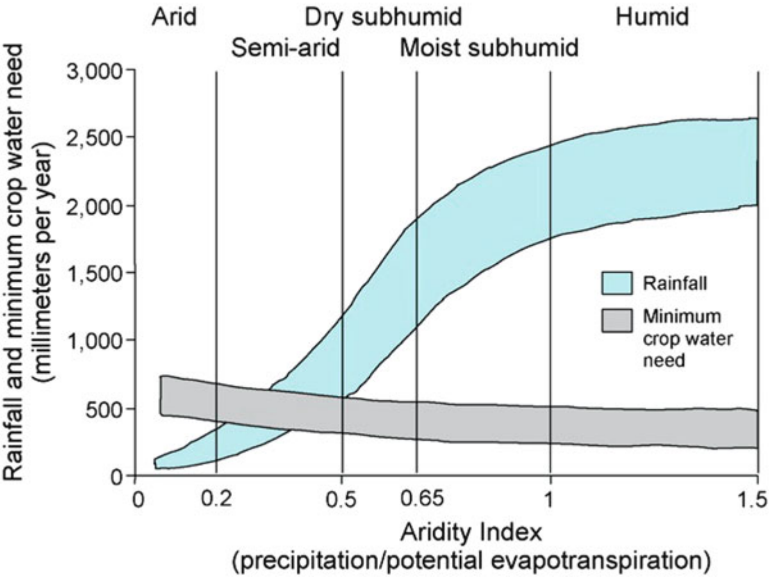

Fig. 2.1 Range of rainfall variability across hydro-climatic zones from arid to humid agroecosystems (Source: Rockström et al. (2010) with permission from Elsevier B.V.)

and India). About 75% of Australia is arid or semi-arid. In South America, semi-arid lands are mainly located in Argentina. Semi-arid lands of North America extend from Mexico to Canada: Great Plains, Pacific Northwest region, Southwest Pacific region of California (Ellis and Ramankutty 2008). The expanse of major drylands in the developing regions of the world are the Sahel countries of Senegal, The Gambia, Mauritania, Mali, Burkina Faso, Niger and Chad, more northerly and drier regions of the West African coastal states. The Greater Horn of Africa – Sudan, Ethiopia, Eritrea, Djibouti, Somalia, and parts of Kenya, Uganda and Tanzania. Southern Africa: Namibia and Botswana, large parts of Zimbabwe, South Africa and Mozambique and parts of neighboring states. West Asia and North Africa: a belt from Morocco to Iran, Yemen and Oman. In South Asia: some arts of Pakistan and major parts of India. Significant populations live in rainfed farming areas such as peninsular India, and in arid zones of the Thar Desert on the border between the two countries. Central and Inner Asia: Countries of the former Soviet Union, Mongolia and parts of China face cold winters as well as hot, dry summers. Latin America includes several distinct dryland regions, including Northern and some parts of Southeastern Mexico, parts of the Andes (that face rather different challenges as dry highland regions), the Chaco region and the densely populated, poor and drought-prone areas of Northeastern Brazil (Biradar et al. 2009; Table 2.1).

Table 2.1 The global dryland areas by continent

	Extension (Mha)			Percentage		
	Arid	Semi-arid	Dry subhumid	Arid	Semi-arid	Dry subhumid
Africa	467.60	611.35	219.16	16.21	21.20	7.60
Asia	704.30	727.97	225.51	25.48	26.34	8.16
Oceania	459.50	211.02	38.24	59.72	27.42	4.97
Europe	0.30	94.26	123.47	0.01	1.74	2.27
North/central America	4.27	130.71	382.09	6.09	17.82	4.27
South America	5.97	122.43	250.21	7.11	14.54	5.97
Total	1,641.95	1,897.74	1,238.68			

Source: FAO (2009)

2.2.1 India

India has about 85 million hectares of rainfed area which constitutes nearly 58% of the total 141 million hectares of arable land. Most dryland areas in India have more than 7 months with essentially no precipitation. A monsoon season of varying length is prevalent when the precipitation greatly exceeds the potential evapotranspiration for at least a portion of the growing season. In such areas crop production becomes comparatively difficult as it mainly depends upon intensity and distribution of rainfall. Crop production, consequently, in such areas is primarily rainfed as there is no facility to give any irrigation, and even protective or lifesaving irrigation is often difficult. These areas get an annual rainfall between 400 and 800 mm which is unevenly distributed, highly uncertain and erratic (Krishnamurthy and Shukla 2000). In certain areas the total annual rainfall does not exceed 500 mm (Guhathakurta and Rajeevan 2008). The crop production, depending upon this rain, is technically called dry land farming and areas are known as dry lands. India has about 47 million hectares of dry lands out of 85 million hectares of total rainfed area. Rainfed areas contribute 40% of the total food grain production of the country. These areas produce 75% of pulses and more than 90% of sorghum, millet, groundnut and pulses from arid and semi-arid regions (Chattopadhyay 2011). Thus, dry lands and rainfed farming will continue to play a dominant role in agricultural production. Scientific study of dryland farming was started by the Government of India in 1923. Research mainly was focused on improving crop yields. Bunding to conserve soil and water and deep ploughing once in 3 years for better intake and storage of water, proper use of farmyard manure to supply plant nutrients; and low seeding rate with intercultivation for weed and evaporation control were the main practices recommended in early part of the twentieth century. By the mid-1950s, the emphasis had shifted to soil management. Soil conservation research and training centres were established at eight locations, focusing on contour bunding. The importance of shorter-duration crops to match the soil-water availability period was recognized in the 1960s. It was also in the mid-1960s that high-yielding cereal hybrids and cultivars became available that were responsive not only to fertilizers but also to management. An All India Co-ordinated Research Project for Dryland Agriculture (AICRPDA) was launched by the Indian Council of Agricultural Research (ICAR) in 1970 in collaboration with Government of Canada and later Central Research Institute for Dryland Agriculture (CRIDA) was established at Hyderabad.

2.2.2 China

Arid and semi-arid lands in China cover 52% of the country located mainly between 30°N and 50°N (Li and Xiao 1992). Rainfall is very variable; annual totals average 300–500 mm, and increase gradually from northwest to southeast (Li et al. 2007; Liu et al. 2005). Rainfall from June to September accounts for 70–80% of the annual total. Dryland agriculture (crops and pastures) accounts for more than 70% of total farmland in northern and north-western China, including the vast rainfed areas to the north of the Qinling Mountains and Huaihe River. Twenty five million hectares are located in the Loess Plateau, while the North China Plain has 16 million hectares of arable land and produces about 20% of the nation's food (Bai et al. 2009). Li and Xiao (1992) reviewed the characteristics, and management of the drylands in China. Management was concentrated on controlling erosion, and making effective use of the precipitation. In China, the drylands make up more than half of the land area, they account for only 30% of the arable land. Increase in soil-surface roughness was the basic tillage principle followed for conserving soil and water in China. This was done largely by: contour ploughing, contour planting, digging pits for seeding, contour plough furrows, and cultivation in pits or furrows. Increasing crop cover was another important practice used in designing soil- and water-conserving cropping systems. Narrow crop rows, intercropping and interplanting are widely used. These practices help produce dense crop canopy, in turn reducing raindrop impact on the soil surface thus maintaining soil permeability, and reducing runoff and erosion. Fallowing has also been considered an important practice for restoring soil water and fertility in China. Ploughing to a depth of about 20–30 cm has been used widely in dryland farming as an effective method for storing precipitation. Deep ploughing is generally carried out in the summer, but not in the spring. As in India, soil mulching is commonly used in China for conserving soil water, and research has shown it to be beneficial (Wang et al. 2007). The use of plastic mulches in China has expanded rapidly. Ma Shijun (1988) reported that there were more than 1.3 million hectares of plastic mulch in use, mainly in the northern provinces of Liaoning, Shanxi, and Shandong, and in the Xinjiang Autonomous Region. Plastic-film mulch was used on about half of the cotton and peanut fields and accounted for more than three-quarters of the fields using plastic mulch. The use of plastic mulches in China has continued to increase water use efficiency in drylands at a rapid rate. However, the driving factor for this practice has been the desire to increase soil temperature rather than reduce soil-water evaporation. The key features of modern technological interventions to combat climate change related effects in China include selection for drought resistance with crops having low water requirement and high yield potential, intercropping systems with high yield and high returns. Nutrient management with emphasis on balance of fertilizer and water use efficiency, tillage management and polythene film mulching techniques, establishment of water storage tanks for harvesting runoff to provide lifesaving/critical irrigation, ammonia-treated wheat straw and silage (corn stem) as livestock forage have been recommended and adapted widely in China. China has the world's largest national agricultural research system which has added a new Center of Excellence for Dryland Agriculture in 2009 with active collaboration with Consultative Group on International Agricultural Research (CGIAR) system.

2.2.3 Australia

Dryland agriculture is practiced in Australia generally between the latitudes 21°S–37°S (with some extending to 42°S) in the semi-arid to dry sub-humid regions, which receive 300–600 mm of annual rainfall. Dryland agriculture has been tried in the semi-arid tropics of northern and north-western Australia (i.e. latitudes of 15–20°S) with little success due economic, social and technical limitations, these are primarily extensive beef production areas (Carberry et al. 2010). Dryland agriculture largely fits the warm, seasonally wet/dry agro-climates in Australia's semi-arid areas, where mixed crop/livestock systems dominate and comprise varied enterprises where wool production, dual-purpose flocks, prime lamb and beef production are integrated with cereal, pulse and oilseed crops and where forage crops are incorporated into the cropping sequence. Dryland agriculture supports 80% of Australia's sheep, 50% of its meat cattle and 93% of its grain production. The cropping of pasture legumes, particularly subterranean clover, fertilized with superphosphate, resulted in substantially improved productivity of Australian dryland agriculture since the 1950s. Later developments to cropping practices such as stubble retaining, crop rotation, and soil fertility management, combined with disease resistance and improved quality of crop and pasture varieties, has led to increased productivity of mixed farming systems. Improved grain yield under limited water supply is one of chief constraints in food grain production in Australian drylands (Carberry et al. 2010). Novel farming practices have been introduced to counter the highly erratic nature of the rainfall; the examples of which are the use of fallows in the northern cropping region and timing and adjustment of fertiliser inputs in the southern and western regions. Molecular breeding programs and conventional breeding has contributed significantly to increased crop production (Fleury et al. 2010). Conservation agriculture has been widely adopted in most regions of Australia which has raised crop productivity over the past 30 years. Conservation tillage practices have resulted in increased water use efficiency of crops in Australian dryland farming. Efficient crop nutrition, improving soil biota, residue management and crop rotation, are the key aspects of conservation agriculture followed in Australian drylands. Dryland agricultural research in Australia has not only resulted in achievement of higher yields, but also agronomic and business management practices that reduce production risk (Bowmer 2011). The Commonwealth Scientific and Industrial Research Organisation (CSIRO) Sustainable Agriculture Flagship has played an important role in the generation of technology suited to dryland farming in Australia.

2.2.4 West Africa

Major Drylands in this region constitute of the Sub-Sahelian part of West Africa which lies between 10°N and 14°N of the equator and is largely semiarid, with 300–800 mm annual rainfall. Soils are mostly Aridisols, Inceptisols and Alfisols which are highly susceptible to wind and water erosion. An estimated soil loss of 10–50 tons/ha takes place from wind alone. Rains are highly erratic; with frequent droughts that may continue for several consecutive years. The erratic character of the rains makes dryland farming very unreliable and risky despite the fact that high rainfall during some parts of the year is usually seen (Mortimore 2001). Sorghum and millets are the principal crops in the African summer rainfall zones in the sub-Sahelian area. The region is characterized by soils with low soil fertility, acid soils, low nutrient holding capacity, and high phosphorus fixation as well as compaction and hardening of soils leading to poor water infiltration. One of the major innovation in dryland agriculture in this region has been the introduction of the "New Rice for Africa", a hybrid between Asian and African species, bred to fit the rainfed upland rice environment in West Africa. It produces over 50% more grain than current varieties when cultivated in traditional rainfed systems without fertilizer. In addition to higher yields, these varieties mature 30–50 days earlier than current varieties and are far more disease and drought tolerant than previous varieties (Kishine et al. 2008). This region is also characterized in general, by high population growth which has led to extensification of arable farming and heightened the competition between grazing and cropping systems. In addition to this the cultivation of marginal lands and changes in farming systems (e.g. no or shorter fallows) have rendered farmers more susceptible to climatic risks. In general there has been declining land productivity in this region. Yield growth of cereals such as maize, millet, and sorghum, rarely exceeds 1% in a year in most countries of these regions (Woodhouse 2009). There is a concentrated research and development effort by the countries themselves and also significant inputs from CGIAR institutes which has shown some promise for increasing in productivity in future.

2.3 Climate Change Scenario in Drylands

The Intergovernmental Panel on Climate Change (IPCC) Working Group 1 marked changes in precipitation over large regions in the drier areas of the world particularly in the Sahel, the Mediterranean, Southern Africa and parts of southern Asia. The intensity and duration of droughts have become longer in these areas since the

1970s, particularly in the tropics and subtropics. This analysis of climate change is associated with higher temperatures, reduced precipitation, changes in sea surface temperatures, erratic wind patterns, and to some extent decreased snow cover (Solomon 2007). IPCC Working Group 2 has noted that, in the Sahel, warming plus reduced rainfall has resulted in reduced length of the vegetative period of various millet varieties no longer allowing them to complete their cycle. Increased occurrence of droughts, longer dry spells and periods of abnormally heavy rainfall is a noteworthy feature of climate change trend (Miyazaki et al. 2010). These when combined with the well-known negative impacts of drought across dryland farming regions of the world will compound the observed impacts of climate change. This is bound to result in drastic fall in food grain production unless combated effectively. Climate constitutes of several inter-related variables. Temperature, evaporation and precipitation are strongly correlated variables which induce erratic changes in the hydrological cycle. This change in combination with the projected pressure on land and water use will become a key issue in sustaining production in rainfed areas of the world. The historical climate record for Africa shows warming of approximately 0.7°C over most of the continent during the twentieth century; a decrease in rainfall over large portions of the Sahel (the semi-arid region south of the Sahara); and an increase in rainfall in east central Africa. Over the next century, this warming trend and changes in precipitation patterns are expected to continue and be accompanied by a rise in sea level and increased frequency of extreme weather events (Patt et al. 2010). In Africa, which is expected to be worst affected by climate change, the estimated projection is increase of Global mean surface temperature between 1.5°C and 6°C by 2100. Sea levels are projected to rise by 15–95 cm (6–37 in.) by 2100. Climate change scenarios for Africa indicate future warming across the continent ranging from 0.2°C per decade (low scenario) to more than 0.5°C per decade (high scenario) (Collier et al. 2008). This warming will be greatest over the interior of semiarid margins of the Sahara and central and southern Africa. In India analysis of data by the Indian Meteorological Department (IMD) in Pune for the period 1901–2005 suggests a rise of 0.51°C annual mean temperature for the country as a whole. Since 1990, minimum temperature is steadily rising and rate of its rise is more than that of maximum temperature. Season-wise, maximum rise in mean temperature was observed during the Post-monsoon season (0.7°C) followed by winter season (0.67°C), Pre-monsoon season (0.50°C) and Monsoon season (0.30°C). During the winter season, since 1991, rise in minimum temperature is appreciably higher than that of maximum temperature over northern plains (Kumar et al. 2006). Upper air temperatures have shown an increasing trend in the lower troposphere, and this trend is significant at 850 hPa level, while decreasing trend (not significant) was observed in the upper troposphere. In general the climate change trend in Indian dryland areas will have no significant change in total rainfall but increased frequency of torrential rainfalls and extreme events are predicted (Joshi and Pandey 2011; Sivakumar and Stefanski 2011). The El Nino-induced irregular weather tends to be increasing in its incidence of occurrence, scale, extent and irregularity in recent years in many parts of the world. Heavy rainfall and flooding in Europe, Russia and China and on the other hand, the drought in some regions of China and South Asia are also some of the projected climate change scenarios. The analysis of the 160-station climate data over China indicated that the temperature over North China in the 1980s increased by 0.54°C when comparing with the 1950s (Yanxiang et al. 2007). There is a warming band from east to west across China's Main land and the warming mode still remains in the eastern China, with the warming center in North China. It is anticipated that the warming range of North China in winter will ascend step by step in the future 30 years of twenty-first century and the warming center will be about 2.5°C in 2030 (Shi et al. 2007). In the winter of 2030, the distribution of precipitation in China will be wetter in north and drier in south, and relative less precipitation in central China, while there will be more precipitation in North China (Yanxiang et al. 2007; Fu et al. 2009). In Australia, the scenario is equally bad with adverse implications for rainfed cultivation. Temperature increases of 0.5–1°C by 2030 and of 0.5°C–2°C by 2070 are projected. This compares with a warming of 0.7°C from 1950 to 2006, so Australia warms faster over the next two decades than it has done over the previous five with at least 0.2°C per decade over the next two decades compared with about 0.1°C per decade over the last five. The very hottest days are forecast to increase in frequency in regionally variable amounts with the number of days with over 40°C could double by 2040. Under an optimistic scenario the number of days above 40°C in Melbourne is expected to double by 2030 and cold days and nights will occur less often (Beazley 2007). Most climate models show that the frequency of rainfall events across Australia is likely to reduce and the amount of extreme rainfall will increase, in addition tropical cyclones are also predicted to increase as a result of climate change (Murphy and Timbal 2008; Nicholls and Collins 2006).

2.4 Challenges to Agricultural Production in Drylands Under Changing Climate

The most important challenge faced by rainfed farm lands of the world is the management of the resource of these areas to sustain the current needs without compromising the resource quality to meet the needs of generations which can be termed as Sustainable Natural Resource Management (SNRM). Adoption of SNRM is all the more difficult under changing climate, in other words, we need climate resilient dryland farming. A schematic framework for SNRM is represented in Fig. 2.2. Resilience can be described as the ability of an ecosystem (drylands in this case) to recover from and also to resist abiotic and biotic stresses that undermine crop production. In order for dryland and rainfed framing ecosystems to endure under unclear and changing environments they must be resilient (Chapin III et al. 2010). This resilience can only be brought about by strategic deployment of technology and innovations on a spatial and temporal context. Thus a major objective of SNRM will be to impart resilience to dryland and rainfed agro-ecosystems.

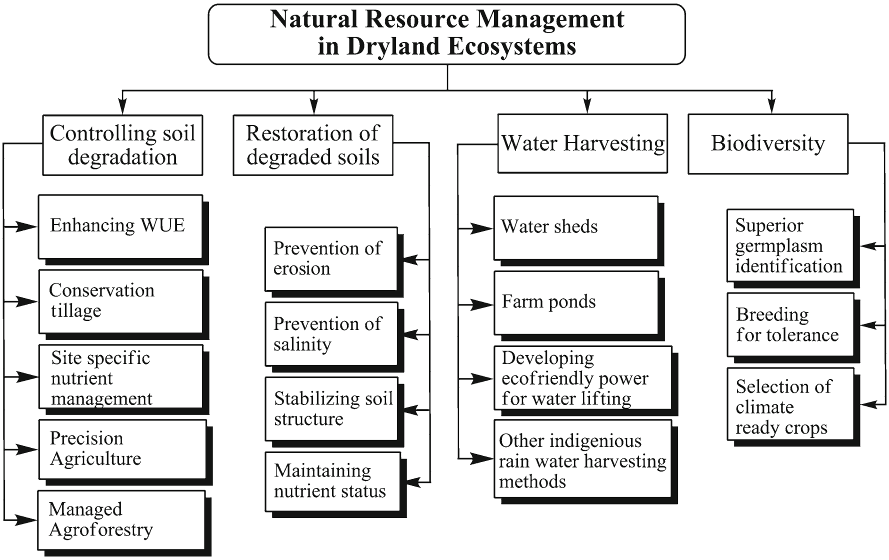

Fig. 2.2 Schematic framework of natural resource management in dryland ecosystems

2.4.1 Water

Increased water stress to crops is predicted as a result of climate change in many drylands, thus water and its effective use present one of the foremost challenges to crop production in the rainfed agriculture. A mean value of 1,300 cubic meters per capita per year (m^3 cap^{-1} $year^{-1}$) of fresh water are required to produce the food for a healthy diet of 3,000 kcal cap^{-1} day^{-1} which includes a fifth of this provided by meat (Rockström et al. 2007). This implies that of about 8,000 km^3 $year^{-1}$ of water is consumed in terms of evapotranspiration from rainfed and irrigated land to feed the current world population (Rost et al. 2008), and in addition to this 5,000 km^3 $year^{-1}$ will be necessary if the population increases to ten billion in 2050 as advocated by the IPCC's SRES A2r scenario (Rost et al. 2009). The above statistics emphasizes the importance of water in rainfed farming in the coming years. Productive dryland landscapes rely on redistribution and storage of water in the soil. Drastic decrease in water availability for agricultural use is one of the most intense and negative impacts of climate change predicted for many parts of the world. These areas will become affected by recurrent droughts, higher evapotranspiration, changes in rainfall patterns and increased salinization. Atypically, a rise in heavy precipitation events is anticipated with a drop in the evenness of rainfall distribution adding to the risk of both flooding and drought for the main crops in these areas (Thomas 2008). Under this condition if appropriate adaptation measures are not correctly followed crop yields in the arid and semi-arid regions of South East Asia, Northern

Africa, Southern Europe and the Middle East are expected to decrease by as much as 10–30% by the 2080 (Tubiello and Fischer 2007). In most of the dryland areas, there is an evident lack of irrigation water – rainfed crop and pasture yields are both low and uncertain. Runoff, evaporation and deep percolation from the soil surface severely reduce the quantity of rainfall available for plant growth. Rainfall has an important role in maintaining groundwater; the relatively inadequate recharge of groundwater resources is dependent largely on the quantity, intensity and period of the rainfall. On the other hand soil properties like infiltration and water holding capacity also influence the amount of surface runoff. Much of the rainfall is lost by evapotranspiration or runoff in drylands resulting in poor recharge (Langhans et al. 2011). Surface runoff, soil-moisture storage, and groundwater recharge rates in dryland regions are highly vulnerable to changing climate.

2.4.2 Temperature

Temperature is a fundamental environmental variable of climate that influences the growth and yield of crops. The phenology and development of crop plants is controlled by air and soil temperature. Temperature stress is defined as prevalence of higher than normal temperatures during crop growth period that cause irreversible damage to plant function or development. Furthermore, high temperatures can accelerate the rate of reproductive development, which in effect curtails the time for photosynthesis to contribute to fruit or seed production in terms of dry matter accumulation (Zinn et al. 2010). The degree and the duration of heat stress and increased night temperatures that occurs in dryland ecosystems as a result of changing climate is a matter of concern. Crop growth and yield can be impaired in diverse ways by either high day or high night temperatures and in addition, to this soil temperatures also play an important role in the response of crops to heat stress. Additional challenge to temperature increase stems from the fact that higher temperatures will increase the rate at which plants lose moisture resulting in increased transpiration and water loss. Temperature affects the stages of development of crops during its progress to physiological maturity, the main stages in food grain crops that are sensitive to temperature are (i) germination (ii) canopy and leaf area development (iii) flowering and reproductive development (v) grain development – anthesis to maturity (Zacharias et al. 2010; Körner and Basler 2010). In general development quickens as temperature increases, a phenomenon called as a linear function of daily mean temperature. The growing degree day concept is an example of a linear model of developmental response to temperature. While a linear model describes the development of a crop when temperatures stay within the optimum range of 10–30°C, a non-linear model is used to describe growth when a crop is exposed to high temperature stress for longer durations (Schlenker and Roberts 2009). Rice is sensitive to high temperature at anthesis inducing sterility if temperatures exceed 35°C and lasts for more longer times, similarly high temperatures can cause spikelet sterility in dry and monsoon season crops in parts of Asia, and in tropical Africa

(Peng et al. 2004). Variations in minimum and maximum temperature as well as day and night temperature are expected due to climate change in dryland areas of the world, wheat for example responds in a complex way to these changes with results of studies showing that the negative effects of a rise in mean air temperature on simulated yields were smallest when the minimum temperature increased more than maximum temperature (You et al. 2009).

2.4.3 Soils

Soils of the drylands are by far the most affected by changing climate which includes changes in rainfall pattern, soil temperature and altered patterns of runoff because soils are basically multi-dimensional porous systems in which numerous physical, chemical and biological/biochemical processes take place. Apart from minerals, organic matter, salts, water and air, soils contain fauna up to 0.25 kg m^{-2} and 30 cm depth, primarily consisting of bacteria, actinomycetes, fungi, earthworms, and other animals (Blum 2005). Higher temperature increases the rate of chemical and biochemical reactions which in turn has a direct effect on soil respiration. Increased respiration can contribute to more carbon dioxide from soil microbes and roots (Davidson and Janssens 2006). First generation climate–carbon cycle models suggest that climate change will suppress carbon accumulation in soils, and could even lead to a net loss of global soil carbon over the next century. Changes in soil carbon status is also a matter of concern under changing temperature and rainfall regimes in drylands since soil carbon not only is important for growth and development of the crop but also for retention of water and nutrients and as an energy source for decomposition process in the soil (Davidson and Janssens 2006; Luke and Cox 2011). The risk of increased erosion is imminent in soils of dry agro ecosystems. High and extreme precipitation will increase runoff primarily due to the inability of the soils to absorb and hold water (Nearing et al. 2004). Extended dry periods will reduce vegetation cover which again will result in substantial runoff. Such erosion events occurring frequently will lead to ecosystem change and also loss of soil nutrients. In addition aridity can hinder surface decomposition and nutrient recycling, thereby affecting crop productivity (Clair and Lynch 2010). Climatic warming will impact soil food webs through a rise in annual mean temperatures, via warmer winter temperatures, or by increasing the frequency of temperature extremes in the soil environment. Soil temperature increases predicted under changing climate may have relatively benign effects on soil biology, however, temperature extremes could bring about substantial negative effects by triggering periods of critical stress for soil organisms (Darby et al. 2011).

2.4.4 Pests and Diseases

In general crop pests and diseases could potentially deprive humanity of up to 50% of the attainable yield in major crops and, when combined with postharvest spoilage and deterioration in quality, these losses become critical, particularly for resource-poor dryland and rainfed regions of the world (Newton et al. 2011). In effect, devastations from plant diseases can be far reaching and alter the course of society and political history as seen in the cases of Irish potato famine and the Bengal famine (Swaminathan 2010). The nature of pathogen interaction with crop productivity under changing climate is complex because every component of the hostpathogen/microbe interaction and the pathogenic complex will have a diverse interaction with the environment, and consequently will alter under different climates. Direct effects of pathogens or other organisms can be the induction of resistance or susceptibility and its associated cost or benefit to the host plant (Newton et al. 2011). The likelihood of most damaging impacts of diseases and pest can be expected in especially cereals like wheat and rice. These are likely to have a large impact in terms of food security under climate change scenarios as seen in the case of wheat (Chakraborty et al. 2010). Changes in levels of CO_2, ozone and UV-B will have an influence on diseases by modifying host physiology and resistance mechanisms. Furthermore, changes in temperature, precipitation and the frequency of extreme events will influence disease epidemiology. An acute change that may arise in the host as an outcome of climate change and the subsequent indirect effects on the pathogen is a possible outcome. Changes in geographical distribution will possibly alter the comparative importance and range of diseases and may give rise to new disease complexes. Evolution of pathogen populations may hasten from enhanced UV-B radiation and increased fecundity under elevated CO_2. Consequently, host resistances may be overwhelmed more swiftly. Specifically increases in leaf waxes and epidermal thickness as a result of increased CO_2 atmospheres can result in the host exhibiting higher physical resistance to some pathogens. Carbon dioxide induced alterations in the architecture of a crop, could lead to increased humidity inside the canopy and can create additional favorable condition for pathogen survival (Luck et al. 2011). High speed winds and cyclones can contribute to increased dispersal of airborne plant pathogens such as rusts, splash-borne pathogens such as bacteria, and wind-borne insects and vectors such as aphids and psyllids (Qureshi 2010). Changes in climatic parameters are well-known to have a direct effect on insect population dynamics through the modulation of survival, growth and development rates, fecundity and dispersal, indirect effects of climate change through hosts, competitors and natural enemies are also well known (Estay et al. 2009). Temperature is the most important variable which alters pest infestation and damage, while measurable changes in humidity, periodicity and intensity of rainfall including extreme events, solar radiation, elevated CO_2, O_3 and ultraviolet light are also factors that can induce changes in pest scenario in crops (Robinet and Roques 2010). Closely related insects can vary distinctly in their survival of climatic stresses in addition to their capability to reproduce and expand in diverse thermal regimes, inducing species distributions and richness. A change in timing of reproduction is one of the behavioral responses to temperature and humidity that can potentially cause changes in pest dynamics in dry areas of the world. These changes will encompass plastic responses, morphological variations and the capability to withstand extremes (Laws and Belovsky 2010). Changing climate in warmer areas can have varied effects on natural enemies of pest species. The fitness of natural enemies can be changed in response to changes in crop growth and development induced by temperature and CO_2 effects. The predisposition of herbivores to predation and parasitism can be reduced due to increased or decreased plant foliage or changed timing of herbivore life cycles in response to plant phenological changes. The efficacy of natural enemies in controlling pests will decline if pest distributions shift into regions outside the distribution of their natural enemies, although a new community of enemies might then provide some level of control (Thomson et al. 2010). Interaction of elevated CO_2 and increased temperature can potentially increase crop damage by insect infestations. In addition climate change can compromise the resistance capabilities of the crop due to changes in physiology of the plant itself, for example in soybean elevated CO_2 and increased temperature caused a down-regulation of genes that produce toxins in the plants that deter insect pests (Zavala et al. 2008; Rao et al. 2009).

2.5 Strategies to Increase Resilience to Climate Change in Dryland Agriculture

Strategic adaptive management of natural resources forms the key to increase the capacity of the dryland agro ecosystem to adjust, to variation in atmospheric and edaphic factors, thus bringing about resilience in the system (Table 2.2). The key to achieve a resilient system is to recognize that dryland systems are highly variable and so deployment of management should be tactically timed to withstand and adjust to not only the gradual climate change but also to the shocks of extreme events. Adaptation measures should be initiated at regional and local levels to lessen the adverse effects of climate change; this should be irrespective of the scale of climate change mitigation undertaken (Reid 2009). Practices and techniques that protect soil, water and biodiversity are paramount to improving resilience of the

Table 2.2 Effects from land management practices or land use on carbon sequestration potential in drylands

Technological options	Sequestration potential (tons C/ha/year)
Croplands	
Conservation tillage	0.10–0.20
Mulch farming	0.05–0.10
Compost	0.10–0.20
Elimination of bare fallow	0.05–0.10
Integrated nutrient management	0.10–0.20
Restoration of eroded soils	0.10–0.20
Restoration of salt-effected soils	0.05–0.10
Agricultural intensification	0.10–0.20
Water conservation and management	0.10–0.30
Afforestation	0.05–0.10
Grassland and pastures	

Source: FAO (2004)

dryland system as whole and increase the ability of the system to adapt to the projected impacts of climate change. Both mitigation and adaptation to climate change have the potential to increase resilience (Singh and Venkateswarlu 2009). Here it is important to note the subtle difference between mitigation and adaptation. Mitigation and adaptation are related to the temporal and spatial scales on which they are effective. The benefits of mitigation activities carried out today will be evidenced in several decades because of the long residence time of greenhouse gases in the atmosphere, whereas the effects of adaptation measures should be apparent immediately or in the near future (Venkateswarlu and Shanker 2009).

2.5.1 Efficient Soil, Water and Nutrient Management

Management of soils, water resources and nutrients are closely entwined in dryland agriculture. Integrated soil and water management which includes on farm water storage, water shed approach and agronomic practices to conserve soil moisture are important to overcome water scarcity during cropping season. Other large scale measures like integrated management of river basins, aquifers, flood plains, and their associated vegetation to provide water storage and flood regulation services are important initiatives that also need to be followed hand in hand. One of the foremost technological interventions in managing natural resource is water shed management in drylands (Sastry et al. 2004). A watershed is defined as any spatial area from which rainwater or irrigation water is collected and drained through a common point and it serves a storage structure for future use. The watershed management involves, the sensible use of all the natural resources available which include land, water and vegetation cover to relieve drought, moderate flooding, thwart soil erosion, increase water availability and thereby increase food, fodder, fuel and fiber on sustainable way. Watershed management includes the treatment of land by using most suitable biological and engineering measures in such a manner that rainfall is conserved *in situ* as much as possible and the surplus is safely disposed or stored in ponds or check dams to recharge ground water. The success of the technology will depend on utilization of the land based on its capability with emphasis on protecting fertile top soil. Watersheds should be built to minimize silting in tanks, reservoirs and lower fertile lands. The presence of protecting vegetative cover throughout the year will be an added advantage in watersheds. Cropping intensity should be increased through intercropping, multitier cropping as in agroforestry and also sequence cropping. Watersheds provide alternate land use systems for effective use of marginal lands; water harvesting can be done for supplemental irrigation during dry spells and critical periods of crop growth like anthesis and grain filling. Quality of water is also of serious concern especially in coastal dry areas. One of the successfully tested technologies in controlling saline water intrusion in coastal saline areas is the Doruvu/Kottai technology practiced effectively in Andhra Pradesh and Tamil Nadu in India. This mainly involves digging of deep (upto 6 m) open wells, which allows horizontal flow of underground water enabled in to the well

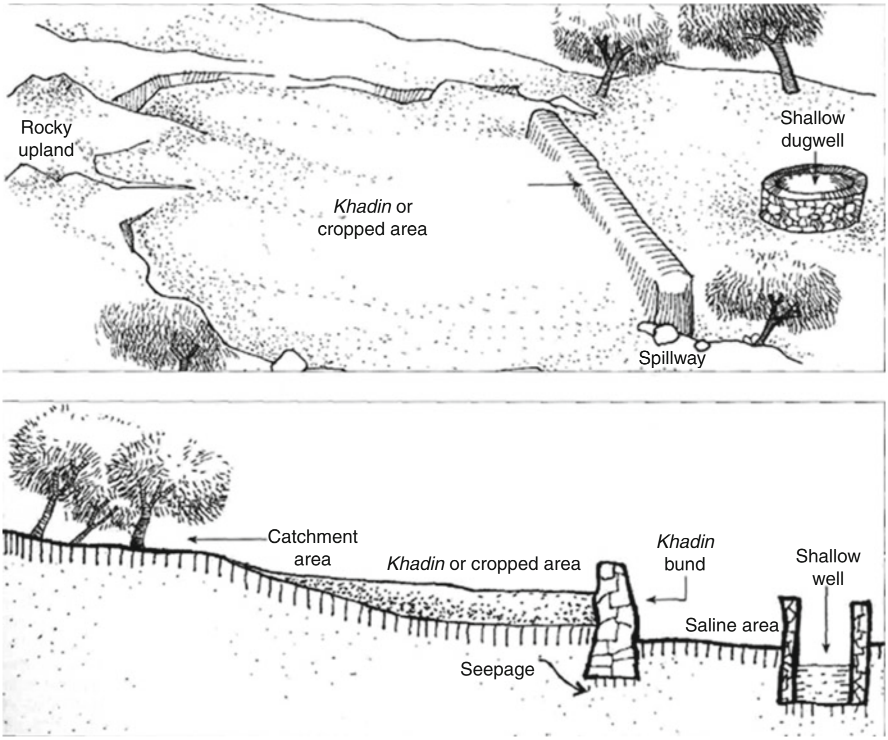

Fig. 2.3 Khadin method of cultivation followed in Rajasthan, India

through pipes. This technology helps in increased fresh water storage in comparatively lesser area giving more water to pump and irrigate crops (Venkateswarlu and Shanker 2009). A runoff farming land-use system, locally called Khadin in Rajasthan (Fig. 2.3), India has also proven to be successful in countering water scarcity (Kolarkar et al. 1983). It is practiced where rocky catchments and valley plains occur in proximity. The runoff from the catchment is stored in the lower valley floor enclosed by an earthen 'bund'. Any surplus passes out through a spillway. The water arrested stands in the khadin throughout the monsoon period. It may infiltrate in the soil or evaporate by the first week of November, leaving the surface moist. If standing water persists longer, it is discharged through the sluice before sowing. Wheat or chick-peas are then planted. These crops mature without irrigation. The soils of the khadins are extremely fertile because of the frequent deposition of fine sediment, while the water that seeps away removes salts. The khadin is, therefore, a land-use system which prevents soil deterioration (Oweis and Hachum 2009; Goyal 2008). Rainwater management can be done to increase plant water availability by *in situ* and *ex situ* water harvesting systems. *in situ* methods will help concentrate rainfall and divert runoff to cropped area structures like bunds, ridges, beds and furrows and basins. Terracing, contour cultivation, conservation agriculture, furrows,

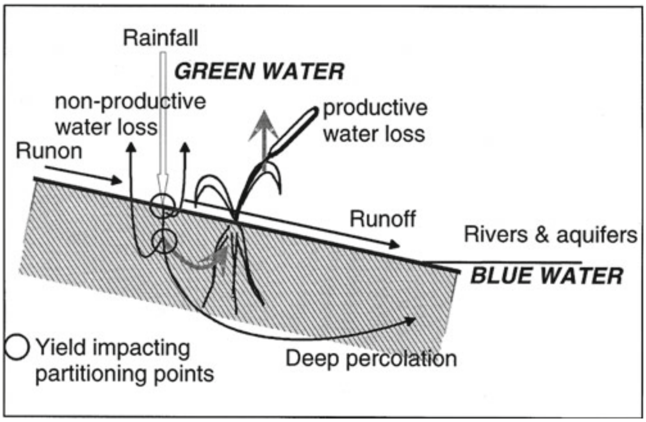

Fig. 2.4 Field-scale water balance showing the distinction between green and blue water flows. Partitioning points are shown (*circles*) impacting crop productivity by determining soil water availability (*upper circle*) and plant water uptake (*lower circle*). Green water flows are divided into productive transpiration flow and nonproductive evaporation flow (Source: Rockström and Falkenmark (2001). with permission from Taylor and Francis)

staggered trenches will also help to maximize rainfall infiltration. On the other hand ex situ methods construction of subsurface tanks, farm ponds, percolation dams and tanks like will be helpful in mitigating dry spells, protecting resource, increase recharge, and allow the luxury of off-season irrigation (Hoff et al. 2010).

Soil water infiltration capacity can be improved by improving soil aggregation, increasing total and macro-porosity, and reducing crusting and compaction. Soil application of biosolids, gypsum, manure, compost and other soil amendments that enhance aggregation and create biopores improves water infiltration capacity. Higher infiltration will help in capturing more water and allowing it to reach the root zone and this in turn will increase the plant water uptake capacity and reducing soil evaporation (Lal 2009).

Soil management is instrumental in management of green water as against water harvesting which involves conservation of blue water (Fig. 2.4). Soil management increases resilience through water retention decreasing soil evaporation, and increasing root system development. Deep tillage and no tillage are two contrasting methods that can be deployed depending on the climate, crop and season to manage soils to use water effectively. Deep tilling in compact soils with low soil organic carbon will prove useful in improving infiltration rate, enhance plant-available water capacity, and increase crop yields under rainfed or dry-farming conditions (Kahlon and Lal 2011). On the other hand, no tilling involves minimum disturbance of the soil. Plowing is substituted with a practice such as ripping the soil where seeds will be planted, deep ripping the soil to break up hard or compacted layers (subsoiling), or using direct planting techniques such as no-till. Any of these techniques, when used in combination with mulching to build organic matter are considered to be a part of conservation agriculture (Rockstrom et al. 2010).

Integrated Nutrient Management (INM) and Site-Specific Nutrient Management (SSNM) also have high potential in rainfed farming systems. Demonstrated benefits of these technologies are; increased yields and 30–40% increase in nitrogen use efficiency. Green manuring has numerous advantages in rainfed areas, the growing and incorporation of a post-rainy season legume crop is a low-cost simple practice that small and marginal farmers can adopt in semi-arid regions. Adoption of this practice, at least in alternate years, can restore the productivity of degraded soils and improve crop yields (Venkateswarlu et al. 2007). It is critical to note here that higher CO_2 concentrations in future will result in temperature stress for many rice production systems, but will also offer a chance to obtain higher yield levels in environments where temperatures are not reaching critical levels. This effect can only be tapped under sufficient integrated and site directed nutrient supply, particularly nitrogen (N). Phosphorus (P) deficiency, for example, not only decreases yield, but also triggers high root exudation and increases CH_4 emissions. Judicious fertilizer application, a principal component of SSNM approach, thus has 2-fold benefit i.e. reducing GHG emissions; at the same time improving yields under high CO_2 levels. Use of neem-coated urea is another simple and cost effective technology which can be practiced by small farmers.

2.5.2 Improved Agronomic Practices

Promotion of integrated farming systems for marginal and small farmers will also be a viable and effective alternative in combating climate change. Improved agronomic and crop production practices like adjustment of planting dates to minimize the effect of high temperature increase-induced spikelet sterility can be used to reduce yield instability, by avoiding flowering to coincide with the hottest period (Kamara et al. 2009). Adaptation measures to reduce the negative effects of increased climatic variability as normally experienced in arid and semi-arid tropics may include changing the cropping calendar to take advantage of the wet period and to avoid extreme weather events during the growing season. Crop varieties that are resistant to lodging may withstand strong winds during the sensitive stage of crop growth. In addition, improved crop management through crop rotations and intercropping, integrated pest management, supplemented with agroforestry and afforestation schemes will be an important component in strategic adaptation to climate change in drylands. In grazing lands, pasture improvement is essential to combat impeding changes through planned grazing processes, enclosures for recovery, or enrichment planting. Intercropping is an efficient strategy that can be followed with desirable outcome in the present climate change scenario (Morton 2007). Grain-legume intercrops have many potential benefits such as stable yields, better use of resources, weeds, pest and disease reductions, increased protein content of cereals, reduced N leaching as compared to sole cropping systems (Mucheru-Muna et al. 2010). Establishment of seed banks are of crucial importance in highly variable and unpredictable environments. This will provide a practical means to re-establish crops obliterated by major disasters and extreme climate events. This will also help in plant community dynamics, as differential plant germination strategies to buffer against inter-annual variability in growing conditions. The promotion of scientific agroforestry forms a key component in the war against climate change. Agroforestry systems buffer farmers against climate variability, and reduces atmospheric loads of greenhouse gases. Agroforestry can both sequester carbon and produce a range of economic, environmental, and socio-economic benefits. For example, trees in agroforestry systems improve soil fertility through control of erosion, maintenance of soil organic matter and physical properties, increased N accretion, extraction of nutrients from deep soil horizons, and promotion of more closed nutrient cycling (Venkateswarlu and Shanker 2009).

2.5.3 Tapping Appropriate Genetic Resources and Breeding for Abiotic Stress

Agricultural biodiversity and crop germplasm exploration for favorable traits is an important area that needs to be tapped to the fullest extent. Seeds, plants and plant parts exhibiting tolerance to temperature, water and other atmospheric stresses caused by climate change needs to collected and conserved to aid crop breeding research. A thorough revisit and re-evaluation of crop germplasm including wild relatives, land races, extant varieties, modern varieties and breeding stocks could help in unraveling previously unknown or ignored traits than could prove more useful in the present scenario. Genetic resources could well prove to be the most important cost effective basic raw material which will allow agriculture to adapt to climate change (Dawson et al. 2011). Considerable progress has been made in the genetic dissection of flowering time, inflorescence architecture, temperature, and drought tolerance in certain model plant systems and by comparative genomics in crop plants (Jung and Müller 2009). Breeding for improvement under climate stresses with traits such as temperature and drought tolerance and high yield in various important crops should be initiated urgently, so that the desired varieties are available when climate change effects are experienced consistently. The climate 'hotspot' areas are more prone to risks due to weather extremes have to be identified and the possible stress environments be quantified. The genetic resources, especially land races and wild relatives from areas where past climates mimicked the projected future climates for agriculturally prime areas, could serve as the starting genotypes for breeding crops for tolerance, maturity and yield attributes. A combination of conventional, molecular marker directed, mutational and transgenic-breeding approaches will be required to evolve the desired crop cultivars. To develop crop genotypes which can perform better under the predicted climate change, it is essential to understand the plant traits that are linked to adaptation (Fig. 2.5). Plant traits which favour yield and also which have a direct effect on the mechanism of tolerance is one of the important characteristic that has to be considered when developing climate ready crops. The primary considerations when selecting traits that have to

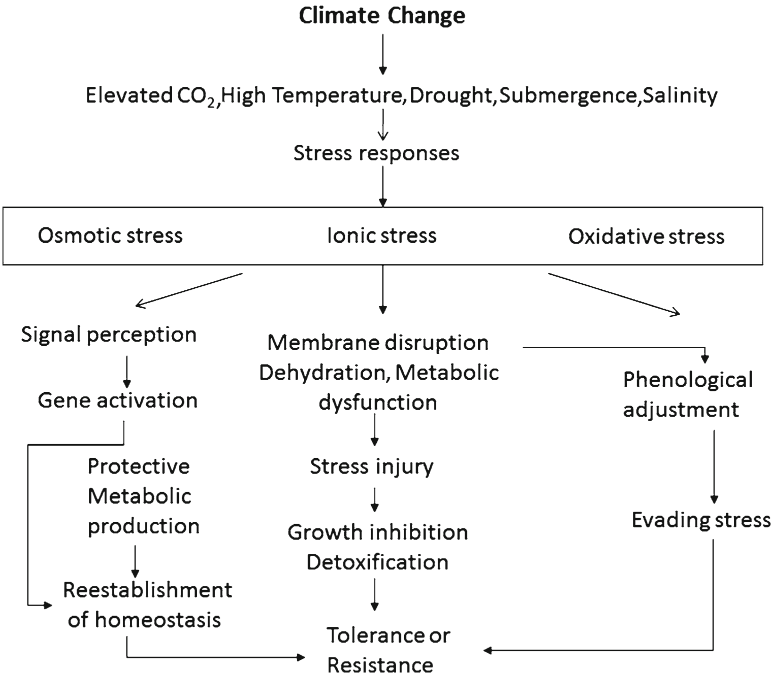

Fig. 2.5 Physiological and biochemical events resulting in tolerance or resistance to abiotic stress caused by climate change in plants

be improved in crops bred for dryland environments are that it must be genetically correlated with grain yield in the target environment. It should have greater heritability than yield itself, and so, less genotype environment interaction (Messina et al. 2011). The traits must show wide variability within the species. These traits have the ability to directly or indirectly control yield over a time scale influencing either water use, water use efficiency and partitioning of biomass to grain. The traits associated with stress tolerance and yield increase will not be usually the same in most crops hence the breeder is presented with a paradox of choices to select from. One of the important traits for drought tolerance is better root system with high biomass production. Abscisic acid (ABA) plays a central role in root-to-shoot and cellular signaling in drought stress and in the regulation of growth and stomatal conductance (Cutler et al. 2010). Osmotic adjustment (OA) is another important mechanism facilitating plants under drought to continue water absorption and maintenance of tissue turgor, thus contributing to continued higher photosynthetic rate and expansion growth. Proline and glycine betaine are important compatible solutes accumulating under drought. Similarly stay green trait is relevant in several crops such as maize, rice and sorghum which can be used effectively for yield improvement in dry, warm environments, caused by climate change. One of the major approaches to crop improvement has been to assess drought tolerance on the basis of yield stability or drought susceptible index (Du et al. 2009). There is a close relation between the available water in soil profile in post flowering period and yield. Significant progress has also been made in using the variability in water use efficiency for breeding for drought tolerance (Tuberosa et al. 2007). Since biomass production is tightly linked to transpiration, the effective use of water (EUW) and not high water use efficiency (WUE) is proposed to be the important determinant of plant production under drought (Blum 2009). Development of transgenics with an enhanced drought tolerance is another significant achievement and most of these plants are under advanced stages of testing in several crops. Several genes have been identified to express in response to drought stress, which include mainly (i) those, which encode products that directly protect cellular metabolism under water deficit and (ii) those regulating gene expression and signal transduction (Maheswari et al. 2010). Stress inducible genes identified so far include functional genes with protective roles in osmoprotection, Reactive Oxygen Scavenging, Ion transport, LEA and HSP. Regulatory genes imparting stress tolerance are those encoding signaling molecules, such as enzymes of phospho-lipid metabolism and various kinases (MAP, CDPKs, histidine kinases etc.) and several transcription factors such as DREB, ABRE etc. Temperature affects most plant-and crop-level processes fundamental to yield determination and hence the development of the final yield response. The impact of increasing temperatures can vary widely between crop species. Species with a high base temperature for crop emergence, such as maize, sorghum, millet, sunflower and some of the legumes such as mungbean and cowpea could benefit from increasing temperatures in cool regions. Most of the small-grain cereals, legumes such as field pea and lentil, linseed and oilseed Brassica spp. with a low base temperature could result in an advancement of phenophase with increased temperatures. Canopy Temperature Depression (CTD), membrane thermo stability, chlorophyll fluorescence, flag-leaf stomatal conductance, as well as photosynthetic rates were shown to correlate with field performance in several crops (Saint Pierre et al. 2010). Macromolecules, such as Heat Sock Proteins (HSPs) form the integral part of tolerance to high temperature in crops. HSPs are believed to be important for the protection of cells against heat injury both in basal thermo-tolerance as well as in acquired thermo tolerance responses. Many drought-adaptive traits may be useful under heat stress as well. Examples would include leaf glaucousness, awn photosynthesis and early maturity (Balkan and Genctan 2009; Salekdeh et al. 2009).

2.6 Conclusions and Future Directions

Rainfed agriculture will continue to play a central role in food security and sustaining livelihoods, chiefly in the developing countries. The resilience in the face of adverse effects of climate change is not just bouncing back but permanently retaining the capability to bounce back. The challenge before us is to continue to develop management practices with the goal of increasing resilience. The global hotspots in terms of water, food and livelihoods are in the dryland regions. Resilience to predicted climate change will depend on increasing agricultural productivity with available water resources; refining technologies and timely deployment of affordable strategies to accomplish potential levels of arable land and water productivity. The need of the hour is to adopt a holistic approach to dryland farming wherein soil and water conservation practices are combined with integrated site specific nutrient management at the watershed or catchment level which has the potential to increase sustainability and agricultural productivity. Reducing water loss is the main strategy in rainfed agriculture, to attain this; an integrated approach will have to be taken to blue and green water. Research in multiple simultaneous abiotic stresses is of paramount significance to understand plant responses to stresses that disturb the homeostatic equilibrium at cellular and molecular level and will help to identify a common mechanism for multiple stress tolerance. A very critical role is envisaged here for biotechnology in identifying metabolic alterations and stress signaling pathways, metabolites and the genes controlling these tolerance responses to both biotic and abiotic stresses and in engineering and breeding more efficient and better adapted new crop cultivars for dry areas of the world. Research on adaptive capability of crops by increasing their resilience to abiotic stresses, pests and diseases will have to expand to new horizons with systems biology perspective.

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Chapter 3
Stress in Plantation Crops: Adaptation and Management

K.K. Vinod

Abstract Plantation crops include perennials grown over large areas in monoculture, excepting fruit trees grown in orchards. These crops face both abiotic and biotic stresses, incited by factors those coexist in plantations. Although Plantation crops are well adapted and are grown mostly in the tropics, where innumerable stress factors operate. Historical significance of few stress havocs in plantation species is remarkable. A wide range of anatomical, physiological and biochemical features contribute to various stress adaptation in plantation crops. Notwithstanding, improvement of stress resistance in plantation species has been mandated to combat unfriendly factors that jeopardize intensive and extensive cultivation. Conventional breeding is cumbersome in plantation crops, where in the process has to involve many generations running for decades, and expensive in terms of time, space and large volume of individuals handled. Recent developments in molecular genetics and biotechnology are aiding acceleration of breeding process in plantation species. Integration of proper crop management strategies with improved cultivars is essential to meet the goals of stress management. This review presents a comprehensive coverage of various adaptive mechanisms and mitigation strategies for several biotic and abiotic stresses affecting major plantation crops like cardamom, cashew, cocoa, coconut, coffee, date, eucalyptus, oil palm, rubber and tea.

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3.1 Introduction

Although the term “plantation” is loosely defined, it generally includes perennial crops, grown in larger areas in monoculture, producing a wide array of produces such as spices, condiments, beverages, oilseeds, other allied materials, industrial and medicinal products, but does not ordinarily include fruit trees grown in larger orchards. They essentially differ from field crops for size of the holding, size of the individual plants, perennial nature and vegetative propagation. Plantation crops are remarkably well adapted plant species grown mostly in tropical regions of the world. These crops are predominantly cross-pollinated and produce heterozygous and heterogeneous population. Inherent genetic variability in the cultivated gene pool of these crops is generally very narrow (Simmonds 1989; Motamayor et al. 2000; Bandyopadhyay 2011). This chapter covers common plantation crops (Table 3.1) having various growth forms (trees, palms or shrubs) and grown for various purposes (oil nuts, industrial raw materials, spices, beverages and in timber industry).

The tropical regions harbor oldest ecosystems on earth, which are diverse, highly evolved, but poorly understood (Ploetz 2007), where plant stress is a common feature. Stress is a disadvantageous influence on a plant, exerted by external factors and stress adaptation or tolerance is the plant’s fitness to cope with an unfavorable environment or an invasion (Taiz and Zeiger 2006; Mittler 2006; Li et al. 2008). When exposed to unfavorable environments, plants show departures from its normal growth and metabolism, which collectively contribute to crop performance and productivity (Blum 2011). Like any other crops of the domesticated environment, plantation crops too are affected by various stress factors.

Stresses faced by plantation crops are either abiotic or biotic (Fig 3.1), originated from factors that coexist in the plantation environment. Abiotic stresses are the most common stress causing physical factors, which occur in all stages of crops’ lifespan. It is estimated that up to 82% of potential crop yields are lost to abiotic stress annually (Bray et al. 2000). Among the biotic factors, diseases are predominant than pests. In some species like cocoa (*Theobroma cacao*), pests and fungal diseases are responsible for more than 40% yield losses (Argout et al. 2008), of which pod rot caused by *Phytophthora* spp. (*P. palmivora* and *P. megakarya*) claims 30-90% of the total global crop loss (Bowers et al. 2001). A great deal of geographic variation exists among various forms of stresses that affect plantation crops, as well as in the adaptation of crops. Stresses can occur from single factor, or from combination of factors in annual cycles. Furthermore, because of perennial nature, accumulated stress effects often act detrimental in plantation crops. Predictions for the global agrarian future warn of rising temperature and humidity fluctuations, water logging, salinity and other problems that can adversely affect crop plants. In addition, these factors may affect diversity and virulence of agricultural pests and diseases leading to epidemics (Gregory et al. 2009). Fortunately, in plantation crops, very few stress factors are known to cause serious concerns in the industry and some are historically documented. During second half of 19th century, coffee plantations of Ceylon was devastated by leaf rust disease caused by *Hemileia vastatrix*, leading to the

Table 3.1 Details of major plantation crops

Crop	Botanical name	Family	2n[a]	Center of diversity	Geographic distribution	Economic produce	Reference
Cardamom	Elettaria cardamomum (L.) Maton	Zingiberaceae	48	Indian center	Tropical	Spice	Ravindran and Madhusoodanan (2002)
Cashew	Anacardium occidentale L.	Anacardiaceae	42	Tropical Americas	Tropical	Culinary nut	Ohler (1979)
Cocoa	Theobroma cacao L.	Sterculiaceae	20	Amazon	Tropical	Chocolate, cocoa	Wood and Lass (2001)
Coconut palm	Cocos nucifera L.	Arecaceae	32	South America, Southeast Asia (?)	Tropical	Oil nut	Thampan (1993)
Coffee	Coffea arabica L. C. canephora Pierre.	Rubiaceae	44 22	East Africa Central and West Africa	Subtropical, tropical	Beverage	Wrigley (1988)
Date palm	Phoenix dactylifera L.	Arecaceae	36	Iran and Arabian region	Arid tropical	Oil nut	Zaid and Jimenez (2002)
Eucalyptus	Eucalyptus sp.	Myrtaceae	22	Australia	Subtropical, tropical	Wood, medicinal oil	Coppen (2002)
Oil palm	Elaeis guineensis Jacq.	Arecaceae	32	West Africa	Tropical	Oil nut	Hartley (1988)
Rubber tree	Hevea brasiliensis Muell. Arg.	Euphorbiaceae	36	Amazon basin	Tropical	Natural rubber	Webster and Baulkwill (1989)
Tea	Camellia sinensis	Camelliaceae	30	Indo-china	Subtropical, tropical	Beverage	Eden (1976)

[a] $2n$ Diploid chromosome number

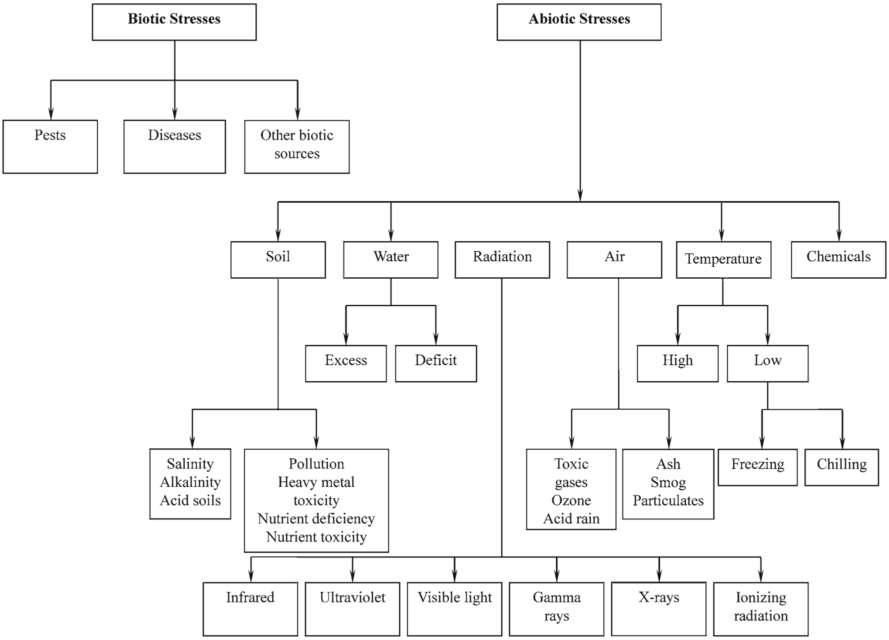

Fig. 3.1 Environmental stresses in plantation crops

closure of entire coffee plantation industry, paving way to the development of tea industry (Ward 1882a, b). Similarly, entire natural rubber plantations were shifted to South and Southeast Asia from Brazil in the late 19th century due the epidemics of South American leaf blight (SALB) disease caused by *Microcyclus ulei* (Labroy and Cayla 1913; Leiberei 2007). In cocoa, two serious diseases (witches' broom by *Crinipellis perniciosa* and frosty pod rot caused by *Moniliophthora roreri*) devastated cocoa production in South and Central Americas (Pereira et al. 1996; Borrone et al. 2004). Recently, during 1980s, a leaf spot disease caused by *Corynespora cassiicola* caused total replanting of high yielding rubber clones in Sri Lanka (Silva et al. 1998; Manju et al. 2001; Fernando et al. 2009). Among these, serious threat of SALB to the world economy has led to the decision to include *M. ulei* as a potential biological weapon by the U.S. National Research Council (Madden and Wheelis 2003) and by the United Nations Office on Drugs and Crime (Lieberei 2007).

3.2 Adaptation to Abiotic Stresses

Abiotic stress occurs from the factors of physical environment and plays a major role in survival and reproduction in plants. Survival under abiotic stress requires specific adaptation that takes place at two levels (a) individual and (b) population.

Table 3.2 Natural adaptation and sensitiveness of plantation crops to abiotic stresses

Crop	Adaptation	Sensitivity	Reference
Cardamom	Low temperature >10°C	High rainfall Wind Drought	Murugan et al. (2007)
Cashew	Drought Well drained acid soils	Water logging Low temperature, Frost	Paiva et al. (2009)
Cocoa	Shade Warm humid climate High rainfall	Drought Low temperature	Herzog (1994) Amanor (1996) Nair (2010)
Coconut	Warm humid climate High rainfall Salinity Strong wind	Water logging Drought Tropical storms	Lakmini et al. (2006) Batugal et al. (2009) Nair (2010) Remison and Iremiren (1990)
Coffee	Cool mountain climate to Warm humid climate Shade	Drought	Van der Vossen (2001) Lashermes et al. (2008) Klein et al. (2002)
Date palm	Drought High temperature Low temperature Salinity	Rain and humidity UV radiation	Zaid and Jimenez (2002)
Eucalyptus	Low temperature	Drought Frost Wind	Grattapaglia (2008)
Oil palm	High rainfall Long photoperiod	Drought Salinity Heavy metal toxicity High temperature	Jalani et al. (1997) Henson et al. (2005) Cha-um et al. (2010) Nair (2010)
Rubber	Long photoperiod Acid soils	Water logging Low temperature Wind Hailstorm	Bolton (1964) Vinod et al. (1996b)
Tea	Low temperature	Wind Frost Drought Flooding Hailstorm	Mondal (2009) Singh (1980)

Individual phenotypic adaptation (phenotypic plasticity) softens the impact of natural selection among genotypes (Bradshaw 1965; Schlichting 1986; Sultan 1987) and by itself forms the chief objective of natural selection (Wright 1931). However, at population level, adaptive plasticity of a plantation species (Table 3.2) will reflect on the environmental stresses it had faced repeatedly in its evolutionary history. One of the best examples for adaptive response is the deciduous nature of rubber trees in the native evergreen rainforests of Amazon, which remains warm and wet throughout the year. Apart from the popular understanding that deciduous trees shed leaves in order to avoid a winter or a tropical dry season, it is postulated that trees might also shed leaves to have a break in their parasitism, and to resuscitate their biochemical locks. This possibly explains why the rubber tree became deciduous, owing to continuous parasitism by *M. ulei*, the SALB pathogen (Robinson 1995).

Adaptation to environmental stress in plants is determined by their genetic make up. The difference in regulatory mechanisms of defense related genes decide whether a plant is resistant or susceptible. These differences are ubiquitous arsenals that help plants to combat stresses of various kinds.

3.2.1 Drought

Drought ranks foremost among the abiotic constraints for production in plantation crops because most of them are grown in tropical semi-arid conditions. Generally, plants adapt themselves to survive drought through two different ways viz., drought escape and drought tolerance. Perennial plantation crops have no scope for drought escape (Kozlowski and Pallardy 1997), while they withstand water stress by drought tolerance. Drought tolerance is manifested either through desiccation avoidance or desiccation tolerance. Desiccation or dehydration avoidance is achieved by maintaining high water potential within plants. In plants, primary mechanisms that maintain high water potential are reduction in transpiration through stomatal regulation, reduction in transpiring area, reduction in radiation absorption and maintaining water uptake by increasing root growth and root hydraulic conductivity. These mechanisms operate through morphological adaptations involving roots, stem and leaves and physiological regulation of transpiration; thereby plants increase internal water storage and prevent tissue dehydration during drought. Reduction in tissue hydration is primarily achieved by maintaining turgor through osmotic adjustment by accumulating solutes and inducing stomatal closure by increasing abscisic acid (ABA) content in leaf tissues, while maintaining near normal physiological activities (Repellin et al. 1994). Maintenance of low water potential primarily requires maintenance of turgor achieved through osmotic adjustments. Desiccation tolerance is rather a limited choice for majority of woody plants (Kozlowski and Pallardy 1997).

Crop adaptation depends on the degree of plant sensitivity to drought. The degree of drought sensitivity varies among plantation crops at species, genotype, phonological and organ levels. For example, at species level, crops like rubber and cashew are very sensitive to water logging; and at organ level, leaves are found to be more vulnerable than stem in evergreen trees (Chen et al. 2009).

Systematic investigations on drought tolerance in plantation crops reveal that a wide range of anatomical, physiological and biochemical adaptability contributes to the level of tolerance (Rajagopal et al. 1990). Therefore, many physiological parameters such as pre-dawn and midday leaf water potential, net photosynthesis, stomatal conductance, transpiration and intercellular CO_2 concentration and biochemical attributes like ascorbic acid, glutathions, tocopherols, chlorophylls, carotenoids, free amino acids and soluble carbohydrates have been used to assess drought stress in these crops. Physiological and morphological characteristics, such as stomatal conductance, crown architecture, root depth and water use efficiency may be used as potential traits for selecting coffee genotypes with superior performance under drought conditions.

3.2.1.1 Anatomical Adaptations

Majority of the anatomical adaptations to drought are modifications to reduce water loss from plants during dehydrating conditions. This is mainly achieved by controlling transpiration loss through stomatal regulation. Stomatal response is probably one of the most complex behaviors in plants. External factors like light, air humidity, soil water content, nutrient status etc. and internal factors like ABA concentration, leaf water status etc. are known to exert direct influence on stomatal behavior. Stomatal behavior is a key indicator in assessment of drought tolerance in plantation crops. Striking differences in stomatal function during drought has been reported in rubber (Sangsing et al. 2004a), cocoa (Gomes et al. 1987; Willson 1999), coconut (Prado et al. 2001; Passos et al. 2005; Gomes et al. 2008) and many other woody plant species (Kozlowski and Pallardy 1997).

Cavitation Resistance

Under drought situations, xylem cavitation occurs when sap pressure within the vessels fall below a threshold resulting in irreversible collapse of the xylem conduits. In cavitation resistance, stomatal closure takes place well in advance as a response to increase in plant hydraulic resistance that prevents water loss, thereby maintaining sap pressure above the threshold (Sperry and Tyree 1988). Sangsing et al. (2004b) have found (i) relatively high vulnerability of xylem conduits to cavitation, (ii) associated responses between stomatal behavior and occurrence of cavitation and (iii) substantial genotypic variation in vulnerability to cavitation suggesting that whole plant level drought adaptive mechanisms are in force in rubber trees. Cavitation resistance therefore is a parameter that pertains more to drought survival. Analysis of xylem embolism, especially in the petioles, may provide a sound criterion for evaluation of genotype behavior under drought conditions.

As that of rubber tree, coffee can also adapt to drought by regulation of hydraulic conductance. Hydraulic conductance in coffee is found to be directly associated with transpiration rate, that may help in regulation of water loss though transpiration and to maintain the sap pressure above threshold to avoid xylem cavitation (Tausend et al. 2000). A drought tolerant genotype therefore must be able to maintain high relative water content (RWC) under moisture stress conditions. It has been reported that by efficient stomatal control on transpiration coupled with low cell-wall elasticity (Pinheiro et al. 2004) coffee plants could retain high leaf RWC (DaMatta et al. 1993) under drought. A slight shift in turgor due to the loss of little water from ensuing drought can signal leaves to maintain a high RWC and retain a high symplast volume. Further, conservation of a high symplast volume may be crucial for maintaining gas exchange under drought as seen in arabica coffee (Meinzer et al. 1990), and hence maintenance of a high RWC is crucial in conferring drought tolerance in coffee (DaMatta 2004).

Foliar Abscission

Leaf abscission occurs in deciduous plantation crops like rubber when they approach annual period of water deficit. Leaf abscission may be considered as a whole-plant mechanism to limit water loss through transpiration in these plants. Leaf shedding was reported in field grown robusta coffee in response to water deficit (DaMatta and Rena (2001) occurring sequentially from older to younger leaves; and more drought-sensitive the genotype is, greater would be the extent of leaf shedding. However, DaMatta (2004) suggested that at least in robusta coffee senescence might be merely a consequence of and not a defense against stress since drought-sensitive clones, which considerably lose their foliage, also show most impaired water status in the remaining leaves.

Leaf Anatomy

Leaf anatomical adaptations such as cell size and number, stomatal frequency, stomatal resistance and epicuticular wax content have been described as essential anatomical indicators for assessing moisture stress in coconut (Gomes and Prado 2007). Waxes are involved in plant's first defense against abiotic stress of which hydrophobic waxes play an important role in water retention by limiting non-stomatal water loss (Kerstiens 1996; Riederer and Schreiber 2001; Jenks 2002). Drought adaptive anatomical features in coconut includes leaflets with upper epidermal waxy cuticle two-times thicker than the lower epidermis, thicker cuticular edges, xylem tracheids with thick lignification and tracheids with scalariform thickening. Further, water tissue with thin-walled cells at the upper and lower angles of straightened leaflet margin and fibrous sheet encircling seven to eight large vascular bundles in a strong midrib are also seen in leaflet lamina. Presence of two layers of large hypodermal cells below the upper epidermis and a multi-layered and closely packed palisade tissue also seen in contrast to scanty spongy parenchyma exist between the upper and lower hypodermis. Drought tolerant coconut cultivars had more scalariform thickening on tracheids and large sub-stomatal cavities (Kumar et al. 2000). A negative relationship between the epicuticular wax content of coconut leaves and transpiration rate has been reported by Kurup et al. (1993) substantiating the presence of high epicuticular wax content among drought tolerant than susceptible genotypes (Riedel et al. 2009).

Stem Vascular Systems

Palms like coconut, oil palm and date have tall singular stems developed from the apical meristem. Being monocotyledons, vascular bundles of xylem and phloem appear scattered throughout the ground parenchyma, and there is no distinction between cortex and pith in these plants. Vascular bundles have an anastomosing or weaving pattern running longitudinally throughout the length of the stem. Stem anatomy is organized in such a way to minimize isolated functional sectors of mass flux from roots to canopy. The transport capacity of this massive hydrosystem increases with the stem diameter, and is functional throughout the lifespan of the palm that can extend well above a century. Hence, the ability to withstand water stress in palms like coconut is presumably coordinated by stem processes (Tomlinson 2006) as well. This complex process may contribute to higher drought adaptation of tall coconut varieties than the dwarf ones. Passos and Silva (1991) found that stem girth of tall coconut decreases between dawn and midday before increasing again during afternoon suggesting stem organized control of water transport. Variation in stem diameter as a response to drought stress also has been described in coconut. Stresses due to drought, flooding, mineral deficiency and some diseases can cause decreases in stem diameter, which reverses to normal when environmental conditions improve. Therefore, stem girth and form make a good record of palms' stress history. Phenological variations in drought sensitivity in oil palm indicate that young palms are more susceptible since they do not possess extensive root system and voluminous stem as in adult palms (Villalobos et al. 1992).

3.2.1.2 Physiological Adaptations

Behavioral Adaptations

Rubber as a deciduous tree has a different phenological adaptation than the evergreen trees. Annual natural defoliation known as 'wintering' occurs at the onset of winter and extends roughly for 4-6 weeks through the season (Vinod et al. 1996a). Leaf flushing occurs at the end of wintering period and well before the arrival of the rains that coincides with dry months. Flushing process requires large quantity of water for leaf development and expansion (Elliot et al. 2006; Williams et al. 2008). Root zone activities show significant deep root water uptake during wintering and flushing of rubber trees (Guardiola-Claramonte et al. 2008). Significant reduction in transpiration (Priyadarshan and Clément-Demange 2004) and increase in root water uptake helps trees to maintain stem water potential required for subsequent leaf flushing. Rubber trees can conserve extracted water without being released to atmosphere until the new foliage is grown (Guardiola-Claramonte et al. 2010).

Differential adaptation to drought among major sub-types of coffee, arabica and robusta appears to be governed by changes in rates of water use and/or efficiency of extraction of soil water (Meinzer et al. 1990; DaMatta et al. 2003; Pinheiro et al. 2004). During prolonged drought, reduction in leaf area and alternate assimilate partitioning new foliage the major adaptive mechanism for survival in arabica coffee (DaMatta 2003). Behavioral difference among the tolerant and susceptible genotypes was reflected in relative drought tolerance, governed by adaptation in root depth, plant hydraulic conductance and stomatal control of water loss (Pinheiro et al. 2005). Accordingly, better drought adaptation among genotypes with deeper root systems is reported among robusta coffee (Pinheiro et al. 2004). Similarly rooting depth influenced drought tolerance has been reported in tea wherein shallow rooted clones were found drought susceptible than the deep-rooted clones. Moreover, drought tolerance was found to increase with rooting depth in shallow rooted clones, whereas no much variation was found in deep-rooted clones (Nagarajah and Ratnasuriya 1981).

Canopy Architecture

Canopy architecture has been found to play a significant role in drought adaptation in trees. Canopy structure partially determines the hydraulic architecture of a tree (Herzog et al. 1998). In coffee, dwarf cultivars with dense crowns are better able to withstand drought by delaying dehydration than cultivars with open crowns. Canopy compactness is also achieved by reducing the size of the leaves and altering the crown shape, that result in better energy dissipation with reduced transpiration (Kozlowski and Pallardy 1997). In rubber trees, adaptation to prolonged drought has been reported to produce compact canopy. This prevents high light intensity (which can be photoinhibitory) from reaching the lower canopy leaves and blocks penetrating radiation from reaching the plantation floor that can heat up surface soil, change vapor pressure deficit (VPD) and alter microclimate leading to evapotranspiration loss. Compact canopy has been implied as an ideal phenotypic feature for drought tolerance in rubber clones (Devakumar et al. 1999). DaMatta (2004) has suggested microclimate associated transpiration loss as one of the probable reasons of crop failure, in spite of sufficient supplemental irrigation, at locations with high evaporative demands planted with coffee cultivars with open crowns.

Stomatal Control – Hydraulic Signaling

Stomatal regulation takes place in plants as a response to signals arising primarily from roots. These signals trigger a cascade of events such as induction of differential gene expression, changes in cell metabolism and development of defensive systems in the above ground organs (Jackson 2002; Kholodova et al. 2006). Primarily systemic signals are hydraulic in nature spreading along the xylem that coordinates physiological responses (Jackson 2002) especially in leaves. As available soil moisture falls, a gradual reduction ensues in shoot water status, initiating direct hydraulic signals to leaves. Several studies suggest ABA accumulation as the primary candidate for hydraulic signaling in plants (Davies and Zhang 1991; Comstock 2002; Kholodova et al. 2006). Root tissues synthesize ABA as an initial response to diminishing soil moisture availability. Hydraulic signals are instantaneously spread through the continuous water phase within plants culminating in foliar stomatal regulation.

As a reliable indicator of stomatal performance, stomatal conductance has been used in plantation crops for assessing drought sensitivity (Rajagopal and Ramadasan 1999; Nainanayake and Morison 2007; Lakmini et al. 2006; Carr 2011), because stomatal conductance is highly correlated with transpiration rate and photosynthetic health. Stomatal conductance has been used as an early indicator of stress in arabica coffee, because decline in stomatal conductance is found to occur even at one-third depletion of available soil water. Evidences suggest that during short-term water deficit, yield reduction in coffee genotypes may be associated with stomatal conductance and net carbon assimilation (Nunes 1976). Although poor stomatal control was found during drought (DaMatta et al. 1997) the mechanisms leading to clonal tolerance to drought in robusta coffee still remain largely unknown (Pinheiro et al. 2005). In coconut, strong evidence of stomatal regulation of plant water status has been documented at mild to moderate drought (Prado et al. 2001; Passos et al. 2005; Gomes et al. 2008) making it a useful parameter to differentiate drought sensitive and tolerant genotypes in association with tissue water potential (Lakmini et al. 2006). VPD sensitive stomatal regulation was reported in cocoa (Willson 1999), wherein leaves exhibit reduced water use efficiency at high VPD (Gomes et al. 1987), leading to tissue water deficit under conditions of limited soil water supply.

Stomatal Control – Non-hydraulic Signaling

Several studies have shown that signals of moisture depletion reaches leaves from roots, unassisted by shoot water status, indicating the existence of a non-hydraulic route of signal transport (Croker et al. 1998). Although perceived as an important component of plant responses to drought, exact mechanisms involved in non-hydraulic root to shoot signaling still remain unclear (Davies et al. 1994). Recently, a non-hydraulic chemical mediated signaling has been reported in coconut, in which chloride ions are involved in sensing soil water depletion in the root zone and sending stomata-closing signals to the leaves. In coconut, chloride ions are found to play two important functions in regulating water balance; first, they regulate stomatal closure by coordinating water flow between six neighboring cells (two guard cells and four subsidiary cells) of the stomatal apparatus, and secondly, chloride ions improve osmoregulation capacity under water stress (Gomes and Prado 2007).

Photosynthesis

Variation in photosynthesis and associated systems has also been used as indicators of drought tolerance in plantation crops. Largely, photosynthetic performance of plants is determined by environmental variables under field conditions. Photosynthesis is closely associated with stomatal function since gas exchange in plants takes place through stomata. Drought-induced stomatal closure limits CO_2 diffusion from the atmosphere to the intercellular spaces resulting in reduced photosynthetic activity (Repellin et al. 1994, 1997). Genotypic variations have been reported in coconut in rates of gas exchange recovery and internal dehydration on exposure to drought. In dwarf coconut varieties, photosynthetic acclimation was observed after repeated drying and recovery cycles (Gomes et al. 2008). Use of rate of photosynthesis and instantaneous water use efficiency as reference parameters has been reported promising in coconut (Nainanayake and Morison 2007). Chlorophyll fluorescence transients have been implicated in differentiating and screening of coconut seedlings that can adapt to water stress condition (Bai et al. 2008)

3.2.1.3 Biochemical Adaptations

Osmoregulation

Osmotic adjustment (OA) has been found associated with maintenance of gas exchange under drought (Turner 1997) in plants. OA occurs in cells in response to drought stress signals, where in osmolyte accumulation takes place to prevent cellular dehydration. Osmolytes are low molecular weight metabolites that are able to compensate high osmotic pressure without interfering with plant metabolism, even at elevated concentrations. They include sugars, polyols, amino acids and quaternary ammonium compounds. Proline is a typical osmolyte, synthesized in plants under different stress conditions. Although drought sensitive, cocoa plants exhibit active osmotic adjustments when exposed to dehydrating environments (de Almeida and Valle 2007), but suffer yield loss under stress (Moser et al. 2010). Osmotic adjustments and stomatal regulation have been reported as one of the mechanisms operating in drought tolerant coconut varieties (Rajagopal and Ramadasan 1999).

Other Biochemical Indicators

The plant hormones, ABA and ethylene play significant role in plant adaptation to environmental stress. Two key multigene families, 9-cis-epoxycarotenoid dioxygenase (NCED) genes (Seo and Koshiba 2002) and 1- aminocyclopropane-1-carboxylate synthase (ACC synthase) genes (Yang and Hoffman 1984) respectively regulate the biosynthesis of these hormones. Late embryogenesis abundant (LEA) proteins are a distinct group of proteins that are induced during dehydration stress caused by extreme temperatures, drought, salinity and certain developmental events such as seed maturation (Close 1996, 1997). LEA proteins are believed to be a subgroup of dehydrins that have extreme hydrophilic nature and are soluble at high temperature. LEA proteins are believed to act as a novel form of molecular chaperone to help prevent the formation of damaging protein aggregates during water stress (Goyal et al. 2005). Dehydrins in general are structural stabilizers, that protect nuclear, cytoplasmic, and membrane macromolecules from dehydration-induced damage, thus maintaining cell structure and integrity. In plantation crops, dehydrin like proteins have been identified in coffee (CcDH1, CcDH2 and CcDH3; Hinniger et al. 2006) and eucalyptus (Bocca et al. 2005). Another important and highly diverse set of proteins implicated in dehydration stress are heat shock proteins (HSPs). HSPs too functions as molecular chaperons minimizing the aggregation of proteins and targeting aggregated proteins for degradation, while assisting in protein folding, assembly and transport.

Various adaptations to drought include a stress-induced cascade of reactions in plants, including scavenging of reactive oxygen species (ROS) produced during oxidative stress. To prevent oxidative damage, cells contain antioxidants that scavenge the free radicals (Yamasaki et al. 1997). Phenolic compounds are cellular compounds with antioxidant properties (Rice-Evans et al. 1997); and several studies have shown that production of compounds with efficient antioxidant structures, such as additional hydroxyl groups on ring B of the flavonoid skeleton is accelerated (Ryan et al. 1998) during drought stress. Plant cells also contain enzymes, such as superoxide dismutase (SOD) and catalase that protect them by scavenging superoxide radicals and hydrogen peroxide respectively (Takeuchi et al. 1996). Transcriptome profile of the rubber tree latex contains many genes related to water stress in abundance, whose actual role in stress defense is not yet known. Transcripts of two genes encoding for *Hevea brasiliensis* ASR (abscisic acid, stress and ripening) like proteins, HbASRLP1 and HbASRLP2 were the most abundant next to those of rubber elongation factor (REF) and small rubber particle protein (SRPP) in the latex. These genes are homologous to tomato ASR gene family (Rossi et al. 1996) and the putative proteins coded by these genes have a domain similar to those proteins induced by water deficit stress, ABA stress and ripening. Further, a gene family of HbRLPs (REF like proteins) was also found expressed at higher levels in rubber latex. Both HbRLPs and SRPP are structurally closer to stress related proteins (Ko et al. 2003).

In tea, elevated polyphenol content has been reported as an indicator for drought tolerance (Hernández et al. 2006; Cheruiyot et al. 2007). Drought tolerant tea clones had higher catalase activity for scavenging hydrogen peroxide formed in the photorespiratory pathway (Jeyaramraja et al. 2003a). Furthermore, drought induced reduction in catechin content has been reported in tea clones (Singh et al. 2009a, b). This behavior is attributable either to an instability of the catechins under drought (Jeyaramraja et al. 2003b) or to possible of loss of catechins due to enhanced cellular injury or to down-regulated pathways leading to limitation in the availability of precursor molecules (Singh et al. 2008, 2009a, b; Jeyaramraja et al. 2003b; Sharma and Kumar 2005).

Although mechanisms are many, each plantation crop has its own combination of adaptive mechanisms to counter drought stress. Overall strategy appears to be to reduction or cessation of leaf-area development, maintenance of good water use efficiency with stomatal regulation, maintenance of efficient photosynthesis and tolerance of additional stress with osmotic adjustment of mature leaves, fruits and roots. Foliar abscission appears to be the choice during severe stress. Osmotic adjustment of the functional leaves is effectively carried out to maintain the overall canopy function and leaf longevity is maintained for a considerably long period.

3.2.2 Temperature

Most of the plant processes are temperature dependent. In plantation crops, apart from growth and establishment, significant temperature influence may be seen on reproductive growth such as floral initiation, release of bud dormancy, anthesis, fruit-set and fruit development. Thermal adaptation varies widely among tropical, subtropical and temperate plantation crops, since the temperature plays a leading role in limiting plant distribution between tropical and temperate regions. In general, flowers and fruits are injured by extremely low or high temperatures. When temperature falls, molecular activity gets decreased and essential biochemical processes involved in sustaining growth are arrested. Low temperatures also decrease the permeability of membranes and increase protoplasmic viscosity. On the other hand, when temperatures goes excessively high, molecular activity may get impaired and the enzymes controlling metabolic processes are denatured or inactivated.

3.2.2.1 Low Temperature

Plant stress occurring due to lowering ambient temperature has been a common phenomenon in all the crops. Degree of variation in plant responses to low temperature depends primarily on the basic adaptation pattern of the crops. Temperate species can tolerate very low temperature, which are fatal to tropical crops. This is the reason why tropical and sub-tropical species fail to establish under temperate climates. In temperate species, low temperature favors shoot growth through its effects on bud formation, bud dormancy and bud expansion. Low temperature is an essential requirement for these crops to break bud dormancy, and elevated temperature during mild winter impairs bud break resulting in crop failures (Weinberger 1950).

However, excessive freezing causes various stress reactions in temperate plantation species. Freezing injury occurs mainly during autumn (winter) season, while frost injury inflicts damages to flowers, buds and young fruits during spring season. Furthermore, rootstocks are more susceptible to freezing injury than scion. Freezing injury occurs when low temperature spells are followed by a period of mild weather that permitted growth to start and caused loss of cold resistance. Hence, repeated mild freezing spells are more deleterious than a single spell of deep freezing. In addition, a few temperate zone plants are susceptible to chilling injury that occurs when they are exposed to temperatures a few degrees above freezing. Freezing injury can be caused either directly by intracellular freezing or indirectly by dehydration of tissues resulting from extracellular freezing. Plants are usually killed by ice crystal formation within the cells, but formation of ice crystals within the intercellular spaces is not necessarily fatal. In frost hardy plants, cellular freezing is prevented through a cell sap concentration mechanism. Concentrated cell sap has lower freezing point than water, and hence ice formation is prevented. Although, slower cooling causes ice to form initially in the intercellular spaces, and as the temperature decreases gradually, water moves out of the cells to the intercellular ice nuclei, making the cell sap thicker. However, rapid cooling is normally fatal because there is little time for water to move out leading to a sudden intracellular freezing resulting in mechanical disruption of protoplasm. In tree species, whole plant level freezing damage occurs when crown and roots are affected; of this, crown damage is usually irrecoverable and fatal. In *Eucalyptus globulus*, it was reported that freezing tolerance of provenances occurred due to their ability to evade the formation of ice in their leaves, suggesting that supercooling is the most probable mechanism conferring resistance in these plants. In addition, accumulation of soluble carbohydrates in the leaves provides cryoprotective mechanisms (Moraga et al. 2006) as soluble sugars are known to function as cryoprotectants in plant tissues, especially at membrane level (Tinus et al. 2000).

Frost cracks can develop on tree trunks when alternating freezing and thawing occur during the winter and early spring. These cracks occur with a loud bang and can be of several feet long. The bark at the affected region occasionally peels off exposing the underlying wood, which becomes site for secondary infection. Since these lesions damage phloem, downward translocation of organic solutes is impeded. Although less hardy, root tissues are relatively less injured than stems during winter, because soil and snow cover protect them from exposure to freezing air temperature. However, freeze damage occurs in roots, when dry soil sucks moisture out of roots during winter, and if the soil is wet, no damage is found to occur.

Spring frost during growing season often injures cambium, causing the formation of abnormal stem frost rings. These rings usually have an inner part of frost-killed cells and an outer layer of abnormal xylem cells produced after the frost. In forest plantations of eucalypts, frost injury is a common problem in temperate regions throughout the world making most of the eucalypt species unsuitable for commercial plantings. *E. nitens* is a fast-growing species widely adapted for planting in high altitudes where occurrence of severe frost is a common occurrence (Turnbull and Eldridge 1984) in contrast to *E. grandis*, use of which is limited due to its lesser frost tolerance (Eldridge et al. 1994).

On the contrary, tropical species are vulnerable to sub-optimal temperature due to intrinsic oxidative stress occurring from the exposure to low temperature. Cold susceptibility occurs from reduction in photosynthetic efficiency and increased photoinhibition of photosystem (Fryer et al. 1998). Reduction in photosynthetic functions is observed in rubber trees grown under cold climate (Alam et al. 2005). Moreover, rubber is reported to have potential for acclimation of photosynthesis to temperature in the growing environs (Kositsup et al. 2009). This photosynthetic plasticity at a reference temperature is associated with flexible response in net CO_2 assimilation rate. Although rubber trees grown at 18°C were not able to maintain net CO_2 assimilation rate, photosynthetic capacity and leaf nitrogen status close to those of trees grown at 28°C, they had potential to survive low temperature stress. Metabolic flexibility of rubber was reported to confer less intrinsic oxidative damage in plants grown under elevated CO_2 in encountering low temperature stress (Alam et al. 2005). Some rubber varieties were found to have greater resilience to chilling up to 96 hours without any damage to photosynthetic apparatus (Mai et al. 2009). Moreover, variation in membrane injury caused by low temperature has also been reported in rubber clones (Ray et al. 2004). Phenological adaptations for low temperature tolerance in rubber clones include shorter reflushing time and faster wintering (Vinod et al. 2010). Abundance of stress responsive proteins, like low temperature- and salt-responsive protein and low-temperature-induced protein has been identified in latex transcriptome (Ko et al. 2003).

Most of the palm species are acclimatized to tropical rain forests and are grown in wide range of habitats outside freezing environments (Gomes and Prado 2007). An exception is the date palm that is cultivated in arid and semi-arid regions characterized by long, hot and dry summers and winters with sub-freezing temperatures. Date palm can withstand wide temperature fluctuations up to its zero vegetation point of 7°C, below which growth stops and plants enters into a resting stage. Freezing injury occurs when the temperature fall below 0°C resulting in metabolic disarrays, leading to partial or total damage of leaves. Inflorescences are also heavily damaged by frost. Pinnae margins turn yellow and dry out at -6° C and exposed leaves of the canopy dry out from -9 to -15°C. If freeze occurs for a long period (12 hours to 5 days), entire palm looks burnt with all leaves showing frost damage (Mason 1925; Nixon 1937). Even if the entire crown is damaged, palms survive and grow after normal conditions are returned, because the meristematic area of a date palm is well protected against frost.

3.2.2.2 High Temperature

Exposure to relatively high temperatures causes heat injury in plantation crops that reduces growth and diminishes chance of survival. Heat injuries may be direct or indirect. Direct injury is the immediate plant response during the exposure of elevated temperature or immediately thereafter. Direct heat injury is relatively rare when compared to indirect injury in planation crops. Indirect heat injury in plants occurs slowly and may not be apparent for many hours or even days after exposure to high temperature. High temperature causes rapid decline in apparent photosynthesis beyond a critical high temperature, while continuing to increase respiration resulting in rapid depletion of carbohydrate reserves and sometimes death. Formation of toxic compounds, production of biochemical lesions, and breakdown of protoplasmic proteins (Levitt 1972) are also common.

Most common direct heat injury observed in rubber is severe sun scorching (Vinod et al. 2003) that occurs when the collar region of young plants is damaged by sun exposed surface soil layer heated well in excess of 55-60°C. In grown-up rubber plants, excessive heating up of stems facing afternoon sun develops cracks and lesions called heat canker. Rubber plants are damaged due to sun scorch mostly when they are very young. After three years of growth expanding crown provides shade protection from scorching. Generally, sun scorch at the collar region results in girdling and death of the plants, while bark lesions results in partial drying up of the bark exposing the stem underneath. Since cambial cells are sensitive to high temperature, they die due to desiccation and oxidative stress. Exposed bark becomes brittle and cracks develop resulting in oozing of latex from crack wounds. This enhances internal drying and secondary pathogen damage ultimately resulting in complete death of the affected bark region. Among the palms grown in plantation sector, betel-nut palm (*Areca catechu* L.) is highly prone to sun scorch (Staples and Bevacqua 2006).

Of the tropical plantation crops, cashew is a hardy tree (Purseglove 1986) that is adapted to a wide range of environmental constraints. It grows well in areas of high temperature, where diurnal temperature can exceed 40°c and can survive seasons of drought. Cashew has an extensive root system with which it can forage for moisture and nutrients and can thrive in poor and marginal soils (Kembo and Hornung 1999).

3.2.3 Salinity

Salinity is a common problem in all parts of the world, especially secondary land salinization that occurs mainly due to clearing of forests and shrub lands for agriculture and excessive irrigation (Cramer et al. 2004). Plant stress due to salinity occurs when soluble salt concentration in soil and water exceeds limits of plant tolerance. Salt affected areas can be of two types, sodic and saline. They have excess sodium and high concentration of carbonate or bicarbonate anions with greater pH ranging from 8.5 to 10.8. They have very poor structure and their sodium absorption ratio (SAR) is high. Saline soils are rich in sodium ions, but with chloride and sulfate as dominant anions; pH and SAR are much lower than sodic soils, but have higher electrical conductivity (>4 dS m^{-1}). Salt-affected soils contain high concentrations of soluble salts that reduce growth in most of the plant species (Flowers and Flowers 2005).

Plants that are sensitive to natural salinity are called glycophytes, and those resistant are called halophytes. Some halophytes can tolerate extreme salinity because of their anatomical, physiological and morphological features or avoidance mechanisms (Flowers et al. 1986).

Salinity induces stress in plants in many ways. It affects numerous physiological or biochemical processes, many of which are seen at cellular level. Primarily high salt concentration reduces osmotic potential of the soil solution creating water stress to root cells even under sufficient soil water availability. Salts interact with nutrient ions and make them unavailable to plants. Further, ions such as sodium cause internal toxicity in plants and they are not readily sequestered into vacuoles in glycophytes (Sairam and Tyagi 2004).

Plant adaptation to salt varies among plantation species in the degree of tolerance to a range of salt concentration that is achieved through one or more different mechanisms. The most important mechanisms are the exclusion of ions at root zone and suppression of ionic translocation to the shoot that help plants to keep levels of ions in growing meristem and young shoots low. Sequestering of salt ions into vacuoles or deposition into bark, ray cells, tracheid walls and lumens, or older senescent leaves, also help tolerant species to prevent salts from interfering with normal metabolic activity in the cells. Some plants show ability to maintain ion exclusion from young shoots under hypoxic conditions of waterlogging and maintain the ability to uptake water continuously in presence of high salt concentrations (Niknam and McComb 2000). Leaf and root Na/K ratio as well as stomatal resistance of plants exposed to salinity proved appropriate indexes of whole plant response to salt stress. Among the eucalypts, *E. grandis* tolerates low to medium levels of soil salinity through salt exclusion mechanism, until a threshold concentration, above which the tolerance fails. Once broken, tissues become very sensitive to salt. On the other hand, *E. maculata* shows less tolerance than *E. grandis* at the lower salt levels, but survives higher salt levels at which *E. grandis* becomes susceptible (Sun and Dickinson 1993).

Coconut palms are moderately tolerant to salinity (Remison and Iremiren 1990). Although grown in wide range of ecological conditions, coconut is considered to possess halophytic properties (Purseglove 1975). Traditionally planters apply sea salt in coconut groves in India, Java and Columbia (Child 1964; Manciot et al. 1979), and it is experimentally proved that sodium chloride (NaCl) application improved development of inflorescence, number of female flowers and nut yield (Fremond et al. 1966; Roperos and Bangoy 1967). Coconut is naturally adapted to maritime shores and is capable of absorbing chloride ions rather easily (Magat et al. 1975; Manciot et al. 1979; Remison and Iremiren 1990). In coastal areas, chloride accumulation in coconut leaves is a common phenomenon. Until recently, importance of chloride ions in coconut physiology was not understood. Evidences indicate that chloride ions play a significant role in non-hydraulic signaling of stomatal control in palms. It is now found that palms deficient in chloride had impaired stomatal function. The physiological role of chloride in coconut nutrition was earlier suspected to be associated with the water economy of the plant. Coconut belongs to a group of plants that lack chloroplasts and starch in their guard cells. For stomatal movements, such plants require chloride ions (Von Uexkull 1985).

3.2.4 Radiation

Adaptations of plantation species to local environments include the efficiency to harvest radiation for optimal growth and development. Most of the radiation in the atmosphere is infrared radiation (700-3000 nm, 67% of the photons) and visible light (400-700 nm, 28%; Nobel 1983). Ultraviolet (UV) radiation (200-400 nm), on the other hand, reaches the atmosphere in smaller amounts (5% of the photons). Biologically most hazardous part of UV radiation, i.e. UV-C (200-280 nm) and UV-B (280-320 nm) are completely absorbed by the stratospheric ozone layer and by other oxygen molecules in the atmosphere (Frederick 1993). In addition, ozone layer absorbs some of the longer-wave UV-B and UV-A radiations (320-400 nm). Therefore, of the photons reaching earth's surface, only about 2% are in the ultraviolet range (Nobel 1983). However, of the total solar energy, UV-B comprises about 1.5% and UV-A radiation about 6.4% (Frederick et al. 1989). Infrared region is responsible for the thermal stress in plants discussed in the previous section. Effect of other types of radiations such as X-rays, gamma rays and other ionizing radiation are negligible in plantation crops.

3.2.4.1 Visible Light

Plants use radiation in the regions approximately from 400 to 700 nm for photosynthesis, the region from 660 to 730nm has important qualitative, photo-morphogenic effects on growth. Physically, effects of light on plant growth depend on irradiant quality and duration (photoperiod) of which the former depends on the intensity and quantum of light exposure. Hence, high and low light exposures can make different effects on plants.

Plantation crops such as cocoa, cardamom, coffee and tea are heliophobes, which prefer low light. Heliophobes are naturally adapted to shade and does not tolerate heavy sunlight. Shade tolerance in trees varies with the age of the tree and with environmental conditions. Trees and shrubs vary widely in their capacity to grow in shade and this often becomes a decisive factor in their success under low light conditions. Trees tend to show higher degree of shade tolerance in their youth, and those growing on nutrient rich soils are more tolerant (Daniel et al. 1979). Shade effect is a complex mechanism in cocoa and is often associated with tree nutrition. Notwithstanding, young cocoa leaves grow well under shade than under full exposure (Wessel 1985). Furthermore, cocoa trees grown under shade have a bushy appearance with small leaves, shorter internodes and dense crown, which is desirable for commercial cultivation.

Coconut and rubber prefer good light, and low light interception showed adverse stress effect in growth of rubber (Khasanah et al. 2006). Shade grown young rubber plants had asynchronous variation in growth between roots and leaves that was attributed to competition for photosythate between secondary roots and leaves (Thaler and Pagès 1996). On contrary, in its natural habitats, young rubber plants grow and establish under the shades of overstorey canopy of rain forests. Rubber plants are found to show differential adaptation to light intensities at different phenological stages. They show natural adaptation to shade only when they are young, and lose this trait when the tree crown brushes overstorey canopy. Under commercial planting, rubber seedlings grown on cleared lands are found to show better growth when put under shades of intercrops (Rodrigo et al. 1997, 2001), possibly because of reduced thermal and/or photooxidative stresses. Senevirathna et al. (2003) have concluded that shade adaptation and shade-induced reductions in dynamic photoinhibition account for the enhanced early growth of rubber under light shade. Shade provides greater protection against diseases and weeds in coffee, cocoa and cardamom than under exposed conditions. There are many reports to suggest that shade increases fruit weight and bean size in coffee, but not quality and fruit-bean ratio (Muschler 2004).

Extensive efforts have been made to understand the factors responsible for shade tolerance in plants. Stomata of shade tolerant species were found to open more rapidly in sun flecks than those of intolerant species (Woods and Turner 1971; Davies and Kozlowski 1974), allowing the former to carry out photosynthesis during short periods of illumination. However, significant relation between stomatal response and shade tolerance could not be established (Pereira and Kozlowski 1976), because stomatal response not only varied with fluctuations in light intensity, but also was greatly modified by water stress preconditioning, temperature and mineral deficiency (Davies and Kozlowski 1974). This implies that many other factors like anatomical changes in leaves (Jackson 1967), changes in chlorophyll-protein ratio, enzyme activity of chloroplasts (Holmgren et al. 1965), rate of respiration

(Loach 1967), rate of photosynthesis (Kozlowski and Keller 1966) and various metabolic changes (Durzan 1971) influence shade tolerance.

Most of the tropical plantation species are adapted to high light exposure. For instance, radiation requirement of rubber (Ong et al. 1998) and coconut (Ochs 1977; Ohler 1999) ranges from 6 to 9 hours per day and exceed 2000 hours annually. Even though cocoa and coffee are heliophobes naturally, in cultivated conditions high productivity is recorded in coffee when grown under sunshine duration of 1900-2200 hours per annum (Descroix and Snoeck 2004; Wintgens 2004). In cocoa, mature trees yield well under full exposure but only in fertile soils. In poor soils, higher yield is obtained only under shade. In rubber, high irradiance especially in association with drought, high or low temperature can cause depression of photosynthetic productivity due to induction of photooxidative stress (Jacob et al. 1999). Oxidative stress induced chlorophyll degradation was also reported in rubber under high irradiance (Ray et al. 2004).

Apart from light intensity, photoperiod variations influence both vegetative and reproductive phases of plant growth (Garner and Allard 1920). At equator, days are of equal length during the entire year, but as we move away, large seasonal differences occur in the length of the daylight period. In temperate species, short days arrest shoot expansion and trigger a dormant state, whereas long days delay or prevent dormancy. Progressive slowing down of shoot elongation takes place under short-day conditions, successively producing shorter internodes until growth eventually ceases. Nevertheless, tropical plantation crops also show varied responses to day length. Experiments under controlled environments show greatly increased shoot growth during days longer than normal in coffee, cocoa and many other woody species (Longman and Jeník 1987).

3.2.4.2 UV Radiation

One of the growing concerns of present day is that the quantum of UV-B radiation reaching earth is increasing. Predictions based on stratospheric chemistry and climate-change models estimate that maximum springtime UV-B radiation will increase dramatically by 2020 (Shindell et al. 1998; Taalas et al. 2000). The intensity of UV-B radiation, in particular, is regulated by the thickness of the ozone layer that is rapidly being depleted by alarming concentration of anthropogenic atmospheric contaminants like chlorofluorocarbons and nitrogen oxides (Crutzen, 1972; Molina and Rowland, 1974).

Plants have several mechanisms for UV-B evasion, which include many UV-B absorptive pigments, reflective modifications and leaf thickness (Caldwell et al. 1983; Beggs et al. 1986). Thicker leaves may have an internal protective influence (Johanson et al. 1995; Newsham et al. 1996) by slight increase in the thickness of upper epidermis, spongy parenchyma and spongy intercellular space (Kostina et al. 2001). This feature is common in most of the plantation tree species. In addition, scattering and reflection of UV-B radiation is achieved through epidermal (epicuticular) wax and leaf hairs (Karabourniotis et al. 1999; Kinnunen et al. 2001). High level of cuticular wax content is a common feature in plantation crops. Changes in crystalline structure of the epicuticular wax with concomitant changes in light reflection may protect plants from damaging effects of UV light or aid them in the adaptation to different light intensities (Riedel et al. 2009). Protective role of epicuticular wax under abiotic stress is reported in coconut (Kurup et al. 1993).

Notwithstanding, most efficient mechanism of UV-B evasion is the accumulation of UV-B absorbing compounds in the epidermal cells of leaves (Burchard et al. 2000). Plants contain many phenolic compounds with anti-oxidant properties. Phenolics like light absorbing flavonoid compounds have been implicated in protecting plants from the damaging effects of UV-B radiation (Li et al. 1993). Flavonoid compounds are a group of phenylalanine derived aromatic secondary products, synthesized via phenylpropanoid pathway. Anthocyanins are the most common flavonoid compounds in higher plants. Temporary reddening of mature leaves seen on exposure to UV-B radiation has been attributed to anthocyanin accumulation (Steyn et al. 2002; Close and Beadle 2003; Gould 2004; Kytridis and Manetas 2006). Although not many studies have been reported on the effects of UV-B radiation in plantation crops, still it is prudent to believe that they have natural adaptation to this kind of stress. Leaves of tropical plantation crops like cashew, rubber and cocoa appear copper brown when young, and often turn reddish in the event of stress due to the accumulation of flavonoid pigments. Phenolic accumulation under photooxidative stress is a common feature in tea (Hernández et al. 2006) and coffee.

3.2.5 Nutrient Stress

Even in the presence of adequate levels of nutrients, crops might not reach optimal growth and productivity when grown in soils that contain phytotoxic levels of some metals / nutrients. Some of the micronutrients that can be potentially toxic at higher concentrations are copper, cobalt, iron, molybdenum, nickel and zinc. Even essential elements at excess levels can reduce plant growth. Plants therefore experience two types of stress in presence of soil elements, (i) deficiency and (ii) toxicity.

3.2.5.1 Nutrient Deficiency

Most of the plantation crops are adapted to nutrient deficient situations of marginal lands, a notable exception being coffee. Annual nutrient requirements of these crops are lower than many of the annual field crops. Plantation crops are large plants with extensive root system, which capacitate them to forage for nutrients from a larger soil volume. However, severe nutrient deficiency can be deleterious to these crops. Among the major nutrients, potassium is required for physiological development of trees, while phosphorus is required for adequate development of root system, meristem growth and wood formation. Magnesium is of particular importance for latex production in rubber trees. Plants under nutrient deficient stress exhibit characteristic external symptoms of starvation.

Plantation crops like rubber, cocoa, oil palm (Fageria and Baligar 2008), and cardamom (Krishnakumar and Potty 2002) are suitable for acid soils. Low pH or acidity favors complex interactions of plant growth-limiting factors involving physical, chemical and biological properties of soil. Calcium, magnesium and phosphorous deficiencies are considered major nutrient constraints that limit plant growth in acidic soils. Phosphorus limitation occurs due to fixation by oxides of iron and aluminum found in abundance in acidic soils. Young plants may require addition of fertilizers when grown in poor soils, but for established trees, there are many reports of adequate growth and cropping without any additional fertilization. The absolute phosphorus requirements of trees are small and deficiency symptoms are seldom found in fields. However, low availability of potassium can be serious and severe deficiency can induce compensatory uptake of other positively charged (especially magnesium and calcium) ions. Therefore, potassium deficient trees are therefore often characterized by unusually high leaf magnesium concentrations. Among plantation crops coffee, tea and cardamom are sensitive to low nutrient levels, resulting in low yields, poor quality and predisposal to pathogen damage.

3.2.5.2 Nutrient Toxicity

Direct nutrient toxicity due to high availability of major nutrients is not common in plantation crops. However, excess quantities of micronutrients and beneficial elements can result in serious toxic stresses limiting crop yield and profitability. Excess levels of nitrogen, phosphorus and potassium may boost vegetative growth, favoring pest and pathogen attacks, and the additional vegetative growth often occurs at the expense of reproductive phase. Sulfur toxicity occurs when sulfur dioxide adsorbed by leaves reacts with water to form bisulfate, inhibiting photosynthesis and degrading chlorophyll (Marschner 1995). Excess application of copper fertilizers or copper-based fungicides can result in copper toxicity, while high zinc fertilization causes zinc toxicity. Boron toxicity was reported to cause serious leaf damage in many crops. Aluminum toxicity is a common problem in acid soils. Tea plants show aluminum tolerance and have high internal tolerance to aluminum which is directly associated with aluminum accumulation (Rout et al. 2001).

3.2.6 Pollution

Pollution stress occurs in plants when pollutants such as toxic substances, gases, particulate matter, acids and radioactive substances contaminate their primary-growing environments (air, water and soil). Pollutants interfere with photosynthesis and respiration, enzyme activity and metabolic processes and causes membrane damage and cell death. Pollution stress is a growing problem in populated and industrialized regions of the world today. Natural pollution does however occur, due to seismic and volcanic activities and acid rains. Pollution being not a regular natural process and mostly of anthropogenic origin, natural adaptation to pollutants is seldom found in plantation crops. However, being large plants they do tolerate moderate and short period pollutions without much damaging effects. However, continuous and heavy exposure may not be tolerated.

3.2.6.1 Air Pollution

Air pollution is a major cause of concern in plantation crops in the recent times. Major air pollutants are carbon oxides, nitrogen oxides, sulfur oxides, ozone, fluorides, ammonia and particulate matter. Ozone, nitrogen oxides, sulfur dioxide and peroxy acetyl nitrates (PANs) can cause direct damage to leaves when they enter stomata. Chronic exposure of leaves to air pollutants can also break down waxy layer that protects plants from excessive water loss, diseases, pests, drought and frost (Miller 1990).

Ozone is produced in the atmosphere during a complex reaction involving nitrogen oxides and reactive hydrocarbons in the presence of sunlight. Reactive hydrocarbons are components of automobile exhausts, fossil fuel combustion and volatile organic compounds emitted by biosphere. Ozone is a main component in the oxidant smog complex (Thomas 1961) and is regarded as a serious air pollutant that affects crop productivity, climate change, human and animal health (Royal Society 2008). Many species of tropical rain forests and plantation crops like oil palm are known to emit isoprene (2-methyl-1, 3-butadiene), a potential reactive volatile organic compound. Emission of isoprene by oil palm is a biological phenomenon that is believed to be under strong circadian control (Wilkinson et al. 2006). In addition, oil palm plantations emit oxides of nitrogen from fertilized soils in significant quantities. Therefore biological emission of volatile organic compound and non-biological emission of nitrogen oxides aid ground level ozone build-up in and around oil palm plantations (Hewitt et al. 2009). Regional ozone pollution (Pierce et al. 1998) can cause vegetation injury in plantation surroundings due to oxidant build-up in the air.

Other major air pollutants are sulfur dioxide and fluorides. Major sources of these pollutants are coal-burning operations, burning of petroleum and the smelting of sulfur containing ores. Sulfur dioxide is converted to bisulfate, which is oxidized to sulfuric acid by the reaction with water and form acid rain. Fluorides are dissolved readily in atmospheric moisture that turns acidic. Sulfur and fluoride toxicity occurs on leaves however, species, varietal and phenological level differences in sensitivity are observed in plants (Griffiths 2003). Nevertheless, scientific information on sulfur dioxide or fluoride toxicity in plantation crops is hardly available.

Other air pollutants like ammonia, chlorine, hydrogen chloride, hydrogen cyanide etc. does not contaminate atmosphere unless in the event of accidents involving the storage, transportation or application of these materials. Release large quantities of these compounds into the atmosphere for brief periods can cause severe injury to vegetation in the immediate vicinity. In rubber plantations, gaseous ammonia is used as an anticoagulant for long-term latex preservation, but no adverse effect of exposure has been reported.

Particulate air pollutants such as cement dust, magnesium-lime dust and carbon soot is deposited on plantations near such industries. Particle deposits on the leaves inhibit normal respiration and photosynthesis. Cement dust can cause chlorosis, necrosis and death of leaves by the combined effect of a thick crust and alkaline toxicity produced in wet weather (Griffiths 2003). Similar stress effects can also happen when volcanic ash is deposited on the leaves in the event of volcanic activity.

3.2.6.2 Water and Soil Pollution

Water pollution occurs when industrial and domestic effluents are mixed with surface and/or ground water or when the effluents are used directly for irrigation. Effluents may contain a wide variety of toxic elements in varying concentrations. Prolonged usage of contaminated water causes elements to accumulate in the soil to levels toxic to plants. Common toxic elements are boron, chloride, copper, nickel, zinc, mercury, arsenic or cadmium. In addition, agricultural soils gets polluted by heavy metals by the soil addition of industrial and urban wastes, sewage sludge, fertilizers and pesticides and products from burning of fossil fuels. Studies show that toxic elements can incite almost all kinds of stress responses in plants, from altered respiration and photosynthesis, oxidative damage, membrane damage, impairment of enzyme activity and metabolism, anatomical and ultrastructural changes, poor growth and quality (Setia et al. 2008).

Limited studies are now available on natural adaptation of plantation crops to water and soil pollution, especially for heavy metal contamination. Arsenic accumulation in oil palm has been reported when grown in arsenic rich soils (Amonoo-Neizer and Amekor 1993) apparently without any stress symptoms. No direct stress was found in crops like coffee, rubber, coconut, eucalyptus and tea grown in soils with high content of lead, chromium, cadmium and nickel, except for the presence of these ions in elevated quantities (Abreu et al. 2005). There are very few evidences of heavy metal sequestration in plantation species so far. Metallothionein like genes (Abdullah et al. 2002) and stress inducible metallothionein promotors have been reported in oil palm, which may play a role in heavy metal detoxification. Metallothioneins are cysteine-rich low molecular weight proteins capable of sequestering metallic ions by binding (Omidvar et al. 2010). Similarly, metallothionein proteins have been isolated from rubber, which have been induced under oxidative stress situations (Zhu et al. 2010). Another low molecular weight protein family, small cysteine rich ligands called phytochelatins, which are involved in accumulation, detoxification and metabolism of heavy metal ions in plants (Maiti et al. 2004) have been identified in tea (Yadav and Mohanpuria 2009) and oil palm (Teoh et al. 2003). Radical scavenging proteins such as dehydrins that binds metals using a histidine-rich domain have been suggested to reduce metal toxicity in plant cells under water-stressed conditions (Hara et al. 2005).

3.2.7 Flooding

Flooding can be accumulation of water at the site of plant growth either by submerging the plants, fully (inundation) or partially (waterlogging), or for a shorter (flash flood) or longer (submergence) period. During waterlogging soil is saturated and root zone is affected, while during inundation stems are affected in addition to roots (Mullan and Barrett-Lennard 2010). Generally, inundation is not a problem in plantation species, but water logging can affect plantations grown at low-lying areas. Depletion of oxygen from soil (hypoxia) results in reduction in aerobic soil activities and anaerobic decomposition of organic matter is induced resulting in accumulation of carbon dioxide. Although rubber tree is sensitive to waterlogging, genotypic variations in adaptive as well as responsive behavior were recorded in rubber seedlings subjected to flooding (Gomes and Kozlowski 1988) ranging from downward curving of leaves (epinasty), reduced chlorophyll content, accelerated ethylene production, inhibited growth, induced decay of roots and stimulated production of lenticels and adventitious roots with large cells (hypertrophy). These adventitious roots contain extensive aerenchymatous cells that would facilitate diffusion of atmospheric oxygen to reduce root zone hypoxia, conferring flooding tolerance. Alternatively, these new roots may restore root to shoot communication and export essential mineral ions and hormones to the shoot system. Therefore it was suggested that, in rubber, flood adapted genotypes can be used as rootstocks in flood affected areas. In cocoa, although trees can withstand flash floods of mild intensity, they are particularly sensitive to water logging, but genotype variation in survival rate has been observed (Bertolde et al. 2010).

3.2.8 Fire

Fire occurrence in plantations is either anthropogenic or natural. Fire damage is a common occurrence in rubber and oil palm plantations, when people set fire to clear the undergrowth or deliberately to settle disputes. Oil palm plantation floors of South East Asia are infested with obnoxious weeds like cogon grass (*Imperata cylindrica*), which are generally cleared by burning (Yassir et al. 2010) occasionally causing fire accidents in plantations. In rubber, during wintering, plantation floors are carpeted by dry leaves that aid the fires to rage through plantations. Oil palms generally survive fire damage (Friday et al. 1999; Hairiah et al. 2000), but rubber trees suffer severe damage, rendering affected bark unusable for latex extraction.

3.2.9 Wind

Wind and hailstorms are natural phenomena that can affect agricultural crops adversely. Perhaps plantation crops are most affected by wind among agricultural crops. Degree of damage depends on the force at which wind blows through the plantations. Although storms and tornados can destroy entire plantation, mild to severe winds can result in branch snaps, trunk snaps and uprooting. Steady winds can give constant strain on the trees. In rubber plantations, when trees are exposed to steady winds from one direction, trees develop a leaning appearance with trunk bent along the direction of wind. Constant strain on the wood tissues leads to tension wood formation on the upper (windward) side (Savill 1983; Reghu et al. 1989). In spite of being susceptible to strong winds, rubber clones show a great degree of variation in wind tolerance (Vinod et al. 1996b; Priyadarshan et al. 1998). Some clones achieve wind fastness by the help of altered crown structure and branching pattern (Cilas et al. 2004) that allow wind to pass through the plantation without inflicting much damage. Palms are more adapted to wind than the trees. In some palms as the root system develops, stem base enlarges giving additional stability to withstand strong winds, storms and hurricanes (Tomlinson 1961). When grown under optimum environments, coconut palms develop enlarged base in tall as well as in dwarf genotypes (Satyabalan 1997).

Hails occurs commonly in tropics, at higher altitudes and latitudes with the onset of summer. There are reports of hailstorm damage to plantation crops like rubber, coffee, tea, cardamom and coconut. In northeast India where rubber is grown extensively, hail shower is a normal annual occurrence during summer. Although mild hail showers do not pose any problem to mature rubber trees, they are capable of inflicting damage in young plants. Notwithstanding, hailstorm of severe intensity can cause severe damage to rubber trees, by shattering the bark by falling hailstones (Meenattoor et al. 1995). Trees that survive damage take a longer time to recoup and it is reported that even after eight years affected bark has not returned to full anatomical features required for commercial latex collection (Vinod and Thomas 2006).

3.2.10 Lightning

Lightning is a spectacular natural phenomenon, occurs commonly along with thunderstorms, volcanic eruptions and dust wind. A lightning is most likely to hit a tall green tree, with an upward stroke of current in excess of 100,000 A (Ahrens 2007) passing through the tree, causing excessive internal heating, resulting in splitting up of vascular tissues and cracking of trunk (Thomas et al. 1995). Extensive observations of lightning damages are reported in crops like coconut and rubber. In rubber, direct lightning strikes cause trees to wilt within few hours of the incident. In partial strikes die-back of branches occur after few days, and in few cases, bark on one side of the trunk is seen damaged on entire length of the tree. Direct lightning strike on rubber trees are normally fatal, and those survive partial strikes remain unsuitable for tapping (Steinmann 1925). Direct fatality may occur only on one or more trees however, many trees around are subsequently killed, from secondary infections of pathogens and more conspicuously from wood boring ambrosia beetles (*Xyleborus* sp., *Xylosandrus* sp., *Platypus* sp). Wood of the affected trees generally shows dark streaks of vascular burning. However, in coconut palms, lightning leaves no palm to survive. Depending on the degree of electrical discharge, the death may occur instantly or after few days. In severe direct strikes, the treetop may be burnt, or decapitated, but such occurrences are relatively rare (Ohler 1999).

3.2.11 Soil Erosion

Soil erosion is a serious problem in plantation crops, since these crops are grown on cleared lands, and are generally planted in wider spacing. Soil disturbances during planting activity and removal of natural ground cover enhance steady depletion of soil layers either by run-off, landslides and wind erosion. Erosion causes loss of fertility that result in poor development of roots in addition to susceptibility to pest and diseases. Poor root anchorage affects absorption of soil water and nutrients and increases susceptibility to wind damage. It is reported that eroded soils under coffee were more acidic than non-eroded soils, with considerable loss of fertility (Hartemink 2006). Plantation crops like rubber grown in sloppy areas are more susceptible to run off and landslides during rainy seasons. In mature oil palm plantations, soil erosion depends chiefly on slope of the site and soil management practices. Intercropping with soil disturbing crops increases the chance of erosion, while those require minimal soil disturbance may help to prevent erosion. In young oil palm and rubber tree, the practice of growing cover crops helps to limit erosion. As the trees mature, cover crop disappears due to canopy or crown closure, resulting in increase of run off and erosion (Chew et al. 1999). Furthermore, the risk of soil erosion is more in surface feeders like rubber and oil palm wherein top soil erosion exposes most of the feeding roots that subsequently dry up and die, resulting in poor uptake of water and nutrients (Ferwerda 1977; Howell et al. 2005). Plant stress attributable to soil erosion can be seen in coffee plantation without adequate shade, having low planting density and grown on slopes. Erosion occurs in tea plantations after pruning.

3.2.12 Climate Change

Climate change is a matter of concern of twenty-first century that warns of rising temperature, unprecedented drought, flood, desertification, radiation, cyclones, forest fires, extreme low temperature that can adversely affect agriculture and human life. Predictions for the first three decades indicate that developing nations will be affected most because of following three reasons. Firstly, most of the developing nations are in tropical and sub-tropical regions where the negative impacts of climate change are expected to be more. Secondly, a large proportion of the global population growth will be happening in the developing world, and the third, more than half of the population in developing nations is dependent on agriculture (Reynolds and Ortiz 2010). This is of particular concern to plantation crops, because most of them are cultivated in the tropics. Being perennial crops, adverse effect on these crops will have long-term repercussions - the production decline and commodity price escalation.

Counting on natural adaptations to climate change in plantation crops is a difficult task. Being perennials, long-term survival can be the best index of adaptation. Fortunately, there are no reports of serious influence of climate change on plantation crops so far. However, one recent report from Ethiopia suggests of a shift in cropping pattern attributable to climate change, which reports of conversion some marginal coffee areas for cultivation of khat (*Catha edulis*), a hardy drought resistant plant, due to prolonged dry periods (Labouisse et al. 2008). A comprehensive review on current knowledge and future perspectives on diseases in tropical and plantation crops as affected by climate changes can be found in Ghini et al. (2011).

3.3 Adaptation to Biotic Stresses

Like abiotic stresses, most of the biotic stresses are seasonal in nature. Therefore, perennial crops are subjected to all kinds of biotic stress irrespective of the seasons. These crops undergo *in situ* natural selection against recurring factors, years after, and become capable of buffering the damage and only the fittest is carried forward to future generations. In practice, most of the biotic factors co-exist in cropping environments in this fashion, but the limit of buffering become apparent when varieties are tested in different environments or when a 'new' disease or pest arrives.

3.3.1 Defense Against Pathogenesis

Pathogenesis is associated with onset of favorable weather conditions, and therefore is limited to certain locations and seasons. Plants have active surveillance mechanisms either constitutive or induced against pathogens that are genetically programmed into every species (Bittel and Robatzek 2007). Basal resistance or innate immunity is the primary level of plant adaptation against diseases, triggered when microbe-associated molecular patterns (MAMPs) are recognized by pattern recognition receptors (PRRs) of host plant cells. MAMPs include specific proteins, lipopolysaccharides and cell wall components of pathogens. Activation of PRRs leads to active defense responses, both in basal and non-host resistance (Jones and Dangl 2006).

3.3.1.1 Constitutive Defense

Constitutive defense in planation crops are manifested through morphological, physiological and biochemical adaptations involving cuticular modifications, accumulation of wax and other organic compounds, production of specialized secretions and stomatal, bark or lenticel adaptations. Most of the morphological modifications also provide structural rigidity and strength to the plants (Freeman and Beattie 2008).

Structural defense

Cuticle acts as the primary physical barrier of plant's defense against pathogens. Recent evidences indicate cuticular modifications that confer disease resistance by release of fungitoxic substances as well as changes in gene expression that form a multifactorial defense response (Chassot et al. 2008). In plantation crops, cuticular defense against *Colletotrichum kahawae*, the coffee berry disease (CBD) pathogen has been known for a long (Nutman and Roberts 1960) in resistant varieties. Cuticular extracts (Nutman and Roberts 1960), wax extracts (Steiner 1972; Lampard and Carter 1973) and hypocotyl extracts (Masaba and Helderman 1985) of resistant varieties have shown clear suppression in conidial germination and mycelial growth of *C. kahawae*, however the exact components responsible for resistance have not been identified yet.

Waxes form a part of the preformed plant defense system against biotic stresses such as fungi, bacteria and insects (Gülz et al. 1991; Yoon et al. 1998; Marcell and Beattie 2002). Epicuticular wax of coconut predominantly contains lupeol methyl ether, isoskimmiwallin and skimmiwallin (Escalante et al. 2002) and other compounds such as lupeol, 3-β-methoxy lupane (lupane methyl ether) and acetates of lupeol, skimmiwallinol and isoskimmiwallinol. Epicuticular wax constitute 95% of the total wax content in coconut, while the remaining 5% include triterpenoids, sterols, primary alcohols, fatty acids and unidentified compounds (Riedel et al. 2009). However, significant genotype variation in epicuticular wax content is found in coconut as well as in rubber (Rao et al. 1998).

Biochemical defense

Majority of the phytochemicals belongs to three major classes of terpenoids, alkaloids and phenolics, and all of them are secondary metabolites. The basic terpene is isoprene, and depending on the number of isoprene units present in the molecules, terpenoids are classified as mono-, sesqui-, di-, tri-, tetra- and polyterpenes. One of the most versatile and abstruse compound produced by the rubber tree, the elastomer called rubber is a polyterpene known as *cis*-poly isoprene. Why rubber tree and many other species produce this compound expending so much of energy remains a mystery. Further, rubber is said to be at the metabolic dead-end since no enzymes are capable of breaking it down (Bonner and Galston 1947). Rubber particles are produced in latex, the cytoplasm of a specialized anastomosing network of laticifer cells. Notwithstanding, there seems to be a general agreement on the role of latex in plant defense against diseases and pests (Dussourd and Eisner 1987; Farrell et al. 1991). In support to this, antifungal activity of latex has been demonstrated *in vitro* (Moulin-Traffort et al. 1990; Giordani et al. 1999).

Alkaloids are nitrogenous compounds, derived from amino acids tryptophan, tyrosine, lysine and aspartates, possessing antibiotic and antifungal properties. Caffeine is a predominant alkaloid found in coffee, tea and cocoa. Highly toxic to fungi and insects, caffeine can even cause allelopathy in other plant species (Freeman and Beattie 2008). The phenolics are another class of defense-related secondary metabolites commonly found in plantation crops that are produced through shikimic acid - phenylpropanoid pathways. Shikimate pathway converts carbohydrate precursors into phenylalanine and tyrosine. Phenylalanine is the precursor molecule for the phenylpropanoid pathway. The phenylpropanoids commonly found in plants are anthocyanins, flavonoids, furanocoumarins, lignins and tannins (Saltveit 2010).

Polypeptide defense

Passive polypeptide defense is achieved in plants through preformed proteins and enzymes. They include defensins, protease inhibitors, amylase inhibitors and lectins. Defensins are cysteine-rich cationic proteins showing antipathogenic activity that are believed to form multimeric pores in the plasma membrane of pathogens leading to membrane disruption and depolarization by affecting ion-channels resulting in cellular ion imbalance (Thomma et al. 2002; Ganz 2003). Plant defensins are of three types, hevein type, thionin type and knotting type of which hevein and thionin types have eight cysteine residues while knotting type has six. Further, hevein and knotting type have continuous sequence of cysteines at the center while thionone type has cysteine sequences at the N-terminal end (Fujimura et al. 2005). Among the defensins, thionines show high affinity to chitin, the major cell wall component of pathogenic fungi (Oita et al. 2000).

The rubber tree latex contains hevein, a single-chain serine protease that has been described as an antifungal protein (Koo et al. 1998, Ko et al. 2003). It is an acidic protein with a chitin-binding domain belonging to lectin super family and is encoded by a multigene family consisting of at least five members (Pujade-Renaud et al. 2005). 50-70% of the total soluble proteins in rubber tree latex are composed of hevein (Van Parijs et al. 1991). Hevein contains 18% cysteine in its amino acid sequence (Lucas et al. 1985) and are found in two forms viz., prohevein (Hev b 6.01) and hevein (Hev b 6.02). Prohevien is actually the hevein preprotein that matures to hevein. Although hevein promoter is constitutively expressed in rubber (Montoro et al 2008), induced production also has been reported during wound related stress (Broekaert et al. 1990). Therefore, hevein is believed to play an important role in plant defense (d'Auzac et al. 1995). Besides heveins, rubber latex contain many proteins that belong to the class of pathogenesis related (PR) proteins. They are Hev b 2 (endoglucanase), Hev b 11 (*Hevea* endochitinase), Hev b 12 Hevea Lipid transfer protein (LTP) and hevamine (Hoffmann-Sommergruber 2002). Unlike that of all known class I chitinases, which are vacuolar proteins Hev b 11 is identified to be a cytosolic (C-serum) protein (Beintema 2007). Hevamine is another chitinase from rubber tree belonging to the family 18-glycosyl hydrolases. This enzyme has a unique property of cleaving the chitin molecule, and the sugar moiety of peptidoglycan (Bokma et al. 1997). Hevamine was the first enzyme found with chitin cleavage specificity (Bokma et al. 2000). Apart from rubber tree, expression and characterization of a putative defensin (EGAD1) in oil palm inflorescences has also been reported (Tregear et al. 2002).

3.3.1.2 Inducible Defense

Successful pathogens overcome primary defense systems and establish contact with the host. From this point onwards, inducible defense decides how plants survive the attack. Induction of defense does not occur until imminence, perhaps to conserve high energy and nutrient required in production and maintenance of defense mechanisms.

Structural fortification

Pathogen induced structural reinforcement of cell wall has been implicated in defense, accomplished through accumulation of glucan polymers such as (1-3)-β-glucan callose (Aist 1976), lignins, suberins (Guest and Brown 1997) or through cross linking of cell wall proteins (Bradley et al. 1992). Induction of callose and other defense related transcripts has been observed in coconut calli as a suitable alternative to characterize both biochemical and molecular interactions that occur between the coconut and its biotic stress factors (Uc et al. 2007). Callose rich haustorial encasements are induced in resistant coffee genotypes in incompatible reaction with leaf rust pathogen, *Hemileia vastatrix*. This encasement is less permeable thus preventing the flow of nutrients to the fungal pegs, thereby arresting the pathogen entry (Silva et al. 2006).

Root rot infection by *Rigidoporus lignosus* and *Phellinus noxius* in rubber tree starts with an increase of cell layers below the point of penetration resulting from a stimulated activity of cork cambium. Cell walls thicken concomitantly due to the accumulation of suberin or lignin. Callose formation occurs in young cork cells as well as in the phloem, obstructing pores of sieve tubes. Heterogeneous lignification takes place in xylem vessels. Tyloses resembling cell islands are formed in the young phloem in order to slow down the progression of invading hyphae (Nicole et al. 1985).

Rapid cork formation below the site of infection has been attributed for the resistant reaction in coffee to berry disease pathogen, *C. kahawae* (Masaba and van der Vossen 1982). Phellogen is formed in few cell layers rapidly, and the fungal invasion is arrested by development of suberized cells (Gichuru 1997).

Secondary metabolites

Many phenolic compounds are induced following pathogenesis. Phytoalexin accumulation in coffee genotypes resistant to *Hemileia vastatrix* and *Pseudomonas syringae* has been implicated in resistance reaction (Rodrigues et al. 1975; Guedes et al. 1994).

A fluorescent blue phytoalexin identified as scopoletin (7-hydroxy-6-methoxy coumarin; Giesemann et al. 1986) is produced in rubber trees during resistant reaction with *Colletotrichum gloeosporioides* (Tan and Low 1975), *Microcyclus ulei*

(Garcia et al. 1995) and *Phytophthora palmivora* (Churngchow and Rattarasarn 2001). MAMPs from *P. palmivora* induced production of scopoletin, *o*-dianisidine (a peroxidase) and accumulation of phenolic compounds in rubber seedlings and in cell suspensions under resistance reaction (Dutsadee and Nunta 2008). Scopolectin was also isolated from leaves of mature rubber trees uninfected with *Corynespora cassiicola* and was found at elevated levels in young leaves post infection. Further, it was demonstrated that scopoletin could fully suppress conidial germination of many rubber tree pathogens viz., *Corynespora cassiicola*, *Cylindrocladium quinqueseptatum*, *Phytophthora* spp., *Colletotrichum acutatum* and *Helminthosporium heveae* (Churngchow and Rattarasarn 2001; Silva et al. 2001). On the contrary, cyanogenesis, the liberation of high amounts of hydrocyanic acid (HCN), is observed from leaves of susceptible in rubber clones, following infections with *M. ulei* while resistant clones released only very little HCN. This indicated that cyanogenesis *per se* does not lead to defense against fungal pathogen but impair resistance reaction (Lieberei 1986).

Lignins are polymers of phenolic monomers commonly found in cell wall, especially in the secondary cell wall. In woody plants, cell wall lignification takes place as cells mature. Lignification provides rigidity to the cell, thereby imparting structural strength to the woody stem. Furthermore, lignins act as versatile defense compounds during pathogen attack. High level of lignin accumulation is a common phenomenon in the renewed bark of the rubber tree, following tapping and other wound induced reactions (Thomas et al. 1995). Gum exudations containing phenolic components occur in coconut and cashew following wounding possibly as a defense against imminent pathogen attack. Apart from lignins, plantation crops such as tea contain water-soluble polymers of flavonoids known as tannins. Tannins are commonly found stored in vacuoles and are toxic to pests rather than pathogens.

Inducible polypeptide defense

Evidences suggest that stress signaling in plants is modulated by multiple hormone-signaling pathways of jasmonic acid, salicylic acid, ethylene and ROS (Fujita et al. 2006) under the regulatory control of transcription factors (TFs) and *cis*-acting elements. TFs are proteins that regulate gene expression by recognizing and binding to the *cis*-elements in the promoter regions of the upstream target genes. Major plant TFs involved in biotic defense belong to five major gene families. Among these, ethylene responsive element binding factors (ERF) zinc finger proteins are involved in ROS signaling, while MYB (myeloblastosis) and MYC (myelocytomatosis) proteins are involved in jasmonic acid signaling. Further, WRKY (tryptophan, W; arginine, R; lysine, K; tyrosine, Y) TFs with zinc finger motifs are active in salicylic acid signaling, whereas, NAC (no apical meristem, NAM; Arabidopsis transcription activation factor, ATAF; cup-shaped cotyledon, CUC) factors activate jasmonic acid signaling and basic-domain leucine-zipper (bZIP) proteins. However, regulatory activity of TFs depends on plant specific adaptation to stress as well as on the types of stresses plants may experience. Recent developments in functional genomics have revealed presence of these gene families in some of the plantation crops. In coffee, most of the isolated genes showed homology to known plant genes suggesting cross-genome conservation of signaling pathways and pathogen resistance mechanisms (Silva et al. 2006).

The WRKY TFs are now known to play key regulatory functions in plant defense against salinity, mechanical damage, drought, cold, pest and diseases as well as in development of seeds, trichome and in the biosynthesis of secondary metabolites. Gene expression studies in *C. arabica* plants challenged with the rust fungus (*Hemileia vastatrix*), the root-knot nematode (*Meloidogyne exigua*) and abiotic treatments showed concomitant expression of *CaWRKY1a* and *CaWRKY1b* with same altered patterns in both biotic and abiotic treatments (Petitot et al. 2008). WRKY gene based markers have been found useful in delineating genetic diversity in coconut germplasm, indicating genome wide ubiquity and variation of these genes (Mauro-Herrera et al. 2007). In cocoa, *TcWRKY* markers have been developed for genome scanning for stress resistant genes (Borrone et al. 2004). Recently, a set of five WRKY TF members have been described in coffee as possible regulators of defense responses (Ramiro et al. 2010).

The rubber tree latex accumulates high levels of induced plant defense gene transcripts and hydrolytic enzymes such as cellulases, polygalacturanses (Kush et al. 1990), β-1,3 glucanase (Chye and Cheung 1995) and peroxidases (Dutsadee and Nunta 2008) suggesting a functional role for the latex in defense (Dussourd and Eisner 1987; Farrell et al. 1991). A detailed transcriptome profiling (Ko et al. 2003) reveals latex abundance of a gene, HbPI1 (*H. brasiliensis* protease inhibitor 1) that share conserved domain with potato inhibitor 1 family and share high level sequence homologies with serine proteinase inhibitor, protease inhibitor and PR protein. Other putative plant defense genes highly expressed in latex included LTI6B (low temperature Induced 6B - a low temperature and salt responsive protein, Blt101 (barley low temperature induced protein), and RTM1 (restricted TEV movement - disease resistance protein.

Increased activities of phenylalanine ammonialyase (PAL), NADPH oxidases and peroxidases were reported in coffee (Silva et al. 2008) during incompatible (resistant) reaction with *H. vastatrix*. Presence of PAL, the key enzyme in the phenylpropanoid pathway indicates phenolic production, while rise in lipoxygenase (LOX) activity (Rojas et al. 1993) indicate peroxide synthesis. However, LOX activity remained constant in a compatible interaction leading to disease. LOX are believed to provide hydroperoxide substrates that can be metabolized to compounds that play important roles in plant defense (Baysal and Demirdöven 2007). Peroxidase production is also reported in defense reaction against *C. kahawae* (Gichuru et al. 1997).

Hypersensitive response

Pathogenesis occurs when successful pathogens break down induced defense systems of the plant and inject a range of effector molecules that suppress host responses at different defense levels of perception, signaling and suppression. Notwithstanding, plants have evolved a sophisticated immune system to detect these effectors using cognate disease resistance proteins, a recognition that is highly specific and often elicits rapid and localized cell death (Xing et al. 2007). The hypersensitive response (abbreviated as HyR to distinguish from horizontal resistance, HR) is a deliberate sacrifice of cells around the site of infection, locally confining the pathogen and deprives it of water and nutrients to stop it from further advancing. HyR is, therefore, a type of programmed cell death (PCD) akin to apoptosis in mammalian cells (Heath 1998). The most common features of HyR are the autofluorescence of dead cells under UV exposure and subsequent browning of cells caused by oxidized phenolic accumulation. Although ion fluxes and generation of ROS commonly precede cell death, direct involvement of ROS may vary with the plant-pathogen combination (Park 2005). Oxidative burst also occur in many instances, but it is not necessarily a requirement for PCD (Groover et al. 1997). HyR is an effector specific reaction and therefore is more pathogen specific. The effector recognition follows signal transduction for the production of specific resistant (R) gene products or R proteins that induce HyR. On successful confinement of pathogens, plants set off effector-triggered immunity (ETI) that produces visible symptoms of HyR. ETI follows classical gene-for-gene theory and a strong ETI is responsible for an incompatible interaction or specific resistance (Göhre and Robatzek 2008).

Reactions similar to HyR, in which the cells around the penetration hyphae collapse, have been reported from rubber clones that are resistant to *Microcyclus ulei* infection. Deposition of autofluorescent compounds and callose, activation of ROS, scopoletin accumulation and cell death are found to occur leaving a limited necrotic patch surrounding the penetrating hyphae (Garcia et al. 1999; Lieberei 2007). Responses similar to HyR were also reported from rubber roots infected with root rot pathogen, *Phellinus noxius* (Nicole et al. 1992).

Naturally rust resistant coffee varieties too show HyR, (Lam et al. 2001; Morel and Dangl 1997) manifested by death of subsidiary and guard cells of stomata around the site of fungal penetration (Martins and Moraes 1996: Silva et al. 2002).

Systemic acquired resistance

The activation of ETI enables host tissues to combat a broad range of pathogens for an extended period, known as systemic acquired resistance (SAR) or 'whole-plant' resistance (Hunt and Ryals 1996; Neuenschwander et al. 1996; Ryals et al. 1996). The chemical activation of SAR requires accumulation of endogenous salicylic acid (SA) that induce a wide range of genes including PR genes (Durrant and Dong 2004; Zhang et al. 2010a) leading to production of chitinases, β-(1, 3)-glucanases, lysozyme, permatins and PR proteins (Ryals et al. 1996; Schneider et al. 1996).

There are many reports suggesting the presence of SAR in plantation crops, but conclusive evidences are still at large. If direct measurement of enzyme activities

(Heil 1999) is to be taken as SAR indicator, rubber tree latex has prominent chitinase activity (Van Parijs et al. 1991) and presence of β-(1,3)-glucanases and many PR proteins (Hoffmann-Sommergruber 2002). Recently, many artificial elicitors such as benzothiadiazoles and harpin protein are being introduced to mimic host-pathogen interaction leading to SAR (Gorlach et al. 1996; de Capdeville et al. 2001). Chemical elicitors such as salicylic acid (Mayers et al. 2005), jasmonic acid (Cohen et al. 1993), DL-β-aminobutyric acid (BABA) (Hong et al. 1999), oxalic acid (Mucharromah and Kuć 1991) and acibenzolar-S-methyl benzo-(1,2,3)-thiadiazole-7-carboxylic acid S-methyl ester (ASM) (Gullino et al. 2000) have been successfully used as SAR elicitors. Significant increase in PAL, peroxidase and β-1,3-glucanase activities on elicitor treated tea leaves challenged with pathogen and 25% decrease in disease intensity have been reported under field conditions. They are preferred because of relatively lesser risk they pose to non-target organisms and environment than toxic chemical fungicides.

Induced systemic resistance

Tree endophytes are silent colonizers of healthy internal plant tissues (Sieber 2007). Although some of the colonizers are potentially pathogenic, during symbiotic existence, disease is not generally incited, perhaps due to the endosymbiotic suppression of disease induction factors (Giordano et al. 2009; Botella et al. 2010). However, endosymbionts stimulate resistance reaction in host plants similar to SAR known as induced systemic resistance (ISR). ISR is therefore just another type of adaptation among crops plants wherein symbiotic relations between the host and colonizing species has been exploited, as against antagonistic relations as in SAR. Notwithstanding, ISR and SAR are believed to be in crosstalk in host plants. Observations during the establishment of successful colonization between the host (cocoa) and the colonizer (*Trichoderma*) (Bailey et al. 2006) indicate high induction of fungal genes such as glucosyl hydrolase family, serine protease and alcohol oxidase. This suggests of host-colonizer genetic crosstalk that results in endophyte-stimulated production of many secondary metabolites, including those involved in induced defense in host (Ryan et al. 2008; Gao et al. 2010).

Of different type of resistant mechanisms, ISR has been the least known in plantation crops. The first report of ISR in a woody species was made in citrus, against *Phoma trachetiphila* that causes mal secco disease (Solel et al. 1995). Among endophytic fungi, many species of *Trichoderma* are prominent colonizers in cocoa and in few other plantation crops (Holmes et al. 2004). Rubini et al. (2005) demonstrated that *Gliocladium catenulatum*, an endophytic fungus, could reduce the incidence of witches' broom disease in cocoa seedlings up to 70% accentuating their usefulness against fungal pathogens as biological control agents (Mejía et al. 2008). Comprehensive reviews on endophytic fungal diversity and use as biocontrol agents in cocoa and coffee are available (Mejía et al. 2008; Vega et al. 2009).

Use of two endophytic bacteria (*Burkholderia cepacia* and *Pseudomonas aeruginosa*) have been reported from oil palm, which could reduce the incidence of basal stem rot disease caused by *Ganoderma boninense* to a tune of 76% (Sapak et al. 2008). Plant growth promoting rhizobacterial (PGPR) bioformulations of *Pseudomonas fluorescens* was successfully used in tea to induce ISR to counter blister blight pathogen under field conditions with treated plants developing high accumulation of PAL, peroxidases and polyphenol oxidases (Saravanakumar et al. 2007). Further, *in vitro* tea plantlets when treated with two native rhizosphere bacterial isolates of *Azospirillum brasilense* and *Trichoderma harzianum* on hardening process, did not develop root rot and wilt diseases commonly found to infect *in vitro* seedlings during hardening process. These inoculated seedlings were found to have higher activities of defense enzymes like PAL, peroxidase and β-1,3-glucanase (Thomas et al. 2010).

In rubber trees, induction of systemic tolerance was demonstrated through lesser incidence of *Corynespora cassiicola* infection when endophytic bacteria isolated from different organs were inoculated artificially (Philip et al 2005). Rocha et al. (2011) have isolated and characterized endophytic fungi from cultivated *Hevea brasiliensis*, and found that these endophytic fungi exhibit marked inhibitory activity on *M. ulei* conidia germination *in vitro*. Moreover, arbuscular mycorrhizal fungi (AMF) were also found to improve plant growth and survival during greenhouse acclimatization of rubber seedlings (Rodríguez et al. 2009). AMF are naturally occurring group of soil fungi that exhibit symbiotic relationships with the roots of most vascular plant species (Vestberg et al. 2002). Further, AMF are suspected to induce systemic resistance, apart from improving plant growth by increasing nutrient uptake, especially phosphorus (Elsen et al. 2008).

RNA silencing

Recent evidences show that plants are capable of identifying and degrading pathogenic viral RNA by sophisticated mechanism of post-transcriptional gene silencing (PTGS) called RNA silencing, a process similar to RNA interference (RNAi) in animals. Plants contain small double-stranded RNA of 20 to 26 nucleotides in length, named short interfering RNA (siRNA) and endogenous small non-coding single stranded micro RNAs (miRNA) of an average 22 nucleotides. These classes of RNAs are related to several RNA biological processes such as the defense against viral invaders, developmental control, cell signaling, transposon silencing and heterochromatin formation (Vrettos and Tournier 2007). During viral infection, siRNAs bind to a complementary viral RNA and induce its degradation, while miRNAs are capable of inhibiting messenger RNA (mRNA). On degradation, plants may retain the digested viral template so that in case future infections they will be able to initiate quick action (Carbonell et al. 2008).

Successful isolation of two siRNAs from coconut leaves infected with coconut cadang-cadang viroid (CCCVd) was reported (Vadamalai 2005). Computational prediction of several miRNA from oil palm expressed sequence tag (EST) sequences has also been reported (Nasaruddin et al. 2007).

3.3.2 Defense to Herbivory

Plantation species in general have well-organized adaptation systems against the most common herbivores, the insect pests. These crops have inbuilt fortifications to ward off most of the insect feeders, and a few very specific pests can survive on them. Some crops like rubber have no serious pest at all. Pest damage does not pose serious threat to these crops and damages seldom surpass economic threshold. However, few sucking pests in cardamom, cashew and tea, borers and spider mites in coffee and cocoa can sometimes become exceptionally serious. In overall, pest resistance is of marginal importance in commercial plantation crops.

Similar to that of pathogen defense, plant mechanisms for insect resistance can be either constitutive or inducible. Among the three mechanisms, antixenosis and antibiosis are prominent in plantation crops, while the requirement of tolerance is relatively low. Plantation crops are rich in alkaloids, volatile monoterpenes, polyterpene compounds, tannins, lignins, cyanogenic compounds, other phenolic compounds and allergens, through which defense against pests are arbitrated. These biomolecules, otherwise known as allelochemicals induce a variety of reactions in pests such as repulsion, toxicity, allergy and indigestion, jeopardizing their normal metabolism, reproduction and survival. For instance, leaf minor (*Leucoptera coffeella*) resistance in coffee species viz., *Coffea racemosa, C. setenophylla* and *C. kapakata* seems to be of antibiosis type wherein phytochemicals interfere with normal growth of the insects. While in *C. canephora*, the resistance is exhibited through antixenosis (Aviles et al. 1983). It has been reported that *C. arabica* leaves has larger concentrations of p-cymene, a monoterpene derivative, and lower levels of beta cymene that greatly enhanced oviposition preference to the leaf miner, which was not the case in *C. canephora* and *C. racemosa* leaves (Filho 2006). Maintaining higher levels of phenolics in the face of attack was found to be one of the strategies for the observed tolerance of certain tea varieties to insect attack especially to *Helopeltis* (Chakraborty and Chakraborty 2005). This feature is also observed in rubber tree making it a less preferred host by most of the insects.

Herbivore induced defense gene activation takes place throughout the plants within hours after suffering the injury. Systemins, a group of plant peptide signal molecules comprising of short 18 amino acid long peptides (Pearce et al. 1991) are known to play a major role in systemic wound signaling (Ryan and Pearce 2003). Similar to systemins, short hydroxyproline rich systemin glycopeptides (HypSys) processed from longer precursor molecules have also been discovered in plants actively involved in defense reaction. HypSys activates methyl jasmonate, amplifying defense response. Presence of HypSys (*Cc*HypSys I, II and II) genes are reported in robusta coffee (Pearce et al. 2008).

Moreover, rubber tree belongs to euphorbiaceae, a large plant family in which cyanogenesis is a common occurrence. Cyanogenesis is the ability to synthesize cyanogenic glycosides, which when enzymatically hydrolyzed by β-glucosidase (Poulton 1990; Francisco and Pinotti 2000) produces sugars and a cyanohydrin compound that spontaneously decomposes to HCN and a ketone or aldehyde.

This step is catalyzed by hydroxynitrile lyase enzyme. Both the enzymes, β-glucosidase and hydroxynitrile lyase are common in cyanogenic plants (Harborne 1993; Gruhnert et al. 1994). The cyanogenic glycosides are usually sequestered into vacuoles, while enzymes are present entirely in the mesophyll tissues (Poulton 1990). On injury, cell disruptions bring both glucoside precursor and the enzyme in contact resulting in the release of HCN (Gruhnert et al. 1994). Rubber leaves contain a broad substrate specific β-glucosidase, linemarase, and cyanogenic glucosides, linamarin and lotaustralin (Lieberei et al. 1985). Since the cyanogenesis is an obligate feature in rubber trees, all living tissues, including that in seeds are strongly cyanogenic and contain both accumulated cyanogenic precursor and respective β-glucosidase. In seeds, endosperm alone contains more than 90% of linamarin (Poulton 1990). Montoro et al. (2008) found that cyanogenesis provides very good defense against herbivores in rubber.

Furthermore, rubber tree is rich in compounds that are potential allergens to herbivores. Rubber latex contain chitinases (Type I and III), β-(1,3)-glucanases and lipid transfer proteins, which are both allergenic and pathogenesis related (Hoffmann-Sommergruber 2002), besides many other *Hev b* allergenic proteins. Thirteen types of allergenic proteins found in latex include rubber elongation factor, small rubber particle proteins, hevein, prohevein, acid transfer proteins, endochitinases and superoxide dismutase (Yeang 2004).

Mechanical defense against herbivory is also found in plantation species, especially in palms. Coconut and other palms protect nuts by providing multiple layers of thick and strong husk. The chemical makeup and abundance of external protectives like waxes keeps pests away from preferring these crops for colonizing (Kolattukudy 1987). Structural adaptations for pest resistance are observed in few crops. Coconut palms with small nuts are more susceptible to eriophyid mites, than palms with larger nuts. Studies by Mariau (1977) revealed that in smaller nuts perianth is less firmly attached to nut enabling mites to access interspace between nut and lower perianth lobes for colonization, whereas in larger nuts this gap is mostly impenetrable. Further, it was observed that on infestation, some palms with smaller nuts do resist the damage by increasing the perianth-nut gap that makes the mites uncomfortable to settle, while exposing them to predators (Aratchige et al. 2007).

3.3.3 Allelopathy

Allelopathy is a natural adaptation to defend plant-plant competition, which causes difficulties in replanting orchards and vineyards and causes poor establishment, stunted growth and even complete mortality in the subsequent plantings. Autotoxicity induced by caffeine has been reported in coffee plants (Waller et al. 1989) and by other alkaloids from coffee such as theobromine, theophylline, paraxanthine and scopolectin (Peneva 2007). Allelopathic effects have also been reported in Eucalyptus (Sasikumar et al 2001; Liu et al. 2008).

3.3.4 Physiological Disorders

Sustained physiological stress due to factors other than pathogens and pests brings out visible symptoms in plantation crops. Although the term physiological disorder is very broad and can include symptoms of abiotic stresses, in a narrow perspective it is used to denote disorders of unknown etiology. Physiological disorders due to known factors can be revert back to normal by stress alleviation, while unknown factors can bring more severe permanent disturbances. Tapping panel dryness (TPD) of rubber is one of the widely known physiological disorders among plantation crops. Hot and cold disease of coffee is a physiological disorder caused in higher elevations due excessive cooling followed by heating. Other disorders of unknown etiology in coconut are pencil point disease, bristle top, dry bud rot, finschafen disease, frond rot, leaf scorch decline and Malaysia wilt.

3.3.4.1 Tapping Panel Dryness

The TPD, also known as 'brown bast' is a serious condition that affects productivity in rubber plantations. TPD is generally manifested as sudden drying up of latex vessels in tree bark at lower side of the tapping panel, rapidly spreading to wider areas on the tree trunk. Bark necrosis occurs usually after a period of prolonged flow of diluted latex, but sudden drying up without this symptom is also seen. As necrosis advances affected bark turn dry, crack and peel off either from the entire trunk or on from one side. Degeneration of normal bark happens simultaneously with the production new bark underneath, which lacks uniform structure and texture. Woody malformations are commonly found interspersed in the new bark rendering it unsuitable for tapping. Apart from the bark degeneration, the affected tree otherwise remain healthy and grow normally.

Observations suggest that TPD is a disorder resulting from continuous wounding of latex tapping that may trigger a non-compensated oxidative stress (Faridah et al. 1996). The disorder may be associated with nutrient depletion (Fan and Yang 1994), metabolic destabilization leading to bursting of lutoids followed by internal latex coagulation, followed by oxidative stress (Chrestin 1989). Apparent differences in metabolic profile between affected and normal bark are observed (Krishnakumar et al. 2001) including putative changes in protein profiles (Sookmark et al. 2002). Genotype variations in onset of TPD are also observed implying the existance of natural adaptation to the unknown causal factors (Yan and Fan 1995; Chen et al. 2003).

Recent evidences indicate association of Myb TFs with TPD (Chen et al. 2003), along with many differentially expressed water-stress-related genes (Mongkolsuk and Schumann 2009). Further, Venkatachalam et al (2007) reported up-regulation genes like cysteine protease like mRNA (CP), PR-osmotin precursor gene (PRO), ethylene biosynthesis related gene (EB), annexin-like protein RJ4 (ALP), phosphatidic acid phosphatase-related gene (PAP) and ASR (abscisic acid, stress and ripening) like protein 2 (ASR-2) in TPD affected bark. However, putative MyB TF and translationally controlled tumor protein (TCTP) were found down regulated. Besides, transcripts of genes *Hb*TOM20 (*Hevea brasiliensis* translocase of the outer mitochondrial membrane) and *Hb*TK (*H. brasiliensis* thymidine kinase), a putative plant thymidine kinase were found significantly down regulated in TPD-affected trees when compared to healthy ones (Venkatachalam et al. 2009, 2010).

3.3.4.2 Stem Tapering

Narrowing of the stem or stem tapering is a common phenomenon in palms. In coconut plantations, it is commonly known as pencil point disease. Although there are no specific factors implicated to cause stem tapering in coconut, any condition that deprives water and nutrients to the growing meristem can cause this symptom. Therefore, associated factors can be drought, disease, pest, mineral deficiencies, inadequate drainage and competition from weeds or any combination thereof. Palms recover in full when the causal factors diminish, but symptoms already produced on trunks do not revert. Stem tapering is also common in palms that are transplanted or relocated. Since the tapered portion remains mechanically weak, wind damage is an associated risk with stem tapering (Ohler 1999).

3.3.5 Wounding

Wounds are common occurrence in biotic and abiotic stresses. Plant tissues are damaged in and around the site of wound, exposing underlying healthy tissues to pest and pathogen invasion. In a controlled experiment in cocoa using conidial suspension of *Verticillium dalhiae*, it was demonstrated that stem puncture predisposes quicker pathogenic infection than soil application (Resende et al. 1995). Therefore, plants need to activate quick expression of defense during wounding. Wound healing is the primary response triggered, during which damaged cells die, turn necrotic due to the action of lytic enzymes and form a protective barrier. The superficial layers become lignified and suberized and cambial activity is accelerated. Wound healing is a stressful and energy expensive process, which falls outside the normal metabolism of plants. Moreover, the nature and duration of healing process depends on type and extent of wound (Thomas et al. 1995). Although natural wounding occurs in varying degrees throughout plants' lifespan, deliberate and continuous mechanical wounding causes perpetual stress in plantation crops like rubber and coconut. Wounding stress occurs in rubber while latex harvesting, whereas it occurs when palm wine is tapped from coconut palm. Agronomic practices such as plucking of tea buds and pruning of coffee can also develop wound related stress.

Wounding induces different types of signals in plants, targeted towards defense and healing process (de Bruxelles and Roberts 2001). Ethylene synthesis is found to occur in rubber in response to wound healing, wherein ACC oxidase (ACO) production is upregulated (Kuswanhadi et al. 2010). When Ethephon (2-Chloroethylphosphonic acid), an ethylene releasing stimulant was applied, in a positive feedback mechanism, ethylene produced was found to enhance expression of genes in the ethylene biosynthesis itself. Further, among three ACO genes, *Hb*ACO1, *Hb*ACO2 and *Hb*ACO3, basal levels of ethylene production appeared to be under the control of *Hb*ACO1, while *Hb*ACO2 and *Hb*ACO3 were responsible for the positive feedback mechanism and wounding response in leaves, but not in the latex. Whereas, a cysteine protease gene, *Hb*CP1 (*Hevea brasiliensis* cysteine protease) was induced in latex during wound healing, suggesting that *Hb*CP1 may be actively involved in protecting rubber plants against pathogen invasion and environmental stresses that involve ethylene signaling (Peng et al. 2008).

Studies using an isolated cDNA sequence of isoflavone reductase-like protein, namely *CaIRL*, from coffee (*C. arabica*) leaves showed that they encode for a novel type of phosphoinositide (PIP) family of NADPH-dependent reductases, which are known to be involved in biosynthesis of defense signaling molecules. Expression studies showed enhanced accumulation of these gene products in coffee leaves following mechanical wounding and fungal exposure (Brandalise et al. 2009).

Catechins are synthesized in tea, as an adaptive response to protect against tissue damage (Jaakola et al. 2002; Liu et al. 2006). During wounding, enhanced catechin production was found to occur mediated through the expressions of *Camellia sinensis* dihydroflavonol 4-reductase (*CsDFR*; Singh et al. 2009a), *Camellia sinensis* CoA ligase (*Cs4CL*; Singh et al. 2008; Rani et al. 2009), PAL enzymes (*Cs*PAL) and cinnamate 4-hydroxylase (*Cs*C4H) (Singh et al 2009b). Harvesting of tea is done by plucking out of terminal bud with two internodes below it, which has meristematically most active cells. These cells have highest activity of N assimilation enzymes like cytosolic glutamine synthetase, *Cs*GS (*Camellia sinensis* Glutamine synthetase; Rana et al. 2010), cell cycle proteins like histones, *Cs*H3 (Singh et al. 2009c) and ribosomal proteins like QM protein, CsQM (Singh et al. 2009d). Plucking process therefore, drains vital nutrients and compounds involved in growth and development and put tea plants into tremendous stress resulting in accumulation many stress related compounds.

Palm wine extraction from inflorescences of coconut and other palms also drains off vital nutrients, sugars and biochemical compounds. Palm tapping is a common practice in South and Southeast Asian and African countries. In coconut, although this practice adversely affects growth and production of palms, no systematic studies are available on stress effects of this traditional practice.

3.4 Management of Stresses

Stress management is a major imperative in production systems of plantation crops. In plantations, ultimate adaptability of a crop species may be associated with several interacting and interrelated factors, as well as their carryover effects. Therefore, unlike that of annuals, plantation species require counterbalancing mechanisms for more sustained stress management.

Sustainable adaptation to stress factors needs to be built-in the genotypes to tide over stress impact. External management can leverage the genetic adaptation potential so that the crops do not suffer beyond economic threshold. So stress management in plantation species needs primarily be genetic and then agronomic.

3.4.1 Crop Improvement

Crop improvement plays a vital role in enhancing stress adaptation potential of crops. Adaptive flexibility of genotypes not only can help in intensive and extensive cultivation but can aid in enduring unforeseen stresses also. For example, increasing the crop area under irrigation has several limitations such as unreliable rains and non-availability of perpetual irrigation, therefore, most feasible approach is to develop varieties that can sustain under limited moisture. These varieties have an added advantage to withstand unexpected moisture stress conditions in the field.

Being crops of perennial nature, focused attempt for breeding against stresses is seldom practiced in plantation crops because of limited genetic variability and long breeding cycle. Conventional breeding is highly cumbersome in plantation crops, because it involves many generations running for decades and expensive in terms of time, space and large volume of individuals handled. Except in case of palms, however, fixation of a superior individual identified at any stage of breeding is rather easy by vegetative propagation. Therefore, integrated breeding aimed at improvement of yield, quality and resistance, is the ideal approach in plantation crops. Notably, improved tolerance to abiotic stress must prove to be stable and inheritable unlike that against biotic stress wherein the tolerance breaks with the evolution of a new biotype. It is a challenge to plant breeders to generate crop plants that can stand, reproduce and set seeds in mild to moderate levels of abiotic stress, if not in extremes. The concern now is to consolidate these advancements in different crops and make further in-roads in raising the genetic level of stress tolerance. Notwithstanding, modern biotechnological tools promise of accelerated breeding to incorporate resistance through gene transformation, marker assisted introgression in many of the plantation crops.

3.4.1.1 Diversity and Genetics of Stress Resistance

In recently domesticated plantation crops like rubber, introduced genetic variation is relatively very limited when compared to wild progenitors. Furthermore, attempts to create of artificial variability by induced mutation and polyploidization has met only with limited success. Therefore, it is prudent to depend on natural diversity to target genetic adaptation to environmental constraints. There are several attempts to identify the genes and their control imparting resistance to various stress factors. While most of them are turned out to be polygenically controlled, there are few reports of monogenic and oligogenic inheritance as well.

The genus *Coffea* has about 90 species, with only two cultivated species, *C. arabica* (arabica) and *C. canephora* (robusta). *C. arabica* is a natural tetraploid, while *C. canephora* is a diploid (Chevalier 1948) with a lot of inter and intraspecific diversity among them, However, *C. arabica* has relatively low diversity when compared to *C. canephora* (Lashermes et al. 2000) and lack resistance to major diseases and pests. Low genetic diversity of *C. arabica* has been attributed to its allotetraploid origin, reproductive biology and evolution process (Etienne et al. 2002). *C. canephora* on the other hand, has wider adaptability to different agro-climatic conditions and show tolerance to leaf rust pathogen, *H. vastatrix*. Besides, *C. canephora* provides the main source of resistance to other disease and pest including CBD (*C. kahawae*), and root-knot nematode (*Meloidogyne* spp.). There are at least nine dominant genes (*SH1-SH9*) conferring resistance against leaf rust in coffee (Bettencourt and Rodrigues 1988). Of these genes *SH1*, *SH2*, *SH4* and *SH5* comes from *C. arabica*; *SH3* from *C. liberica*; and *SH6*- *SH9* from *Hibrido de Timor* (HDT, a spontaneous natural hybrid between *C. arabica* x *C. canephora*) derivatives (Rodrigues et al. 1975; Bettencourt and Rodrigues 1988). Moreover, *C. liberica* also provides resistance against leaf rust (Srinivasan and Narasimhaswamy 1975), while *C. racemosa* provides coffee leaf miner resistance (Filho et al. 1999). CBD resistance is complete in *C. canephora* and partial in *C. arabica*. Genetic studies on CBD resistance conclude that three major genes *viz., R*, *K* and *T* are responsible for resistance. Partial resistance observed in arabica and HDT derivatives were due to recessive nature of these genes at least at any one locus (van der Vossen and Walyaro 1980). Recent reports, however, suggests of oligogenic and quantitative resistance to CBD (Silva et al. 2006). Recently, a major dominant gene, *Ck-1* has been mapped for CBD resistance in *C. arabica* (Gichuru et al. 2008), which may perhaps be synonymous to the *T* locus. Inheritance studies on high-level resistance found in *C. canephora* against root-knot nematode, *Meloidogyne exigua* reveals that a simply inherited major gene, *Mex-1* (Noir et al. 2003), controls resistance.

The tea genus, *Camellia*, seems to be a genetically obscure one because of inconsistent and subtle genetic variation at species level and of genetic instability due to high out-breeding nature. The cultivated tea, *C. sinensis* and its progenitors have originated from Irrawaddy river basin in Myanmar extending between Southeast China and Assam in Northeast India (Eden 1976). Recorded diversity history shows that species count increased from 82 species in 1958 (Sealy 1958) to more than 325 species in 2000 (Mondal 2002). Presently, more than 600 varieties are under cultivation world over, of which some are unique in caffeine content and disease tolerance. Cultivated tea hybridizes well with wild relatives, creating a myriad of variants in tea genetic pool. One of the particularly interesting wild species is *C. irrawadiensis* whose morphological distribution overlaps with that of cultivated tea (Banerjee 1992) and a few desirable traits such as anthocyanin pigmentation and special quality characters of Darjeeling tea might have originated from these species (Wood and Barua 1958). It is widely accepted that three species i.e. *C. assamica*, *C. sinensis* and *C. irrawadiensis* have predominantly contributed to the cultivated gene pool of tea that includes progenies and the hybrids between them (Mondal et al. 2004). The information available on genetic resistance to various stresses in tea is rather limited.

Resistance to grey blight caused by *Pestalotiopsis longiseta* is high among *C. assamica* and two dominant genes, *Pl1* and *Pl2* are reported so far. The *Pl1* gene, imparts a higher level of resistance, and has an epistatic action with the *Pl2* gene, which has a moderate level of resistance (Takeda 2002, 2003). Besides, there are partial resistant sources to blister blight (Premkumar et al. 2008), anthracnose and cold damage (Takeda et al. 1987).

The genus *Hevea* to which rubber tree belongs has ten recognized species, of which *H. brasiliensis* alone is cultivated. Major world production of natural rubber takes place in its introduced home in the South and Southeast Asian countries because of a potentially devastating disease, SALB, in its native land. Therefore, rubber cultivation in Asia is virtually on a vulnerable threat from SALB because of two reasons, (a) the variability of the introduced genetic pool is very narrow and (b) no genetic resistance to SALB is available. Further, a little information is available with respect to genetics of resistance for other diseases of rubber. Various parameters indicative of possible resistance has been described in the case of SALB such as incubation time, latency, lesion size and number, sporulation, time taken for stromata to appear, and time from inoculation to leaf fall. Simmonds (1990) suggested that complete resistance to SALB was likely to be monogenic. However, no conclusive evidence on this claim is available so far. Possibility of combining durable resistance to SALB looks remote, as the pathogen can adapt so quickly to the host resistance, much faster than the development of a resistant combination (Rivano et al. 2010). Notwithstanding, it is reported that resistance to *Corynespora cassiicola* in rubber was articulated by two dominant genes *A* and *B*. Gene *A* is in epistatic interaction with *B* in which recessive form of *A* suppresses expression of *B* (Hadi et al. 2004). Le Guen et al. (2000) used isozyme marker based linkage mapping approach to map a qualitatively inherited dominant gene, *Phr* lying at 14.7 cM from the *adh* locus, conferring resistance to *Phyllachora huberi*, which causes black crust disease. Genotypic variations exist for tolerance to disease as well as environmental variations in rubber clones (Raj et al. 2005).

Among the palms, coconut is the only species in the genus *Cocos*. Although there are many genetic variants in cultivated coconuts, exact evolutionary relations between them are still obscure. Genetic studies on economic traits show quantitative inheritance, while there are only a few attempts to study genetics of disease resistance. Studies on complex diseases like lethal yellowing, root wilt etc. are particularly difficulty because of the difficulty in effectively inducing diseases under controlled situations. However, there are many reports confirming genotype variability in resistance to most of the stress factors in coconut, with some varieties carrying exceptionally high level of resistance.

The genus *Eucalyptus* harbors high inter- and intraspecific genetic variability for resistance to stress factors. Variation in abiotic stress tolerance may extend from frost susceptibility to extreme frost tolerance as well as tolerance to drought. Besides, resistance to diseases enables adequate disease management by planting resistant clones, progenies or species. In *E. grandis*, a single dominant gene, *Ppr-1* (Junghans et al. 2003), governs resistance to rust pathogen, *Puccinia psidii*, while a general level of tolerance is exhibited to *Cylindrocladium* leaf blight.

3.4.1.2 Classical Approaches

Breeding for resistance to stress has not been a major breeding objective in plantation crops until the beginning of 20th century. However, stress resistance breeding has become a major objective in breeding most of these crops now, because of following reasons. Catastrophic events of crop destruction around the world due to various stress factors has led to better understanding of the biological events related to stress. Shrinking resources for cultivation and increasing demand for various plantation commodities had led to perpetuation of plantation crops to non-traditional regions. Further, better understanding of genetics of stress adaptation and modern tools in crop improvement has opened new avenues for crop improvement.

The utilization of vertical resistance (VR) in breeding for stress resistance especially for biotic stresses in annual and seasonal crops had been very successful, occasional disasters notwithstanding. However, in plantation crops that grow in nearly non-seasonal environments, effective use of VR is not an ideal option. This is because any attempt to use VR in these crops had resulted only in transient, non-durable resistance. The experiences shows that pyramiding of VR only helped in delaying in the development of new pathotypes a little. Further, introgression of VR genes from wild species would lead a breeder to a very low genetic purity level in respect of other characters, which would require several backcrosses and many decades of efforts for improvement.

Nevertheless, the use of horizontal resistance (HR) is promising as the only choice in plantation crops because HR is reasonably heritable in all these crops. Further, offspring produced between ideal parents are slightly more resistant than the parents are. Notwithstanding, directed selection for resistance is not only done individually, but done also at a particular location in overall, discarding unhealthy and susceptible genotypes, and selecting only resistant ones. This method of selection has been the most successful breeding strategy in plantation crops for stress resistance as it accumulates HR, albeit in congruence with yield and quality traits. For effective selection, however, pre-breeding is an essential necessity for developing best lineages and for accumulating favorable alleles in the breeding population.

In coffee, one of the main breeding objectives worldwide is to transfer disease resistance from diploid species such as *C. canephora* or *C. liberica* into cultivars of *C. arabica* without affecting coffee quality (Etienne et al. 2002). Several stages are involved in the improvement of *C. arabica*, of which the first stage is selection and testing of superior individuals. These superior individuals are seed propagated, and two cycles of selfing is done before testing the characters in each line for stability. Thereafter, superior lines are intercrossed, in double or multiple crossings, and selection is done from progenies for improved individuals with respect to resistance and allied traits. Backcrossing may produce varieties that are more superior. The best selections emerging out of the selection process are cloned for further multiplication and released for commercial cultivation. Robinson (1976) employed a crash-breeding program in Ethiopian coffee, by screening and selection for CBD resistance among naturally occurring variation in planter's crop. The seeds collected from the selections were grown and progeny tested for durability of resistance along with yield and quality. This intense screening resulted in superior populations with balanced domestication in seven years. Clonal selection is the most important procedure followed in rubber breeding. Because of longer selection cycle and possibility of occurrence of multiple stresses, stress endurance is used as a selection parameter in rubber along with yield traits. Clones can be evolved at any stage of breeding. Selective hybridization of promising clones is further done either among themselves or with wild germplasm lines. The progenies are directly selected from seedling nurseries and cloned for further evaluation. Natural open pollinated half-sib populations are also screened for desirable characters including resistance. Susceptible and poor performing clones are normally discarded while selection. Polycross gardens comprising of pre-potent clones are also utilized and the selection is generally exercised in the polyclonal seedling orchards, even at maturity stage. In India, increased resistance to biotic and abiotic stresses has now been re-emphasized in rubber breeding and selection (Venkatachalam et al. 2006), in particular, for low temperature tolerance and resistance to *Corynespora* leaf fall disease. In the case of SALB, no durable resistance has been reported so far in rubber, although source of putative total resistance, characterized by absence of spores on leaves, are found in some wild clones of *H. brasiliensis* and in other species such as *H. benthamiana, H. guianensis*, *H. pauciflora* and *H. spruceana* (Simmonds 1990). Introduction of unexploited genetic variability from wild to augment narrowing genetic base of the cultivated gene pool was carried out by the International Rubber Research and Development Board (IRRDB) in 1981. An expedition, carried out in the Amazon basin spread across three districts of Acre, Rondonia and Mato Grosso of Brazil, collected 194 high yielding trees which were not affected by *Phytophthora* and SALB along with a total of 63768 seeds, 1413 meters of budwood and 1160 seedlings (Ong et al. 1983). These collections show continuous variation for SALB resistance in French Guyana (Clement-Damage et al. 1998) and for *Phytophthora* resistance in India (Mercy et al. 1995).

In tea breeding, mostly conventional approaches are being practiced (Chen et al. 2007), with yield and quality as the prime targets. Nevertheless, breeding for resistance against stress factors has received only little emphasis, because it has not been largely successful. Natural selection plays an important role in adaptation of tea clones to a set of stress factors by eliminating susceptible clones during early stages of multiplication. Since only tolerant clones survive, a well-buffered population survives under every geographical niche where tea is cultivated. Even if any related species is identified with a great degree of resistance to a particular stress, such species are not included in breeding programs because, most of the wild cross produce very inferior quality tea that is not acceptable (Bezbaruah 1987) and difficult to improve upon. Therefore, a compromise on quality with respect to resistance can be more economically devastating than the stress factor itself. Since available genetic base of cultivated tea is narrow, particularly with respect to quality, scope for further improvement for resistance within the available base remain limited (Willson 1999). Notwithstanding, breeding attempts for stress resistance have not been without any success. In Japan, distant hybridization of cultivated and 26 wild species, resulted in isolation of an interspecific hybrid, Chatsubaki (*C. sinensis* × *C. japonica*), with high resistance to tea anthracnose, grey blight and low temperature besides having low caffeine content (Takeda et al. 1987). Chatsubaki is now used in regular tea breeding programs. In India, a high yielding standard tea variety has been developed by interspecific hybridizations involving *C. irrawadiensis*, *C. assamica* and *C. sinensis* (Bezbaruah 1987). Recently, it has been reported from China that one excellent new clone with high cup quality, resistant to disease, suitable for fine green tea and very early sprouting in the spring has been selected from the Cobalt-60 γ-ray irradiated offspring of Longjing 43 cuttings (Yang et al. 2003). This clone is undergoing adaptability tests and may become the first clone bred using induced mutagenesis in plantation crops (Chen et al. 2007).

As a crop of economic importance, at one time, coconut was considered neglected in terms of breeding and genetics (Harries and de Poerck 1971; Williams et al. 1975). Owing to various practical constraints in palm breeding, therefore, genetic improvement in coconut had made a slow progress. However, declining nut production and shrinking cultivation in the major production environments has restored the efforts for breeding for resistance in coconut recently. Further, widespread incidences of lethal yellowing in the Caribbean and Africa, cadang-cadang in the Philippines, root wilt disease and recent outbreak of eriophyid mites (*Aceria guerreronis*) in India have raised serious concerns, because most of these threats are beyond control by conventional plant protection measures. Although a long-term process, development of resistant/tolerant genotypes is the only practical solution to combat these stress factors.

In coconut, screening of exotic germplasm had resulted in identification of Malaysian dwarfs (yellow, red and green) tolerant to lethal yellowing in Jamaica in 1950s. These were recommended for planting on a large scale in Jamaica and Florida, only to be withdrawn sooner due to their susceptibility to many other climatic constraints. Nevertheless, this has led to the development of a resistant hybrid, Maypan, obtained from the cross Malaysian yellow dwarf (MYD) × Panama tall (Harries and Romney 1974). Maypan could revive the Jamaican coconut industry largely, until it was massively destroyed by fresh outbreak of the disease (Broschat et al. 2002). Recent reports suggest genetic contamination among parents as the reason for loss of resistance (Lebrun et al. 2008), but other reasons like development of virulence in pathogen and/or vector and very narrow window of genetic variation among the cultivars cannot be ruled out (Baudouin et al. 2008).

In root wilt screening program in India, disease escapes are selected from ‘hot spot’ areas belonging to tall (T) as well as dwarf (D) types and serologically tested for the presence of phytoplasma. Disease free plants are then intermated in combinations of T × T, T × D, D × T and D × D. Seedlings raised from these crosses were understory planted in ‘hot spots’ to subject them for vigorous natural selection. Disease escapes were further tested serologically to ensure absence of root wilt pathogen. Some D × T hybrids involving disease-free Chowghat green dwarf (CGD) palms and west coast tall (WCT) palms planted in 1991 remained disease free a long period (Nair et al. 2006). This program has now resulted in the development and release of two selections Kalparaksha (Nair et al. 2009) and Kalpasree and one hybrid Kalpa Sankara with field resistance/ tolerance to root (wilt) disease and yield potential.

3.4.1.3 In Vitro Approaches

In plantation crops, several tissue culture techniques such as somatic embryogenesis, meristem and axillary bud culture, induction of adventitious buds, androgenesis and protoplast culture are used for plant regeneration and micropropagation. One of the extreme advantages of a cell culture system is the facility for artificial screening of germplasm and mutant lines to identify stress tolerant genotypes, mutants and somaclonal variants those can augment accelerated breeding programs. Stress factors that are introduced in the culture media include increased salinity, pathogenic toxins, low nutrient content, heavy metal and induced water deficit. Regeneration of genetically engineered plants (transgenics) is yet another possibility under *in vitro* systems. Besides, micropropagation helps in developing disease free planting materials in plantation spices like cardamom (Babu et al. 1998).

In vitro selection

Most widely explored stress factor using *in vitro* systems in crop plants is salt tolerance. Cell culture systems offer several unique advantages in studying cellular level mechanisms and functions of salt tolerance and provide alternative methods for screening, selecting, and characterizing salt tolerance at the cellular level. Sodium chloride (NaCl) is the most frequently used salt for salt tolerance screening, although use of other salts and dilutions of seawater have been reported. Spontaneous variability generally appears in cell culture, sufficient to allow effective selection; use of mutagens such as ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS) helps to increase mutation frequencies. Selected salt tolerant cell lines are further evaluated to see if the tolerance remains stable after the cells had been moved to salt-free culture systems. There are several reports detailing mechanisms of salt tolerance as well as successful isolation of tolerant cell lines in many plantation species. In *Eucalyptus microtheca*, nodal segments cultured *in vitro*, showed varying degree of ion accumulation, with decreasing K^+/Na^+ ratio with increase in salinity, implying a simple way of *in vitro* screening than the cumbersome *in vivo* screening (Morabito et al. 1994). Pollen germination *in vitro* in the presence of elevated salt concentrations has been implicated as a reliable index of pollen tolerance to salinity in olive, a Mediterranean tree crop similar to tropical plantation crops. Further, a close correlation between pollen (gametophyte) tolerance and whole plant (sporophyte) responses to salinity was also found in olive (Soleimani et al. 2010).

Induction of artificial water deficit by incorporating polyethylene glycol (PEG-6000), mannitol and NaCl into *in vitro* culture of excised coconut embryos revealed that embryos from putative drought tolerant lines were able to withstand NaCl, but not PEG and mannitol (Karunaratne et al. 1991). The survival of embryos in high NaCl concentrations is perhaps indicative of the halophytic nature of coconut and it is assumed that same mechanism plays a role in drought tolerance as well.

Selection for disease resistance under *in vitro* systems is reported in coffee, oil palm, cardamom and date palm. Partially purified culture filtrate (PPCFs) containing

C. kahawae phytotoxins was used in varying concentrations for screening hypocotyl explants obtained from CBD resistant (HDT) and susceptible arabica (N39) genotypes, showed that calli from HDT showed rapid growth and no necrosis, while N39 calli had varying degree of necrosis and growth suppression. Selections from the surviving calli of N39 on regeneration had shown increased CBD resistance (Nyange et al. 1995). Dorcas et al. (2010) isolated basal rot resistant somaclones using PPCF of *Fusarium oxysporum* from a series of *in vitro* screenings in cardamom. They used somaclones derived from plants that withstood an initial pathogenicity test in the screen house. These somaclones were then cultured through a series on PPCF concentrations, selecting only those survived, until the selected lines survived undiluted PPCF. In date palm, pathotoxins such as fusaric, succinic, 3-phenyl lactic acids and their derivatives, marasmins and peptidic toxins as selective agents in cell culture are being used for isolating resistant lines against *Fusarium oxysporum* f. sp. *albedinis* that caused fusariosis wilt or bayoud (El Hadrami et al. 2005; El Modafar 2010).

Shoot cultures of *Eucalyptus camaldulensis* clones with different levels of salt tolerance were exposed to NaCl and ABA *in vitro* showed that proline accumulation increased significantly in all the clones. However, when the cultures were exposed to NaCl alone, resistant clones had significant high proline accumulation whereas in susceptible clones proline content remained unchanged (Woodward and Bennett 2005). Substantial accumulation of proline, polyamines (PA) and ABA occurs in plants during adaptation to various environmental stresses such as salinity, drought and high and low temperatures. Proline is a known osmoprotectant during drought stress in plants, and can serve as a nitrogen and carbon source during stress recovery process (Galiba 1994), and its accumulation is positively correlated to the salinity level or to the intensity of water stress (Heuer 1999). Elevation of endogenous ABA in response to cold treatment is hypothesized to induce synthesis of proteins that are responsible for the increase of frost hardiness (Chen et al. 1983). ABA dramatically increases freezing tolerance of the cells cultured *in vitro*, and enables cultured plant cells to survive freezing temperatures without previous cold treatment (Galiba et al. 1995).

Genetic Transformation

One of the remarkable achievements in plant cell culture is the opportunity to transfer genes across organisms through plant transformation employing somatic embryogenesis. Transgenic technology has now been tried in almost all cultivated species and is one of the remarkable technologies in the area of biotic stress management. The most successful event of genetic transformation in plants is transferring of *cry* (crystalline protein) genes from the soil bacterium, *Bacillus thuringiensis* (Bt) for imparting insect resistance. Although herbicide tolerance has also been achieved through transgenic technology, nevertheless, it is of little importance in plantation crops, except in the case of cardamom. Herbicide tolerance is incorporated in coffee and eucalyptus, primarily as selective agents during transgene development or as model transgenesis system rather than for agronomic use.

Agrobacterium tumefaciens mediated genetic transformation is widely used in plantation crops. Although *A. rhizogenes* and biolistic methods have been tried, the popularity of *A. tumefaciens* system is attributed to its simplicity and ease of generating transgenic derivatives. Coffee was among the early perennials in which successful transformation events for stress tolerance were reported. Transgenic coffee plants containing the *cry1A(c)* gene were produced using both *A. rhizogenes* (Leroy et al. 1997) and *A. tumefaciens* (Leroy et al. 2000) mediated transformation systems. The *cry1A(c)* gene encodes for an insecticidal crystal protein that is toxic to certain insects including the coffee minor, *Perileucoptera coffeela* (Filho et al. 1998). Transformed plants showed high degree of field tolerance to leaf miner in French Guyana (Perthuis et al. 2005). Other than coffee, successful regeneration of transgenic plants with augmented stress tolerance was reported rubber, oil palm, eucalyptus, cocoa and tea (Table 3.3). Successful incorporation of cowpea trypsin inhibitor (*cpTI1*) gene (Abdullah et al. 2003; Ismail et al. 2010) and *Bt* gene (Lee et al. 2006) is reported recently towards achieving insect resistance in oil palm. Transgenic plants obtained are undergoing screening.

Whilst, most of the transformation attempts are targeted against pests, chitinases and antibody linked small chain variable fragment (scFv) genes are used for generating disease resistant transgenes. The scFv genes coding for antibodies specific to pathogenic toxins were recently demonstrated to be efficient against toxin producing pathogens like *Corynespora cassiicola* in rubber (Sunderasan et al. 2009). This provides opportunities to incorporate such genes in tissue transformation systems to incorporate resistance against pathogens. In oil palm, white rot pathogen, *Ganoderma boninense* establishes by destroying lignin fraction of woody tissues and exposing white cellulose, which fungus utilizes by causing rot. Resistance against *G. boninense* is being attempted through transfer of chitinase and ribosome inactivating protein (RIP) genes (Hashim et al. 2002). Lignin content has been genetically modified in plantation crops like eucalyptus, opening the possibility of attempting such systems in oil palm (Price et al. 2007; Paterson et al. 2009).

Transgenesis towards abiotic stress tolerance were reported in rubber against oxidative stress in which superoxide dismutase (*HbSOD*) gene (Jayashree et al. 2003) and Mn-superoxide dismutase (*Mn-SOD*) gene (Sobha et al. 2003) were successfully incorporated. Transgenic development to tackle abiotic stresses has also been attempted for freezing tolerance in eucalyptus (Zhang et al. 2010b) and for salinity tolerance in tea (IHBT 2006). In eucalyptus, cold hardiness is incorporated through a protein TF called C-repeat binding factor (CBF), such as *Eucalyptus gunnii* derived *EguCBF1a* and *EguCBF1b* (El Kayal et al. 2006). CBF are known to regulate expression of a number of genes conferring frost hardiness. Driven by cold inducible dehydrin promoters, CBF transgene expression occurred only during cold stress thereby improving freeze tolerance significantly without negatively influencing other agronomically important traits (Zhang et al. 2010a).

Therefore, outlook on transgenic development now focuses on genes that are expressed only ‘in need’ to tackle stress situations. For instance, if a constitutive promotor such as CaMV35S was used in eucalyptus transformation, CBF mediated negative impacts such as reduced growth, reduced leaf area and increased thickness associated with a decrease in cell size, higher oil gland density and a wax deposition on the cuticle could have encountered in the transgenic plants (Navarro et al. 2011). Constitutive gene expression of a stress related transgene at non-target sites and at time of no stress can therefore be unavoidable. Further, this will effect in channeling of metabolic energy and accumulation of unnecessary proteins besides other adverse side effects. For that reason, a stress-inducible promoter would be ideal when the transgenic plants are targeted to deal with abiotic stresses (Zhang et al. 2000). Following the successful use of stress inducible promoters in eucalyptus, leaf specific promoters are being tested in coffee against leaf miners and leaf rust (Brandalise et al. 2009).

Haploid Recovery

Haploids and doubled haploids (DH) have been identified as a tool in crop breeding, because they aid in understanding genetic mechanisms of trait expression, including stress resistance. Notwithstanding, they provide opportunity to develop homozygous lines, those are useful as parental lines for hybrid development as well as in inducing mutations and transformations. DH lines are also used in linkage map development using various marker systems, and in mapping putative quantitative trait loci (QTL) linked to various agronomic traits. Harnessing of gametoclonal variants can be an additional benefit from cultured gametes.

In plantation crops, attempts to recover haploids from anther and ovule culture have been reported in coffee (Lashermes et al. 1994; Carneiro 1999), rubber (Cheng 1983), tea (Srivastava and Chaturvedi 2008), coconut (Perera et al. 2009) and date palm (Chaibi et al. 2002), although the success of plant regeneration in woody species was not encouraging (Peixe et al. 2004). Recently, isolation of spontaneous haploids and DH lines has been reported in oil palm, by high throughput screens of naturally occurring population (Dunwell et al. 2010). Although haploids and DH lines have not been greatly employed in developing stress resistant lines in plantation crops, they can still be useful auxiliary tools in studying genetics and development of new breeding lines in the future.

3.4.1.4 Molecular Breeding

Genetics of stress resistance in crop plants is relatively simple and is under regulatory control of fewer genes. However, adaptive genetic regulation for complex factors such as drought, which influences plant metabolism both in temporal and spatial dimensions, necessarily needs to be multigenic. Additionally, mutagenic stress factors such as UV radiation are capable of inducing adaptive mutations to the same stress that caused them. Since crop adaptation to various stress factors is as old as the crop itself, evolutionary lineages among resistance genes are a common feature, occurring across either species or genus. A great deal of biotechnological research is underway at identifying, mapping, isolating and transferring resistance genes in cultivated species, because of the power and versatility of molecular marker technology.

Table 3.3 Summary of transformation studies for stress tolerance in plantation species

Crop	Species	Stress	Target gene	Promoter	Transformation	Tissue system	Reference
Cocoa	*T. cacao*	Fungal pathogens	TcChi1	CaMV 35S (E12-Ω)	A. tumefaciens	Cotyledon explants	Maximova et al. (2003, 2006)
	T. cacao	Pod borer	CryIBa, CryIIa	CaMV 35S	A. tumefaciens	Somatic embryos	Chaidamsari (2005)
Coffee	*C. arabica*	Herbicide – Chlorsulfuron	Csr1–1	CaMV 35S	A. tumefaciens	Leaf explants	Leroy et al. (2000)
	C. arabica	Leaf miner	Cry1Ac	CaMV 35S	A. tumefaciens	Leaf explants	Leroy et al. (2000)
	C. arabica	Berry borer	α-AI1	PHA-L	Biolistic	Embryogenic calli	Barbosa et al. (2010)
	C. canephora	Leaf miner	Cry1Ac	CaMV 35S	A. rhizogens	Leaf explants	Leroy et al. (1997)
	C. canephora	Herbicide – Chlorsulfuron	Csr1–1	CaMV 35S	A. rhizogens	Leaf explants	Leroy et al. (1997)
	C. canephora	Herbicide – Chlorsulfuron	Csr1–1	CaMV 35S	A. tumefaciens	Leaf explants	Leroy et al. (2000)
	C. canephora	Leaf miner	Cry1Ac	CaMV 35S	A. tumefaciens	Leaf explants	Leroy et al. (2000)
	C. canephora	Herbicide – Glufosinate	bar	CaMV 35S	Biolistic	Leaf explants	Ribas et al. (2006)
	C. canephora	Herbicide – Glufosinate	bar	CaMV 35S	A. tumefaciens	Leaf explants	Ribas et al. (2006)
Eucalypt	*E. grandis*	Herbicide – Glyphosate	?	?	A. tumefaciens	?	Llewellyn (1999)
	E. camaldulensis	Leaf beetle	Cry3A	CaMV 35S	A. tumefaciens	Cotyledon explants	Harcourt et al. (2000)
	E. camaldulensis	Herbicide – Glufosinate	bar	CaMV 35S	A. tumefaciens	Cotyledon explants	Harcourt et al. (2000)
	Eucalyptus sp.	Freezing	EguCBF1a, EguCBF1b	CaMV 35S	A. tumefaciens	Leaf explants	Navarro et al. (2011)
	Eucalyptus sp	Freezing	CBF	Dehydrin	A. tumefaciens	Somatic explants	Zhang et al. (2010a)
Oil palm	*E. guinensis*	Basal stem rot	Chitinase	?	?	?	Hashim et al. (2002)
	E. guinensis	Basal stem rot	RIP	?	?	?	Hashim et al. (2002)
	E. guinensis	Bagworm	CpTI	?	Biolistic	Immature embryos	Abdullah et al. (2003)
	E. guinensis	Insect	CryIAb	Rubisco promoter	Biolistic	Immature embryos	Lee et al. (2006)
	E. guinensis	Herbicide – Glufosinate	bar	Ubiquitin	A. tumefaciens	Embryogenic calli	Masli et al. (2009)
	E. guinensis	Insect	CpTI	ACT1-5′	Biolistic	Embryogenic calli	Ismail et al. (2010)

Rubber tree	*H. brasiliensis*	Pathotoxins	scFv	CaMV 35S	A. tumefaciens	Somatic embryos	Yeang et al. (2002)
	H. brasiliensis	Oxidative	SOD (HbSOD)	CaMV 35S	A. tumefaciens	Anther derived	Jayashree et al. (2003)
	H. brasiliensis	Oxidative	SOD (Mn-SOD)	FMV 34S	A. tumefaciens	Anther derived	Sobha et al. (2003)
	H. brasiliensis	TPD	IPT	ipt promoter	A. tumefaciens	Somatic embryos	Kala et al. (2003)
	H. brasiliensis	TPD	SOD antisense	CaMV 35S	A. tumefaciens	Somatic embryos	Rekha et al. (2006)
	H. brasiliensis	Oxidative	SOD (CuZnSOD)	CaMV 35S	A. tumefaciens	Somatic embryos	Leclercq et al. (2007)
Tea	C. sinensis	Blister blight	Chitinase	?	Biolistic	Somatic embryos	IHBT (2006)
	C. sinensis	Abiotic	Osmotin	?	Biolistic	Somatic embryos	IHBT (2006)
	C. sinensis	Insect	CryIAc	?	?	?	Mondal (2008)

CaMV cauliflower mosaic virus, *FMV* Figwort Mosaic Virus, *PHA-L* phytohemagglutinin promoter, *SOD* superoxide dismutase, *bar* bialaphos resistance, *cry* crystal protein, *TcChi1 Theobroma cacao* Cass I chitinase, *Csr* Chlorsulfuron resistant, *CBF* C-repeat binding factor, *CpTI* cowpea trypsin inhibitor, *ACT1* actin promotor, *scFv* small chain variable fragment, *α-AI1* α-amylase inhibotor1, *IPT* isopentenyl transferase, *RIP* ribosome inactivating protein, ? no data available

Quantitative Trait Loci and Marker Assisted Selection

Inheritance pattern of molecular marker alleles between two contrasting individuals, which is visualized as differentiable banding patterns in their progenies, is the basis of molecular mapping in plants. Since many markers are found in close physical proximity to each other on a chromosome, generating a linkage map using marker segregation is rather easy. Further, by analyzing segregation pattern of markers with that of agronomic traits, putative associations between them can be estimated. Therefore, co-segregating markers with a trait should be linked to the genes responsible for the trait expression. Hence, marker can be linked to a qualitative gene (a major gene) as in the case of disease resistance or to a polygene (a quantitative trait locus, QTL) as in the case of quantitative traits. Presently, most widely used markers are microsatellites (simple sequence repeats, SSRs), candidate gene markers and more recently single nucleotide polymorphisms (SNPs). Many recent reviews detailing marker based linkage map development, QTL mapping and marker assisted selection (MAS) in plantation crops are available - Ceashew (Cavalcanti and Wilkinson 2007; Paiva et al. 2009), cocoa (Figueira and Alemanno 2005; Bhattacharjee and Kumar 2007; Guiltinan et al. 2008), coconut (Hocher et al. 2005; Batugal et al. 2009), coffee (Lashermes and Anthony 2007; Lashermes et al. 2008), eucalyptus (Myburg et al. 2007; Grattapaglia 2008), oil palm (Rival and Parveez 2005; Price et al. 2007; Mayes et al. 2008; Sambanthamurthi et al. 2009; Billotte et al. 2010), rubber tree (Priyadarshan 2007; Priyadarshan et al. 2009) and tea (Tanaka and Taniguchi 2007; Kamunya et al. 2010).

In plantation crops, many QTLs are mapped for tolerance to various stresses (Table 3.4). Early successful QTL mapping for a stress tolerant trait was reported from interspecific hybrids of *Eucalyptus gunnii* and *E. globulus* for frost tolerance (Vaillancourt et al. 1995). They identified one random amplified polymorphic DNA (RAPD) marker linked to the trait that explained 18.5% of the phenotypic variation among interspecific F2 progenies. Subsequently, Byrne et al. (1997) identified two QTLs for frost tolerance that explained 7.7 to 10.8% of the phenotypic variation from a controlled bred family of *E. nitens*, using restriction fragment length polymorphism (RFLP) markers. QTLs related cuticular wax content has been mapped in coconut using interval mapping, on a fairly robust linkage map developed from a population between East African Tall (EAT) and Rennell Island Tall palms (Riedel et al. 2009). Since waxes are implicated in plant defense against abiotic stresses, this study offers a large number of markers useful for coconut breeding as well as having potential for oil palm and other related palm species.

In rubber tree, Lespinasse et al. (2000b) identified eight QTLs associated with SALB resistance from a F1 population, for which a dense linkage map was constructed earlier (Lespinasse et al. 2000a), by controlled inoculation of different strains of *M. ulei*. They mapped a large effect QTL on linkage group g13, which was subsequently remapped by Le Guen et al. (2003) as a major QTL (*M13-1bn*) for SALB resistance. This QTL explained 36 to 89% of the phenotypic variance for field resistance under natural conditions rather than under controlled inoculation.

Many QTLs were mapped in cocoa for resistance to black pod disease, followed by witches' broom and frosty pod diseases. Lanaud et al. (2009) carried out a meta-analysis of disease resistance QTLs by analyzing sixteen QTL experiments, and projected 76 QTLs detected on a progressively established consensus map. Several hot spots, with QTLs for resistance to different diseases, were observed. They identified thirteen consensus QTLs for *Phytophthora* resistance, six for witches' broom and five for frosty pod resistance, suggesting that these QTLs would be unambiguous choice for MAS programs (Goffinet and Gerber 2000). Although few QTL mapping studies have been reported in coconut, most of them were targeted to agronomic traits. A bulked sergeant analysis (BSA) was carried out within three native coconut populations of lethal yellowing susceptible West African Tall (WAT), and two resistant populations of Malayan Yellow Dwarf (MYD) and Atlantic Tall (AT) palms to differentiate tolerant and susceptible palms using RAPD markers (Cardeña et al. 2003). Recently, Shalini et al. (2007) examined a native palm population differing in eriophyid mite tolerance, using simple sequence repeat (SSR) and RAPD markers, and reported marker-trait association for mite resistance. In date palm, presence of mitochondrial plasmids, designated as R and S were reported to be reliable molecular markers for resistance to vascular wilt (Bayoud disease) caused by the fungus *Fusarium oxysporum* f. sp. *albedinis* (Quenzar et al. 2001). However, recent report suggests that these molecular markers have failed to distinguish resistance and susceptible date palms (El Modafar 2010).

Discovering More Stress-Related Genes

Genes responsible for stress resistance can be identified by searching for genes that regulate key proteins and biochemical pathways responsible for proactive mechanisms. A more recent approach is to look for TFs that are part of signaling network, because they constitute the initially activated genes in response to stress. Publically available annotated crop specific gene databases are now developed in plantation crops (Table 3.5). Interestingly, observations from most of the crop plants show similarities in gene expression in response to stresses of similar kinds. Although plant response to stresses remains a complex multigenic event, number of genes isolated, cloned and characterized so far is scanty. Therefore, there is an urgent need to enrich the databases with novel stress responsive genes. With advances in functional genomics and microarray technology, having a complete gene expression profile of stress response is not remote (Zhang et al. 2000).

There are many unknown genes conferring biotic resistance in crop plants. These resistance (R) genes are found to have conserved sequence motifs shared between a variety of plants resistant to diverse pathogens (Richter and Ronald 2000). Using degenerate primers based on the conserved motifs, nine distinct classes of R gene like sequences have been identified in coffee (Noir et al. 2001). These sequences or resistant gene analogues (RGAs), represent substantial variability, and share closely related sequences (Etienne et al. 2002).

Table 3.4 Stress resistance associated quantitative trait loci and genomic regions mapped in plantation crops

Crop	Stress	Population	QTLs	Marker	References
Cashew	Black mould	CP 1001/CP 96	1	AFLP, SSR	Cavalcanti and Wilkinson (2007)
Cocoa	Black pod	UPA402/UF676	2	AFLP	Lanaud et al. (2000)
	Black pod	Catongo/Pound 12	6	RFLP	Crouzillat et al. (2000)
	Black pod	IMC57/Catongo	3	–	Motilal et al. (2000)
	Black pod	Na34/IMC60//IFC2, Na34/IMC60//IFC5	5	AFLP	Flament et al. (2001)
	Black pod	Scavina/H//IFC1	13	AFLP	Risterucci et al. (2003)
	Black pod	DR1/Catongo, ICM78/Catongo	1	AFLP	Clement et al. (2003)
	Black pod	UPA402/UF676	4	SSR, RGA	Lanaud et al. (2004)
	Black pod	Pound 7/UF273	3	SSR, RGA	Brown et al. (2007)
	Witches' broom	Scavina6/ICS1	1	AFLP	Queiroz et al. (2003)
	Witches' broom	Scavina6/ICS1	2	SSR, RGA	Brown et al. (2005)
	Witches' broom	Scavina6/ICS1	1	RAPD, AFLP, SSR	Faleiro et al. (2006)
	Frosty pod	Pound7/UF273	5	SSR, RGA	Brown et al. (2007)
Coconut	Lethal yellowing	WAT, MYD, AT	5(?)	RAPD	Cardeña et al. (2003)
	Mite	Local population	5	RAPD, SSR	Shalini et al. (2007)
	Abiotic stress	East African Tall/Rennell Island Tall	46	AFLP, SSR	Riedel et al. (2009)
Coffee	Berry disease	Matari/S.288	21	AFLP	Prakash et al. (2004)
	Berry disease	Matari/S.288, Matari/S.288//2* Matari	10	AFLP, SSR	Mahé et al. (2008)
	Berry disease	Caturra/DI200	7	AFLP, SSR	Herrera et al. (2009)
	Berry disease	Hibrido de Timor/Catuai Amarelo	3	AFLP	de Brito et al. (2010)
	Berry disease	Caturra/DI200	5	RGA	Romero et al. (2010)

Eucalyptus	Frost	E. gunni/E. globulus	1	RAPD	Vaillancourt et al. (1995)
	Frost	E. nitens	2	RFLP	Byrne et al. (1997)
	Frost	E. globulus	3(?)	RAPD	Fernández et al. (2006)
	Leaf spot (MLD)	E. globulus	2	SSR	Freeman et al. (2008a)
	Herbivory	E. nitens	45	RFLP, SSR, EST	Henery et al. (2007)
	Herbivory	E. globulus	3	SSR	Freeman et al. (2008b)
	Herbivory	E. globulus	11	AFLP, SSR	O'Reilly-Wapstra et al. (2010)
	Puccinia rust	E. grandis	1	RAPD	Junghans et al. (2003)
	Puccinia rust	E. grandis/E. tereticornis, E. grandis/E. camaldulensis	2	SSR	Mamani et al. (2010)
Rubber tree	SALB	H. brasiliensis/H. benthamiana	9	RFLP, AFLP, SSR, ISO	Lespinasse et al. (2000b)
	SALB	H. brasiliensis/H. benthamiana	6	RFLP, AFLP, SSR, ISO	Le Guen et al. (2003)
	SALB	H. brasiliensis/H. benthamiana	8	RFLP, AFLP, SSR, ISO	Le Guen et al. (2007)
Tea	Drought	TRFCA SFS150/AHP S15/10	13	AFLP, RAPD	Kamunya et al. (2010)
	Nematode	TRFCA SFS150/TRFK 303/577	1	RAPD	Kamunya et al. (2010)

AFLP amplified fragment length polymorphism, *RAPD* random amplified polymorphic DNA, *RFLP* Restriction fragment length polymorphism, *SSR* simple sequence repeats; *IRAP* inter-retrotransposon amplified polymorphism, *RGA* resistant gene analogues, *EST* expressed sequence tags, *ISO* isozymes, *SCAR* sequence characterized amplified regions, *DArT* diversity array technology, *SALB* South American leaf blight, *MLD Mycosphaerella* leaf disease, *WAT* West African Tall, *MYD* Malayan Yellow Dwarf, *AT* Atlantic Tall, *BSA* bulked segregant analysis, *IM* interval mapping, *SMA* single marker analysis, *SMRA* step-wise multiple regression analysis, ? data in conclusive

Table 3.5 Online databases on genomic resources of plantation crops

Crop	DataBase name	Maintained by	Web address
Cardamom	NCBI	NCBI	http://www.ncbi.nlm.nih.gov
Cashew	NCBI	NCBI	http://www.ncbi.nlm.nih.gov
Cocoa	TropGENE DB	CIRAD	http://tropgenedb.cirad.fr/en/cocoa.html
	CocoaGenDB	CIRAD	http://cocoagendb.cirad.fr/
Coconut	TropGENE DB	CIRAD	http://tropgenedb.cirad.fr/en/coconut.html
Coffee	TropGENE DB	CIRAD	http://tropgenedb.cirad.fr/en/coffee.html
	CoffeeDNA	University of Trieste, Italy	http://www.coffeedna.net/
	MoccaDB	IRD	http://moccadb.mpl.ird.fr/
Date plam	NCBI	NCBI	http://www.ncbi.nlm.nih.gov
Eucalyptus	TreeGenes	UCDAVIS	http://dendrome.ucdavis.edu/treegenes/
Oil palm	TropGENE DB	CIRAD	http://tropgenedb.cirad.fr/en/oilpalm.html
	PalmGenes	MPOB	http://palmoilis.mpob.gov.my/palmgenes.html
Rubber tree	TropGENE DB	CIRAD	http://tropgenedb.cirad.fr/en/rubbertree.html
Tea	NCBI	NCBI	http://www.ncbi.nlm.nih.gov

MPOB Malaysian Palm Oil Board, Selangor; *IRD* Institut de Recherche pour le Développement, Marseille; *UCDAVIS* University of California, Davis; *CIRAD* Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Paris, *NCBI* National Center for Biotechnology Information, Maryland

3.4.2 *Crop Management*

Crop improvement is no panacea for stress management. For effective mitigation, improved cultivars need to be agronomically facilitated to cope up with the stress situation. For instance, tree survival can be increased by deep plowing or subsoiling before planting to improve aeration, and by soil fumigation, which destroys nematodes and fungi. Rational irrigation scheduling is a way of management of drought stress in irrigated plantation crops.

3.4.2.1 Plant Protection

Various methods are practiced to protect plants directly from drought, high temperature, sunlight exposure and wounding. In rubber, mulching prevents damage to the collar region in the nursery from sun scorch and prevents moisture lose from plant base. Placing shade baskets and plaited coconut leaves, or simply covering with hey or thatch grass around the stem is practiced generally to protect young plants in initial years of planting. When plants reach a height of more than one meter, lime washing of stem is done to protect the plants from sun scorch. If sun scorch, fire or lightning damage occurs, affected areas are carefully scrapped, cleaned and wound dressing compounds such as petroleum jelly or bitumen is applied along with fungicides (Kothandaraman and Idicula 2000). Similar management systems prevail in crops like coffee, cocoa, tea (Whitmore 2000).

Detailed and recent comprehensive coverage of chemical and cultural methods of plant protection against diseases and pest is available for all plantation crops (Shanmugavelu et al. 2010; Nair 2010). A recent review on diseases of tropical perennial crops (Ploetz 2007) discusses on strategies and techniques in management of various diseases under diverse environments. Besides, individual crop compilations are also available dealing at length on many of these agronomic practices; cardamom (Ravindran and Madhusoodanan 2002), cashew (Ohler 1979), cocoa (Wood and Lass 2001), coconut (Thampan 1993), coffee (Wrigley 1988), date palm (ECSSR 2003), eucalyptus (Coppen 2002), oil palm (Hartley 1988), rubber (Webster and Baulkwill 1989) and tea (Eden 1976).

3.4.2.2 Biocontrol

Control of plant pathogens and pests using biological organisms has been accepted as a most natural and environment friendly alternative to the chemical treatments. Entomopathogenic agents such as bacteria and viruses as well as entomophagous agents and parasitoids have been used for the control of insect pests while hyperparasites and mycophagous agents have been employed in targeting pathogens in crop plants as supplementary modes to tackle biotic stresses (Shah and Pell 2003; Pell et al. 2010; Lundgren 2009). Details of many of the endophytic entomopathogens have been described in a recent review (Ownley et al. 2010). To a very limited extent, phytophagous agents are used in control of weeds.

Entomopathogens

Most widely tested entomopathogenic agents against lepidopteran and coleopteran pests are *Bacillus thuringiensis*, nuclear polyhedrosis virus (NPV) and fungi such as *Metarhizium anisopliae* and *Beauveria bassiana*. *M. anisopliae* is one of the first biocontrol agents to be used against insects (Sorokin 1883). Also popularly known as ‘green fungi’, use of *M. anisopliae* for controlling the larvae of coconut black beetle, *Oryctes rhinoceros* has been a popular practice in all coconut growing regions (Nirula et al. 1955; Fernando et al. 1994). Subsequently, *B. bassiana* was found effective against coconut weevil, *Pantorhytes plutus* (Prior et al. 1988) and the cocoa mirid bug, *Helopeltis theobromae* (Tong-Kwee et al. 1989). In Indonesia, both *M. anisopliae* var. *anisopliae* and *B. bassiana* were reported as promising natural enemies of *Brontispa longissima*, the coconut hispid beetle (Hosang et al. 2004). Many entomopathogenic fungi are now described (Vega 2007). A comprehensive review on entomopathogenic fungi for coffee berry borer has been provided by Vega et al. (2008). Bacterial isolates of *Pseudomonas alcaligenes* was found to cause septicemia in black beetle grubs (Gopal et al. 2002). In coconut, use of entomopathogenic viral agents, such as *Oryctes* specific non-occluded virus resulted in large-scale suppression of black beetles (Bedford 1980; Mohan and Pillai 1993). In oil palm, control of a defoliating pest, *Latoia viridissima* (Lepidoptera: Limacodidae) was reported by spraying a picornavirus suspension (Fedière et al. 1990). Nakai (2009) has reported use of granuloviruses in control of two important tea pests, *Adoxophyes honmai* and *Homona magnanima* (Tortricidae: Lepidoptera).

Entomophagy

Predators and parasitoids of insect pests have been known for a long. In Indonesia, a pupal parasitoid, *Tetrastichus brontispae* (Hymenoptera: Eulophidae) of the coconut hispid beetle, *Brontispa longissima* was reported as one of the promising natural enemies in coconut (Hosang et al. 2004). Among the predatory mites on coconut eriophyid mite, *Aceria guerreronis*, the most frequently found species was *Neoseiulus baraki* (Acari: Phytoseiidae) (Fernando et al. 2003; de Moraes et al. 2004) in Sri Lanka. *N. baraki* has a flat and elongated idiosoma (de Moraes et al. 2004), that help these mites to creep into narrow spaces (Aratchige et al. 2007). Stylops or strepsipterans (Insecta: Strepsiptera) are endoparasites on other insects such as bees, wasps, leafhoppers, silverfish, and cockroaches (Whiting 2009). Many strepsipteran parasites have been reported in plantation pests. For example, *Halictophagus palmae* is a strepsipteran parasite on the plant hopper, *Proutista moesta*, a vector of many pathogens of coconut, oil palm and betel nut. Females of *Stichotrema dallatorreanum* parasites on the long-horned grasshoppers, *Segestes decoratus* and *Segestidea novaeguineae* (Orthoptera: Tettigoniidae), two severe pests of oil palm in Papua New Guinea. Similarly, *S. dallatorreanum* is used as a biocontrol agent against two grasshopper species that cause severe defoliation of oil palm, *Segestidea defoliaria defoliaria* in West New Britain and *S. gracilis gracilis* in New Ireland Provinces (Kathirithamby and Caudwell 2003). For biocontrol of coffee berry borer, three hymenopteran parasitoids, *Prorops nasuta*, *Cephalonomia stephanoderis* and *Phymastichus coffea* were introduced in many coffee producing countries (Espinoza et al. 2009). Recently, a species of predatory thrips, *Karnyothrips flavipes* (Thysanoptera: Phlaeothripidae) is reported from Kenya that were found feeding on coffee berry borer eggs and larvae (Jaramillo et al. 2010). Parasitoids against two common pests in tea plantations, *Adoxophyes honmai* and *Homona magnanima* has been reported from Japan (Nakai 2009). In eucalyptus, successful use of a parasitoid in minimizing the target pest population below economic threshold, resulting in total withdrawal of chemical use, was reported from California. The blue gum psyllid, *Ctenarytaina eucalypti*, a pest of *Eucalyptus pulverulenta* was successfully suppressed by the introduction of a parasitoid wasp, *Psyllaephagus pilosus* from Australia in 1992. By 1994, recommendation for chemical control of blue gum psyllid could be withdrawn owing to the establishment of parasitoid population throughout California (Dahlsten et al. 1998). Similarly, another wasp *P. bliteus* has been released to control spotted gum psyllid, *Eucalytolyma maideni* (Dahlsten et al. 2003).

Hyperparasitism and mycophagy

Most widely used anti-pathogenic agents are *Trichoderma*, *Bacillus* and *Pseudomonas*. These have been established as useful biocontrol agents against *Ganoderma boninense* (Susanto et al. 2005), *Ceratocystis paradoxa* (Eziashi et al. 2006) in oil palm and coconut (Srinivasulu et al. 2008). Among *Trichoderma* species, *T. viride* (Bastos 1996) and *T. stromaticum* (Samuels et al. 2000) are identified as efficient epiphytic mycoparasites in controlling the witches' broom disease in cocoa. Anatagonistic suppression on *Schizopyllum commune*, a soil borne pathogen in oil palm, using bacterial isolates of *Burkholderia cepacia* and *Serratia* sp. (Dikin et al. 2003) have been identified, suggesting the usefulness of these bacteria as biocontrol agents. In rubber, biological control of SALB pathogen using a hyperparasitic fungus, *Dicyma pulvinata* that colonizes ascostromata of *M. ulei* is reported (Mello et al. 2005, 2006; Melo et al. 2008). Kim et al. (2009) demonstrated anatgonism of a phylloplane bacterial isolate of *Bacillus subtilis* against tea anthracnose pathogen, *Colletotrichum theae-sinensis*. Control of soil borne pathogens like *Pythium*, *Rhizoctonia* and *Phytophthora* using mycophagous soil fauna comprising of a nematode (*Aphelenchus avenae*), mite and a collembolan insect was reported recently (Taylor 2010). Further, microorganisms implicated in ISR including AMF are widely used as biocontrol agents.

Phytophagy and smothering

Although the use of phytophagous insects is seldom practiced in plantations, there had been attempts in the past to introduce some defoliating agents on noxious weeds. A project initiated by the CABI Bioscience (then Commonwealth Institute of Biological Control) in 1966, identified about 200 natural enemies of *Chromolaena odorata* (=*Eupatorium odoratum*), a common noxious weed in plantations. An arctiid moth, *Pareuchaetes pseudoinsulata* was one of the first natural feeders on *C. odorata* to be introduced in Asia and Africa; subsequently followed by other natural enemies (Muniappan and Bamba 2000). Attempts to identify and release of fungal pathogens as biocontrol agents to control another noxious weed in plantations, *Lantana camera* is detailed by Thomas and Ellison (2000).

Smothering of weed flora, by the use of fast growing beneficial plants as cover crops is a common practice in rubber, oil palm and cocoa plantations. In addition to weed suppression, these leguminous plants enrich soil by nitrogen fixation, add large quantities of biomass, conserve moisture and support many beneficial microflora. Most commonly used cover crops in rubber and oil palm plantations are *Peuraria phaseoloides* and *Mucuna bracteata*, while *Indigofera spicata* is grown widely in cocoa plantations. Other leguminous species used are creeping peanut (*Arachis pintoi*), *Calopogonium mucunoides*, *Centrosema pubescens*, *Macroptilium atropurpureum* and *Stylosanthes guianensis*. They are also grown in cashew and coconut plantations for their beneficial effects.

3.4.2.3 Agronomic Management

Agronomic management of plantation crops facilitates them to grow in most adequate environments, with optimal water and nutrient support, besides helping them to tide over stress situation greatly. Keeping up of soil health by addition of organic manures and mulches can help in the build-up of beneficial microflora, which helps in release of nutrients and suppress soil borne pathogens in the rhizosphere. In plantations and nurseries, providing windbreaks was often useful in preventing wind damage.

Cropping Systems

Selecting of cropping patterns based on the agro-ecological suitability is a very important proactive management strategy against stresses. In coffee, large scale planting of mixtures of coffee genotypes has been done as an effort to reduce anticipated infection by *Hemileia vastatrix* in Colombia (Ruiz et al. 1990). Region wise cropping recommendations are issued from time to time in all major rubber growing countries, which include proportional mixing of clones of different origin. In India, polyclonal (open pollinated seedlings collected from designated pre-potent clones grown together) gardens and use of polyclonal rootstocks are also recommended in extensive rubber culture to non-traditional regions.

Intercropping of more than one plantation crop is also practiced in India, China and Sri Lanka; such as rubber intercropped with banana, pineapple, medicinal plants, spices, vegetables, fodder crops, coffee, cocoa and tea (Punnoose et al. 2000). Three tier systems of rubber - coffee - pineapple are reported from China (Zheng and He 1991). In Ghana, a carefully designed intercropping system with coconut and cocoa was found mutually beneficial, with both crops showing better flowering, higher yield and without serious disease problems (Osei-Bonsu et al. 2002).

3.4.2.4 Soil and Plant Diagnosis

Soil and plant diagnosis are done to assess the nutrient deficiency and sufficiency, to evaluate crop quality and to assess stress effects. Although, the plant itself is the final proof of soil health in plantation species, plant analysis helps to predict the immediate crop nutrient requirements. A rationalization of fertilization may be beneficial in accordance with productivity-linked factors such as cultivation, growth and production. Plant nutrient analysis may be augmented with tests on soil pH, available nutrient status, elemental constitution and physical properties. Plant samples for analysis are collected according to the sampling methods described for each plantation crop (Table 3.6).

Foliar nutrient diagnosis must always be accompanied by soil analysis. Soil analyses require careful soil sampling with good judgment. It is better to collect soil samples annually (if required periodically) based on age, duration and rooting

Table 3.6 Plant tissue sampling techniques in plantation crops

Crop	Growth stage/season	Plant part to sample	N[a]	Reference
Cardamom	Just before flowering	Fifth pair of leaves from panicle initiation tillers	25	Sadanandan et al. (2000)
Cocoa	5–10 week old leaves	Third or fourth leaf of the last maturing flush	30	Pushparajah (1994)
Coconut	At the start of dry season	Leaf # 14	30	Pushparajah (1994)
Coffee	Just before flowering	Third or fourth pairs of leaves from tips of fruit-bearing branches at mid-height of tree	30	Pushparajah (1994)
Oil palm	Before dry season	Leaf # 17	30	Pushparajah (1994)
Rubber	100 days after leaf formation	Basal leaves of top whorl of shoots of branches in shade of canopy	30	Pushparajah (1994)
Tea	Before the first harvest of the season	Third mature leaf from tip of the young shoot	30–40	Eden (1976)
Cashew	Just before flowering	Last fully matured leaf of the current season flush	30	Bhaskar et al. (1995)
Date palm	Late summer or early fall	2–3ranks in the ‘spiral of 13’ from the top of the tree, 4–6 central leaflets	10	Krueger (2007)
Eucalyptus	First growing season	First fully expanded leaf	20	Shedley et al. (1995)

[a] *N* Number of plants to sample

pattern of the crops. Before sampling a field, its agricultural history, soil types etc. may be recorded. In plantation crops, a composite sample can represent up to 5 hectares in a field with apparent uniformity, else the field may be divided as per the judgment for taking composite samples.

Mitigation of Nutrient Toxicity

Although nutrient deficiency can be managed easily by supplementing the deficient nutrient, amelioration of nutrient toxicity is a formidable task. In plantation crops, excess quanta of essential nutrients are not known to create any problem, but that of supplementary elements can be problematic. Toxicity due to micronutrients, except Mo, can be corrected to a certain extent the addition of organic matter. This will help in bringing the soil pH to a level above 5.5 or to the optimum of 6.5. Application of green manures and growing cover crops can be sustainable practices that greatly reduce adverse effects of excess elements. Further, excess levels of Zn can be reduced by artificial inoculation of AMF into soil. However, excess B and Mo may require leaching with clean water, a difficult proposition to practice in plantation crops.

3.4.2.5 Remote Sensing

Technological advancements in space science and computation have extended applications in agriculture. One of the core applications is to assess plant stress caused by various biotic and abiotic stresses through analysis of spectroradiometry. Variations in spectral reflectance from crop canopy in response to stress can be easily detected and interpreted than by the field-based investigations. For this, aerial photography using multispectral imaging, thermal imaging or air-borne radar are used. Remote sensing has an added advantage of looking at a vast area, while comparing data between healthy and stressed crops all at the same time. Moreover, it can help in rapid and repetitive assessment of the extent of spread of drought, disease, pest, migration of locusts. In plant disease management, this new technique is called phytopathometry. Although application of remote sensing has varying success in agriculture, many potential and encouraging results are reported especially in the field of plant disease management (James and Barret 1989)

One of the pioneering demonstrations of the use of remote sensing technology in agriculture was reported in a plantation crop. In 1970, scientists from Indian Space Research Organization could identify root wilt affected coconut palms using aerial infrared photography (Dakshinamurti et al. 1971; Pisharoty 1972). They suggested that early detection of disease incidence could help in reducing crop loss by timely control measures. Remote sensed mapping and monitoring of disease incidence and damage assessment are reported in rubber. Successful plantation scale mapping of incidence and spread of leaf fall diseases caused by *Corynespora cassiicola* (Manju et al. 2001) and *Colletotrichum gloeosporioides* using satellite data during critical periods showed spread of the diseases throughout the South Canara region in India with varying severity. Remote sensed data showed more than 90% agreement with the ground truth data. There was a significant reduction of near infrared, but an increase in red and short wave infrared reflectance in diseased plantations when compared to healthy, which helped in accurate measurements (Ranganath et al. 2004).

Remote sensing techniques have now been perfected in detecting physiological disturbances, caused by pests and parasites, by recording changes in foliar reflectance not perceivable by naked eye or near infrared photography. Using this technique, Nandris et al. (1985) could establish relationship between spectral reflectance and root disease in rubber. Spectral libraries for various leaf diseases and other stress factors are being developed for remote sensing applications in plantation crops. Further, using a ground-based handheld field spectroradiometer, spectral libraries of individual trees affected by diseases are also being developed in rubber (Jusoff et al. 2010).

3.4.2.6 Simulation and Modelling

In the modern era, computational advancements are being translated into practical predictions of abiotic and biotic stresses in plantation crops. These decision making systems, incorporate complex models evolved from multivariate relations of various parameters that define the stress reaction in plants. Simulation and modeling help in making predictions on the ensuing stress well in advance so that corrective measures can be initiated even before the onset of actual stress. Models incorporate various methodologies such as time series analysis, multivariate linear stepwise regressions, exponential computations and neural networks. Models are made in multidimensional patterns, either temporal, spatial or both. For instance, temporal development of adult population can be used to determine insecticide application timing, while spatial changes in the soil characteristics can be used to assess the dynamics of soil borne pathogens and pests such as root weevils and cockchafer beetles. In tea, drought sensitivity for individual clones could be predicted from a curvilinear yield reduction model related to advancing water deficit. This could help in determining appropriate time of drought sensitivity in each clone (Burgess and Carr 1996). Phenological models are used for accurate prediction of water requirement during dry periods as being done in rubber tree plantations (Guardiola-Claramonte et al. 2010). Nowadays, remotely sensed data on crop health, soil characteristics and weather parameters are being incorporated into modeling systems for improving precision of the forecasts.

3.5 Conclusions

Birth of the third millennium of the contemporary era, had witnessed dramatic advancements in scientific knowledge on stress biology of plants: genetics and breeding, physiology and biochemistry, molecular biology, genetic engineering, bioinformatics, computational biology, nano-biotechnology, agrometeorology, agronomy, remote sensing and crop modeling. While plant physiology has provided new tools to understand complex network of stress related responses, a plethora of organic compounds have been identified in various biochemical pathways in stress related reactions. Advances in molecular genetics and breeding has led to the development of marker linkage frameworks of chromosomes, which enable tagging of genes on the crop genome, aiding selection and introgression of beneficial alleles based on marker segregation (Cattivelli et al. 2008). Automated methods for discovery of molecular markers and new technologies for high-throughput, low-cost molecular marker genotyping in plants have been developed. Genotyping methods include multiplexing of SSRs using Multiplex-Ready® marker technology (MRT); DArT genotyping; SNP genotyping using the Invader® assay, single base extension (SBE), oligonucleotide ligation assay (OLA) SNPlex ™ system and Illumina GoldenGate ™ and Infinium ® methods. These techniques can be used in genetic linkage analysis, QTL mapping, diversity analysis, association mapping and marker-assisted selection (Appleby et al. 2008). Further, advances in molecular biology help in analyses of proteomes and transcriptomes of stress related gene expression by identifying the up and down regulated gene products; cloning of beneficial genes and development of transgenics. Bioinformatics has amassed voluminous genomic databases, which enable searching, comparing and recognizing genes across genomes and tools to manipulate genome information into marker sequences, gene constructs and single nucleotide polymorphisms. Still, use of these technologies need to be perfected in plantation crops to reap the benefits they promise. Development of most competent genotypes to tackle unknown threats of the future is the major challenge in plantation crops. Integration of scientific information accrued so far would leverage judicious use of technologies for the production of most competent genotypes that are environment friendly and conducible to agronomic management.

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Chapter 4
Enhancing Productivity and Performance of Oil Seed Crops under Environmental Stresses

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Abstract Oilseed crops, like other plants are sedentary and therefore, prone to adversities of the locale; be it unfavorable levels of cold, heat, drought, frost or salt. All of these significantly impact plant growth and development. In economically important oilseed crops, there is a reduction in yield and/or oil level, or quality that affects the growers and consumers. Some plants have been reported to survive adverse growth conditions, and how they sustain in such conditions has been a matter of interest to plant researchers. Plants are conscious of their growth environment, and have developed a mechanism to sense the external environment and defend adversities. Typically, stress (biotic and abiotic) is sensed and the signal transmission results in physiological and biochemical changes at the cellular level. These changes apart from triggering production of several metabolites also cause induction of genes involved in stress response. Many of these involved mechanisms shadow the ABA-dependant or the ABA-independent pathways and in partnership with transcription factors and cis-elements activate a series of genes. Some of the mechanistic examples involve modifying stomatal function, altering turgor pressure, amongst other ways to conserve nutrients and water. Brassinosteroids are being discovered as involved in stress response. It is possible to classify the response pathways as those involving regulatory proteins and functional proteins. While some of these changes involve calcium flux, recent evidences indicate a role for SnRK family of protein kinases. Knowledge of the response pathways, genes, quantitative trait loci and evolution are helpful in enhancing our understanding of stress responsiveness and engineering to augment stress tolerance of crop plants, including oilseeds.

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4.1 Introduction

Since plants typically remain in one location during their life, they have developed numerous ways to deal with environmental stresses such as drought, salt, cold, heat, intense light, and excessive ozone or CO_2. Environmental stresses are a major factor for crop loss accounting for average yield losses of more than 50% for major crops (Boyer 1982; Bray et al. 2000). Stress conditions normally limit the plants from expressing their full genetic potential. Understanding physiological, biochemical, metabolic and molecular processes that are altered by stress and the responsive mechanisms developed by plants to tackle stress would aid environmental stress management for improving crop productivity. Under natural field conditions plants are often subjected to a combination of different stress conditions (Craufurd and Peacock 1993). Research should also focus on the aspects of stress combinations to facilitate development of crop plants with enhanced tolerance to field stress conditions (Mittler et al. 2006).

Soybean (*Glycine max*) and Canola (*Brassica napus*; canola is not rapeseed though similar) occupy first two positions in terms of worldwide oilseed production accounting for ~250 million metric tons and ~60 million metric tons respectively, in 2009 (USDA 2009). Several reports highlight the influence of environmental stresses on oil seed crops; for instance environmental stress during seed fill in oil seed crops can alter the chemical composition of the seed and reduce yield, viability, and vigor (Dornbos and Mullen 1992). Soybean seed yield, seed number and seed size were reduced dramatically by high air temperature and drought stress during seed fill (Dornbos and Mullen 1992). Majority of the Canola (*Brassica napus*) growing areas of Canada are often affected by extreme frost during seedling development in the spring and seed maturation in the fall (McClinchey and Kott 2008). Like frost, drought has a great impact on canola yield in Canada (Wan et al. 2009). The winter annual forms of canola in Europe produce an average yield of about 3,000 kg ha^{-1}, which is approximately double the average yield of the Canadian and Australian spring canola, the key reason believed to be that the European canola are typically grown under cooler temperatures with sufficient precipitation during the growth season (Rakow 2004).

Drought occurs throughout the world on a regular basis, in fact, annually. High temperature combined with water deficit affects crop production (Boyer 1982). As crop losses due to drought stress most likely exceed losses from all other causes combined (Chaves et al. 2003), this will be the major focus of this chapter. Plants experience drought stress when water supply to roots decreases and transpiration rate exceeds uptake of water. Decreased water conditions lead to many adverse effects on plants such as disruption of water potential gradients, loss of turgor, denaturation of proteins, disruption of membranes, and other detrimental effects (Kozlowski and Pallardy 2002). Some of these bioprocesses occur rapidly, such as a change in protein phosphorylation, while other responses occur over few hours or days such as the gene expression profile of a plant. The severity of plant response to drought depends on many factors, such as plant species, the length of time exposed to decreased water conditions and soil conditions. Some species of plants are able to maintain better growth in drought conditions. Studying these tolerant plants can help identify genes and pathways that could be introduced into agricultural crops to increase their resistance to drought. Genetically engineering drought tolerant crop plants has significant economic importance. In order to successfully modify plants, details of the molecular mechanisms involved in drought tolerance need to be understood better (Vinocur and Altman 2005; Shinozaki and Yamaguchi-Shinozaki 2006; Valliyodan and Nguyen 2006). Here, we review some molecular aspects of the stress mechanisms and components including sensory molecules, signal transduction, oxidative/osmolytic stress signaling, calcium-mediated signal pathways, and then expand in to mechanisms specific to drought stress.

4.2 The Relay: Sensing and Signalling

Plant traits associated with stress resistance mechanisms are usually multigenic, and thus difficult to control and engineer (Valliyodan and Nguyen 2006). The –'omics studies have identified the activation and regulation of numerous stress-related transcripts and proteins that can be broadly divided into two major categories; sensory molecules and protective molecules (osmoprotectants, antioxidants, reactive oxygen species etc). Sensory molecules perceive stress signal and initiate a cascade to transmit the intracellular signal. In many cases, this involves activation of nuclear transcription factors to induce the expression of a set of genes. A sensory molecule could regulate branches of the signaling cascade that are initiated by one aspect of the stress condition. For instance, a low-temperature detecting sensor could initiate a signaling cascade responsive to membrane fluidity but would not necessarily control signaling initiated by an intracellular protein despite its conformation and activity being influenced directly by low temperature. Drought, salt and cold stresses have been shown to induce transient Ca^{2+} influx into the cell cytoplasm derived from either influx from the apoplastic space or released from internal stores. Channels responsible for the Ca^{2+} influx represent one type of sensor for the stress signals. Internal Ca^{2+} release is controlled by ligand-sensitive Ca^{2+} channels; with these ligands being the second messengers. Repetitive Ca^{2+} transient generated by both (first and second) messengers, including abscisic acid (ABA) is a characteristic of the Ca^{2+} signal cascade. Receptor-like kinases consist of an extracellular domain that may function in ligand binding or protein-protein interactions, a transmembrane domain and an intracellular kinase domain. The two-component sensor-response regulator systems involving histidine kinases is another signaling system. The extra-cellular sensor domain perceives a signal, autophosphorylates the cytoplasmic histidine residue, and the phosphoryl moiety is passed to an aspartate receiver in a response regulator that constitutes a part of the sensor or a separate protein. Sensors may also couple with a downstream mitogen-activated protein kinase (MAPK) cascade or directly phosphorylate specific targets to initiate cellular responses.

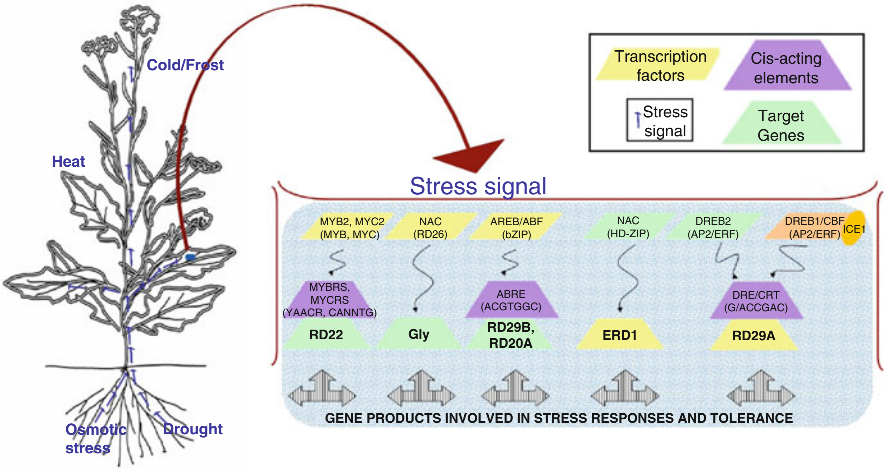

Fig. 4.1 Transcriptional regulatory networks of abiotic stress signals and gene expression in *Brassica napus*. A Brassica plant with known stresses are highlighted in the left. Leaf being one of the important components of stress responsiveness is magnified to highlight the changes occurring at the cellular level. Known signal transduction pathways involving drought, salinity, and cold-stress responses are shown. At least three are ABA-dependent and others are ABA-independent. ABRE functions as a major ABA-responsive element in the ABA-dependant pathway. MYB2 and MYC2 function in ABA-inducible gene expression of the RD22 gene. The RD26 NAC transcription factor is involved in ABA-responsive gene expression in stress responses. In one of the ABA-independent pathways, DRE is mainly involved in the regulation of genes by drought, cold and osmotic stress. DREB1/CBFs/ICE1 are involved in cold-responsive gene expression. DREB2 transcription factors are involved in dehydration and high salinity stress-responsiveness. The NAC-involving ABA-independent pathway is controlled by drought and salt. The NAC and HD-ZIP transcription factors are involved in ERD1 gene expression

Signal transduction pathways for cold, drought, and salt stress can be divided into three major signaling routes, namely: (a) osmotic/oxidative stress signaling via the MAPK modules – this involves the generation of ROS scavenging enzymes and antioxidant compounds as well as osmolytes; (b) Ca^{2+}–dependent signaling that activates the late embryogenesis abundant (LEA)-type genes (such as the DRE/CRT set of genes) – this involves the production of stress-responsive proteins, many with undefined functions, and (c) Ca^{2+}–dependent salt overlay sensitive (SOS) signaling that regulates ion homeostasis – this involves the osmotic stress-specific SOS pathway which is specific to ionic stress (Xiong et al. 2002). A schematic of signal transduction pathways involving osmotic and ionic stress in plants is shown (Fig. 4.1). It may be noted that a signal transduction pathway commences with perception of the stress signal, followed by the generation of second messengers such as inositol phosphates and reactive oxygen species, and or alteration in the intracellular Ca^{2+} levels leading to a protein phosphorylation cascade that targets proteins directly involved in cellular protection or transcription factors controlling stress-regulated genes. Numerous and detailed reviews on this topic are available (Xiong and Zhu 2001; Zhu 2001, 2002; Chinnusamy et al. 2004).

Arabidopsis thaliana has been used as a model species for the dissection of stress responsive pathways in plants. Numerous genes that are responsible for defense against stress have been discovered in Arabidopsis. The monocot *Oryza sativa* (rice) is another model species that has particularly large economic impacts worldwide. These two species have been studied comparatively to determine mutual stress responsive pathways. However, in Canada, North Americas, and parts of Europe, oil seed rape (*Brassica* and related species, Brassicaceae) and soybean are widely cultivated. Canada is one of the largest rape oilseed crop producers and plans to boost canola production by 65% (to 15 million tons) by 2015 (Canola Council of Canada 2009). This along with the demand, and nutritional quality makes it an economically significant oil seed crop in North America and Europe. Given the genetic proximity it shares to *Arabdiopsis* and its wide geographic range and the extreme environments it thrives in (−30°C to +30°C), it serves as an excellent candidate for gene exploration for the possible transfer of genetic information into crop plants for enhanced resistance to drought.

4.2.1 Influence of High Temperature and Drought in Brassica

Heat injury to seedlings occasionally occurs on hot days, with air temperatures in the range of 30–35°C and soil temperatures of 37°C. Heat injury is commonly associated with drought injury, but excessive heat also causes death of plants even if moisture is optimal. When in the blooming stages, heat blasting and/or flower abortion is a strong possibility. This can vary from field to field and is very dependent on time of flowering, soil moisture and humidity during the hot periods. Usually in this situation, no or limited pod growth occurs and thus no seed is produced. Pods are seen in patches on the main stem and branches as related to time of flowering and the heat stress. It may be possible to minimize flower abortion by providing optimal soil moisture for *B. napus* growth.

Both low and high temperatures can adversely affect plant development prior to and during flowering. Low, but nonfreezing temperatures prior to flowering stage could slow down the rate of plant development. The start of flowering is delayed or, if begun, the rate of flower opening is slowed and the amount of pollen shed is reduced. High temperatures at flowering will hasten the plant's development, reducing the time from flowering to maturity. High temperatures during flowering shorten not only the pollen receptivity of the flower, but also the duration of pollen release and its viability. This can decrease the number of pods that develop and the number of seeds per pod, resulting in lower yields. Once pods are formed, canola is more tolerant than at flowering to high temperatures. Cool night temperatures at this time also help the plant recover from extreme heat or dry weather. However, during this stage, a combination of heat and extreme drought will severely affect the pod and seed development, including formation of seeds, seed size and oil content. Literature indicates that seed oil content is highest when seeds mature under lower

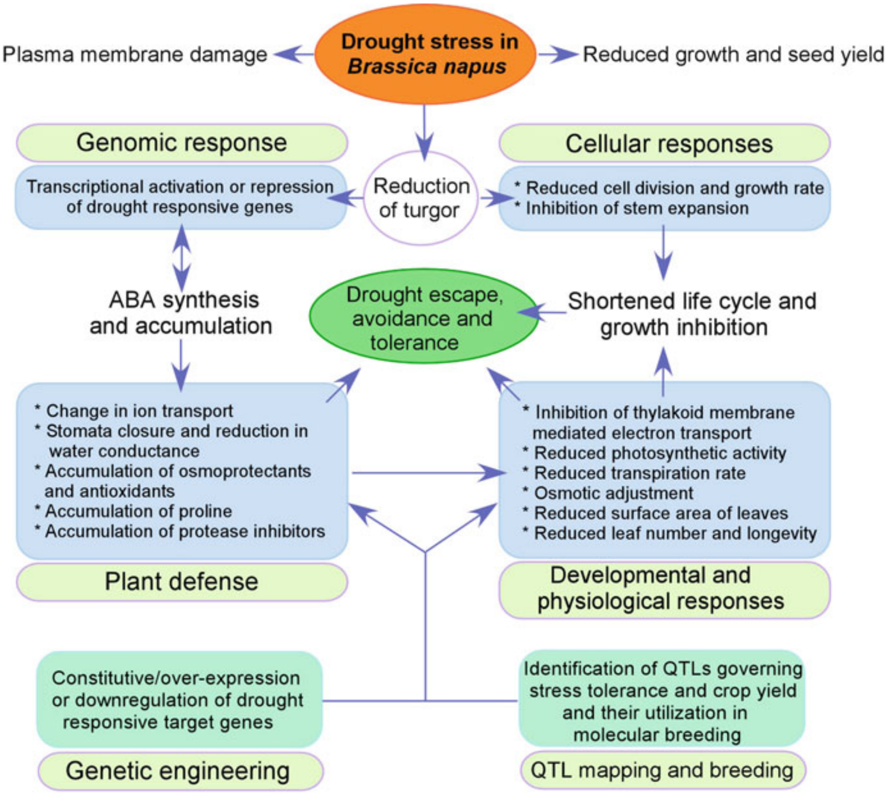

Fig. 4.2 Drought stress responses in *Brassica napus*

temperatures as high temperatures during seed maturation results in reduced oil content (Valliyodan and Nguyen 2006).

Drought stress affects several developmental and physiological processes in oil seed rape including reduced cell division and growth rates, inhibition of stem expansion, stomata closure, inhibition of thylakoid membrane mediated electron transport, plasma membrane damage, reduced photosynthetic activity, changes in lipid metabolism, and reduction in seed yield and quality (Fig. 4.2; Benhassaine-Kesri et al. 2002; Sinaki et al. 2007; Naderikharaji et al. 2008; Wan et al. 2009). The effect of drought stress varies with the genotype, and growth and developmental stage of oilseed rape (Robertson and Holland 2004). The drought occurrence time is often more important than the stress intensity. In oilseed rape, the growth stages most sensitive stages to drought stress are from flowering to early and middle phases of seed filling (Champolivier and Merrien 1996; Gan et al. 2004; Sinaki et al. 2007). Plants under water stress are also more prone to heat stress. For the canola plant life cycle from seed to seed, the rate of water consumption increases in a linear fashion to reach its highest plateau level around flowering, and then decreases rapidly during grain filling and seed maturation (Canola Council of Canada 2009). Also, in soybean, stress during any stage of growth leads to yield reduction; with the most yield reduction ranging from 20% to 50% when subject to stress at the time of flowering. Stress also led to dramatic reduction of seed oil content, as observed in many grain crops (Bruce et al. 2002).

4.3 Defending Drought Stress

Plants have developed numerous defenses to tackle drought, which are categorized by three basic mechanisms; drought escape, drought avoidance and drought tolerance (Fig. 4.2; Levitt 1972). Some plants have evolved to escape drought conditions by completing their life cycle and reproducing while the soil still retains some moisture, and before drought conditions are present commence (Mooney et al. 1987). Avoiding tissue dehydration due to drought is a challenge met in many different ways, with the ultimate goals being minimization of water loss and maximization of water uptake. Some avoidance mechanisms are closure of stomata following the onset of drought stress even in the presence of light (Luan 2002), reducing the surface area of leaves to sunlight exposure (Ehleringer 1992), and increasing trichome level (Larcher 2000). Others plants attempt to avoid dehydration by various physical mechanisms such as regulating their transpiration rate. Others decrease their leaf surface area, leaf number, or leaf longevity that in turn reduces the photosynthesis rate, resulting in a reduction in crop yield. When escape and avoidance to drought are impossible, a plant must become accustomed to reduced water availability and adapt to tolerate the low water availability within the environment. As indicated earlier, this is done in several ways such as osmotic adjustment (Morgan 1984), accumulation of osmolytes (Williams and Leopold 1989), and stomata regulation, (Jones 1973; Luan 2002). The specific response to drought is dependent on the type of plant and conditions present in the environment.

4.3.1 Stomata Regulation

It has long been debated how photosynthesis is down-regulated due to drought and whether it is due to stomata closure or metabolic blockage (Jones 1973; Luan 2002). In an effort to reduce the transpiration rate when exposed to arid conditions, some plants have evolved ways to regulate stomata size. Stomata closure reduces transpiration rate and leads to a lowered level of carbon uptake, resulting in lower level of photosynthesis in leaves (Chaves 1991; Mullet and Whitsitt 1996). A reduction in the number of leaves reduces water loss due to transpiration and can also be viewed as a way to redistribute nutrients in older leaves and stems before they are shed. The cascade of events that leads to stomata closure has long been sought and some interesting findings have been made. For instance, it is known that stomata often close in response to drought before any change in water content in the leaf is detected (Socias et al. 1997). A major player in the signal to close the stomata is abscisic acid (ABA; Wilkinson and Davies 2002). Abscisic acid is produced in the root where, most likely, the first responses to drought occur (Davies and Zhang 1987; Schachtman and Goodger 2008). The rapid accumulation of ABA, and the sequence of subcellular physiological events in the ABA-induced stomatal closure is well understood (Assmann and Wang 2001; Schroeder et al. 2001). ABA induced rapid Ca^{2+} influx in the guard cells, and the increase of Ca^{2+} concentration in the cytosol inhibits the membrane proton pumps and K+ inward channels, while activating the anion and K+ outward channels in the plasma membrane. This results in rapid efflux of anions and cytosolic solutes and membrane depolarization, leading to loss of guard cell turgor and stomatal closure.

ABA is not the only factor that induces stomata closure. Also, stomata are seldom completely open or closed (Rogers et al. 1979), and the stomata status depends on numerous environmental cues (Neil et al. 2002; Hunt et al. 2003). Some factors include; leaf water potential (Tardieu and Davies 1992), xylem sap pH (Davies and Wilkinson 1997), and leaf-to-air vapor pressure deficit regardless of the available water present in the soil (Oren et al. 1999). Evidence for a hydraulic signal in root to shoot communication of water stress also exists in the literature (Christmann et al. 2007). The regulation of stomata in relationship to drought, decreased leaf water potential, relative water content, available water, ABA, in addition to other factors, varies widely between different species and genotypes. Due to this fact, some species are better adapted to drought conditions and could be studied to further improve knowledge of the signal cascade leading to drought resistance. For instance, small reduction in stomatal conductance due to increased ABA sensitivity led to a marked reduction only in the rate of transpiration but not in photosynthesis in the drought-tolerant, *SNAC1*-overexpressing rice (Hu et al. 2006).

4.3.2 Role of Abscisic Acid

The hormone ABA helps regulate various stress responses in plants to drought, cold and salinity (Leung et al. 1997; Davies and Wilkinson 1997; Christmann et al. 2005; Chinnusamy et al. 2008). During drought conditions ABA alters ion transport of guard cells, which in turn promotes stomata closure and prevents stomata opening, thus allowing the plant to slow the transpiration process. The stomatal aperture determines the rate of CO_2 diffusion into chloroplast for photosynthesis. During the day, stomata are opened for an extended period for maximum uptake of CO_2 while ~90% of water absorbed by plants is lost by transpiration *via* stomatal openings. ABA-mediated stomata closure is a dosage-dependant process and can be achieved by increasing ABA levels within guard cells by elevating ABA production *in vivo* (Iuchi et al. 2001) and/or by modulating guard cells responsiveness to ABA

Table 4.1 Examples of canola transgenics produced by genetic engineering for enhanced drought tolerance

Target gene	Source	Approach	Phenotypes	Reference
COX (Choline oxidase)	*Arthrobacter pascens*	Constitutive expression	Moderate abiotic stress resistance	Huang et al. (2000)
ERA1 (Enhanced response to ABA1)	Arabidopsis	Antisense down regulation	Enhanced drought tolerance and yield protection	Wang et al. (2005)
BnFTA (*B. napus* farnesyl transferase)	*B. napus*	RNAi	Yield protection under drought stress	Wang et al. (2009)
Ptdlns-PLC2 (Phosphotidylinositol-phospholipase C2)	*B. napus*	Overexpression	Enhanced drought tolerance, early flowering and maturation	Georges et al. (2009)

(Wang et al. 2005, 2009). The 9-cis-epoxycarotenoid dioxygenase (NCED), an enzyme involved in ABA biosynthesis, plays a key role in drought stress-regulated ABA biosynthesis (Qin and Zeevaart 1999; Iuchi et al. 2001). The ectopic expression of *AtNCED3* in *Arabidopsis* results in increased endogenous ABA levels and enhanced drought tolerance in the transgenic plants (Iuchi et al. 2001). At least four orthologues of AtNCED3 in *B. napus* have been identified (Wan et al. 2009). AtNCED3 and/or its corresponding orthologues could be valuable to improving drought tolerance of oilseed rape through genetic engineering.

The reduction in stomatal conductance by targeting protein farnesylation has also been achieved in plants (Wang et al. 2005, 2009). Protein farnesylation is a post-translational modification involving the attachment of C_{15}-farnesyl residues to the carboxyl termini of specific target proteins. Farnesylation has been implicated in negative regulation of ABA signaling pathway in guard cells (Pei et al. 1998). In plants, protein farnesylation is performed by the action of farnesyltransferase (FTA), which is a heterodimer consisting of an α and a β subunit. In Arabidopsis *AtFTA1* and *ERA1* (*Enhanced Response to ABA1*) encode the α and β subunits of farnesyltransferase, respectively (Pei et al. 1998; Wang et al. 2005, 2009). The deletion of *ERA1* in Arabidopsis decreases the transpiration rate of leaves by ABA hypersensitive regulation of stomata closing and consequently slows down desiccation during drought stress (Pei et al. 1998). The utility of farnesyltransferases as biotechnological targets for enhancing drought tolerance in *B. napus* has been examined (Table 4.1; Wang et al. 2005, 2009). Transgenic *B. napus* expressing an *ERA1* antisense construct driven by a drought-inducible rd29A promoter enhanced ABA sensitivity as well as significant reduction in stomatal conductance and water transpiration under drought stress (Wang et al. 2005). Similarly, the down-regulation of *FTA* in Canola using *AtHRP1* (*Arabidopsis hydroxy pyruvate reductase 1*) promoter driving *BnFTA* hairpin RNAi expression cassette resulted in yield protection under drought stress in the field (Wang et al. 2009).

Abscisic acid-activated protein kinase (AAPK) is detected in guard cells but not in roots, leaf epidermal cells, mesophyll cells, flowers, or seeds (Assmann et al. 2000). The AAPK protein is activated by ABA, but not by darkness or elevated CO_2 level – the conditions that trigger stomata closure (Assmann et al. 2000). The increased level of ABA in water-stressed plants stimulates the release of potassium by stomata guard cells leading to stomata closure (Hsiao et al. 1985). It has been noted that moderate starvation of potassium inhibits water stress induced stomata closure and resistance to low water conditions. This may be one of the main causes of dehydration in plants growing in low potassium soil (Benlloch-Gonzalez et al. 2007).

4.3.3 Role of Brassinosteroid in Stress Resistance

In the recent years, brassinosteroid, another class of phytohormone has been implicated in stress responses in numerous plants including oil seed crops. Brassinosteroids are a class of plant polyhydroxy steroids that are structurally similar to animal and insect steroid hormones, and control a broad range of responses in plants such as cell division and expansion, xylem differentiation, seed germination, vegetative growth, and apical dominance (Sasse 2003). Though the effects of brassinosteroid on plant development were known as early as the 1970s (Mandava 1988), it is the recent molecular genetic studies of brassinosteroid-deficient mutants and brassinosteroid-insensitive mutants that have established an essential role for brassinosteroids in plant growth and development (Clouse and Sasse 1998; Clouse 2002). A remarkable feature of brassinosteroids is their potential to increase plant resistance to a range of stresses, including high and low temperature stress, drought, salinity, herbicidal injury, and pathogen attack (Khripach et al. 2000; Krishna 2003; Kagale 2007; Kagale et al. 2007). The exogenous treatment of *A. thaliana* and *B. napus* with 24-epibrassinolide, a brassinosteroid analogue, enhances seedling tolerance to high and low temperature stress, and drought stress (Dhaubhadel et al. 1999, 2002; Kagale et al. 2007; Kagale 2007). Investigation into the mechanism by which 24-epibrassinolide confers thermotolerance revealed that heat shock proteins (HSP) accumulate to higher levels in 24-epibrassinolide-treated *B. napus* seedlings (Dhaubhadel et al. 1999), which correlates with higher HSP as well as global protein synthesis due to a modified translational machinery in these seedlings (Dhaubhadel et al. 2002). In compliance with enhanced drought tolerance, 24-epibrassinolide-treated *B. napus* seedlings accumulated higher levels of transcripts of the transcription factors such as *BnCBF5* and *BnDREB*, and structural drought stress marker genes such as *BnPIP1* and *BnD22* that are proposed to function in better distribution of water, and efficient defense against the large number of proteases produced during stress conditions, respectively (Kagale et al. 2007). Evidence from these as well as several other recent studies has unequivocally established an essential role for brassinosteroids in stress responses of *B. napus*. Brassinosteroids are thus excellent biotechnological targets for genetic manipulation to potentially improve plant architecture, stress tolerance and crop productivity. Indeed, modulation of endogenous brassinosteroid levels by overexpressing *DWARF4*, encoding a key enzyme involved in brassinosteroid biosynthesis, improves plant size and seed yield in *A. thaliana* and *B. napus* (Choe et al. 2001; Kagale 2007) and stress tolerance in *B. napus* (Kagale 2007).

4.3.4 Osmolytes

The morphological effects of water deficits are well known and can be easily visualized in a plant, though the causes of these symptoms are less clear at a biochemical and molecular level (Bruce et al. 2002). One of the first responses to water deficit is osmotic adjustment (Leung et al. 1997). Plants increase their osmotic potential leading to enhanced stress resistance by accumulating osmolytes such as proline, glycine betaine and non-reducing sugars and polyols. Indeed, constitutive expression of a bacterial choline oxidase (*COX*), a gene responsible for production of glycinebetaine by oxidation of choline, in *B. napus*, Arabidopsis and tobacco resulted in moderate enhancement of abiotic stress resistance in these plants (Huang et al. 2000). Osmolyte accumulation allows additional water to be taken up from the environment buffering the immediate effect of decreased water within the plant. Osmolytes do not interfere with normal cellular biochemical reactions (Williams and Leopold 1989; Jaleel et al. 2007), but osmolytes increase thermodynamic stability of folded proteins and provide protection against denaturing stresses (Yancey 2001). The accumulation of soluble sugars such as sucrose, glucose and fructose is often associated with drought tolerance in plants. Soluble sugars may help protect the plant during drought either by using the hydroxyl group of sugars to act as a water molecule to maintain hydrophilic interactions in membranes and proteins by hydrogen bonding with them (Williams and Leopold 1989), or by forming a highly viscous layer in the cytoplasm of the dehydrated cells. Viscosity reduces the molecular movement and helps maintain the structural stability of the macromolecules (Sun and Leopold 1997). Several genes involved in metabolism of these sugars have been found to increase the ability of different transgenic plants to resist stress (Garg et al. 2002; Shinozaki 2006). Over-expression of drought-inducible galactinol synthetase gene (*AtGolS2*) in *Arabidopsis* yielded increased drought resistance because of the excess accumulation of galactinol and raffinose (Shinozaki and Yamaguchi-Shinozaki 2006). Also, transgenic plants that express the DREB1A/C- repeat binding factor 3 (CFB3) accumulate more galactinol and raffinose than wild type plants that help in improving their drought tolerance (Avonce et al. 2004).

In addition to the sugars mentioned above, trehalose is often accumulated in large quantities in response to desiccation. Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is an alpha-linked non-reducing disaccharide found in nature from a wide variety of sources such as bacteria, fungi, yeast, plants, and invertebrate animals (Elbein 1974). Trehalose is a factor involved in tolerance to environmental conditions such as drought, heat, and salt stress (Thevelein 1996) and functions as a reserve carbohydrate and stress protectant, stabilizing proteins and membranes and protecting them from denaturation (Goddijin and van Dun 1999). The high water retention capacity of trehalose helps stabilize proteins and membranes enabling the cells to recover when rehydration conditions are present. Tobacco transformed with the trehalose phosphorylase (TP) gene from *Pleurotus sajor-caju* showed an increased ability to withstand drought. The trehalose synthetase gene (*Tsase*) from *Grifola frondosa* also yielded an increase in the ability to withstand drought in tobacco (Han et al. 2005). Trehalose phosphate synthase (TPS) and trehalose-6-phosphate-phosphatase (TPP) have been used to study and engineer stress resistance in plants (Valliyodan and Nguyen 2006) including Arabidopsis (Avonce et al. 2004).

4.3.5 Proline Accumulation

The amino acid, proline, is thought to play a role in a plant's ability to withstand drought stress by helping regulate osmotic adjustment, stabilizing subcellular structures, scavenging free radicals, and acting as a stress signal. Other suggested functions of proline are the detoxification of ROS and interaction with the hydrophobic residue of proteins. The proline biosynthesis pathway has been well characterized (Nanjo et al. 1999). In Arabidopsis, over-expression of proline synthetase gene produced plants with an elevated resistance to drought, while suppression of this gene results in plants with a decreased ability to withstand drought (Bartels and Sunkars 2005). A strong correlation between the accumulation of proline and drought tolerance has been demonstrated by over-expressing the *Arabidopsis* pyrroline-5-carboxylate synthase gene (*AtP5CS*) and rice P5CS gene (*OsP5CS*) in petunia (Yamada et al. 1999). Deficiency of proline from antisense suppression of the *P5CS* gene resulted in decreased drought tolerance in *Arabidopsis*. Gene knockout of proline dehydrogenase (*ProDH*) led to growth inhibition, indicating that excessive proline is detrimental to plant growth (Chinnusamy et al. 2005).

So far, we described the various components of stress pathways, and their mechanisms in plants. It is clear that physiological and biochemical changes at the cellular level that are associated with drought stress include turgor loss, change in membrane fluidity and composition, changes in solute concentration, and interaction between and among proteins and lipids (Chaves et al. 2003). Plants maintain tissue turgor either by avoiding or tolerating dehydration, or both (Kramer and Boyer 1995). Such stress tolerance and/or resistance mechanisms are developmentally regulated including by morphological changes such as root thickness, the ability of roots to penetrate compacted soil layers, and root depth and mass (Pathan et al. 2004). While some phenotypes are constitutive (i.e., present even in the absence of stress conditions), the adaptive traits such as osmotic adjustment and dehydration tolerance appear in response to water deficit conditions (Serraj and Sinclair 2002). Reduction of photosynthesis, accumulation of organic acids and osmolytes, and changes in carbohydrate levels are typical responses to stress, which are a result of numerous coordinated events such as stomatal closure, and reduced photosynthetic enzyme activity. Synthesis of osmoprotectants, osmolytes or compatible solutes is one of the mechanisms that plants have evolved to overcome water deficit. These osmotic balancing agents accumulate in plant cells in response to drought stress and disappear post-stress (Tabaeizadeh 1998), and include amino acids, polyols, and quarternary ammonium and tertiary sulfonium compounds (Rontein et al. 2002).

4.4 SnRK Family of Protein Kinases and Stress Response

The sucrose non-fermenting 1-related protein kinases (SnRKs) are protein kinases containing catalytic domains with sequences similar to SNF1 (sucrose non-fermenting 1, Celenza and Carlson 1986) protein kinase in yeast (Halford et al. 2003). SNF1 is activated in response to low glucose levels in yeast and is required for the derepression of a battery of genes that are repressed by glucose (Ronne 1995; Dickinson 1999). Though the SNF1 family of protein kinases are a distinct group within the protein kinase super family, they are closely related to the calcium-dependent protein kinase (CDPK) group (Hardie 2000). SNF1-related protein kinases in plants comprises of three subfamilies of protein kinases: SnRK1, SnRK2 and SnRK3. The SnRK1 catalytic domain is similar to SNF1, however catalytic domains of SnRK2 and SnRK3 have only 42–45% amino acid sequence identity with SnRK1 and SNF1 in the domain region (Halford et al. 2003). The SnRK2 and SnRK3 gene subfamilies appear to be unique to plants and are relatively large and diverse compared to SnRK1.

In animals and yeast, SNF1 family members are involved in protecting cells against nutritional and environmental stresses (Halford and Hardie 1998). SnRK1 is considered as one of the global regulators of plant metabolism, in addition to their involvement in plant development and stress responses (reviewed in Polge and Thomas 2007). Transgenic potato plants suppressed with SnRK1 subunit StubGAL83 showed hypersensitivity to salt accumulation in transgenic plants, suggesting that SnRK1 may activate protection systems against such stress (Lovas et al. 2003). The ability of SnRK1 to phosphorylate class 1 heat shock proteins (HSP) (Slocombe et al. 2004) indicates that they could play a role in regulating mechanisms against high temperature stress. SnRK2 group seems to be largely involved in environmental stress signalling in plants. Several reports convincingly prove the role of SnRK2 in the regulation of drought response mechanisms. Osmotic stress activation of a 42-kD kinase, ASK1, a member of the SnRK2b subfamily in tobacco cells phosphorylates transcription factors ATF-2 and c-Jun (Mikolajczyk et al. 2000). Phosphorylation status change in turn triggers the activation of these transcription factors in response to osmotic stress (reviewed in Treisman 1996). Shukla and Mattoo (2008) suggested that SnRK2 kinases have a major role in regulating gene expression during hyperosmotic stress. Protein phosphorylation, has pivotal roles in ABA and osmotic stress signaling in higher plants. Two protein phosphatase genes, *ABI1* and *ABI2*, are known to regulate these signaling pathways in *Arabidopsis* (Leung et al. 1994, 1997). Yoshida et al. (2002) reported that mutation in SRK2E, a member of SnRK2 family in Arabidopsis resulted in a wilty phenotype mainly due to loss of stomatal closure in response to a rapid decrease in humidity. Expression of ABA inducible genes *rd22* and *rd29B* were suppressed in *srk2e* mutants. These results show that SRK2E plays an important role in ABA signalling in response to water stress. In another study *srk2d/e/i* triple mutant plants showed greatly reduced tolerance to drought stress and highly enhanced ABA insensitivity in addition to globally and drastically impaired expression of ABA- and water stress-dependent genes (Fujita et al. 2009). They also observed that in the triple mutant, SRK2D, SRK2E and SRK2I (SRK2D/E/I), and AREB1, one of the Arabidopsis cDNAs encoding the basic-domain leucine zipper (bZIP) transcription factors, co-localized and interacted in nuclei *in vivo*. In addition, their downstream genes substantially overlap. AREB1is one of the transcription activators of ABRE-dependent ABA signalling that enhances drought stress tolerance in Arabidopsis plants (Fujita et al. 2005). This extensive study also reported that almost all dehydration-responsive LEA (late embryogenesis abundant) protein genes and group-A *PP2C* (protein phosphatase 2C) genes are strongly down-regulated in the *srk2d/e/i* triple mutants which establishes the pivotal roles of SRK2D/E/I in ABA signalling in plants. It has also been shown that PKABA1 from wheat, a member of the SnRK2 subfamily is involved in mediating ABA-induced changes in gene expression (Anderberg and Walker-Simmons 1992; Gómez-Cadenas et al. 1999). SnRK2s have also been reported to have roles in sulfur-deprivation response in *Chlamydomonas reinhardtii* (Gonzalez-Ballester et al. 2008). The SnRK3 gene family includes SOS2, an *Arabidopsis* protein kinase involved in conferring salt tolerance (Halfter et al. 2000; Liu et al. 2000). It is reasonable to assume that SnRKs would be implicated in numerous stress responsive regulatory functions and roles given the fact that they have been partially characterized. Evidence for involvement of these groups of protein kinases in regulation of drought response in Arabidopsis also points to the fact that it likely would have similar regulatory roles in close relatives like *Brassica sp.*

The signal perception and response in higher plants to environmental stimuli involves the phosphotidyl-specific phospholipase C (ptdlns-PLC), a hydrolytic enzyme that utilizes phosphotidylinositol 4,5-bisphosphate to produce second messengers such as Sn-1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate (Hirayama et al. 1995; Kim et al. 2004; Das et al. 2005; Vergnolle et al. 2005). The overexpression of *Bnptdlns-PLC2* in transgenic *B. napus* seedlings leads to early flowering and maturation and results in enhanced drought tolerance (Table 4.1; Georges et al. 2009).

4.5 Quantitative Trait Loci and Temperature Stress

Crop yield has considerably improved through the indirect manipulation of quantitative trait loci (QTLs) that control heritable variability of the traits and physiological mechanisms that determine biomass production and its allocation. QTL-based approaches enhance our understanding of the genetic basis of crop performance under stress conditions because this knowledge can assist breeders with release of plant cultivars that can cope with abiotic stresses.

Heat stress often accompanies drought stress, and the two interact in the way they affect plants. Excessive heat perturbs many cellular and developmental processes and directly affects grain production by reducing fertility and grain quality (Barnaba's et al. 2008). Male gametophyte development is the facet of reproduction that appears most prone to disruption by drought, heat, and cold stresses (Saini 1997; Mamun et al. 2006). It is however, unclear at present whether there is any common basis to the sensitivity of male reproduction to the various stresses. The genetic basis of heat stress tolerance in crop plants is poorly understood. In wheat, two QTLs were identified that controlled grain-filling duration, a trait thought to be correlated with heat tolerance (Yang et al. 2002). In maize, QTLs were identified that controlled pollen heat tolerance (germinability and pollen tube growth), a factor influencing heat-induced sterility (Frova and Sari-Gorla 1994). Studies investigating multiple parameters related to heat tolerance in wheat provided evidence for genetic variability and multiple tolerance mechanisms (Dhanda and Munjal 2006). Mutants for seven 'hot' loci in Arabidopsis, which are compromised in their ability to acquire thermotolerance may further our understanding of heat tolerance in crop species (Hong et al. 2003).

Maize and rice originate from tropical/subtropical regions and are relatively cold sensitive. Cold exposure leads to poor seedling establishment and, at booting stage, results in reduced fertility mainly due to the arrest of microspore development. QTLs controlling sensitivity to chilling have been identified in maize (Hund et al. 2005; Jompuk et al. 2005; Presterl et al. 2007), sorghum seedlings (Knoll and Ejeta 2008), and in rice at the seedling and booting stages (Andaya and Tai 2006; Kuroki et al. 2007; Lou et al. 2007). In cultivated tomato (*Solanum lycopersicum*), a QTL allele introgressed from a wild relative (*Solanum hirsutum*) increased chilling tolerance (Goodstal et al. 2005).

Freezing damage arises from dehydration and membrane damage is caused by the growth of ice crystals. Full expression of frost tolerance at the vegetative stage typically requires a prior period of acclimation during which plants are exposed to low, nonfreezing temperatures (Xin and Browse 2000). Loci controlling vegetative frost tolerance have been identified at corresponding positions across the Triticale species at two locations on the long arms of group 5 chromosomes. The proximal loci (Fr-1) are close to or coincident with the Vrn-1 loci involved in flowering response to vernalization. Clusters of C-REPEAT BINDING FACTOR (CBF) genes map at the distal (Fr-2) loci and represent likely candidates for controlling genes (Galiba et al. 2005; Baga et al. 2007; Francia et al. 2007). CBFs, also known as dehydration-responsive element-binding 1 (DREB) factors, are cold-responsive transcription factors with likely roles in coordinating cold responses leading to cold/freezing tolerance in plants (Shinozaki and Yamaguchi-Shinozaki 2000). A CBF gene (CBF2) also maps at a freezing-tolerant QTL in Arabidopsis and appears to be the controlling gene (Alonso-Blanco et al. 2005). Frost at flowering time can cause sterility or shriveled grains in many cereal crops, leading to sporadic episodes of severe losses (Fuller et al. 2007). QTLs for reproductive frost tolerance have been reported in barley, near the Fr-H1 locus, and on chromosome arm 2HL (Reinheimer et al. 2004). However, efforts to breed for reproductive frost tolerance have met with comparatively little success, most likely due to the lack of sufficient genetic variation, the confusing effects of tiller developmental stage on susceptibility, and the dependence on frost simulation chambers for phenotyping. QTLs for winter hardiness have also been identified in lentil (Kahraman et al. 2004), rapeseed (Asghari et al. 2007), and rye grass (Xiong et al. 2007). Characterization of such QTLs will be useful in identifying additional gene players involved in stress responsiveness in plants.

4.6 Summary and Future Perspectives

Development and commercialization of oil seed crops have been successful mainly due to the intensive and collective breeding endeavors. While several improvements with regards to the oil and non-meal aspects have been achieved, one area of oil seeds that needs improvement is developing means to overcome stress tolerance. Using the understanding gained from how a stress signal is perceived and passed on to activate a specific pathway, it should be possible to implement directed engineering of pathways to manipulate response systems. Though numerous approaches to achieve drought tolerance through genetic engineering exists, the approach via ABA and Brassinosteroid will be interesting, and efficient, given the fact that these are natural players in the plant metabolism. Stomatal movement is natural, so is the presence of ABA and brassinosteroids. Changes in ABA levels lead to opening and closure of stomata (control transpiration loss). Similarly, the ability of brassinosteroids to impart resistance to numerous stresses is appealing, and therefore, function efficiently if modulated effectively.

Some questions remain, such as when and how do plants sense drought stress in the roots and communicate to the shoot to initiate ABA-mediated stomatal movement and control? While reports indicate ABA itself serves as a chemical signal (Wilkinson and Davies 2002), others indicate that shoot-generated ABA may be ineffective or insufficient to stimulate adequate stress response (Christmann et al. 2007). With brassinosteroids, their role in stress sensing and signalling as well as the molecular mechanisms by which they induce stress resistance in plants is not fully understood. Likewise, it will be interesting to probe the mechanistics of SnRK-mediated stress regulation in plants.

Genomics-assisted improvement of abiotic stress tolerance of crops will increasingly rely on the QTL approach. The systematic dissection of QTLs governing the naturally occurring variation relevant for crop yield and its sustainability will facilitate targeted and effective tailoring of cultivars with an improved performance under abiotic constraints. Nonetheless, it is difficult to predict to what extent QTL-based MAS will affect breeding practices aimed at improving tolerance to abiotic stresses. However, given the constant search for novel genes and means to fight stresses, there will be a growing interest in wild relatives of crops and landraces to identify superior alleles among those that the domestication bottleneck and modern agriculture have provided in the present era.

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Chapter 5
Applications of Machine Learning in Breeding for Stress Tolerance in Maize

Leonardo Ornella, Gerardo Cervigni, and Elizabeth Tapia

Abstract Corn is one of the world's most important cereals and a major source of calories for humanity, along with rice and wheat. Climate change and the use of marginal land for crop production require the development of genotypes adapted to stressful environments, particularly drought tolerant plants. Among the new technologies currently available for accelerate the releasing of new genotypes there is an emerging discipline called Machine Learning (ML). A primary goal of ML algorithms is to automatically learn to recognize complex patterns and make intelligent decisions based on data. This work reviews several strategic applications of ML in maize breeding. Quantitative trait loci mapping, heterotic group assignment and the popular genome-wide selection are some of the key areas currently addressed by the literature. Results are encouraging and propose ML algorithms as a valuable alternative to traditional statistical techniques applied in maize, even the more recently introduced linear mixed models.

5.1 Introduction

Corn is one of the world's most important cereals and a major source of calories for humanity, along with rice and wheat. Annual yield losses due to drought average around 15% of potential yield (Edmeades 2008). Even water deficits act as a significant

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limitation to yield in the US Corn Belt in 20% of years; losses are somewhat greater in tropical countries that rely on a relatively unpredictable rainy season for crop growth (La Rovere et al. 2004; Edmeades 2008). Climate change and population growth suggest that the production of major crops will move to marginal areas, mainly with water deficit (Edmeades 2008). It is clear that one of the main objectives of plant breeding is the development of genotypes adapted to stressful environments. Furthermore, the pressure of both climate change and food demand makes necessary the development and/or the application of new strategies in order to accelerate the process of breeding (Bänziger and Araus 2007; Edmeades 2008; Prasanna et al. 2010).

Advances in genomics led to the identification of numerous DNA markers in maize during the last few decades. Restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR) and single nucleotide polymorphisms (SNPs) are some of the most common markers used in plant breeding (Kumar et al. 2009). It is reported that with the emergence of new disciplines such as structural genomics, transcriptomics, proteomics and metabolomics[1]; and their integration with plant physiology and plant breeding, DNA markers will become less important in analytical breeding (Araus et al. 2008). However, DNA marker technology is still advantageous regarding cost/benefit (Bernardo 2008).

In the two past decades, with the adoption of these new technologies, molecular breeding (which includes MAS or Marker Assisted Selection) had considerable impact in maize improvement (Araus et al. 2008). Molecular marker can applications can be partitioned (roughly) into four groups:

- DNA fingerprinting and analysis of genetic diversity.
- Assigning inbred lines to heterotic groups.
- Prediction of hybrid performance.
- QTL analyses.

For most of these areas it has been extensive research in order to identify the appropriate statistical method (Kearsey and Farquhar 1998; Mohammadi and Prasanna 2003; Piepho et al. 2008). However, and particularly for drought tolerance, results are not completely satisfying and more research on methodologies is needed (Ribaut and Ragot 2007; Araus et al. 2008; Bernardo 2008; Collins et al. 2008).

Machine Learning (ML) is an emerging discipline with a revolutionary impact on a variety of areas (Duda et al. 2001; Mitra and Aharya 2003). Algorithms of ML allow recognizing complex patterns and making intelligent decisions based on data. Due to their high generalization capabilities and distribution-free properties they are presented as a valuable alternative to traditional statistical techniques applied in maize breeding, even the more recently introduced linear mixed models (Maenhout et al. 2007).

The purpose of this revision is to evaluate some ML applications currently available to molecular breeding and their potential utilization to drought tolerance breeding. The remainder of this revision is organized as follows. Section 2 introduces ML and

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[1] Regarding applications of ML in these areas, the reader can see Zhang and Rajapakse (2009).

some key issues on supervised and unsupervised learning algorithms. In Sect. 3 we discuss several ML applications in molecular breeding, especially for phenotypic prediction and/or heterotic assignation. In Sect. 4 we discuss experimental results relative to QTL mapping and, concluding this paper, in Sect. 5 we present some considerations regarding further implementations.

5.2 Machine Learning: An Overview

ML is a branch of artificial intelligence concerned with the design and development of algorithms that allow computers to evolve behaviors based on empirical data, such as from sensor data or databases (Breiman 2001). It may be considered as a natural outgrowth of the intersection of Computer Science and Statistics (Cunningham 1995; Mitchell 2006). A learner can take advantage of examples (data) to capture characteristics of interest of their unknown underlying probability distribution (Du and Swamy 2006).

Learning problems can be roughly categorized as either supervised or unsupervised (Hastie et al. 2009). In supervised learning, the goal is to predict the value of an outcome measure based on a number of input measures. A distinction in output type has led to a naming convention for the prediction task: regression when we predict quantitative outputs, and classification when we predict qualitative outputs (Hastie et al. 2009). In unsupervised learning or learning without a teacher, there is no outcome measure, and the goal is to describe associations and patterns among a set of input measures (Theodoridis and Koutroumbas 2003; Hastie et al. 2009). ML researchers have already proposed many different types of algorithms. It can be mentioned nearest-neighbour methods, decision tree induction, error backpropagation, reinforcement learning, lazy learning, rule-based learning, etc. (Witten and Eibe 2005; Caruana and Niculescu-Mizil 2006).

Many of these algorithms are implemented in open source statistical and data mining libraries. Most of these libraries, such as Weka (Bouckaert et al. 2010) or R-project (R Development Core Team 2008), run on a wide variety of platforms, mainly Windows or Linux; and more important, Open Source Licenses allow research groups to copy and modify the codes to their own specific necessities without paying royalties or fees (Sonnenburg et al. 2007; Bouckaert et al. 2010). Some successful areas of application are: text mining (Witten and Eibe 2005), bioinformatics (Li et al. 2006); image analysis (Zhong et al. 2009) and medical diagnosis (Kukar and Groselj 2005).

Researchers also have produced several different theoretical frameworks for understanding these methods. These theories provide insight into experimental results and help to guide the development of improved learning algorithms. For example, PAC learning framework allowed the development of boosting algorithms (Schapire 2003), whereas Statistical Learning Theory is the backbone of Support Vector Machines (SVM) (Burges 1998; Gunn 1998). Since many of applications discussed in this review are instances of SVMs, we will focus on this last framework.

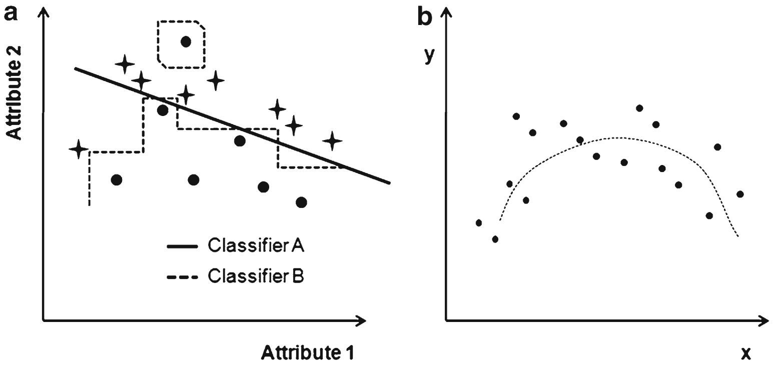

Fig. 5.1 Two examples of supervised learning. (**a**) Binary classification in two dimensional feature space: the algorithm has to find a decision boundary between two classes. (**b**) Regression

5.2.1 *Supervised Learning*

Learning from examples is a paradigm in which systems (natural or artificial) pick up a functional relationship from a training set of examples. Within this paradigm, a learning algorithm is a map from the space of training sets to the hypothesis space of possible functional solutions. A central question for the theory is to determine conditions under which a learning algorithm will generalize from its finite training set to novel examples (Poggio et al. 2004; Hastie et al. 2009).

The training set can be expressed as $\{(\mathbf{x}_1, y_1); \ldots; (\mathbf{x}_n, y_n)\}$, where $\mathbf{x} \in X$ (Input Space) and $y \in Y$ (Output Space), in regression, $y \in R$. Besides, $\mathbf{x}_i$ can be represented as a vector $(\boldsymbol{x}_1, y_1); \ldots; (\boldsymbol{x}_n, y_n)$ or equivalently as a point in a p-dimensional feature space whose coordinates are specified by $\boldsymbol{x}_i$. The j-th co-ordinate of a point in the feature space corresponds to the value of the i-th feature for an item represented by that point (Fig. 5.1). The features may be continuous, categorical or binary (Witten and Eibe 2005).

It is assumed that the pairs $(\mathbf{x}, y) \in X \times Y$ are random variables distributed according to an unknown distribution P. If we observe a sequence of n i.i.d. pairs $(\boldsymbol{x}_i, y_i)$ sampled according to P, the goal is to construct a function g: $X \rightarrow Y$ which predicts y from $\boldsymbol{x}$ (Bousquet et al. 2004). The function g is an element of some space of possible functions G: the hypothesis space (Bousquet et al. 2004; Poggio et al. 2004). A criterion to choose this function g is needed; therefore, the risk of g can be defined as (Bousquet et al. 2004):

$$R(g) = P(g(\mathbf{x}) \neq y) = E(1_{g(x)\neq y}) \tag{5.1}$$

Notice that P can be decomposed as $P(\mathbf{x}) \times P(y/\mathbf{x})$ (Bousquet et al. 2004).

Since P is unknown we cannot directly measure the risk. We can only measure the agreement of a candidate function with the data. A common criterion is the empirical risk (Bousquet et al. 2004):

$$R_n(g) = \frac{1}{n}\sum_{i=1}^{n} 1_{g(x_i)\neq y_i} \tag{5.2}$$

Classical learning theory was developed around the study of ERM (empirical risk minimization). One of its main achievements is a complete characterization of the necessary and sufficient conditions for generalization of ERM and its consistency. Consistency requires that the expected error of the solution converges to the expected error of the most accurate function in the hypothesis class H, i.e., for ERM, generalization is equivalent to consistency (Poggio et al. 2004). However, ERM is not the only approach to choosing g. Other successful frameworks are: Structural Risk Minimization, Regularization, etc. (Bousquet et al. 2004; Hastie et al. 2009).

5.2.2 Major Issues in Real World Applications

Many, if not most, ML algorithms can deal with heterogeneity of the data, redundancy and presence of interactions and non-linearities, each one in their own way. There is no universally best learning algorithm. Even the best models perform poorly on some problems, and models that have poor average performance perform well on a few problems or metrics (Caruana and Niculescu-Mizil 2006; Witten and Eibe 2005; Kotsiantis 2007). So, the choice of the best classifier for a particular domain is a critical step and there is abundant literature about the topic (Bouckaert and Eibe 2004; Kim 2009).

When the data-set is very large, it can be split randomly in two data-sets (training and test set) without loss of information, obtaining both a stable classifier and a reliable estimation of the prediction error (Borra and Ciaccio 2005). In most real situations, data set in not sufficiently large, so prediction error is estimated by resampling techniques (Witten and Eibe 2005; Kim 2009). Most reported are: repeated cross-validation, repeated hold-out and bootstrap methods (Borra and Ciaccio 2005; Witten and Eibe 2005; Kim 2009). The most used measure of prediction capability of a nonparametric classification model is the error-rate, defined as the ratio between the number of misclassified cases, given by the classifier, and the total number of cases examined. There are several alternatives such as Kappa Coefficient or AUC, the area under the ROC curve (Witten and Eibe 2005). Finally, you need an appropriate statistical test if you want to compare the behavior of multiple classifiers (Bouckaert and Eibe 2004). Several heuristic versions of the t-test have been reported (Nadeau and Bengio 2003). Also, there are available non-parametric alternatives such as Kolmogorov-Smirnov test (Ornella and Tapia 2010).

Many supervised learning tasks have to face *large p, small n* problems, i.e., where the number of parameters or features (dimensions) exceeds the number of instances to learn from. This is generally called "*the curse of dimensionality*" (Hastie et al. 2009). Due to several reasons, it severely affects the performance of algorithms (Hastie et al. 2009). For example, standard multiple linear regression cannot be used without variable selection (Jannink et al. 2010). Feature (or attribute) subset selection is the process of identifying and removing as many irrelevant and redundant features as possible (Duda et al. 2001; Liu and Motoda 2008). This reduces the dimensionality of the data and enables data mining algorithms to operate faster and more effectively (Kotsiantis 2007). There are many feature selection methods reported in the bibliography (Liu and Motoda 2008). Only in WEKA library there are implemented more than seven different filter metrics which in turn can be combined with different search strategies (Witten and Eibe 2005).

Filters methods select subsets of variables as a pre-processing step, independently of the chosen predictor (the algorithm) and using a predetermined metric (Witten and Eibe 2005; Liu and Motoda 2008). There are other alternatives. Wrapper methods, for example, assess subsets of variables according to their usefulness to a given predictor (Witten and Eibe 2005). In other words, wrappers utilize the learning machine of interest as a black box to score subsets of variables according to their predictive power (Witten and Eibe 2005).

5.3 Molecular Breeding for Drought Tolerance

The unpredictability of drought, geographically and across seasons, has emphasized the importance of drought tolerance as a breeding objective (Bänziger and Araus 2007; Makumbi et al. 2011). The designation of heterotic groups and the prediction of phenotypic performance is a primary objective in breeding. The correct selection of parents can maximize gains through breeding, using fewer resources.

The definition of heterotic groups and heterotic patterns, an empirical task in hybrid maize breeding, has contributed to large increases in yield of temperate maize germplasm (Hallauer and Miranda 1988). Classifying inbred lines into heterotic groups is critical to optimize resources in the development of high yielding hybrids and synthetic varieties. Particularly for drought, the designation of proper heterotic groups is really important since heterosis has been recognized as a important source of stress tolerance (Bruce et al. 2002). The use of testers (lines, synthetics, etc.) allows the placement of a new inbred into the appropriate heterotic group using only a small number of field crosses (Hallauer and Miranda 1988; Warburton et al. 2005). However, if the pool is large and/or diverse, it would be necessary to have more than two testers to adequately represent the diversity present in each heterotic group, or to form more than two groups (Warburton et al. 2005).

Also, the prediction of phenotypic performance from molecular marker data has received increasing attention from plant breeders, as the cost of phenotyping was gradually overtaking the cost of genotyping (Bernardo 2008). Molecular marker genotypes that are either within genes or tightly linked to QTL influencing traits under selection can be employed as a supplement to phenotypic observations in a selection index (Moose and Mumm 2008). In cases where genetic correlations are high, further efficiencies can be gained by substituting phenotypic selection during some selection cycles, which can reduce phenotyping efforts and cycle times by permitting the use of off-season nurseries (Moose and Mumm 2008).

Traditionally, breeder's criteria of selection were based on analysis of variance (ANOVA) of fixed effects and least squares estimation (Hallauer and Miranda 1988). Rex Bernardo (1994) introduced Linear Mixed Modeling (LMM) (Henderson 1984) to predict the performance of untested cross based on field trial results of related hybrids. LMM allows the inclusion of a matrix containing genetic (additive and/or dominant) relationships between parents (Piepho et al. 2008), which can be generated whether from molecular (Bernardo 1994) or pedigree data (Piepho et al. 2008). LMM generates unbiased estimations of heritability, mainly because it takes account correlations between observations due to covariance between relatives (Balzarini 2002) and, not least important, it can handle unbalanced field trial data typically originated from hybrid breeding programs (Balzarini 2002; Piepho et al. 2008).

It has been shown that LMM are superior when hybrids originate from crosses between unrelated inbred lines, which is most likely the case in commercial breeding programs (Charcosset et al. 1998). Although correlations between predicted and observed SCA values are too low to allow for an effective selection towards high heterosis hybrids (Bernardo 1995) the methodology cannot be discarded since it shows very high performance with secondary traits, with higher heritability. Regarding this last issue, in breeding for drought tolerance is really important the selection of secondary traits related to a higher yield potential and/or to improved behavior of the crop when grown in a stressful environment (Bänziger et al. 2000), this is known as analytical or physiological breeding (Araus et al. 2008). Yield is a quantitative inherited trait under multigenic control, and it is characterized by low heritability and a high genotype-by-environment (GE) interaction. Hence, as traditional (i.e., empirical) breeding appears to be reaching a plateau; several approaches, which complement traditional with analytical selection methodologies, may be required to further improve grain yields (Araus et al. 2008). Finally, with the utilization of a selection index, whose weights are chosen by the breeder based on the phenotypic and genotypic covariance between the trait and grain yield, predicted progress for grain yield can be maximized (Bänziger et al. 2000). The proper estimation of the function relating secondary traits and yield also could be obtained using appropriate supervised learning algorithms. To the best of our known, there's no literature about it.

5.3.1 Genomic Selection

While MAS has been effective for the manipulation of large effect alleles with known association to a marker, it has been at an impasse when many alleles of small effect segregate and no substantial, reliable effects can be identified (Jannink et al. 2010). Association mapping has been successfully applied to breeding populations to mitigate the lack of relevance of biparental populations in QTL identification and QTLs have been mapped in this way (Ersoz et al. 2009). This practice nevertheless retains the disadvantage of biased effect estimates and therefore poor prediction of line performance (Jannink et al. 2010).

Genomic selection (GS) (or genome-wide selection) is an approach proposed by Meuwissen et al. (2001) for improving quantitative traits that uses all available DNA maker information across the genome to estimate genetic values (Bernardo 2008; Jannink et al. 2010). It emerged from high density parallel genotyping technologies (Jannink et al. 2010); at such high densities, it is assumed that linkage phase between markers or haplotype blocks of markers and causal polymorphisms would be consistent across families so that population-wide estimates of marker effects would be meaningful (Meuwissen et al. 2001). In the original work (Meuwissen et al. 2001) the authors decided to avoid marker selection in the development of a prediction model so that estimated marker effects would be unbiased. A consequence of that decision was that more predictor effects (p) need to be estimated than the number (n) of available observations. Furthermore, there may be a high degree of correlation or multicollinearity between the predictors (Jannink et al. 2010).

GS uses a training population of individuals that have been both genotyped and phenotyped to develop a model that takes genotypic data from a candidate population of untested individuals and produces genomic estimated breeding values (GEBVs), these GEBVs say nothing of the function of the underlying genes but they are the ideal selection criterion (Jannink et al. 2010). In the plant breeding context, untested individuals would belong to a broader population defined as a crop market class or the breeding program as a whole (Jannink et al. 2010). According to Bernardo and Yu (2007), GS can be described as a black-box procedure as well as a brute-force procedure for exploiting markers to improve a quantitative trait and it is superior to MARS, which involves finding a subset of markers with significant effects. By brute force, it is mean that large numbers of markers are used as a surrogate for the phenotype and large numbers of individual plants are evaluated for their marker data. By black box, it is mean that the procedure does not involve dissecting the mechanisms underlying the control and inheritance of quantitative traits.[2]

In so-called "*large p, small n*" problems, which in machine learning literature is known as "*the curse of dimensionality*", standard multiple linear regression cannot be used without variable selection, which conflicts with the original goal of avoiding marker selection (Jannink et al. 2010). An important danger in the development of a prediction model is overfitting: an overfitted model can exaggerate minor fluctuations in the data and will generally have poor predictive ability (Duda et al. 2001; Jannink et al. 2010). Machine learning offers several alternatives to deal with this problem. For example, Gonzalez-Recio et al. (2009) proposes a two-step approach

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[2] Both black box procedure and brute-force inference are paradigms in machine learning (Hastie et al. 2009; Mitra and Aharya 2003).

with machine learning and a Bayesian threshold least absolute shrinkage and selection operator (BL-LASSO) model to detect interactions between relevant SNPs associated with rheumatoid arthritis (RA). In the first step, redundancy and co-linearity in the feature set were reduced whether by Information Gain procedure (a filter method) or by a wrapper procedure, both implemented in WEKA. In wrapper, the attribute evaluator was Naive Bayes and the search method was bidirectional hill-climbing (Gonzalez-Recio et al. 2009).

Another challenge is how models can accommodate the complexity of quantitative traits (e.g., diverse forms and degrees of interaction between genes) as well as the peculiarities of breeding populations in which the standard assumptions of an infinitesimal model (such as linkage equilibrium, artificial selection, and assortative mating) do not hold. Parametric and non-parametric procedures address these problems differently (Piepho et al. 2008; Crossa et al. 2010; Jannink et al. 2010).

Due to their capabilities to cope with the above-mentioned difficulties, machine learning methods, such as SVMs or Random Forest (RF), have been proposed to GS (Gianola and van Kaam 2008; Jannink et al. 2010). RF (Witten and Eibe 2005) is an ensemble predictor consisting of a collection of tree structured predictors, where each tree in the ensemble is grown on the basis of a bootstrapped sample of the training dataset. Each tree individually predicts the target response and the forest (i.e., the ensembles of trees) predicts the target response as an average of individual tree predictions. Since both SVM and RF build a non-linear prediction model, they may be especially useful when the relationships between predictors and responses are nonlinear, as would occur if epistatic effects account for a significant amount of genetic variation of a target trait (Jannink et al. 2010).

Gianola and van Kaam (2008) discuss from a theoretical perspective RKHS (Reproducing Kernel Hilbert Spaces) regression procedures for prediction of total genetic value for quantitative traits, using phenotypic and genomic data simultaneously. They argued that a nonparametric treatment is needed for capturing the multiple and complex interactions potentially arising in whole-genome models, based on thousands of markers. They also stated that machine learning offer exciting avenues for whole genome analysis of quantitative traits and perhaps a change in analytical paradigms (Gianola and van Kaam 2008).

de los Campos et al. (2009) and Crossa et al. (2010) evaluated, with good results, BL-LASSO and GS using maize or wheat datasets with SNP information. Although authors don’t mention the discipline, LASSO, and other regularization methods like RIGDE, is extensively treated in machine learning literature (Zhao and Yu 2006; Hastie et al. 2009). Regularization involves introducing additional information in order to solve an ill-posed problem or to prevent overfitting. This information is usually of the form of a penalty for complexity, such as restrictions for smoothness or bounds on the vector space norm (Duda et al. 2001).

Schulz-Streeck and Piepho (2010) proposed using geostatistical models for GS. They highlight that the gaussian model is essentially equivalent to reproducing kernel Hilbert spaces regression (Gianola and van Kaam 2008) and also to least squares support vector machine (LS-SVM) regression. All these models may have particular relevance when inheritance is not merely additive (Schulz-Streeck and Piepho 2010).

It is recognized that GS will dramatically change the purpose of phenotyping in plant breeding (Bernardo 2008; Jannink et al. 2010). Phenotyping currently serves to determine which lines to select; under GS, phenotyping will serve primarily to train prediction models.

While it is well known that GEBV accuracy increases as the size of the training population increases, according to Jannink et al. (2010) no research has been conducted on training population design to develop accurate GEBV models while minimizing resources consumed by phenotyping.

As expressed before, different GS methods use substantially different approaches to address the *large p-small n* problem (Jannink et al. 2010). The methods may therefore capture different aspects of the marker genotype to phenotype map and could complement each other. If such complementation occurs, a synthesis of methods might be superior to any single method. In the same way that RF averages a number of predictors to achieve more accurate predictions, combining methods may be valuable (Jannink et al. 2010). Several ensembles methods had been successfully applied to combine different methods for regression, yet none of them have been reported in maize (Brown et al. 2005; Pardo et al. 2010).

Finally, with respect to SVM regression, Maenhout et al. (2007) have done extensive analysis on ε-insensitive support vector machine regression (ε-SVR) and phenotypic prediction using molecular markers. Their results will be discussed in the next section.

5.3.2 ε-SVM for the Prediction of Maize Hybrid Performance

Maenhout et al. (2007, 2008) evaluated several scenarios of ε -insensitive support vector machine regression (ε-SVR) to screen for genetically superior inbred lines based on their molecular marker profiles. Molecular and phenotypic data were generated from a maize breeding program of the private company (105 inbred lines from Iowa stiff stalk synthetic heterotic group and 93 lines from the complementary Iodent group) (Maenhout et al. 2007). Molecular data consisted in information from SSR (75 loci) and AFLP (569 polymorphic bands) (Maenhout et al. 2007).

In ε-SVR the goal is to find a function $f(\boldsymbol{x})$ that deviates at most ε from the target value y for each training sample in the data set (See Appendix). Under this context, each training sample i can be represented as a couple consisting of a vector $\boldsymbol{x} \in \boldsymbol{X}$ (molecular data) and a scalar $y \in R$ (phenotypical response of hybrid i for the trait under study). Initially, $\boldsymbol{X}$ was considered as a binary space of n dimensions where n is the total number of possible alleles that make up a molecular fingerprint. Consequently, the entries in the vector $\boldsymbol{x}_i$ were set to 1 if one of the homozygous parents carries the corresponding allele or −1 otherwise (Maenhout et al. 2008). Due to several reasons, the binary input features of inbreds where replaced by allele frequencies which resulted in a faster convergence of the training algorithm while achieving the same prediction accuracy (Maenhout et al. 2008). The advantage of ε-SVR lies in the use of kernel functions that allow exploring nonlinear models for hybrid prediction. Three (3) different kernel functions were evaluated: linear (Maenhout et al. 2007; Maenhout et al. 2008), Gaussian (Maenhout et al. 2007; Maenhout et al. 2008; Maenhout et al. 2010), and a specific kernel function derived from commonly applied genetic similarity measures for dominant and co-dominant markers (Maenhout et al. 2007) (See Appendix for more details).

Unbalanced phenotypical measurements of three traits: yield (Y), moisture content (MC) and days until flowering (FD), were corrected using linear mixed modeling (Bernardo 1994) and the corrected phenotypical values (without trial, location or block effects) of all hybrids were used as a training set for constructing an ε-SVR in which the molecular fingerprints of each hybrid serve as predictor variables (Maenhout et al. 2008). These models were used to predict whether corrected total genetic value, GCA or SCA values of unknown inbred lines and hybrids.[3] Prediction accuracy was evaluated by means of cross-validation routines and by Pearson correlation between the actual and the predicted values as well as the standard error which is defined as the square root of the sum of the squared deviations between the actual and the predicted values (Maenhout et al. 2008). Results of ε-SVR prediction were compared with those obtained using the prediction system proposed by Bernardo (1994, 1995).

Overall, results indicated that both methods (ε-SVR and BLUP) allow the routine screening of new inbred lines despite the fact that predicting the SCA value of an untested hybrid remains problematic with the available molecular marker information and standard kernel functions (Maenhout et al. 2008). The genotypical performance of a testcross hybrid, originated from a cross between an untested inbred line and a well-known complementary tester, can be predicted with moderate to high accuracy while this cannot be said for a cross between two untested inbred lines. The ε-SVR framework, however, allows for a greater flexibility in combining different kinds of predictor variables (Maenhout et al. 2007). Also, non-linear kernels perform better than linear for every combination of trait and marker type (Maenhout et al. 2007, 2008, 2010).

In Maenhout et al. (2010), both prediction methods (BLUP and ε-SVR) were compared under several scenarios regarding number of training instances or number of loci. The prediction's accuracy of BLUP was less sensitive to a reduction of the number of training examples compared with that of ε-SVR. The latter was, however, better at predicting hybrid performance when the size of the molecular fingerprints was reduced, especially if the initial set of markers had low information content.

The probability of retaining a line belonging to the top 1%, based on BLUPs calculated from testcross results, when choosing the 20%, best ε-SVR based genotypical values without performing any field trial is 89% (Maenhout et al. 2008). If we want to select the earliest hybrids this probability increases to 97% for moisture content (Maenhout et al. 2008). It should be clear that when selection cycles with a

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[3] Total genetic value of the cross i×j can be expressed as: $g_{ij} = GCA_i + GCA_j + SCA_{ij}$. GCA_i is the general combining ability of the ith parent and SCA_{ij} is specific combining ability of crossing the i-th with the j-th parent.

minimum of field trials are envisioned, ε-SVR models allow for the accurate identification of superior inbred lines without field trial data. This conclusion, however, presumes that enough testcross training examples for building the ε-SVR model are available (Maenhout et al. 2008).

To construct an ε-SVR or BLUP model for the prediction of phenotypic response based on a hybrids molecular fingerprint, training data that contains a vector of marker scores and a single response value for every hybrid is needed. The best prediction accuracy is achieved by constructing these hybrid response values by summing the appropriate GCA and SCA BLUPs, obtained from a LMM analysis with a random genotypic effects assumption. If prediction accuracy is determined by means of a validation trial, both ε-SVR and BLUP perform close to the theoretical limit for the traits grain moisture content and days until flowering while they both fall short for grain yield, a trait with a low heritability in advanced breeding pools. The accuracy of SCA predictions was insufficient for all three traits.

This lack of predictive power is not reflected in the prediction accuracy measures obtained through cross-validation procedures, as these do not take into account the uncertainty introduced by G×E effects. As stated before, if only a limited set of training examples is available but the genotyped markers are either numerous or very informative, BLUP is more accurate than ε-SVR. If on the other hand, if the set of molecular markers is either restricted in size or information content, ε-SVR is the preferred prediction method (Maenhout et al. 2010).

5.3.3 Heterotic Assignation Using Molecular Data

Guinand et al. (2002) compared different nonparametric machine learning techniques with parametric likelihood estimations commonly employed in population genetics for purposes of assigning individuals to their population of origin (assignment tests). They conclude that the relative performance of each machine learning classifier improved relative likelihood estimators for empirical data sets, suggesting an ability to learn and utilize properties of empirical genotypic arrays intrinsic to each population.

Ornella et al. (2008) suggest that, instead of predict the performance of untested hybrids, molecular fingerprint could be used directly to assign inbreds to heterotic groups previously established. The idea is to explore the ability of classifiers to "mimics" the breeder's decision when assigning lines to heterotic groups using test crosses or other field assay (a black box procedure).

Unlike the SVR approach (Maenhout et al. 2007), and since this approach is a classical classification problem (Witten and Eibe 2005), each training line is represented as the classical pair $\boldsymbol{x}_i \in \boldsymbol{X}$ and $y \in Y$ but here $\boldsymbol{X}$ is a categorical (nominal) space of p dimensions, being p the number of marker loci, Y is also categorical and its value (the class) is the known heterotic group. Once trained, the classifier just has to assign the potential heterotic group to a new line based only his molecular profile (Ornella and Tapia 2010).

Several well known multiclass classifiers were evaluated: RF, Decision Trees (J48), Naive Bayes and Bayes Nets, among others (Ornella et al. 2008; Ornella and Tapia 2010). It was also assessed the performance of SVM classifiers, these classifiers have particularly high generalization capabilities and have become very popular in recent years; nevertheless, they are inherently binary classifiers and a combination scheme is necessary to extend SVMs for problems with more than two classes (Rifkin and Klautau 2004). Two combination schemes were used; the One Against All (OAA) (Rifkin and Klautau 2004) and the Error Correcting Output Coding (ECOC) approach (Dietterich and Bakiri 1995). The success of the classification was estimated by means of error rate and Cohen's Kappa Coefficient (Witten and Eibe 2005) and by repeated montecarlo 10-cv crossvalidation (Kim 2009). The choice of the Kappa coefficient was motivated by its ability to better measure the agreement between binary inter-annotators than the traditional classification error; it is well suited for unequal class distribution datasets (Witten and Eibe 2005). Results were variable, depending on the dataset evaluated. For example, on the smallest dataset (26 lines and 21 SSR), best results were obtained using Simple logistic (Landwehr et al. 2005) whereas on medium (73 lines and 83 SSR) and large datasets (197 lines and 94 SSR), Bayes Nets (Witten and Eibe 2005) and ECOC-SVM approach get the highest performances. Two feature selection methods, both implemented in WEKA, were utilized in order to improve classification. Correlation based Feature Subset selection or CFS (Hall 2000), which is fully automatic and does not require a priori specification of the number of features to be included in the final subset (Hall 2000), and Relief-F, which is based on a completely different metrics for select attributes (Kononenko 1994). This second algorithm is not automatic so it was calibrated in order to retain (10, 20, …, 90)% of the original number of attributes (Ornella 2010). A slight improvement of classification was observed on all datasets when using Relief-F. However, Kappa values were never higher than 0.6, which would have indicated a substantial agreement between observed and predicted values (Landis and Koch 1977). None improvement of classification was observed when using CFS for filtering, however; CFS removes noisy attributes non-correlated between them and theory suggests that interactions between genes associated with molecular markers could play an important role in the generation of the observed heterosis (Fiévet et al. 2010) so filters that contemplate this situation remain to be explored. General results direct application on breeding. Still, alternative scenarios remain to be explored. For example, if a breeder has an heterotic pattern (A, B, C) and want to improve only a particular heterotic group (let's say A) with new germplasm, he/she can optimize parameters of classifiers and utilize other statistical measures of the performance, such as sensitivity or specificity (Witten and Eibe 2005), in order get better results for that particular objective. Only in the newest version of WEKA there are implemented more than 30 different algorithms, each one with its own characteristics; also the performance of many of them can be improved by using meta-learner (Rokach 2009; Yang et al. 2010) so, it also rest to evaluate the behavior of these alternatives.

To conclude, and as it was previously mentioned for GS, in heterotic classification it is also crucial to determine the effect of number of marker and number of training instances in order to optimize resources. It was observed a significant relation when evaluating the performance of several classifiers and different number of training instances (Ornella 2010). All classifiers perform poorly when $n < 30$ lines were used to train the algorithm and show a relatively good behavior when $n > 50$. On the contrary, a less important difference was observed when varying the number of features by randomly selecting a subset of markers (Ornella 2010). However, these results were preliminary and with the objective to optimize a protocol useful to third world breeding programs, with a very low budget; it would be necessary to validate these observations with other datasets.

5.3.4 Applications of Unsupervised Learning: Cluster Analysis

With increases in the sample sizes of breeding materials and germplasm accessions used in crop improvement programs, methods to classify and order genetic variability are assuming considerable significance (Mohammadi and Prasanna 2003; Reif et al. 2005). As previously stated, the assignment of new lines into heterotic groups is generally performed by crosses with testers representatives of each group (Warburton et al. 2005). However, to identify testers to classify germplasm to heterotic groups when such groups do not exist already is exceedingly difficult, and testers have been selected based mainly on their general combinability rather than their genetic background (Warburton et al. 2005). Molecular markers have been also proposed to suggest new heterotic groups using cluster analysis (Warburton et al. 2005; Choukan et al. 2006). Cluster analysis (CA) is a collection of statistical methods that can be used to assign cases to groups (clusters), group members will share certain properties in common and it is hoped that the resultant classification will provide some insight into the research topic (Theodoridis and Koutroumbas 2003). By CA, molecular data is used to allocate inbreds into groups or clusters (Mohammadi and Prasanna 2003). Clusters containing many lines (more than 3–5) which show a fairly low average genetic diversity within the cluster can be considered to form a good potential heterotic group, and may have a higher probability of showing good mid-parent heterosis when crossed to any other line in any other proposed heterotic group (Warburton et al. 2005). Although this hypothesis must still be confirmed with field studies, it would be result in considerable savings in time and money (Warburton et al. 2005).

Most works in maize utilize distance-based methods, in which a pair-wise distance matrix is used as an input for analysis by a specific number of clusters that are required for accurate clustering algorithm into a graphical representation (such a tree or dendrogram)in which clusters may be visually identified (Mohammadi and Prasanna 2003). There is many similarity (or distance) coefficients, most reported are Jaccard (J) and Modified Rogers' distance (MRD) (Mohammadi and Prasanna 2003; Reif et al. 2005; Balestre et al. 2008). Among the most used algorithms for cluster it can be mentioned UPGMA (unweighted pair-group method using the arithmetic average) and Ward's method (Mohammadi and Prasanna 2003; Balestre et al. 2008).

It is well known that groups obtained by molecular analysis can be affected by either the similarity coefficient (Reif et al. 2005; Warburton et al. 2005; Balestre et al. 2008) or by the cluster algorithm (Mohammadi and Prasanna 2003; Warburton et al. 2005). For instance, most algorithms do not provide an objective definition of what constitutes an optimal tree or dendrogram, and systemic errors are likely to be introduced. Bootstrap analysis has been proposed to estimate the confidence limits of the groups (Mohammadi and Prasanna 2003; Zheng et al. 2008). An alternative, model-based, approach (Pritchard et al. 2000) has been increasingly used in grouping inbreeds using molecular markers (Mohammadi and Prasanna 2003; Xie et al. 2008). Given the number of clusters ($\boldsymbol{K}$) and assuming Hardy Weinberg and linkage equilibrium within clusters, the software estimates allele frequencies in each cluster and population memberships for every individual. The general "admixture" model also estimates admixture proportions for each individual; it uses Markov Chain Monte Carlo to integrate over the parameter space and make cluster assignments (Pritchard et al. 2000). Although the value of $\boldsymbol{K}$ must be provided to the algorithm, a heuristic method for selecting most probable $\boldsymbol{K}$ is often used (Evanno et al. 2005).

Historically, CA was included in Multivariate Analysis (Mohammadi and Prasanna 2003; Reif et al. 2005; Warburton et al. 2005). It is also included in machine learning and statistical pattern recognition as unsupervised learning because it does not rely on predefined class-labeled training examples (Duda et al. 2001; Theodoridis and Koutroumbas 2003; Hastie et al. 2009). According to Ye (2003), even thought the long term use of CA, serious efforts to perform effective and efficient clustering on large data sets started only in recent years with the emergence of data mining.

As an example of these new applications we will briefly describe the $W-k-means$ algorithm (Huang et al. 2008). In this procedure a new step is added to the standard $k-means$ clustering process to calculate the feature weights from the current partition of data in each iteration (Huang et al. 2008). The weight of a feature is determined by the sum of the within-cluster dispersions of the feature: the larger the sum, the smaller the feature weight. The weights produced by the $W-k-$means algorithm measure the importance of the corresponding features in clustering. The small weights reduce or eliminate the effect of insignificant (or noisy) features. Therefore, the feature weights can be used in feature selection. Since the $k-means$ clustering process is not fundamentally changed in $W-k-$means, the efficiency and convergence of the clustering process remain (Huang et al. 2008). $W-k-$means could be considered a valuable alternative to explore, since most distance-based methods doesn't considerate the information content of each loci.

Other multivariate techniques, such as Principal Component Analysis (PCA) and multidimensional Scaling (MDS), are also included in several books of machine learning (Duda et al. 2001; Hastie et al. 2009).

5.4 Mapping of QTLs (Quantitative Trait Loci)

Use of MAS for improving complex traits remains a challenge for crop breeders, at least in the public sector (Xu et al. 2009) and/or abiotic stress (Collins et al. 2008). While the genetic dissection of major crop performance in drought-prone environments has greatly benefited from the use of DNA markers, it has not been implementation in real breeding (Araus et al. 2008; Xu et al. 2009). The challenge for molecular breeders is to identify QTLs of major effect that are independent of genetic background and to devise more effective breeding approaches for the application of the resultant markers, such as pedigree selection (Xu et al. 2009). Molecular markers linked to QTLs are used to estimate the QTL genotype, location and effects (QTL architecture) and get a less biased estimate of the number of genes / QTLs underlying traits. Improving mapping QTL methods is always necessary to marker-assisted selection (MAS). Also, QTL cloning can be successfully applied (Collins et al. 2008). Geldermann (1975) define quantitative trait loci (QTL) as a chromosomal region linked to or associated with a marker gene which affects a quantitative trait. This nomenclature is really appropriate to understand the process of QTL detection. QTLs are nothing more than statistical associations. Therefore, in addition to marker technologies, phenotyping methods and statistical tools are critical to establish quality QTLs (Araus et al. 2008).

5.4.1 *Traditional QTL Mapping Methods*

The first model describe to QTL detection with various progenies type is the Single Marker (SM) model (Beckmann and Soller 1988). In this case, the use of a genetic map is not necessary, although it helps on interpretation of the results. The possibility of identifying a QTL depends on the genetic distance (frequency of recombination or r) between the marker and the QTL as well as the magnitude of the additive (a) and dominance (d) effects. Only if $r < 0{:}5$ and QTL genetic effects are pronounced, the association QTL-marker could be detected. ANOVA and regression have allowed the identification of markers linked to QTLs, but have some limitations: (1) do not indicate if there is one or more QTLs linkage to the marker, (2) do no estimate QTL position and (3) QTL effect may be underestimated because it is mixed with the frequency of recombination.

The interval mapping (IM) originally proposed by Lander and Botstein (1989) is the basis of all QTL mapping methods currently used. Unlike SM, in the IM is essential to have a genetic map because the analysis unit is the genetic interval and not a single molecular marker (Lander and Botstein 1989). QTL location is performed through the whole genome testing the null (H_0: no QTL) and alternative (H_A: QTL linked) hypotheses. Likelihood ratio (LR) or LOD score are used to test H_0 vs H_A, a QTL is present when the value of LR or LOD obtained is greater than a previously established cutoff point.

In maximum-likelihood analyses, a combined test for the presence of p parameters are estimated (full model) compared with the maximized likelihood (L_0) from which the parameters are omitted or set at some value (reduced model). Then 2log(L1/L0) provides a *LOD* score which should be asymptotically distributed as a with *p* degrees of freedom (Wilks 1938). Regression is maximum-likelihood when errors are independent and normally distributed (Draper and Smith 1966). In Haley and Knott regression model (Haley and Knott 1992), *LR* test can be written in terms of the residual sum of squares of the full model (fitting the regression), the reduced model (omitting the regression) and the number of observations; $\mathrm{RSS}_{\mathrm{full}}$, $\mathrm{RSS}_{\mathrm{reduced}}$ and *n*, respectively. According to, Haley and Knott (1992), LR can be expressed as:

$$LR = n \ln \frac{RRS_{(reduced)}}{RRS_{(full)}} \tag{5.3}$$

IM have several advantages compared with SM analysis (Beckmann and Soller 1988; Soller and Beckmann 1990). However, the statistic for a specific interval can be very affected for QTLs at some nearby point on the chromosome, i.e., the estimated positions and effects of QTLs identified by this method are likely to be biased (Haley and Knott 1992; Martinez and Cumow 1992). It is not efficient to use only two markers at a time to do the test, as the information from other markers is not utilized. Lander and Botstein (1989) also proposed a simultaneous search strategy for multiple QTLs on multiple intervals to alleviate some of these problems. However, mapping can be still biased, because the number of QTLs on a chromosome is unknown. To refine the IM, the composite interval mapping method (CIM) was development (Jansen 1993; Zeng 1993, 1994); CIM use the partial regression coefficient as test statistic, which dependent only on those QTLs that are located in the interval. The partial regression coefficient is conditioning on unlinked markers in the analysis to reduce the sampling variance of the test statistic by controlling some residual genetic variation and thus increase statistical power of the test. Conditioning on linked markers, the statistic reduce the chance of interference of possible multiple linked QTLs on hypothesis testing and parameter estimation. It potentially increases the precision of the test and estimation, but with a possible decrease of statistical power of the test. Therefore, there is a trade-off between precision and efficiency of the mapping by using an interval test. Finally, two sample partial regression coefficients of the trait *y* on markers *i* and *j*, are generally uncorrelated unless the two markers are adjacent markers. There are also several Bayesian methodology proposals to use in QTL mapping (Satagopan et al. 1996; Sen and Churchill 2001; Yi 2004). In summary, Bayesian QTL mapping methods (BIM) try to take full account of the uncertainties in QTL number, location, and effects by studying their joint distributions. Such a method takes the prior knowledge about these parameters as a prior distribution, reduces the uncertainty by integrating the information from the data, and expresses the remaining uncertainty as a posterior distribution of parameters.

Controlling Type I error for detecting and locating QTL has received considerable attention. A problem common to many methods is the difficulty of determining appropriate significance thresholds (critical values) against which to compare test statistics (LOD or LR). Basically, there are two problems to solve. First, the chi-square distribution of the test statistic under an appropriate null hypothesis, in most cases, is not satisfied (Hartigan 1985). Second, as multiple hypothesis testing is implicit in the genome searches used for locating QTL (Zeng 1993, 1994); a large number of tests may be carried out, many of which are not independent. In (Lander and Botstein 1989) is discussed the issue of the appropriate significance value of the test statistic using the LOD score for mapping procedure covering a whole genome. The authors showed that an appropriate threshold for LOD score was $(2\log10)t\infty$ where $t\infty$ solve the equation $t\infty=(C+Gt\infty?)\chi2(t\infty)$. C is the number of chromosomes of the organism. G is the length of the genetic map, measured in Morgans, $\chi2(t\infty)$ is the probability that a random variable from a $\chi2$ distribution is less than $(t\infty)$. The authors suggest that a typical LOD threshold should be between 2 and 3, to ensure an overall false positive (type I error) rate for QTL detection of 5%. The threshold value of the test statistic for CIM is different from IM. Using multiple regression, the test statistics in CIM are almost independent between different intervals but highly correlated within intervals. Therefore, appropriate markers should be selected as a background control. If too many markers are fitted in the model it could reduce significantly the number of degrees of freedom and increase the threshold value of the test statistic, particularly when the sample size is small (Zeng 1993). In Zeng (1993, 1994) is advocated the use of an approximate one degree of freedom chi-square threshold for his method when the sample size is large, and the number of evenly spaced markers is small. Other researchers have advocated the use of "conservative" threshold values based on chi-square distributions with either one or two degrees of freedom. The method presented by Jansen and Stam (1994) relies on weighted sum of squared residuals for the case of mixture models. Admittedly, it is stated "*as an ad hoc approximation we used the chi-squared distribution with one degree of freedom, multiplied by the residual variance*" (Jansen and Stam 1994). Churchill and Doerge (1994) proposed permutation test to found the genome-wide threshold to control Type I error rate for mapping a single QTL. Permutation is performing shuffling the data N times by randomly pairing trait values with genotypes. The shuffled data are then analyzed for QTL effects.

The resulting test statistics at each analysis point are stored and the entire procedure (shuffling and analysis) is repeated N times. The critical value is an experiment-wise threshold that provides an overall $100(1-\alpha)\%$ critical value that is valid simultaneously for all analysis points. Results of the QTL analysis on the original data can be compared to these critical values to determine statistical significance and thus to detect QTL effects. According to Doerge and Rebai (1996), for a 5% and 1% level of significance would be necessary to perform 1,000–10,000 permutations, respectively.

5.4.2 QTL Analysis by Statistical Machine Learning

An algorithm based on statistical machine learning (SML) was reported recently by Bedo et al. (2008). The algorithm performance was compared on backcrossing synthetic data set and a real genotype and phenotype data obtained from 94 DH (double haploids) of barley measured in 16 environments. The algorithm consist in a linear predictive model, recursive feature elimination, bootstrap re-sampling for estimation of model performance and marker effects, and generation of QTL profiles by local summation. Contribution from markers was assumed to be additive according the follow linear model:

$$f(x;\,\beta,b)\sum_{b\in K} x_{ik}\,\beta_k + b \tag{5.4}$$

Where x_{ij} be the genotype of plant *i* at marker *j*, and be the vector consisting of all markers from plant *i*. Under the linear assumption, the estimate of y_i for plant I is where K is a set of markers, x_{ik} is the genotype of marker *k* for plant *i*, $\boldsymbol{\beta}$ is the associated weight vector, and *b* is the bias parameter.

The algorithm estimates the generalization performance of a QTL model by splitting the data into independent training and testing subsets that used for model induction and evaluation, respectively. The parameters β and b are estimated from the training data using the Ridge Regression algorithm (Hastie et al. 2009). Recursive Feature Elimination (RFE) is performed to select a model with a low number of markers for accurate phenotype prediction. It commences with the full model using all features and then discards the least important feature. This process is recursively applied until a model of desired size is reached.

Performance of models was evaluated using bootstrap resampling (Hastie et al. 2009). Models are built on the training set, with a test set reserved for the evaluation of model performance. The fraction of variance explained was the criterion to evaluate the performance (Bedo et al. 2008). Additionally, the contribution of individual markers incorporates to the model was also estimated. This is, quantify the amount of additional predictive power provided by each marker given some already selected set of markers. As RFE-RIDGE produces nested subsets of selected makers, attributing the change in variance explained to the marker that was removed. The Area under Receiver Operating Characteristic or AUC (Witten and Eibe 2005) was incorporate to the algorithm as a general measure of classification performance. It was used to evaluate QTL profiles for simulated data where the QTL positions are known (Bedo et al. 2008).

5.4.3 Comparation Among QTL Analysis Methods

Using the real data set (94 DH) QTLs for nine traits were mapped using SML, MR and CIM (Bedo et al. 2008). The size model of SML was evaluated for each trait. As expected, the simplest characters need few markers in the model to explain the most of the variance. The large numbers suggest a complex genetic basis. Pubescent leaves and plant high could be modeled with one and seven markers, showing the low genetic complexity. However, 100 markers were necessary in diastatic power model, while 400 were required for α-amylase. Accuracy of genetic complexity was also studied by BIM and SML. The median difference in the number of detected QTL for SML was zero, with low variance, demonstrates that the genetic complexity could be detected more exactly by SML. The genome profile of the QTL effects was also compared. SML and CIM showed the most correlated results above them (Pearson's coefficient correlation: $\rho = 0{:}8$), a very high value considering that SML uses marker information only, while the CIM requires the construction of a genetic map and additional information to the genotype of markers for genetic interval (markers as cofactors). At significance level of $P < 0.05$, SML reported less than half that obtained with MR and CIM. This result shows the power of the bootstrap validation step, eliminating those spurious QTL, those that cannot be cross-validated by a second method. By contrast, 95% of the QTL detected by SML also were detected for MR and CIM, showing that the SML was more robust.

When the synthetic data set was used, MR method performs worse than SML and BIM with a low median and large variance. BIM achieved a high median, but a large variance than SML. As the difference between the means of the BIM and SML was not significance, the authors conclude that both methods are similar respect of to locating QTL. However, SML provides better estimates of QTL strength (variance explained) that BIM, perhaps due to the lack of the bootstrapping step in BIM, re sampled will account for the upward bias of BIM (Bedo et al. 2008).

The precision with which a QTL can be mapped is an important aspect in the context of marker-assisted selection (MAS) and gene cloning in particular. SML generated the narrower peaks and better and more consistently defined the QTL signal than MR and CIM. The resolution of QTL was evaluated in (Bedo et al. 2008) on chromosome 7H in a region where previously the gene Amy2 was mapped, this gene encodes an α−amylase. SML showed two peaks of QTL, one locus to 4.6 cM from Amy2 locus suggesting the existence of two different loci. These results are consistent with fine-mapping study performed in this region where recombinants between Amy2 and the QTL were identified (Han et al. 1997). Conventional mapping methods have low accuracy if the coefficient of determination is low (Kearsey and Farquhar 1998). The CIM typically generates large regions of QTL that can be narrow using closer markers to control genetic background. This approach, however, reduces the statistical power of CIM (Zeng 1993) and QTL detection depends on an "ad hoc" test in the study region. BIM provides a similar degree of resolution to SML but overestimates the effects of QTL, and its location could not be validated by other methods. The models generated by SML are less biased and have greater power to define because recursive feature elimination.

Markers are evaluated in the context of other markers. If multiple markers contain a similar level of information, then those that explain lower proportion of the variance are eliminated. The remaining markers will still explain most of the variance, as consequence a well-defined QTL peaks is obtained (Bedo et al. 2008).

The SML algorithm was also evaluated for their sensitivity to accuracy of the marker record and order on genetic linkage (Bedo et al. 2008). Two genetic maps were used to compare methods (MR, SML and CIM). One of them with 0.4% potential genotyping errors (97% call rate) and other re-optimized version of the map with potential genotyping errors removed (99.6% call rate). QTL profiles obtained from the maps were highly correlated using MR, lower correlation value was obtained for SML and lower still for CIM. Although a highly correlated profile was obtained with MR, only 67% of the mapped QTL were consistent between the two maps. Of those identified with the CIM, the percentage was even lower (64%). The SML detected less than half of the QTL that CIM or MR, as a result of this validation step by bootstrapping. However, 81% of the QTL were mapped in both maps. Although SML can operate without the construction of a genetic map, errors in the order of the markers affects the performance of SML, but only marginally in the final process of plotting of variance explained *vs* genome position. Map curation affected the power of QTL detection and the estimated QTL effects. In the case of SML, the variance explained by QTL was consistent between the two maps. CIM was less consistent; map curation reduced the explanatory power of the most important QTL on chromosome 7 from 25% to 10% of the variance explained. These data show that the SML is more robust to genotyping and linkage errors than both MR and CIM. SML is a quite different strategy to map QTL from that already published. As stated before, to apply SML is not necessary to construct a genetic map which sometime is a laborious task. However, it can help researcher to get a better interpretation, especially genome location. Other important advantage of SML is that produces narrower peaks than MR and CIM. Similar precision was obtained by BIM but it underestimated the genetic complexity. SML also provides better estimation of variance explained and other QTL parameters (Bedo et al. 2008).

To the best of our knowledge this is the only report on ML and QTL mapping in plant breeding. Wang et al. (2010) propose address the QTL mapping problem using a semiparametric regression model and a collaborative targeted maximum likelihood estimator (C-TMLE). However, the method was evaluated in mice data (Wang et al. 2010).

5.5 Final Considerations

The need to accelerate breeding for increased yield potential and better adaptation to drought and other abiotic stresses is an issue of increasing urgency (Araus et al. 2008).

The pressure of both climate change and population growth demands for the development and/or the application of new strategies in order to accelerate the process of maize breeding, especially in stress environments. Machine Learning (ML) is an emerging discipline with a revolutionary impact on a variety of areas. Due to their high generalization capabilities and distribution-free properties they are presented as a valuable alternative that can overcome limitations of current statistical approaches in maize breeding.

ML algorithms have been reported in several maize applications, i.e., phenotypic prediction, heterotic group assignment and QTL mapping, with a relatively high level of success; sufficient to generate rapid gains in selection cycles. However, literature is still limited and further research is needed.

Although the emergence new molecular techniques such as transcriptomics and proteomics propose a revolutionary impact in analytical breeding, DNA marker technology is still advantageous regarding cost/benefit and a potential partner for this recently introduced discipline. Moreover, there is a huge literature regarding maize molecular breeding, and their associated databases (Baenziger et al. 2009). Therefore, it might be worth to analyze all of this information under the light of this new paradigm.

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Appendix: Support Vector Machines

The SVM algorithm is based on the statistical learning theory and the Vapnik-Chervonenkis (VC) dimension introduced by Vladimir Vapnik and Alexey Chervonenkis (Cortes and Vapnik 1995). VC theory characterizes properties of learning machines which enable them to generalize well to unseen data (Smola and Schölkopf 2004). Its formulation embodies the Structural Risk Minimization principle; it minimizes an upper bound on the generalization error, as opposed to ERM which minimizes the error on the complete training data (Gunn 1998). Due to this industrial context, SVM research was orientated towards real-world applications. Initial work focused on OCR (optical character recognition). Excellent performances were also obtained in regression and time series prediction applications (Smola and Schölkopf 2004).

With N training cases, p predictors, and m support vectors (See below) a support vector machine requires $m^3 + mN + mpN$ operations, assuming $m \approx N$ (Hastie et al. 2009). Several computational shortcuts are available. Extensive explanations on SVMs can be found in Burges (1998), Gunn (1998) or Hastie et al. (2009).

Support Vector Machines Classification

The underlying idea is to calculate a maximal margin hyperplane (the decision function) separating two classes of the data (Smola and Schölkopf 2004), such decision function is fully specified by a usually small subset of the data (the support vectors) which defines the position of the separator (Fig. 5.2a). New samples are classified according to the side of the hyperplane they belong to (Burges 1998; Gunn 1998).

In the case of non separable data, the "ideal boundary" must be adapted to tolerate errors for some object i:

$$minimize \frac{1}{2} \| \mathrm{w} \|^2 + C \sum_{\mathrm{i}=1}^{n} \zeta \,\mathrm{i} \tag{5.5}$$

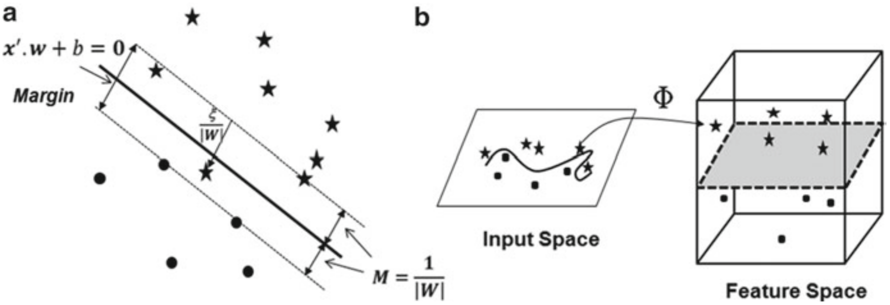

Fig. 5.2 Support vector classifiers. (**a**) A non ideal (separable) case. The decision boundary is the solid line. (**b**) Rather than fitting nonlinear curves to the data, training data can be mapped from the instance space to feature space where a hyperplane can be used to do the separation

under the constraints $\zeta i \geq 0$ and $\zeta i + yi\ (w \cdot x_i + b) - 1 \geq 0$ w and b are respectively the normal vector and the bias of the hyperplane, and each ζi corresponds to the distance between the object *i* and the corresponding margin hyperplane (Gunn 1998).

The parameter C is a regularization meta-parameter, when *C* is small, margin maximization is emphasized whereas when *C* is large, the error minimization is predominant (Cortes and Vapnik 1995).

To learn non-linearly separable functions, data are implicitly mapped to a higher dimensional space by means of mercer kernels (Fig. 5.2b). They can be decomposed into a dot product, $K(x, y) = \phi(x) \cdot \phi(y)$ (Burgues 1998). The simplest kernel, the linear kernel can be defined as: $K(x,y) = \phi(x)$. Other commonly used kernels are the Gaussian (Radial Basis Function) kernel:

$$K(\mathrm{x},\mathrm{y}) = exp(-\gamma \parallel \mathrm{x} - \mathrm{y} \parallel^2); \quad \gamma > 0\left(\gamma = \frac{1}{2\sigma}\right) \tag{5.6}$$

and the dth-degree polynomial (inhomogeneous) kernel, defined as:

$$K(\mathrm{x},\mathrm{y}) = (1 + \mathrm{x} \cdot y)^d \tag{5.7}$$

Support Vector Machines Regression

SVMs can also be applied to regression problems by the introduction of an alternative loss function (Smola and Schölkopf 2004), which must be modified to include a distance measure (Fig. 5.3). In ε-SVR the goal is to find a function $f(\mathbf{x})$ that deviates at most ε from the real value *y*. Initially one can restrict the possible set of solutions to linear functions like:

$$f(\mathrm{x}) = \langle \mathbf{w}, \mathbf{x}_1 \rangle + \mathrm{b}, \mathrm{w} \in \mathrm{R}^{\mathrm{h}} \text{ and } \mathrm{b} \in \mathrm{R} \tag{5.8}$$

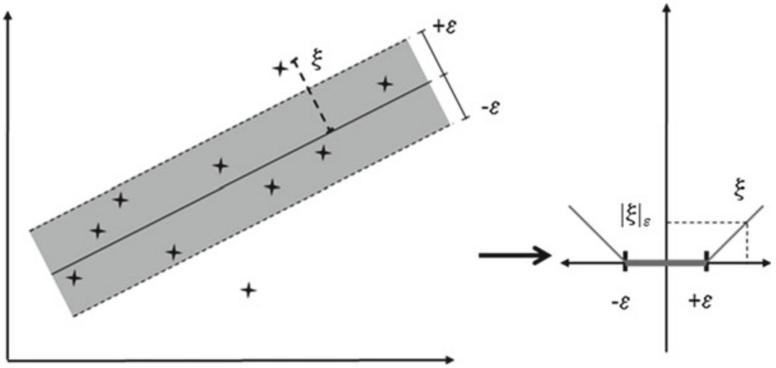

Fig. 5.3 ε-SVR insensitivity tube of width 2ε for a one-dimensional linear function *f* and its corresponding loss function

The norm of the weight vector w should be as small as possible; this generates simple (flat) solutions which avoid overfitting the training data. Figure 5.3 depicts a regression problem for which no linear solution exists for the given width ε of the insensitivity tube. Each training sample *i* is therefore allowed to have slack variables ξ_i and ξ_i^* which allow under or overestimation of training examples outside the insensitivity tube ε. These training errors should be minimized together with the euclidean norm of the weight vector **w** which allows for the formulation (Smola and Schölkopf 2004):

$$minimize \frac{1}{2} \|\mathbf{w}\|^2 + C\sum_{i=1}^{n} \xi_i \tag{5.9}$$

$$subject\ to \begin{cases} y_i - \langle \mathbf{w}, \mathbf{x}_1 \rangle - b \le \varepsilon + \xi_i \\ \langle \mathbf{w}, \mathbf{x}_1 \rangle - b - y_i \le \varepsilon + \xi_i^* \\ \xi_i, \xi_i^* \ge 0 \end{cases} \tag{5.10}$$

The constant *C* determines the trade-off between the flatness of *f* and the extent to which deviations larger than ε are tolerated. ε>0 is a predefined constant which controls the noise tolerance. This is the ε -insensitive error measure, ignoring errors smaller than ε (Hastie et al. 2009). There are other loss functions: conventional least squares error, laplacian loss function, etc. (Gunn 1998). Best values of both *C* and ε can be determined by means of a simple grid search in combination with some crossvalidation routine or more elaborate strategies like gradient descent methods (Maenhout et al. 2007; 2010).

One can create non-linear functions *f* mapping the training samples into a higher dimensional space named the feature space F(ϕ: X→F) and solving the linear regression there (Hastie et al. 2009; Smola and Schölkopf 2004). As in classification, to make this approach computationally feasible, one can use a symmetric kernel function K(x, y) = ⟨ϕ(x),ϕ(y)⟩ that gives directly a dot product in feature space (Gunn 1998; Smola and Schölkopf 2004).

Not all symmetric functions over $X \times X$ are kernels that can be used in SVMs (Gunn 1998; Smola and Schölkopf 2004). Since a kernel function K is related to an inner product it has to satisfy some conditions that arise naturally from the definition of an inner product and that are given by Mercers theorem: the kernel function has to be positive semi-definite (PSD)(Gunn 1998; Smola and Schölkopf 2004).

Most kernels like Gaussian (Eq. 5.6) or polynomial (Eq. 5.7) kernels require the knowledge of one or several additional kernel parameters (Maenhout et al. 2007). The use of context specific kernel functions can avoid the computationally exhausting grid searches needed to identify these parameter values that allow a minimal generalization error (Maenhout et al. 2008). Since dot products in feature space are in fact measures of similarity between cases, the use of PSD genetic similarity measures as kernel functions is a valid option (Maenhout et al. 2007, 2008). Maenhout et al. (2007, 2008) proposed the use of a kernel derived from Jaccard similarity measure when using a dominant marker (AFLP), a kernel derived from the complement of the Modified Rogers distance (Reif et al. 2005) when a codominant marker (SSR) and a weighted sum of the two PSD matrices when using combined data. The functioning of other genetic distances remains to be explored.

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Chapter 6
Heat Stress in Rice – Physiological Mechanisms and Adaptation Strategies

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Abstract Heat stress is a present day hot topic in the world as it throws great challenges before the scientific world by adversely affecting the crop plants and their yield, the need for resilience in all aspects of the crop and resilient varietal identification and improvement are the need of the hour. Here in this review, plant responses to heat stress morpho-anatomical and biochemical changes along the phenology were reported. Importance of physiological parameters in identifying heat tolerant varieties is a necessary prerequisite and is reliable and superior to all the screening procedures. The importance of the photorespiration and its role in final yield loss, as it has interwoven metabolic links with carbon and nitrogen metabolisms are specially focussed on evolutionary aspects. The changes in the hormonal ratio with phenology and molecular responses to heat stress, mechanism of heat tolerance and genetic improvement for heat-stress tolerance, fertigation role in tolerance is discussed.

6.1 Introduction

Rice is world's second highest produced grain crop, after maize. The major rice producing countries are China, India, Pakistan, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, and Japan. Asian farmers still account for 92% of the world's total rice production. Among cereal crops, rice is a major source of food after wheat for more than 2.7 billion people on daily basis. In view of current levels of growing world population, it is estimated that there will be a need to increase

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food production upto 38% by 2025 and 57% by 2050 to maintain the food supply (Bennet 2001; Naheed et al. 2007). But the changes in the climate bringing some hurdles in achieving the goal.

Owing to anthropogenic, biotic and abiotic factors, emission of Green House Gases (GHGs) [CO_2, CH_3, Chloro Floro Carbons (CFCs) and NO_2] leading to the global warming and allied problems which directly affects the crops on the earth. The chronological release of GHGs and the possible effects on rice crop dynamics were critically reviewed (Solomon 2007; Wahid et al. 2007; Subrahmanyam et al. 2011) respectively. Atmospheric CO_2 is expected to exceed 600 μl 1^{-1} by the twenty-first century and is predicted to increase global mean temperature by 0–8°C. Varietal differences in response to CO_2 and temperature and a significant interactive effect of CO_2 and temperature on development of lowland rices may provide options to minimize adverse effects of future climate changes. The combination of increased CO_2 and increased temperature resulted in a small increase in biomass and yield in the dry season and a small decrease in the wet season. A number of studies have examined the effects of elevated atmospheric CO_2 or combinations of elevated temperature and CO_2 can have marked effects on rice growth and yield. (Imai et al. 1985; Ziska et al. 1996; Kim et al. 2003; Baker 2004; Yang et al. 2006; Sakai et al. 2006). Hence, exists a need to control either emission of “GHGs” or improve our cultivars for the modified conditions. Climate change will bring quality and yield losses to rice growers unless counter-measures are developed. Interactions among plant structure, function and the environment need to be investigated at various phases of plant development at the organism, cellular as well as molecular levels in order to achieve the targeted food production in the coming years. Tolerance to a combination of different stress situations, particularly those that mimic the field environment, should be the focus of future research programmes aimed at developing transgenic crops with enhanced tolerance to naturally occurring environmental conditions.

Rice genotypes have been screened for tolerance to high temperature during flowering (Satake and Yoshida 1978; Matsui et al. 2001; Matsui and Omasa 2002; Prasad et al. 2006a) however; these studies did not explicitly separate tolerance from avoidance. Heat stress is one of the abiotic stresses that limit plant biomass production and productivity, especially in tropical and subtropical countries (Boyer 1982). High temperature is detrimental to both the vegetative and reproductive stages of rice (Pareek et al. 1995). Plant resistance to heat can be subdivided into escape (successful reproduction before the onset of severe stress), avoidance (maintenance of a high plant water status during stress e.g. caused by stomatal closure, trichomatous nature, reduced leaf area, senescence of older leaves, etc. or by increased root growth) and tolerance (the maintenance of plant function at limited water availability and/or the recovery of plant water status and plant function after stress) may involve osmotic adjustments, but may also be the result of rigid cell walls or small cells. Heat/drought tolerance may also be associated with the efficient scavenging of reactive oxygen species (ROS) formed as a consequence of disturbed metabolism (Sairam and Saxena 2000).

Peng et al. (2004) reported that the yield of dry season rice crop decreased by 15% for each 1°C temperature increase in the growing season mean temperature. A correlation study showed a yield decline with higher night temperatures due to global warming from 1979 to 2003 (Peng et al. 2004). Hence, counter measures are to be made as quickly as possible to improve yield safety in crops. The whole developmental process, from grain to grain, needs to be considered and appropriate strategies may target several developmental stages (Triboï and Triboï-Blondel 2002). To increase the varietal yield potential and in order to reduce the yield gap by improving the varietal adaptability and quality improvement to fit the purpose. Increase in yield stability and reduction in cost of cultivation, under these circumstances, rice varietal improvement program has to play a major and definite role with clear objectives and relevant strategies. The strategies should be somaclonal variations, anther culture, *in vitro* fertilization, embryo rescue, protoplast fusion between distantly rice varieties and genetic markers to identify specific genes and gene blocks to accelerate the breeding process.

Today, yield safety has gained more significance because of the forecasted climatic changes, high temperature is chiefly considered as key stress factor with high potential impact on crop yield (Barnabas and Katilinjager Feher 2008). Yield safety can only be improved if future breeding attempts will be based on the valuable new knowledge acquired on the processes determining plant development and its responses to stress. High temperature is often accompanied with low water supply, so the primary aim of breeding must be to develop cultivars tolerating both the types of stresses (Tester and Bacic 2005). However, advanced techniques of molecular breeding and genetic engineering have provided additional tools, which could be employed to develop crops with improved heat tolerance and to combat this universal environmental adversary.

This review attempts to summarize and evaluate the pros and cons of the heat and its allied problems in rice and potential encountering encountering/ adaptation strategies to attain yield safety. Biotechnological tools to mitigate the problems of climate change (heat stress) with special reference to photorespiration and fertilization doses.

6.2 Threshold Temperature

The point of temperature at which the performance of a crop declines is heat stress threshold. As the rice crop is concerned, the optimum temperature is 33˚C (Jagadish et al. 2007). Changes in the global climate, notably in regional spatial and temporal temperature patterns (Houghton et al. 1990) are predicted to have important consequences for crop production (Parry 1990). Higher plants exposed to excess heat, at least 5°C above their optimal growing conditions, exhibit a characteristic set of cellular and metabolic responses required for the plants to survive under the high-temperature conditions (Guy 1999).

6.3 Different Changes along the Phenological Phases

Plant stress responses are very complex. The response to heat stress differs with phenology which include morpho-anatomical and cellular to molecular level (Wahid et al. 2007). Exposure to heat will cause senescence and sterility (Siddique et al. 1999). The response depends on the species and genotype (Rampino et al. 2006), the length and severity of water loss (Araus et al. 2002; Bartels and Souer 2004; Zhu et al. 2005b), the age and stage of development (Zhu et al. 2005a), the organ and cell type (Verdoy et al. 2004; Cominelli et al. 2005; Zhou et al. 2007) and the sub-cellular compartment (Battaglia et al. 2007). High night temperatures (HNT) has recently become a major rice research area. A very narrow critical range of 2–3°C has been shown to result in drastic grain yield reduction in the tropics (Nagarajan et al. 2010) and subtropics (Peng et al. 2004).

6.3.1 Morpho-Anatomical and Phenological Changes

Different phenological stages differ in their sensitivity to high temperature, which can reveal a better understanding of interactions between stress atmosphere and the plant. The phenological stage at which plant undergoes stress will determine the damage severity. High temperature induces sterility, if the sensitive physiological processes (anther dehiscence, pollination, pollen germination on the stigma, pollen tube growth or the early events of fertilization) are affected.

6.3.1.1 Morphological Changes

High temperature can cause considerable pre and post harvest damages, including scorching of leaves, sunburns on leaves, branches and stems, leaf senescence shoot and root growth inhibition, reduced TDM and grain filling. Both plant growth and developments are affected by temperature (Porter and Moot 1998). Different stresses might require conflicting or antagonistic responses. It is quite often observed that during the heat stress, plants open their stomata to cool their leaves by transpiration. However, if heat stress is combined with drought the plants are unable to open their stomata, as a result leaf temperature remains higher (Rizhsky et al. 2002). Many hydrophilic globular proteins accumulate in seeds during the maturation phase, when the seeds are developing desiccation tolerance. The plant responses to high temperature vary with plant species/varieties and phenological stages. Reproductive processes are remarkably affected by high temperatures, which ultimately affect fertilization and post fertilization process leading to meagre crop yield. Satake and Yoshida, (1978) reported that the spikelets at anthesis that were exposed to more than 35°C temperatures about 5 days during the flowering period were sterile and set no seed. At high temperature the anther dehiscence production of pollen is very poor, hence low numbers of germinating pollen grains on the stigma (Matsui et al. 2000, 2001; Prasad et al. 2006). There is genotypic variation in spikelet sterility at high temperature (Satake and Yoshida 1978; Matsui et al. 2001; Prasad et al. 2006) that can be defined by different temperature thresholds (Matthews et al. 1995; Nakagawa et al. 2002). Responses to high temperature spikelet sterility increases in response to daily maximum temperature (Matthews et al. 1995; Horie et al. 1996; Nakagawa et al. 2002). Previous reports have shown that the high air temperatures can reduce grain yield even under CO_2 enrichment (Matsui et al. 1997a; Prasad et al. 2006) increased temperature induced spikelet sterility (Satake and Yoshida 1978; Kim et al. 1996; Matsui et al. 1997b; Ohe et al. 2007; Jagadish et al. 2007). Transpiration was more influenced than photosynthesis at the beginning of drying period. Irrigation induced a rapid resumption of transpiration and photosynthesis, simultaneously with the leaf unrolling and progressive stomatal opening.

6.3.1.2 Anatomical Changes

During heat stress generally cell number as well as cell size reduces, reduction in stomatal aperture size and xylem vessels in roots and shoots, enhanced density of trichomes on both the surfaces of the leaf are some of the common adaptive features. (Anon et al. 2004). Increased plasma membrane permeability, disturbed thylakoids, empty mitochondria and spoiled PS-II were also observed (Zhang et al. 2005).

6.3.1.3 Biochemical Changes

The increase in CO_2 concentration stimulates increase in RuBisCO activity as a consequence photorespiration is reduced. Hence, increasing temperature could result in higher net photosynthesis and CO_2 uptake (Potvin 1994). In high temperature, the carbohydrate availability for developing wheat florets as it is a major factor in the grain number was reported (Mishra and Mohapatra 1987; Abbate et al. 1995; Demotes-Mainard and Jeuffroy 2004). Although grain crops show sensitivity to drought during floral initiation and the pre-meiotic differentiation of floral parts (Barlow et al. 1977; Winkel et al. 1997), the most remarkable effects on yield have been recorded when stress coincided (Westgate and Thomson Grant 1989; Saini 1997). When carbon assimilation during stem elongation is reduced by drought stress, the storage capacity in the stems also decrease. Disturbances in the carbohydrate metabolism of the ovary because of inhibited photosynthate influx during early development can be presumed to be responsible for these developmental anomalies in maize (Zinselmeier et al. 1995; Boyer and Westgate 2004).

6.3.2 *Physiological Responses*

6.3.2.1 Water Relations

Consequence of short term heat stress results in stomatal closure followed by leaf rolling. This helps the plants to avoid heat stress for a shorter duration. Also, heat flux is being regulated at the leaf surface by the trichomes. The long term influence of he stress needs regulation of transpiration. Stomatal regulation of transpiration is one of the strategies used by the plant to delay the occurrence of plant water stress due to soil water shortage. The reduction in transpiration occurs to the detriment of the photosynthesis since the stomata are the common path way for water vapor and CO_2 exchange. For rice few studies deal with the incidence of water stress on gaseous exchange with atmosphere; this is due to the aquatic mode of rice cultivation (Yoshida and Shioya 1976). But in Western Africa, rice is cultivated on non-saturated soil (rainwater cultivation). Rice is susceptible to water stress and estimated loss due to drought is 50% of the world rice production (Bouman et al. 2005; Tao et al. 2008; Yang et al. 2008). Drought severely impacts on yield at reproductive stage of the rice plant (Wang et al. 2005). It is not easy to oppose the combined effect of drought and heat on more sensitive stages like reproduction as these two quite often go hand-in-hand. Drought stress was found to reduce dry weight, root traits, water potential, photosynthetic parameters, basal quantum yield (Chen et al. 2010).

High temperature results in water loss in plants more at daytime. Water loss, if perceived by the cell, may trigger several cellular signal transduction pathways. A transmembrane histidine kinase and its associated proteins form an osmosensor which senses the water deficiency in *Arabidopsis* (Urao et al. 1999, 2001). However, there are unknown mechanisms by which plant cells sense water deficiency (Chaves et al. 2003). Water stress up regulates the phosphorilation/dephosphorylation with the help of kinases or phosphatases (Bray 2002; Kaur and Gupta 2005; Mishra et al. 2006). In *Leymus chinensis* a perennial grass, it was suggested that high temperature, combined with severe soil drought, might reduce the function of PS-II, weaken nitrogen anabolism, strengthen protein catabolism and provoke lipid peroxidation (Xu and Zhou 2006). Though, ABA and antioxidants synthesize and cellular structures spoil in drought, it has a less drastic effect on the structure and functioning of the female sexual generation than on the male gametophyte, it may still cause severe yield losses when associated with high temperature (Saini et al. 1983; Maestri et al. 2002; Weis and Berry 1988). Heat stress as well as limited water availability can significantly impair photosynthesis (Harding et al. 1990a, b; Sharkey 2005; Subrahmanyam et al. 2006), reducing the amount of assimilates available to the grain.

Water – High temperature – Grain filling: In the chloroplasts, heat reduces the photochemical efficiency of photosystem-II (PS-II), the most heat-sensitive component in photosynthesis (Al-Khatib and Paulsen 1999). Temperatures higher than 35°C significantly decrease the activity of ribulose 1-5-bisphosphate carboxylase

(RuBisCO), results in the reduced photosynthesis (Crafts-Brandner and Law 2000; Griffin et al. 2004). Water deficiency disturbs photosynthetic processes in vegetative plant tissues, particularly in leaves, resulting in a reduction in the water-soluble carbohydrate level in the anthers and in the expression of the gene responsible for the synthesis of the acidic invertase enzyme (Saini 1997). At the time of grain filling water stress reduces photosynthesis, induces early senescence and shortens the grain-filling period, but increases the remobilization of assimilates from the straw to the grains (Blum 1998; 2005; Gebbing and Schnyder 1999; Plaut et al. 2004; Yang and Zhang 2006). However, under controlled soil drying, when plants could sufficiently rehydrate during the night, enhanced whole-plant senescence and faster and better stem reserve remobilization could be observed (Yang and Zhang 2006). The current photosynthetic assimilates and reserve carbohydrates of vegetative tissues in leaves, stem and panicles play main role in grain-filling of cereals (Plaut et al. 2004; Yang and Zhang 2006). Stem reserve mobilization is an important process supporting grain filling under heat stress conditions (Blum et al. 1994). Therefore, under stress conditions, stored carbohydrates may become the predominant source contributing to as much as 75–100% to the grain yield (van Herwaarden et al. 1998). The mobilization of total non-structural carbohydrates from the stem was enhanced at high temperatures during wheat grain filling (Plaut et al. 2004; Tahir and Nakata 2005). In contrast, the limited assimilate supply to the grain was suggested to be the main factor limiting grain weight under high-temperature stress in rice (Kobata and Uemuki 2004). Exogenous ABA remobilizes the pre-stored carbon from the leaves and stems, far superior to cytokinins (Ahmadi and Baker 1999; Yang et al. 2003a). High ABA concentrations (0.1 M), however, reduced the transport of sucrose into the grains and lowered the starch synthesis ability of intact grains, resulting in low grain weight (Ahmadi and Baker 1999). On the other hand, under certain circumstances, limited water availability may promote nutrient remobilization from the leaves and stems and increase the rate of grain filling (Yang and Zhang 2006), thus compensating for its shortened duration. The activation of the key enzymes of sucrose-to starch conversion resulted in increased sink activity, finally leading to an increased grain-filling rate under these specific conditions (Yang and Zhang 2006). High temperature induced net assimilation rate as a result of reduced grain filling, delaying senescence and increased dry weight (Egeh 1991).

6.3.2.2 Accumulation of Compatible Osmolytes

Either light or heat or both together can generate oxidative stress by accumulating the reactive oxygen species. At the photosynthetic apparatus, heat stress generates ROS, which could damage both PS-I and PS-II. The destructive effect of ROS might be alleviated by antioxidant enzymes and the sequestration of other components in plant cells. Under stress, different plant species may accumulate a variety of osmolytes such as sugars and sugar alcohols (polyols), proline, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds (Sairam and Tyagi 2004).

γ-4-aminobutyric acid (GABA) GABA is a non protein amino acid, synthesized from the glutamic acid as a result of glutamate decarboxylase (GAD) catalytic activity. Episodes of high temperature increase the cytosolic level of Ca, which leads to calmodulin mediated activation of GAD (Taiz and Zeiger 2006). The conventional breeding, marker assisted selection (MAS) and genetic engineering (GE) techniques focus to increase the accumulation rate of osmolytes in stressed plants are in need.

6.3.2.3 Photosynthesis

In thermally contrasting climates, plants generally exhibit photosynthetic temperature responses that reflect an adaptation to the temperature regime of their respective habits. Field studies on several mosses indicate that the optimum (80%) temperatures (5°C–20°C) for photosynthesis are somewhat lower than those obtained with higher plants (Oechel 1976; Ogren and Hunt 1978). Categorically thermophillic photosynthetic characteristics exhibited by the hot desert C_4 species which represent the opposite extreme among vascular plants. Some authors attempted on different species to note the temperature acclimations ex., *Amaranthus edulis* (El-Sharkawy et al. 1968), *Atriplex lentiformis* (Pearcy 1977). *Hammada scoparia* (Lange et al. 1974) and many tropical origin C_4 grasses (El-Sharkawy and Hesketh 1964), it is not yet clear what happens exactly to rate of photosynthesis above 40°C.

Effect of Temperature on CO_2 Photosynthesis: As the leaf temperature increases the CO_2 uptake increases up to (25°C) in C_4 plants, as the leaf temperature increases, the CO_2 uptake increases upto 45°C (Bjorkman et al. 1975). A theoretical analysis of the effect of day temperature on photosynthesis indicated that the enhancement of photosynthesis due to elevated CO_2 is larger at higher temperature (Long 1991; Morison and Lawlor 1999). Berry and Bjorkman, (1980) tried to find out the photosynthetic response and adaptation to temperature in higher plants.

What happens with photosynthetic apparatus: Chlorophyll fluorescence, the ratio of variable fluorescence to maximum fluorescence (F_v/F_m) and the base fluorescence (F_0) are physiological parameters that have been shown to correlate with heat tolerance (Yamada et al. 1996). Degradation of chlorophyll and photosynthetic apparatus were suggested to be associated with the production of active oxygen species (Camejo et al. 2006; Guo et al. 2006). PS-II is highly thermolabile and its activity is greatly reduced or even partially stopped under high temperatures (Bukhov et al. 1999). Heat stress may lead to the dissociation of oxygen evolving complex (OEC) resulting in an imbalance between the electron flow from OEC toward the acceptor side of PS-II in the direction of PS-I reaction centre (De Ronde et al. 2004). This implied that the degradation of the impaired PS-II units occurred in the light during this period of time. Following this, *de novo* synthesis of PS-II units in the light gave a gradual rise to the observed PS-II activities. On the other hand, under high temperature, PS-I stromal enzymes and chloroplast envelops are thermostable and in fact PS-I driven cycle electron pathway, capable of contributing to thylakoids proton gradient, is activated (Bukhov et al. 1999), these results stands in marked contrast to the inferences made from results of previous studies (Pearcy 1978; Raison et al. 1982), in which the heat stability of the photosynthetic machinery increased in parallel with the saturation of fatty acids in the thylakoid membrane lipids. However, because such changes in the composition of membrane lipids in these studies were achieved by altering the growth temperature, which might also affect the levels of other cellular metabolites and enzymes, the proposal of a direct link between the changes in lipids and the thermal stability of photosynthetic activities seems unjustified.

Photosynthetic temperature acclimatization: The temperature dependence of photosynthesis for a given plant as observed in its natural habitat is the result of a complex interaction between the prevailing environment and the characteristics inherent in the species or genotypes. Many investigations have been understood to determine the relative importance of genotypic and environmental factors in photosynthetic temperature adaptations for this review we shall use the expression photosynthetic acclimation to denote environmentally induced changes in photosynthetic characteristics that result in an improved performance under the new growth regime. The genetically determined ability to so acclimate will be termed acclimation potential. There were little seasonal changes in the maximum photosynthetic rate. Regardless of seasonal shift in the temperature addiction of photosynthesis, the optimum temperature was considerably higher than the prevailing tissue temperature for most of the time as an explanation for this observation.

Oechel (1976) advanced the hypothesis that "C_3 plants have not evolved to the full range of thermal regimes in which they are found" as this statement may well be true in many cases, especially in relation to hot arid habitats, maximum photosynthetic effectiveness is not necessarily obtained at the temperature optimum but rather below it. Altitudinal gradients will act harmfully on the photosynthetic temperature adaptation through seasons, as the elevation increases, the optimum temperature declined (Slatyer 1977). The photosynthetic acclimation is a predominant basis of the altitudinal shift in optimum temperature, although, ecotype differences among the populations are also important (Slatyer and Ferrar 1977a, b). The seasonal shifts in temperature optima occurs both in irrigated plants and in plants growing under natural water stress conditions, and cannot be explained in stomatal responses.

Phenotypic plasticity and acclimation potential of different species: The potential for such photosynthetic acclimation to growth temperature is quite variable among species. It is very important to discuss recent advances of the effect of temperature, photosynthetic efficiency of leaves, to evaluate the extent of photosynthetic temperature adaptation in higher plants and to consider the underlying physiological and biochemical mechanisms. Some of the considerable aspects such as the heat exchange between plant canopies and environment, the effect of temperature on photosynthates partitioning and on whole plant productivity as well as heat injury and interactions between the effects temperature and water stress on whole plant photosynthesis are also the major concerns.

In all cases, high growth temperature causes an upward shift in the optimum temperature for light saturated photosynthesis; however, it varies from deserts to coasts. But, a considerably greater acclimation potential is found among evergreen desert shrubs that are subjected to large seasonal temperature variation. The plants have higher photosynthetic rates at low measurement temperatures when grown under a cool regime and higher rates at high measurement temperatures when grown under a hot regime. The rates at the respective optimum temperatures were similar, irrespective of growth temperature. Acclimation to low temperature may be considered to involve an increase in the capacity of temperature limited enzymatic steps of the photosynthetic process, whereas, acclimation to high temperature involves an increased heat stability of the photosynthetic apparatus. Changes in either of the two factors may result in a shift in the optimum temperature. The position in this optimum is also affected by the point at which largely temperature independent reactions (Such as diffusive transport of CO_2 into the leaf) become limiting and the rate of counteracting reactions.

Stomatal effects on the temperature response of photosynthesis: There are some reversible temperature responses, stomata respond to a number of major environmental factors such as temperature, light, humidity and CO_2 concentration, feedback control via photosynthesis may also occurs and internal water status may have profound effects (Cowan 1977; Hall et al. 1976; Raschke 1975; Sheriff 1979). There are a number of reports on the responses of stomata to temperature some indicate that stomata tend to open with increasing temperature (Crookston et al. 1974; Drake et al. 1970; Drake and Salisbury 1972; Hofstra and Hesketh 1969) others indicate that they close at high temperature (Downess 1970; Heath and Meidner 1957; Heath and Orchard 1957; Rees 1961; Wuenscher and Kozlowski 1971). There are also many reports that support the maximal opening of stomata at intermediate temperatures (Hofstra and Hesketh 1969; Losch 1977, 1979; Neilson et al. 1972; Raschke 1970; Whiteman and Koller 1967).

Several studies have concluded that CO_2-induced increases in crop yields are much more believable in warm than in cool season crops. Despite its other potential negative implications, global warming may not greatly affect the overall net photosynthesis (Wahid et al. 2007). This CO_2 induced increase in plant high temperature tolerance may have an ample impact on both the productivity and distribution of many crop species in future.

How grain filling and grain quality gets affected: Grain filling is closely linked to whole-plant senescence and stem reserve utilization. Pre-anthesis assimilate reserves in the stems and sheaths of wheat and rice contribute to 10–40% of the final grain weight (Yang and Zhang 2006). Based on the elegant experiments conducted by Fitzerald (----) it can be concluded that plants growing at 33°C had 14 days grain filling duration compared to plants grown at 26°C (30 days). Because of the shorter duration for grain-filling, there is more immature grain at higher temperatures, with immaturity expressing as chalk. "So overall, rice is a weak perennial with a limited amount of time devoted to grain production and that time is reduced by high temperature."

6.3.2.4 Respiration

For every organism, survival is more important than the closing the life cycle, they respire more to withstand the situation i.e. survival strategy. Increased respiration requires greater carbon fixation for sustained growth and survival if not the increased respiration will contributes for the final yield loss. In the mitochondrial electron transport chain, an NADH: ubiquinone oxidoreductase has been identified as one of the thermolabile components (Downs and Heckathorn, l998).

6.3.2.5 Photorespiration

Photorespiration is a accompanying metabolic process that is essential to allow photosynthesis to occur in O_2 containing environment and it probably co-evolved with oxygenic photosynthesis in cyanobacteria that lived 3.8–2.5 billion years ago in the nearly O_2 free environment of the Precambrian ocean (Eisenhut et al. 2008; Allen and Martin 2007; Canfeld 2005). In due course, heterotrophic protists uptaken the cyanobacterium initiated the evolution of algae and higher plants (Reyes-Prieto et al. 2007). Some workers believe that the predecessors of present day C_4 plants, in which CO_2 is initially fixed by an O_2 insensitive process into four carbon compounds channeled the high photorespiratory CO_2 fluxes to specific leaf cells, the bundle sheath, paving the way for the evolution of C_4 photosynthesis (Sage 2004).

Photorespiration is the second most important process in the biosphere, exceeded only by photosynthesis. Photorespiratory CO_2 losses are respectively high; they amount to about 20% of C_3 plant net photosynthesis in moderate conditions (Cegelski and Schaefer 2006) and can be even higher in increased temperatures. So, photorespiration has been a key target for crop improvement (Peterhansel et al. 2008; Kebeish et al. 2007) as the increasing population and challenges from climate changes are threatening.

In high temperature, we can observe photosynthetic and respiratory imbalances. Generally, the rate of photosynthesis decreases while dark and photorespiration rates increase. The most dramatic effects of photorespiration on leaf gas exchange properties are O_2 inhibition of CO_2 fixation and O_2 stimulation of CO_2 evolution in the light. Hence, for many years photorespiration was considered almost fully in terms of carbon metabolism.

Keys et al. (1984) pointed out, however, that the rate of NH_3 released in photorespiration equals the rate of CO_2 release, and so photorespiration must also be regarded as a major pathway of plant nitrogen metabolism. They also showed through inhibition of glutamine synthetase activity by methionine sulphoximine, that photorespiratory NH_4 is recycled by the sequential action of glutamine synthetase and glutamate synthase and not as had been generally assumed by mitochondrial glutamate dehydrogenase. It was confirmed by the isolation of *Arabidopsis* photorespiration mutants deficient in glutamate synthase activity (Somerwille and Ogren 1980).

Grove et al. (1983) reviewed on photosynthesis and photorespiratory nitrogen metabolism. The requirement for coupled glutamine synthetase-glutamate synthase activity in photorespiration was also evident from the isolation of an *Arabidopsis* mutant with a defective chloroplast dicarboxilic acid transporter (Somerville and Ogren 1980). Thus, this transporter catalyses the entry of 2-oxoglutarate, formed from glutamate in the amination of glyoxylate to serine, into chloroplast. The influx of oxoglutarate is presumably coupled to the efflux glutamate. The isolation of this mutant strain also demonstrated that the primary function of the chloroplast dicarboxilase transporter is to shuffle photorespiration 2-oxyglutarate and glutamate, and indicated that the proposed role of this transporter in shuttling of reducing equivalents between the chloroplast and cytoplasm (Heber 1974) is not a significant function.

At normal air levels of CO_2, the high temperature performance of the C_4 species is decidedly superior over C_3. However, if the C_3 plants grown in high CO_2 concentration, the photosynthetic performance may equal the C_4 plant as if both C_3 and C_4 plants were heat adapted (Moony 1978; Moony et al. 1978; Hell muth 1971).

Higher plant species can be separated into two general categories based on their respiratory responses to illumination. Those species which fix CO_2 primarily by the photosynthetic carbon reduction cycle (Calvin and Bassham 1962). Apparently have very sizable respiratory rates in the light. Photorespiration is much more difficult to detect in the second group which fix CO_2 via the C_4 dicarboxilic acid pathway (Hatch and Slack 1970; Hatch et al. 1967), whereas, Maheswari et al. (1988) reported that higher glutamine synthetase activity in C_3 plants may be necessary adaptation to a higher photorespiratory NH^{4+} production and explain the similar or higher N losses in C_4 as compared to C_3 plants. These species are generally extreme efficient in photosynthesis (Bisalputra et al. 1969; Downess and Hesketh 1968; Downton and Tregunna 1968; Tregunna and Downton 1967). The second group referred as low compensation species (Downton and Tregunna 1968) because of their capacity to deplete the ambient atmosphere to very low CO_2 concentrations. The first group will be referred to as high compensation species.

Enzymes and pathways and notable observations: photorespiration requires eight enzymes and two transporters of the photorespiratory N_2 cycle in the main photorespiration process (Woo et al. 1987; Reumann and Weber 2006) but none involved in the photorespiratory carbon cycle.

In chloroplast: Plants also have a cytosolic PGP (2PG phosphatase), but only deletion of the plastidial enzymes results in a 'photorespiratory phenotype' such mutants die in normal air and require elevated CO_2 for normal growth (Schwarte and Bauwe 2007).

In peroxysomes: Glycolate oxidase is a flavinmononucleotide dependent peroxysomal enzyme that consumes glycolate and molecular O_2 to produce glyoxylate and H_2O_2 in an irreversible reaction. Similar to photorespiratory phenotype observed in GOX antisense rice (*O. sativa*) plants (Xu et al. 2009). GOX deficient maize (*Zea mays*) also requires elevated CO_2 for survival finally providing clear evidence for the long disputed need of an intact photorespiratory metabolism in C_4 (Zelitch et al. 2009). Steps required for the depletion of H_2O_2 will be observed in continued steps.

In mitochondrion: Two molecules of Glycine yields one molecule of serene by the combined action of Glycine decarboxylase (GDC) and serine hydroxyl methyltransferase (SHMT). Particularly, GDC null mutants do not survive even in a non photorespiratory environment (1% CO_2), which is due to essential role of GDC in one carbon metabolism (Engel et al. 2007)…After many sequential steps, the plastidial enzyme glycerate 3-kinase completes the C_2 cycle by returning the 3PGA carrying three out of four 2PG carbon atoms back to the calvin cycle in the chloroplast.

Interacting secondary metabolisms: Requisite intertwining of photorespiration with photosynthesis, N_{2-}assimilation (GS and GOGAT path ways), respiration, one carbon metabolism and associated purine biosynthesis and redox signaling are the main metabolic processes.

Co-evolution of photorespiration and oxygenic photosynthesis: Cyanobacteria were the first prokaryotes that produce reducing equivalents for CO_2 assimilation by the oxidation of water and hence were the first organisms in which RuBisCO was directly exposed to O_2. All this suggests that the 2PG metabolism is essential for the viability of all organisms that perform oxygenic photosynthesis, regardless of whether they are prokaryotes, eukaryotic algae or higher plants.

Challenges with regard to photorespiration: Awareness about participating membrane translocators is meager. The complexity is more as the photorespiration has more connections with different metabolisms need to be addressed. So far, the majority of these studies have been carried out with C_3 land plants with focus at a single molecular level such as transcripts, proteins or metabolites and as such do not provide a fully integrated view of photorespiration.

6.3.2.6 Cell Membrane Thermostability

Fatty acid metabolism related genes may participate in the repair of stress-induced damage in membranes, to regulate permeability to toxic ions and fluidity of the membrane (Torres-Schumann et al. 1992; Holmberg and Bülow 1998). High temperature causes modifications in membrane functions mainly because of the alteration of membrane fluidity. In plant cells, membrane–based processes such as photosynthesis and respiration are especially important. Three commonly used assays of heat tolerance in plants (Blum 1988) are related to the plasmalemma (cell membrane stability or CMS assay), the photosynthetic membranes (chlorophyll fluorescence assay) and the mitochondrial membranes (cell viability assay based on 2, 3, 5-triphenyl-tetrazolium chloride (TTC) reduction).

6.3.2.7 Protein Synthesis

Heat shock proteins are the lion share of the total proteins synthesized during the heat stress. Starch accumulation in grains is the result of complex enzymatic processes, with sucrose synthase (SuSase), AGPase, soluble starch synthase (SSS) and SBE as key players (Morell et al. 2001). In contrast, in the case of controlled soil drying, allowing the recovery of the stressed plants during the night, the activities of SuSy, SSS and granule-bound SBE were substantially enhanced in rice and wheat grains (Yang et al. 2001b, 2003b, 2004b).

6.3.2.8 Hormonal Changes

The plant undergoes a great differential plethora of hormones, that varies from cytokinins to auxins and abscisic acid (ABA) to gibberellins etc., the involvement of phytohormones in the thermotolerance is still unknown. Oxidative damage is one of the consequences of high temperature in plants caused by the heat-induced imbalance of photosynthesis and respiration (Fitter and Hay 1987). Some of the heat-induced processes at the cell, organ and whole-plant levels may be hormone regulated; others may be the consequence of a new hormonal status, altered by heat stress (Hoffmann and Parsons 1991; Maestri et al. 2002). King and Evans, (1977) found the relations among the photoperiod, inhibition of flower induction, increased ABA level. The plant hormones (ABA and cytokinins) play major roles in regulating the link between senescence and assimilate remobilization as observed in both wheat and rice (Ahmadi and Baker 1999; Yang et al. 2001a, 2002, 2003a, 2004a; Yang and Zhang 2006). In rice, water stress substantially increases ABA but reduces the zeatin and zeatin riboside concentrations in the root exudates and leaves (Yang et al. 2002).

Cytokinins: Inflorescence is a modification of shoot, when the plant attains maturity in its vegetative parts, it will tend to flower. In specialized conditions like heat and allied stresses, the ratio of the plant growth regulators (PGRs) greatly varies with the environmental signals.

ABA: ABA induction is an important component of thermotolerance where water shortage and high-temperature stresses frequently occur simultaneously (Gong et al. 1998). Plant responses to drought may rely on several mechanisms that sense water status, turgor, bound water, hormones (e.g. ABA), alteration in cell membranes, etc. (Chaves et al. 2003). ABA is implicated in osmotic stress responses and mediates one of the intracellular dehydration signaling pathways (Davies and Jones 1991). ABA had site specific activity; ABA signal transduction pathway probably comprises a protein kinase/phosphatase cascade interacting with Ca^{2+} (Bray 2002). The rate of photosynthesis and the chlorophyll content of the flag leaf (as parameters of senescence) were negatively correlated with ABA and positively with cytokinin levels. Whereas, the increased concentrations of ABA in the root, induced by soil drying, may favors root growth and increase root hydraulic conductivity. Both of these processes lead to an increase in water uptake and postpone the development of water deficit in the shoot. It causes stomatal closure and reduces leaf expansion, thereby preventing the dehydration of leaf tissues. ABA mobilizes the reserves under drought conditions (Yang et al. 2001a). Xylem-borne ABA reaches reproductive structures and influences their development, presumably by regulating the gene expression that controls cell division and carbohydrate metabolic enzyme activity under drought conditions (Liu et al. 2005). The ABA concentration of the ovary was found to increase substantially as the result of severe, long-lasting stress prior to flowering compared with irrigated maize plants, but this difference disappeared by flowering (Ash et al. 2001). But workers did not exclude the possibility that ABA may play a role in the abortion of female flowers. However, increasing the ABA concentration without lowering the water supply did not induce low grain numbers in wheat (Dembinska et al. 1992), in the case of low water supplies the ABA concentration increased in the developing floral organs of both maize and wheat (Ober et al. 1991; Westgate et al. 1996). Whereas, Yang et al. (2001a) states that, high ABA levels in early reproductive structures caused by environmental stresses may inhibit cell division and impair floret and then seed development.

Gibberellins: The effect of gibberellins on high temperature tolerance is the reverse of that of ABA (Maestri et al. 2002). An inherently heat-resistant dwarf mutant of barley (*Hordeum vulgare*) with impaired gibberellins synthesis was made heat sensitive by the application of exogenous gibberellic acid, whereas the application of a gibberellin-antagonist compound conferred heat tolerance (Vettakkorumakankav et al. 1999). Damage of seed fertility by cooling treatment and endogenous gibberellins in ears of rice plants was observed (Yoshioka and Suge 1996).

6.3.2.9 Oxidative Stress and Antioxidants

Genes encoding plant catalases are expected to play an important role in antioxidant defence in response to environmental and physiological oxidative stress (Scandalios 1990; Iwamoto et al. 1998). Elevated temperatures may reduce the activities of antioxidant enzymes, as observed in maize (Gong et al. 1997). Accordingly, in a set of wheat (*Triticum aestivum*) genotypes, the capacity to acquire thermotolerance was correlated with higher activities of catalase and superoxide dismutase, higher ascorbic acid content and less oxidative damage (Sairam et al. 2000; Almeselmani et al. 2006). Ascorbate and glutathione acts against the active oxygen and keeps under control (Noctor and Foyer 1998).

6.3.2.10 Stress Proteins

Synthesis of stress proteins results in the decrease in the synthesis of normal proteins and the accelerated transcription and translation of heat shock proteins (HSPs) (Bray et al. 2000), Heat stress transcription factors (HSFs) are the terminal components of signal transduction pathways mediating the activation of genes responsive to heat stress.

6.4 Mechanism and Induction of Heat Tolerance

Weerakoon et al. (2008) using a combination of high temperature (32–36°C) with low (60%) and high (85%) RH recorded high spikelet sterility with simultaneous increase in temperature and RH. Hence it can be concluded that the reduction in spikelet temperature in relation to RH is avoidance while the performance of a variety at a given spikelet temperature to be true tolerance.

The higher growth rate of *O. meridionalis* at 45°C compared with *O. sativa* ssp. *japonica*, as well as a lesser impact of heat on photosynthesis, indicated tolerance of *O. meridionalis* to the extreme heat typical of its natural range. RuBisCO activase and the regulation of the large and small isoforms found in rice are a striking aspect of the heat stress response of *O. meridionalis*. The RuBisCO activase large isoform, in particular, is selectively up-regulated in response to heat. Multiple enzymes of the Calvin Cycle increased in abundance with heat. A fall in FNR, an important component of the light reaction, implies a susceptibility of electron transport at 45°C for *O. meridionalis*. The consistent increase in expression of a THI1 homologue at high temperatures was notable because both THI1, an enzyme involved in thiamine biosynthesis, and thiamine have been linked to the heat stress response in plants. Interestingly, thiamine levels fell in heat-stressed *O. meridionalis* even though the abundance of THI1 increased (Scafaro et al. 2010). The increase in the grain-filling rate might be attributed to enhanced enzyme activities and metabolic processes. As a result, the overall temporal program of grain development is accelerated and compressed at high temperatures. The increase in the rate of grain dry matter accumulation may even compensate for the decrease in its duration (Dupont and Altenbach 2003).

Among the different methods to make the crop tolerant, foliar application or pre-sowing seed treatment of low concentrations of inorganic salts, osmoprotectants, signaling molecules (PGRs) and oxidants (e.g., H_2O_2) as well as preconditioning of plants are common approaches. It was observed that the heat acclimated, compared to non-acclimated, turf grass leaves manifested higher thermostability, lower lipid peroxidation product malondialdehyde (MDA) and lower damage to chloroplast upon exposure to heat stress (Xu et al. 2006). The researchers proposed that the higher heat tolerance was due to enhanced glutathione synthase activity, promoting binding of the ammonia accumulated during exposure to high temperature.

The role of Ca^{2+} is crucial in attaining the tolerance; it takes care of antioxidant activity and not for osmotic adjustment (Jiang and Haung 2001). Under heat stress, Ca^{2+} requirement for growth is high to mitigate adverse effects of the stress (Kleinhenz and Palta 2002). Application of Ca^{2+} in the form of $CaCl_2$ prior to the stress treatment elevated the content of lipid peroxidation product, MDA and stimulates the activities of guaiacol peroxidase, SOD and catalase, which could be the reasons for the induction of heat tolerance (Kolupaev et al. 2005).

6.5 Breeding Strategies

Breeding rice varieties tolerant to high temperature has so far received little attention as compared to other abiotic stresses like drought and salinity. The breeding process is very complex and involves several steps like genetic donor identification, hybridization and recombination, phenotypic or MAS of desired genotypes from segregating population, preliminary evaluation, multi environment testing, on-farm trials, varietal release procedures and promotion of approved cultivars.

Due to conventional selection and breeding methodologies to obtain high agro-economical important traits, mankind ignored select adapted traits like drought and heat stress tolerance. Apart from this the narrow gene pool (10–20%) of domesticated rice (*Oryza sativa* L.) has very little scope to cultivate in the hotter climatic zones when compared to its wild progenitors (Zhu et al. 2007). Hence, cross with its wild relatives which possess a diverse array of stress-related genes definitely opens the possibility of rapid genetic improvement through breeding and transgenics. The overall potential and drawback of modern genetic and genomic approaches in cereal improvement and in deciphering the regulatory mechanisms of abiotic stress tolerance in plants have recently been thoroughly reviewed (Snape et al. 2005; Langridge et al. 2006; Varshney et al. 2006; Sreenivasulu et al. 2007). Lafitte et al. (2006) Improvement of rice drought tolerance through backcross breeding: evaluation of donors and selection in drought nurseries.

6.5.1 Modern Approaches – Yield Safety Under High Temperature

Recent advances in plant biotechnology and use of molecular marker have contributed significantly better understanding of the genetic basis of heat tolerance and accelerated the precession of rice breeding. Genetic studies on heat stress in crop plants revealed that, the heat tolerance is multigenic trait and controlled by different sets of genes, at different stages of plant development and in different tissues (Howarth 2005; Bohnert et al. 2006).

To dissect out the genetic basis of the heat stress tolerance at differential stages of development in rice, recent researchers used DH, RIL and BIL population derived from the cross between susceptible and tolerant verities of rice, of these most of the population developed by using indica/japonica or indica/indica crosses (Zhang et al. 2008; Chen et al. 2008). Genetic research of heat tolerance in rice using molecular marker was first initiated by Cao et al. (2002), further experiments to identify QTLs on heat stress was extended several others (Cao et al. 2003; Zhu et al. 2005a, b; Zhao et al. 2006; Zhang et al. 2008; Chen et al. 2008; Wang et al. 2011). Most of the QTLs associated with heat stress tolerance identified during flowering stage of development. Heat and its associated traits like yield, stay green and floral sterility QTLs in rice were mapped on all 12 linkage groups and mainly on the chromosome 1, 4, 7 and 10 (Cao et al. 2002, 2003; Zhu et al. 2005a, b; Zhao et al. 2006; Zhang et al. 2008, 2009; Chen et al. 2008; Jagadish et al. 2010; Wang et al. 2011).

Many of the traits determining abiotic stress tolerance and the quality and quantity of yield are controlled by a large number of genes, which have both major and minor individual effects. The studies combined with genetic approaches to identify and map genes (or QTLs) conferring thermo tolerance will not only facilitate marker – assisted breeding for heat tolerance but also pave the way for cloning and characterization of underlying genetic factors which could be useful for engineering plants with improved heat tolerance (Wahid et al. 2007).

Pyramiding of poly genes or quantitative trait loci responsible for yield by marker aided selection using molecular techniques is the only strategy identified at present. This process may lead to small incremental yield improvement but not quantum jumps in the yield potential. Hence, only a long term gradual improvement in yield potential can be anticipated.

6.5.2 Non Conventional Breeding Strategies

Hidden diversity for abiotic and biotic stress tolerances in the primary gene pool of rice revealed by a large backcross breeding program (Ali et al. 2006). Brar and Khush (1997), focused mainly on the alien introgression in rice. Genome-wide transcriptome analysis has identified hundreds of genes encoding transcription factors that are induced or repressed by environmental stresses (Chen and Zhu 2004). Chen et al. (2002) identified two groups (biotic and abiotic) of transcription factors. Experiments using micro-array technology have identified several genes that are induced by abiotic stresses, including drought, and these genes have been classified into two major groups. The heat tolerance of plants is a complex trait, most probably controlled by multiple genes. The comparative molecular biological analysis of heat-sensitive and heat tolerant genotypes of rice varieties may give the heat-tolerant genotypes responded to stress by increasing the expression of genes participating in photosynthesis, protein synthesis and the preservation of cell status and of those related to transcription factors.

6.5.3 Genetic Manipulations for Heat Tolerance

Genetic improvement entails development of cultivars which can tolerate environmental stresses and produce economic yield. Rice has the smallest cereal genome consisting of just 430 Mb across 12 chromosomes. In the past, the proteomic analysis was used as an alternative technique to determine the molecular responses of rice (Salekdeh et al. 2002; Komatsu et al. 2003; Komatsu and Tanaka 2004; Lee et al. 2007). Genome plasticity in plants, including genetic (e.g., directed mutation) and epigenetic (e.g., methylation, chromatin remodeling, histone acetylation) changes, allows long-term adaptation to environmental changes/conditions (Joyce et al. 2003). However, genetic improvement of plants for stress tolerance can be an economically viable solution for crop production under stressful environments (Blum 1988). The information regarding the production of heat tolerance rice using transgenic approach is found to be scarce, though the use of traditional plant breeding protocols and contemporary molecular biological techniques, including molecular marker technology and genetic transformation, have resulted in genetic characterization and/or development of plants with improved heat tolerance.

Several genes are involving in synthesis of heat stress proteins (HSPs) are switching on when plants exposed to either abrupt or gradual increase in temperature

(Nakamoto and Hiyama 1999; Schoffl et al. 1999; Liu et al. 2006; Sun et al. 2006; Momcilovic and Ristic 2007) and play pivotal role in plant recovery from heat stress (Ristic and Cass 1992). Genetic engineering of heat shock factors (HSF) and antisense strategies are instrumental to the understanding of both the functional roles of HSPs and the regulation of HSFs. Manipulations of the HS-response in transgenic plants have the potential to improve common abiotic stress tolerance and this may have a significant impact on the exploitation of the inherent genetic potential of agronomically important plants like rice. The increased acquired thermo tolerance of transgenic lines is attributed to the higher levels of HSP chaperones. Experimental data obtained from transgenic, reverse-genetics and mutation approaches in cereal and non-cereal species confirm causal involvement of HSPs in thermo tolerance in plants (Queitsch et al. 2000).

Transformation technology for improving plant stress tolerance is limited and just beginning. In rice, Agarwal et al. (2003) introduced *Arabidopsis thaliana* hsp101 (Athsp101) cDNA into the *Pusa basmati* 1 cultivar of rice (*Oryza sativa* L.) by *Agrobacterium* mediated transformation. In this experiment, they compared the survival of T2 transgenic lines after exposure to different levels of high temperature stress with the untransformed control plants. The transgenic rice lines showed significantly better growth performance in the recovery phase following the stress. In this experiment they observed thermo tolerance appeared to acquire solely due to over-expression of high molecular weight heat shock proteins (Hsp101). A proteomic analysis of heat responsive proteins in rice leaves was done by Lee et al. (2007) which can lead to a better understanding of the molecular basis of heat-sensitivity in rice plants.

6.6 Fertigation – A Novel Strategy

Development of varieties with a high level of fertilizer use efficiency has already been initiated focusing on root type development rather than above ground plant type. Emphasis would be placed on developing varieties that can produce comparatively a bigger and longer root mass with thick strong roots having the ability to penetrate deep into the soil. This will not only improve fertilizer use efficiency but also the tolerance to heat that may in turn improve yield stability.

Yadav et al. (2000) reported, Rice yield trends in the treatments receiving complete doses of fertiliser NPK were generally not significant, but in the treatments having fertilize with manure combination, positive and significant trends in yield of rice and inferred that the soils of rice growing areas having low organic matter and total nitrogen contents may not be able to support continuous cropping of rice system unless adequate supply of nutrients through fertilizers or manures is ensured.

Nitrogen: Nitrogen has significance in vegetative growth which determinates the final yield, emphasizing the importance of nitrogen availability for floret development. Maheswari et al. (1993) reported that the N remobilization from senescing tissues is important for the N economy of the plant. Limited nitrate uptake from the dehydrated soil and the decreased nitrate concentration in the xylem could lead to the alkalinization of the xylem sap, thus affecting ABA accumulation both in the leaves and the reproductive structures, resulting in reduced yield (Liu et al. 2005). The role of nitrogen in the life cycle of a crop is enormous; the contribution of saturated lipids and protein components to membrane function under high-temperature stress needs further study. Essentiality of nitrogen availability at anthesis/blooming is supported by (Fischer 1993). Experimental results indicate that changes in the protein fraction composition under heat and drought stress are mainly caused by the altered quantity of total N accumulated during grain filling (Triboï et al. 2003). In accordance, the post-anthesis application of nitrogen fertilizers reduced the effect of high temperature on the storage protein composition of wheat (Dupont and Altenbach 2003) whereas Maheswari et al. (1992) reported that the higher levels of applied N to wheat not only increased leaf and grain N concentration but also led to higher free NH^{4+} levels in leaves and in developing ears.

Higher amounts of nitrogen uptake by roots lead to better yield under elevated CO_2 (Rogers et al. 1996; Kimball et al. 2002; Kim et al. 2003). Elevated CO_2 generally induce stomatal closing and reduce transpiration (Kimball et al. 2002); this situation can decrease nitrogen uptake (McDonald et al. 2002). There are several reports in relationship between transpiration and nitrogen uptake (Delhon et al. 1995; Cerezo et al. 1999; Kiyomiya et al. 2001). Thus, elevated CO_2 could affect nitrogen uptake capacity both positively and negatively through variations in photosynthesis and transpiration.

Potassium: Potassium is required for a wide range of functions within the plant metabolism such as leaf area and leaf chlorophyll content, enzyme activation, osmotic turgor regulation and transportation of assimilates. It has role in protein synthesis including HSPs, stomatal articulation. An optimum level of potassium is important throughout the span of the rice plant as it plays major role in the water uptake from the soil. Sufficient potassium levels in plants can greatly lower the ROS production by reducing activity of NAD(P)H oxidases and maintaining photosynthetic electron transport. Potassium deficiency causes severe reduction in photosynthetic CO_2 fixation and impairment in partitioning and utilization of photosynthates. Such disturbances result in excess photosynthetically produced electrons and thus stimulation of ROS production by intensified transfer of electrons to O_2 (Cakmak 2005). So potassium is essential in withstanding the elevated temperature.

6.7 Energy Economics Under Heat Stress

Plant growth gets effected severely due to a reduction in net photosynthesis rate and generation of reducing powers as well as disturbances with mitochondrial functions. It is suggested that during light reactions increased leaf temperature induces ATP synthesis to balance ATP consumption under heat stress possibly by cyclic electron flow (Bukhov et al. 1999).

Heat tolerance in plants is a cost-intensive process and consumes considerable cellular energy to cope with adversaries of high temperature. A strategy of improving stress tolerance in plants by maintaining the plant's energy homeostasis under stress is the production of transgenic plants with lowered poly (ADP) ribosylation activity; such transgenics appear to be tolerant to multiple stresses by preventing energy overconsumption under stress, thereby allowing normal mitochondrial respiration (De Block et al. 2005).

6.8 Concluding Remarks

The ability of the plant to cope with or adjust to the heat stress varies with the species and developmental stages, in particular anthesis and grains filling are more susceptible. Apart from them, Pollen viability, embryo development, patterns of assimilate partitioning, growth and development of seed/grain, structural changes in tissues and cell organelles, disorganization of cell membranes, disturbance of leaf water relations, impedance of photosynthesis and lipid peroxidation are major concerns in this elevated temperature climate.

Plants manifest numerous adaptive changes. Signal transduction mechanism need to be focused from heat stress point of view where Ca^{2+} role is very important. The heat shock proteins and molecular chaperons which ensure three-dimensional structure of membrane proteins helps in understanding the sustained cellular functions and survival strategies under heat stress, a clear cut idea on proteomics is the need of the hour. Applications of exogenous osmoprotectants play a major role in the heat tolerance. Execution of traditional breeding practices should take place keeping phenotypic flexibility in mind. Identification of temperature signal perceiving regions, Understanding the signal transduction pathway cross talk, Temperature stress regulators, Understanding post transcriptional and translational changes, Identification of genes with specific function, Understanding the nature of cross protection and Identifying targets of injury.

Furthermore, applications of genomics, proteomics and transcriptomics approaches to a better understanding of the molecular basis of plant response to heat stress as well as rice plant heat tolerance are of the essence.

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Chapter 7
Improvement of Drought Resistance in Crops: From Conventional Breeding to Genomic Selection

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Abstract Drought stress is the major factor limiting yield and yield stability of crops. To improve plant performances under drought conditions direct selection for yield over multiple locations has traditionally been employed. This approach is hampered by low heritability and high G × E interaction influenced by differences arising from soil heterogeneity and others environmental factors. The indirect selection using secondary traits has succeeded only in a few cases, due to problems with repeatability and lack of high-throughput phenotyping strategies. During last years, considerable efforts have been directed towards identifying physiological traits associated with yield and drought resistance. With the availability of whole genome sequences, physical maps, genetics and functional genomics tools for many crops, integrated approaches using molecular breeding and genetic engineering offer new opportunities for improving yield in drought prone conditions. The identification of the genetic bases of important physiological traits and the cloning of the genes sustaining yield in drought-prone environments will move the selection toward a "breeding by design" approach that will accumulate an increasing number of useful traits into elite genotypes that, in turn, will result in a reduction of the gap between yield potential and actual yield.

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7.1 The Complexity of Drought Environments and Plant Adaptation

Drought is by far the most important environmental stress in agriculture, causing important crop losses every year. Temperature increase and more erratic rains predicted as consequences of climate change could cause more frequent drought events. As irrigation is not considered a sustainable option to drought everywhere, losses of crop yield under water scarcity require a genetic solution. Furthermore increase in crop demand for food to feed the world population also mandates that future genetic gain in yield both in well watered and stress prone conditions need to be utilized. The development of elite cultivars for drought prone areas has been hampered by difficulties inherent to the complexity of drought environments and to the complexity of plant responses to drought.

A drought event can have different faces. A critical factor is the timing of the drought episode in relation to the crop developmental stage. Drought events are most harmful at flowering, when the reproductive process can be drastically affected by insufficient supply of photosynthates and changes in phytoregulators. Furthermore, often drought is not the only stress event, the contemporary presence of different kinds of stresses, like diseases or high temperatures, mineral toxicities or poor nutrition, can exacerbate the effect of water scarcity. The International Maize and Wheat Improvement Center (CIMMYT) defined three main patterns of drought stress: grain-filling stress, pre-flowering stress, and continuous stress (Reynolds et al. 2005). Considering the wheat crop, the first pattern describes the typical water distribution profile of Mediterranean countries and Australian regions, where crop experiences water deficit during grain-filling, while average precipitation exceeds average pan evaporation prior to flowering. The second adverse scenario occurs in South America where drought stress is most likely to occur prior to wheat flowering. The third scenario is experienced by wheat grown after monsoonal rains, such as in regions of South Asia where irrigation water is unavailable, and in some Australian regions. In this context, there is no rainfall during the growth season, so the crop must survive on whatever moisture is stored in the soil profile from the summer rainfall. Nevertheless, unpredictability is the main characteristic of drought, for instance in the Mediterranean environment beside the typical "terminal drought", early drought during vegetative growth and flowering as well as years with ample water availability during the whole cereal growing season can also occur.

A number of physiological studies have identified many traits whose presence/expression is associated with plant adaptability to drought prone environments. They span a wide range of vital processes and/or pattern of organ development, some are constitutive, others activated upon exposure to stress. In the 1972, Levitt defined plant responses to stress according to three main categories. The escape strategy is based on the modulation of plant development timesheet on the climate trend to conclude the life cycle before the environmental adversities show up and it is distinct from features influencing resistance to drought. The latter is defined avoidance which is based on maintenance of plant water status through improved water balance often by means of constitutive adaptations, the most important of which concerns root architecture and leaf morphology, in terms of increased water uptake by deeper roots and/or reduced water loss by increased leaf waxyness. Osmotic adjustment is also a typical physiological mechanism for drought avoidance. Dehydration tolerance involves biochemical mechanisms (e.g. accumulation of compatible solutes to preserve membrane integrity, water-soluble carbohydrates relocation, etc.) activated upon stress exposure to enable the cell to tolerate the negative effects caused by cellular dehydration. At whole plant level, biomass partitioning and other traits associated to plant reproductive biology enable the plants to mitigate the negative effects of drought on yield (Cattivelli et al. 2008).

From the yield point of view the plant behaviour towards limited water and energy resources can be summarized by Passioura's equation $Y = WE \times WUE \times HI$, where WE = Water Extracted from soil, WUE = Water Use Efficiency and HI = Harvest Index. According to this rule, the drought adapted ideotype is an opportunistic plant able to exploit all water resources in the most efficient manner and avoid the stress. It will perfectly balance the need of water of the yield-related physiological processes with the pattern of water supply. Such an ideotype will be characterised by high yield potential and high yield stability (minimum G × E interaction), ensuring high production both in stress free environment as well as under moderate drought.

The effectiveness of selection for drought related traits is not well defined, due to high G × E interaction and also there is no single response well correlated with yield under all drought situations. For example, small plant size, reduced leaf area, early maturity and prolonged stomatal closure leading to a reduced total seasonal evapotranspiration can improve plant survival conferring the ability to tolerate extreme stress. Even if this conservative and protective strategy can ensure a minimal yield in dry environments, it can result into reduced yield in stress-free and mild to moderate stress environments. Since rainfall patterns and evaporative demand over a crop cycle show geographic variation, traits conferring drought-adaptation in specific environments may differ. In general, a plant designed to cope with a specific type of drought may under-perform when stress conditions are different. For example, WUE (Water Use Efficiency) is a physiological trait often considered as an important determinant of yield under stress and even as a component of crop drought resistance. Indeed, Rebetzke et al. (2002) reported a successful breeding program for wheat in drought-prone environments, based on the carbon isotope discrimination (Δ) as a surrogate for WUE (Farquhar and Richards 1984). However the association between WUE and plant production looked very elusive for various crops under different growing conditions and depending on soil water availability (Condon et al. 2004). Therefore even if strong relationships between physiological traits associated to drought tolerance and yield are found, they can be limited to narrow environmental conditions within a given crop phenology (Blum 2009). The strong heterogeneous features of drought events can reduce or even overturn the positive effect also of traits associated to increased water availability. For example, root meristems capable of adjusting osmotically at given water potential and deeper roots to improve the extraction of additional moisture from the soil will positively impact root mass and final yield. Nevertheless, if additional moisture is unavailable at deeper soil layers, a condition quite common in many drought-prone environments (e.g. the Mediterranean basin), growing larger/deeper roots will not be advantageous and might even negatively influence final yield due to excessive partitioning of photosynthates to the roots and the high metabolic cost required for sustaining the functions of a large root system.

7.2 Assessment of Drought Tolerance in Crops

A crucial aspect in all studies dedicated to plant water relationship is the assessment of the degree of drought resistance of different genotypes. In many studies the identification of resistant and susceptible cultivars is based on few physiological processes related to drought response. The difficulty to identify a physiological parameter as a reliable indicator of yield in dry conditions had suggested using yield performance over a range of environments as the main indicator for drought tolerance (Voltas et al. 2005). Furthermore, a number of regression techniques of yield against environmental indices as independent variables were developed to evaluate genotype adaptability. Several indices were proposed to describe the yield performance of a given genotype under stress and non-stress conditions or in comparison with the average yield or the yield of a superior genotype (Finlay and Wilkinson 1963; Fischer and Maurer 1978; Soika et al. 1981; Lin and Binn 1988; Yadav and Bhatnagar 2001). Some authors have expressed the yielding capacity with regard to an environment-related physiological trait, such as canopy temperature or water potential. As an estimate of the water stress experienced by crop plants, Idso et al. (1981) suggested a "crop water stress index" (CWSI) derived from the increase in average canopy temperature in relation to that of a well-watered reference plot and evaluated by infrared thermometry. In a study on *Triticum aestivum* and *Vicia faba* the water potential index (WPI) was suggested as a measure of the total water stress experienced by a crop in a given environment for a specific time interval (Karamanos and Papatheohari 1999). Other approaches have been proposed to quantify the degree of drought based on specific environmental factors (such as weather, soil water availability) independent on the G × E interaction. By providing yield analysis as function of an environmental index, these different approaches allowed comparison of genotype performances under different degrees of water limitation. Araus et al. (2003) found that yield was well correlated with water input under different water stress conditions. Motzo et al. (2001) proposed a seasonal water stress index based on soil-plant-atmosphere interaction, where stress was quantified as 1-(fraction of transpirable soil water). Rizza et al. (2004) proposed an integrated WSI, based on a simple soil water balance and the integrated reduction of plant transpiration relative to potential transpiration. They proposed to analyze yield potential and adaptability of cultivars to water stress by means of the intercept and slope of a linear regression of yield vs. WSI.

7.3 Achievement of the Conventional Breeding in the Last Century

Despite yield in drought-prone environments has a low heritability due to a high G × E interaction, high intra-site variability and low genetic variation (Blum 1988), conventional breeding has obtained some positive achievements in the last century. Indeed, retrospective analyses show that a genetic gain from 10 to 50 kg ha^{-1} $year^{-1}$ has been recorded for cereals and legumes over the last Century in all countries, including those characterized by vast drought-prone regions (Calderini et al. 1995; Calderini and Slafer 1998; Abeledo et al. 2002). Moreover, when yield increases were expressed as percentage, similar values were recorded in environments with different degrees of water stress (Araus et al. 2002). Several experiments have compared large sets of cultivars characterized by different year of release under different water regimes, allowing a direct comparison of the performance of the same cultivars in drought and well watered conditions (Tollenaar and Lee 2002; Araus et al. 2002). For most crops these analyses showed a linear relation between yield and year of release, the slope of which gives an estimate of the genetic improvement. These studies show that conventional breeding had bred cultivars adapted to stressed environments also selecting for high yield in stress free environments which ensure higher heritability (Cattivelli et al. 2008). Very often, modern cultivars are selected based on the absolute performance of the genotypes across a number of different environments. This breeding strategy has contributed to reduced interaction of genotypes with environments, selecting genotypes with better stability across a wide range of locations and years. As a result, very often modern genotypes outperformed the old ones in all test environments including those with moderate drought stress with a stronger responsiveness to improved fertility (De Vita et al. 2010). This suggests that some of the traits selected to improve yield potential also increase yield at least in mild to moderate drought conditions ensuring yield stability.

In barley, a study carried on a sample of the germplasm grown in Europe identified several modern genotypes with high yield potential and minimal G × E interaction which ranked among the best in both rainfed and irrigated treatments with highly significant correlation between yield in rainfed and irrigated conditions (Rizza et al. 2004). Notably, the authors observed that while old varieties were characterized by low yield in rainfed conditions and by a minimal ability to improve yield when water became available, modern cultivars showed a higher yield in rainfed conditions and strong yield increases in response to irrigation. Moreover, among superior genotypes there was a large predominance of varieties selected in regions usually not affected by drought (e.g. Northern Italy, Sweden, France, and Netherlands), further suggesting that selection under favourable environments allows the identification of genotypes with a wide range of adaptability. Analogous experiments on sugar beet genotypes representing diverse genetic backgrounds grown in drought prone and irrigated conditions led to similar results (Ober et al. 2004).

The comparison of different cultivars allowed the identification of the main morpho-physiological traits modified during selection in association with yield improvement. Many reports suggest that increases in yield in water-limited environments are mainly determined by the inherent yield potential, instead of traits associated to drought adaptation. Under mild to moderate drought conditions, traits maximizing productivity normally expressed in the absence of stress can still sustain a significant yield improvement (Blum 1996; Slafer et al. 2005; Tambussi et al. 2005). This can be explained considering that the main targets of selection (high harvest index in wheat and barley, stay green in maize and sorghum, resistance to pests and diseases, nitrogen use efficiency) are equally beneficial under dry and wet conditions and, often, the best performances for these traits were overriding the differences in drought adaptability. In soybean for example, modern cultivars characterized by high yield under irrigated conditions, also outperformed in rainfed trials, despite the greater capacity of old cultivars to retain water when measured in terms of water potential and stomatal conductance (Frederick et al. 1990, 1991). This rationale can explain the success of wheat and rice varieties bred at CIMMYT and IRRI where selection under stress free environments identified genotypes with high yield in a wide range of conditions including regions with a low yield potential (van Ginkel et al. 1998; Trethowan et al. 2002). Selection for high yield potential alone would therefore be sufficient, however high yield potential, that is the maximum conversion of environmental inputs into grain output, can be considered the ideal genetic background where to introgress relevant adaptive traits for genotypes intended for more stressed environments. Van Ginkel et al. (1998) provided evidence that in wheat yield potential explains only part of the yield variance under drought conditions and that adaptive traits also contribute significantly to performance under drought. In maize, retrospective studies on the genetic yield improvement have underlined that adaptation to stress conditions is the main feature of modern hybrids. Comparison of short-season hybrids representing yield improvement from the late 1950s to the late 1980s showed that genetic yield improvement was 2.5% per year and that most of the genetic yield improvement could be attributed to increased resistance to stress, like high plant population density, weed interference, low night temperatures during the grain-filling period, low soil moisture, and low soil N (Tollenaar 1989; Cattivelli et al. 2008; Tollenaar and Wu 1999; Tollenaar and Lee 2002). Yield improvement is the result of more efficient capture and use of resources, both the seasonal incident radiation as well as nutrients and water. The improved resource capture is associated with increased leaf longevity, a more active root system, and a higher ratio of assimilate supply by the leaf canopy (source) and assimilate demand by the grain (sink) during the grain-filling period. The improved efficiency in resource capture and use of the new hybrids is most clearly evident under stress. Indeed, improvements of resource use under optimum conditions have been small, as leaf photosynthesis, leaf-angle distribution of the canopy, grain chemical composition, and the proportion of dry matter allocated to the grain at maturity (i.e., harvest index) have remained virtually constant. These findings suggest that the new avenues for selection cultivars with high yield potential and high yield stability should associate both traits for yield per se as well as traits for stress avoidance/tolerance to maximize the exploitation of available resources. Yield potential and adaptive traits can be combined by alternating segregating generations between an optimum moisture location to select for yield potential, and water-stressed sites to add traits contributing to adaptation under drought. This strategy can allow the identification of superior genotypes with broad adaptation capability, yielding well under the relevant type of drought, but also exploiting eventual improved conditions of the target environment. A selection index could be effective to combine information from both environments (Rosielle and Hamblin 1981; Edmeades et al. 1999; Monneveux et al. 2006).

Screening for yield potential improves yield under drought if yield under drought and yield under well water conditions are positively correlated. This is usually the case for moderate drought conditions. A crossover effect in the yield of genotypes of high and low yield potential when regressed against the environmental index over a wide range of environments is found when drought conditions become extreme. An ideal genotype would combine the highest yield (highest intercept) with the lowest sensitivity to water stress (lowest slope); nevertheless very often a high yield performance under wet and dry conditions of superior genotypes was found to be associated with a high sensitivity to water stress (high slope) (Pantuwan et al. 2002; Ober et al. 2004; Rizza et al. 2004; Pidgeon et al. 2006). The threshold generally valuable for all crops has been identified when stress reduced yield to less than 50% of non-stress yield (Pantuwan et al. 2002), conditions that are rare in commercial agriculture. Recent studies in tropical maize (Banziger et al. 2006), bean (Beebe et al. 2008), and rice (Venuprasad et al. 2007) suggest that under extreme stress conditions, the response to selection for yield in low-yielding environments is greater when selection is performed in the target environment than in a high-yielding environment. These achievements have been obtained with germplasm showing higher genetic variance for yield in low than high yielding environments, probably because association of traits specific for drought tolerance.

Selection in drought stress environments can be achieved either by screening under natural drought stress in the target environments or by screening in managed-stress environments. While the first approach is often less efficient because of the sporadic and unpredictable occurrence of drought events, screening in managed-stress environments, including dry-season trials, rain-out shelters, and drained upper paddies, is more reliable. However in this case, the effectiveness depends on the accuracy of predictions of the performance under natural drought stress, therefore on yield heritability in the selection environment and its genetic correlation with yield under stress in the target environment (Atlin and Frey 1990). In upland rice, the effectiveness of direct selection for yield under severe drought using natural and artificially imposed conditions was recently assessed on segregating populations obtained by crossing high-yield cultivars, developed for irrigated lowland conditions, with drought-tolerant lines adapted to upland conditions (Venuprasad et al. 2007). This study demonstrated that direct selection for yield under stress is likely to be more effective if conducted in a well managed screening environment effectively controlling intra-trial field heterogeneity. Indeed, under these conditions, yield is usually heritable as yield estimated under non stress conditions (Bernier et al. 2007; Atlin 2004; Venuprasad et al. 2007). Moreover direct selection for yield under drought stress in the dry season was compared with selection for yield under non-stress conditions. Direct selection for yield was effective, notwithstanding yield gains under severe stress occurred only in a population having a highly drought tolerant parent. On the other hand, although yields under stress and non-stress conditions were positively correlated, selection under non-stress conditions resulted in significant gains in stress environments where mean yield was reduced by less than 60%.

7.4 Selection by Secondary Traits

As discussed, traditional breeding for stress adaptation is handicapped since genetic variation and heritability for yield generally decline under stress (Blum 1988). Notwithstanding physiological analyses of yield components and retrospective studies comparing genotypes released in different breeding eras have led to the identification of many traits associated to yield under water scarce conditions, opening the era of the analytical breeding. Provided that these “secondary traits” have higher heritability under stress than yield, they can be exploited to improve selection efficiency during the analytical breeding of new cultivars. These secondary traits should be genetically associated with yield under drought conditions, highly heritable, cheap and easy to measure by accurate phenotyping methods suitable to screen large populations over multiple environments, and should carry no yield penalty under favourable conditions (Ludlov and Muchow 1990). Secondary traits correlated to yield under drought could be used alone to drive the selection, otherwise phenotyping of secondary traits associated to drought-resistance can be combined with yield evaluations to identify higher drought resistant cultivars avoiding penalties for yield potential.

The secondary traits most commonly and successfully used by breeders are those associated to plant phenology, like flowering date and plant height, or disease resistance, which are easily visually determined. Secondary traits for adaptation to drought prone conditions have been just sporadically used, notwithstanding Richards (2006) reported some successful examples, demonstrating that physiological knowledge can result in a more precise targeting of genetic variation. These traits impacted on yield under drought by influencing components of the Passioura’s equation both constitutively or in response to drought. Successful examples are based on extended crop duration and osmotic adjustment for wheat to increase the water use and increased axial resistance to water transport and carbon discrimination to increase water use efficiency, while reduced anthesis silking interval (ASI) and delayed leaf senescence have been considered respectively in maize and sorghum to affect harvest index. In maize, the value of secondary traits in breeding for drought resistance has been also demonstrated by examining the genetic correlations with grain yield, applying divergent selection for specific secondary traits, or estimating the correlated response after selecting for grain yield (Edmeades et al. 1992; 1997).

In groundnut, it has been demonstrated that physiological trait-based breeding can help to change the target of selection, effectiveness being the same of the empirical approaches. Indeed, while yield advantage in empirical selections comes largely from greater water uptake ability (probably through deeper root system exploiting more water from the soil), the trait-based approach allowed to largely improve the water use efficiency (Nigam et al. 2005).

Secondary traits for breeding drought tolerant crops can be chosen within three broad strategies: phenology (escape), yield potential and drought resistance depending on the specific features of the target drought environment (stress timing, frequency and intensity). The traits for drought escape can be considered for yield improvement under drought if they allow plants to cope with a predictable severe stress event, usually occurring every year at the same growth stage. The escape traits usually act at phenology level, i.e. flowering time, photoperiod sensitivity and vernalization requirement. Shortening crop duration can be useful to synchronize the crop cycle with the most favourable environmental conditions. Indeed earliness is an effective breeding strategy for enhancing yield stability in Mediterranean environments where wheat and barley are exposed to terminal drought stress or for rice in eastern India, Thailand, and Laos where the monsoon tends to withdraw predictably and sharply. It is known, however, that an extreme earliness leads to yield penalty also in Mediterranean environment and, in fertile conditions, earliness is not correlated with grain yield (Cattivelli et al. 1994). Late heading and flowering, followed by a short grain-filling period can be associated to higher yield when the drought stress is experienced in early season, during the vegetative phase (van Ginkel et al. 1998).

When soil water is no longer available through roots to sustain evapotranspiration, stomata close saving water, but also limiting entry of CO_2 for photosynthesis. Nevertheless high yield requires high stomatal conductance to sustain a great CO_2 fixation. Therefore every response to water scarcity whose effect is a bigger capture of soil water or a better maintaining of plant water status will maximise soil water use thus sustaining transpiration and yield. Therefore breeding for higher yield potential plus traits conferring stress avoidance may be a good option in case of moderate stress conditions. For instance, when rainfall matches the early phase of plant cycle, a vigorous crop establishment is agronomically desirable as it helps to shade the soil thus avoiding loss of water by evaporation directly from the soil and suppressing weed competing for water. Early vigour depends on large seed and embryo size, while wider leaves and a more prostate growth habit sustains a rapid ground cover. The early vigour can produce assimilates partitioned in a better root system to relieve subsequent moisture stress. To avoid premature exhaustions of soil water, early dry matter could be successfully combined with early flowering. Where soil moisture is still available at maturity or deep soil moisture is available, deep rooted cultivars have demonstrated a clear yield advantage. A vigorous root system could be constitutive or adaptive. Osmotic adjustment (OA) is a frequently cited adaptive mechanism based on the accumulation of solutes whose effect on osmotic pressure helps to maintain a favourable gradient of water potential. It allows plants to maintain water absorption, to sustain cell expansion for root growth or flow of water into the plant itself from a relatively dry soil for higher photosynthetic rate. Questions remain with respect to the positive contribution of OA in plant productivity (Serraj and Sinclair 2002). Although intraspecific variability for OA and the association between OA and grain yield maintenance under drought stress have been reported in a number of crop species (Chimenti et al. 2006), the correlation between osmotic adjustment and yield increases with increasing drought stress, being positive under severe water deficit when crop survival is threatened but irrelevant for most agriculture conditions (Serraj and Sinclair 2002). A proven benefit of OA is the maintenance of root development, while the increase in water potential gradient develops when yield capacity has been lost and crop survival is threatened as little additional water can be extracted (Serraj and Sinclair 2002).

Leaf permeability also is crucial, as leaves can lose water through cuticle, increasing crop transpiration without an associated benefit in CO_2 fixation. Glaucousness which is caused by the presence of epicuticular wax can prevent these losses (Kerstiens et al. 2006). Recent insights from physiology studies have indicated a critical role of mesophyll permeability in controlling the movement of CO_2 into the leaf as well as the factors affecting its extent. It is expected that further progress on this topic will suggest specific leaf traits to be modified to increase the availability of CO_2 into the leaf under drought conditions.

The traits discussed above mainly contribute to greater yields through increase in total biomass. Then, biomass is converted into yield based on harvest index (HI). Two factors influence HI: the number of grains and the quantity of assimilate to fill the grains. In all crops, drought events during specific stages of floral development can severely damage seed set, through pollen sterility and abortion of ovary and/or embryos or can prematurely end grain filling. Low water potential around the time of anthesis is particularly damaging in maize and rice. Maize yields failed completely because of excessive delayed ASI which results in ovary abortion and severe embryo abortion (Boyle et al. 1991; Bolanos and Edmeades 1993) when water deficit decreases photosynthate flux to developing organs. In rice sterility increases when drought stress prevents panicle exertion (Saini and Westgate 2000). Moreover drought causes premature leaf senescence thus further limiting photo-assimilation. To allow more assimilate supply to the growing florets, thus reducing the very high rate of floret abortion just before anthesis, an option is the increase of duration of spike growth by a longer stem elongation period (Miralles et al. 2000; Slafer et al 2001). Under post-anthesis stress, the storage of water soluble carbohydrates in the stem of small grain cereals and their subsequent remobilization to grain can directly improve HI. Stay green phenotype resulting in a delay of senescence is therefore an important trait in several crops (e.g. maize, sorghum) exposed to drought during the grain-filling (Campos et al. 2004; Tollenaar and Wu 1999) since it improves assimilate production and storage.

A “xerophytic” breeding strategy which improves plant survival through the limitation of the evapotranspiration can be applied in extremely harsh environments where survival is jeopardised, according to the indicated crossover limit. In such conditions, progress in improving drought tolerance is usually slower than using direct selection for yield in unfavourable environments because several negative correlations between tolerance traits and yield may limit genetic progress using the indirect selection method (Ceccarelli 1994; Bänzinger et al. 1997; Simmonds 1991). On the other hand, in Australia the comparison of "indirect" (trait) selection with the conventional "direct" (yield) selection method to improve drought resistance in navy bean indicated that seed yield has more G × E interaction than some yield components such as transpiration, water use efficiency, and HI (Dowkiw et al. 2000).

7.5 Assessment of Secondary Traits

Many secondary traits for breeding in drought prone conditions, like root systems, OA and stomatal conductance, are difficult to measure directly. Nevertheless, as they improve the plant water status, they can be indirectly assessed through other traits correlated to plant water status. As transpiration causes leaf cooling, canopy temperature and its reduction relative to ambient air temperature are an indication of transpiration and thus of stomatal conductance, both factors favoring net photosynthesis and crop duration. Canopy Temperature Depression (CTD) has been used to assess plant water status since it represents an integrated physiological response to drought and high temperature (Amani et al. 1996). An imaging technique, Infrared Red Termography (IRT), has been used to easily assess whole leaf temperature based on IR for selecting rice genotypes with higher conductance associated with higher yield (Horie et al. 2006).

In plants, the heavy isotope of carbon 13C is discriminated compared to 12C during diffusion and biochemical reactions, as a result, in several C3 species, the carbon isotope discrimination (Δ) is positively correlated with the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration (Ci/Ca) and negatively associated with transpiration efficiency (TE, which corresponds to WUE at leaf level). Thus, a high Ci/Ca leads to a higher Δ and a lower TE (Farquhar and Richards 1984). Both measure of canopy temperature and carbon discrimination are suitable for large screening, however both present some shortcomings. Several methodological limitations have been found for measure of CTD due to not homogeneous canopy or growing conditions (Royo et al. 2002; Lafitte et al. 2003), while carbon discrimination is limited by the cost of its determination. As Δ13C is not applicable to C4 species due to differences in the photosynthetic pathway, oxygen isotope composition in plant tissues might be an alternative since it reflects evaporative enrichment of leaf water caused by transpiration (Barbour 2007). Isotope discrimination can be applied on leaf or seed material for integrated measures during and after organ growth. To look at the dynamics of photosynthetic fixation as stress occurs, soluble and starch carbohydrate components can also be considered (Ghashghaie et al. 2001; Fresneau et al. 2007). Since the ash content is physiologically associated to carbon discrimination through the control of leaf water content, the mineral or the total ash content of leaves or grains represents a cheaper option to carbon discrimination analysis also used in C4 plants (Voltas et al. 1998; Araus et al. 2001a).

Chlorophyll fluorescence emissions from intact plants are easily and rapidly measured, and have been shown to be directly related to photosynthetic activity

(Baker and Rosenqvist 2004). The fluorescence parameter Fq′/Fm′, which estimates the quantum yield of photosystem II photochemistry, has been shown to be directly related to the rates of linear electron transport and CO_2 assimilation in leaves (Baker and Oxborough 2004). Consequently, this parameter potentially provides an effective tool for rapidly screening for differences in photosynthesis and thus assimilation, or for differences in the response to stresses like drought. As photosynthetic performance across the area of individual leaves can be extremely heterogeneous, especially for stressed plants, to screen for effects on leaf photosynthesis using fluorescence on the whole leaf imaging instruments using charge coupled device (CCD) cameras should be adopted (Nedbal and Whitmarsh 2004; Oxborough 2004).

The leaf water use efficiency (assimilation/transpiration) is a critical component of yield under drought, but it is generally effective in breeding practise when its increase is associated to an increase in assimilation, while traits reducing the transpiration have often negative consequences on yield. By coupling fluorescence and thermal imaging techniques it is now possible to rapidly evaluate both assimilation and stomatal conductance for high-throughput genotype screening (Chaerle et al. 2007).

Spectroradiometers measure the spectra of light reflected by the canopy both at the crop (Aparicio et al. 2000; Araus et al. 2001b; Royo et al. 2003) and at the plant levels (Alvaro et al. 2007). As drought strongly impairs leaf expansion (Royo et al. 2004) and thus plant growth (Villegas et al. 2001), these instruments can therefore be used to indirectly evaluate total green biomass at a critical plant stage (i.e., anthesis) or its change over time. In addition, other physiological characteristics of plants, such as canopy architecture, plant water status, nitrogen concentration and even photosynthetic efficiency are captured in the spectra (Araus et al. 2001b; Aparicio et al. 2000; Tambussi et al. 2002; Babar et al. 2006). A wide range of spectroradiometrical indices have been identified to evaluate field plots for yield and their adaptation to environmental conditions, the most famous being the “classical” vegetation indices (VI) related to green biomass such as the normalized difference vegetation index (NDVI) or the simple ratio (SR). However, up to now spectral reflectance measurements such as the water index (WI) or the photochemical reflectance index (PRI) have been scarcely used in breeding programs particularly due to the strongly influence of differences in green biomass (Araus et al. 2001b).

A secondary trait routinely evaluated is leaf color acquired by portable chlorophyll meters such as the Minolta SPAD (http://www.specmeters.com/Chlorophyll Meters/Minolta SPAD 502 Meter.html) which measures the total chlorophyll content. Besides the physiological significance of the trait per se, total chlorophyll is an indicator of early senescence such that caused by drought stress (Araus et al. 1997; Rharrabti et al. 2001), as well as N fertilization, protein content of grains and it is positively correlated with yield in wheat (Rharrabti et al. 2001).

Recently, the development of modern phenomics technologies based on high throughput analysis using visible, near infra-red, far infra-red and fluorescence imaging, allows us to reduce the “phenotyping bottleneck” which has, until now, limited our ability to capitalize on substantial investments already made in plant functional genomics and modern breeding technologies (Tester and Langridge 2010).

7.6 Molecular Markers-Assisted Genetic Improvement

Molecular markers have the capability of revealing polymorphisms in the nucleotide sequence and allowing therefore discrimination between different alleles at a given locus. Such information can be used for a number of studies aimed to confirm identity between parents and progeny, to determine evolutionary relationships and genetic distances and to construct genetic and physical maps to localize genes or genomic regions responsible for the expression of a trait of interest. Once an association between a molecular marker and the gene(s) responsible for a trait of interest has been established, it can be employed in a plant breeding program in order to significantly increase the speed and the effectiveness of selection (Marker Assisted Selection – MAS). The fact that they are largely unaffected by environmental factors and developmental stages makes them even more suitable for this pourpose.

Molecular markers characteristics and applications have been recently and extensively reviewed (Korzun 2002; Röder et al. 2004; Francia et al. 2005; Varshney et al. 2005; Khlestkina and Salina 2006; Borrelli et al. 2009); virtually, every kind of molecular marker can be used for MAS; nevertheless, they should meet the five main requirements for utilization in MAS defined by Collard and Mackill (2008): flexible and high-throughput detection methods, low quantity and quality of DNA required, low cost per assay, tight link to target loci and a high level of polymorphism in breeding material. PCR-based markers, particularly microsatellites (SSRs), and Single Nucleotide Polymorphisms (SNPs), are much more suitable for use in MAS programs than other techniques, SNPs are expected to became the best choice in the next years.

7.6.1 SNP and Genomic Selection

The new DNA sequencing technologies, made available in the last years, rapidly are producing huge amounts of sequences with a number of applications including genome resequencing and polymorphism detection, mutation mapping, DNA methylation and histone modification studies, transcriptome sequencing, gene discovery, alternative splicing identification, small RNA profiling and DNA-protein interactions (Lister et al. 2009; Delseny et al. 2010). Currently, there are three widely deployed deep sequencing platforms, Genome Sequencer FLX from 454 Life Sciences/Roche, Illumina Genome Analyzer, and Applied Biosystems SOLiD. Each instrument essentially massively parallelizes individual reactions, sequencing hundreds of thousands to hundreds of millions of bases in distinct, relatively short (50–400 bases) DNA fragments, in a single run. The development of new molecular markers in particular is taking advantage of these new technologies that offer therefore a new perspective to applications in the field of genetic improvement of crops. To benefit fully from this major breakthrough in genome sequencing, bioinformatic tools that analyze huge amounts of sequences for the automated design of molecular markers have been developed.

Single nucleotide polymorphism (SNP) markers are increasingly becoming the marker system of choice. They occur in virtually unlimited numbers: several millions of SNPs have been developed based on the sequence of the human genome (Frazer et al. 2007), and similar amounts of SNPs are expected to be produced also in crops in the next future.

Even if the use of SNPs in plants is still in its infancy with respect to results obtained in animal species, an increasing number of large-scale genetic variant discovery initiatives are being undertaken by means of next-generation sequencing platforms, allowing for drastically quicker and cheaper variant discovery, and leading towards a far more comprehensive view of the genome (Ganal et al. 2009). All SNP detection approaches are based on resequencing of different genotypes to determine allelic variants at the highest number of loci as possible. Large projects aimed to identify a very high number of SNPs have been undertaken for crops. About 2,000 and more than 125,000 SNPs were identified by 454 resequencing in two independent projects in maize with a rate of about 85% of validated SNPs (Barbazuk et al. 2007; Gore et al. 2009). In rice, the reference sequences of cultivars Nipponbare and 93–11 were utilised to identify SNPs and to map them by resequencing a set of 150 recombinant inbred lines derived by the cross between the two varieties on an Illumina Genome Analyzer (Huang et al. 2009). New platforms are now available for the rapid genotypization of individuals with SNPs. The Infinium assay by Illumina (http://www.illumina.com/technology/beadarray_technology.ilmn) can provide the analysis with several hundred thousands SNPs in a single assay. The KBiosciences Competitive Allele Specific PCR SNP genotyping system (KASPar) is a novel homogeneous fluorescent genotyping system (http://www.kbioscience.co.uk) providing very fast and cheap analyses.

The possibility to develop and analyze so huge numbers of SNPs in crop species opens new perspectives for the use of these molecular markers to accelerate selection of improved genotypes. While the current MAS strategies use only markers for which a significant association with a trait has been identified, a new method called genomic selection and developed by Meuwissen et al. (2001) predicts breeding values using data deriving from a huge number of molecular markers with a high coverage of the genome. Genomic selection uses all marker data as predictors of performance and consequently delivers more accurate predictions with respect to current MAS approaches. Marker effects are first estimated on a training population for which genotypic data (with very dense marker coverage) and a deeply accurate phenotypic evaluation are available. Based on these data, prediction models for marker effects are developed in order to estimate the genomic breeding value of any individual in breeding programs for which only genotypic data are available. Simulation studies have shown that genomic selection can lead to high correlations between predicted and true breeding value over several generations without repeated phenotyping (Meuwissen et al. 2001; Habier et al. 2007). Therefore, genomic selection can result in lower costs and increased rates of genetic gain (Zhong et al. 2009).

7.6.2 Identification of Phenotype-Associated Markers

The search for molecular markers strictly associated with a locus controlling important phenotypic traits is a key objective in molecular genetics and can be carried out with methods based on segregation mapping, genomic introgression, and association mapping (Morgante and Salamini 2003). Segregation mapping is based on the availability of large mapping populations such as F2s, Recombinant Inbred Lines (RILs), Backcross Inbred Lines (BILs) or Doubled Haploid Lines (DHLs). These segregating populations are used to develop linkage maps in which the relative position of hundreds of molecular markers is indicated on each chromosome. Then, the gene(s) and QTL(s) position can be determined through the combination of phenotypic characteristics and segregation data of molecular markers. A plethora of studies have been carried out in order to identify genomic regions involved in determining high yield in stress and non stress environment in crops. The wide range of physiological and biochemical mechanisms involved in dehydration response explains the complexity of plant response to drought, for which a high number of QTLs widespread on many chromosomes have been found (Cattivelli et al. 2002, 2008). QTLs for yield and yield-related traits most frequently account for between 2% and 10% of the total phenotypic variation; major QTLs with R2 values higher than 15% have seldom been described, especially when evaluating segregating materials obtained from elite accessions (Quarrie et al. 2005; Dilbirligi et al. 2006). In durum wheat, Maccaferri et al. (2008) identified 16 QTLs in which two major QTLs on chromosome arms 2BL and 3BS of durum wheat affecting grain yield and showing significant effects in multiple environments (rainfed and irrigated). Some recent papers have reviewed in details the QTLs identified for traits related to drought stress tolerance (Maccaferri et al. 2009; Ashraf 2009), furthermore, for many crop plants information on drought-related QTL findings have been collected in open source databases, such as GRAMENE (http://www.gramene.org/) or GRAINGENES (http://wheat.pw.usda.gov/GG2/quickquery.shtml#qtls). In particular, Courtois et al. (2009) extracted information from about 60 papers published between 1995 and 2007 and compiled a database containing QTLs for drought resistance traits and for 29 root parameters. The data describe 2,137 root and drought QTLs, out of which 675 for root traits detected in 12 mapping populations.

Once identified, a QTL needs to be validated in order to provide reliable QTL-associated markers for breeding. Near Isogenic Lines (NILs) can be produced backcrossing a donor plant with a recurrent parent. As each NIL carries only one target QTL in the genomic background of the recurrent parent, behaving as a single Mendelian factor, the effect of the target QTL can be precisely evaluated, and the gene/QTL finely mapped and isolated (Salvi and Tuberosa 2005).

The whole QTL analysis process, from the construction of the primary mapping population to the achievement of NILs, is labour intensive and requires many generations and years. An alternative and effective approach for identification of target QTLs is the development of Introgression Lines (ILs), a set of lines obtained through the marker assisted backcross of a donor parent, usually a wild relative, with a recurrent parent. After 2–3 backcross generations, each line will contain a specific genomic region of donor parent into the recurrent genetic background and the whole set of ILs is organized to represent the whole donor genome (Zamir 2001). Because the identification of QTLs using ILs does not require a pre-existing linkage map, this is a more user-friendly method for practical breeding works. Different examples are available, in which genomic segments carrying QTLs for drought resistance traits from *O. rufipogon* were identified in a set of introgression lines in rice (Zhou et al. 2006; Zhang et al. 2006). Xu et al. (2005) evaluated a large set of 254 introgression lines in an elite indica genetic background for grain yield and related traits under the irrigated and drought conditions in two consecutive years. They found 36 QTLs, some being expressed in both treatments, and other specific of the irrigated or drought condition. Once a QTL has been identified, the IL carrying the region of interest can be used for fine mapping and cloning of QTL/gene as well as donor line in breeding programmes.

Introgression of useful alleles from wild species has highlighted the problem of the effectiveness of a gene in a particular genetic background. It is infact necessary to confirm that QTLs discovered in a given mapping population will improve drought tolerance when introduced into high yielding elite genotypes. This is particularly difficult when the traits are governed by interaction with other genes and/or environment. In these cases the effect of the QTL alleles can differ depending on the genetic structure of the current germplasm set in the breeding program (Wade 2002), and the continuous change of the genetic background during the breeding process can lead to a change in the value of the QTL allele that is transferred by MAS. The “Mapping As-You-Go” strategy (Podlich et al. 2004) involves repeated re-estimation and validation of the QTL effects throughout the breeding process to ensure that they remain relevant throughout. This method results in substantial increases in MAS efficiency compared with standard approaches based on the evaluation of the QTL effects only at the beginning of the breeding program, particularly when epistasis and/or genotype-environment interactions play a significant role.

Besides linkage map studies, an emerging approach for the identification of genes underlying phenotypic variations in complex traits is represented by linkage disequilibrium (LD, i.e. non random association between alleles at linked loci) mapping (Syvänen 2005). LD infers associations between genotypes (haplotypes) and phenotypic variations examining the genetic polymorphisms that have been generated into different genetic backgrounds through thousands of generations of recombination. A whole genome may be scanned to identify regions that are associated with a particular phenotype by using molecular markers, covering the whole genome. Otherwise, alleles at a few selected candidate genes may be tested for association with a phenotype (Rafalski 2002). Candidate genes for a trait of interest are usually referred to sequences whose expression profile or protein function can be associated to the trait and whose position on the genome co-maps with a QTL controlling the trait. The extent of LD is affected by many factors, including the population history and the frequency of recombination in the examined genome segment. In plant species where population bottlenecks are common, such as sugarcane and sugarbeet, LD extending for several cM was found (Jannoo et al. 1999; Kraft et al. 2000). On the contrary, maize studies have shown that LD decays within 1,500 bp distance, allowing successful identification of QTLs associated with variation in flowering date (Thornsberry et al. 2001). LD has been successfully employed in finding marker-trait associations for yield and yield stability in a collection of 146 modern two-row spring barley germplasm: the association was evaluated on a mean of 15 environments for each variety in Northern Europe (Kraakman et al. 2004), and for kernel morphology and milling quality in a selected sample of 95 cultivars of soft winter wheat (Breseghello and Sorrells 2006), indicating that association mapping approaches can be a viable alternative to classical QTL approaches based on segregating population studies. Association mapping has been also employed to study drought resistance determinants, in particular in sunflower and *Pinus taeda* (Kane and Rieseberg 2007; Gonzalez-Martınez et al. 2006). 46 SNPs from 41 disease and abiotic stress-inducible genes were tested for their genetic association with carbon isotope discrimination with 961 clones by Gonzalez-Martınez et al. (2008). Two particularly promising candidates for their genetic effects on carbon discrimination were dhn-1, involved in stabilization of cell structures, and lp5-like, a glycine rich protein putatively related to cell wall reinforcement proteins, both of which already shown to be water-deficit inducible.

LD estimates can also be used to determine target marker densities for genomic selection, as dense marker coverage is needed to maximize the number of QTLs in LD with at least one marker, thereby also maximizing the number of QTLs whose effects will be captured by markers (Heffner et al. 2009).

7.6.3 Critical Factors for Success of MAS

The success of a MAS breeding programme depends first of all on the nature of the trait to be improved. While the transfer of monogenic traits is simple and well documented in literature (Campbell et al. 2002; Bonafede et al. 2007), MAS programmes for traits with a complex genetic basis are much more difficult due to the high cost, the genotype × environment and gene-gene (i.e. epistasis) interactions, trade-offs between different organization levels, which in turn, result in a low efficiency, as the case of breeding for drought resistance. An important factor is also the degree of association between the markers and the major gene(s) or quantitative trait loci (QTLs) of interest; the closer are the marker and the gene, the lower is the probability of marker and gene being separated by recombination. If the gene controlling the trait has been isolated, it is possible to design a so called “perfect” marker directly within the gene; in this way the association between the gene and the marker cannot be lost.

The high interaction of G × E is one of main constraints in application of MAS for improved drought resistance. The ability of an organism with a given genotype to change its phenotype in response to changes in the environment is known as phenotypic plasticity (Schlichting 1986; Pigliucci 2005). When QTLs are detected in a stressed environment vs. optimal conditions they are considered as related to adaptive traits, while little attention is paid to phenotypic plasticity. Reymond et al. (2003, 2004) have proposed a new approach in which QTLs are identified through the correlation between the molecular data and the parameters describing the response curve of the trait to the environmental conditions, rather than the raw phenotypic data per se. This approach allows the characterization of the genotype per se rather than its behaviour in a given environment. The authors have successfully applied this method to the evaluation of leaf elongation rate (LER) in response to temperature, evaporative demand and soil water status. Response curves of LER to meristem temperature, water vapor pressure difference, and soil water status were established in 100 RILs of maize in six experiments carried out in the field or in the greenhouse, and a QTL analysis was carried out on the slopes of these responses. Most QTLs were specific of one response only.

Another critical factor for traits explained by a number of QTLs, each of them controlling a part of the observed variability, is an accurate phenotyping. Capacity for precise phenotyping under reliable conditions probably represents the most limiting factor for the genetic analysis of quantitative traits. Phenotypic measurements are needed, giving a good precision level on a large number of genotypes (Cattivelli et al. 2008). Designs of laboratory or field experiments defining stress combinations pertinent to specific target crops and their environment should take into account as many environmental factors of the target environment as possible, including macro- and micro-environmental factors (Salekdeh et al. 2009). To achieve a careful phenotyping, it is also important to control timing, intensity and duration of stress. To reduce the signal-to-noise ratio in field based experiments there is a need to select research plots with low spatial variability in soil properties, good management of nutrients, weeds and pests, and experimental design that should control within-replica variability (Edmeades et al. 2004).

Uniformity of conditions in both control and stressed plots is a key factor in screening large number of accessions for a conditional trait like drought tolerance. This problem is particularly important when accessions with different morpho-phenological traits are compared, making difficult to apply the same intensity of stress to a specific stage of the crop lifecycle. For this reason, there is great interest in methods that reduce systematically the number of accessions to be screened without significant loss of diversity in traits of interest. The use of taxonomical, geographical and morphological descriptors, as well as molecular markers, can help in constituting mini-core collections composed by a lower number of individuals, but retaining the genetic diversity for the trait of interest. Bidirectional selective genotyping is another method of reducing the number of lines in a mapping population that requires genotyping and refined phenotyping. A previously detected large-effect QTL for yield under drought stress in rice (Bernier et al. 2007) was confirmed in a 436-line population by selective genotyping just 20 lines at each phenotypic extreme of the population (Bhatnagar-Mathur et al. 2008).

7.6.4 MAS for Drought Tolerance

Environmental variability and low stability of QTLs for grain yield make difficult the choice of genomic regions to be transferred by MAS to improve grain yield in drought prone environments. This is the main reason why the MAS-mediated improvement of grain yield in drought prone environments has yield limited success by transferring QTLs for yield. MAS programs for drought-related morphological and physiological traits may represent a better strategy for improvement of drought resistance. During the last 15 years, the application of QTL analysis allowed the identification of many chromosome regions involved in control of variation in almost all the physiological, morphological and developmental changes observed during plant growth in water-limiting conditions. Particular attention has been paid to: (i) genetic variation of the OA (Teulat et al. 1998; Robin et al. 2003), (ii) genetic bases of phenological traits – e.g. stay green phenotype (Sanchez et al. 2002; Jiang et al. 2004; Verma et al. 2004) and anther-silking interval (ASI increases in response to drought) (Hall et al. 1982; Ribaut et al. 1996); (iii) the ability of the roots to exploit deep soil moisture to meet evapotranspirational demand (Johnson et al. 2009; Nguyen et al. 2004); (iv) the limitation of water-use by reduction of leaf area and shortening of growth period (Anyia and Herzog 2004); (v) isotope discrimination (Martin et al. 1989; Juenger et al. 2005; Saranga et al. 2004); (vi) the limitation of non-stomatal water loss from leaves – e.g. through the cuticle (Lafitte and Courtois 2002) and (vii) the response of leaf elongation rate to soil moisture and evaporative demand (Reymond et al. 2003). Comparative analysis of QTL results clearly shows that chromosomal regions determining variation in agronomic and physiological drought-related traits cover a large proportion of the whole genome.

Examples of successful MAS programmes for water stress-related traits have been reported for different species and with different outcomes. In a marker-assisted backcross experiment carried out in maize, the drought tolerant line Ac7643, donor parent, and the drought susceptible line CML247, recurrent parent, characterized by a very large male-female flowering asyncrony interval, were crossed. Five QTLs for ASI, located on different chromosomes and co-localized with QTLs for yield components, were selected, each of them explaining >5% of the phenotypic variance for ASI. Plants with the highest proportion of CML247 alleles at 60 markers distributed throughout the genome, and with good agronomic traits and yield performance were selected in two backcrosses. When compared with controls, hybrids obtained from these selected lines, even if with no significant differences in grain yield under mild water stress and well watered conditions, showed a consistently higher mean grain yield under severe water stress conditions, the best five MAS-derived hybrids yielding at least 50% more than control hybrids (Ribaut and Ragot 2007).

In rice, MAS was used to transfer several QTLs for root depth from the japonica upland cultivar “Azucena”, adapted to rainfed conditions, to the lowland indica variety “IR64”. MAS selected lines showed a greater root mass in low rainfall trials (Courtois et al. 2003; Steele et al. 2006). More recently, four Near-Isogenic Lines (NIL) derived from a previous study (Steele et al. 2006), were evaluated in field trials for 3 years and showed to excel as compared to parents not only for development of root apparatus, but also grain yield (Steele et al. 2007). All these efforts resulted in the release of a highly drought tolerant variety, Birsa Vikas Dhan 111 in India, characterized by early maturity, high drought tolerance and high grain yield with good grain quality (Steele 2009).

Isolating the gene(s) behind a QTL and designing a marker within it (them) could represent the best approach to pyramid different QTLs into the same genotype through MAS. For instance, QTLs explaining some grain yield components have also been cloned in rice, as grain number (Gn1a) (Ashikari et al. 2005) and grain size (GS3) (Fan et al. 2006). Candidate genes can be identified by association mapping studies, by confirming the co-segregation between the gene and QTLs for drought resistance or by analysing with bioinformatic tools the genes present in QTL-underlined genomic regions. A study in *Arabidopsis* has led to the cloning of the ERECTA gene, a sequence beyond a QTL for transpiration efficiency (Masle et al. 2005). Sequencing of entire genome of crop species is expected to provide a huge opportunity to clone QTL for drought-related traits replacing the previous approach based on the construction of functional genetic maps with candidate genes to identify the genetic determinants of QTLs based on cosegregation between the candidate and the QTL (e.g. Zheng et al. 2003; Nguyen et al. 2004; Diab et al. 2004; Tondelli et al. 2006).

7.7 Transgenic-Assisted Genetic Enhancement

Another possible breeding practice consists of the introduction of relevant genes via genetic transformation. Molecular biology studies have identified a number of genes involved in plant response to drought. Usually, expression studies, carried out at level of one/few genes or of the whole transcriptome, are utilised to identify genes whose transcription profile is regulated by the dehydration stress.

Plant transformation provides new variability and a wide array of novel genes not previously accessible to plant breeders, also from related plants or other species even in other taxonomic phyla, which can be easily inserted and functionally expressed into agronomic background in a single event, without associated deleterious genes (Sharma et al. 2002). This process also allows changing the level and the spatial and temporal pattern of transgene expression by choosing suitable promoters, constitutive or inducible, or adding introns into the constructs (Sahrawat et al. 2003). The engineered genetic enhancement may solve many plant breeding problems particularly when the gene controlling the trait of interest is not present or is very difficult to access in the gene pool of the major commercial crops. Pest, disease and herbicide resistance provide examples in which transgenic crops have been obtained and successfully adopted worldwide (Borrelli et al. 2009). Anyway, in the case of more complex traits, transgenic events are at a pre-commercial phase and many more are still at laboratory level and most likely few of them will be successfully validated in field trials.

A drought stress event triggers at cellular level the activation of signal transduction pathways in which transcription factors, RNA-interacting proteins and protein kinase/phosphatases play a key role and which in turn regulate the expression of a number of downstream stress-related genes involved in different metabolic and physiological pathways, as free-radicals and toxic-compounds scavenging, osmolyte accumulation, water and ion uptake and transport (Apse and Blumwald 2002; Wang et al. 2003; Zhang et al. 2004; Vinocur and Altman 2005; Cattivelli et al. 2008). On the basis of this knowledge it is possible to develop transgenic plants in which the general stress response is up regulated, or in which specific metabolic or physiological processes related to stress tolerance are enhanced (Parry et al. 2005; Bohnert et al. 2006; Umezawa et al. 2006).

An example of metabolic engineering successful in several plants subjected to stress consists of the design of transgenic plants for higher osmolyte contents (Wang et al. 2003). In particular, an improvement of tolerance to different abiotic stresses was observed in transgenic rice lines showing stress-inducible accumulation of trehalose (Garg et al. 2002), in wheat showing a small increase in mannitol (Abebe et al. 2003), tobacco and maize plants with increased content of glycine betaine (Shen et al. 2002; Quan et al. 2004; Zhang et al. 2008), and in soybean, petunia and tobacco plants with enhanced accumulation of proline (Ronde et al. 2004; Yamada et al. 2005; Gubis et al. 2007). Other strategies to broaden stress tolerance in plants provided for controlling the amount of reactive oxygen species (ROS – Ashraf 2009), maintaining energy homeostasis under stress conditions (De Block et al. 2005), or, specifically for water deficit, for the production of phenotype with decreased conductance and higher water use efficiency by over expression of a maize NADP-malic enzyme (Laporte et al. 2002), by regulating the ABA synthesis and response (Wang et al. 2005; Hu et al. 2006), by improving the root system (Gaxiola et al. 2001; Park et al. 2005) or delaying water stress induced leaf senescence (Rivero et al. 2007).

Many examples are also available demonstrating that it is possible improving the resistance to drought stress by acting on genes involved in signal transduction and regulation of the expression of downstream genes. Transgenic plants with overexpression of OsMAPK5, a mitogen-activated protein (MAP) kinase as well as of OsCDPK7, a calcium-dependent protein kinase, resulted in enhanced levels of stress-responsive genes allowing a more sustained stress tolerance (Saijo et al. 2000; Yang et al. 2001; Xiong and Yang 2003).

Among the best characterized transcription factors acting in response to abiotic stresses are the members of the DREB/CBF gene family as well as sequences coding for proteins with basic leucine-zipper (bZIP), MYC and MYB DNA binding domains (Abe et al. 1997, 2003; Jacoby et al. 2002; Dong et al. 2003; Dubouzet et al. 2003; Shen et al. 2003; Li et al. 2005; Agarwal et al. 2006; Hu et al. 2006; Ito et al. 2006; Olsen et al. 2006; Sakuma et al. 2006; Kim et al. 2007). Many crops were engineered with genes encoding the DREB/CBF transcription factors (tomato – Hsieh et al. 2002; rice – Dubouzet et al. 2003; Oh et al. 2005; Ito et al. 2006 and wheat – Pellegrineschi et al. 2004), showing increased stress tolerance as well as the over induction of downstream stress related genes. A common constraint encountered with plants over expressing drought-related genes and transcription factors in particular, consisted of abnormalities in plant growth and development in absence of the stress factor when transformed plants were realized with the transgene under the control of constitutive promoters. This problem is usually solved by utilising a stress-inducible promoter.

The majority of these studies were carried out in controlled conditions, but it is necessary to perform field trials in order to collect data on the real effect of the transgene on drought resistance and on yield in stress conditions. Under water limited environments both in controlled and field trials, transgenic maize plants with increased expression of the transcription factor ZmNFYB2 showed tolerance to water stress when evaluated using stress-related parameters such as chlorophyll content, stomatal conductance, leaf temperature, reduced wilting, and maintenance of photosynthesis (Nelson et al. 2007). Encouraging field trial data are available for also transgenic rice plants over-expressing the rice stress responsive transcription factor SNAC1 (Hu et al. 2006).

The involvement of the farnesyltransferase (ERA1) gene in the regulation of ABA sensing and water stress tolerance was assessed by molecular studies, but field trials carried out for 3 years on Brassica carrying an ERA1 antisense construct have shown that, under moderate water stress conditions, a significant reduction in stomatal conductance and water transpiration was found at flowering, and the yield of the transgenics was significantly higher than the control (Wang et al. 2005). Water stress could accelerate leaf senescence leading to a decrease in canopy size, loss in photosynthesis and reduced yields. Transgenic tobacco plants expressing a gene encoding isopentenyltransferase showed reduced drought-induced leaf senescence and resulted in improved water stress tolerance, with a minimal yield loss when watered with only 30% of the amount of water used under control conditions (Rivero et al. 2007).

Improved biomass productivity and water use efficiency under water deficit conditions were detected in transgenic wheat constitutively expressing the barley HVA1 gene, encoding a member of the group 3 late embryogenesis abundant (LEA) proteins. When these plants were tested in nine field experiments over six cropping seasons (the largest field test for transgenic plants with improved tolerance to water stress published so far) the data have shown that the HVA1 protein confers a significant protection from water stress (Bahieldin et al. 2005). Very promising results have been showed also for genes coding for aquaporins. In field tests, tomato plants overexpressing the gene SlTIP2 developed significantly higher (26%) plant biomass than the control plants and this was also associated with a significant (up to 21%) increment of fruit yield under relatively mild and more severe regimens of water deficiency stress (Sade et al. 2009). Finally, Jeong et al. (2010) performed a 2-years field trials with rice transgenic plants overexpressing the gene OsNAC10, a transcription factor containing the NAC domain, under the control of the constitutive promoter GOS2 and the root-specific promoter RCc3. While the transgene under the control of the constitutive promoter did not improve grain yield in field with respect to wild types in both normal and drought conditions, the transgene under the control of the root-specific promoter was effective in increase the plant tolerance to drought, high salinity and low temperature at the vegetative stage. More importantly, the RCc3:OsNAC10 plants showed significantly enhanced drought tolerance at the reproductive stage, increasing grain yield by 25–42% and 5–14% over controls in the field under drought and normal conditions, respectively.

In the last decade many differently engineered plants have been proposed and tested for improved performance under abiotic stresses. Even though about 3,000 patents and patent applications emerge in CAMBIA's BiOS patent database by searching for "drought tolerance" and "drought resistance", however a large proportion is concerned with metabolic or stress-induced genes having doubtful functional significance under field conditions, as indicated by Passioura (2007). The evaluation of "drought tolerance" of transgenic plants has often been based on survival capacity, with very limited analyses of the transgene effects on yield potential. In general, the majority of abiotic stress studies are performed under controlled conditions, where a single constrain is applied as 'shock' treatment, and do not reflect the real conditions that occur in the field where the crops are routinely subjected to a combination of different constrains. Then the response of plants in field cannot be directly inferred from the response to each of different stresses applied individually (reviewed by Mittler 2006). This gap might explain why some transgenic plants, that showed enhanced tolerance to a particular stress when analysed in controlled conditions, failed to show this tolerance when tested in field (Borrelli et al. 2009). Very recently, the agriculture company Monsanto announced that its first-generation drought-tolerant corn has moved to the fourth, and final, phase before an anticipated market launch early next decade (http://monsanto.mediaroom.com/index.php?s=43&item=676). In field trials drought-tolerant corn have shown 6–10% yield enhancement in some of the key drought-prone areas in the United States.

7.8 Conventional Versus Molecular Breeding

To be advantageous with respect to classical phenotypic selection, MAS should either (i) provide an increased genetic gain per unit of time, which is the case when MAS permits selection at DNA level at an early stage in the development and/or when it permits reduction of the generation interval, or (ii) provide an increased genetic gain per unit of cost (i.e. molecular genotyping cheaper than phenotypic evaluation) (Hospital 2009).

A direct comparison of MAS vs CB (Conventional Breeding) for yield and yield-components improvements was run in cucumber (Robbins and Staub 2009). Four inbred lines were intermated and then maternal bulks were used to create four base populations for recurrent mass selection. Both MAS (genotyping at 18 marker loci) and CB (open-field evaluations) were practiced for yield indirectly by selecting for four yield-component traits that are quantitatively inherited with 2–6 quantitative trait loci per trait. These traits included multiple lateral branching, gynoecious sex expression (gynoecy), earliness, and fruit length to diameter ratio. Both MAS and CB provided improvements in all traits under selection in at least one population, except for earliness, which did not respond to MAS. Generally, CB was most effective for gynoecy, earliness, and fruit length to diameter ratio, while MAS was most effective for multiple lateral branching and provided the only increase in yield (fruits per plant). Therefore, both MAS and CB were useful for multi-trait improvement, but their effectiveness depended upon the traits and populations under selection.

Reliable information regarding comparative advantage of MAS over CB in breeding is scarcely available. There is a certain agreement in affirming that the cost of MAS is much lower than those of CB depending on the choice of markers. In a recent study, Abalo et al. (2009) carried out a comparative study in order to determine the efficiency of both methods in breeding for maize streak virus (MSV) resistance in Uganda. They found that, although both breeding approaches were effective in generating MSV-resistant lines, disease incidence was higher in populations under CS (79%) than MAS (62%). However, an equal number of lines generated by MAS and CS displayed high yield potential and MVS resistance in testcrosses. In this evaluation, MAS was cheaper than CS by 26%, and authors concluded that when laboratory facilities are already established, MAS would be recommended in breeding for MSV resistance (Abalo et al. 2009). Anyway, with the new technologies for high-throughput SNP development and analysis, costs of genotypization are expected to decrease significantly in the next future. In this scenario, the limiting step for marker assisted selection will be the identification of markers strongly linked to traits of interest and robust prediction models for genomic selection.

7.9 Concluding Remarks

Thanks to new molecular technologies, breeders now have new perspectives for plant improvement. Markers for single loci and QTLs as well as genes for plant transformation will provide an opportunity to integrate classical phenotype-based selection with selection on the basis of genotype. Based on all knowledge of the traits sustaining yield and drought resistance, randomly dispersed QTLs, transgenes or both can be accumulated into elite genotypes in a “breeding by design” strategy (Peleman and Van der Voort 2003). The understanding of the genetic bases of all agronomic traits and the analysis of allele variations at the corresponding loci would enable the breeders to design new ideotypes first in silico, then to construct the new genotypes *in planta*. For instance a combination of different approaches has been followed to develop new rice cultivars referred to as “Green Super Rice”, possessing resistance to multiple insects and diseases, high nutrient efficiency, and drought resistance, promising to greatly reduce the consumption of pesticides, chemical fertilizers, and water (Zhang 2007). Nevertheless, the polygenic basis of complex traits and the difficulty to follow minor QTLs with molecular markers strongly limit the application of MAS to traits related to drought resistance. Furthermore, the biparental mapping populations used in most QTL studies do not readily translate to breeding applications. However, high throughput sequencing has made marker discovery affordable for most crop species, and the continued reduction of genotyping costs will facilitate dense genome wide marker coverage for all crop species. The availability of large SNP panels for crops on one hand will accelerate the QTL discovery and transfer in MAS programs already in course; on

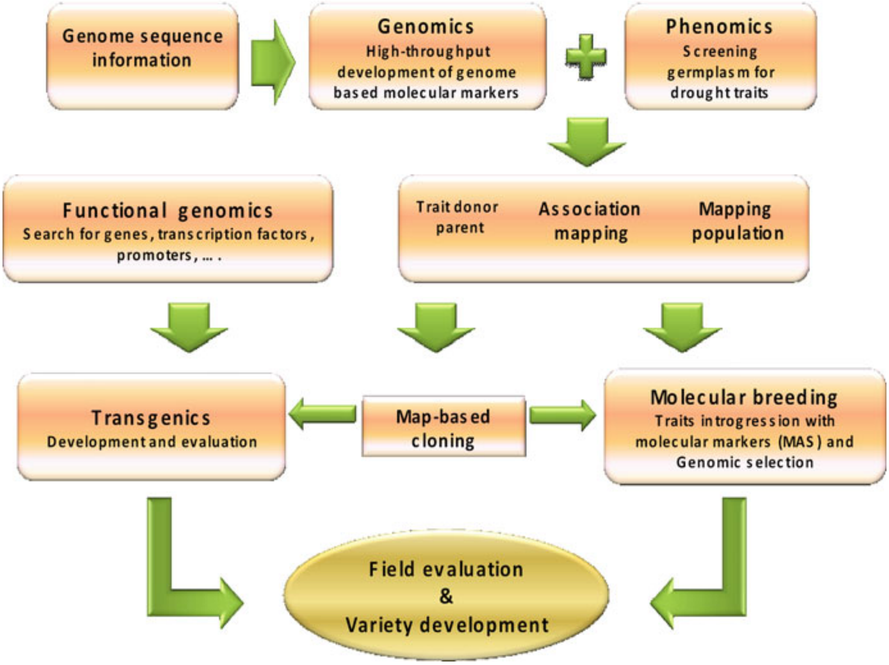

Fig. 7.1 Flowchart illustrating the relationships among the different genomic tools available to identify superior alleles for plant improvement

the other hand it will make possible genomic selection to accelerate the breeding cycle, and introduce greater flexibility in the relationship between phenotypic evaluation and selection (Fig. 7.1).

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Chapter 8
Plant Response and Tolerance to Abiotic Oxidative Stress: Antioxidant Defense Is a Key Factor

Mirza Hasanuzzaman, Mohammad Anwar Hossain, Jaime A. Teixeira da Silva, and Masayuki Fujita

Abstract In a persistently changing environment, plants are constantly challenged by various abiotic stresses such as salinity, drought, temperature extremes, heavy metal toxicity, high-light intensity, nutrient deficiency, UV-B radiation, ozone, etc. which cause substantial losses in the yield and quality of a crop. A key sign of such stresses at the molecular level is the accelerated production of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•). ROS are extremely reactive in nature because they can interact with a number of cellular molecules and metabolites, thereby leading to irreparable metabolic dysfunction and death. Plants have well-developed enzymatic and non-enzymatic scavenging pathways or detoxification systems to counter the deleterious effects of ROS that include the enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione *S*-transferase (GST), glutathione peroxidase (GPX) and peroxidases (POX) as well as non-enzymatic compounds such as ascorbate (AsA), glutathione (GSH), carotenoids and tocopherols. In plant cells, specific ROS-producing and scavenging systems are found in

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different organelles and the ROS-scavenging pathways from different cellular compartments are coordinated. Recent studies in plants have shown that relatively low levels of ROS act as signaling molecules that induce abiotic stress tolerance by regulating the expression of defense genes. Additionally, numerous results have shown that plants with higher levels of antioxidants, whether constitutive or induced, showed greater resistance to different types of environmental stresses. In this chapter we attempt to summarize recent researches on the mechanisms and possible regulatory roles of ROS in abiotic stress tolerance. Further, we discuss the progress made during the last few decades in improving the oxidative stress tolerance of plants through genetic engineering by different components of ROS detoxification systems in plants.

Abbreviations

ABA	abscisic acid
APX	ascorbate peroxidase
AsA	ascorbic acid
ATP	adenosine triphosphate
CAT	catalase
DHA	dehydroascorbate
DHAR	dehydroascorbate reductase
ETC	electron transport chain
GAP	glycerinaldehyde-3-phosphate
GO	glycolate oxidase
GPX	glutathione peroxidase
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidized glutathione
GST	glutathione *S*-transferase
HM	heavy metal
LOOH	lipid hydroperoxides
MDA	malondialdehyde
MDHA	monodehydroascorbate
MDHAR	monodehydroascorbate reductase
NADPH	nicotinamide adenine dinucleotide phosphate
NADPHox	NADPH oxidases
NO	nitric oxide
PC	phytochelatins
PCD	programmed cell death
PEG	polyethylene glycol
POX	peroxidases
ROOH	organic hydroperoxides
ROS	reactive oxygen species
RuBisCO	ribulose-1,5-bisphosphate carboxylase/oxygenase
Se	selenium
SNP	sodium nitroprusside

TG	total glutathione
XO	xanthine oxidase

8.1 Introduction

Plants are frequently exposed to a plethora of unfavorable or even adverse environmental conditions, termed abiotic stresses (such as salinity, drought, heat, cold, flooding, heavy metals, ozone, UV radiation, etc.) and thus they pose serious threats to the sustainability of crop yield (Bhatnagar-Mathur et al. 2008). Abiotic stresses remain the greatest constraint to crop production worldwide. It has been estimated that more than 50% of yield reduction is the direct result of abiotic stresses (Rodríguez et al. 2005; Acquaah 2007). Abiotic stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang et al. 2001). However, the rapidity and efficiency of these responses may be decisive for the viability of the given species.

Oxygen supports aerobic life of land plants granting them great energetic benefits but on the other hand challenges them through an endless formation of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•). However, certain environmental stresses or genetic defects cause the production of ROS to exceed the management capacity. ROS play two divergent roles in plants: at low concentrations, they act as signaling molecules for the activation of defense responses under stresses, whereas at high concentrations, they cause exacerbating damage to cellular components. If prolonged, abiotic stresses, through enhanced production of ROS, can pose a threat to cells by causing the peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of the programmed cell death (PCD) pathway and ultimately cell death (Mittler 2002; Sharma and Dubey 2005, 2007). Oxidative stress is essentially a regulated process, and the equilibrium between ROS and antioxidative capacity determines the fate of the plant. The enhanced production of ROS is, however, kept under tight control by versatile and cooperative ROS-scavenging antioxidant mechanisms that modulate intracellular ROS concentration (Apel and Hirt 2004). These mechanisms can be conveniently divided into two groups, viz. non-enzymatic and enzymatic antioxidants (Fig. 8.4). Under control conditions the antioxidant defense system provides adequate protection against active oxygen and free radicals (Asada and Takahashi 1987). However, under stressful situations the equilibrium between the production and scavenging of ROS may be perturbed and the response becomes moderate or low (Gill and Tuteja 2010). Several reports confirmed that enhanced antioxidant defense combats oxidative stress induced by abiotic stressors like salinity (Hasanuzzaman et al. 2011a, b; Hossain et al. 2011), drought (Selote and Khanna-Chopra 2010; Hasanuzzaman and Fujita 2011), heat (Chakrabortty and Pradhan 2011; Rani et al. 2011), cold (Zhao et al. 2009; Yang et al. 2011), flooding (Li et al. 2011a), heavy metal toxicity (Hossain et al. 2010; Gill et al. 2011a), UV-radiation (Kumari et al. 2010; Li et al. 2010b; Ravindran et al. 2010) and ozone (Yan et al. 2010a, b). As increasingly extreme environmental factors are having an ever greater effect on agriculture, plant biologists are facing with the urgent task of developing genotypes capable of tolerating environmental changes with the least possible damage. Hence it is first necessary to obtain knowledge on the defense and regulatory processes of plants. Developing plants with higher antioxidative potential provides an opportunity to develop plants with enhanced tolerance to abiotic stresses.

This chapter attempts to present an overview of our recent understanding on the physiology and molecular biology of plant tolerance mechanisms in response to abiotic stress factors. Special emphasis has been given to abiotic stress-induced ROS metabolism and differential regulation of the antioxidative defense system (both enzymatic and non-enzymatic) in inducing abiotic stress tolerance.

8.2 Abiotic Stressors in Plants

Most crops grown under field conditions are frequently exposed to various abiotic stresses. The complex nature of the environment, along with its unpredictable conditions and global climate change, are increasing gradually, which is creating a more adverse situation (Mittler and Blumwald 2010). A number of abnormal environmental parameters are collectively termed abiotic stress (Fig. 8.1). Abiotic stresses modify plant metabolism leading to harmful effects on growth, development and productivity. If the stress becomes very high and/or continues for an extended period it may lead to an intolerable metabolic load on cells, reducing growth, and in severe cases, result in plant death. However, plant stress may vary depending on the types of stressor and on the prevailing period. In nature, plants may not be completely free from abiotic stresses. They are expected to experience some degree of stress by any factor(s). Some environmental factors, such as air temperature, can become stressful in just a few minutes; others, such as soil water content, may take days to weeks, and factors such as soil mineral deficiencies can take months to become stressful (Taiz and Zeiger 2006).

8.3 Production of Reactive Oxygen Species in Plants

In plant cells, ROS are continuously produced as a consequence of aerobic metabolism in all the intracellular organelles, in particular in the chloroplast, mitochondria and peroxisomes (Apel and Hirt 2004). The chloroplast is the main source of ROS in plants. Insufficient energy dissipation during photosynthesis can lead to the formation of a chlorophyll triplet state that can transfer its excitation energy onto O_2 to make $^1O_2$ (Logan 2005). $O_2^{\bullet-}$ is produced by the photosynthetic electron transport chain (ETC) via the reduction of O_2 (Mehler reaction) (Apel and Hirt 2004), which is subsequently converted to H_2O_2 by superoxide dismutase (SOD) (Foyer and Noctor 2000). The photoproduction of ROS is largely affected by physiological and environmental factors, including high light intensity and drought stress (Asada 2006). Under conditions those impair CO_2 fixation in the chloroplast, the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) increases and the glycolate that is produced moves from chloroplasts to peroxisomes (Takahashi and Murata 2008;

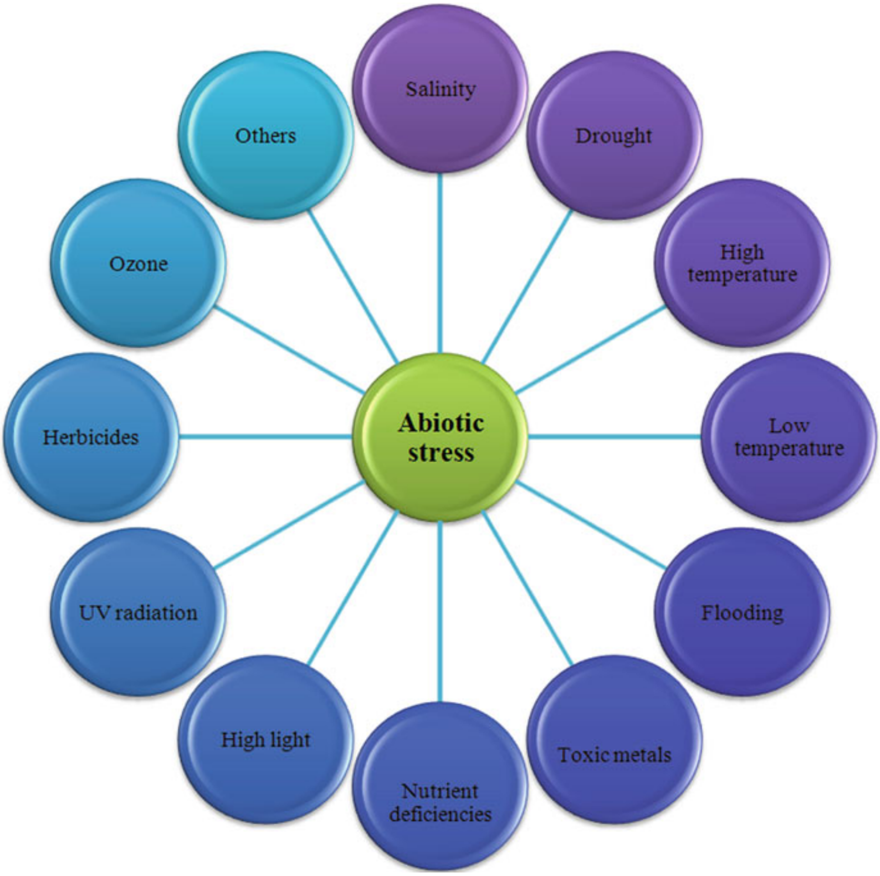

Fig. 8.1 Different types of abiotic stressors in plants

Fig. 8.2). In peroxisomes, the generation of H_2O_2 involves glycolate oxidation catalyzed by glycolate oxidase (GO), the β-oxidation of fatty acids and catabolism of lipids (Halliwell 2006). On the other hand, the generation of $O_2^{\bullet-}$ involves both the reaction of xanthine oxidase (XO) in the organelle matrix and a small electron transport chain at the peroxisomal membrane level. The plant mitochondrial electron transport chain is also an important source of ROS production in plant cells (Fig. 8.2) and consists of several dehydrogenase complexes that reduce a common pool of ubiquinone (Q). ROS production is likely to occur mainly in complex I (NADH dehydrogenase) and the Q zone (Møller 2001; Blokhina et al. 2003; Fig. 8.2). Although mitochondrial ROS production is much lower compared to chloroplasts, mitochondrial ROS are important regulators of a number of cellular processes, including stress adaptation and PCD (Robson and Vanlerberghe 2002). In glyoxysomes, acyl-CoA oxidase is the primary enzyme responsible for the generation of H_2O_2. Plasmamembrane-bound NADPH oxidases (NADPHox) as well as cell-wall associated peroxidases (POX) are the main sources of $O_2^{\bullet-}$ and H_2O_2 producing apoplastic enzymes activated by various forms of stress (Mittler 2002; Mhamdi et al. 2010).

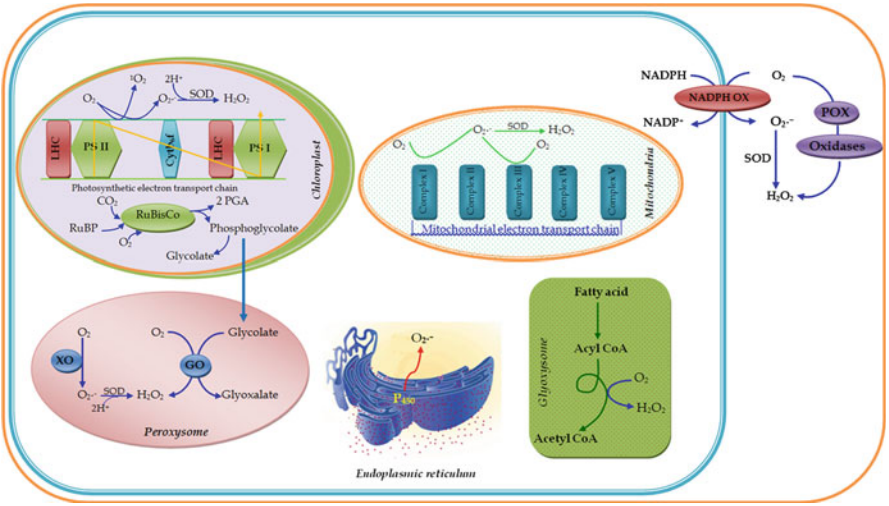

Fig. 8.2 Mechanisms of ROS production in different cell system (Adapted from Mhamdi et al. 2010)

Additional sources of ROS in plant cells include the detoxifying reactions catalyzed by cytochromes in both the cytoplasm and the endoplasmic reticulum (Urban et al. 1989).

8.4 Detoxification of ROS by the Antioxidant Defense System

In general, plant cells are adequately equipped to keep ROS within the limits that are generated as a consequence of normal cellular metabolic activities. Under different stress conditions, however, ROS generation often exceeds the overall cellular antioxidative potential leading to stress-induced adverse effects on plant growth and physiology. A steady state balanced is required to protect plant cells from oxidative damage (Fig. 8.3). Plants possess an efficient non-enzymatic (ascorbate, AsA; glutathione, GSH; α-tocopherol; phenolic compounds, alkaloids and non-protein amino acids) and enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione reductase, GR; glutathione peroxidase, GPX; glutathione *S*-transferase, GST and peroxidases, POX) antioxidant defense systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging ROS (Mittler et al. 2004; Gill and Tuteja 2010; Fig. 8.4). These antioxidant defense systems are found in almost all cellular compartments (Table 8.1, Fig. 8.5), demonstrating the importance of ROS detoxification for cellular survival (Mittler et al. 2004). These defenses are not restricted to the intracellular compartment, but are also found in the apoplast to a limited extent (Mittler 2002; Gill and Tuteja 2010).

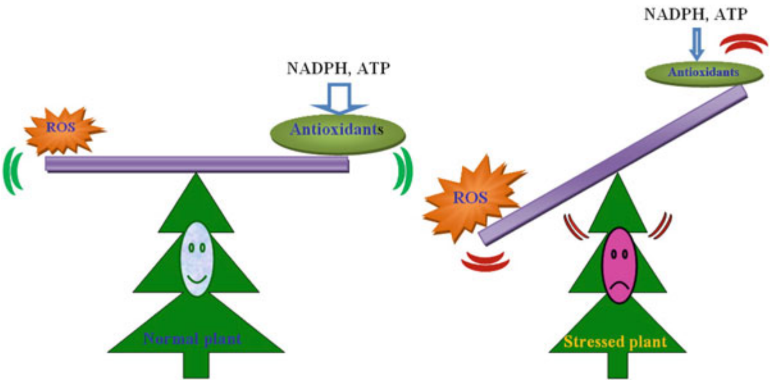

Fig. 8.3 The equilibrium and imbalance between ROS and antioxidants. Energy support also plays an important role in this equilibrium

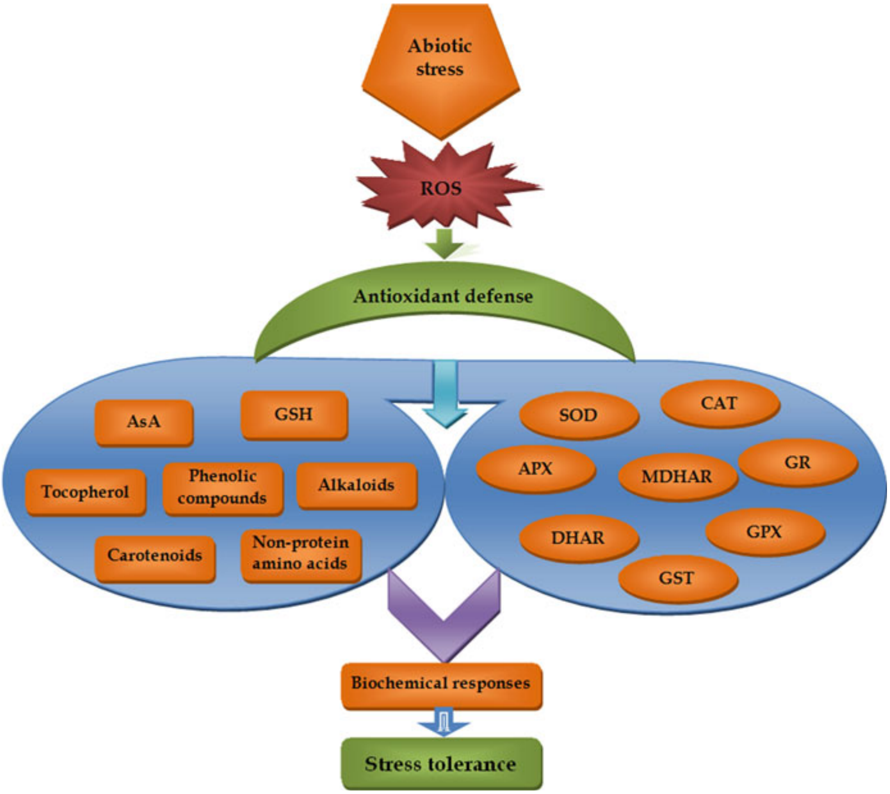

Fig. 8.4 Major non-enzymatic and enzymatic antioxidants involved in antioxidant defense system

Table 8.1 Different ROS-scavenging antioxidants and catalyzed reactions involved

Antioxidants	Enzyme code	Major reactions catalyzed	Site of reaction[a]
SOD	EC 1.15.1.1	$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$	Chl, Cyt, Apo, Mit, Per
CAT	EC 1.11.1.6	$H_2O_2 \rightarrow H_2O + ½O_2$	Per, Chl, Mit
APX	EC 1.11.1.11	$H_2O_2 + 2AsA \rightarrow 2H_2O + 2MDHA$	Chl, Cyt, Apo, Mit, Per
MDHAR	EC 1.6.5.4	$NADPH + H^+ + 2MDHA \rightarrow 2AsA + NADP^+$	Chl, Cyt, Mit
DHAR	EC 1.8.5.1	$DHA + 2GSH \rightarrow AsA + GSSG$	Chl, Cyt, Mit
GR	EC 1.6.4.2	$NADPH + H^+ + GSSG \rightarrow 2GSH + NADP^+$	Chl, Mit, Cyt
GPX	EC 1.11.1.9	$2GSH + ROOH\ (H_2O_2) \rightarrow GSSG + ROH + H_2O\ (2H_2O)$	Cyt, Mit
GST	EC 2.5.1.18	$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$ $RX + GSH \rightarrow HX + GS\text{-}R$	Chl, Cyt, Mit
AsA	–	Scavenges $O_2{\cdot}^-$, H_2O_2, OH·, and $^1O_2$	Chl, Cyt, Apo, Mit, Per
GSH	–	Scavenges H_2O_2, OH·, and $^1O_2$	Chl, Cyt, Apo, Mit, Per
Tocopherols	–	Scananges $^1O_2$, OH·, ROO· and ROOH	Membranes

Adapted from Mittler (2002), Blokhina et al. (2003), Ashraf (2009), and Gill and Tuteja (2010)

[a] *Chl* chloroplast, *Cyt* cytosol, *Mit* mitochondria, *Apo* apoplast, *Per* peroxisome, *R* may be an aliphatic, aromatic or heterocyclic group, *X* may be a sulfate, nitrite or halide group

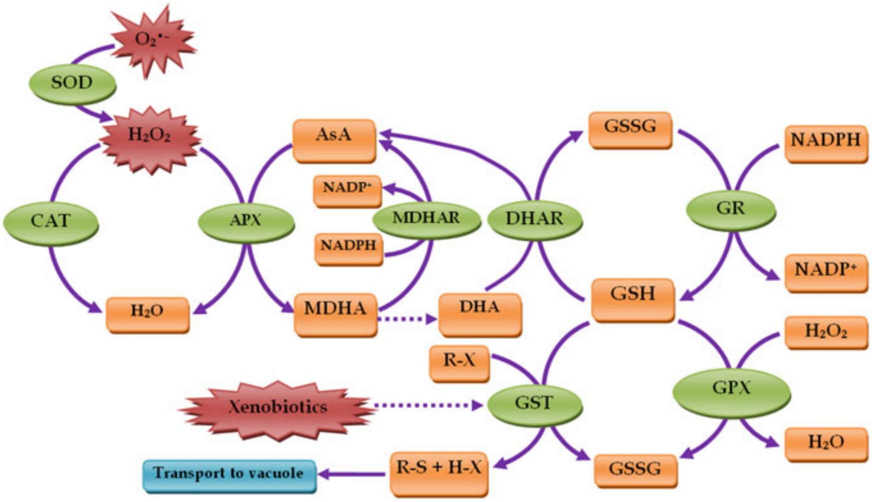

Fig. 8.5 Mechanisms of ROS detoxification by different antioxidant enzymes. *Dotted lines* denote non-enzymatic conversions. *R* may be an aliphatic, aromatic or heterocyclic group; *X* may be a sulfate, nitrite or halide group

8.4.1 Non-enzymatic Components

8.4.1.1 Ascorbate (AsA)

Ascorbate (AsA) is an important antioxidant in plant tissues which is synthesized in the cytosol of higher plants primarily from the conversion of D-glucose to AsA. It reacts with a range of ROS such as H_2O_2, $O_2^{\bullet-}$ and $^1O_2$, which are the basis of its antioxidant action. AsA, the terminal electron donor in these processes, scavenges free radicals in the hydrophilic environments of plant cells. It also scavenges OH• at diffusion-controlled rates (Smirnoff 2005). In the AsA-GSH cycle, two molecules of AsA are utilized by APX to reduce H_2O_2 to water with the concomitant generation of MDHA. MDHA is a radical with a short life span that can disproportionate into DHA and AsA. The electron donor is usually NADPH and the reaction is catalyzed by MDHAR or ferredoxin in a water–water cycle in the chloroplasts (Gapper and Dolan 2006). In plant cells, the most important reducing substrate for the removal of H_2O_2 is AsA (del Río et al. 2006; Wu et al. 2007). AsA is also thought to maintain the reduced state of the chloroplastic antioxidant, α-tocopherol. AsA in plants may be involved in the synthesis of zeaxanthin, which dissipates excess light energy in the thylakoid membranes, preventing oxidative damage (Conklin et al. 1996). AsA is also responsible for keeping prosthetic metal ions in a reduced form, thereby maintaining the activity of various antioxidant enzymes (De Tullio 2004). AsA plays an important role in plant stress tolerance (Sharma and Dubey 2005; Hossain et al. 2010; Hasanuzzaman et al. 2011a; Hossain et al. 2011). Exogenous application of AsA influences the activity of many enzymes and minimizes the damage caused by oxidative processes through synergic function with other antioxidants (Shalata and Neumann 2001).

8.4.1.2 Glutathione (GSH)

Glutathione (GSH) acts as an antioxidant and is involved directly in the reduction of most ROS (Noctor and Foyer 1998). Additionally, GSH plays a key role in the antioxidative defense system by regenerating other potential water-soluble antioxidants like AsA via the AsA-GSH cycle (Foyer and Halliwell 1976). It also plays an indirect role in protecting membranes by maintaining α-tocopherol and zeaxanthin in a reduced state. GSH prevents the denaturation of proteins caused by the oxidation of protein thiol groups under stress. In addition, GSH is a substrate for GPX and GST, which are also involved in the removal of ROS (Noctor et al. 2002a). Other functions for GSH include the formation of phytochelatins (PCs), which have an affinity to HM and are transported as complexes into the vacuole, thus allowing plants to have some level of resistance to HM (Sharma and Dietz 2006). GSH also takes part in the detoxification of xenobiotics and acts as a storage and transport form of reduced sulfur (Srivalli and Khanna-Chopra 2008). The role of GSH in the antioxidant defense system provides a strong basis for its use as a stress marker. The change in the ratio of its reduced (GSH) to oxidized (GSSG) form during the degradation of H_2O_2 is important in certain redox signaling pathways (Li and Jin 2007). GSH acts as a redox sensor of environmental cues, and an increase in GSH provides resistance to plants against oxidative stress. Recent reports suggest that an increase in GSH content enhances protection to various abiotic stresses (Hossain and Fujita 2010; Hossain et al. 2010; Hasanuzzaman et al. 2011a, b; Hasanuzzaman and Fujita 2011).

8.4.1.3 Tocopherol

Tocopherols is very abundant in the thylakoid membranes, which contain polyunsaturated fatty acids (PUFA) and are in close proximity to ROS produced during photosynthesis (Fryer 1992) and circumstantial and correlative evidence strongly suggest an antioxidant role for tocopherol (Munne-Bosch and Alegre 2003). There are four tocopherol and tocotrienol isomers (α, β, γ and δ). Relative antioxidant activity of the tocopherol isomers in vivo is $\alpha > \beta > \gamma > \delta$ and hence α-tocopherol has the highest antioxidant activity (Garg and Manchanda 2009). Tocopherols contribute to reduce ROS levels (mainly $^1O_2$ and OH•) in photosynthetic membranes and limits the extent of lipid peroxidation by reducing lipid peroxyl radicals (LOO•) to their corresponding hydroperoxides (Maeda et al. 2005). Tocopherols can physically quench and therefore deactivate $^1O_2$ in chloroplasts. Before being degraded, one molecule of α-tocopherol can deactivate up to 120 $^1O_2$ molecules by resonance energy transfer (Munné-Bosch 2007). Furthermore, tocopherols are part of an intricate signaling network controlled by ROS, antioxidants, and phytohormones, and are therefore good candidates to influence cellular signaling in plants (Munné-Bosch 2007).

8.4.2 Enzymatic Components

Antioxidant enzymes are located in different sites of plant cells and work together to detoxify ROS. The major antioxidant enzymes are SOD, CAT, GPX, GST and AsA-GSH cycle enzymes. The AsA–GSH cycle involves four enzymes (APX, MDHAR, DHAR and GR) as well as AsA, GSH and NADPH which work together to detoxify H_2O_2 in a series of cyclic reactions and further regenerate AsA and GSH (Fig. 8.5).

8.4.2.1 Superoxide Dismutases (SOD)

In plant cells, SODs constitute the frontline of defense against ROS. It removes $O_2^{\bullet-}$ by catalyzing its dismutation, one $O_2^{\bullet-}$ being reduced to H_2O_2 and another oxidized to O_2. SODs are classified based on the metal ion in their active site, namely copper and zinc (Cu/ZnSOD), manganese (MnSOD), and iron (FeSOD). Cu/ZnSOD is localized in the cytosol and chloroplasts, MnSOD in the matrix of mitochondria and peroxisomes, and FeSOD in the chloroplasts of some higher plants, but they are also generally found in prokaryotes (Scandalios 1993). The enhanced activity of SODs minimizes abiotic oxidative stress and has a significant role in the adaptation of a plant to stressed environments (Mobin and Khan 2007; Singh et al. 2008).

8.4.2.2 Catalases (CAT)

Catalases (CATs) are tetrameric heme-containing enzymes that use H_2O_2 as a substrate and convert it to H_2O and O_2, thus preventing cells from oxidative damage (Sanchez-Casas and Klesseg 1994). CATs are present in peroxisomes, glyoxysomes, and related organelles where H_2O_2-generating enzymes are located (Agarwal et al. 2009). CAT has one of the highest turnover rates of all enzymes: one molecule of CAT can convert around six million molecules of H_2O_2 to H_2O and O_2 per minute. Thus, CAT is important in removing H_2O_2, which is generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration, and purine catabolism (Gill and Tuteja 2010). It has also been reported that apart from its reaction with H_2O_2, CAT also reacts with some hydroperoxides (Ali and Alqurainy 2006). CAT activity shows variable trends under different abiotic stresses (Singh et al. 2008; Hasanuzzaman et al. 2011a, b; Hasanuzzaman and Fujita 2011).

8.4.2.3 AsA-GSH Cycle Enzymes

The AsA–GSH cycle is the major defense system against ROS in chloroplasts, cytosol, mitochondria, peroxisomes and apoplasts. The AsA–GSH cycle involves four enzymes (APX, MDHAR, DHAR and GR) as well as AsA, GSH and NADPH which work together to detoxify H_2O_2 in a series of cyclic reactions and further regenerate AsA and GSH (Fig. 8.5). In this cycle APX catalyses the reduction of H_2O_2 to H_2O with the simultaneous generation of monodehydroascorbate (MDHA), which is converted to AsA by the action of NADPH-dependent MDHAR or disproportionates nonenzymatically to AsA and dehydroascorbate (DHA) (Asada 1992). DHA undergoes irreversible hydrolysis to 2, 3-diketogulonic acid or is recycled to AsA by DHAR, which uses GSH as the reductant (Chen et al. 2003). This results in the generation of GSSG, which is regenerated to GSH by GR.

Ascorbate Peroxidases (APX)

The scavenging of H_2O_2 by APX is the first step of the AsA-GSH cycle and may play the most essential role in scavenging ROS and protecting cells in higher plants (Asada 1994). APXs are heme-containing enzymes involved in scavenging H_2O_2 in water-water and AsA-GSH cycles using AsA as the substrate, catalyzing the transfer of electrons from AsA to H_2O_2, producing DHA and water (Raven 2000; Pang and Wang 2010). The APX family consists of at least five different isoforms including mitochondrial (mAPX), thylakoid (tAPX) and glyoxisome membrane forms (gmAPX), as well as chloroplast stromal soluble form (sAPX), cytosolic form (cAPX) (Noctor and Foyer 1998). APX activity is enhanced in plants in response to during different abiotic stress conditions (Singh et al. 2008; Hossain et al. 2010; Hasanuzzaman and Fujita 2011).

Monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR)

The univalent oxidation of AsA leads to the formation of MDHA. If MDHA is not reduced again to AsA by MDHAR, it will spontaneously disproportionate into AsA and DHA. DHA is then reduced to AsA by DHAR in a reaction requiring GSH (Chen et al. 2003). Rapid regeneration is necessary in order to maintain the antioxidative capacity of AsA. The regeneration of AsA could be regulated in this cycle mainly by NADPH-dependent MDHAR activity (Mittova et al. 2000) and thus it is crucial for AsA regeneration and essential for maintaining a reduced pool of AsA (Martínez and Araya 2010). Although there are also a few reports about MDHAR activity in other physiological processes that are related to oxidative stress, research on different crops under environmental stresses revealed the regulatory role of MDAHR during oxidative stress tolerance and acclimation (Mittova et al. 2003; Hossain et al. 2010, 2011). MDHAR and DHAR are equally important in regulating the level of AsA and its redox state under oxidative stress (Eltayeb et al. 2006, 2007). DHAR is also a key component of the AsA recycling system (Martínez and Araya 2010) which regenerates AsA from the oxidized state (DHA) and regulates the cellular AsA redox state. It is thus crucial for tolerance to various abiotic stresses leading to the production of ROS. Increased DHAR activity was reported in response to various ROS-inducing stresses (Lee et al. 2007; Hossain et al. 2010; Hasanuzzaman et al. 2011a).

Glutathione Reductase (GR)

Glutathione reductase (GR) is a potential enzyme of the AsA-GSH cycle and plays an essential role in the defense system against ROS. Increased GR activity confers stress tolerance and has the ability to alter the redox state of important components of the ETC. This enzyme catalyzes the reduction of GSH, involved in many metabolic regulatory and antioxidative processes in plants where GR catalyses the NADPH-dependent reduction of disulphide bond of GSSG and is thus important for maintaining the GSH pool (Chalapathi Rao and Reddy 2008). Thus GR also maintains a high ratio of GSH/GSSG in plant cells, also necessary for accelerating the H_2O_2 scavenging pathway, particularly under stress conditions (Pang and Wang 2010). GR plays a crucial role in determining the tolerance of a plant under various stresses by maintaining the antioxidant machinery of the cell, confering stress tolerance (Sumithra et al. 2006; Hossain et al. 2011; Hasanuzzaman et al. 2011a).

8.4.2.4 Glutathione Peroxidases

Glutathione peroxidases (GPXs) are a large family of diverse isozymes that use GSH to reduce H_2O_2 and organic and lipid hydroperoxides (LOOHs), and therefore protect plant cells from oxidative stress (Noctor et al. 2002a). GPX is also a principal cellular enzyme capable of repairing membrane lipid peroxidation and is an important protectant against oxidative membrane damage (Kühn and Borchert 2002). In recent years, a number of GPXs genes have been identified from plant species (reviewed by Kumar et al. 2010). Apart from H_2O_2-detoxifying activity, GPX functions as an oxidative signal transducer (Miao et al. 2006).

8.4.2.5 Glutathione *S*-Transferases (GST)

Plant GSTs are a superfamily of multifunctional enzymes which catalyse the conjugation of electrophilic xenobiotic substrates with GSH (Dixon et al. 2010). Among the enzymes related to GSH metabolism, GST isoenzymes account for approximately 1% of a plants total soluble protein (Marrs 1996). GSTs catalyse the binding of various xenobiotics (including numerous pesticides) and their electrophilic metabolites with GSH to produce less toxic and more water-soluble conjugates (Edwards et al. 2000). Besides catalyzing the conjugation of electrophilic compounds to GSH, GST isoenzymes also exhibit POX activity (Gullner and Kömives 2001). Various abiotic stresses are powerful inducers of GST activity in plants (Dixon et al. 2010). Plant GSTs are also associated with responses to various forms of abiotic stress (Hossain et al. 2006; Dixon et al. 2010; Hossain and Fujita 2010; Hossain et al. 2010, 2011) and confer stress tolerance in plants.

8.5 Plant Responses and Antioxidant Defense Under Major Abiotic Stresses

8.5.1 Salinity

Soil salinity, one of the most severe abiotic stresses, limits the production of nearly over 6% of the world's land and 20% of irrigated land (15% of total cultivated areas) and negatively affects crop production worldwide. On the other hand, increased salinity of agricultural land is expected to have destructive global effects, resulting in up to 50% land loss by the middle of the twenty-first century (Mahajan and Tuteja 2005). Some of the adverse effects of salinity have been attributed to an increase in sodium (Na^+) and chloride (Cl^-) ions and hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms. Both Na^+ and Cl^- produce many physiological disorders in plants but Cl^- is the most dangerous. Due to the accumulation of Cl^-, relative salt tolerance has been linked to plant growth water use efficiency and transpiration. In addition to upper plant parts, salinity also effects root growth and physiology and ultimately their function in nutrient uptake.

8.5.1.1 Plant Responses to Salt Stress

A plant's response to salt stress depends on the genotype, developmental stage, as well as the intensity and duration of the stress. Increased salinity has diverse effects on the physiology of plants grown in saline conditions and in response to major factors like osmotic stress, ion-specificity, nutritional and hormonal imbalance, and oxidative damage. The outcome of these effects may cause the disorganization of cellular membranes, inhibit photosynthesis, generate toxic metabolites and decline nutrient absorption, ultimately leading to plant death (Mahajan and Tuteja 2005). In general, the response of a crop plant to salinity is reduced growth (Tavakkoli et al. 2011). Osmotic stress due to salinity leads to a slow growth rate and developmental characteristics such as vegetative development, net assimilation capacity, leaf expansion rate and leaf area index (Zheng et al. 2008; Hasanuzzaman et al. 2009). A reduction in photosynthesis is also one of the most conspicuous effects of salinity stress (Leisner et al. 2010; Raziuddin et al. 2011). To cope with salt stress, plants exhibit some morphological, anatomical, and physiological, or biochemical adaptive features which help them to sustain and thrive under saline conditions. Physiologically, a common mechanism in plants is the accumulation of certain compatible solutes such as glycerol, sucrose, trehalose, pinitol, proline and quaternary ammonium compounds such as glycinebetaine (Ashraf and Harris 2004). Generally, these compatible solutes protect plants from stress injury through different means, including the protection of cytoplasm and chloroplasts from salt-induced damage and scavenging ROS, stabilization of proteins and general maintenance of physiological stability of plants under stressful conditions (Galinski and Truper 1994; Ashraf and Harris 2004).

8.5.1.2 Oxidative Stress in Plants Under Salinity

In plants, salt stress can lead to the reduction of CO_2 availability and inhibit carbon fixation, exposing chloroplasts to excessive excitation energy which in turn could increase the generation of ROS (Gill and Tuteja 2010). Under salt stress, stomatal conductance in plants decreases to avoid excessive water loss which leads to a decrease in the internal CO_2 concentration (Ci) and slows down the reduction of CO_2 by the Calvin cycle. This response causes the depletion of oxidized NADP+, which acts as a final acceptor of electrons in photosystem I, and alternatively increases the leakage of electrons to O_2 forming $O_2^{\bullet -}$. In addition, Na^+/Cl^- toxicity resulting from salt stress could disrupt photosynthetic electron transport and provoke electron leakage to O_2. The decrease in Ci slows down the reactions of the Calvin cycle and induces photorespiration, resulting in the generation of more H_2O_2

in the peroxisome. The cell membrane-bound NADPHox and apoplastic diamine oxidase are also activated during salt stress and contribute to the generation of ROS (Ashraf 2009; Abogadallah 2010). In fact, it is not possible to determine the contribution of all sources to the generation of ROS under salt stress. Enhanced ROS production under salt stress induces phytotoxic reactions such as lipid peroxidation, protein degradation, and DNA mutations (Tanou et al. 2009). Several reports showed the overproduction of ROS in plants under saline conditions and ROS-induced membrane damage is a major cause of cellular toxicity by salinity (Mittova et al. 2004; Hossain et al. 2011; Hasanuzzaman et al. 2011a, b).

8.5.1.3 Antioxidant Defense in Plants Exposed to Salt Stress

When ROS increases in response to salinity, plants use a scavenging mechanism involving non-enzymatic and enzymatic antioxidants (Demiral and Turkan 2005). In tomato (*Lycopersicon esculentum*) seedlings, exogenous AsA increased the capacity to recover from salt stress (Shalata and Neumann 2001). The addition of exogenous AsA to the root medium remarkably increased seedling survival under NaCl stress (300 mM for 7, 8 or 9 h). In addition, exogenous AsA also partially inhibited increases in lipid peroxidation. Exogenously applied AsA partially or completely countered the inhibitory effects of salt stress in maize (Hamada and Al-Hakimi 2009). Under salt stress, the AsA-deficient *Arabidopsis* mutant *vtc-1* contained 30–60% of the AsA content of wild-type (WT) plants and accumulated a much higher level of H_2O_2 than WT (Huang et al. 2005), which coincides with a greater decrease in the ratio of reduced AsA to total AsA and with reduced activity of the AsA–GSH cycle enzymes. Likewise, GSH also plays a protective role in salt tolerance by maintaining the redox state. Investigation on the enzymatic pathways leading to GSH synthesis during the response to salt stress of WT and salt-tolerant *Brassica napus* L. (canola) plants showed that salt stress induced the assimilation of sulfur and the biosynthesis of cysteine and GSH in order to mitigate salt-induced oxidative stress (Ruiz and Blumwald 2002; Hussain et al. 2008). Sumithra et al. (2006) found that GSH concentration in the salt-stressed mung bean leaves of cv. Pusa Bold was higher than cv. CO 4, whereas GSSG concentration was higher in the leaves of CO 4 than in those of Pusa Bold, indicating that Pusa Bold was more tolerant than CO 4 as the levels of lipid peroxidation and H_2O_2 concentration in Pusa Bold was lower than in CO 4 under salt stress. In addition, maintaining a high ratio of GSH/GSSG plays an important role in salt tolerance (Hossain et al. 2011). Salt-tolerant cultivars of cotton had a higher GSH/GSSG ratio than salt-sensitive lines under saline conditions (Gossett et al. 1996).

The activity of ROS-scavenging enzymes is highly correlated with antioxidant defense and salt stress tolerance. However, the activities vary with plant cultivar, stress duration and dose. The generation of ROS and increased activity of many antioxidant enzymes during salt stress have been reported in different plant studies with several reports indicating that the activity of antioxidant enzymes of salt-tolerant genotypes increased in response to salinity whereas salt-sensitive species failed to do so (Mittova et al. 2002; Heidari 2009; Ghosh et al. 2011; Hasanuzzaman et al. 2011a, b; Hossain et al. 2011). El-Bastawisy (2010) concluded that salt tolerance was related to the endogenous levels of the enzymatic and the non-enzymatic antioxidants in wheat seedlings. Among the three wheat cultivars (H 168, Gimmeza 7 and Beni swif 1) under observation, the activities of SOD, CAT, APX and GR as well as the non-enzymatic antioxidants (AsA and GSH) increased mostly in H 168, but declined in Gimmeza 7 and particularly in Beni swif 1. H 168 had a superior antioxidant defense system and was more tolerant to NaCl than the other two cultivars due to the higher enzymatic and non-enzymatic antioxidants. Mittova et al. (2002) reported that, compared with cultivated tomato (L. *esculentum*), the better protection of wild salt-tolerant tomato (L. *pennellii*) root plastids from salt-induced oxidative stress was correlated with increased activities of SOD, APX and GPX. In another study, Vaidyanathan et al. (2003) investigated the immediate responses to salinity-induced oxidative stress in two major rice (Oryza sativa L.) cultivars, salt-sensitive Pusa Basmati 1 (PB) and salt-tolerant Pokkali (PK). Upon exposure to NaCl stress, PK showed higher activity of ROS-scavenging enzymes as well as enhanced levels of AsA and GSH than PB. Although SOD activity was lower in PK, it showed less lipid peroxidation and lower levels of H_2O_2 than PB under stress. Mandhania et al. (2006) observed that the activities of CAT, POX, APX and GR increased with an increase in salt stress in both sensitive and tolerant wheat cultivars, although SOD activity declined. Upon desalanization, partial recovery of the activity of these enzymes was observed in the salt-tolerant cultivar but a very slow recovery in the sensitive cultivars. Azooz et al. (2009) reported that the activity of CAT, POX, APX and SOD in salt-tolerant maize cultivars increased markedly during salinity stress but mostly decreased after salinity stress in the salt-sensitive cultivar. In another study, Dai et al. (2009a) indicated that the NaCl-induced gene expression and increased activities of SOD, CAT and POX enhanced the tolerance of oilseed rape plants against NaCl stress. In our study, mung bean seedlings, salt tolerance was correlated with higher activities of AsA-GSH cycle enzymes, including CAT and GPX (Hossain et al. 2011). GR, GPX and GST activities increased in response to salt stress (200 mM, 48 h), while the activities of MDHAR, DHAR and CAT decreased sharply with an associated increase in H_2O_2 and lipid peroxidation (expressed as malondialdehyde, MDA) level. Importantly, proline or betaine pretreated salt-stressed seedlings showed an increase in the activities of APX, DHAR, GR, GST, GPX and CAT involved in the ROS detoxification system compared to the untreated control and mostly salt-stressed plants with a simultaneous decrease in H_2O_2 and MDA level. Hasanuzzaman et al. (2011a) confirmed that the antioxidative system was enhanced by the application of exogenous selenium (Se), which induced oxidative stress in rapeseed (*Brassica napus*) seedlings subjected to salt stress (100 and 200 mM NaCl for 48 h). The AsA content of the seedlings decreased significantly with an increase in salt stress. The amount of GSH and GSSG increased with an increase in the level of salt stress, while the GSH/GSSG ratio decreased. In addition, APX and GST activity increased significantly with increased salt concentration (both at 100 and 200 mM NaCl), while GPX activity increased only at moderate salt stress (100 mM NaCl). GR activity remained unchanged at 100 mM NaCl, while it decreased under severe (200 mM NaCl) salt stress. The activity of MDHAR, DHAR and CAT decreased after salt stress was imposed whereas a sharp decrease in their activities was observed under severe salt stress (200 mM NaCl). A concomitant increase in the levels of H_2O_2 and MDA was also observed. More importantly, Se treatment in the salt-stressed seedlings increased the contents of AsA and GSH, the GSH/GSSG ratio, and the activities of APX, MDHAR, DHAR, GR, GST, GPX and CAT which led to a reduction in the levels of H_2O_2 and MDA compared to salt stress alone. The application of exogenous Se rendered the plants more tolerant to salt stress-induced oxidative damage by enhancing their antioxidant defense. In wheat seedlings, Hasanuzzaman et al. (2011b) further showed that modulation of ROS detoxification systems by exogenously applied SNP (an NO donor) improved oxidative stress tolerance of wheat seedlings subjected to salt stress (150 and 300 mM NaCl, 4 days). Salt-stressed seedlings pretreated with NO (1 mM SNP, 24 h) showed an increase in the AsA and GSH contents and the GSH/GSSG ratio as well as the activities of MDHAR, DHAR, GR, GST and GPX. Although different studies have established that the antioxidant defense system plays a crucial role in salt-stress tolerance in plants, defining salt tolerance has been quite difficult until now because of the complex nature of salt stress and the wide range of plant responses.

8.5.2 Drought

Drought is one of the most devastating environmental stresses that affects the growth and development of plants. The effects of drought stress are expected to increase with climate change and a growing water crisis (Harb et al. 2010). Thus, a better understanding of the effects of drought on plants is vital for improved management practices and breeding efforts in agriculture and for predicting the fate of natural vegetation under climate change. A plant suffers from drought stress due to the unavailability of water to the root zone or excessive transpiration rate. However, the adverse effects of drought stress on growth and development of crop plants are multifarious in nature.

8.5.2.1 Plant Responses to Drought

Plant responses to drought differ considerably depending on the intensity and duration of stress as well as plant species, cultivar and growth stage (Jaleel et al. 2008a, b). In general, drought stress affects the growth, dry matter production and economic yield of plants. Drought stress is characterized by a reduction of water content, decreased leaf water potential, turgor loss, stomatal closure and decrease in cell elongation and expansion (Jaleel et al. 2009; Mingchi et al. 2010; Din et al. 2011). However, water stress inhibits cell enlargement more than cell division (Jaleel et al. 2009). Reduced growth under drought stress is attributed to the impairment of various physiological and biochemical processes, such as photosynthesis, respiration, translocation of nutrients, ion uptake, and carbohydrate metabolism (Jaleel et al. 2008a, b, c). Drought stress followed by desiccation can potentially lead to gross disruption of metabolism and cell structure and eventually to the cessation of enzyme-catalyzed reactions (Smirnoff 1993). A reduction in chlorophyll content was reported in different crops grown under drought stress (Kiani et al. 2008). The leaf photosynthetic rate of higher plants under drought stress decreases due to the lower relative water content and leaf water potential (Lawlor and Cornic 2002). Drought stress also negatively affects dry matter partitioning and temporal biomass distribution (Petropoulos et al. 2008; Wu et al. 2008). In drought tolerance, plants are able to tolerate water deficiency by manipulating the biochemical and physiological parameters and thus avoiding the injurious effects of drought. Adaptation to drought is a complex process involving numerous changes including attenuated growth, the activation/increased expression or induction of genes, transient increase in abscisic acid (ABA), accumulation of compatible solutes and protective proteins, increased level of antioxidants and suppression of energy-consuming pathways (Bartels and Sunkar 2005).

8.5.2.2 Oxidative Stress in Plants Under Drought

Drought stress may lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of ROS and induce oxidative stress (Mittler 2002; de Carvalho 2008). The excess production of ROS during drought stress results from impaired electron transport processes in the chloroplasts and mitochondria (Smirnoff 1993). Down-regulation of PSII results in a disproportion between the generation and utilization of electrons, resulting in changes in quantum yield. These changes in the photochemistry of chloroplasts in the leaves of drought-stressed plants result in the dissipation of excess light energy in the PSII core and antenna, thus generating free radicals like O_2^-, $^1O_2$, H_2O_2 and OH•, which are potentially dangerous under drought stress (Li et al. 2010a; Faize et al. 2011). In fact, under drought stress, ROS production is enhanced in different ways. However, it is quite complicated to assess the part of ROS generated by the Mehler reaction to that generated by photorespiration. Photorespiration is one of the major causes of ROS production under drought stress; more than 70% of total H_2O_2 is produced due to photorespiration (Noctor et al. 2002b). ROS accumulation and oxidative stress increase under drought stress (Li et al. 2010a; Faize et al. 2011; Sorkheha et al. 2011) and drought-induced oxidative stress significantly increases lipid peroxidation (Pandey et al. 2010; Hasanuzzaman and Fujita 2011).

8.5.2.3 Antioxidant Defense in Plants Exposed to Drought Stress

The enhancement of antioxidant defense mechanisms is considered to be an adaptive mechanism of plants to drought stress and the strengthening of these defense mechanisms, through the enhanced functions of antioxidant components (enzymatic and non-enzymatic), may reduce or prevent oxidative damage and improve the drought resistance of plants (Sharma and Dubey 2005; de Carvalho 2008; Jaleel et al. 2009). AsA is one of the strongest non-enzymatic antioxidants that provides better protection against drought stress (Yazdanpanah et al. 2011). Increased AsA content in mung bean seedlings supplemented with proline and glycinebetine conferred better protection against drought stress (Hossain and Fujita 2009). Under drought stress, the protective action of the GSH system against oxidation of sulfhydryl groups of soluble proteins is established (Loggini et al. 1999). Selote and Khanna-Chopra (2006) demonstrated that mild water deficit stress did not change the GSH pool but the GSH/GSSG ratio was altered in the leaves of drought-acclimated wheat seedlings. Exposure to severe drought stress, however, resulted in a drastic decline in GSH redox pool in the leaves of non-acclimated plants than that of drought-acclimated ones (Selote and Khanna-Chopra 2006). This might be due to either higher biosynthesis or regeneration of GSH accompanied by the enhanced activities of the AsA–GSH cycle enzymes in the leaves of drought-acclimated wheat seedlings during severe water stress. In addition to AsA and GSH, several reports showed that drought stress resulted in an increase in α-tocopherol levels that enhanced stress tolerance (Munné-Bosch et al. 2009).

In parallel to non-enzymatic antioxidants, the activity of antioxidant enzymes also play a significant role in drought stress tolerance of many plants (Sharma and Dubey 2005; Hossain and Fujita 2009; Selote and Khanna-Chopra 2010; Hasanuzzaman and Fujita 2011). SOD and the enzymes of the AsA-GSH cycle (APX, MDHAR, DHAR and GR) appear to function as important components of the antioxidative defense system under drought-induced oxidative stress in rice (Sharma and Dubey 2005). Hossain and Fujita (2009) also observed short-term enhanced drought tolerance with increased activities of APX, MDHAR, DHAR, GR, GPX, GST and CAT in mung bean seedlings, confirmed by lower levels of H_2O_2 and MDA. Mohammadkhani and Heidari (2007) observed a positive and strong correlation between antioxidant enzymes and drought stress while investigating the responses of *Zea mays* L. var. 704 (drought-tolerant) and var. 301 (drought-sensitive). With 40% polyethylene glycol (PEG), the activity of GPX, APX and CAT in the roots and shoots of 704 plants was higher than in the control. However, enzyme activity decreased in 301 sensitive plants under drought than in 704 tolerant plants. Shehab et al. (2010) reported an increase in the activity of various antioxidant defense enzymes (SOD, APX, GR and CAT) in rice, representing protective activity to counteract the oxidative injury caused by drought. Selote and Khanna-Chopra (2010) demonstrated that drought acclimation induces oxidative stress tolerance of wheat seedlings, attributed to a well-coordinated induction of the ROS detoxification system. Direct exposure of severe water stress to non-acclimated seedlings caused greater water loss, excessive accumulation of H_2O_2 followed by elevated lipid peroxidation due to the poor response of antioxidant enzymes, particularly APX, MDHAR, DHAR, GR and the AsA–GSH redox balance. Drought-acclimated wheat roots during subsequent severe water stress conditions enhanced systematic up-regulation of SOD, APX, CAT, POX, and AsA–GSH cycle components at both the whole cell level as well as in mitochondria and maintained a higher relative water content and lower level of H_2O_2. Furthermore, termination of stress followed by rewatering led to a rapid enhancement of all the antioxidant defense components in non-acclimated roots, suggesting that the excess levels of H_2O_2 during severe water stress conditions might have inhibited or down-regulated the antioxidant enzymes. In a study with ten cultivars of oilseed rape (*B. napus*), Abedi and Pakniyat (2010) reported that the oilseed rape variety with the highest level of enzyme activity under both optimum and limited irrigation regimes (drought) was considered to be the most tolerant cultivar while the varieties with the lowest enzymes activities were considered to be sensitive to drought stress. Filippou et al. (2011) suggested that CAT has a primary role in H_2O_2 detoxification in Medicago plants: CAT was significantly induced in leaves after imposing water stress as well as in roots after 3 days of water stress in comparison with cAPX, which was differentially regulated (suppressed expression). Sečenji et al. (2010) demonstrated that the expression levels of cAPX and tAPX variants increased significantly in a drought-tolerant wheat cultivar while cytosolic and stromal APX-coding transcripts were higher in a drought-sensitive cultivar after a 4 week-long water deficit stress. However, Sofo et al. (2005) suggested that CAT may be less important than APX in scavenging H_2O_2 in roots in long-term stress. Sánchez-Rodríguez et al. (2010) further demonstrated that drought tolerance of a tolerant tomato cultivar (*L. esculentum* cv. Zarina) is attributed to a higher antioxidant defense system. Five tomato cultivars were subjected to mild water stress (50% field capacity) and maintained for 22 days. Drought stress significantly increased the H_2O_2 and MDA level in all the cultivars except for Zarina. Analysis of antioxidants revealed that the activities of APX, DAHR, MDHAR and GR increased sharply in the tolerant cultivar more than in others. Importantly, Zarina maintained a higher level of AsA under drought stress. Therefore, appropriate induction of both enzymatic and non-enzymatic antioxidant defense systems allowed cv. Zarina to be tolerant to drought-induced oxidative stress.

The importance of well coordinated antioxidant defense in inducing drought tolerance was also observed in a study with rapeseed seedlings (*B. napus* cv. BINA sharisha 3) by using exogenous Se (Hasanuzzaman and Fujita 2011). Drought stress (mild or severe) caused a significant increase in GSH and GSSG content; however, the AsA content increased only under mild stress. The activity of APX was not affected by drought stress. MDHAR and GR activities increased only under mild stress. The activities of DHAR, GST and GPX significantly increased under any level of drought stress, while CAT activity decreased. Importantly, the Se-pretreated (25 μM Na_2SeO_4, 48h) seedlings exposed to drought stress showed a rise in AsA and GSH content, maintained a high GSH/GSSG ratio, and evidenced increased activities of APX, DHAR, MDHAR, GR, GST, GPX and CAT accompanied by lower levels of H_2O_2 and MDA. Coordinated induction of AsA and GSH and their metabolizing enzymes, a consequence of the application of exogenous Se, rendered the plant tolerant to drought-induced oxidative stress. Based on the above reports we concluded that better antioxidant protection is vital for plant growth and development under drought stress conditions.

8.5.3 *High Temperature*

High temperature or heat stress results from temperatures high enough to damage plant tissues, substantially influencing the growth and metabolism of plants (Balla et al. 2009). Although variable for different plant species, temperatures in the range of 35–45°C produced heat stress effects on tropical plants (Hall 1992). However, the extent to which this occurs in specific climatic zones depends on the probability and period of high temperatures occurring during the day and/or at night. Different global circulation models predict that greenhouse gases will gradually increase the world's average ambient temperature and lead to global warming (Meehl et al. 2007). Therefore, plants' responses and adaptation to elevated temperature and the mechanisms to develop heat-tolerant cultivars should be examined.

8.5.3.1 Plant Responses to High Temperature

Plant responses to high temperatures vary with the degree of temperature, duration and plant type. At very high temperatures, cellular damage or cell death may occur within minutes, which may lead to a catastrophic collapse of cellular organization (Schöffl et al. 1999). However, at moderately high temperatures, cell injury or death may occur only after long-term exposure. These injuries ultimately lead to starvation, inhibited growth, reduced ion flux, and the production of toxic compounds and ROS (Howarth 2005). The main symptoms of high temperature stress on plants may include scorching of leaves and twigs, sunburn on plant organs, leaf senescence and abscission (Guilioni et al. 1997; Ismail and Hall 1999). High temperature causes a delay in seed germination and a loss of vigor (Egli et al. 2005) as well as reduced plant emergence. Shoot dry mass, relative growth rate and net assimilation rate are significantly reduced by high temperature (Wahid 2007). High temperatures can cause fruit discoloration and damage, and reduce yield (Wahid et al. 2007). More importantly, heat stress, singly or in combination with drought, is a common constraint during anthesis and grain-filling stages in many cereal crops of temperate regions. Heat stress reduces kernel growth, ultimately causing the loss of kernel weight and density (Guilioni et al. 2003; Monjardino et al. 2005). A familiar consequence of high temperature in plants is the heat-induced imbalance in photosynthesis and respiration (Wahid et al. 2007). Normally, photosynthetic activity remains stable up to 30°C but decreases sharply above this temperature to reach complete inhibition at about 40°C (Bar-Tsur et al. 1985). However, high temperature influences the photosynthetic capacity of C3 plants more strongly than in C4 plants. High temperature stress is often associated with reduced water availability in field conditions (Simões-Araújo et al. 2003). Under heat stress, different plant species may accumulate a variety of osmolytes such as sugars and sugar alcohols (polyols), proline, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds. Accumulation of such solutes may contribute to enhanced high temperature stress tolerance of plants (Wahid et al. 2007).

8.5.3.2 Oxidative Stress in Plants Under High Temperature

High temperature stress impaired mitochondrial functions and resulted in induced oxidative damage (Suzuki and Mittler 2006). Extreme temperature stress accelerates the generation and reactions of ROS including $^1O_2$, $O_2^{\bullet -}$, H_2O_2 and OH•, thereby inducing oxidative stress (Mittler 2002; Yin et al. 2008). Under high temperature, RuBisCO can lead to the production of H_2O_2 as a result of its oxygenase reactions (Kim and Portis 2004). The main effects of ROS include autocatalytic peroxidation of membrane lipids and pigments, modification of membrane permeability and functions (Xu et al. 2006). A number of research works has revealed high temperature-induced oxidative damages in plants (El-Shintinawy et al. 2004; Yin et al. 2008). The drastic increase in lipid peroxidation due to high temperature stress was reported by many scientists (Mo et al. 2010; Wu et al. 2010).

8.5.3.3 Antioxidant Defense in Plants Exposed to High Temperature Stress

Tolerance to high temperature stress in crop plants is associated with an increase in antioxidants (Almeselmani et al. 2006; Babu and Devraj 2008; Almeselmani et al. 2009) Studies on heat-acclimated versus non-acclimated turfgrass species suggested that the former had lower production of ROS as a result of enhanced synthesis of AsA and GSH (Xu et al. 2006). In wheat, heat stress induced the accumulation of GSH levels and increased the activity of the enzymes involved in GSH synthesis and the GSH/GSSG ratio (Kocsy et al. 2002). Chauhan (2005) observed a heat stress-induced increase in the levels of GSH in the flag leaf of two wheat genotypes with contrasting behavior in heat tolerance at all stages of grain development. Almeselmani et al. (2006) found a significant increase in the activity of SOD, APX and CAT in late and very late planting and at all growth stages of wheat; however, GR and POX activity decreased in late and very late planting (exposed to high temperature) compared to normal planting. Later on, Almeselmani et al. (2009) observed that the activities of SOD, APX, CAT, GR and POX increased significantly at all stages of growth in heat-tolerant cultivars (C 306) in response to heat stress while the susceptible cultivar (PBW 343) showed a significant reduction in CAT, GR and POX activities in the high temperature treatment. A significant increase in the APX-mRNA level under heat stress at the vegetative and anthesis stages was also observed; expression was greater in C 306. Several authors also reported the involvement of SOD in temperature stress tolerance (Lu et al. 2008; Zhao et al. 2009). Badawi et al. (2007) reported higher GR activity in heat-tolerant wheat cultivars compared to sensitive cultivars. Babu and Devraj (2008) observed that heat stress drastically reduced the activities of GR and CAT in French bean (*Phaseolus vulgaris*). However, no variations were observed in APX, POX, and CAT isozymes. Zhao et al. (2009) reported that the co-expression of GST and CAT in heat-induced plants had an important effect on the antioxidant system, in particular, the whole AsA–GSH cycle. Rani et al. (2011) exposed a 5-day-old thermo-tolerant genotype, namely BPR-542-6, and a thermo-susceptible genotype, namely NPJ-119, of *Brassica juncea* to high temperature stress (45.0 ± 0.5°C) and observed that the activities of SOD, POX, CAT, APX and GR increased, although the increase was significant only in the tolerant genotype. On revival, SOD and CAT began to decrease but the activities of POX and GR continued to increase in both the genotypes. APX, however, continued to increase in the tolerant genotype but started to decrease in the susceptible genotype. Chakraborty and Pradhan (2011) observed that CAT, APX and SOD showed an initial increase before declining at 50°C, while POX and GR activities declined at all temperatures ranging from 20 to 50°C. In addition, total antioxidant activity was maximum at 35–40°C in the tolerant varieties and at 30°C in the susceptible ones. Clearly, an increase in temperature leads to the increased expression of these antioxidative enzymes until a pre-determined temperature after which they decline, this temperature varying in tolerant and susceptible varieties. Tolerant varieties could maintain increased activities at higher temperatures than susceptible ones (Chakraborty and Pradhan 2011).

8.5.4 Low Temperature

About two-thirds of the world's land is annually subjected to temperatures below freezing point and about half of it suffers from temperatures below −20°C (Larcher 2001). Thus, in most regions around the world, plants are exposed to low temperature at least part each year. Among the abiotic stresses, low temperature stress is a serious threat to the sustainability of crop yield. Chilling stress results from temperatures cool enough to produce injury without the formation of ice in plant tissues whereas in freezing stress ice forms in plant tissues. Both chilling and freezing stresses are together termed cold stress. Chilling stress usually occurs at temperature between 0 and 10°C but a few tropical species such as rice and sugarcane are exceptionally sensitive to chilling and show injury signs up to 15°C (Thomashow 1999).

8.5.4.1 Plant Responses to Low Temperature Stress

There are various effects of low temperature stress depending on the species, plant age, and the duration of exposure. Low temperature may impose stress on a plant in two ways: By the effects of low temperature alone, and by dehydration of the cells and tissues when cellular water freezes. Low temperature stress affects seedlings more than mature plants with noticeable symptoms on plants including surface lesions, a water-soaked appearance, desiccation, discoloration, tissue breakdown, accelerated senescence and faster decay due to leakage of plant metabolites (Sharma et al. 2005; Solanke and Sharma 2008). Another major negative effect of low temperature stress is that it induces severe membrane damage which is largely due to acute dehydration associated with freezing (Yadav 2010). Low temperature-sensitive plants show a physical transition of the cell membrane from a flexible liquid-crystalline to a solid gel phase thereby affecting the cellular function in a number of ways. Thus the immediate effect is higher membrane permeability and ion leakage (Farooq et al. 2009). In extreme cases, chilling stress results in accelerated senescence and eventually plant death (Sharma et al. 2005). Low temperature stress also severely hampers the reproductive development of plants which may cause floral sterility (Nahar et al. 2009; Yadav 2010). Chilling stress also affects the root growth of plants (Einset et al. 2007; Farooq et al. 2009). These changes limit the roots' capacity for water and mineral uptake and ultimately overall plant growth (Ercoli et al. 2004; Farooq et al. 2009). Low temperature reduces dry matter production and partitioning in crop plants (Verheul et al. 1996).

8.5.4.2 Oxidative Stress in Plants Under Low Temperature

With decreasing temperature, the solubility of a gas increases, which leads to a higher concentration of O_2 and thus enhances the risk of oxidative stress at low temperature which leads to the increased production of $O_2^{\bullet-}$, H_2O_2, $^1O_2$, and OH• (Guo et al. 2006). Low temperature conditions aggravate the imbalance between light absorption and light use by inhibiting the activity of the Calvin–Benson cycle. In addition, enhanced photosynthetic electron flux to O_2 and the over-reduction of respiratory ETC causes ROS accumulation during chilling (Hu et al. 2008). During cold treatment, the enzymes of the Calvin-Benson cycle are slowed by simple thermodynamics, thus limiting the supply of $NADP^+$ for reduction and ADP and Pi for phosphorylation. Incoming light energy continues to be channeled into ETC as long as the pigment beds remain intact and connected to the photosystems (PS I and PS II). These two factors, a slowing of the dark reactions and continuing energy absorption, over-reduce the photosynthetic ETC leading to the leakage of absorbed energy in an uncontrolled manner from the thylakoid membrane. As the light-independent reaction of photosynthesis is very temperature sensitive, the energy leaked during chilling in light causes the formation of ROS (Wise 1995), whose increased concentration causes damage to membrane lipids, proteins and nucleic acids, leading to PCD (Apel and Hirt 2004). Low temperature increases MDA content as a result of oxidative stress (Mo et al. 2010).

8.5.4.3 Antioxidant Defense in Plants Exposed to Low Temperature Stress

The improvement of low temperature stress tolerance is often related to the enhanced activities of enzymes of antioxidant systems in plants. Plants exposed to low temperatures use several non-enzymatic and enzymatic antioxidants to cope with the harmful effect of oxidative stress; higher contents of antioxidant defense enzymes are correlated with higher chilling tolerance (Kang and Saltveit 2002; Huang and Guo 2005). Antioxidant enzymes have higher activity in chilling-tolerant cultivars than in susceptible ones (Guo et al. 2005).

Zhang et al. (2008) observed a significant increase in AsA and GSH levels in maize plants during low temperature stress. In addition, increases in GSH levels and/or GR activity during low temperature stress have been reported in different plant studies (Kocsy et al. 2000, 2001; Bhowmik et al. 2008). Guo et al. (2006) tested four rice cultivars under chilling conditions and concluded that chilling tolerance was well correlated with the enhanced antioxidant capacity of the cultivars, which was attributed to the higher AsA content and increased activity of antioxidant enzymes like APX and GR. Several studies reported enhanced TG levels in low temperature-tolerant plants like maize, tomato, and turf grass (Bhowmik et al. 2008) more than in sensitive plants. During low temperature acclimation, the maintenance of a high GSH/GSSG ratio is very important to ensure the functionality of GSH in the AsA-GSH cycle and other physiological processes (Kocsy et al. 2000). During cold-acclimation, ROS scavenging enzyme systems are activated, which help to detoxify ROS and increase tolerance to cold stress. A number of experiments comparing different species have reported that low temperature-tolerant plants showed greater activities in antioxidant enzymes than in sensitive ones. Huang and Guo (2005) found the higher efficiencies of antioxidant enzymes in chilling-tolerant rice cultivars than in chilling-susceptible cultivars. They observed that the activities of SOD, CAT, APX and GR, as well as AsA content of tolerant cultivar (Xiangnuo-1) remained high, while those of a chilling susceptible cultivar (IR-50) decreased under chilling. Zhao et al. (2009) observed a strong relationship between chilling sensitivity and the activities of antioxidant enzymes of postharvest tomato fruits exposed to short-term (24 h) or long-term (20 days) chilling stress. They observed that the chilling tolerance of tomato cultivars could obviously be indicated by higher activities of CAT, APX, POX and SOD. Dai et al. (2009b) reported that the elevated activities of APX and GR allow the cell to cope with oxidative stress due to chilling. Yang et al. (2011) observed that the enhanced activities of SOD, CAT, APX and POX in *Cucumis sativus* plants reflected better tolerance to chilling injury. Prasad (1997) observed higher GR and GPX activities in acclimated seedlings compared with non-acclimated seedlings during low-temperature stress and recovery. Gechev et al. (2010) reported that CAT and DHAR are most strongly affected by chilling (5°C) and may be the rate-limiting factor of the antioxidant system at low temperatures. In chickpea cultivars the activities of SOD, APX, GR and POX increased in cold-acclimated plants and subsequent chilling stress (2 and 4°C for 12 days), which indicated the enhanced chilling tolerance capacity of this cultivar to protect plants from oxidative damage (Turan and Ekmekçi 2011).

8.5.5 Waterlogging

Due to the increased frequency of extreme climate events, waterlogging has become an important constraint to crop production globally, causing a significant reduction in yield (Wollenweber et al. 2003). Waterlogging stress may develop due to several direct (improper irrigation practices) and indirect (global warming) anthropogenic and natural consequences (meteorological) leading to altered plant metabolism, architecture and ecogeographical distribution depending upon a plant's responses.

Waterlogging induces the progressive reduction in soil O_2 concentration and in redox potential (Ruiz-Sánchez et al. 1996), which contribute to the appearance of several reduced compounds of either chemical or biochemical origin (Kozlowski 1997). Alarming changes in the earth's average temperature, erratic rainfall, and rise in sea level due to increasing melting glaciers could exaggerate waterlogging or flooding problems in the near future.

8.5.5.1 Plant Responses to Waterlogging

The growth and development of most of the higher plant species are hampered by soil flooding, and particularly by complete submergence, both of which can result in death (Jackson and Colmer 2005). During waterlogging, the gas exchange between soil and the upper atmosphere decreases, and as gas diffusion in water decreases many fold, O_2 in the soil declines rapidly, and the soil may become hypoxic or anoxic within a few hours (Malik et al. 2002). One of the initial responses to waterlogging stress appears to involve the closing of stomata to avoid water loss, with a subsequent down-regulation of the photosynthetic machinery (García-Sánchez et al. 2007). Under submerged conditions, there is a decrease in total chlorophyll content in plants (Damanik et al. 2010), which sometimes respond to waterlogging by reducing leaf water potential, stomatal conductance, gas exchange and plant growth (Arbona et al. 2008). During long-term soil submergence, root hydraulic conductance decreases, which impairs water uptake and eventually leads to leaf wilting and chlorosis in citrus (Arbona et al. 2008). However, many wetland plant species can sustain themselves in flood-prone areas, achieved by a combination of genetic potential and some major physiological adaptations and acclimation such as physical 'escape' from a submerged environment (Voesenek et al. 2003), avoidance of O_2-deficiency through effective internal aeration (Jackson and Armstrong 1999), tolerance to anoxia (Gibbs and Greenway 2003), and the capacity to prevent or repair oxidative damage (Blokhina et al. 2003).

8.5.5.2 Oxidative Stress in Plants Under Waterlogging

Waterlogging, like other abiotic stresses, also leads to oxidative stress through an increase in ROS, such as $O_2^{\bullet -}$, $^1O_2$, H_2O_2 and OH• (Arbona et al. 2008). ROS are produced at the transition when a plant or any of its parts either enters to hypoxia/anoxia from normoxic conditions or returns to an aerobic environment (Irfan et al. 2010). Kumutha et al. (2009) and Sairam et al. (2009) showed that hypoxia-induced ROS are due to induction of membrane-linked NADPH oxidase. Higher accumulation of H_2O_2 and increased lipid peroxidation under anaerobic conditions has been reported by several researchers (Hossain et al. 2009; Kumutha et al. 2009; Sairam et al. 2011).

8.5.5.3 Antioxidant Defense in Plants Exposed to Waterlogging Stress

In many plant systems, the involvement of oxidative stress in flooding-induced damage and the antioxidant response was studied and a direct relationship between an increase in antioxidant activity and stress tolerance was observed (Arbona et al. 2008; Bin et al. 2010). Waterlogging stress resistance may depend, at least in part, on the enhancement of the antioxidative defense system which includes antioxidant enzymes such as SOD, CAT, APX, MDHAR, DHAR, GR, GPX as well as other non-enzymatic antioxidant compounds such as AsA, GSH, carotenoids and α-tocopherol (Arbona et al. 2008; Hossain et al. 2009; Bin et al. 2010).

Under waterlogging, leaves of citrus showed a significant increase in total ascorbic acid, AsA, DHA, and AsA/DHA ratio in stressed plants than in control conditions (Arbona et al. 2008). Likewise, TG, GSH and GSH/GSSG ratio also increased with a concomitant decrease in GSSG content. High levels of some antioxidant enzymes were important to survive oxidative stress after plants were subjected to different levels of waterlogging. Waterlogging stress increased SOD, CAT, APX and GR activities, although some differences were observed among genotypes. In general, the stress-sensitive cultivars showed lower activities than tolerant cultivars (Arbona et al. 2008). Hossain et al. (2009) demonstrated that coordinated antioxidant activity involving increased activities of SOD and CAT, together with a modulation of the AsA–GSH cycle, allowed citrus plants to cope with flooding-induced oxidative stress up to a certain point. Among the different antioxidant enzymes, SOD and CAT showed early responses whereas APX exhibited a late response with the de novo synthesis of AsA under walerlogging which was maintained by unaltered or decreased MDHAR and DHAR activities during the entire period of anoxia and post-anoxia; there was no positive correlation between DHAR activity and AsA/DHA ratio (Hossain et al. 2009). Kumutha et al. (2009) showed that an increase in the activity of antioxidant enzymes during waterlogging of pigeonpea (*Cajanus cajan*) is required to scavenge not only the post-hypoxic ROS build up, but also to detoxify the cellular system of ROS produced during hypoxia itself. The activity of antioxidant enzymes such as SOD, APX, GR and CAT increased under waterlogging. The comparatively greater antioxidant enzyme activities resulting in less oxidative stress in ICP 301 (waterlogging tolerant) could be one of the factors determining its higher tolerance to flooding than Pusa 207 (susceptible to waterlogging). The higher expression of SOD, POX and APX, predictive of waterlogging tolerance, reduced the level of ROS in Chrysanthemum (Yin et al. 2009). The more effective expression of antioxidant mechanisms in the tolerant cultivar also contributed to its lower level of lipid peroxidation (Yin et al. 2009). In rice seedlings, Damanik et al. (2010) suggested that tolerance to submergence stress might be proven by increasing the capacity of the antioxidative system. Following 8 days of complete submergence, they observed higher activities of antioxidative enzymes (SOD, CAT, APX, and GR) in waterlogging-tolerant varieties. Bin et al. (2010) suggested that in maize seedlings, increased POX, APX, GR, CAT and SOD activities led to an efficient H_2O_2 scavenging system and enhanced protection against oxidative stress caused by waterlogging. However, the activities were higher in waterlogging-tolerant genotypes. Sairam et al. (2011)

observed that under waterlogging, the activity of three antioxidative enzymes (SOD, APX and GR) showed a continuous increase up to 8 days of waterlogging in waterlogging-tolerant mung bean genotypes; in susceptible genotypes the increase in the activity of these enzymes was observed only following 2–4 days of waterlogging. In all subsequent stages there was a decline in activity of all three enzymes compared to the control and plants waterlogged for 2–4 days. Li et al. (2011a) showed that waterlogging pretreatment or hardening applied before anthesis can effectively improve the tolerance of wheat to waterlogging occurring during the generative growth stage, also effectively alleviating the oxidative damage to flag leaf cells by maintaining relatively higher activities of ROS-scavenging enzymes (SOD, CAT and APX) than the non-hardening treatment.

8.5.6 Heavy Metals

Heavy metals (HMs) are defined as metals with a density higher than 5 g cm^{-3}. Among the 90 naturally occurring elements, 53 are HMs (Weast 1984), only 17 HMs are available to living cells and are of importance for organisms and ecosystems based on their solubility under physiological conditions (Weast 1984). Although some elements have an importance as micronutrients, at higher concentrations they are toxic to plants and other organisms (Nies 1999). In nature, there are two main sources of HMs: the underlying parent material and the atmosphere. The HM content in soils depends on the weathering of rocks and on atmospheric metallic pollution. In addition to natural sources, viz. volcanoes and dusts, anthropogenic activities like mining, metal industries, agrochemicals, waste dumping, power houses, combustion of fossil fuels, etc., cause the emission of HMs and the accumulation of these compounds in ecosystems (Galloway et al. 1982; Angelone and Bini 1992). In recent years, substantial amounts of HMs have been released by geological activities or by accelerated anthropogenic impacts causing serious environmental problems (Sun et al. 2008). Since HMs are often found both in soil and water as contaminants, studies on complex HM toxicity in different plant species have come into focus.

8.5.6.1 Plant Responses to Heavy Metal

Making a generalization about the effect of HMs on plants is difficult due to the multidimensional variations in parameters under different concentrations, types of HMs, duration of exposure, target organs of plants, plant age, etc. Several physio-biochemical processes in plants cells are affected by HMs (Dubey 2011). Direct phytotoxic effects of HMs include their direct interactions with proteins, enzymes, displacement of essential cations from specific binding sites, causing altered metabolism, inhibiting the activities of enzymes, etc. (Sharma and Dubey 2007;

Sharma and Dietz 2008). Initially, a HM interacts with other ionic components present at the entry point of a plant root system. Later, the HM ion reacts with all possible interaction partners within the cytoplasm, including proteins, other macromolecules and metabolites. After that, HMs influence homeostatic events, including water uptake, transport and transpiration and thus symptoms start to develop and become visible, eventually leading to the death of plant cells (Fodor 2002; Poschenrieder and Barceló 2004). The most obvious plant reaction under HM toxicity is the inhibition of growth rate (Sharma and Dubey 2007). HMs also cause chlorosis, necrosis, leaf rolling, inhibition of root growth, stunted plant growth, altered stomatal action, decreased water potential, efflux of cations, alterations in membrane functions, inhibition of photosynthesis, altered metabolism, altered activities of several key enzymes, etc. (Sharma and Dubey 2007; Dubey 2011). Seed germination is also severely affected by HMs (Ahsan et al. 2007). Higher levels of HMs usually decrease photosynthesis (Heckathorn et al. 2004). HM inhibit the rate of photosynthesis and respiration (Llamas et al. 2000; Vinit-Dunand et al. 2002) and also inhibit carbohydrate metabolism and their partitioning in growing plants. Physiological adaptation of plants in response to HM stress also involves the production of different types of organic solutes, which includes small molecules such as proline, betaine which protect plants from stresses by cellular adjustment through the protection of membrane integrity and enzyme stability (Hossain et al. 2010). In addition, some signaling molecules like NO also increase under HM stress (Hsu and Kao 2004). Plant responses to HM also caused significant induction of sulfur assimilation (Gill and Tuteja 2011)

8.5.6.2 Oxidative Stress Under Heavy Metal Toxicity

There is enough evidence that exposure of plants to excess concentrations of redox active HM results in oxidative injury. HM uptake by transporters and distribution to organelles is followed by ROS generation, stimulated either by HM redox activity or by the effects of an HM on metabolism in a subcellular site-specific manner. HM-dependent activation of plasma-membrane-localized NADPH oxidase also contributes to the release of ROS. In contrast to the physiologically non-redox-active HM, such as Zn^{2+} and Cd^{2+}, the redox-active HMs Fe, Cu, Cr, V and Co enable redox reactions in the cell. They are involved in the formation of OH• from H_2O_2 via Haber-Weiss and Fenton reactions and initiate non-specific lipid peroxidation (Sharma and Dietz 2008). Lipid peroxidation is also specifically induced by HM-dependent activation of lipoxygenases (LOX) (Montillet et al. 2004). Among the HMs, Cd is the most widely studied in plants. The presence of Cd led to excessive production of ROS causing cell death due to oxidative stress such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage to nucleic acids (Gill and Tuteja 2010; Hossain et al. 2010; Gill et al. 2011b). Hossain et al. (2010) observed a 130% increase in H_2O_2 and a 103% increase in MDA content in mung bean seedlings when exposed to Cd stress (1 mM $CdCl_2$, 48 h).

8.5.6.3 Antioxidant Defense in Plants Exposed to Heavy Metals Stress

To repair the HM-induced inhibitory effects of ROS, plants employ a ROS-detoxifying antioxidant defense machinery which includes non-enzymatic (GSH, AsA, α-tocopherol and carotenoids) and enzymatic (SOD, CAT, APX, GR, MDHAR, DHAR, GPX and GST) antioxidants (Gill and Tuteja 2010; Hossain et al. 2010; Gill et al. 2011a). Exposure to HMs provoke prominent responses of antioxidative systems, but the direction of the response dependends on the plant species, plant organ, the HM used and the intensity of the HM stress (Schützendübel and Polle 2002).

In a comprehensive study on mung bean, Anjum et al. (2011) observed a decrease in AsA, the AsA/DHA ratio, GSH, the GSH/GSSG ratio in both tolerant and sensitive cultivars exposed to Cd treatment (100 mg kg^{-1} soil). However, the decreases were lower in the tolerant cultivar compared to the sensitive cultivar. This suggests the protective role of the AsA and GSH pools toward Cd stress tolerance. Under HM stress, GSH serves in bio-reductive reactions as an important defense line against ROS to protect cells from oxidative stress damage, and to modify metal toxicity by altering the rates of metal uptake, elimination and by chelating metal ions in cells (Lima et al. 2006). GSH have a stronger ability to directly scavenge metal-induced ROS (Gill and Tuteja 2010, 2011). There are several possible ways for GSH to be involved in HM tolerance and sequestration (Wójcik and Tukiendorf 2011). Cai et al. (2010) studied the effect of exogenously applied GSH on the performance of rice cultivars under Cd stress and noted that exogenous GSH significantly alleviated Cd-induced growth inhibition and markedly reduced Cd uptake in both genotypes. In contrast, Wójcik and Tukiendorf (2011) indicate that the natural content of endogenous GSH in WT *Arabidopsis* plants is sufficient for Cd-tolerance. This decrease in GSH content led to lower Cd-tolerance of plants although an increase in GSH content could not enhance Cd tolerance, even showing toxicity.

As for non-enzymatic antioxidants, the protection against oxidative stress caused by toxic HMs is also greatly achieved by the production of enzymatic antioxidants such as SOD, CAT, enzymes of the AsA-GSH cycle (APX, MDHAR, DHAR and GR), GST and GPX; cumulatively, these biochemical attributes serve as an index of HM sensitivity or tolerance in different plant species (Hossain et al. 2010; Anjum et al. 2011; Gill et al. 2011a, b). Gill et al. (2011b) reported that higher tolerance to Cd is due to better coordination between the antioxidative enzymes, which help to protect the photosynthetic machinery. The enzymatic antioxidant system involves the sequential and simultaneous action of a number of enzymes for the removal of ROS under HM stress (Gill et al. 2011b). El-Beltagi et al. (2010) observed substantial increases in antioxidant enzymes, such as CAT, GST and POX in Cd-stressed plants in comparison with the control. The specific activity of CAT increased by increasing the Cd concentration, and reached a maximum value with 25 ppm of Cd in leaves, while at the highest concentration of Cd (50 ppm), CAT activity decreased relative to 25 ppm Cd in both leaf and root tissues. Cd treatment caused a significant increase in GST-specific activity in both roots and leaves. GST activity at the highest concentration of Cd (50 ppm) reached 459% in leaves and 756% in roots, relative to control plants (El-Beltagi et al. 2010). Domínguez et al. (2010) confirmed that the antioxidant system efficiently achieves tolerance to Cd toxicity, allowing normal plant development, even in the presence of the highest Cd concentration. They showed that the activation of GPX, CAT, APX and SOD, coupled with the activation of AsA-GSH cycle enzymes (APX, MDHAR, DHAR and GR), was sufficient to reduce Cd-induced ROS accumulation and oxidative damage caused by the lower Cd concentrations (10 and 100 μM), but not by the highest Cd concentration (1 mM). Anjum et al. (2011) observed a protective role of AsA–GSH cycle metabolism in two mung bean cvs. Pusa 9531 (Cd–tolerant) and PS 16 (Cd–susceptible) under Cd stress. The changes in the AsA–GSH redox state and an increase in AsA–GSH–regenerating enzymes, such as APX, MDHAR, DHAR and GR and other antioxidant enzymes, such as SOD strongly supported over-utilization of AsA–GSH in Cd–treated plants. They observed that the oxidative stress caused by Cd toxicity was partially overcome by an AsA–GSH–based detoxification mechanism in the two genotypes studied because increases in lipid peroxidation and H_2O_2 content were accompanied by a corresponding decrease in reduced AsA and GSH pools. APX has an important role in the scavenging of H_2O_2 under stressed conditions but its activity depends on the Cd concentration applied (Gill et al. 2011a). Hossain et al. (2010) observed that Cd stress caused a significant increase in GSH and GSSG content in mung bean seedlings, while the AsA content decreased significantly with a sharp increase in H_2O_2 and MDA. APX, GST and GPX activities increased in response to Cd stress (1 mM $CdCl_2$, 24 h), while the activities of CAT, MDHAR, DHAR and GR were sharply decreased. Exogenous application of betaine or proline, resulting in an increase in GSH and AsA contents, maintenance of a high GSH/GSSG ratio and increased activities of APX, DHAR, MDHAR, GR, GST, GPX and CAT involved in the ROS detoxification system compared to the control and mostly also Cd-stressed plants, with a concomitant decrease in the levels of GSSG, H_2O_2 and MDA. They concluded that up-regulation of the antioxidant machinery provided protection against Cd-induced oxidative stress.

Kachout et al. (2009) reported that Atriplex plants cultured in soil polluted with HMs (Cu, Ni, Pb, Zn) showed varietal differences in HM tolerance, correlated with differences in antioxidant enzyme activities. Ahmed et al. (2010) reported that enhanced activity of POX, CAT and SOD may be of great significance for scavenging oxidative stress caused by excessive Cu in safflower plants and that these antioxidant enzymes served as good predictors for the evaluation of HM tolerance. It was suggested that the antioxidative activity seems to be of fundamental importance for the adaptive response of Atriplex plants to HM stress. Verma and Dubey (2003) indicated that SOD, POX and GR could serve as important components of the antioxidative defense mechanism against Pb-induced oxidative injury in rice seedlings. They observed a 1.9–2.0-fold increase in SOD activity, a 1.2–5.6-fold increase in GPX activity and a 1.2–1.9-folds increase in APX activity in the roots of rice seedlings exposed to 1 mM Pb for 15 days. GR activities showed an about 128–196%

increase in roots and 69–196% in shoots compared to control seedlings, while Pb treatment resulted in a decline in CAT activity in roots. Qureshi et al. (2007) observed that AsA content declined under Pb stress in a dose-dependent manner while DHA content increased. Similarly Pb-treated plants showed a rapid decline in GSH content, while the GSSG and total glutathione contents increased rapidly. Pb-treated plants showed a dose-dependent increase in SOD and APX activities, while CAT activity declined under severe stress (500 μM Pb-acetate) (Qureshi et al. 2007). Singh et al. (2006) observed that the levels of AsA and GSH, AsA/DHA and GSH/GSSG ratios in the fronds of *P. vittata* were much greater than in *P. ensiformis* under As stress indicating that *P. vittata* has a greater antioxidant capacity than P. ensiformis. The lower levels of antioxidant compounds (AsA, GSH and carotenoids) in *P. ensiformis* than in *P. vittata* are correlated with its greater exposure to ROS and lower scavenging ability. In wheat, Li et al. (2007) reported that APX and SOD activities decreased at low concentrations of As, and increased at high concentrations of As, while CAT activity displayed an increasing trend when the concentration of As was lower than 1 mg kg^{-1}, and then decreasing trend. Gupta et al. (2009) observed a significant increase in the activities of SOD, GPX and CAT in two varieties (Varuna and Pusa Bold) of *B. juncea* at lower concentrations of As stress (50 μM). The increased tolerance in Pusa Bold may be due to the higher activity of antioxidant enzymes. Shri et al. (2009) observed enhanced activity of antioxidant enzymes and isozymes of SOD, APX, POX and GR in rice seedlings subjected to As exposure. Contradictorily, Sun et al. (2008) reported that the activities of SOD and POX in rice leaves were significantly decreased under As stress (40 mg kg^{-1}) which resulted in a higher accumulation of ROS in As-stressed leaves, causing lipid peroxidation. In rice seedlings, the activities of all isoforms of SOD (Cu-ZnSOD, MnSOD and FeSOD), GPX and APX increased in Ni-treated (200 and 400 μm $NiSO_4$) seedlings, while no clear induction of CAT was observed (Maheshwari and Dubey 2009). The activity of AsA-GSH cycle enzymes (MDHAR, DHAR and GR) significantly increased in Ni-treated seedlings. In another study, Wang et al. (2010) observed significant increases in SOD, CAT and GPX activity of cotyledons, stems and roots of *Luffa cylindrica*, suggested that treatment with different levels of Ni may enhance the activity of these antioxidants, thus alleviating Ni-induced oxidative damage and enhancing Ni tolerance. Shanker et al. (2004) demonstrated the role of ROS-scavenging enzymes in plant parts under chromium (Cr) stress. Scavenging enzymes were not induced by a lower concentration of Cr because there is controlled ROS production. However, the combined action of SOD and CAT showed a major role in minimizing the effects of oxidative stress due to their capacity to scavenge $O_2^{\bullet-}$ and H_2O_2 under Cr stress. Shiyab et al. (2009) reported that Indian mustard (*Brassica juncea*) showed an efficient metabolic defense and adaptation system to mercury (Hg)-induced oxidative stress due to antioxidant defense. A lower level of H_2O_2 was observed in shoots with higher Hg concentrations due to the effective generation of an enzymatic antioxidant defense system (especially CAT) to scavenge H_2O_2.

8.5.7 UV Radiation

In the past few decades there has been a depletion of the stratospheric ozone (O_3) layer due to emissions of halogen-containing compounds of anthropogenic origin. This has resulted in a concomitant increase in solar ultraviolet-B radiation (Mpoloka 2008) because a 1% loss of O_3 leads to a 2% increase in UV radiation. This increase is predicted to increase in the near future, which may cause a negative impact on plants and other biological organisms. Extended exposure to UV-B radiation is especially harmful to all photosynthetic organisms due to their requirement for light (Sinha et al. 2003). Plants use solar radiation for photosynthesis and accordingly are also exposed to UV-B radiation.

8.5.7.1 Plant Responses to UV Radiation

Plants exhibit tremendous variability in their sensitivity to UV-B radiation (Mpoloka 2008). Under exposure to UV-B radiation, different kinds of morphological, biochemical and physiological responses of plants have been reported. UV-B radiation has detrimental effects such as reduced photosynthesis, biomass reduction, decreased protein synthesis, impaired chloroplast function, damage to DNA, etc. (He et al. 2003; Zhang et al. 2003). Enhanced UV-B radiation significantly decreases plant height and leaf area, and increases leaf thickness (Ren et al. 2007). Increased leaf thickness suggests the possibility of a lower penetration of UV-B radiation into the deeper mesophyll layer (Bornman and Vogelmann 1991). The photosynthetic system is also a sensitive component to increased exposure to UV-B (Sharma et al. 1998). However, the response of plants to changes in UV-B radiation also depends upon associated stresses e.g., low light, temperature extremes, atmospheric pollutants, metal toxicity, drought and nutrient deficiencies (Correia et al. 2005).

8.5.7.2 Oxidative Stress in Plants Under UV Radiation

Exposure to UV-B leads to the generation of ROS such as $^1O_2$, $O_2^{\bullet-}$, H_2O_2 and OH• (Moldau 1999). An increase in ROS by UV-B radiation has been observed in several plant species (Agrawal and Rathore 2007; Du et al. 2011; Singh et al. 2011), leading to the oxidative destruction of cell components through oxidative damage of nucleic acids, membrane lipids, proteins and enzymes (Roleda et al. 2006a, b). Uncontrolled generation of ROS in plant cells induced by UV-B also causes detrimental effects on enzymatic activities and gene expression, which eventually leads to cellular injury and PCD (Mackerness et al. 2001).

8.5.7.3 Antioxidant Defense in Plants Exposed to UV Radiation-Induced Stress

Plants contain a complex biochemical defense system which is considered to play a major role in protecting plants from UV-B damage (Liang et al. 2006). However, the available reports on the effect of UV-B radiation and their antioxidant response indicate considerable differences between plant tissues and/or plant species (Rao et al. 1996; Mackerness et al. 2001). The antioxidant defense system includes non-enzymatic antioxidants and antioxidant enzymes. Jain et al. (2003) found that UV-B enhanced the level of AsA in cucumber cotyledons; however, treatment of cotyledons with UV-B radiation reduced the α-tocopherol content. Enhancement of the AsA level under UV-B stress was observed in wheat leaves (Sharma et al. 1998) and *Arabidopsis thaliana* (Rao et al. 1996). UV-B radiation evidently induces a signal transduction that enhances the in vivo level of AsA (Jain et al. 2003). Costa et al. (2002) reported that UV-B radiation induced an antioxidant defense system in sunflower cotyledons and plant survival was higher, despite the oxidative stress. They observed that the GSH/GSSG ratio was significantly increased in response to UV-B treatments (15.0 and 30.0 kJ m^{-2}) while the AsA/DHA ratio was not affected. The activity of the antioxidant enzymes CAT and GPX increased under UV-B radiation while the activities of APX and GR were not altered. Tocopherols are involved in the reduction of PUFA radicals that are formed in plants during UV-B stress. Acute exposure of UV-B led to a decrease in α-tocopherol levels in plants (Jain et al. 2003; Agrawal et al. 2009) reflecting reactions with lipid radicals. α-tocopheroxyl radicals are immediately produced in UV-B-irradiated liposomes and thus result in the instant breakdown of the antioxidantive role of α-tocopherol which is not regained without the presence of AsA/thiols (Agrawal et al. 2009). Activation of antioxidant enzymes (SOD, CAT, APX, POX and GR) by UV-B has been reported in several plant species (Rao et al. 1996; Sharma et al. 1998). Ren et al. (2007) concluded that *Populus* trees respond to enhanced UV-B radiation with changes in the levels of antioxidant enzymes, especially APX and SOD. Recently, Ravindran et al. (2010) observed that CAT, POX and SOD activities were inhibited under supplemental UV-B radiation treatment in *Indigofera tinctoria* L. seedlings. Kumari et al. (2010) observed that the activities of SOD, CAT, APX and GR in *Acorus calamus* (sweet flag) plants were stimulated under elevated UV-B, while no definite trend of change was observed for AsA. They suggested that UV-B radiation may stimulate the enzymatic and non-enzymatic defense systems of Acorus plants, showing its better adaptation at a lower dose of UV-B (+1.8 kJ m^{-2} day^{-1}). Li et al. (2010b) reported the increased activities of SOD and POX in *Corallina officinalis* L. exposed to UV-B, while APX and CAT activities remained stable. Those enzymes worked together as ROS scavengers at a low dose of UV-B. However, at a high dose of UV-B, the antioxidant capacity decreased. In cucumber cotyledons, UV-B enhanced the activity of SOD, APX and GPX (Tekchandani and Guruprasad 1998; Jain et al. 2003).

8.5.8 Ozone

Ozone (O_3) is a secondary air pollutant formed by photochemical oxidation of primary pollutants such as nitrogen oxides, hydrocarbons and carbon monoxide (Lelieveld and Crutzen 1990) that are released into the atmosphere mainly by fossil fuel combustion, biomass burning, and biogenic emissions (Kesselmeier and Staudt 1999). It is predicted that significant crop losses due to O_3 damage will increase by 25% in background O_3 concentration over the next 30–50 years (Meehl et al. 2007). In many industrialized countries, tropospheric ozone (O_3) reaches to such high concentrations which is harmful for the plant species (Schraudner et al. 1997). Therefore, considering the predicted effect of O_3, it is necessary to explore the multifarious responses of plants and their adaptation under elevated O_3.

8.5.8.1 Plant Responses to O3

Ozone is the most damaging air pollutant to plants (Ashmore 2005). Ozone stress has been characterized as either acute or chronic, depending on the O_3 concentration and the duration of exposure (Sandermann 1996). While the actual concentration and duration threshold for O_3 damage varies from species to species and even among genotypes of the same species (Burkey et al. 2000), it is commonly accepted that acute damage is caused by a very high concentration of O_3 (>150 ppb) within a short period of time while chronic O_3 damage occurs by a lower concentration of exposure over a longer period of time (Gillespie et al. 2011). Many reports indicate that O_3 leads to a general reduction of growth and competitive fitness of plants (Gillespie et al. 2011) in which elevated O_3 concentrations cause oxidative injury in living tissues and may result in negative long-term effects on the vitality of plants, leaf damage, biomass reduction, altered metabolism and accelerated senescence, which lead to losses in yield (Ashmore 2005; Li et al. 2010c; Feng et al. 2011).

8.5.8.2 Oxidative Stress in Plants Induced by O_3

Being a strong oxidant, O_3 can interact with constituents of the apoplast to generate ROS such as H_2O_2, $O_2^{\bullet-}$, OH• and HOO• (Yan et al. 2010a, b). Significant increases in protein carbonylation, increased lipid peroxidation and changes in cellular permeability are a consequence of O_3 exposure (Gillespie et al. 2011). Wohlgemuth et al. (2002) reported that blocking H_2O_2 and $O_2^{\bullet-}$ accumulation markedly reduced O_3-induced cell death in various plant species. Different studies indicated that elevated O_3 remarkably increased the levels of H_2O_2 levels and MDA in plants (Li et al. 2010c; Yan et al. 2010a, b; Feng et al. 2011).

8.5.8.3 Antioxidant Defense in Plants Exposed to O_3 Stress

The antioxidant system that is responsible for controlling the level of ROS in plant tissues plays an important role in conferring O_3 tolerance to plants (Tausz et al. 2007). Increasing the level of endogenous antioxidants such as AsA or flavonoids could limit the deleterious effects of oxidative stress caused by O_3 (Vickers et al. 2009). Application of chemical antioxidants for protecting vegetation from O_3 injury has been extensively studied over the past four decades. Apoplastic AsA forms the first line of defense against O_3 (Didyk and Blum 2011), which participates in a series of cell wall reactions targeted to prevent O_3 from generating free radicals. At least in some crops, O_3 tolerance has been shown to be a heritable trait involving the antioxidant system and high apoplastic AsA content (Fiscus et al. 2005) and apoplastic AsA was found to detoxify up to 30–50% of O_3 taken up by leaves (Turcsanyi et al. 2000). Burkey et al. (2000) observed that in *Phaseolus vulgaris* L., AsA was the only variable identified as a potential factor in O_3 tolerance in which tolerant genotypes contained more AsA than sensitive lines. In addition, *Arabidopsis* mutants containing diminished concentrations of AsA were more susceptible to O_3 than WT plants (Conklin et al. 1996), suggesting that a minimum level of AsA is required to protect plants against O_3 stress. Sen Gupta et al. (1991) observed higher GSH and GSSG levels in poplar leaves following a 3-h exposure to 180 nmol mol^{-1} of O_3 compared to the control. Ozone fumigation of clover leaves induced a decrease of TG content, mainly due to the strong increase in GSSG, while GSH decreased (Scebba et al. 2003). The increase in GSSG could, therefore, be an index of O_3-induced oxidative stress. Borowiak et al. (2009) showed that SOD activity increased with an increase in O_3 concentration in sensitive as well as resistant cultivars. However, O_3-sensitive and O_3-resistant poplar clones exhibited different patterns of SOD activity. Bandurska et al. (2009) reported a positive correlation between O_3 level and APX activity in a resistant tobacco cv. Bel B, which did not reveal visible symptoms, indicating that this enzyme may contribute to the detoxification of H_2O_2 and alleviation of O_3-induced oxidative damage. Strohm et al. (2002) demonstrated that in poplar leaves, the total ascorbate contents and the activities of APX, MDAR and DHAR were not significantly affected by acute O_3 exposure in all poplar lines. They also showed that in developing leaves of transgenic plants, over-expressing GR in the cytosol or chloroplasts, APX and DHAR activities were higher than in WT plants. Scebba et al. (2003) demonstrated that in clover leaf, the activities of POX, APX and MDHAR increased in both clover species, but always more than in tolerant species, confirming again its higher level of protection against oxidative-induced stress. Pukacka and Pukacki (2000) found GPX to play a regulatory role in scavenging H_2O_2 under O_3 stress. Both resistant and sensitive cultivars showed a positive and highly significant correlation between GPX activity and the degree of leaf damage. Gillespie et al. (2011) indicated that soybean grown at chronic elevated O_3 concentrations (90 ppb) increased the total antioxidant capacity of plants which were matched by changes in AsA content, but not phenolic content. In their study, DHAR activity more than doubled in plants grown at elevated O_3 levels than in controls, whereas GR activity was significantly lower.

8.6 Transgenic Approaches to Enhance Oxidative Stress Tolerance

Recently, understanding of the role of ROS-scavenging systems in plant stress tolerance has increased through the use of gene transfer technology to manipulate the antioxidative capacity of plants. Several studies clearly demonstrated that enhancement of ROS-scavenging systems in plants through transgenic approaches can provide partial protection from oxidative damage, indicating that this strategy could be used to improve plant stress tolerance (Roxas et al. 2000; Ruan et al. 2011). Several successful approaches to achieve tolerance through the genetic engineering of specific genes have been studied and the improvement of the antioxidant defense system, enhanced abiotic stress tolerance and increased productivity. Often, non-enzymatic and enzymatic components of antioxidative defense systems are significantly up-regulated in transgenic plants compared to non-transformed or WT plants.

Hemavathi et al. (2010) showed that transgenic potato (*Solanum tuberosum* L. cv. Taedong Valley) over-expressing the L-gulono-c-lactone oxidase (*GLOase*) gene showed enhanced basal levels of AsA content (141%) than non-transgenic tubers and showed better survival under various abiotic stresses caused by methyl viologen, NaCl and mannitol. There was also a direct correlation between elevated levels of AsA accumulation in transgenics and their ability to withstand abiotic stresses. Alteration in GSH levels by transgenic approaches also conferred enhanced stress tolerance in plants. *B. juncea* (mustard) plants overexpressing *GS* or γ-*ECS* showed enhanced tolerance to a variety of HMs (Cd, Zn, As and Pb) due to the higher capacity of GSH synthesis as well as PC synthesis (Reisinger et al. 2008). Liu et al. (2008) suggested that the overexpression of α-tocopherol can increase the tolerance of plants to oxidative stress caused by abiotic stresses. Tocopherol cyclase (*VTE1*, encoded by *VTE1* gene) catalyzes the penultimate step of tocopherol synthesis. Transgenic tobacco plants overexpressing *VTE1* from *Arabidopsis* showed decreased lipid peroxidation, electrolyte leakage, and H_2O_2 content compared to the WT when exposed to drought conditions (20% PEG) (Liu et al. 2008).

Faize et al. (2011) showed that simultaneous overexpression of Cu/Znsod and *apx*, or at least *apx*, in the cytosol of transgenic tobacco plants alleviated the damage produced by water stress (3–5 days). In general, oxidative stress parameters such as lipid peroxidation, electrolyte leakage, and H_2O_2 levels, were lower in transgenic plants than in non-transformed plants suggesting that, at least, overexpression of cytapx protects tobacco membranes from water stress. Moreover, an increase in the activity of some antioxidant enzymes was also observed in the chloroplasts of transgenic plants overexpressing cytsod and/or cytapx. Artlip et al. (2009) observed that SOD overexpression (*SOD-OX*) leaves exhibited improved resistance to both acute (30 min) and longer-term exposure (2 to 24 h) to elevated temperatures (40 and 45°C) compared to the non *SOD-OX* lines. In sweet potato (*Ipomoea batatas*) plants, expression of Cu/ZnSOD and APX in chloroplasts enhanced drought resistance and the capacity to recover from drought stress (Lu et al. 2010). Compared with non-transgenic plants, the expression of antioxidant enzymes (SOD, APX and CAT) in transgenic plants was profoundly increased under drought stress and rewatering periods resulted in low levels of MDA and electrolyte leakage. Transgenic plants also exhibited better growth, photosynthetic activity (Fv/Fm) and water status under drought stress compared with non-transgenic plants. Zhao and Zhang (2006) showed that co-expression of the GST and *CAT1* genes resulted in a greater increase of CAT and SOD activity in transgenic compared to non-transgenic rice seedlings exposed to both salt (200 mM NaCl) and paraquat while a significant increase of GST activity in transgenics occurred only in paraquat-stressed plants. The generation of H_2O_2 and MDA decreased in the transgenics than in non-transgenics under the same conditions. Moreover, the transgenic seedlings showed markedly enhanced tolerance to salt stress upon 200 mM NaCl treatment compared with non-transgenics. They concluded that enhancement of the ROS-scavenging system that led to increased oxidative stress protection in GST + *CAT1*-transgenic rice plants could result not only from increased GST and CAT activity but also from the combined increase in SOD activity. CAT activity of transgenic *Brassica juncea* plants overexpressing the *BjCAT3* gene was approximately 2-fold higher than that of WT which was correlated with enhanced tolerance under Cd stress (Guan et al. 2009). Transgenic rice plants (cv. Nipponbare and cv. BR5) overexpressing a CAT gene from *Escherichia coli, katE*, were more tolerant to NaCl (100 mM) than WT plants (Nagamiya et al. 2007; Moriwaki et al. 2008).

Rice plants overexpressing *OsAPXa* showed increased APX activity under cold stress (Sato et al. 2011). In their study, the levels of H_2O_2 and MDA increased by 1.5- and 2-fold, respectively in WT plants subjected to a 12°C treatment for 6 days. In contrast, transgenic lines showed significantly lower levels of H_2O_2 and MDA than WT plants. While studying different ROS signals, Miller et al. (2007) generated a double mutant lacking thylakoid ascorbate peroxidase (*tylapx*) and cytosolic ascorbate peroxidase 1 (*apx1*) genes. Two different signals were likely generated in plants lacking cytosolic *APX1* or *tylAPX*. The absence of a chloroplastic H_2O_2-removing enzyme triggered a specific signal in cells that resulted in enhanced tolerance to heat stress (38°C) (Miller et al. 2007). Sun et al. (2009) found that the thylakoid-bound APX gene (*LetAPX*) from tomato, when overexpressed in tobacco, improved salt (200 mM NaCl) tolerance.

The simultaneous expression of multiple antioxidant enzymes, such as CuZnSOD, APX, and DHAR, in chloroplasts was more effective than single or double expression for developing transgenic plants with enhanced tolerance to multiple environmental stresses (Lee et al. 2007). Transgenic tobacco plants expressing both CuZnSOD and APX in the chloroplast (CA plants), or DHAR in chloroplast showed enhanced tolerance to oxidative stresses such as paraquat and salt. Later, they introduced the gene encoding DHAR into CA transgenic plants and observed that mature leaves of transgenic plants expressing all three antioxidant genes (CAD plants) had approximately 1.6–2.1-fold higher DHAR activity, and higher ratios of reduced AsA/DHA, and GSH/GSSG compared to CA plants. Thus CAD plants were more resistant to paraquat-induced stress, exhibiting only an 18% reduction in membrane damage relative to CA plants. In addition, seedlings of CAD plants had enhanced tolerance to NaCl (100 mM) compared to CA plants. The manipulation of DHAR expression is important for the genetic engineering of stress-tolerant plants (Amako and Ushimaru 2009). The results obtained by Yin et al. (2010) indicate that plants overexpressing DHAR showed better root growth than WT plants and showed lower H_2O_2 content, less lipid peroxidation and a lower level of oxidative DNA damage than WT plants under Al stress (300–500 μM). Compared with WT plants, DHAR-overexpressing plants showed a higher AsA level and APX activity which contributed to their higher antioxidant capacity and higher tolerance to Al stress. The overexpression of MDHAR minimizes the deleterious effects of environmental stresses (Eltayeb et al. 2007). In their study, transgenic tobacco plants overexpressing the *A. thaliana* MDHAR gene (*AtMDAR1*) in the cytosol exhibited up to a 2.1-fold higher MDHAR activity and a 2.2-fold higher level of reduced AsA compared to non-transformed control plants. The transgenic plants showed enhanced stress tolerance under O_3, salt, and PEG stresses and greater PSII effective quantum yield under O_3 and salt stresses. Furthermore, these transgenic plants exhibited significantly less H_2O_2 when tested under salt stress. Thus, overexpressed MDHAR confers enhanced tolerance to O_3 (2 ppm), salt (300 mM NaCl), and drought (10% PEG) stress (Eltayeb et al. 2007).

Martret et al. (2011) observed that tobacco chloroplast transformants expressing genes encoding DHAR, GR, and GST exhibit altered anti-oxidant metabolism and improved tolerance to salinity and chilling. This improved protection could be explained by synergistic effects of DHAR with GR or GST with GR. The expression of these combinations of transgenes also increased the regeneration of AsA (1.6-fold) and GSH (2.4-fold) and participated in a more rapid scavenging of $O_2^{\bullet -}$ and H_2O_2 prior to their interaction with target molecules. In both chilling and salt stresses, the protective effect could be observed when DHAR or GST levels were enhanced independently and in the case of chilling stress, a further improvement was observed when these were combined with increased GR activity. Plants overexpressing DHAR and GST did not differ from WT in their tolerance to methyl viologen, while DHAR:GR and GST:GR did. These different results illustrate the fact that in some cases, overexpression of a single antioxidant enzyme does not provide protection against oxidative stress and simultaneous expression of multiple antioxidant enzymes is more effective than single expression for enhancing tolerance to environmental stresses. Roxas et al. (2000) showed that overexpression of a tobacco GST with GPX activity in transgenic tobacco (*Nicotiana tabacum* L.) enhanced seedling growth under heat and salt stress. In addition to increased GST and GPX activity, transgenic seedlings expressing GST/GPX had elevated levels of MDHAR activity and higher levels of AsA and GSH than WT seedlings. When stress was imposed, overexpression of GST/GPX in transgenic tobacco seedlings provided increased GSH-dependent peroxide scavenging ability and alterations in AsA and GSH metabolism that led to reduced oxidative damage, indicated by a decrease in lipid peroxidation. Jha et al. (2011) showed the significant induction of the plant-specific *Tau* class *GSTU* genes by different abiotic stresses provided better protection of plants against oxidative damage. A transcript study of *SbGST* gene expression under salt, cold, drought with time period point and concentration point revealed that the expression of the *SbGST* gene was up-regulated under all stress conditions. Consequently, the transgenic lines showed higher seed germination and survival than WT, confirming that over-expression of the *tau* class *SbGST* gene in transgenic tobacco plays a vital role in abiotic stress tolerance. Herbette et al. (2011) reported that transgenic tomato showing higher GPX activity was more resistant to an abiotic stress (mechanical injury) but more susceptible to biotic stresses (such as pathogen attack). They also suggested that overexpression of GPX provoked opposite effects in biotic and abiotic challenges, suggesting a key role for this scavenger enzyme in controlling both types of stress responses.

Plant resistance to abiotic stresses is genetically complex and multigenic, and thus more difficult to control and engineer. Plant engineering strategies for abiotic stress tolerance rely on the expression of genes that are involved in signaling and regulatory pathways or genes that encode proteins conferring stress tolerance or enzymes present in pathways leading to the synthesis of functional and structural metabolites (Vinocur and Altman 2005). Though it is possible to confer partial tolerance to a certain abiotic stress by overexpressing a single component of the antioxidant defense system, only limited improvement in stress tolerance has been achieved (Lee et al. 2009). As the ROS detoxification system is a coordinated process, overexpressing one enzyme is not sufficient to counterbalance the ROS levels. Therefore, increases in one component might not result in an overall increase in protection against abiotic stresses. Transgenic plants will, however, continue to be extremely useful tools in biotechnology and will lead to an improved understanding of the gene networks and molecular physiology of plant responses to abiotic stresses.

8.7 Conclusion and Future Perspectives

The unquestionable importance of abiotic stress in world agriculture is demonstrated by the fact that abiotic factors cumulatively account for major limitations in crop production worldwide. Therefore, further steps to understand the molecular and physiological mechanisms of abiotic stress tolerance and to find the ways that would increase stress tolerance in plants are crucial in agriculture. It is possible to minimize losses in agricultural production due to abiotic stresses by a judicious blend of knowledge in crop physiology and crop husbandry procedures. The production, metabolism and detoxification of ROS are essential processes in plant growth, adaptation and survival. The generation and scavenging of ROS are vital parts of plant defense mechanisms and regulation, and over-expression of novel isoforms of genes coding for ROS-detoxifying enzymes increase tolerance against environmental stresses. Although ROS were initially recognized as toxic by-products of aerobic metabolism, recently, it has become apparent that ROS also play an important signaling role in plants' processes such as growth, development, and responses to adverse environmental conditions. However, to evaluate the negative effects caused by potential stressors, it is important to understand mechanisms of resistance and tolerance. The potential of engineering plants that overexpress genes for antioxidants provides an opportunity to develop plants with enhanced tolerance to abiotic stresses. With advancements in molecular biology and the availability of advanced genetic tools, considerable progress has been made in improving stress-induced oxidative stress tolerance in crop plants by developing transgenic lines with altered levels of antioxidants (Lee et al. 2007; Ashraf et al. 2008). Use of exogenous chemical protectants like proline, glycinebetaine, Se and signaling molecules like NO has also showed significant up-regulation of antioxidative defense and thus better alleviation of oxidative stress due to efficient co-regulation of both enzymatic and non-enzymatic antioxidant defense systems (Hossain et al. 2010; Hasanuzzaman et al. 2011a, b; Hasanuzzaman and Fujita 2011). In fact, the ROS detoxification system is very complex and multileveled controlled, and changing one component of the antioxidative defense system might not modify the capacity of the pathway as a whole defense system (Lee et al. 2009). Furthermore, overexpression of combinations of antioxidant enzymes in transgenic plants has been shown to have synergistic effects on stress tolerance. Therefore, increased emphasis is being placed on producing transgenic plants overexpressing genes – i.e., gene stacking – associated with more than one antioxidant in order to achieve tolerance to multiple environmental stresses.

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Chapter 9
Transcription Factors and Genes in Abiotic Stress

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Abstract Plants are constantly exposed to changes in environmental conditions. When these changes are rapid and extreme, plants generally perceive them as stresses. Abiotic stresses are the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination, plant vigour and crop yield. Responses to abiotic stresses are not linear pathways, but are complicated integrated circuits involving the interaction of additional cofactors and/or signalling molecules to coordinate a specified response to a given stimulus. The regulation of these responses requires proteins operating in signal transduction pathways, such as transcriptional factors, which modulate gene expression by binding to specific DNA sequences in the promoters of respective target genes. This type of transcriptional regulatory system is called regulon. At least four different regulons that are active in response to abiotic stresses have been identified. Dehydration-responsive element binding protein 1 (DREB1)/C-repeat binding factor (CBF) and DREB2 regulons function in ABA-independent gene expression, whereas the ABA-responsive element (ABRE) binding protein (AREB)/ABRE binding factor (ABF) regulon functions in ABA-dependent gene expression. In addition to these major pathways, other regulons, including the NAC and MYB/MYC regulons are involved in abiotic stress-responsive gene expression. Transcription factors (TFs) are powerful targets for genetic engineering in abiotic stress resistance in crop plants and many studies have been done in the last two decades on this topic. The aim of this book chapter is to give a comprehensive and up-to-date literature review in this field.

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Abbreviations

ABA	ABscisic Acid
ABF	Abscisic Binding Factor
ABI	Abelson interactor
ABRE	ABA-responsive element
ADP	Adenosine diphosphate
AP2	APETALA2
APX	Ascorbate Peroxidase
AREB	ABA-responsive element binding
ATAF	Arabidopsis Transcription Activation Factor
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
bHLH	basic Helix-Loop-Helix
bZIP	Basic Leucine Zipper Domain
CBF	C repeat Binding Factor
CBL	Calcineurin B-Like
CDPK	calcium-dependent protein kinase
cGMP	cyclic Guanosine MonoPhosphate
CIPK	Calcineurin B-like -Interacting Protein Kinase
CKII	Casein kinase II
Cor	Cold responsive
CRT/DRE	C-repeat /Dehydration Responsive Element
CRY	Cryptochrome Photoreceptors
CUC	Cup-shaped Cotyledons
DHN	Dehydrin
Dof	DNA binding with one finger
DREB	Dehydration-Responsive Element Binding protein
EL	Excess Light
ERD	Early Responsive Dehydration
EREBP	Ethylene Responsive Element Binding Protein
ERF	Ethylene Responsive element binding Factors
ESK1	Eskimo1
FCA	Flowering CA
GBF1	G-box Binding Factor 1
GR	Glutathione Reductase
H2O2	Hydrogen Peroxide Superoxide
HOS1	High expression Osmotically responsive gene 1
HY	Hypocotyl
HYH	Hypocotyl 5-like
ICE1	Inducer C-repeat binding factor Expression
JA	Jasmonic Acid
LAF	Long After Farred
LBD	Ligand Binding Domain

Lea	Late embryogenesis abundant
LHC	Light Harvesting Chlorophyll
LRE	Light-Responsive cis-Element
MAPK	Mitogen Activated Protein-Kinase
MYB	Myeloblastosis
MYBRS	Myeloblastosis Recognition Sequence
MYC	Myelocytomatosis
MYCRS	Myelocytomatosis Recognition Sequence
NAC- NAM	No Apical Meristem
NAD	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCED3	9-cis-epoxycarotenoid dioxygenase
NCP	Nuclear Pore Complex
NHE	Na + H + Exchanger
NO	Nitrous Oxide
NOS	Nitrous Oxide Systems
$^1O_2$	Singlet Oxygen
O_2^-	Hydroxyl radicals
Pfr	Phytochrome Active
PHD	Plant Homeodomain
Phy	Phytochrome
PIF3	Phytochromes Interacting Factor 3
PL	Pyridoxal
PLP	Pyridoxal-5-Phosphate
PR	Pathogens Related
PS	Photosystem
RbcS1A	Ribulose bisphosphate carboxylase Small subunit
RD	Responsive Dehydration
ROS	Reactive Oxygen Species
SOS	Salt Overly Sensitive
SUMO	Small Ubiquitin-related Modifier
TFBS	Transcription Factor Binding Sites
TFs	Transcriptional Factors
UV	UltraViolet
UVR	Ultraviolet Resistance

9.1 Introduction

Plant growth and productivity are under constant threat from environmental changes in the form of various stress factors. The most common abiotic stresses are drought, flooding or submergence, salinity, extreme temperatures (heat and freezing) and high light. In addition, deficits of inorganic nutrients, such as nitrogen, phosphorus and potassium, to name only the most important ions for plant growth, may restrict plant development. Furthermore, residuals of chemicals used in normal agricultural practice to improve yield in specific crops may generate stress, because such compounds may reach the underlying aquifer or persist in the soil. Finally, the continued modification of the atmosphere by human activities is of some concern. One example is the increase in the concentration of ozone in the troposphere, because it can generate oxidative stress, which leads to the destruction of proteins and cells, premature ageing and reduced crop yields. A final point to make is the consideration of compounding damaging effects by multiple stress factors which act simultaneously. For example, high light combined with low temperature, conditions that may coincide during early spring at high latitudes, constitute a composite stress because the energy captured by photosynthesis cannot be converted into carbon biosynthesis, because the biochemical process is slower at low temperature. Low temperature, in fact, restricts biochemical reactions that accomplish biosyntheses and reduces the transport of nutrients and water from the soil to the photosynthetically active tissues (Bohnert 2007).

Tolerance or susceptibility to these abiotic stresses is a very complex phenomenon, both because stress may occur at multiple stages of plant development and more than one stress simultaneously affects the plant. Therefore, the perception of abiotic stresses and signal transduction to switch on adaptive responses are critical steps in determining the survival and reproduction of plants exposed to adverse environments (Chinnusamy et al. 2004).

During the past few years, transcriptome analysis has indicated that distinct environmental stresses induce similar responses. Overlap between stress responses can explain the phenomenon known as cross-tolerance, a capability to limit collateral damage inflicted by other stresses accompanying the primary stress.

9.2 Role of Transcription Factors in the Activation of Stress Responsive Genes

Responses to abiotic stresses require the production of important metabolic proteins such as those involved in synthesis of osmoprotectants and regulatory proteins operating in signal transduction pathways, that is kinases and transcription factors (TFs). In addition, new transcripts are synthesised and within a few hours a steady-state level of stress adaptation has been reached. In general, the transcriptional regulation of genes is directly controlled by a network of TFs and transcription factor binding sites (TFBS) (Singh and Foley 2002; Chaves and Oliveira 2004).

In the past two decades, important advances have been made in the understanding of transcriptional changes induced by environmental constraints and in identification of signalling proteins and TFs which regulate the stress-induced gene expression.

Gene induction occurs primarily at transcriptional level, the temporal and spatial regulation of stress-induced expression patters is an important part of the plant response (Rushton and Somssich 1998).

Responses to abiotic stresses are not linear pathways, but are complicated integrated circuits involving the interaction of additional cofactors and/or signalling

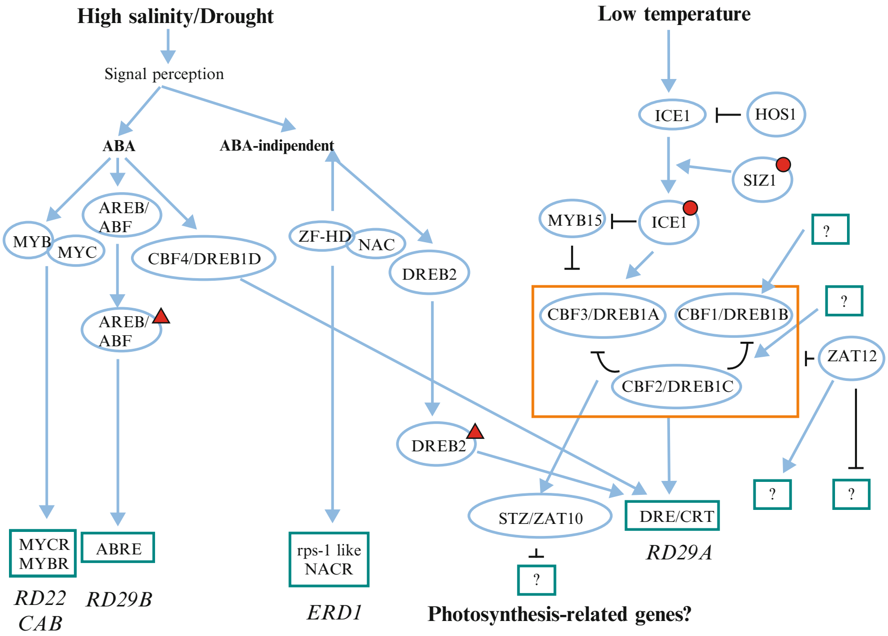

Fig. 9.1 Transcriptional network of abiotic stress responses. Transcription factors are shown in *ovals*. Transcription factor-modifying enzymes are shown in *circles*. The *small triangles* correspond to post-translational modifications. *Green squares* with question marks represent putative MYC ICE1-like transcription factors that may activate CBF1/DREB1B and CBF2/DREB1C. The *green boxes* represent the *cis*-elements present in stress-responsive genes. The *red dot* corresponds to the sumoylation modification by SIZ1 of the ICE1 transcription factor. The *dashed black line* from SIZ1 to HOS1 represents competition for binding places on the ICE1 transcription factor. SIZ1 blocks the access of HOS1 to the ubiquitination sites on the ICE1. CBF4/DREB1D is a DRE cis-element binding factor that is ABA dependent

molecules to coordinate a specified response to a given stimulus. The regulation of these responses requires proteins operating in the signal transduction pathways, such as transcriptional factors, which regulate gene expression by binding to specific DNA sequences in the promoters of respective target genes. This type of transcriptional regulatory system is called regulon. At least four different regulons that are active in response to abiotic stresses have been identified. Dehydration-responsive element binding protein 1 (DREB1)/C-repeat binding factor (CBF) and DREB2 regulons function in abscisic acid (ABA)-independent gene expression, whereas the ABA-responsive element (ABRE) binding protein (AREB)/ABRE binding factor (ABF) regulon functions in ABA-dependent gene expression (Saibo et al. 2009). In addition to these major pathways, other regulons, including the NAC (or NAM, No Apical Meristem) and Myeloblastosis-Myelocytomatosis (MYB/MYC) regulons, are involved in abiotic stress-responsive gene expression (Fig. 9.1).

As cold, salinity and drought stress ultimately impair the osmotic equilibrium of the cell it is likely that these TFs as well as the major stress genes may cross talk with each other for their maximal response and help in reinstating the normal physiology of the plant.

TFs are powerful targets for genetic engineering in abiotic stress resistance in crop plants and many studies have been done in the last two decades on this topic. The aim of this chapter is to provide a comprehensive and up-to-date review in this field.

9.3 Drought

Water deficit is the most widespread and detrimental abiotic stress factor in agronomic terms (USDA 1989). The amount of land area experiencing drought, about 1%, is expanding and is expected to rise to 30% by the end of this century (Yi et al. 2010). Drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Prolonged water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally plant death (Jaleel et al. 2008, 2009).

Responses to water deficit depend on the species and genotype, length and severity of water loss, age and stage of development, organ and cell type and subcellular compartment (Bohnert and Jensen 1996). The responses to water loss may occur within a few seconds (such as changes in the phosphorylation status of a protein), or within minutes, hours or days (such as changes in gene expression and plant morphology) (Verslues and Bray 2006).

It appears that drought triggers the production of ABA, which in turn induces various genes involved in a signalling cascade for the regulation of downstream biochemical protective mechanisms (Shinozaki and Yamaguchi-Schinozaki 1997). ABA also plays other roles during drought. For instance, ABA accumulates during water-stress conditions in leaf tissues around the guard cells to promote stomatal closure (Garcia-Mata and Lamattina 2003; Little and Eidt 1968). This latter condition dramatically reduces foliar transpiration, in many cases by two orders of magnitude or more, and is a primary defence response of the plant against dehydration (Yamaguchi-Shinozaki et al. 1992). Stomatal closure during drought, however, leads to limited carbon uptake and subsequent reduction in leaf photosynthesis (Chaves 1991, Chaves et al. 2003).

9.3.1 Abscisic Acid as Signalling Molecule

Loss of water is a physical stress in the environment that initiates biochemical events. The mechanism to sense the stress and the signal transduction events which follow are not well understood. However, it is certain that the cell must have a mechanism to recognize a decrease in water content, which is probably related to turgor pressure. One intermediary in the signalling pathway is the plant hormone ABA, which increases in response to water deficit by alterations in ABA biosynthesis and catabolism. It, in turn, induces the expression of various genes involved in a signalling cascade for the regulation of downstream biochemical protective mechanisms playing a pivotal role in response to drought stress (Bray 2007; Shinozaki and Yamaguchi-Schinozaki 1997).

ABA is an apo-carotenoid compound derived from the oxidative cleavage of the 11–12 double bond of 9-cis epoxy carotenoids (nexanthin and/or violaxanthin) (Qin and Zeevaart 1999). During periods of water deficit, the enzyme 9-cis-epoxycarotenoid dioxygenase (encoded by a family of genes named NCED), the key regulatory step of ABA biosynthesis, completes the oxidative cleavage of *cis*-xanthophylls to xanthoxin, which in turn is converted into ABA aldehyde by a short-chain alcohol dehydrogenase.

The Arabidopsis gene family contains nine members, five of them are likely involved in ABA biosynthesis, with the one member, NCED3, playing a major role during stress. The accumulation of ABA initiates a series of events, many of which promote plant adaptation to the conditions of water deficit. First, ABA must be recognized in the cell. One ABA receptor, encoded by a gene controlling flowering time in Arabidopsis, flowering (FCA), has recently been identified (Razem et al. 2006), although this ABA receptor is unlikely to be the only receptor involved in water deficit responses since this receptor does not function in the control of stomatal conductance. ABA recognition initiates a signal transduction pathway that is composed of such signalling components as kinase/phosphatise cascades, RNA-processing proteins and calcium. Finally, signal transduction fulfils the action of ABA by activating gene expression (Bray 2004). Several drought stress–inducible genes have been reported in vegetative tissues, and many of them are also activated by ABA (Ingram and Bartels 1996; Seki et al. 2002).

9.3.2 Genes Regulated by Drought

The information contained within the genome of each species dictates the plant response. The genome controls the regulation of the response to water deficit as well as the effectiveness of the response. Microarrays, largely done using the model plant *Arabidopsis thaliana*, have been used to catalogue the many genes that are induced or repressed in response to conditions that may lead to cellular water-deficit stress (Seki et al. 2002). Induced genes are candidates as those that function in the regulation of the plant response or in the adaptation of the plant to stress. Many research groups, using different methods of exposing Arabidopsis plants to cellular water-deficit stress, have identified more than 800 induced genes (Bray 2004). These genes can be placed in at least four different functional groups: signal transduction, transcriptional regulation, cellular metabolism and transport and protection of cellular structures (Fig. 9.2).

Multiple kinase/phosphatase cascades regulate the stress response, including a subunit of G proteins and type 2C protein phosphatases [e.g. Abelson interactor 1 (ABI1)]. There are at least six different classes of TFs that participate in gene induction or repression in response to water deficit. Homeobox domain and NAC domain

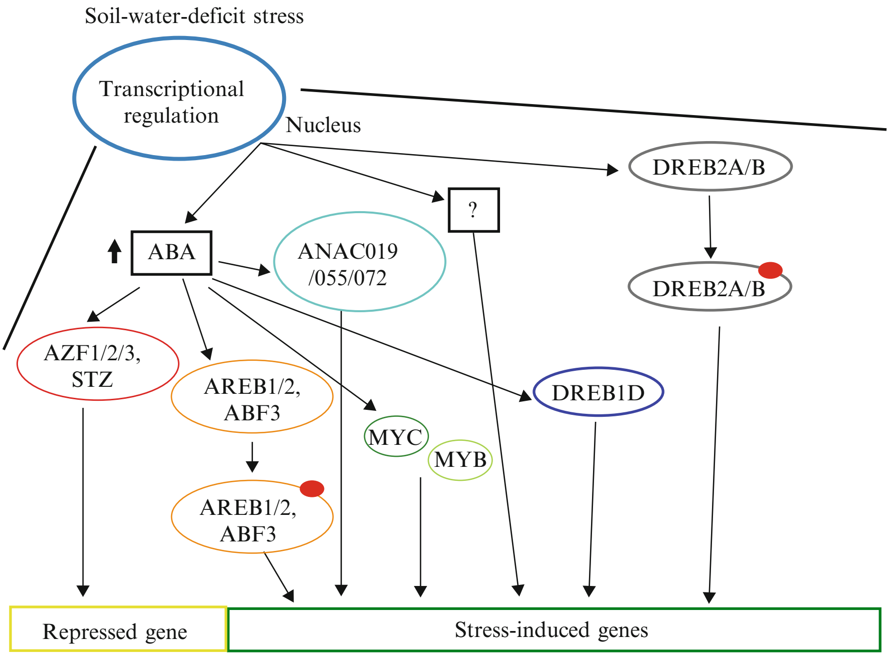

Fig. 9.2 Classes of genes that are induced by water-deficit stress. The *inset* shows the different types of transcription factors that induce/repress sets of genes, called regulons

containing TFs are induced by multiple experimental treatments that mimic water-deficit stress. Accumulations of proteins encoded by genes which have metabolic or structural functions are thought to promote adaptation to stress. One class of genes that could play a role in protection is called the late embryogenesis abundant (*Lea*) genes. The *Lea* genes are also developmentally programmed for expression in desiccating seeds. These genes encode small hydrophilic proteins that are predicted to protect proteins and membranes through chaperone-like functions. These proteins were thought to improve the performance of rice plants by protecting cell membranes from injury (Chandra et al. 2004).

9.3.3 Gene Regulation and Transcriptional Factors in Water Deficit

The expression of genes in response to water deficit can be regulated at the transcriptional, post-transcriptional and translational levels. Most research has been done to explore the mechanisms of transcriptional regulation. In response to water-deficit stress, there are two major transcriptional regulatory pathways of gene expression defined by the involvement of ABA. The ABA-independent pathway is controlled largely by a family of TFs called dehydration response element binding protein (DREB), which contains a DNA binding motif originally identified in a flower patterning protein called APETALA2 (AP2) (Fig. 9.2), while transcription factor families known to be as the most responsive to ABA signalling under drought are NAC, AREB/ABF, and MYB.

9.3.4 ABA-Independent Pathway

DREB are important TFs which induce a set of abiotic stress-related genes and confer stress resistance to plants. The DREB TFs could be dichotomized as DREB1 and DREB2, which are involved in two separate signal transduction pathways under low temperature and dehydration, respectively. They belong to the ethylene responsive element binding factors (ERF) family of TFs. ERF proteins are a sub-family of the AP2/ethylene responsive element binding protein (EREBP) TFs that is distinctive to plants. ERF proteins share a conserved 58–59 amino acid domain (the ERF domain) that binds to *cis*-elements, the GCC box, found in many pathogens related (PR) gene promoters conferring ethylene responsiveness (Gu et al. 2000), and to the C-repeat CRT/dehydration responsive element (DRE) motif involved in the expression of cold and dehydration responsive genes (Agarwal et al. 2006).

The expression of *DREB2A* and its homolog *DREB2B* are induced by dehydration and high salt stress (Liu et al. 1998; Nakashima et al. 2000). DREB2A and B proteins require post-translational modification for their activation by a mechanism that has not been clarified yet. It has been found in some studies that the expression of *DREB* genes is induced by abiotic stresses, however, at different time periods. *AtDREB2A* is induced within 10 min under drought or salt stress (250 mM NaCl) (Liu et al. 1998). In rice, *Os-DREB2A* is induced within 24 h after dehydration and salt stress (250 mM NaCl) and it responds faintly to ABA and cold stress (Dubouzet et al. 2003).

Presently, there is not much information available on the tissue-specific expression of DREBs. The transcription of *GmDREBa* and *GmDREBb* is induced by cold, drought and salt in leaves of soybean seedlings. The expression of *GmDREBc* is not significant in leaves but shows high expression levels in roots under drought, salt and ABA treatments (Li et al. 2005).

The DREB proteins contain an ERF/AP2DNA-binding domain. The ERF/AP2 domain is quite conserved and the TFs containing it are widely found in many plants, including Arabidopsis (Drews et al. 1991; Leon-Kloosterziel et al. 1994; Elliot et al. 1996; Klucher et al. 1996; Wilson et al. 1996; Okamuro et al. 1997), tomato (Zhou et al. 1997), tobacco (Ohme-Takagi and Shinshi 1995), rice (Sasaki et al. 1994; Weigel 1995) and maize (Moose and Sisco 1996). Amino acid alignment of different DREB proteins shows high sequence similarity in the nuclear localization signal at the N-terminal region and some similarity in the C-terminal acidic domain (Agarwal et al. 2006). A conserved Ser/Thr-rich region that is present in the adjacent ERF/AP2 domain is considered to be responsible for phosphorylation of DREB proteins (Liu et al. 1998).

Based on the various studies summarized above, it is clear that the DREB proteins are important TFs involved in the regulation of abiotic stress-related genes and play a critical role in imparting stress endurance to plants.

Another ABA-independent pathway was identified after the observation that Early Responsive to Dehydration Stress 1 (ERD1) gene transcripts accumulated before any increase of ABA in response to dehydration and high salinity (Nakashima et al. 1997). Promoter analysis of ERD1 revealed TFs belonging to the NAC family and zinc finger homeodomain (ZF-HD) as essential to the activation of the ERD1 gene (Tran et al. 2007). The increased drought tolerance may be due both to the reduced transpiration rate (increased stomatal closure) and to an increased ABA sensitivity.

The strong induction of Stress Responsive –NAC1 (SNAC1) gene expression by drought in guard cells suggests an effect in stomatal closure (Hu et al. 2006). Two R2R3-MYB TFs (AtMYB60 and AtMYB61) are known to be directly involved in stomatal dynamics in Arabidopsis (Cominelli et al. 2005; Liang et al. 2005). In addition, the over-expression of SNAC1 upregulates a rice R2R3-MYB gene (UGS5) with a NAC recognition site in its promoter region (Hu et al. 2006). However, the relationship between SNAC1 and the TFs implicated in stomatal closure is unknown. This connection needs to be further investigated in order to understand the regulatory mechanisms underlying stomatal movements under drought stress.

9.3.5 ABA-Dependent Pathway

ABA-dependent gene induction during water deficit is controlled by at least five different classes of TFs. The ABA response element (ABRE) with the consensus ACGTGG/TC is bound by basic Leucine Zipper Domain (bZIP-type) TFs (Fig. 9.2). Three Arabidopsis bZIP TFs (AREB1/ABF2, AREB2/ABF4, and ABF3) are expressed in response to water-deficit stress and ABA treatment. Activation of the TFs requires ABA accumulation and the induction of an ABA-responsive protein kinase which activates the TF through phosphorylation.

Other TFs are also involved in ABA regulation of gene expression during cellular water deficit. Three genes encoding a class of TFs that is unique to plants, the NAC domain proteins ANAC019, ANAC055, and ANAC072 are induced by water deficit and ABA treatment. The NAC domain is a 60 bp DNA binding domain that is predicted to form a helix-turn-helix motif.

MYB, MYC, homeodomain TFs and a family of transcriptional repressors (Cys2/His2-type zinc-finger proteins) are also involved in the ABA response to water deficit. Expression of the drought-inducible gene Responsive to Dehydration 22 (RD22) from Arabidopsis was found to be induced by ABA. The promoter region of RD22 contains MYC (CANNTG) and MYB (C/TAACNA/G) *cis*-element recognition sites. MYC and MYB TFs only accumulate after an increase of ABA concentration. Over-expression of these TFs result in enhanced sensitivity to ABA and drought tolerance (Abe et al. 2003).

9.3.6 *Transcription Factors Involved in Regulation of Stomatal Aperture by Drought*

The first indication that TFs could be involved in stomata regulation appeared when it was found that ABI3 (an ABI3/VP1 B3-type TF) suppressed the effect of the abi1 mutation on stomatal regulation (Parcy and Giraudat 1997). It has been reported that modulation of transcription plays an important role in controlling guard cell activity. Recently two MYB-type TFs were identified as regulators of stomatal movements.

AtMYB60, a R2R3-MYB gene of Arabidopsis was shown to be specifically expressed in guard cells and its expression regulated by light conditions, ABA and water stress. However, elevated CO_2 concentrations, which are known to induce stomatal closure, do not modulate AtMYB60 expression. The expression of this gene is negatively regulated under drought, concomitantly with stomatal closure. Accordingly, the atmyb60-1 null mutant shows a constitutive reduction in stomatal opening and decreased wilting under water stress conditions. Interestingly, only a limited number of genes is altered in the mutant and most of them are downregulated. Many of these genes (e.g. Aquaporin, ERD10, ERD13 and ERF) were already described as being involved in plant response to water stress (Cominelli et al. 2005). AtMYB61, a member of the *A. thaliana* family of R2R3-MYB TFs, is also specifically expressed in guard cells in a consistent manner, being involved in the regulation of stomatal aperture (Liang et al. 2005). However, it has been shown that AtMYB60 and AtMYB61 have distinct expression patterns and functions: AtMYB60 gene expression is induced by light, leading to an increased stomatal aperture; AtMYB61 gene expression causes reduction in stomatal aperture. Moreover, Liang et al. (2005) showed that AtMYB61 gene expression was not altered in plants treated with ABA, salt and drought, known to induce stomatal closure. Thus, AtMYB61 seems to act via a parallel mechanism to that responsible for stomata closure in response to water deficit.

9.3.7 *Modulation of Gene Expression Involved in Stomatal Closure by Nitric Oxide*

Since the late 1940, there is increasing evidence that nitric oxide (NO), plays an important role in diverse physiological processes in plants, as an inducer of leaf expansion, root growth, phytoalexin production and stomatal closure (Leshem 1996; Noritake et al. 1996). Indeed, recent studies show that NO is involved in signalling functions in plants, especially in defence signalling. Several studies based on plants with altered NO-levels have recently provided genetic evidence for the importance of NO in gene induction. Gene expression levels are altered by NO, that lead to activation of genes involved in signal transduction, disease resistance and stress response, photosynthesis, cellular transport and basic metabolism. Recent evidence suggests that NO plays a role in the wounding/jasmonic acid (JA) signalling pathway. NO donors affect both wounding-induced H_2O_2 synthesis and wounding- or JA-induced expression of defence genes.

Stomatal closure is regulated by ABA-derived guard cell membrane transport to promote osmotic solute loss. Nitrate reductase-produced NO, but not NOS-derived NO is required for the ABA-regulated stomatal closure in Arabidopsis (Desikan et al. 2002). Infact, in ABA insensitive mutants abi1 and abi2, the phosphatases positions in ABA signal transduction cascade were localized downstream of NO. In *Vicia faba*, Ca^{2+}-sensitive ion channels are regulated by NO-derived calcium–release from intracellular stores and are cGMP-dependent (Durner et al. 1998). Indeed, NO directly regulates outward rectifying K^+ channels ($I_{k,out}$) or a closely associated protein, perhaps by protein S-nitrosylation (Grün et al. 2006 and references therein).

9.4 Flooding Stress

Flooding and submergence are two conditions that cannot be tolerated by most plants for periods of time longer than a few days. These stresses lead to anoxic conditions in the root system. At a critical oxygen pressure, mitochondrial respiration that provides the energy for growth in the photosynthetically inactive roots will decrease, then cease and the cells will die (Verslues and Bray 2006). But O_2 shortage (hypoxia/anoxia) is not only restricted to flooding stress. It is a frequent metabolic status of cells during normal development, particularly in tissues with high cell density, a high O_2 demand, and/or restricted O_2 entry, such as meristems, seeds, fruits, and storage organs (Geigenberger 2003).

Anoxia is distinct from hypoxia. In the former condition, there is essentially no oxygen (e.g., 0% O_2 at 20°C) available for respiration, NAD^+ regeneration happens via lactate and ethanolic fermentation, and there is ATP production solely via glycolysis (2–4 mol ATP per mole hexose). Cellular ATP content is low, and ADP content is elevated. In the latter, the oxygen concentration (e.g., <20.9% and >0% O_2 at 20°C) limits respiration and increases anaerobic metabolism, ATP is produced via glycolysis owing to limited availability of O_2 for oxidative phosphorylation, and NAD^+ is regenerated via lactate and ethanolic fermentation. Cellular ATP content is reduced, while ADP content is elevated (Bailey-Serres and Voesenek 2008).

Upon complete submergence several species from flood-prone environments have the capacity to stimulate the elongation rate of petioles, stems, or leaves, for restoring the contact between leaves and air. This amelioration response, called the low oxygen escape syndrome (LOES) facilitates the survival of submerged organs. The SUB1A gene of the polygenic rice (*Oryza sativa* L.) Submergence1 (Sub1) locus was shown to confer submergence tolerance through a ‘quiescence’ strategy in which cell elongation and carbohydrate metabolism are repressed. SUB1A, encodes an ERF domain–containing TF. The lack of SUB1A-1 or the presence of a slightly modified allele is associated with reduced submergence tolerance and the induction of the LOES (Fukao et al. 2006; Bailey-Serres and Voesenek, 2008).

In plants, different cell types exhibit a conserved response to low oxygen levels at the molecular level (Mustroph et al. 2010). This response includes the induction of a core set of genes after 30 min under hypoxia, whose expression is maintained for several hours (Klok et al. 2002; van Dongen et al. 2009). The increased transcript levels of these genes are further correlated with an enhanced association of the mRNAs with polysomes, suggesting that these transcripts are actively translated (Branco-Price et al. 2008).

Much effort has been expended in the search for the oxygen sensing process itself. An oxygen sensor is capable of directly detecting oxygen availability and subsequently triggering a signalling cascade. In animals the perception of O_2 deficit involves O_2-binding proteins, ROS and mitochondria. The O_2-consuming prolyl hydroxylases (PHDs) are direct sensors of O_2 availability. Under normoxia, PHDs target the proteosomal degradation of hypoxia inducible factor 1α (HIF1α), a subunit of a heterodimeric TF that regulates acclimation to hypoxia. The concomitant drop in PHD activity stimulates an elevation in HIF1α as O_2 declines. A paradox is that the production of ROS at the mitochondrial ubiquinone:cytochrome *c* reductase complex (Complex III) is necessary to initiate O_2 deficit responses. On the contrary, plants lack a HIF1α ortholog, although PHDmRNAs are strongly induced by O_2 deficit in Arabidopsis and rice. Furthermore, significant increases in mRNAs encoding enzymes involved in ROS signalling and production have been reported in several species upon transfer to low O_2 conditions (Bailey-Serres and Voesenek 2008 and references therein).

Since it is clear that the mechanism by which oxygen is perceived in plants is still largely unknown, hypoxia-responsive TFs could be a good starting point to investigate the regulation of the hypoxic response, as they represent the last regulatory element in the signal cascade (Licausi and Perata 2009).

Several studies have addressed the role of some hypoxia responsive TFs belonging to different protein classes, including the MYB, NAC, Arabidopsis Transcription Activation Factor (ATAF), Cup-shaped Cotyledons (CUC), Plant Homeodomain (PHD) and ERF families (Hoeren et al. 1998; Bond et al. 2009; Christianson et al. 2009; Licausi et al. 2010b). So a complex of transcriptional regulators is required to activate the molecular response to hypoxia.

9.4.1 Gene Regulation and Transcriptional Factor in Flooding Stress

Microarray data have revealed several transcription factors whose expression increases in response to various regimes of oxygen deprivation in Arabidopsis and rice, such as heat shock factors, ethylene response-binding proteins, MADS-box proteins, AP2 domain, leucine zipper, zinc finger and WRKY factors (Loreti et al. 2005; Lasanthi-Kudahettige et al. 2007). Furthermore, genes that encode putative components of the signal transduction pathway have been identified, such as calcium binding proteins, protein-modifying enzymes (i.e. receptor-like kinases and MAP kinases) and also already known components such as the gp91_phox subunit of the NAD(P)H oxidase (Bailey-Serres and Chang 2005; Magneschi and Perata 2009).

Lasanthi-Kudahettige et al. (2007) demonstrated the induction of several ERF and heat shock factors in anoxic rice coleoptiles. ERF-like TFs are induced both in Arabidopsis and rice, and these genes may play a role in the growth of coleoptiles if the plants regain access to oxygen and the capacity to synthesize ethylene.

Recently Licausi et al. (2010a), using a qRT-PCR platform (Morcuende et al. 2007; Barrero et al. 2009), have identified TFs that are differentially expressed by hypoxic conditions. Among the TFs that have been characterized, members of the AP2/ERF-type family are the most commonly represented in the set of upregulated TFs, followed by Zinc-finger and basic helix-loop-helix (bHLH-type) TFs. TFs belonging to the bHLH family are also highly represented among the downregulated genes, together with members from the bZIP and MYB families. Subsequently, they identified four protein families that included hypoxia-responsive TFs able to bind DNA elements present in the promoter of at least half of the hypoxic responsive core genes. The ERF binding sites ATCTA (Welsch et al. 2007), ATATT (Kannangara et al. 2007) and the DOF TF binding element CTTTT (T/A). In *silico* experiments and trans-activation assays confirmed that some TFs active in flooding stress are able to regulate the expression of hypoxia responsive genes. Particularly, five hypoxia-induced TFs (At4g29190; LBD41, At3g02550;HRE1, At1g72360; At1g69570; At5g66980) from different TF families [Zinc Finger, Ligand Binding Domain (LBD) or Lateral Organ Boundary Domain, ERF, DNA binding with one finger (DOF), ARF] showed this ability (Licausi et al. 2010b).

There is evidence of redox-sensitive TFs, at least one of which might be involved in the adaptive response to low oxygen. ZAT12, a putative zinc finger-containing TF, is recognized as a component in the oxidative stress response signalling network of Arabidopsis (Rizhsky et al. 2004). During oxidative stress, ZAT12 promotes expression of other TFs and the upregulation of cytosolic ascorbate peroxidase 1, a key enzyme in the removal of H_2O_2. Accumulation of ROS is a common consequence of biotic and abiotic stresses, including oxygen deprivation. ZAT12 transcript levels were significantly elevated in response to hypoxia and anoxia in several independent analyses (Branco-Price et al. 2005), indicating involvement of this potentially redox-regulated factor.

Advances have been made in molecular analyses of cDNAs and genes involved in the anaerobic response. Huq and Hodges (2000) reported early activation of a rice (*Oryza sativa* L.) gene by anoxia, the *aie* (anaerobically inducible early) gene. This gene encodes for a putative protein that shows short stretches of similarities to functionally interesting proteins (e. g. DNA binding proteins and nitric oxide synthase), indicating its putative involvement in signalling.

9.5 Salinity

High salinity, partially related to water availability as well (Bohnert, 2007), affects plants in two main ways: high concentrations of salts in the soil disturb the capacity of roots to extract water, and high concentrations of salts within the plant itself can be toxic (Hasegawa et al. 2000; Munns 2002; Munns and Tester 2008). Together, these effects reduce plant growth, development and survival. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to support continued growth (Cramer and Nowak 1992).

High salt concentrations, usually sodium chloride, cause osmotic stress by decreasing water potential within the cells, which immediately causes stomatal closure and reduces cell expansion in young leaves and root tips. Subsequently, ions, in particular sodium (Na^+), accumulate in the leaf blade and in the photosynthetic tissues, affecting photosynthetic components such as enzymes, chlorophylls, and carotenoids (Davenport et al. 2005). These effects, in turn, lead to secondary stresses such as oxidative stress and nutritional disorders (Hasegawa et al. 2000; Zhu 2001; Chinnusamy et al. 2006).

9.5.1 *Salt Overly Sensitive (SOS) Signal Pathway*

One of the main strategies for improving plant salt tolerance is through responses that counteract the osmotic component of the stress and typically, induce compartmentalization of Na^+ and Cl^- at the cellular and intracellular level to avoid toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf (Munns and Tester 2008) and synthesis and accumulation of compatible solutes within the cytoplasm (Fig. 9.3). Compatible solutes play a role in plant osmotolerance in various ways, protecting enzymes from denaturation, stabilising membranes or macromolecules or playing adaptive roles in mediating osmotic adjustment (Ashraf and Foolad 2007).

It has recently been reported that in Arabidopsis the increase of ion homeostasis is mediated mainly by a Salt Overly Sensitive (SOS) signal pathway (Yang et al. 2009). In particular, SOS1, encoding a plasma membrane Na^+/H^+ antiporter, plays a critical role in sodium extrusion and in controlling long-distance Na^+ transport from the root to shoot (Shi et al. 2000, 2002). This antiporter forms one component in a mechanism based on sensing of the salt stress that involves an increase of cytosolic $[Ca^{2+}]$, protein interactions and reversible phosphorylation with SOS1 acting in concert with other two proteins known as SOS2 and SOS3 (Oh et al. 2010 and references therein) (Fig. 9.3). SOS3 encodes a calcineurin B-like (CBL) protein, which is an EF-hand Ca^{2+}-binding protein that functions as a calcium sensor for salt tolerance (Zhu et al. 1998). SOS2 encodes a Ser/Thr protein kinase known as the CBL-interacting protein kinase (CIPK) (Liu et al. 2000). Salt stress elicits a transient increase of Ca^{2+} that is sensed by SOS3. SOS2 interacts with and is activated by SOS3 (Halfter et al. 2000). The SOS2/SOS3 kinase complex phosphorylates and activates SOS1 (Qiu et al. 2002). In yeast, co-expression of SOS1, SOS2 and SOS3 increases the salt tolerance of transformed yeast cells much more than expression of one or two SOS proteins (Quintero et al. 2002), suggesting that the full activity of SOS1 depends on the SOS2/SOS3 complex. Recently, SOS4 and SOS5 have also been characterized. SOS4 encodes a pyridoxal (PL) kinase that is involved in the biosynthesis of pyridoxal-5-phosphate (PLP), an active form of vitamin B6. SOS5 has been shown to be a putative cell surface adhesion protein that is required for normal cell expansion. Under salt stress, the normal growth and expansion of a plant cell becomes even more important and SOS5 helps in the maintenance of cell wall integrity and architecture (Mahajan et al. 2008).

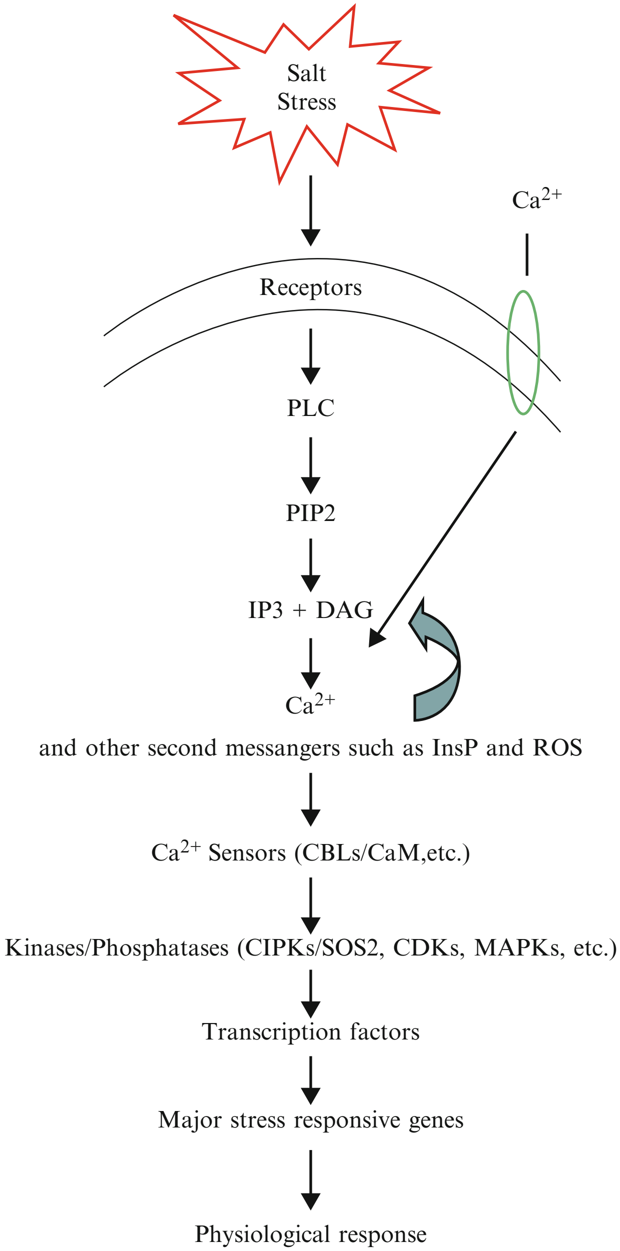

Fig. 9.3 Overview of signalling pathway under salt stress condition. Stress signal is first sensed by the membrane receptor, which activates PLC and hydrolyses PIP2 to generate IP3 and DAG. Following stress, cytoplasmic calcium levels are up-regulated via movements of Ca^{2+} ions from apoplast or from its release from intracellular compartments mediated by IP3. This change is perceived by calcium sensors which interact with their down stream signalling such as kinases and/or phosphatases. These proteins affect the expression of stress responsive genes leading to physiological responses

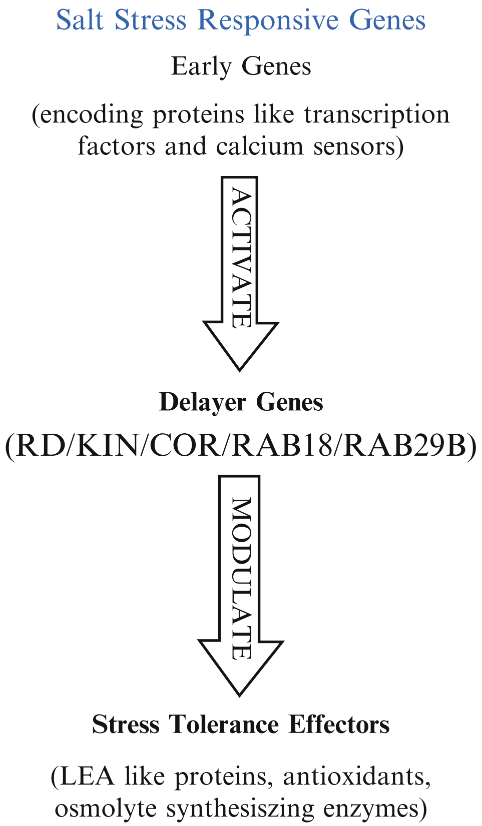

Fig. 9.4 Early and delayed gene expression in response to abiotic stress signalling. Early genes are induced within minutes of stress perception and often express transiently. In contrast, various stress genes are activated slowly, within hours of stress expression and often exhibit a long-term stable expression level. The expression of major stress genes like RD/KIN/COR/RAB18/RAB29B result in the production of various compatible compounds, antioxidants, molecular chaperones and LEA-like proteins which act in stress tolerance

Enhanced salt tolerance can also be achieved by overexpression of *A. thaliana* Na^+/H^+ exchanger 1 (AtNHX1) (Zhang et al. 2001), and Arginine Vasopressin 1 (AVP1) (Gaxiola et al. 2001), genes that regulate ion homeostasis in a similar manner as SOS1.

9.5.2 Late Embryogenesis Abundant (Lea) Proteins

The susceptibility or tolerance to salinity in plants is a coordinated action of multiple stress responsive genes, which also cross talk with other components of stress signal transduction pathways. Overexpression of genes encoding Late Embryogenesis Abundant (*Lea*) proteins, which accumulate to high levels during seed development, such as the barley HVA1 (Xu et al. 1996) and wheat dehydrin-5 (DHN-5) (Brini et al. 2007), can enhance plant salt tolerance, although their function is unknown. Salt and osmotic stress regulation of *Lea* gene expression is mediated by both ABA dependent and independent signalling pathways (Fig. 9.4). Both the pathways use Ca^{2+} signalling to induce *Lea* gene expression during salinity. ABA plays an

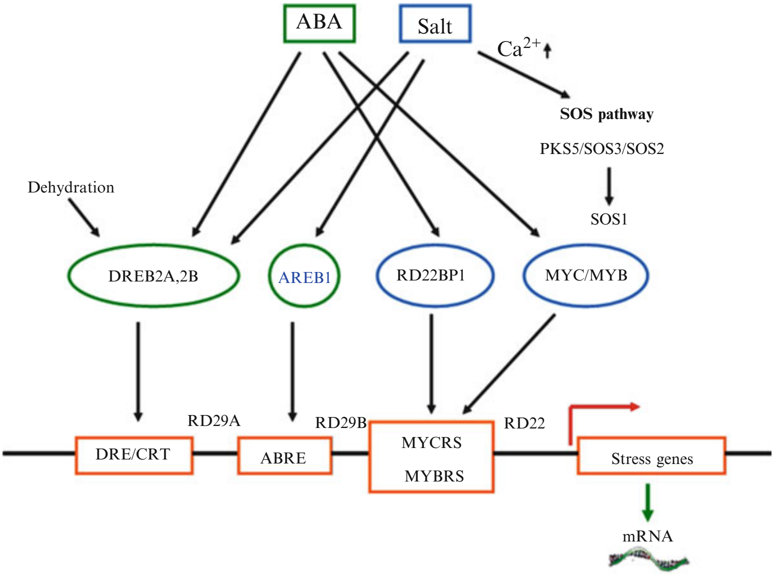

Fig. 9.5 Transcriptional regulatory network of *cis*-acting elements and ABA-dependent transcription factors involvend in gene expression under salt stress. Transcriptional factors involved in salinity stress are DREB2A/DREB2B, AREB1 and MYC/MYB, which interact with DRE/CRT, ABRE and MYCRS/MYBRS elements and activates the expression of respectively stress genes. Transcription factor-binding sites are represented as rectangoles. Salinity works mainly through the SOS pathway to restate cellular ionic equilibrium

important role in the response of plants to salinity, as well as to drought, as confirmed by Xiong and co-workers experiments (2001), who found that ABA-deficient mutants performed poorly under salinity stress. Salt stress signalling through Ca^{2+} and ABA mediate the expression of the *Cor*/*Lea* salinity stress responsive genes by various upstream TFs that activate DRE/CRT, ABREs, MYC recognition sequence (MYCRS) and MYB recognition sequence (MYBRS) *cis*-elements (Fig. 9.5). The regulation of gene expression through DRE/CTR *cis*-elements appears to be mainly ABA-independent, whereas ABRE and MYB/MYC element-controlled gene expression is ABA-dependent. This latter activates bZIP TFs called AREB, which bind to ABRE element to induce the stress responsive gene (*RD29A*). TFs such as DREB2A and DREB2B transactivate the DRE *cis* element of osmotic stress genes and thereby are involved in maintaining the osmotic equilibrium of the cell (Majaian and Tuteja 2005) (Fig. 9.5).

Recently it has been shown that ABA-dependent and -independent TFs may also cross talk to each other in a synergistic way to amplify the response and improve stress tolerance. For example, *RD29A* expression depends on both DRE and ABRE elements and ABA can also induce the expression of C-repeat binding proteins, CBF1-CBF3. Some genes, such as RAD22, lack the typical CRT/DRE elements in their promoter, suggesting an alternative unknown mechanism. The MYC/MYB

TFs could bind MYCRS and MYBRS elements, respectively, and help in activation of the *RD22* gene.

Over expression of regulatory genes in signalling pathways, such as protein kinases (MAPK, CDPK) also increases plant salt tolerance (Kovtun et al. 2000; Saijo et al. 2000; Moon et al. 2003; Teige et al. 2004).

9.6 High Light Stress

Light is one of the most important environmental factors for plants, as it provides the source of energy for plant life. It is therefore not surprising that plants have adopted the ability to sense multiple parameters of ambient light signals, including light quantity (fluence), quality (wavelength), direction and duration. The responses of plants to light are complex: seed germination, seedlings photomorphogenesis, chloroplast development and orientation, photodinesis, stem growth, pigment biosynthesis, flowering and senescence (Kendrick and Kronenberg 1994). Collectively these processes are known as photomorphogenesis. Most of these responses requires changes in both chloroplast and nuclear gene expression, which are mediated by three major classes of photoreceptors: phytochromes (Phy), blue/Ultraviolet A (UVA) and UVB light receptors (Ahmad and Cashmore 1996; Jordan 1996; Quail et al. 1995).

Excess light (EL) is the light absorbed by plants and algae that exceeds their photosynthetic capacity (Fig. 9.6). Although light is needed for photosynthesis, absorption of EL can lead to increased production of highly reactive intermediates and by-products that can potentially cause photo-oxidative damage and inhibit photosynthesis (Hazen et al. 2005).

Transcriptional regulatory networks have a key role in mediating light signalling through the coordinated activation and repression of specific downstream genes (Thompson and White 1991). For each developmental response, the above-mentioned

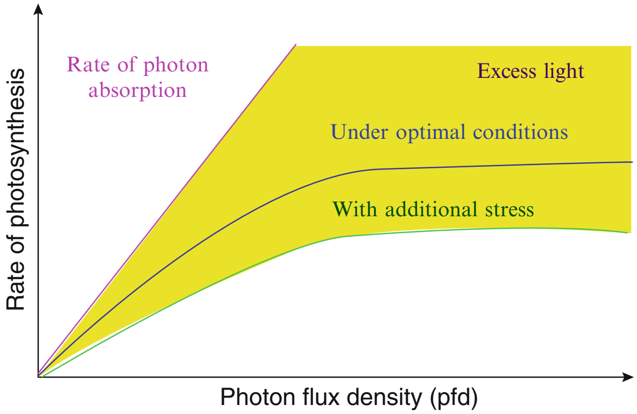

Fig. 9.6 Light response curves for photosynthesis compared with the rate of light absorbiment.

photoreceptors can contribute to the perception. In addition to this complexity there are organ-specific and developmental stage-specific responses to light, which represent a multitude of variations among light-responsive transcriptional networks.

Organisms that perform oxygenic photosynthesis convert light energy into chemical energy (in the form of ATP and NADPH) through the photosynthetic transport of electrons within thylakoid membranes. However, when the rate of absorption of light energy by photosynthetic pigments exceeds the rate of its consumption in chloroplasts, the absorbed light energy accelerates the process of photo-inhibition (Jiao et al. 2003; Tepperman et al. 2004). Therefore, exposure of a plant to light exceeding what is utilized in photochemistry leads to inactivation of photosynthetic functions and the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radicals and singlet oxygen ($^1O_2$; Niyogi 1999, 2000). The effects of these ROS can be the oxidation of lipids, proteins and enzymes necessary for the proper functioning of the chloroplast and the cell as a whole.

Classical genetic and molecular approaches have identified various regulators downstream of photoreceptors. Many of these encode TFs, as well as kinases, phosphatases and degradation-pathway proteins. Although some of these regulators are specific for light quality, others regulate signal transduction networks in response to various light signals, representing potential signal integration points.

Microarray experiments have shown that exposure to EL results in dramatic changes in gene expression in plants (Adamiec et al. 2008; Bechtold et al. 2008) and green algae (Vanderauwera et al. 2005; Fischer et al. 2006).

Light can modulate photoreceptor activity by inducing changes that alter their cellular localization. The best characterized light receptor is Phy, which exists in two photochemically interconvertible forms, Pr and Pfr, and is encoded by a small family of genes in angiosperms. Phytochromes are synthesized in the inactive Pr form, that absorbs red light (660 nm) and are activated on light absorption by conversion to the biologically active Pfr form, that absorbs far-red light (730 nm), (Fig. 9.7). The photoconversion of phytochromes results in their translocation from the cytoplasm into the nucleus, which is crucial for allowing them to interact with transducers in initiating downstream transcriptional cascades (Quail 2002).

In Arabidopsis, a subset of these genes appears to be regulated in EL by the blue/ UV-A light absorbing cryptochrome photoreceptors, CRY1 (Wang et al. 1997). CRY1 and its paralog CRY2 are nuclear flavoproteins which share a similar chromophore-binding domain at their N termini but differ in the variable extensions at their C-termini. CRY1 and CRY2 are known to mediate specific blue light–dependent effects such as inhibition of hypocotyl elongation in response to relatively high and low frequencies of blue light, respectively (Christie et al. 1998; Salomon et al. 2000). CRY2 is constitutively nuclear-localized, whereas CRY1 is nuclear in the dark but largely cytoplasmic under light (Lin and Shalitin 2003). It has been suggested that light activation of the N terminus of CRY1 (CNT) induces a conformational change in its C terminus (CCT), allowing its autophosphorylation and dimerization, and possible interactions with downstream partner proteins (Sang et al. 2005).

Genome-wide gene expression analysis showed that the EL-dependent regulation of 77 genes was altered in a *cry1* mutant, and 26 of these genes were also

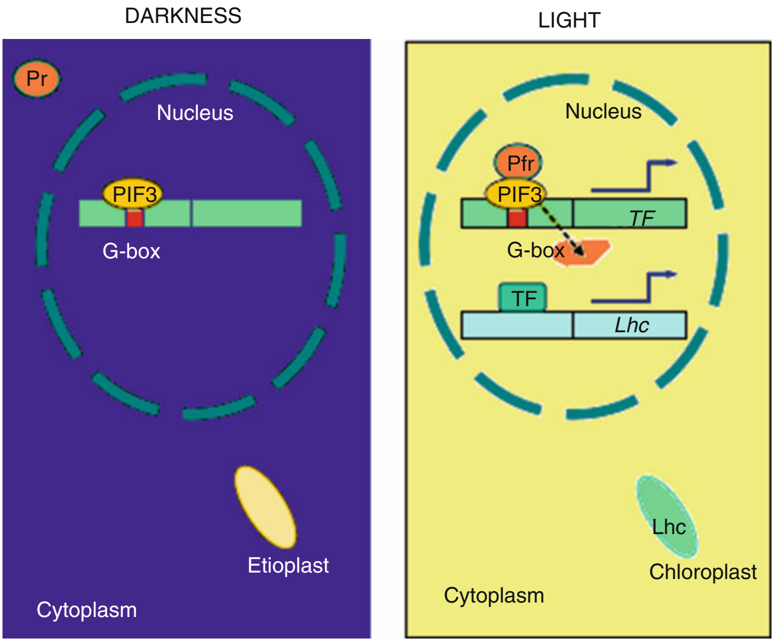

Fig. 9.7 Control of gene expression by the bHLH factor PIF3

misregulated in a *hy5* TF mutant (Wang et al. 1997). For example, induction of *ELIP1* and *ELIP2*, which encode light stress-related genes relatives of the light harvesting chlorophyll *a/b*-binding (LHC) protein family, by either EL or high-intensity blue light was strongly attenuated in *cry1* mutants. However, their induction was unaffected in *cry2*, *phyA*, *phyB*, or *hyh* mutants, indicating that the induction of *ELIP1* and *ELIP2* is mediated specifically by CRY1 in a blue light–dependent manner (Wang et al. 1997).

A picture is emerging in which signals from different photoreceptors are highly integrated, but at the same time light-regulated networks are able to direct organ-specific responses. Besides gene activation, microarray data suggest the existence of at least one other pathway, which is initiated by the early repression of transcriptional cascades, as revealed by the fact that the expression of a large number of TFs is rapidly repressed by light (Jiao et al. 2003; Toledo-Ortiz et al. 2003).

9.6.1 Light-Responsive Transcription Factors and Gene Activation

An important finding from microarray analysis is the enrichment of TFs in light-responsive genes during photomorphogenesis, especially shortly after light exposure when light-stimulated photomorphogenesis is barely observable (Toledo-Ortiz et al.

2003; Jiao et al. 2003). In the case of the photosynthesis-associated nuclear genes (PhANGs) from higher plants, interesting evolutionary aspects of the molecular mechanisms by which transcription is activated by light receptors (e.g. phytochrome) could be addressed through the comparative analysis of promoter sequences.

Light-responsive TFs have been identified through screens for light-responsive *cis*-element (LRE)-binding proteins and through genetic analyses of mutants that are deficient in their response to specific types of light. A combination of various methods has been used to identify these LREs. Traditional deletion and mutagenesis analysis of promoters of known light responsive genes has been used to pinpoint LREs, and footprinting and gel-retardation assays have been used to screen for binding motifs of known light-responsive TFs (Jiao et al. 2007). A range of LREs have been documented in different promoters, many of which positively or negatively mediate gene expression in response to light. Although many LREs and their binding proteins have been identified, no single element is found in all light-regulated promoters, suggesting a complex light-regulation network and a lack of a universal switch (Jiao et al. 2007). Several general conclusions are possible: no single conserved sequence element is found in all light responsive promoters; regulatory elements contain different combinations of *cis*-acting sequences; several protein factors bind sequences within photoregulated promoters, although their actual contribution to light-activated transcription remains uncertain. In fact, some of these TFs are regulated by just one type of light, whereas many more respond to a wide spectrum of light.

Both positive and negative transcriptional control of TFs by light has been documented. For example, the transcription of Common Plant Regulatory Factors 1 (CPRF1) from parsley is rapidly induced by light (Fig. 9.8). CPRF1 has the ability to bind to G-box, a well-defined LRE and its levels increase only transiently after light treatment, while transcription might be blocked by the binding of CPRF1 to its own promoter (Feldbrugge et al. 1994).

Although individual LRE-containing promoters primarily respond to a specific wavelength of light, no reported LRE has activity in an organ-specific manner, suggesting that combinatorial functions of distinct elements are important for light-regulated promoter activities (Degenhardt and Tobin 1996).

Several basic post-translational mechanisms are involved in regulating TF activities and the subcellular localization in response to light. The phosphorylation of TFs is a common modification that can influence their ability to bind to promoters (Fig. 9.8). For example, the level of G-Box Binding Factor 1 (GBF1) is constant but its affinity for the G-box is modulated by its phosphorylation status: its phosphorylation by nuclear Casein Kinase II (CKII) enables G-box binding (Klimczak et al. 1995). Light might also regulate the subcellular localization of TFs through phosphorylation (Harter et al. 1994) (Fig. 9.9). For example, CPRF2 from parsley is localized in the cytosol in the dark and treatment with light causes an import in the nucleus; light-dependent *in vivo* phosphorylation of CPRF2 is probably the key event that triggers its nuclear import (Wellmer et al. 1999).

Several bHLH TFs that bind the G-box have been implicated in phytochrome-mediated responses in genetic experiments. Phytochrome Interacting Factor 3 (PIF3) is a nuclear-localised bHLH protein (Ni et al. 1998). Upon irradiation, phytochrome

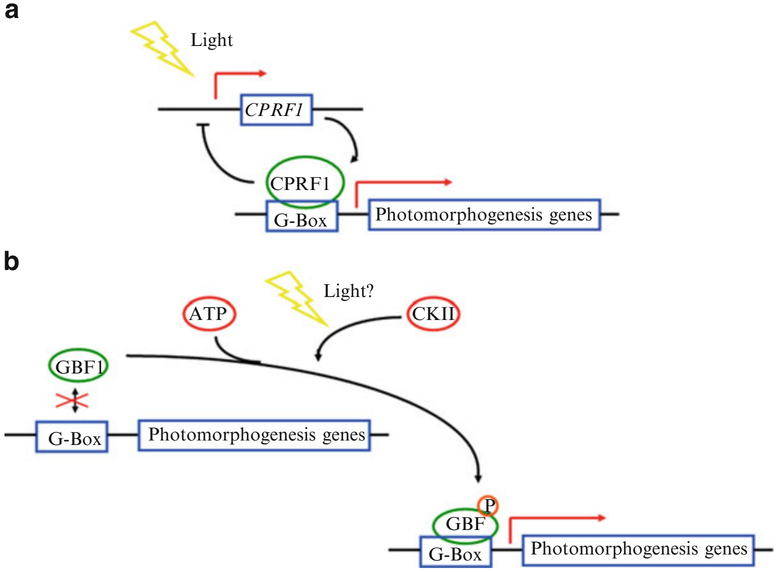

Fig. 9.8 General mechanisms of transcription factor regulation by light. (**a**)Transcription of the G-box-binding transcription factor COMMON PLANT REGULATORY FACTORS 1 (CPRF1) is induced by light. CPRF1 represses its own transcription, resulting in the tight control of its expression. (**b**) Unphosphorylated G-BOX BINDING FACTOR 1 (GBF1) lacks affinity for its target genes. Phosphorylation by CASEIN KINASE II (CKII) allows GBF1 to bind to promoters that contain G-boxes

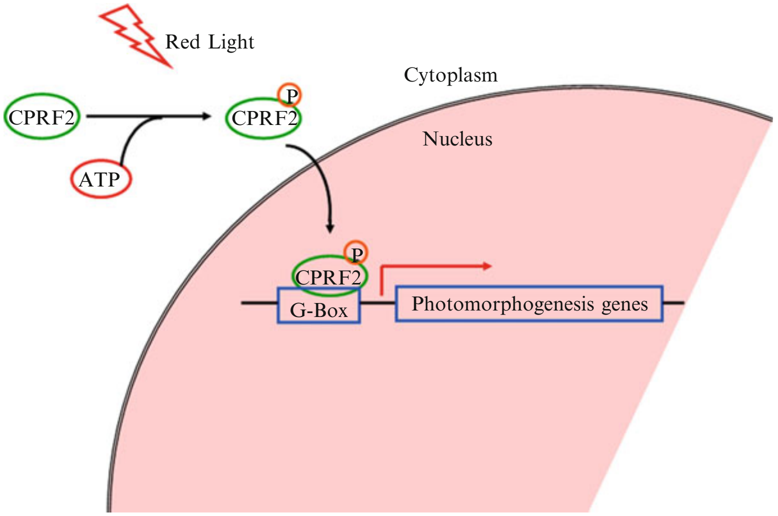

Fig. 9.9 Mechanism of transcription factor regulation by *red light*. *Red light* induces phosphorylation and nuclear translocation of the G-box-binding transcription factor CPRF2

is transformed from Pr to Pfr and part of the phytochrome pool migrates in its Pfr form from the cytosol to the nucleus (Yamaguchi et al. 1999) where it binds DNA-bound PIF3 (Martinez-Garcia et al. 2000). Pfr causes degradation of PIF3 and presumably activates PIF3 by unknown molecular mechanisms. In the presence of Pfr, PIF3 is required for normal expression of several photosynthetic genes and chloroplast development. PIF3 provides a short-cut between phytochrome and gene expression as the *pif3* mutant shows impaired induction by red light of a small group of nuclear-encoded genes of chloroplast proteins as well as impaired accumulation of chlorophyll (Monte et al. 2004) (Fig. 9.7). Light perceived by phytochromes induces rapid degradation of PIF3, but leaves a residual pool (Bauer et al. 2004; Monte et al. 2004).

In the dark, some TFs that positively regulate gene expression in response to light, such as Long After Farred Light 1 (LAF1), are ubiquitylated by Constitutive Photomorphogenic 1 (COP1), a ring-finger-type ubiquitin E3 ligase. In darkness, COP1 acts as E3 ligase in the nucleus, targeting TFs like HY5 and LAF1 to degradation via the 26S proteasome. Upon exposure to light, COP1 migrates from the nucleus to the citosol (Osterlund et al. 2000).

9.7 Ultraviolet-B (UVB) Radiation

UV radiation is an integral part of the sunlight that reaches the surface of the Earth. The UV region of the spectrum is by convention divided into three parts: UVA (320–400 nm), UVB (280–320 nm) and UVC (less than 280 nm). Of these, only UVA and longer-wavelength UVB have biological importance because the stratospheric ozone layer very effectively absorbs UV radiation with wavelengths below 290 nm (McKenzie et al. 2003). It is now agreed that, just like visible light (400–750 nm), the UVB portion of the solar radiation that reaches the sessile plants can act both to cause environmental stress (damaging) and as an informational developmental (non-damaging) signal (Brosche and Strid 2003; Frohnmeyer and Staiger 2003; Barnes et al. 2005). The level of UVB is also dependent on altitude, the degree of cloud cover, dispersal or absorbance by atmospheric aerosols and pollutants, surface reflectance, and the thickness of the vegetation canopy. Absorption of UVB photons can lead to substantial damage wide range of biologically active molecules, such as nucleic acids, aromatic amino acids, lipids and phenolic compounds (Jansen et al. 1998; Gerhardt et al. 1999; Frohnmeyer and Staiger 2003). Because of its absorption spectrum, DNA is a major and long-studied target of UVB irradiation. UVB radiation is known to change gene expression in plants, both by up-regulation and down-regulation. Genes that encode for enzymes of the phenylpropanoid pathway have been shown to be up-regulated at the transcription level, leading to the biosynthesis of UVB protecting pigments. Many other defence related genes are also ‘switched on’ by UVB radiation. In contrast, many genes associated with photosynthetic proteins (32 kDa PSII protein, Lhcb, RuBisco, etc.) are down-regulated.

Transcriptomic analyses in maize (Blum et al. 2004; Casati et al. 2006) and Arabidopsis (Hectors et al. 2007; Kilian et al. 2007; Brown and Jenkins 2008) show that UVB regulates a large number of genes concerned with a wide range of cellular processes. Some of these genes are expressed in specific organs and at particular developmental stages. It is, therefore, important to identify DNA sequence elements and TFs involved in these responses and to understand how transcriptional regulation is coupled to UVB signalling pathways. A significant proportion of the genes, most rapidly induced by UVB, encode TFs and these proteins undoubtedly play key roles in UVB responses (Kilian et al. 2007).

The study by Ulm et al. (2004) established that Long Hypocotyl5 (HY5), a bZIP transcription factor that is one of the key regulators of cryptochrome and phytochrome controlled photomorphogenesis, is an important component of the UVB-induced signalling network. UVB promotes rapid transcriptional activation of HY5 (and its interacting partner Long Hypocotyl5-Like [HYH]) independently of all known photoreceptors, and loss of HY5 results in the impairment of the transcriptional induction of a subset of UVB-responsive genes. Taken together, these observations demonstrate that UVB up-regulates HY5 transcription by yet-unknown signalling pathway (s), and that the signalling cascades that mediate responses to visible light and long-wavelength UVB (300–320 nm) use shared components.

A. thaliana UVB Resistance (UVR8) is a UVB-specific signalling component that orchestrates the expression of a range of genes with vital UVB-protective functions. UVR8 regulates the expression of the transcription factor HY5 when the plant is exposed to UVB (Hectors et al. 2007). Additional studies suggested that HY5 also regulates the transcription of several photosynthesis-related genes, such as the ribulose bisphosphate carboxylase small subunit (RbcS1A) (Lee et al. 2007). Given that HY5 appears to regulate the expression of several Arabidopsis genes known to respond to abiotic stress conditions (e.g. CBF1, DREB2A, RD20 and MYB59) (Lee et al. 2007), it is inferred that HY5 could also be involved in the regulation of photosynthesis by adverse environmental conditions.

In vitro analysis showed that HY5 directly binds to the promoters of several light-inducible genes (Hiltbrunner et al. 2006) and a recent chromatin immuno-precipitation analysis in combination with a whole-genome tiling microarray revealed that HY5 binds directly to a large number of genomic sites, mainly at the promoter regions of annotated genes. HY5 interacts specifically with the G-box (CACGTG) and is required for normal control by light of promoters bearing this sequence (Lee et al. 2007). The *hy5* mutant shows impaired gene expression responses to UVB (Ulm et al. 2004).

9.8 Cold Stress

Cold stress prevents the expression of full genetic potential of plants owing to its direct inhibition of metabolic reactions and, indirectly, through cold-induced osmotic (chilling-induced inhibition of water uptake and freezing-induced cellular dehydration), oxidative and other stresses. Cold stress, which includes chilling (<20°C) and/or freezing (<0°C) temperatures, adversely affects the growth and development of plants. Chilling and freezing are stresses that show different effects on plants: the former leads to slow biochemical reactions, such as enzyme and membrane transport activities; the latter leads to ice crystal formation that can cause the disruption of cell's membrane system (Chinnusamy et al. 2007). Cellular membranes, in fact, are fluid structures, and cold temperatures can reduce membrane fluidity with increased rigidity and fragility.

Cold acclimation is a process by which plants acquire freezing tolerance upon prior exposure to low non-freezing temperatures. Most temperate plants can cold-acclimate and acquire tolerance to extracellular ice formation in their vegetative tissues. Winter-habit plants (winter wheat, barley, oat, rye, oilseed rape, etc.) have a vernalization requirement, which prevents premature transition to the reproductive phase before the threat of freezing stress during winter has passed. However, after vernalization and at the end of the vegetative phase, the cold acclimation ability of winter cereals gradually decreases (Fowler et al. 1996) and many important crops, such as rice, maize, soybean, cotton and tomato are chilling sensitive and incapable of cold acclimation. The molecular basis of this acquired chilling tolerance or chilling acclimation is poorly understood.

Cold stress regulates the plant transcriptome through transcriptional, post-transcriptional and post-translational mechanisms.

9.8.1 Transcriptional Regulation Under Cold Stress

Cold acclimation temperatures induce complex changes in the plant transcriptome. Cold stress induces the expression of AP_2/ERF family TFs, that is, CBFs, which can bind to *cis*-elements in the promoters of COR genes and activate their expression (Fig. 9.10). CBFs regulate the expression of genes involved in phosphoinositide metabolism, transcription, osmolyte biosynthesis, ROS detoxification, membrane transport, hormone metabolism and signalling and many others with known or presumed cellular protective functions (Fowler and Thomashow 2002; Maruyama et al. 2004; Lee et al. 2005).

The first isolated cDNAs encoding DRE binding proteins were DREB1A and DREB2A (Liu et al. 1998) from Arabidopsis and then, DREB genes have been isolated from a wide variety of plants. In wheat and barley, a number of *CBF* homologs have been mapped to low temperature QTLs, *Fr-2* chromosomal region (Skinner et al. 2005; Vágújfalvi et al. 2005; Miller et al. 2006).

Expression of *DREB1* genes is extensively investigated in various crops with regard to different abiotic stresses. It was found that the expression of *AtDREB1* gene is induced by cold, but not by dehydration, or high salt stress (Liu et al. 1998; Shinwari et al. 1998). Similarly, *CBF* genes also showed high expression in response to low temperature treatment and its transcript was detectable after 30 min of exposure to 4 °C, and showed maximum expression at 1 h (Medina et al. 1999). It has been found in some studies that the expression of both the *DREB* genes is induced

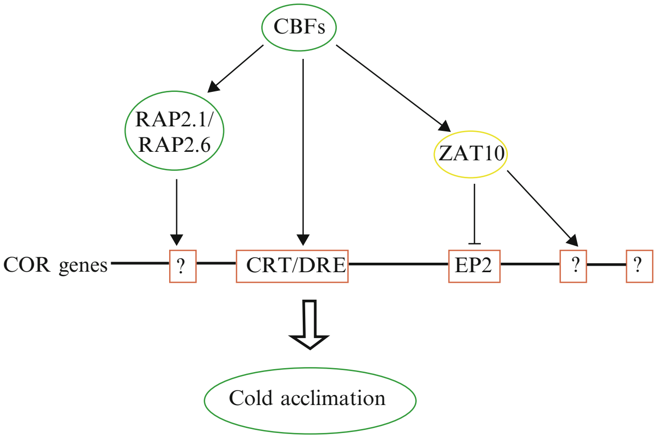

Fig. 9.10 CBFs regulate the expression of COR genes that confer cold tolerance. CBFs might cross-regulate the each other's transcription. CBFs induce the expression of ZAT10 which might downregulate the expression of COR genes. *Arrows* indicate activation; *lines* ending with bar indicate negative regulation; *question mark* (?) indicate unknown *cis*-elements

by abiotic stresses, however, at different time periods. In rice, *OsDREB1A* and *OsDREB1B* were induced within 40 min after cold exposure and did not respond to ABA treatment.

Indeed, CBF regulon could be subregulated by cold-responsive transcription factor genes RAP2.1 and RAP2.7 as shown by microarray analysis of transgenic Arabidopsis plants ectopically expressing CBFs (Fowler and Thomashow 2002).

Transcriptome and metabolome analyses in Arabidopsis accessions differing in constitutive freezing tolerance suggest that the CBF pathway might also have a crucial role in constitutive freezing tolerance (Hannah et al. 2006).

In Arabidopsis, ICE1 (Inducer of CBF Expression1), a MYC-type bHLH TF, can bind to MYC recognition elements in the CBF3 promoter and is important for the expression of CBF3 during cold acclimation. ICE1 is constitutively expressed and localized in the nucleus, but it induces expression of CBFs only under cold stress (Fig. 9.11). This suggests that cold stress-induced post-translational modification is necessary for ICE1 to activate downstream genes in plants (Chinnusamy et al. 2003).

The cold induction of genes involved in calcium signalling, lipid signalling or encoding receptor-like protein kinases are also affected by the ice1 mutation (Lee et al. 2005).

Cold-acclimation transcriptional network investigated by microarray on cold-responsive transcriptome of wild type and mutants of transgenic Arabidopsis show that ICE1 is a transcriptional inducer of CBFs (CBF1–CBF3), ZAT12, NAC072 and the constitutively expressed transcription factor HOS9 in Arabidopsis (Benedict et al. 2006).

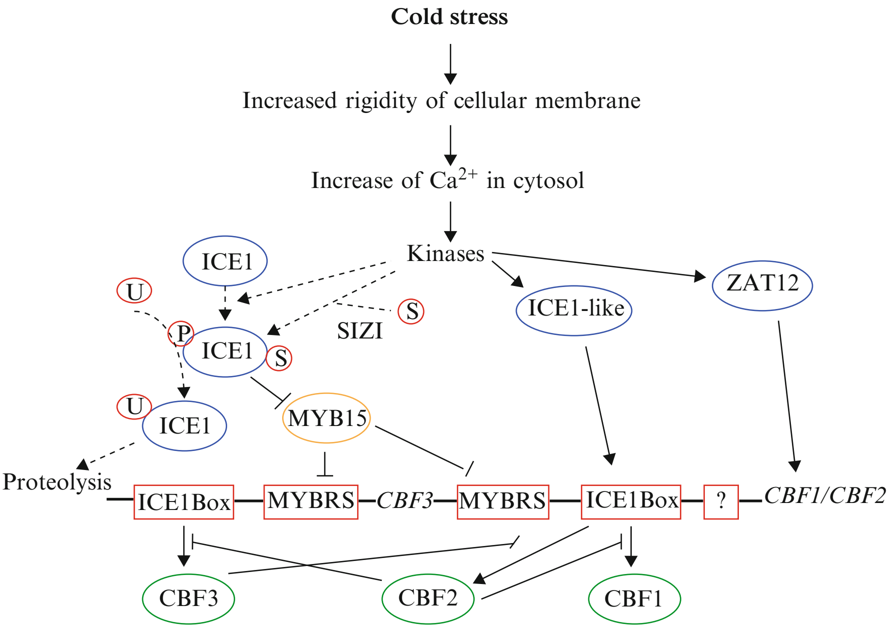

Fig. 9.11 Cold-responsive transcriptional network in Arabidopsis. Costitutive expressed ICE1 is actived throught sumoylation and phosphorylation induced by cold stress. ICE1 actived induce the transcription of *CBFs* and reprime *MYB15*. The expression of *CBFs* is negatively regulated by MYB15 and ZAT12. HOS1 mediates the ubiquitination and proteolysis of ICE1, thus negatively regulates CBF regulons. *Broken arrows* indicate post-translational regulation; *solid arrows* indicate activation; *lines* ending with bar indicate negative regulation; *question mark* (?) indicate unknown *cis*-elements

Thus, CBFs play a pivotal role in gene regulation during cold acclimation in evolutionarily diverse plant species.

9.8.2 Feedback Repression of Transcriptional Factors

Feedback repression of TFs which regulate cold acclimation gene expression appears to be a key for maintaining an optimal cold-induced transcriptome. In Arabidopsis, during cold acclimation, CBF2 is a negative regulator of *CBF1* and *CBF3* expression (Fig. 9.11) and furthermore, *CBFs* are negatively regulated by an upstream TF, MYB15. In fact, *MYB15* is expressed even in absence of cold stress and can bind to MYB recognition elements in the promoters of *CBFs*. An other transcriptional factor gene, always present in Arabidopsis, negatively regulating *CBFs* is *ZAT12*, a cold-induced C2H2 zinc finger (Chinnusamy et al. 2007) (Fig. 9.11).

9.8.3 Post-transcriptional Regulation Under Cold Stress

Gene expression is, also, post-transcriptionally regulated at pre-mRNA processing, mRNA stability, export from nucleous and translation step.

RNA processing is a crucial nuclear step for the synthesis of functional mRNAs and this process is coupled with nuclear export of mRNAs. The transduction of environmental signals into the nucleus to alter transcription and the export of mRNA and small RNAs to the cytoplasm is through the nuclear pore complex (NCP) of the nuclear envelop. In plants, export competent messenger ribonucleoproteins consist of mRNA cargo and nucleocytoplasmic shuttling nuclear proteins, such as the RNA export factors and DEAD-box proteins. The DEAD-box family of RNA helicases is involved in RNA metabolism, such as transcription, RNA processing, RNA decay and nucleocytoplasmic transport. An important role for such helicase in mRNA export and plant cold stress responses is the induction of *CBF1* and *CBF2* (Gong et al. 2002).

Indeed, small non-coding RNAs (microRNAs or miRNAs and short interfering RNA or siRNAs) have potentially a crucial role in plant stress response, because are ubiquitous repressors of gene expression. miRNAs and siRNAs regulate gene expression by directing the cleavage or translational repression of complementary target mRNAs or by inducing transcriptional silencing of target genes.

Abiotic stresses induce miRNAs and siRNAs that seem to downregulate specific target genes.Transcriptome analysis in Arabidopsis revealed that the NRPD1A required for nat-siRNA and heterochromatic siRNA biosynthesis is upregulated by cold stress (Lee et al. 2005). The upregulation of NRPD1A might impact the cold-responsive transcriptome through increased generation of some siRNAs. Microarray analysis shows that about 17% of cold-upregulated genes encode TFs, whereas only 7% of cold downregulated genes encode transcriptional regulators. So microarray analysis suggests that post-transcriptional regulation might serve for downregulation of genes during cold acclimation (Lee et al. 2005; Sunkar et al. 2007).

9.8.4 Post-translational Regulation Under Cold Stress

Controlled proteolysis of transcriptional regulators also plays an important role in shaping the cold-responsive transcriptome in plants. Two important post-translational protein modifications are the ubiquitination and the sumoylation.

Ubiquitination is mediated by High Expression of Osmotically Responsive1 (HOS1). *HOS1* encodes a RING finger ubiquitin E3 ligase, the nuclear localization of which is also enhanced by cold stress. HOS1 physically interacts with ICE1 and mediates the ubiquitination of ICE1 to regulate negatively the expression of ICE1 target genes (Fig. 9.11) and is thus critical for the de-sensitization of plant cells to cold stress (Dong et al. 2006).

Sumoylation is induced by SUMO (Small Ubiquitin-related Modifier) proteins that are conjugated to proteins substrates in a process dependent on SUMO E3 ligases. Sumoylation might protect target proteins from proteasomal degradation preventing the ubiqiutination (Ulrich 2005).

9.8.5 The Importance of Regulons That Are Not Cold Responsive

Two TFs have recently been identified in Arabidopsis, High Expression Of Osmotically Responsive Genes 9 (HOS9) and HOS10, that are required for basal freezing tolerance (Zhu et al. 2004, 2005). The HOS9 and HOS10 genes encode homeodomain and MYB (AtMYB8) TFs, respectively, and their transcript levels are not cold responsive. Loss-of-function mutations in these genes cause significant decreases in basal and acquired freezing tolerance. Interestingly, the mutants show stronger or earlier cold-induction of several CBF-target genes, but no effects on the expression of CBFs. These results suggest a crucial role in freezing tolerance for regulons that are not cold responsive.

The importance of CBF-independent pathways is also supported by analysis of mutants that have increased freezing tolerance. Mutations in Eskimo1 (ESK1), a protein of unknown function, result in constitutive freezing tolerance (Xin et al. 2007).

9.9 Oxidative Stress

Oxidative stress occurs when reactive oxygen species (ROS) are not rapidly scavenged and the rate of repair of damaged cell components fails to keep pace with the rate of damage. If this situation persists, irreversible damage results in a loss of physiological competence and eventual cell death (Mittler 2002; Mullineaux and Baker 2010).

ROS are generated in all organisms accompanying biochemical reactions, for example in mitochondria, peroxisomes or vacuoles. They include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the extremely short-lived hydroxyl radical (OH^-) (Bonhert 2007). Normally, ROS are rapidly removed by a complex defence system, involving enzymes and metabolic intermediates of low molecular weight functioning as antioxidant agents, among these, ascorbate and glutathione (GSH) are of paramount importance (Blokhina et al. 2003). If there is a serious imbalance in any cell compartment between the production of ROS and antioxidant defence, this removal can be impaired and oxidative stress and damage occur (Mittler 2006; Foyer and Noctor 2005).

However, in many cases, the production of ROS is genetically programmed, induced during the course of development and by environmental fluctuations, and has complex downstream effects on both primary and secondary metabolism. In fact, plant cells produce ROS, particularly superoxide and H_2O_2, as second messengers in many processes associated with plant growth and development (Gapper and Dolan 2006), and transmit information concerning changes in the environment via the production of bursts of superoxide at the plasma membrane (Foyer and Noctor 2005).

ROS signalling has been shown to be an integral part of acclimation response to stress. ROS play, a dual role in the response of plants to abiotic stresses functioning as toxic by-products of stress metabolism, as well as important signal transduction molecules integrated in the networks of stress response pathway mediated by calcium, hormone and protein phosphorylation (Miller et al. 2010). Besides being harmful agents, ROS, in fact, also act as signal compounds eliciting a response to the original stress through changes of nuclear gene expression.

During photosynthesis ROS generation occurs via electron transport reactions in the chloroplasts, such as the Mehler reaction, which generates superoxide anion that is converted to H_2O_2. ROS are also produced in the chloroplasts through the photoreduction of the herbicide methyl viologen (MV), which is a superoxide anion propagator. The relatively low reactivity of H_2O_2 suggests that it could diffuse from the chloroplast to initiate signalling events. Alternatively, the accumulation of H_2O_2 could be limited to chloroplasts initiating distinct signalling cascade. Environmental stresses like high light intensity, drought, extreme temperatures, salinity and UV radiations all enhance photosynthetic ROS generation (Scarpeci et al. 2008) and oxidative damage in different cell compartments and, ultimately, cause cell death unless counteracted by photoprotection mechanisms. They, in fact, cause peroxidation and de-esterification of membrane lipids, and also lead to protein denaturation as well as other forms of photo-oxidative damage. Consistently, the chloroplast and in particular the thylakoid membranes, which are susceptible to damage (Moller et al. 2007) are both the target and the checkpoint platform controlling stress responses (Dall'Osto et al. unpublished data).

In plants, different pathways sense ROS from extracellular sources or organelles such as mitochondria, chloroplast or peroxisome. For example, H_2O_2 can play a very important role in mediating signal transduction in response to stress in plant cells. H_2O_2 diffuses rapidly from its site of synthesis within subcellular microdomains, depending on its concentration, and can transmit intracellular signals to the nucleus by oxidizing various upstream components of the signalling pathway, including TFs that ultimately result in changes in gene expression (Mittler 2006).

In the case of heat shock, there is emerging evidence that there is a cross-talk between heat and oxidative stress signalling. A burst of H_2O_2 was reported to occur after very short periods at high temperature, apparently as a result of NADPH oxidase activity. This burst has been correlated with the induction of heat responsive genes, a process assumed to be mediated through direct sensing of H_2O_2 by Heat shock transcription factors (Hsfs) (Desikan et al. 2004).

The transcript level (relative abundance) of some genes encoding proteins with antioxidant functions, such as glutathione reductase (GR) and ascorbate peroxidase (APX), is higher during recovery from water stress and may play a role in the protection of the cellular machinery against photooxidation by ROS (Ratnayaka et al. 2003). Moreover, in response to stress injury, plants trigger a detoxification process, which may include changes in the expression of LEA/dehydrin type genes, synthesis of molecular chaperones, proteinases, enzymes for scavenging ROS and other detoxification proteins. This process functions in the control and repair of stress induced damage and results in stress tolerance.

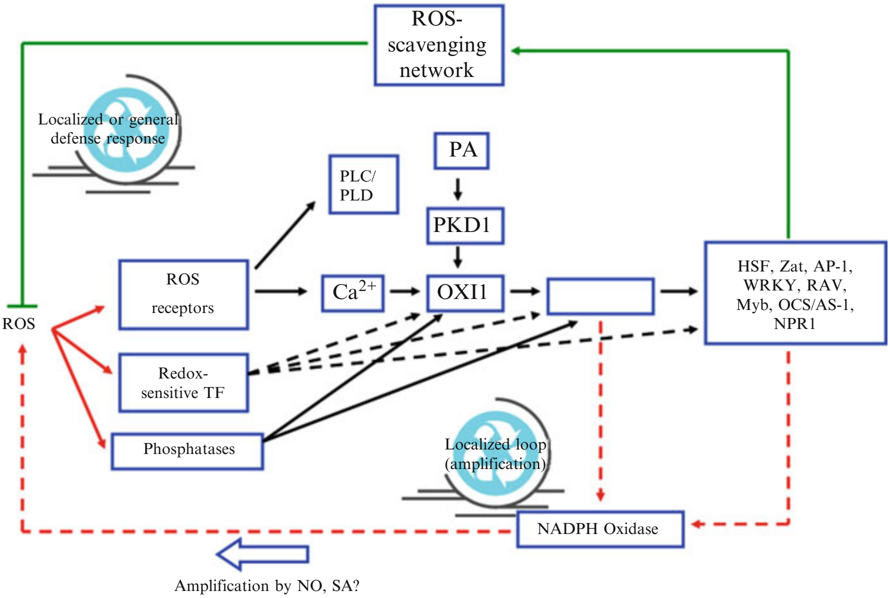

Fig. 9.12 Reactive oxygen species transduction network. Here three mechanisms for ROS detection are shown: ROS receptors, redox sensitive transcription factors and phosphatases. HSF, heat shock factor; PDK, phosphoinositide-dependent kinase; TF, transcription factor

9.9.1 ROS-Scavenging Network

Fernandez et al. (2008), recently found that an EST with homology to a glycolate oxidase (T120) [GenBank: BU671805] was up-regulated under abiotic stresses. So, they hypothesized that this EST is involved in a general production of ROS under different abiotic stresses. These ROS may be signals inducing ROS scavengers and other protective mechanisms, as well as damaging agents contributing to stress injury in plants (Prasad et al. 1994). Many ESTs from leaf and stem cDNA libraries, encoding peroxidases, thioredoxins, catalases and oxygen-evolving enhancer proteins, show transcriptional changes in response to the studied stresses. Most of those proteins are up-regulated in stress conditions due to the accumulation of these products during the oxidative stress. However, a NADH-plastoquinone reductase and a catalytic hydrolase are down-regulated. Genes encoding proteins associated with cellular homeostasis (respiration, cellular biogenesis and DNA repair) show a distinct decline under abiotic stresses (Kawasaki et al. 2001).

In the last decade studies in Arabidopsis have unravelled some mechanisms regarding the key components involved in the ROS signal transduction pathway of plants. Although the receptors for ROS are still mostly unknown at present, it has been suggested that plant cells sense ROS via at least three different mechanisms: (i) unidentified receptor proteins; (ii) redox-sensitive transcription factors, such as NPR1 or HSFs; and (iii) direct inhibition of phosphatases by ROS (Fig. 9.12).

Downstream signalling events associated with ROS sensing involve Ca^{2+} and Ca^{2+}-binding proteins, such as calmodulin, the activation of G-proteins, and the activation of phospholipid signalling, which results in the accumulation of phosphatidic acid. It is possible that the localization of ROS signals in specific cellular sites is similar to that of Ca^{2+} signals in response to stimuli. A recently identified serine/threonine protein kinase (OXI1) has been shown to play a central role in ROS sensing and the activation of MAPKs 3 and 6 by Ca^{2+}. This kinase is also activated by PDK1 through the phospholipase-C/D-phosphatidic-acid pathway. A MAPK cascade involving MAPK3/6 acts downstream of OXI1 and controls the activation of different defence mechanisms in response to ROS stress. The expression of different transcription factors is enhanced by ROS and includes members of the WRKY, Zat, RAV, GRAS and Myb families. Studies on knockout plants have revealed that the zinc-finger protein Zat12 is required for the cytosolic ascorbate peroxidase 1 (Apx1) expression and plant protection during oxidative stress, and that the highly conserved zinc-finger paralogs LOL1 and LSD1 have antagonistic effects on SOD and O_2^- accumulation (Mittler et al. 2004 and references therein).

The promoter of gene coding for the central H_2O_2-scavenging enzyme Apx1, as well as the promoters of many defence genes involved in H_2O_2 signalling and response, contain an heat shock transcription factors (Hsf)-binding motif (Mittler and Zilinskas 1992; Rizhsky et al. 2004; Davletova et al. 2005; Miller and Mittler 2006). Promoter analyses, as well as overexpression studies of AtHsfA1b in Arabidopsis, suggest that the Hsf-binding site at the Apx1 promoter is functional (Panchuk et al. 2002). Furthermore, several other Arabidopsis Apx genes showed enhanced transcript accumulations in response to a short-term heat shock in an AtHsfA1b-dependent mechanism (Panchuk et al. 2002).

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Chapter 10
Chlorophyll a Fluorescence in Abiotic Stress

Lucia Guidi and Elena Degl'Innocenti

Abstract Chlorophyll (Chl) fluorescence is a non-invasive technique employed to measure photosynthesis in leaves, widely used to study plant's response to abiotic stresses. In fact, Chl fluorescence parameters can be efficiently used to evaluate changes in photosystem II photochemistry, linear electron transport rate and CO_2 assimilation in vivo. In this paper also the role of photochemical and non-photochemical quenching involved in the changes in PSII quantum efficiency are discussed. In addition to, it is now practical to use Chl fluorescence imaging to detect heterogeneous patterns of photosynthetic performance across leaves. It is known that photosynthetic apparatus is the main target of many abiotic stresses being chloroplast the organelle which posses pigments that absorb light and drive redox reactions of thylakoids but also the site in the cell where O_2 is evolved from water. Clearly, the availability of O_2 near of the electron transport chain may lead to the formation of reactive oxygen species (ROS) in the cellular environment. For this reason this paper reports how Chl fluorescence has been conveniently used to detect the responses of plants to abiotic stresses such as ozone, drought, temperature, light intensity, UV radiation and salinity.

Abbreviations

AL	actinic light
Amax	CO_2 assimilation rate in light saturated conditions
CCD	charge coupled device
CEF	chloroplast electron flow
Chl	chlorophyll

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DHAP	dyhydroxyacetone-3-phosphate
ETR	electron transport rate
F0	minimum fluorescence yield in dark adapted state
F0′	minimum fluorescence yield in light conditions
Fm	maximum fluorescence yield
Fm′	maximum fluorescence yield in light conditions
Ft = F = Fs	fluorescence yield in steady-state conditions
Fv	variable fluorescence yield
LED	light emission diode
LHC	light harvesting complex
LSD	least significant difference
MB	modulated beam
NPQ	non photochemical quenching
P680	reaction center of PSII
PAR	photosynthetically active radiation
PC	plastocyanin
PGR-5	proton gradient regulation 5
PI	performance index
PPFD	photosynthetically photon flux density
PQ	plastoquinone
PSII and PSI	photosystem II and I
QA	primary acceptor of PSII
qE	quenching due to the electron gradient of pH
qI	quenching due photoinhibition
qL	photochemical quenching coefficient determined by Kramer et al. (2004)
qNP	non-photochemical quenching coefficient
qP	photochemical quenching coefficient
qT	quenching due to the state I-II transition
Rfd	ratio of fluorescence decrease
ROS	Reactive Oxygen Species
Rubisco	ribulose 1,5-bisphosphate carboxylase/oxygenase
SFIN	the energy that is dissipated or lost from photosynthetic electron transport
SFIP	structural and functional PSII events
SOD	superoxide dismutase
SP	saturation pulse
TF	critical temperature for fluorescence
TM	thylakoids membrane
UV	ultraviolet

10.1 Introduction

Since 1940 it has been known that Chl fluorescence emission provides information about photosynthetic activity (McAlister and Myers 1940; Kautsky and Zedlitch 1941) and following Butler (1978) developed a very simple model that describes

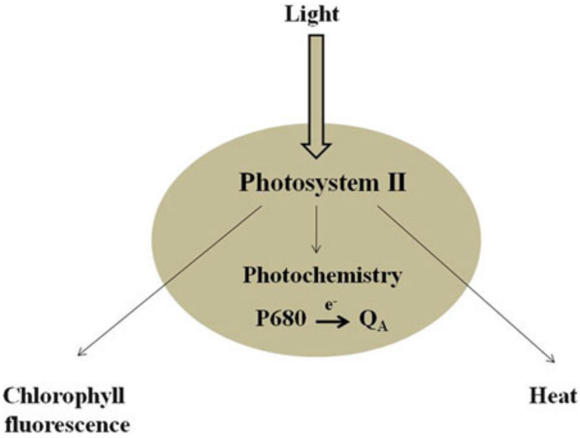

Fig. 10.1 Simple model of the possible fate of light energy absorbed by PSII. Light energy absorbed by chlorophylls associated with PSII can be used to drive photochemistry in which an electron (e^-) is transferred from reaction center Chl, P680, to the primary quinone acceptor of PSII, QA. Alternatively, absorbed light energy can be lost from PSII as Chl fluorescence or heat. The processes of photochemistry, Chl fluorescence, and heat loss are in direct competition for excitation energy. If the rate of one process increases, the rates of the other two will decrease (from Baker 2008).

Photosystem II (PSII) photochemistry (Fig. 10.1). The first event in the photosynthesis in both PSI and PSII, is the generation of an exciton within the associated pigment matrix resulting from the absorption of a photon by Chl a. The exciton is generally lost from the system along one of the three de-excitation pathways: photochemistry, conversion to kinetic energy and fluorescence.

Photochemistry results by the electron transfer from the reaction center Chl of PSII (P680) to the primary quinone acceptor (QA) and the process is typically termed photochemical quenching (qP) because quenches Chl fluorescence yield. The other pathways by which the excitation energy is lost are represented by non-radiative decay which is in competition with photochemistry, fluorescence and other decay process. These fluorescence quenching processes are collectively termed non-photochemical quenching (qNP) (Govindjee et al. 1967; Murata and Sugahara 1969) and consist of several components with the three major ones being so-called (i) high-energy dependent quenching (qE), related to the build-up of a transthylakoidal pH-gradient, (ii) quenching due to the state transition, a process which involves light harvesting complex II (LHCII) phosphorylation (qT) (Allen 1992) and (iii) photoinhibition-dependent quenching (qI) (Powles and Björkman 1982; Krause and Weis 1991).

The usefulness of Chl fluorescence analysis is that the light emission within PSII pigment matrices competes for excitation energy with photochemistry and with non-radiative processes. Because of this competition, it is possible to measure changes in photochemistry and thermal deactivation by measuring Chl fluorescence emission and also the separation of fluorescence quenching into photochemical and non-photochemical components (Schreiber and Bilger 1993) by modulated fluorescence measurements (Schreiber 1986; Schreiber et al. 1986).

Then, Chl fluorescence is certainly a tools that, when appropriately used, provides useful information about leaf photosynthetic performance. Many review reported that Chl fluorescence can be used in laboratory, controlled environment and field conditions, to assay photosynthetic process in leaves (Walker 1992; Maxwell and Johnson 2000; Baker and Rosenqvist 2004; Papageorgiou and Giovindjee 2004; Baker 2008; Henriques 2009).

Many Chl fluorescence parameters have been defined in the literature and many attempts have been made to establish a single nomenclature (Van Kooten and Snell 1990; Maxwell and Johnson 2000; Baker et al. 2001) even if frequently different terms have been used to identify the same fluorescence parameters. For this reason sometimes there is misleading and erroneous interpretation of this non-invasive and certainly useful tool.

10.2 Chlorophyll Fluorescence: Basic Concept

For the first time Kautsky et al. (1960) found that, when a leaf was transferred from the dark to the light, an increase in fluorescence yield was observed over a period of about 1 s (Fig. 10.2). This is due the fact that once PSII reaction centers absorb light and QA has accepted an electron, it is not able to accept another electron until it transfers the electron to the secondary quinone QB. In this state the PSII reaction centers are typically termed "closed".

The most common method to determine fluorescence parameters from Kaustky curve is reported in Fig. 10.2. Starting with a dark-adapted leaf, the minimal fluorescence, F0 is obtained at very low photosynthetically photon flux density (PPFD). The light intensity used to determine F0 is below 1 μmol m^{-2} s^{-1} which ensures that all PSII reaction centers are capable of photochemistry and so in the "open" state, i.e. QA oxidized. A saturating flash of light is then applied, giving the determination of maximal fluorescence Fm in dark adapted leaves when all reaction centers are closed, i.e. decrease their ability for PSII photochemistry. From this fluorescence parameter derives an other important and useful parameter that indicates the intrinsic (or maximum) efficiency of PSII, i.e. quantum efficiency if all PSII reaction centers are open, the ratio Fv/Fm [= (Fm-F0)Fm]. This ratio it has been widely used to assess alterations in the photosynthetic systems induced by stress, because a decrease in the Fv/Fm ratio can be attributed to photodamage to the reaction centers of PSII and/or the development of slowly relaxing fluorescence quenching processes, both of which signify the presence of long-term disturbances in plant physiology (Björkman and Demmig 1987).

When a leaf is illuminated with a saturating pulse of light, there is an overall reduction in the efficiency of photochemistry that induces an increase in the yield of fluorescence. However, following this rise, the fluorescence of illuminated leaves typically decreases and reaches a steady-state level in few minutes. This quenching of fluorescence yield to the steady-state is coincident with the induction of CO_2 assimilation in the leaf (Ireland et al. 1984). Then a part of this fall in Chl fluores-

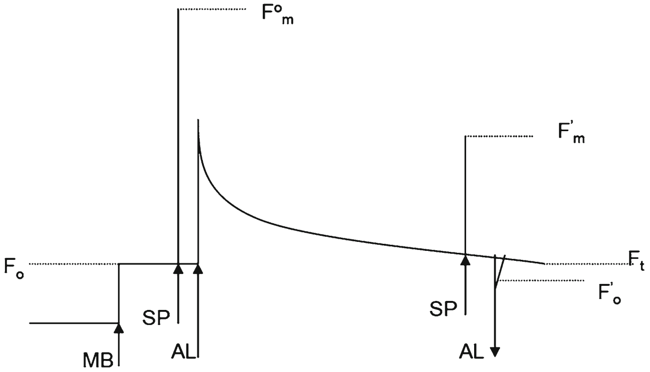

Fig. 10.2 Sequence of a typical fluorescence trace. A measuring light is switched on (MB) and the zero fluorescence level is measured (F_o). Application of saturating flash of light (SP) allows the measurement of maximum fluorescence level (F_om). A light to drive photosynthesis (AL) is then applied. After a period of time, another saturating light flash (SP) allows the maximum fluorescence in the light (Fm′) to be measured. The level of fluorescence immediately before the saturating flash is termed Ft. Turning off the actinic light (AL) typically in the presence of far-red light allows zero level fluorescence in the light to be estimated (from Maxwell and Johnson 2000)

cence is caused by photochemical quenching and is due to the increase in rates of QA oxidation. However, part of this decrease in fluorescence yield is due by the development of non-photochemical quenching. To obtain information from steady-state fluorescence is important to divide photochemical and non-photochemical quenching components and this is now possible thanks to the work of Schreiber et al. (1986). If we assume that photochemical quenching can be completely suppressed by a saturating light pulse and that non-photochemical quenching does not change when we superimpose saturating pulse, we can simple divide the two components of quenching by using the so-called "saturation pulse" method (Schreiber and Bilger 1993; Schreiber 2004). During the saturation pulse, maximal fluorescence yield, Fm′, is achieved and, generally its value is lower than the dark adapted value (Fm). Clearly, the lowering of Fm is a selective measure of non-photochemical quenching. In Fig. 10.3 the principle of quenching analysis by the saturation pulse method is reported following the nomenclature proposed by van Kooten and Snel (1990). The photochemical quenching coefficient qP is calculated as: (Fm′-F)/(Fm′-F0′) where F (=Ft) is the value of fluorescence yield immediately before the saturating flash. The non-photochemical quenching coefficient qNP is determined by the formula: 1-(Fm′-F0′)/(Fm-F0). For both coefficients is important to determine the coefficient F0′.

To determine F0′ in the light state, the leaf tissue has to be transiently darkened and it has to be assured that QA is quickly and fully oxidized, before there is substantial relaxation of non-photochemical quenching. Then far-red light can be applied for selectively excited PSI in order to enhance oxidation in the intersystem electron transport chain. When the determination of F0′ is difficulty, as e.g. in the field, the analysis of non-photochemical energy dissipation can be described

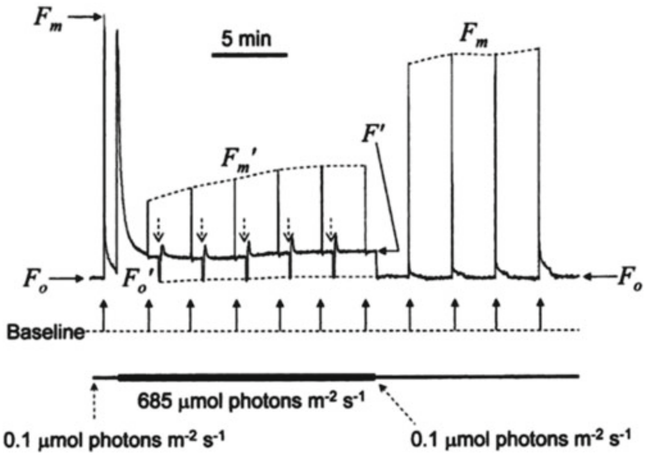

Fig. 10.3 Fluorescence quenching analysis using modulated fluorescence. All parameters denote with a *prime* (′) are from the leaf exposed to actinic light. The parameters without a prime are obtained from a lead in dark-adapted state (see *bar* under the graph) (from Baker and Oxborough 2004)

by the parameter NPQ introduced originally by Bilger and Björkman (1990). This parameter compares the light-induced Fm′ level to the dark-adapted Fm level. In fact, the formula to calculate NPQ is (Fm/Fm′)-1. NPQ assesses increases in non-photochemical quenching in a light-adapted leaf relative to the non-photochemical quenching occurring in the dark-adapted state. For this reason it is valid when we compare samples which have the same quenching characteristics in the dark-adapted state, i.e. similar values of Fv/Fm.

By saturation pulse methods we can determine not only photochemical and non-photochemical quenching but also the effective quantum yield of PSII photochemistry. Under illuminated conditions, the PSII quantum yield is reduced because of PSII reaction centers are closed and because increases heat dissipation by non-photochemical mechanisms. This actual or effective quantum yield of PSII photochemistry is termed ΦPSII or ΔF/Fm′ and is determined by the formula (Fm′-F)/Fm′ where F is the fluorescence yield determined at any time during illumination (see Fig. 10.3) (Genty et al. 1989). Schreiber (2004) reported that the relationship between fluorescence yield and PSII quantum yield is fundamental for practical applications of Chl fluorescence methodologies. An other important parameter that can be obtained from saturation pulse analysis is the ratio Fv′/Fm′, which provides an estimate of the maximum quantum efficiency of PSII photochemistry when all reaction centers are open.

Genty et al. (1989) reported a linear relationship between the effective quantum yield of PSII photochemistry and the quantum yield of CO_2 assimilation rate in mature leaves of C4 plants in which CO_2 assimilation is the main sink for the products of linear electron flux, i.e. ATP and NADPH. This relationship is found over a range of light intensities (Genty et al. 1989; Krall and Edwards 1990, 1991), over a range of atmospheric CO_2 concentrations (Genty et al. 1989; Siebke et al. 1997) and during induction of photosynthesis, when dark-adapted leaves are exposed to actinic light (Genty et al. 1989). However, this linear relationship between PSII operating efficiency and quantum yield of CO_2 assimilation rate is sometimes lost. In the case of C3 leaves in which photorespiration is operating, ATP and NADPH produced by electron transport rate, are utilized not only for CO_2 assimilation rate (Harbinson et al. 1990; Ghannoum et al. 1998). A detailed review on these aspects is written by Baker and Oxborough (2004) and by Schreiber (2004), in which are report some examples where the linearity between PSII operating efficiency and the quantum yield of CO_2 assimilation is lost.

In the 1996, Genty and collaborators presented new expressions based on basic fluorescence parameters that describe the partitioning of absorbed excitation energy in PSII between three pathways, expressed in terms of the complementary quantum yields of photochemical conversion [ΦII = (Fm′-F)/Fm′ = ΦPSII = ΔF/Fm′), regulated thermal energy dissipation related to NPQ (ΦNPQ = F/Fm′-F/Fm) and “primarily constitutive losses” corresponding to the sum of non-regulated heat dissipation and fluorescence emission (ΦNO = F/Fm). The sum of the three components is close to 1 and they can be calculated without knowledge of F0 and F0′.

Other Chl fluorescence-derived parameters are calculated using the so-called “lake model”, which is based upon the assumption that PSII reaction centres are connected by a shared antenna (Cailly et al. 1996), in contrast with the so-called “puddle model” in which each reaction centre in PSII is assumed to possess own independent antenna systems (Lavergne and Trissl 1995; Trissl and Lavergne 1995). Actually, the photosynthetic units do not act as a “puddle” (Butler 1978; Lazar 1999), as PSII reaction centres form functional dimers in the thylakoid membrane (Barber 2003), which makes the “lake model” a good representation of how PSII reaction centres operate in terrestrial plants (Lazàr 1999; Kramer et al. 2004). Then, the photochemical quenching in the lake model, qL, is calculated as reported by Kramer et al. (2004) as qL = qP × F0′/Fs. The lake model allows discriminate the quantum yield of regulated energy dissipation in PSII, ΦNPQ = 1-ΦPSII-1/[NPQ + 1 + qL(Fm/F0-1)] from the quantum yield of non-regulated energy dissipation in PSII, ΦNO = 1/[NPQ + 1 + qL(Fm/F0-1)] as reported in Kramer et al. (2004). Finally, the rates of energy dissipation, Js, were calculated assuming a factor of 0.5 for the proportion of absorbed photons utilized by PSII reaction centres (Melis et al. 1987) and a leaf absorptance of 0.85. The utilization of photons absorbed by the PSII antennae in the photosynthetic electron transport and in the thermal dissipation is assessed from the quantum efficiency (Φ) and the rate (J) of photochemical energy dissipation (ΦPSII, JPSII), light-dependent (ΦNPQ, JNPQ), and both fluorescence and light-independent thermal dissipation (ΦNO, JNO), by considering that 1 = ΦPSII + ΦNPQ + ΦNO.

A new development in Chl fluorescence measurements is represented by the imaging of fluorescence signal using charge coupled device (CCD) camera (Oxborough 2004). This instrument provides a powerful tool to resolve spatial heterogeneity of leaf photosynthetic performance (Nedbal and Withmarsh 2004; Oxborough 2004). In fact, in many situations it has been identified photosynthetic heterogeneity, i.e. during induction of photosynthesis (Bro et al. 1996; Oxborough and Baker 1997), with changes in carbohydrate translocation (Meng et al. 2001), in response to drought (Meyer and Genty 1999; West et al. 2005; Nejad et al. 2006), chilling (Hogewoning and Harbinson 2007), ozone pollution (Leipner et al. 2001; Guidi et al. 2007; Guidi and Degl'Innocenti 2008), wounding (Quilliam et al. 2006), high light (Zuluaga et al. 2008) and infection with fungi (Scholes and Rolfe 1996; Meyer et al. 2001; Scharte et al. 2005; Schwarbrick et al. 2006; Guidi et al. 2007) or virus (Perez-Bueno et al. 2006). Chl fluorescence imaging has also been used in the screening program. For example in the screening of algal mutant colonies with altered thylakoid electrochemical gradient (Bennoun and Beal 1997), screening for non-photochemical mutants of Chlamydomonas sp. (Niyogi et al. 1997) and Arabidopsis sp. (Niyogi et al. 1998) and the detection of herbicide effects on the Fv/Fm in Arabidopsis sp. and Agrostis tenuis several days before any visible effects on the plants were observed (Barbagallo et al. 2003). In addition to, Chl fluorescence imaging is used also to characterize mutants of sunflower with deficient greening program and altered development of leaf mesophyll (Fambrini et al. 2010).

The advance of highly sensitive CCD cameras and the development of extremely strong light emitting diodes (LED) has become possible that some fluorometers are able not only to measure images of Chl fluorescence but are also fully competent in providing all relevant Chl fluorescence parameters using the saturation pulse method. In this way, images of photosynthetic activity and its spatio-temporal variations can be obtained.

This chapter will illustrate the effects of some environmental stresses on plant photosynthetic performance and how Chl fluorescence methodology has been used to study the effects of abiotic stresses on photosynthetic apparatus. In addition, it will be reviewed as Chl fluorescence can be utilized to identify species and/or cultivars that are tolerant or sensitive to stresses.

10.3 Stress in Relations to Chlorophyll Fluorescence

The concept of plant stress is very difficult to define because of the complex interaction between plants and environment. However, the stress is defined, in a very general way, as a biotic or abiotic factor that prevents plants from normal functioning and thus results in a reduction in their growth and reproduction (Osmond et al. 1987). Plants cannot flee from the stressful conditions and then they have developed various strategies to adapt to the changing environment and to counter the stress effects. The adaptation of plants involves three events: stress perception, transduction of stress signals, and the final response (Lichtenthaler 1998).

Most of the environmental stresses induce in green plants an oxidative damage of the cell structure and consequently a loss in the cellular activities. During respiration the transfer of electrons to O_2 and subsequent redox reactions in plant cells may generate various toxic O_2 species. Under normal non-stressed conditions, the cell tends to maintain a redox homeostasis which can be disrupted by various factors. Then a shift of redox state induced by stress factors determines an oxidative environment. In the cells, chloroplast is the organelle which posses pigments that absorb light and drive redox reactions of thylakoids but also the site in the cell where O_2 is evolved from water. Clearly, the availability of O_2 near of the electron transport chain may lead to the formation of reactive oxygen species (ROS) in the cellular environment. On the other hand, chloroplasts are able to produce strong oxidants associated with PSII which are responsible for the splitting of H_2O molecules, but they can also oxidize pigments, proteins and lipid of the thylakoid membranes as well. This characteristic makes the chloroplast a major stress sensor in green plants (Biswal and Biswal 1999). Even the separation charge and the electron transport rate associated represents an other important factor that makes chloroplast sensitive to stress.

Because of chloroplast represents the major sensitive target of environmental stresses, after stress conditions photosynthetic electron flux results from an imbalance between the generation and utilization of photosynthetic electrons. Excess of excitation energy has to be safely removed because it can induce damage to the photosynthetic machinery, primarily to PSII, thus causing photoinhibition that, in turn decreases photosynthetic activity, growth and productivity. However, plants have developed mechanisms aimed to quickly and efficiently repair photodamaged PSII (Aro et al. 2005). Different photoprotective mechanisms are developed by plants to avoid photoinhibition. In Fig. 10.4 are reported some examples of leaf and chloroplast mechanisms involved in minimizing photoinhibition of PSII as illustrated by Takahashi and Badger (2010): light avoidance associated with leaf or chloroplasts movement, screening of photoradiation, ROS scavenging system, dissipation of absorbed light energy as thermal energy (qE), cyclic electron flow around PSI and the photorespiratory pathway.

The antioxidant mechanisms present in the chloroplast, such as the ascorbate-glutathione cycle (Mittler 2002), preserve the damage. Ascorbate is also involved in the xanthophylls cycle, where it facilitates the conversion of violaxanthin in zeaxanthin. This cycle acts at PSII level antenna system where it protects the photosynthetic apparatus from being photo-damaged during high light stress (Demmig-Adams and Adams 1996).

The LHCII can be stabilized also by the action of other antioxidant such as carotene that directly scavenges ROS (Bassi and Caffari 2000) or α-tocopherol, that is involved in the detoxification of ROS and is correlated positively with high-light and drought stress (Munne-Bosche 2005).

Clearly the intimate relationship between the actual photochemical efficiency of PSII and CO_2 assimilation rate in leaves determines that Chl fluorescence can be used to detect differences in the response of plants to changed environmental conditions and, consequently, to screen for tolerance to abiotic stresses.

10.3.1 Ozone

Tropospheric ozone (O_3) is a phytotoxic air pollutant whose concentration has been rising since the industrial revolution and represents a problem affecting both natural plant communities and crops (Ashmore and Marshall 1999). The major way by

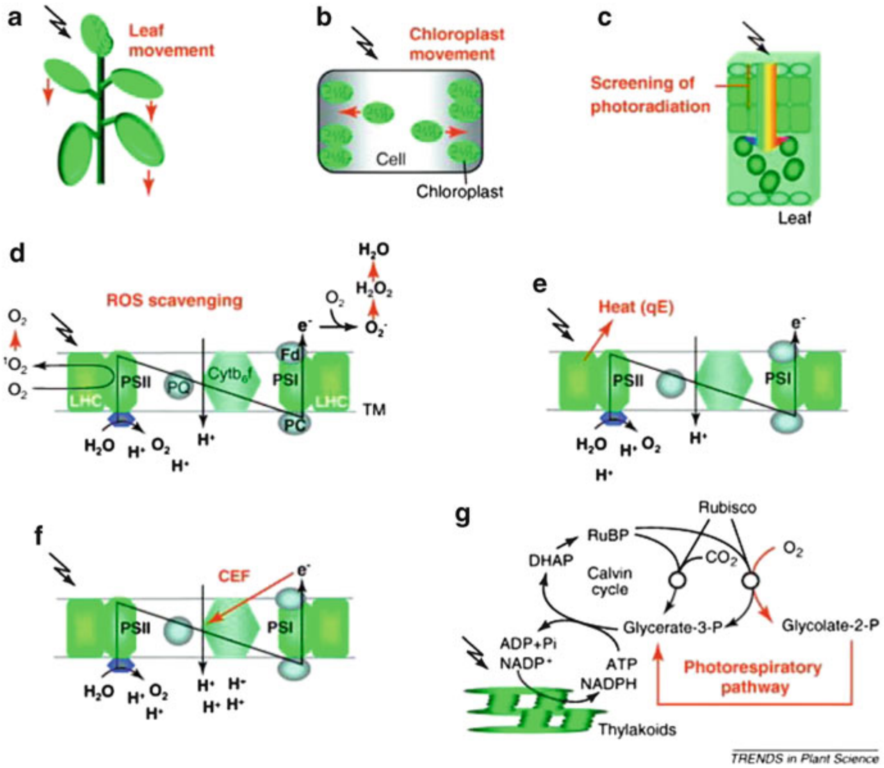

Fig. 10.4 Examples of leaf and chloroplast mechanisms involved in minimizing photoinhibition of PSII. (**a**) Leaf movement. Leaves move to minimize the absorption of excessive light. (**b**) Chloroplast movement. Chloroplasts change their position to minimize the absorption of light. (**c**) Screening of photoradiation, for example, UV screening by phenolic compounds in epidermis cells. (**d**) ROS scavenging. $^1O_2$ produced at PSII is scavenged by membrane-bound a-tocopherol and carotenoids. O_2– and H_2O_2 produced at PSI are scavenged enzymatically and non-enzymatically by ascorbate. (**e**) Thermal energy dissipation of absorbed light energy (qE). qE dissipates light energy absorbed by photosynthetic pigments as heat at minor light-harvesting proteins. (**f**) CEF around PSI. CEF includes both the NAD(P)H dehydrogenase complex dependent and PGR5-dependent pathways and helps to generates ΔpH across the thylakoid membrane. (**g**) Photorespiratory pathway. Glycolate-2-P generated by the oxygenase reaction of Rubisco is recycled into the Calvin cycle intermediate glycerate-3-P through the photorespiratory pathway. Abbreviations: *CEF* chloroplast electron flow; *DHAP* dihydroxyacetone phosphate; *PC* plastocyanin; *PQ* plastoquinone; *TM* thylakoid membrane (from Takahashi and Badger 2010)

which plants absorb O_3 is represents by stomata. When O_3 enters the plants, it quickly reacts with the apoplasts of the mesophyll cells, produces ROS and triggers a series of signaling cascades and plant defense responses which ultimately induce visible leaf damage, decreased photosynthetic activity and/or accelerated senescence (Sandermann 1996; Leipner et al. 2001; Kangasjarvi et al. 2005).

It is well known that the major effect on photosynthesis is that O_3 reduces stomatal conductance. This reduction is due to the damage to the photosynthetic apparatus leading to reduced fixation, increased internal CO_2 concentration, and finally to reduced stomatal conductance (McKee et al. 1995; Fiscus et al. 1997). However, O_3 has also been shown to alter photosynthetic electron transport rate because of reduced the efficiency of excitation capture in plants (Calatayud and Barreno 2001; Calatayud et al. 2002, 2004; Guidi et al. 2002; Zheng et al. 2002; Flowers et al. 2007; Biswas et al. 2008). On the other hand, Guidi et al. (2002) reported that the Fv/Fm ratio decreased in bean stressed leaves exposed to high light intensity and/or an acute O_3 treatment indicating photoinhibition and demonstrated that the corresponding increase in minimal fluorescence, F0, indicates a higher number of deactivating PSII centers. The increase in minimal fluorescence yield determined also an increase in excitation pressure at PSII level (decrease qP).

Chl fluorescence has been useful also to detect the different response of plants to chronic or acute O_3 treatment. Chen et al. (2009a) found that, while the acute and chronic O_3 treatments produced very similar reductions in whole-leaf CO_2 uptake rate, the within-leaf spatial patterns of factors underlying this change differ markedly. Acute O_3 concentration induced a significant spatial heterogeneity and photosynthetic depression especially at the base of the leaf. The reductions in operating efficiency of PSII determined in light conditions under acute O_3 treatment resulted from the decrease in Fv′/Fm′, an estimate of the PSII maximum efficiency within light-adapted material and Fq′/Fv′, an estimate of the PSII operating efficiency within light-adapted material determined by using Chl fluorescence imaging. Similar decreases in operating efficiency of PSII determined in light conditions under chronic O_3 derived from changed only in d Fq′/Fv′.

Frequently Chl fluorescence tool is utilized to detect different sensitivity to O_3 among species or cultivars of the same species. Feng et al. (2011) reported a decrease of Fv′/Fm′ in two winter wheat cultivars subjected to elevated O_3 concentration. In the same leaves of one of the cultivars qP decreased indicating a reduced net rate of re-oxidation of QA which in turn resulted in a larger fraction of closed PSII reaction centers and thus lower PSII quantum efficiency. The obtained results support the notion that the decrease in the quantum yield of PSII electron transport may be a mechanism to down-regulated photosynthetic electron transport so that the production of ATP and NADPH is maintained in equilibrium whit the decreased demand in the Calvin cycle in ozonated leaves as already reported for spinach, lettuce, snap bean and wheat exposed to acute O_3 concentration (Calatayud et al. 2002, 2004, 2010; Flowers et al. 2007).

Chl fluorescence methodology can be conveniently used also to screen in a short time many samples. For example Clark and co-workers (2000) used a Chl fluorescence performance index (PI) to screen O_3 sensitivity of Fagus sylvatica plants. The performance index PI has been defined as the ratio of two other Structure-Function-Indexes (SFI). The first, SFIP responds to structural and functional PSII events leading to electron transport rate within photosynthesis (Tsimili-Michael et al. 1998). The second, SFIN refers to the energy that is dissipated or lost from photosynthetic electron transport (Strasser et al. 1999).

Several factors suggest that the effects of acute O_3 are heterogeneous at the leaf level and the clearest evidence that effects of elevated O_3 are non-uniform across the leaf surface is the characteristic symptom of visible damage. The effects of O_3 are strongly dependent on uptake or flux into the plant, so any variation in the aperture between stomata, i.e. stomatal heterogeneity, would also result in a non-uniform effect of O_3 damage within the mesophyll (Fuhrer 2002). For these reasons Chl fluorescence imaging can be used conveniently to detect the O_3-induced damage. Leipner et al. (2001) by using this technique demonstrated as perturbations of photochemical efficiencies were not observed in cells associated with all of the stomata on the upper leaf surface or within cells distant from the upper leaf surface. The authors concluded that ozone penetrates the leaf through stomata and initially damages only cells close to stomatal pores.

On the contrary Endo and Omasa (2007) found that the change in the distribution of Chl fluorescence after exposure to 300 nL L^{-1} O_3 indicated the inhibition of photosynthesis that had been caused. Furthermore, the sites where the fluorescence intensity decreased were located close to the abaxial leaf surface and were not related to the positions of the stoma. Such a phenomenon may occur as a result of O_3 diffusion through gaps in the cuticle, or O_3 may diffuse rapidly once entering the spongy tissue via open stomata through the air spaces. Based on the fluorescence images, decreases in Chl fluorescence yield were not seen in chloroplast units but were observed at the single cell level, indicating that O_3 first attacks mesophyll cells and then the function of the chloroplasts in the injured cell is reduced.

In an other report Gielen et al. (2007) hypothesized that 2 × ambient O_3 concentration caused accelerated leaf senescence during the sixth year of free-air O_3 fumigation in sequence in the 66-year-old Fagus sylvatica trees. Because both O_3 stress and leaf senescence result in a non-homogeneous distribution of ΦPSII across the leaf, they used Chl fluorescence imaging in addition to spot measurements of Chl fluorescence to quantify the degree of photosynthetic leaf heterogeneity.

Guidi et al. (2007) compared the perturbations in photosynthesis induced in Lupinus albus plants by O_3 and by a pathogen by using Chl fluorescence imaging. The image of Fv/Fm in O_3-treated leaves or artificial inoculated with Pleiochaeta setosa was heterogeneous upon the leaf surface (Fig. 10.5).

In ozonated leaves, several areas with reduced Fv/Fm were distinguished 48 h after fumigation and the severity of damage increased further in the following hours when the major veins were also affected (Fig. 10.5b, f). A different phenomenon was observed in infected leaves which were monitored only until 144 h since they began to wilt badly thereafter. Indeed, no noticeably changes in Fv/Fm ratio were found in the first three days after inoculation, a subsequent marked reduction was found mostly in the area exhibiting visible symptoms (Fig. 10.5g, m).

10.3.2 Drought Stress

Reductions in water availability result in reduced photosynthetic performance in many plants. When the drought stress is mild, decrease in photosynthesis is considered to be the result of reduced availability of CO_2 due to stomatal closure (Kaiser 1987; Cornic 2000; Munns 2002; Buckley 2005).

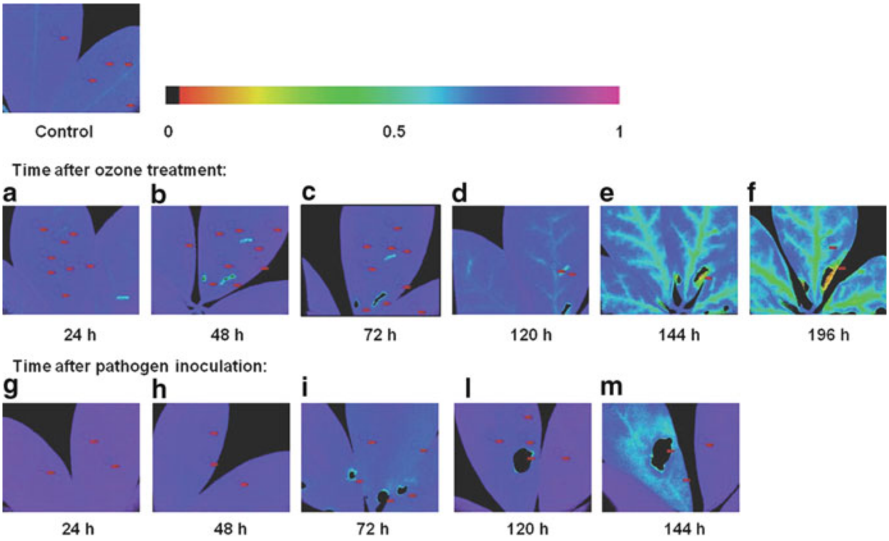

Fig. 10.5 Fluorescence images of the Fv/Fm ratio in leaves of Lupinus albus after a single pulse of ozone (150 nL L^{-1}, for 5 h) (A and F) or after artificial inoculation with Pleiochaeta setosa (G and M). All data are collected from the same group of leaves using the Imaging-PAM fluorometer as described in Sect. 10.2. The images correspond to different measurement times [24 (A and G), 48 (B and H), 72 (C and I), 120 (D and L), 144 (E and M) and 196 (F) after fumigation or inoculation] and are normalized to the false color bar provided. The analysis of Fv/Fm was carried out on dark-adapted leaves. Pixel value display is based on a false color scale ranging from black (0.00–0.040) via red, yellow, green and blue to purple (ending at 1.00) (from Guidi et al. 2007)

Under severe drought conditions, photosynthetic CO_2 fixation is suppressed by enhanced diffusive resistance within leaf (closure of stomata and decline of mesophyll conductance) and by drought-induced impairments of metabolic processes (Flexas et al. 2004, 2006; Reddy et al. 2004). For many years there is a focus debate whether stomatal or nonstomatal limitations play the major role in drought-induced inhibition of photosynthesis (Cornic and Fresneau 2002; Flexas et al. 2002, 2004; Grassi and Magnani 2005). At the end, a widely accepted theory posits that, under mild to moderate drought stress, it is the diffusive resistances that predominantly limits photosynthesis, whereas under severe drought, when stomatal conductance decreases below 0.1–0.05 mol $H_2O\ m^{-2}\ s^{-1}$ metabolic limitations become dominant (Flexas et al. 2002, 2004).

Chl fluorescence is commonly used to study the functioning of photosynthetic apparatus under drought stress. Many works report as drought conditions induced a reduction of the ratio Fv/Fm (Yang et al. 2006; Savage et al. 2009; Zheng et al. 2009; Gimeno et al. 2010; Sommerville et al. 2010; Vilagrosa et al. 2010; Faraloni et al. 2011; Walter et al. 2011) even if other authors reported only small differences in the ratio Fv/Fm recorded in stressed and well-watered plants (Silva et al. 2010; Signarbieux and Feller 2011; Tezara et al. 2011). It has been reported that water stress did not cause a long-term effect on photochemical efficiency of PSII in sunflower, but it decreased actual quantum efficiency of PSII electron transport (ΦPSII) (Maury et al. 1996; Pankovic et al. 1999). However, the response of sunflower photochemical efficiency to drought stress is genotype-dependent (Maury et al. 1996). On the other hand, Gallé et al. (2007) reported in severe drought stress induced in Quercus pubescens that PSII photochemistry (both Fv/Fm and ΦPSII) was reversibly down-regulated and dissipation of excess energy was enhanced (higher NPQ) during the stress phase. Photoprotective mechanisms in oak acted to preserve the potential functionality of PSII during severe drought stress leading to a rapid recovery of photosynthetic activity after rewatering.

Using Fv/Fm values Percival and Sheriffs (2002) selected 30 woody plant species with the ability to maintain photochemical efficiency even after a 70-day drought. The ranking by visual observation was similar to that based on the rate of photosynthesis. As photosynthesis is the primary physiological function that contributes to the productivity of a plant, any species identified as drought tolerant based on Fv/Fm may reflect this tolerance in their yield or productivity parameters. Adopting a similar approach to evaluate adult coconut form/acc, Nainanayake (2007) grouped 6 form/acc as drought tolerant based on high readings for both Fv/Fm and the rate of photosynthetic CO_2 assimilation observed at the end of the drought.

Razavi et al. (2008) reported a gradual reduction of photochemical quenching and the actual quantum yield of PSII in strawberry leaves subjected to drought stress while non-photochemical quenching increased significantly. However, in these leaves the ratio Fv/Fm was not affected by drought stress.

In an other work Massacci et al. (2008) combined measurements of gas exchange and Chl fluorescence in cotton plants and found that moderate drought stress severely affected CO_2 fixation even if the electron transport rate was enhanced. The authors reported that the additional energy produced by electron transport chain in the chloroplasts is used to increase the rate of photorespiration while photosynthesis is kept constant or slightly decreases. The increased photorespiration process represents in cotton a mechanism by which plants prevent an over-reduction of the photosynthetic apparatus and, therefore, the generation of ROS.

Ogaya et al. (2011) in a rain exclusion experiment simulating drought conditions expected in Mediterranean areas (15% decrease in soil moisture) studied the response of forest plants to the forecasted climatic changes. The Fv/Fm ratio was measured in Quercus ilex and Phillyrea latifolia, the co-dominant species of the studied forest, from 1999 to 2009. In both species, the ratio was highly dependent on air temperatures, and in a second term, in water availability.

In natural environment such as the Mediterranean area, water stress is coupled with high solar radiation and the response mechanisms to these combined stresses have been widely investigated (Pereira and Chaves 1993; Chaves et al. 2002, 2003; Guidi et al. 2008). Guidi et al. (2008) observed that, early during the treatment period, water stress acted together with high solar irradiance in reducing PSII photochemistry in Ligustrum vulgare (Fig. 10.6), likely because of the fast imposition of the water-stress treatment in the experiment. However, the decline in both the maximal and the actual efficiency of PSII photochemistry was not the primary cause of the water-stress-induced reductions in CO_2 photoassimilation in light saturated condi-

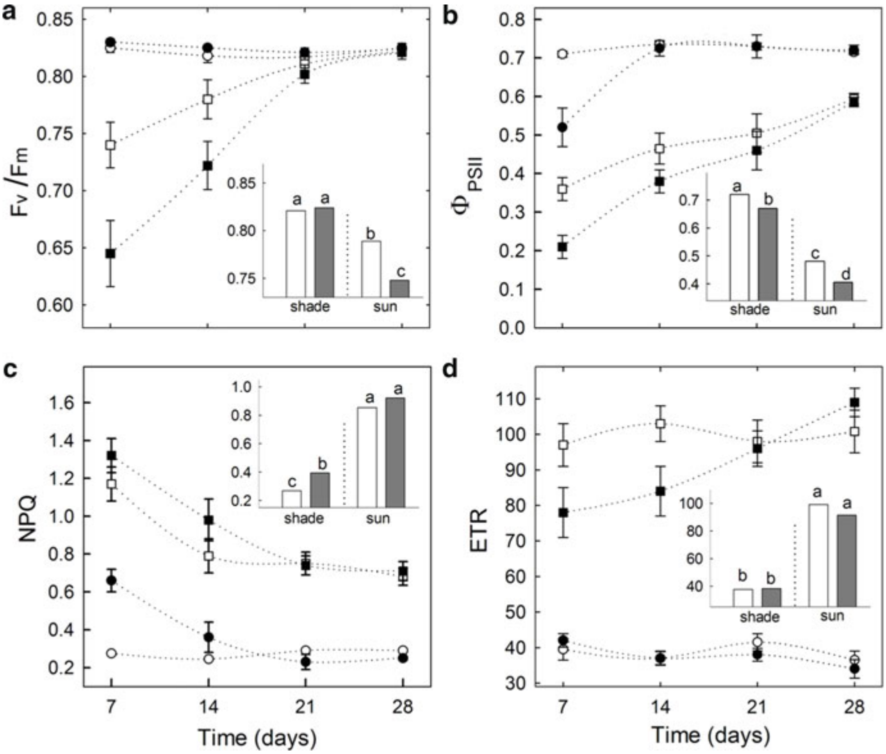

Fig. 10.6 Time-course of (**a**) maximal (Fv/Fm) and (**b**) actual (ΦPSII) efficiency of PSII photochemistry, (**c**) non-photochemical quenching (NPQ) and (**d**) electron transport rate (ETR) in shade (□, ■) and sun (○, ●) leaves of well-watered (open symbols) or water-stressed (closed symbols) Ligustrum vulgare over a 4-week experiment. Values are the means ± sd, n =4 and values not accompanied by the *same letter* are significantly different at $P<0.05$, based on a least significant difference (*LSD*) test. In the insets data resulting from time-course experiments have been pooled and bars (open or *grey bars* refer to WW or WS plants, respectively) are the mean of 16 replicate measurements (from Guidi et al. 2008)

tions (Amax) in sun plants (Flexas et al. 1998, 2006). Other mechanisms for protecting the photosynthetic apparatus from photodamage – namely (1) an increase in NPQ (Fig. 6c); and (2) the consequent down-regulation of ΦPSII (Fig. 10.6b), which should have offset a greater electron transport rate (Fig. 10.6d) by meeting the need for greater reducing power to support carboxylation of Rubisco (Krause and Weis 1991) – likely contributed to the limitation of Amax in full sun (Demmig-Adams and Adams 1996; Müller et al. 2001).

Chl fluorescence imaging is even utilized to detect spatial and temporal changes in rose leaves under progressive and slow water stress (Calatayud et al. 2006). The authors reported also that this non-invasive technique makes possible the early detection of water stress in greenhouse rose plants and it could also be applied to irrigation management.

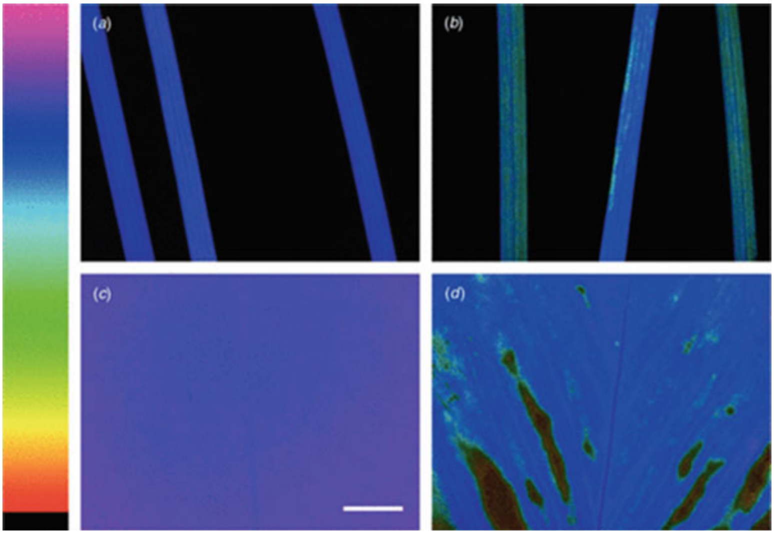

Fig. 10.7 Spatial variation in maximum quantum efficiency of PSII photochemistry (Fv/Fm) in phyllodes of (**a**, **b**) Acacia floribunda and (**c**, **d**) A. pycnantha. Phyllodes (**a**, **c**) before the measurement cycle and the same phyllodes (**b**, **d**) at the day of greatest water stress. Colour scale at left of image ranges from Fv/Fm 0 (*black*) to 1 (*pink*). Spatial scale bar = 0.5 cm (from Sommerville et al. 2010)

Sommerville et al. (2010) used Chl fluorescence imaging to assess changes in the functional status of the photosynthetic apparatus in phyllodes experiencing severe drought (Fig. 10.7). It was hypothesized that the efficiency of PSII photochemistry, Fv/Fm, would be greater in areole regions near the central primary midnerve than in areole regions between secondary nerves with drought.

In comparing two species with contrasting nervation patterns, it was hypothesized that Fv/Fm would be lower in tissue between secondary nerves in the species with lower primary nerve density, owing to greater distance from major hydraulic supply lines and, therefore, increased resistance in the path to the site of water use. The authors concluded that it is difficult to determine whether observed changes in Fm and Fo were due to photo-oxidative damage, quenching processes, or modification of phyllode optical properties. Nevertheless, following the interpretation by Franklin et al. (1992), the higher F0 coincident with the lower Fv/Fm in Acacia pycnantha droughted plants may indicate damage to PSII consistent with the slower rate of recovery of Fv/Fm following rehydration in these plants

Water stress reduced ΦPSII from 0.451 to 0.240 in this experiment. This may be due to the decrease in the rates of consumption of ATP and NADPH by photosynthesis that is not compensated by increase in water-water cycle and photorespiration, or by other electron sinks, and consequently, ΦPSII decreases (Fracheboud and Leipner 2003). This observation was also reported in A. thaliana and C. canephora where water stress reduces actual quantum yield of PSII (Pinheiro et al. 2004; Jung 2004). Water stress significantly increased non-photochemical fluorescence quenching (NPQ) from 1.26 in well-watered conditions to 2.28 in water-stressed one.

Any change in NPQ measures the efficiency of heat dissipation that protects the leaf from light-induced damages (Maxwell and Johnson 2000). Therefore increased NPQ in some recombinant inbred lines suggest their capacity to be protected through thermal energy dissipation. Increased 1-qP values under water-stressed conditions in this study could be due to closure of reaction centers, which is associated to PSII inactivation, resulting from a saturation of photosynthesis and other electron sinks by light (Osmond et al. 1993).

10.3.3 Light Intensity

Irradiation is one of the most important environmental factor affecting plant survival, growth, reproduction, distribution, and yield (Wang et al. 2007). Photosynthetic capacity is different in sun and shade leaves and has usually been determined by measuring CO_2 photoassimilation of leaves and Chl fluorescence ratios (Govindjee 1995).

Under high light conditions there is an increase in the amount of photosystem, electron transport rate, ATP synthase complex and enzymes of Calvin-Benson cycle. On the contrary, under low light there is an increase in the amounts LHC and in the stacking of thylakoid membranes to form grana.

Although plants are supplied by a broad array of photoprotective mechanisms, they are not able to avoid photoinactivation. During the course of a sunny day, many PSII complex can be destroyed and quickly repaired (Chow and Aro 2005). The photoinactivation of PSII in vivo is not limited only to high light but also occurs at low irradiance as reported by many authors (Anderson and Aro 1994; Keren et al. 1995; Park et al. 1995).

Lichtenthaler et al. (2007) carried out a work in which Chl fluorescence ratio RFd (ratio of fluorescence decrease) of green sun and shade leaves of three broadleaf trees (Platanus acerifolia Willd., Populus alba L., Tilia cordata Mill.) was determined. This ratio presented higher values in sun leaves (2.8–3.0) as compared to shade ones (1.4–1.8). The authors used Chl fluorescence imaging technique and demonstrated that RFd values were not uniformly distributed across the leaf area. The possible explanation is that this may cause by or is related to the non-uniform distribution of stomata opening, as already described for beech leaves and for needles (Beyschlag et al. 1994; Kuppers et al. 1999).

In general plants living in full sun conditions exhibit different leaf and photosynthetic characteristics compared to those living in shade. Under high irradiance, plants are predisposed to suffer photoinhibition, the process which is defined as the slow, reversible decline in photosynthetic efficiency that occurs when absorbed light is in excess of that required for CO_2 photoassimilation (Krause et al. 1988; Demmig-Adams and Adams 1992; Long et al. 1994). However, plants have adopted different mechanisms to cope with photoinhibition and this ability varied greatly between different plant species.

Recently in an interesting work Favaretto et al. (2011) reported an investigation on the ability of pioneer and late-successional species to adapt to a strong light irradiance in a reforestation area by measuring various parameter including Chl fluorescence parameters. The authors observed a greater decline in the ratio Fv/Fm under full sun light in the late-successional species that in the pioneer species. The obtained results suggest that pioneer species have more potential tolerance to photooxidative damage that late-successional species associated with the higher superoxide dismutase (SOD) activity and the increase in carotenoid/Chl ratio found in some pioneer species. Reduced photoinhibition in pioneer species probably results from their higher photosynthetic capacities, as been already observed by these authors (Nogueira et al. 2004).

In the Mediterranean area, high light stress usually occurs together with other environmental constraints such as high temperature and salinity stress, as excess soil salinity may rise up during the warm summer season, when clear days and daily irradiance are at the maximum (Chaves et al. 2003). The interactive effects of root-zone salinity and sunlight on Olea europea were studied by using Chl fluorescence analysis (Melgar et al. 2009). Root-zone salinity stress affected neither the actual efficiency of PSII photochemistry ΦPSII nor the non-photochemical quenching qNP in sunny leaves. Instead, ΦPSII and qNP were significantly affected in shade leaves by 5 week of treatment with 125 mM NaCl. The high concentration of violaxanthin-cycle pigments in sun leaves suggests that zaexanthin may protect the chloroplast from photo-oxidative damage rather than dissipating excess excitation energy via non-photochemical quenching mechanisms.

Some researchers (Ma and Cheng 2003; Chen and Cheng 2007; Li and Cheng 2008) showed that the sun-exposed side of apple fruit have higher photosynthetic capacity and higher xanthophyll cycle pool size and antioxidants as compared to the shade-exposed side of the fruit. In a further report, these authors studied the thermo-tolerance of the sun-exposed peel and the shaded peel of 'Fuji' apple (Malus domestica Borkh.) by using Chl fluorescence (Chen et al. 2009b). Maximum quantum yield of PSII, Fv/Fm, decreased to a similar extent in the sun-exposed peel and the shaded peel as temperature rose from 25°C to 44°C, but the sun-exposed peel reached slightly lower values at 46–48°C. It appears that the exposure to higher surface temperature under high light does not make the sun-exposed peel more tolerant of heat stress than the shaded peel of apple fruit.

Chl fluorescence imaging technique not only provides the possibility to screen the differences in photosynthetic CO_2 assimilation between sun and shade leaves but in addition permits detection and quantification of a large gradient in photosynthetic rate across the leaf area existing in sun and shade leaves as reported by Lichtenthaler et al. (2007). These authors concluded that shade leaves are exposed to a more homogeneous environment during most of their existence, therefore enabling more homogeneous leaf properties.

10.3.4 UV Radiation

It is well known as stratospheric ozone depletion determines an increase in UV-B radiation in the biosphere (WMO 2007). The UV-B wavelength band ranges from

280 to 320 nm, though only wavelengths greater than 290 nm can reach the Earth's surface. In sunlight, the ratio of UV-B to photosynthetically active radiation (PAR; 400–700 nm) fluctuates, primarily caused by changes in solar angle and thickness of the ozone layer. For these reasons photosynthetic organisms are thus inevitably exposed to UV-B radiation.

Many researchers have be demonstrated as PSII is particularly sensitive to UV-B radiation (Melis et al. 1992; Allen et al. 1998; Levizou and Manetas 2001; Searles et al. 2001; Xiong and Day 2001; Paul and Gwynn-Jones 2003; Guidi et al. 2011). It has been found that a very sensitive UV-B response is the rapid light-driven degradation of the structurally and functionally similar D1 and D2 proteins in the core of PSII (Jansen et al. 1996). The degradation response is maximal at 300 nm, with shorter wavelengths having less effect (Jansen et al. 1993). Rapid PAR-driven turnover of D1 (D2 is stable under PAR) has been proposed to be part of a damage–repair cycle essential for maintaining PSII function under photoinhibitory conditions (Aro et al. 1993). Jansen et al. (1998) reported that by analogy, it is possible that UV-B-driven D1-D2 turnover is also part of a repair cycle, preventing accumulation of UV-inactivated PSII. However, it has been reported as no changes in both Fv/Fm and ΦPSII in plants exposed to UV-B irradiance as high as 32–40 kJ m^{-2} day^{-1} when PPFD irradiance exceeded 400–500 μmol m^{-2} s^{-1} (Noguès et al. 1995; Allen et al. 1997).

Xu et al. (2010) found that enhanced UV-B radiation caused a great decrease in net photosynthesis rate, but had no significant effect on the maximum efficiency of PSII, Fv/Fm or photochemical quenching coefficient, qP in cuttings of Populus cathayana. Similar results have been previously reported in broad-leaved tree species Fraxinus excelsior, Betula pendula, Quercus robur, Acer pseudoplatanus, Populus yunnanensis, Populus trichocarpa, Populus trichocarpa × P. deltoides, which had lower Amax but no changes in Fv/Fm and qP after exposure to elevated UV-B radiation (Schumaker et al. 1997; Keiller and Holmes 2001; Bassman et al. 2003; Duan et al. 2008).Albert et al. (2010) investigated Salix arctica and Vaccinium uliginosum in a high arctic ecosystem over a period of 6 years. They found that, despite plants responded significantly every year to the current UV improving their UV-screening capacity, the negative effect on PSII performance could not be avoided and, by Chl fluorescence measurements concluded that the decreased PSII performance to be a part of a response decreasing plant carbon uptake.

Yang et al. (2007) investigate the effects of UV-B radiation on photosynthesis of winter wheat seedlings under two different growth temperatures and found a decreased maximal quantum yield of Photosystem II, Fv/Fm, and increased minimum fluorescence Fo in seedlings exposed to high UV-B radiation at both growth temperatures (25/20°C and 10/5°C) and in those subjected to low UV-B radiation and growth at 10/5°C. High UV-B increased total pool size of xanthophyll cycle pigments, but decreased the de-epoxidation state (DEPS) of these pigments at both temperatures, while low UV-B only decreased DEPS at 10/5°C. In conclusion, elevated UV-B radiation but not low temperature (10/5°C) inhibited photosynthesis in winter wheat seedlings. This UV-B induced inhibition was enhanced by higher UV-B intensity and lower growth temperature.

On the other hand, Núñez-Olivera et al. (2006) found no changes in response to UV-B in Chl fluorescence variables and concluded that photosynthesis in acclimated plants growing outdoors, does not appear to be at risk from current levels of UV-B as reported also by other researchers (Allen et al. 1998; Day 2001; Searles et al. 2001). Also, a decline of Fv/Fm in V. vinifera Silvaner under solar UV-B was not found because of protection mechanisms in which the xanthophyll cycle and screening compounds could be involved (Kolb et al. 2001). Diurnal changes occurred only in a few variables (ΦPSII, ETR, NPQ, and xanthophylls cycle) and were mainly dependent on the diurnal variations in PAR, which might be a more decisive factor than the different UV-B irradiances associated with the two radiation regimes imposed in this experiment.

Guidi et al. (2011) conducted a UV-exclusion experiment in which the effects of both UV-radiation and visible-light irradiance were examined on physiological and biochemical traits in Ligustrum vulgare, a species inhabiting partially shaded areas of the Mediterranean Basin. UV-irradiance had a relatively minor impact on most examined traits, as compared with the effect of visible-light irradiance. UV-induced variations on most parameters but the maximal (Fv/Fm) and actual (ΦPSII) efficiency of PSII photochemistry varied to a greater extent because of visible-light than UV-irradiance and full sun leaves had smaller Fv/Fm and ΦPSII than the partially shaded ones. Similarly, also Laposi et al. (2009) found that the values and the extent of diurnal change in Fv/Fm indicated that the photoinhibition that was observed in leaves of beech was mostly due to PAR, but it can be enhanced with a 45% increase in the present ambient level of UV-B radiation measured at midday of unclouded summer days.

Chl fluorescence measurements have been used also by Gilbert et al. (2009) which studied the impact of UV-B radiation on 10 genotypically different barley and tomato cultivars in a predictive study to screen for potentially UV-tolerant accessions and to analyze underlying mechanisms for UV-B sensitivity. The obtained results indicate that photosynthetic light and dark reactions have to play optimally in concert to render plants more tolerant against UV-B radiation.

Jansen et al. (2010) analyzed the role of constitutive and inducible protection responses in decreasing UVB-mediated inactivation of PSII in Arabidopsis thaliana accessions that originate in different geographical regions. For this study they used substantially the Fv/Fm ratio and by using this Chl fluorescence parameter showed the importance of inducible, rather than constitutive, responses in the protection of PSII.

10.3.5 Temperature

Temperature stresses experienced by plants can be classified into three types: those occurring at (a) temperatures below freezing, (b) low temperatures above freezing, and (c) high temperatures. Freezing and thawing of leaves of herbaceous plants leads to damage when the freezing temperature falls below a certain tolerance limit, which depends on the plant species and state of acclimation. Such damage is expressed as an irreversible inhibition of photosynthesis observed after thawing. In frost-damaged leaves the capacity of photosynthetic reactions of the thylakoid membranes is impaired. Particularly, the water-oxidation system, photosystems II and I are inhibited. However, it appears that CO_2 assimilation is more readily affected by freezing stress than the activity of the thylakoids. The inhibition of CO_2 fixation seen in initial stages of damage seems to be independent of thylakoid inactivation. This can be shown by Chl fluorescence analysis made simultaneously with measurement of CO_2 assimilation. The effects of freezing and thawing were studied in detail with isolated mesophyll protoplasts prepared from both non-hardened and cold-acclimated plants of Valerianella locusta (Krause et al. 1988). Freezing damage was characterized by various parameters such as plasma membrane integrity, photosynthetic CO_2 assimilation, Chl fluorescence emission and activities of thylakoids isolated from the protoplasts. All tests indicated a substantially increased frost tolerance of protoplasts obtained from cold-acclimated as compared to non-hardened leaves. CO_2 assimilation and related fluorescence changes were the most freezing-sensitive parameters in both types of protoplasts (Krause et al. 1988).

The effects of chilling on PSII efficiency are well known (Tijskens et al. 1994; Allen and Ort 2001) even if the effects are more relevant when low temperatures are combined with high level of irradiance (Allen and Ort 2001). Hogewoning and Harbinson (2007) reported that in Calathea makoyana leaves chilling (5°C and 10°C) up to 7 days in the dark did not affect PSII efficiency whereas chilling in the light caused severe photoinhibition sometimes followed by leaf necrosis. In the work the authors have been used Chl fluorescence images analysis to detect the different sensitivity to photoinhibition in the different leaf regions. The authors reported that the greater loss of Fv/Fm in the interlobate regions, compared with the chlorophyll-rich lobate regions, may be due to the relatively larger irradiation per unit thylakoid in these areas. Low temperatures exacerbate an imbalance between the source of energy and the metabolic sink thus adjustment of the photosynthetic apparatus to maintain the balance of energy flow during cold acclimation seems to be very important for cold tolerance.

Dai et al. (2007) have been carried out a study in which the change of Chl fluorescence parameter in frozen leaves were examined using two winter barley cultivars differing in cold tolerance to investigate physiological response to low temperature as affected by cold acclimation (under 3/1°C, day/night for 5 days before freezing treatment) and irradiation (high irradiance: 380 μmol m^{-2} s^{-1} and low irradiance: 60 μmol m^{-2} s^{-1}) during recovery. The obtained results underline as some Chl fluorescence parameter during recovery from freezing can be useful used as the indicators in identification and evaluation of cold tolerance in barley.

In an other work (Ehlert and Hincha 2008) it has been reported that Chl fluorescence imaging offers an additional advantage in comparison to other analysis. It provides useful spatial information on chilling-induced damage. The authors reported that freezing damage was indeed not homogenously distributed over the whole leaf and this phenomenon can be exactly detected by Chl fluorescence imaging.

Plants growth is greatly affected by elevated temperature stress and has been directly correlated with decreased photosynthetic efficiency (Georgieva et al. 2000).

Photosynthetic process appears one of the most heat-sensitive processes in plants, leading to numerous changes in structure and function of the photosynthetic apparatus. Elevated temperature-induced detrimental effects are manifested in reduced photosynthetic metabolism (Koves Pechy et al. 1998), photosynthetic capacity and photochemical efficiency (Percival 2005). Within the photosynthetic apparatus, PSII is the most thermolabile component of the electron transport chain (Berry and Bjorkman 1980; Thompson et al. 1989; Mamedov et al., 1993; Cajanek et al. 1998).

Exposure to a short-term high temperature treatment resulted in significant changes in PSII Song et al. (2010) examined the effect of extreme heating (40/35°C for 30 days) on the growth and photosynthesis of an alien invasive species Wedelia trilobata and its indigenous congener (Wedelia chinensis). An obvious decreasing trend of Fv/Fm was observed in these two species, while the value of F0 was increased under high temperature in the later stages of the treatment indicating photoinhibition of PSII activity (Maxwell and Johnson 2000). The obtained results are consistent with the evidence of Sun et al. (2007), which demonstrated that moderate high temperature stress caused a partial inhibition of PSII.

The relationship between source leaf thermostability and prestress antioxidant capacity was quantified by monitoring the actual quantum yield response of PSII, ΦPSII to a range of temperatures in Gossypium hirsutum (Snider et al. 2010). To test this hypothesis, thermosensitive (cv. ST4554) and reportedly thermotolerant (cv. VH260) G. hirsutum plants were exposed to control (30/20°C) or high day temperature (38/20°C) for both cultivars grown under the control temperature regime and measuring antioxidant enzyme activity for those same leaves. VH260 was more thermotolerant than ST4554 as evidenced by Fv/Fm being significantly lower under high temperature for ST4554 but not VH260.

Mathur et al. (2011b) report a study in which Chl fluorescence analysis was used to detect change in photosynthetic machinery in wheat (Triticum aestivum) subjected to elevated temperature. Detached leaves were subjected to elevated temperature stress of 35°C, 40°C or 45°C. At 35°C, there was no effect on photosynthetic efficiency, including the oxygen-evolving complex, and the donor side of PSII remained active. At 40°C, activity was reduced by 14%, while at 45°C, a K intermediate step was observed, indicating irreversible damage to the oxygen-evolving complex. The authors concluded that this analysis can be used to rapidly screen for vitality and stress tolerance characteristics of wheat growing in the field under high temperature stress. In an other work, Mathur et al. (2011a) report that there was a change in the relative amounts of PSII α, β and γ centers in relation to elevated temperatures. It seemed that with increasing temperature the PSIIβ and PSIIγ centers increased at the expense of PSIIα centers. The reducing side heterogeneity was also affected as shown by an increase in the number of QB-non-reducing centers at high temperatures. The reversibility of high temperature induced damage on PSII heterogeneity was also studied. Antenna size heterogeneity was recovered fully up to 40°C while reducing side heterogeneity showed partial recovery at 40°C. An irreversible damage to both the types of heterogeneity was observed at 45°C. The work represents a significant contribution to understand the basic mechanism involved in the adaptation of crop plants to stress conditions.

The responses of photosynthetic CO_2 assimilation rate, dark respiration and Chl fluorescence to supra-optimal temperatures of varying duration and kinetics in Phaseolus vulgaris was studied by the aim to study whether the instability of photosynthesis under severe heat stress is associated with cellular damage (Hüve et al. 2011). Effective quantum yields of PSII were strongly reduced at 42°C in the steady-state experiments and at 46°C in the heat pulse experiments. The decline of CO_2 uptake was associated with reduction in oxygen evolution, and this in turn, was closely correlated with changes in Fv/Fm ratio. In this case, the major damage likely occurred at PSII. However, as has been reported before (Bukhov and Carpentier 2003), not all PSII exhibited the same sensitivity. Rapid temperature response curves (1°C min-1) of dark-adapted minimum fluorescence yields F0 can be used to further pinpoint the thresholds for leaf heat sensitivity. At the so-called critical temperature for fluorescence rise (TF), F0 starts to increase rapidly, and the values of TF are associated with the temperatures indicating loss of chloroplast thermostability (Armond et al. 1978) or with temperatures at which the rates of net assimilation become unstable (Seemann et al. 1984; Havaux 1993). In P. vulgaris the critical temperatures for F0 increase were between 46°C and 48°C.

It is well known that heat stress often inhibits photosynthesis in higher plants. Under natural conditions, plants are often subjected to heat stress and high light simultaneously during the daytime in the summer season. However, most studies on the response of photosynthesis have been performed in the dark (Allakhverdiev et al. 2008; Yamamoto et al. 2008). Although it has been reported that inhibition of photosynthesis induced by heat stress was enhanced when heat stress was imposed by high light (Al-Khatib and Paulsen 1998), it is unclear how photosynthesis, and in particular PSII photochemistry and the dissipation of excess light, response to heat stress under high light conditions. Yin et al. (2010) investigated how high light affected the responses of photosynthesis to heat stress by studying the effects of high temperature (25–42.5°C) either in the dark or in the light (1,000 $\mu mol\ m^{-2}\ s^{-1}$) on PSII photochemistry and the xanthophyll cycle in rice plants. Obtained results show that heat stress alone had no effect on Fv/Fm but resulted in a decrease in CO_2 assimilation rate. A decrease in the CO_2 assimilation rate accompanied by no decrease in Fv/Fm induced by heat stress can potentially expose the plants to excess excitation energy under light conditions, which, if not safely dissipated, may result in photodamage to PSII because of an overreduction of reaction centers (Demmig-Adams and Adams 1992). The inhibition of violaxanthin de-epoxidase resulted in an increase in the sensitivity of PSII to photoinhibition under heat stress. These results suggest that high light has profound effects on the responses of CO_2 assimilation rate and PSII to heat stress.

10.3.6 Salinity

FAO reported that almost 80 million hectares of arable lands worldwide is currently affected by salinity (FAO 2008). As reported by Munns et al. (2006) salt stress imposes both ionic and osmotic stresses on plants that induces reductions in growth (Munns and Tester 2008). Many plants when are subjected to salinity stress show a decline in photosynthetic activity (Munns and Termaat 1986; Brugnoli and Björkman 1992) and a direct consequence to this limitation is the exposure of plants to excess energy, which, if not efficiently dissipated, may be harmful to PSII due to over-reduction of reaction centers (Demmig-Adams and Adams 1992). There are a lot of reports on photosynthetic characteristics under salt stress (Qiu et al. 2003; Koyro 2006; Wei et al. 2006) and, generally, photosynthesis is inhibited by salt stress (Ma et al. 1997; Sultana et al. 1999; Qiu et al. 2003; Sudhir and Murthy 2004; Koyro 2006; Munns et al. 2006; Chaves et al. 2009).

The initial effects of salinity are very similar to those observed when plants are subjected to drought. Decrease in leaf water potential induces stomatal closure and sometimes inhibits photosynthetic metabolism with evident changes in the actual quantum efficiency of PSII (Baker and Rosenqvist 2004; Mehta et al. 2009; Pak et al. 2009; Abdeshahian et al. 2010; Azizpour et al. 2010) while no or little changes are also recorded in Fv/Fm (Shabala et al. 1998; Bosque Sanchez et al. 2003; Smethurst et al. 2008; Seckin et al. 2010). In intact plants, PSII is well protected against salinity stress, and is the last "line of defense" that fails only when nothing else can be done to protect it from the detrimental effects of salinity (Smethurst et al. 2009). Multiple mechanisms such as restriction of the unidirectional Na^+ uptake by roots, active Na^+ extrusion from the cytosol to the external media, efficient cell- and tissue-specific Na^+ compartmentation, prevention of Na^+ transport to the shoot, and recirculation of Na^+ back to the roots through the phloem (Blumwald et al. 2000; Berthomieu et al. 2003; Tester and Davenport 2003) may all contribute to protection of PSII against salt stress.

Some reports of salinity effects on the photochemical efficiency of PSII of different plant organ, tissue, and cell preparation are limited and conflicting. Some researchers have demonstrated that salt stress inhibits PSII activity (Santos 2004, Jiang et al. 2006; Hichem et al. 2009), whereas others have indicated that salt stress has no effect on PSII (Lu et al. 2003a, b; Demiral and Türkan 2006).

The effects of short-term salt stress on gas exchange and the regulation of photosynthetic electron transport were examined in Arabidopsis thaliana and its salt-tolerant close relative Thellungiella halophila (Stepien and Johnson 2009). Plants cultivated on soil were challenged for 2 weeks with NaCl. The inhibition of CO_2 assimilation in salt-stressed Arabidopsis is accompanied by a decrease in electron transport through PSII, indicated by the decline in ΦPSII, and cumulative damage to PSII, indicated by the progressive drop in Fv/Fm. These data were accompanied by an increase in NPQ. In contrast, in Thellungiella, although gas exchanges were marginally inhibited by high salt and PSI was unaffected, there was a large increase in electron flow involving PSII. Overall, the response of Arabidopsis to salt stress is very much in line with that expected from the responses of a variety of other species to a variety of stresses. Down-regulation of linear electron transport limits oxidative stress and increased cyclic flow enhances photoprotective energy dissipation.

Despite the fact that most plants accumulate both sodium (Na^+) and chloride (Cl^-) ions to high concentration in their shoot tissues when grown in saline soils, most researches on salt tolerance in annual plants have focused on the toxic effects of Na^+ accumulation. An experiment was conducted to compare the responses to Na^+ and to Cl^- separately in comparison with the response to NaCl in a soil-based system using two varieties of Vicia faba that differed in salinity tolerance (Tavakkoli et al. 2010). The variety Nura is salt-sensitive and it accumulates Na^+ and Cl^- to high concentrations, while the line 1487/7 is salt tolerant which accumulates lower concentrations of Na^+ and Cl^-. There was a significant reduction in both Fv/Fm and ΦPSII in both genotypes, but line 1487/7 maintained a higher capacity of the PSII system compared with Nura. Fluorescence data also indicate that the decline in photosynthesis in saline soils is likely to be a consequence of toxic Cl^- concentrations. While Na^+ and Cl^- accumulated to high concentration, ΦPSII and Fv/Fm declined markedly in Cl^- and NaCl treated soils. The proportion of PSII reaction centers that remained open (qP) was also reduced, indicating that this decrease in ΦPSII was due to feedback regulation caused by processes such as photoinhibition as well as salt-induced photodamage.

The Fv⁄Fm in Arthrocnemum macrostachyum, a C3 shrub, salt accumulator (Winter et al. 1976) showed a significant reduction at midday compared to dawn, which is indicative of photoinhibition associated with over-reduction of PSII (Redondo-Gomez et al. 2010). This photoinhibition would be caused by a lower proportion of open reaction centers (lower Fm) resulting from saturation of CO_2 photoassimilation by light. This decrease seems to be dynamic photoinhibition, since the low midday values recovered completely by dawn to optimal values for unstressed plants (Björkman and Demmig 1987). The midday depression of Fv⁄Fm was highest in the non-saline treatment, indicating that absence of salt represents the most severe environmental stress for A. macrostachyum. It is possible that a stable quencher was formed in the presence of NaCl that did not decay during the dark adaptation period before measurement. Alternatively, it could be concluded that, under low NaCl conditions, plants are more prone to photoinhibition through damage to PSII reaction centers.

The effect of salinity (0–400 mM NaCl) on photosynthesis, photosystem II efficiency and xanthophylls cycle were investigated in the halophyte Atriplex centralasiatica by Qiu et al. (2003). In the work it has been reported as CO_2 assimilation rate decreased significantly because of salinity stress as well as the efficiency of excitation energy capture by open PSII reaction centers (Fv′/Fm′). In these plants Fv/Fm did not change when measured at predawn indicating that the photoinhibition observed was due to photoprotective processes and not to photoinhibitory damage. On the other hand, in these salt-stressed plants NPQ increased significantly as compared to the controls as well as the level of zeaxanthin by the de-epoxidation to violaxanthin. The authors found a significant correlation between the change in the de-epoxidation state of the xanthophylls cycle and actual PSII efficiency, the efficiency of excitation capture by open PSII reaction centers and non-photochemical quenching. The obtained results underline as the xanthophylls cycle plays a key role in protecting the photosynthetic apparatus from excess energy in salt-adapted plants with decreased CO_2 assimilation capacity. Other authors have been reported as the sensitivity to salt stress in cereals might be associated with both reduction in PSII photochemical efficiency and enhanced qNP to dissipate excess energy (Moradi and Ismail 2007).

It has been reported as mild-salinity levels does not induce sustained photodamage to PSII as revealed by unvaried Fv/Fm ratio in plants (Baker and Rosenqvist 2004; Morales et al. 2005; Redondo-Gomez et al. 2006; Naumann et al. 2007) even if leaf gas exchanges were reduced. However, some studies have shown changes in Fv/Fm ratio after dark-adaptation of the leaf as a result of salinity (Lee et al. 2004; Castillo et al. 2005). In the first stages of salinity stress stomatal conductance decreases and, consequently, the CO_2 fixation leaving PSII unaffected (Baker and Rosenqvist 2004). However, Naumann et al. (2007) found significant changes in the actual efficiency of PSII, ΦPSII at 10 $g\,L^{-1}$ salinity in coastal plant species (Myrica cerifera and Phragmites australis) prior to visible signs of salt stress and in parallel an increase in ΦNPQ in both species. The authors reported that the decrease in ΦPSII and the increase in ΦNPQ with no change in Fv/Fm ratio for both species indicate an enhancement in thermal dissipation in PSII to avoid photodamage.

One way in which plants can counteract the negative effects of salinity depends on the potassium nutrition. In fact, it is known that an increase in potassium supplies limits the reduction in salt-stressed plants' growth (Delgado and Sànchez-Raya 1999; Quintero et al. 2007). The interactive effects of salinity and potassium deficiency on photosynthetic performance were investigated in wild (Hordeum maritimum L.) and cultivated barley (Hordeum vulgare L. var. Manel) (Degl'Innocenti et al. 2009). Potassium deficiency increased the negative effects induced by salt in the photosynthetic process of H. vulgare, and this species seems unable to counteract the negative effects of salinity. Although there was an excess of energy following the limitation to CO_2 photoassimilation and a down regulation of PSII photochemistry in H. maritimum, this species displays mechanisms that play a role in avoiding PSII photodamage aimed to dissipate this excess energy

High sunlight and root-zone salinity may also alter PSII photochemistry and photosynthetic pigment composition (Logan et al. 1998; Lu et al. 2003b), by either decreasing the total Chl concentration or increasing the ratio of violaxanthin-cycle pigments to total Chl. Melgar et al. (2009) reported a work in which Olea europea (cv. Allora) plants were grown under partial shading (15% full sunlight) or full sunlight (sun plants/leaves) and were supplied with 0 or 125 mM NaCl. Obtained results showed as the greater maximal PSII photochemistry (Fv/Fm) at midday, detected in salt-treated than in control plants, growing in full sunshine. By contrast, salt-treated plants in the shade suffered from midday depression in Fv/Fm to a greater degree than that observed in control plants. The high concentration of violaxanthin-cycle pigments in sun leaves suggests that zeaxanthin may protect the chloroplast from photo-oxidative damage, rather than dissipating excess excitation energy via non-photochemical quenching mechanisms.

In addition, other works have shown that there were no changes in PSII photochemistry in salt-stressed roses, sorghum and Artemisia anethifolia when they were grown under relatively low light conditions, and that the interaction between high light and other environmental stresses (low or high temperatures) result in the damage of PSII (Jimenez et al. 1997; Lu and Zhang 1998; Lu et al. 2003c). Boughalleb et al. (2009) evaluated the difference in the responses of Nitraria retusa. Atriplex halimus and Medicago arborea plants to different NaCl salinity levels. Nitraria retusa (a leading shrubs in steppes, deserts and saline soils) PSII photochemistry and carotenoid content were unaffected by salinity, but a reduction in Chl content was observed at 800 mM NaCl. Similar results were found in A. halimus (a Mediterranean xero-halophyte saltbush species, highly resistant to drought and salinity), but with a decrease in the efficiency of PSII (Fv/Fm) occurred at 800 mM. Conversely, in M. arborea (a shrub growth habit that can adapt to extreme ecological conditions such as drought resistance) plants, the authors observed a significant reduction in pigment concentrations and Chl fluorescence parameters.

He et al. (2009) exposed non-grafted, self-grafted and rootstock-grafted tomato plants to different concentration of salt stresses and investigated whether grafted plants could improve tolerance to salinity, and whether the induced tolerance to salt stress was associated with the protection of the photosynthetic apparatus. The ratio Fv/Fm was not affected in tomato plants under mild and moderate salt stress. In non-grafted and self-grafted plants under severe salinity conditions, Fv/Fm decreased to 0.76 and 0.77, respectively, suggesting the occurrence of photoinhibition and this could be a consequence of damage to PSII. The constant Fv/Fm in rootstock-grafted plants might show that photoinhibition of PSII was not triggered. Compared with Fv/Fm, actual quantum yield of photosynthesis (ΦPSII) was more sensitive to salt stress. Moderate and severe salt stress considerably decreased ΦPSII in non-grafted and self-grafted plants, which was attributed to both the decrease of open PSII reaction centers (qP) and the efficiency of the excitation energy capture by open PSII reaction centers (F′v/F′m) (Fig. 10.8), since ΦPSII is a product of qP and F′v/F′m (Genty et al. 1989). In this study, the higher ΦPSII in rootstock-grafted plants was well correlated to the better performance of photosynthesis under moderate and severe salt stress. Under moderate and severe salt stresses, ΦPSII was considerably decreased with a slight or no decrease in Fv/Fm. This might be due to an enhancement in NPQ, which suggested that more energy was dissipated through thermal in PSII.

10.4 Concluding Remarks

In conclusion Chl fluorescence tool provides information on how the light energy absorbed by Chl pigment impacts on PSII. Certainly, the flow of electrons through PSII indicates the overall rates of photosynthesis and so by using Chl fluorescence parameters we have an estimation of photosynthetic performance.

In addition to, the chloroplast, and even more PSII, represents the most sensible part of the photosynthetic apparatus to light-induced damage. In fact, once generated, the excitation energy must be safely dissipated or used, because of excess energy cal also damage the photochemical apparatus. Stress changes the proportion of absorbed light energy which are used for photosynthetic quantum conversion, Chl fluorescence or heat dissipation. So the study of Chl fluorescence provides useful information about leaf photosynthetic performance and the extent to which this performance is limited by photochemical and non-photochemical processes. As reported in this paper this methodology has been conveniently used in stress researches in which provides useful information about stress tolerance mechanisms and, consequently, to screen for tolerance to abiotic stresses.

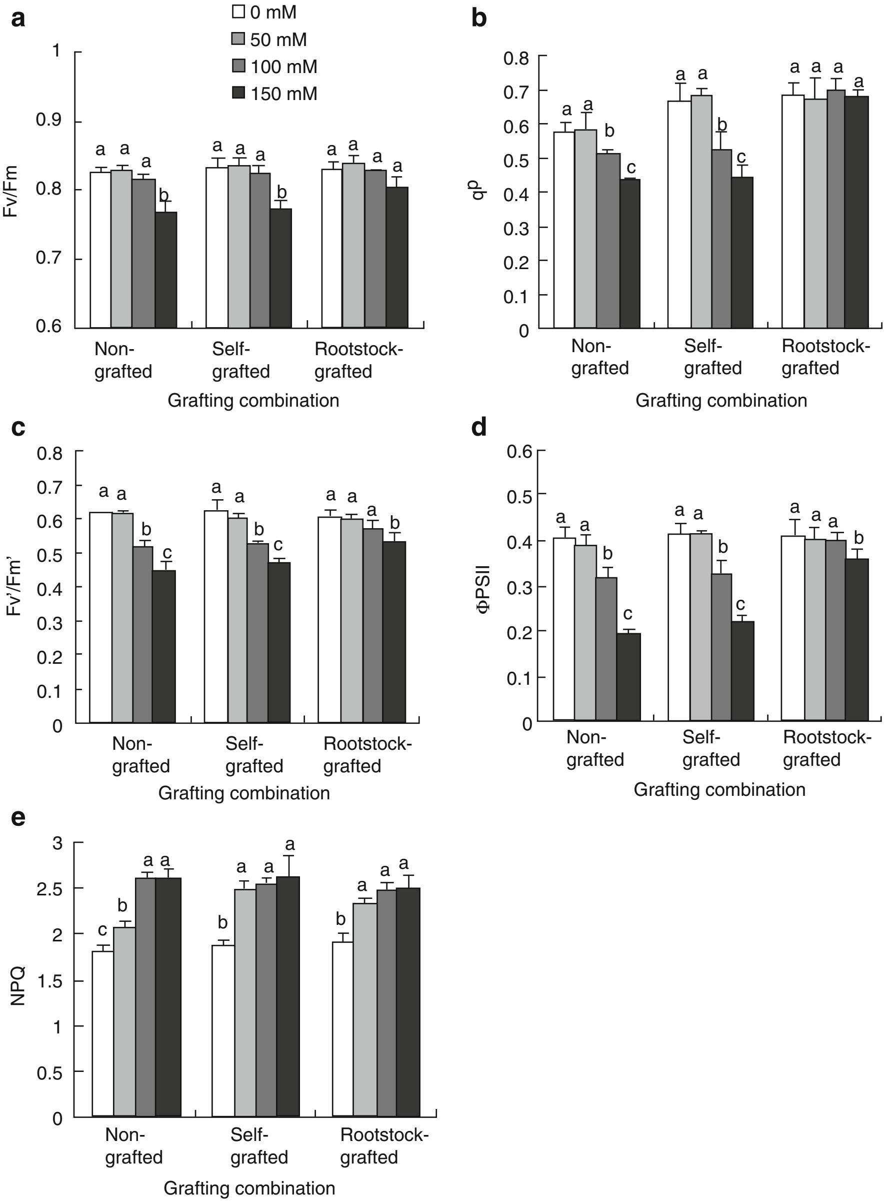

Fig. 10.8 Effects of different NaCl concentrations (0, 50, 100 and 150 mM) in the nutrient solution on the Fv/Fm ratio (**a**), photochemical quenching (qP) (**b**), effective quantum use efficiency of PSII in the light-adapted state (F′v/F′m) (**c**), effective quantum yield (ΦPSII) (**d**) and non-photochemical quenching (NPQ) (**e**) of non-grafted, self-grafted and rootstock-grafted tomato plants. *Different letters* indicate significant differences according to LSD test ($P < 0.05$). Values are the means of four replicate samples (from He et al. 2009)

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Chapter 11
Crop Stress and Aflatoxin Contamination: Perspectives and Prevention Strategies

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Abstract The fungal metabolites called aflatoxins are potent naturally occurring carcinogens, produced primarily by *Aspergillus flavus* and *A. parasiticus*. A. flavus affects many agricultural crops such as maize, cotton, peanuts, and tree nuts. It can contaminate these crops with aflatoxins in the field before harvest. It is a serious concern because of its toxic and carcinogenic properties and also due to the risk of contamination in food and feed on human health and livestock. It is not only a serious food safety issue, but it has significant economic implications for the agricultural industry worldwide because of restrictions limiting the trade of contaminated crop. Host plant resistance is an effective, efficient and dependable tool to protect crops from the preharvest infection and aflatoxin contamination processes. Host plant resistance to aflatoxin contamination is a complex trait, and dissecting this trait is an equally complex task. With the technological breakthrough in genomics and next-generation sequencing, our understanding on the *Aspergillus* biology is greatly enhanced. This chapter aggregates the rich aflatoxin literature and focuses on the factors that cause the stress execrably and increase the aflatoxin contamination of grains before and after harvest. Further discussed are potential future prevention technology and strategies that could be employed to guide future research, such as "next-generation" genetics.

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11.1 Introduction

The fungal metabolites called aflatoxins are among the most potent naturally occurring carcinogens, and are produced primarily by *Aspergillus flavus* and *A. parasiticus* (Squire 1981). The infection and colonization of maize (*Zea mays* L.) and peanut (*Arachis hypogaea* L.) may result in the accumulation of several carcinogenic mycotoxins collectively referred to as aflatoxins. Aflatoxin is not only as a risk factor for cancer but has immunologic and nutritional effects associated modulation with the infectious diseases such as HIV (Williams et al. (2004, 2010). The production of aflatoxin is exacerbated by exposure of *A. flavus* to elevated temperatures, drought and low relative humidity with high net evaporation (Widstrom et al. 2003a). Drought reduces the ability of maize to resist the growth of *A. flavus* because it negatively affects the expression of genes encoding for resistance-associated proteins (Payne et al. 1986; Guo et al. 1995; Payne 1998; Wang et al. 2008; Chen et al. 2009; Guo et al. 2009, Scully et al. 2009; Fountain et al. 2010; Luo et al. 2010), which ultimately leads to increased pre- and post-harvest aflatoxin contamination.

The importance of crop management practices that reduce aflatoxin contamination became apparent when research revealed that most of the agronomic factors that influence *Aspergillus* infection and aflatoxin contamination are those which can be controlled by the producer (Lillehoj 1983). These include: (1) planting date selection (Widstrom 1996); (2)fertilization (Wilson 1989); (3) tillage (Jones 1987); (4) irrigation (Payne et al. 1986; Smith and Riley 1992), and (5) land and hybrid selection (LaPrade and Manwiller 1976; Zuber 1977; Lillehoj et al. 1982), along with disease, insect, and weed management (Widstrom et al. 1975; McMillian et al. 1980; Lillehoj et al. 1980; Wilson et al. 1981; Doupnik 1972; Campbell et al. 1993; Glover and Krenzer 1980). Each of those factors, whether controlled or not, is inextricably linked to plant stress. In general, a healthy non-stressed plant is less likely to have high levels of infection or contamination than one subjected to stress. However, under favorable conditions for aflatoxin contamination no plant is completely immune.

In addition to the management of the agronomic practices, genetic based host plant resistance to biological and environmental stress factors holds much promise. Such biotic such as insects, diseases, weeds and abiotic factors including drought are often interactive that contribute directly to the infection and contamination process. Research on host plant resistance (LaPrade and Manwiller 1976; Lillehoj et al. 1976; Widstrom et al. 1978; Darrah 1987; Scott et al. 1991; Naidoo et al. 2002; Chen et al. 2009; Scully et al. 2009; Guo et al. 2011a) have made great progress in understanding the biology of *Aspergillus* and the regulation of afaltoxin biosynthesis (Bhatnagar and Cleveland 1991; Yu et al. 2008). This research has clearly demonstrated that host plant resistance provides a protective genetic barrier against the development of stress symptom in crop plant. This resistance is influenced by environmental and biological factors that can contribute to ear infection and aflatoxin contamination (Zuber and Lillehoj 1979; Lillehoj 1983). Yu et al. (2008) summarized the biotic and abiotic factors affecting aflatoxin biosynthesis by toxigenic *Aspergilli*.

The molecular mechanisms related to these effects are still unclear (Payne and Brown 1998; Guo et al. 2005; Yu et al. 2008), but some of these stress-factors may affect expression of the aflatoxin regulatory gene, *aflR*, or alter the expression of globally acting transcription factors that respond to external signals. This review aggregates the rich aflatoxin literature and focuses on the factors that cause the stress execrably and increase the aflatoxin contamination of grains before and after harvest. Further discussed are potential future prevention technology and strategies that could be employed to guide future research, such as "next-generation" genetics (Nordborg and Weigel 2008). Complex-trait analysis in plants uses newly developed genome-wide association mapping (Mitchell-Olds 2010), rather than the traditional genetic linkage mapping of the trait of the interest (Qin et al. 2011), to dissect the complex trait such as host plant resistance to aflatoxin contamination in corn and peanut.

11.2 Significance of Aspergilli and Aflatoxins

11.2.1 Biology of Aspergillus flavus and A. parasiticus

The genus *Aspergillus* was first described by a Florentine priest-mycologist named Pier Antonio Micheli (1679–1737) in 1729. He named it *Aspergillus* because the spore-bearing structure characteristic of the genus resembled a devise called aspergillum used by the Catholic Church to sprinkle holy water. *Aspergillus* is the most abundant and widely distributed fungal mold found on Earth, and is especially prevalent in the autumn and winter in the Northern hemisphere on a variety of substrates including soil, plant debris, insect carcasses, atmosphere and even human and animal patients. The positive and negative impacts of these *Aspergilli* to human beings are as diverse as its distribution. The greatest positive economic benefits of these *Aspergilli* have been the production of industrial enzymes and organic compounds such as amylases, glycosidases, pectinasess, proteases and citric acid. *Aspergillus oryzae*, *A. sojae* and *A. tamari* are termed as "koji molds" used for more than a thousand years to produce sake and soy sauce in Asian countries. Some *Aspergilli* possess numerous secondary metabolic pathways that produce compounds with biotechnological and pharmacological properties. A number of antibiotics, antitumor, antifungal agents, and other pharmaceuticals have been derived from *Aspergillus* metabolites, including lovastatin, one of the first successful cholesterol-lowering drug produced by *A. terreus*, penicillin, an antibiotic that saved millions of human lives during World War II in the early 1940s, produced by *A. nidulans*, and cilofungin, a semi-synthetic experimental anti-Candida drug modified from echinocandin B, produced by *A. nidulans* (*Emericella nidulans/rugulosa*).

However, taxa of this genus are seriously deleterious and negatively impact mammalian health causing diseases of humans, animals and the plants they consume by producing toxic and carcinogenic compounds noted previously. *A. fumigatus* is the most common human pathogen accounting for over 90% of both invasive and noninvasive human aspergillosis (Denning 1998; Denning et al. 1991, 2003;

Mori et al. 1998), followed by *A. flavus*, *A. terreus*, *A. niger*, *A. nidulans*, and *A. ochraceus*. Three phyto-pathogens especially including *A. flavus*, *A. parasiticus*, and *A. versicolor* infect agricultural crops and food grains, producing aflatoxins. *A. nidulans*, as a model organism for the study of developmental biology and used for drug development and produces aflatoxin precursors, sterigmatocystin (ST) and dihydrosterigmatocystin (DHST). *A. niger* has industrial importance in the production of organic compounds and industrial enzymes, but is also a plant pathogen that colonizes grape and produces ochratoxins. *A. ochraceus*, *A. carbonarius*, and *A. tubingensis* additionally produce ochratoxins. *A. fumigatus* produces gliotoxin, while *A. clavatus* and *A. terreus* produce patulin. These *Aspergilli* are medical, industrial, and agricultural important organisms and are of significant economic, health and food safety importance worldwide.

Aspergillus flavus and *A. parasiticus* are the most harmful taxa in the genus *Aspergillus* because they produce the most toxic and the most potent carcinogenic compounds (Jelinek et al. 1989). These fungi are saprobe that is capable of surviving on many organic nutrient sources like plant debris, tree leaves, decaying wood, animal fodder, dead insects and animal carcasses, stored grains, and even human and animal patients (Klich 1998). They can survive in a wide range of temperature condition from 12°C to 48°C, but for aflatoxin formation, the optimal temperature is about 30°C, while the optimal temperature for growth is about 37°C which roughly coincides with mammalian body temperatures, which makes humans and warm blooded animals prone to fungal infection (pathogenicity) (Denning 1998; Denning et al. 1991, 2003; Mori et al. 1998). Unlike *A. nidulans*, *A. flavus* and *A. parasiticus* (also many other imperfect filamentous fungi such as *A. fumigatous* and *A. oryzae*) do not have a typical sexual reproductive stage. However, under certain condition, they are able to develop ascospore-bearing ascocarps embedded within stromata (containing sexual spores, cleistothecia) and intercross is possible between strains with opposite mating-type genes MAT1-1 and MAT1-2 (Horn et al. 2009a, b, c). Typically, the fungus survives and over-winters in the form of mycelium or asexual spores known as conidia. Under adverse stressed conditions such as lack of adequated nutrients or water, the fungal mycelium will transform to resistant structures called sclerotia for surviving the extreme harsh environment. The fungus overwinters either as spores or as sclerotia or as mycelium in debris. Under favorable conditions sclerotia germinate directly to produce new colonies or produces conidiophores with conidia (Bennett et al. 1986; Chang et al. 2002; Cotty 1988).

11.2.2 Economic Significance of Aflatoxin Contamination of Grain

A. flavus is a weak and opportunistic plant pathogen, affecting many agricultural crops such as maize (corn), cotton, groundnuts (peanuts), and tree nuts. It can contaminate these crops with aflatoxins in the field before harvest. Because it is a saprophyte, tt also causes the spoilage of post harvest grains during storage. *A. flavus* also lacks host specificity (St Leger et al. 2000) and can attack seeds of both monocots and dicots, and seeds produced both above (corn) and below the ground (peanuts).

The aflatoxins were first identified by a severe animal poisoning incident in 1960, when more than 100 thousand turkey chicks were dead after being fed with *A. flavus* infested peanut meal (Allcroft et al. 1961; Lancaster et al. 1961). There are two groups of aflatoxins including the B group that was named because they fluorescence blue under ultraviolet light and the G group because they fluorescence green. *A. flavus* produces aflatoxin B_1 and B_2, while *A. parasiticus* produces aflatoxins B_1, B_2, G_1, and G_2. Aflatoxin M_1 was named because it was initially identified from bovine milk. Aflatoxin M_1 is a bioconverted hydroxylation product from aflatoxin B_1 within the ruminal system after being fed with toxin contaminated feedstuffs (van Egmond 1989). In addition to aflatoxins B_1 and B_2, *A. flavus* also produces many other mycotoxins such as cyclopiazonic acid, kojic acid, beta-nitropropionic acid, aspertoxin, aflatrem and aspergillic acid (Goto et al. 1996).

Because of the toxic and carcinogenic properties of aflatoxins, the risk and tolerance of contamination in food and feed on human health and livestock productivity are rigorous (Fung and Clark 2004). The International Agency for Research on Cancer (IARC) has designated aflatoxin as a human liver carcinogen (van Egmond 1989; van Egmond and Jonker 2005; van Egmond et al. 2007). In order to minimize potential exposure to aflatoxins, maximum amount of aflatoxins allowed in many commodities have been set (van Egmond 1989; van Egmond and Jonker 2005; van Egmond et al. 2007). Over 100 countries impose specific regulations limiting total aflatoxins in foodstuffs and feedstuffs (van Egmond and Jonker 2005). The U.S. Food and Drug Administration (FDA) has set specific regulatory guidelines to prevent the sale of aflatoxin contaminated commodities. The FDA regulation sets limits at no higher than 20 ppb total aflatoxins for interstate commerce for food and feedstuff and 0.5 ppb aflatoxin M_1 in milk for human consumption. The European Commission has set the limits on groundnuts subject to further processing at 15 ppb for total aflatoxins and 8 ppb for aflatoxin B_1, and for nuts and dried fruits subject to further processing at 10 ppb for total aflatoxins and 5 ppb for aflatoxin B_1. The aflatoxin levels for cereals, dried fruits, and nuts intended for direct human consumption are even more stringent, and the limit for total aflatoxins is 4 and 2 ppb for aflatoxin B_1 (van Egmond and Jonker 2005). It is not only a serious food safety concern, but it has significant economic implications for the agricultural industry worldwide because of restrictions limiting the trade of contaminated crops.

11.2.3 *Aflatoxin Biosynthesis*

Since initial identification in 1960, Research on aflatoxin occurrence, toxicity, and biosynthesis strategies have been extensive (Bennett 1970; Bennett and Goldblatt 1973; Bennett et al. 1976; Papa 1976, 1979, 1984). The discovery of a color mutant that accumulates brick-red pigment, norsolorinic acid (NOR) in *A. parasiticus* (Bennett 1979; Bennett et al. 1976, 1983, 1971) presaged the discovery of the aflatoxin biosynthetic pathway (Bennett et al. 1997). It provided the opportunity to isolate the first aflatoxin pathway gene that encoded a reductase for the conversion from NOR to eventually aflatoxins (Chang et al. 1992). After cloning the important aflatoxin pathway genes, a 75 kb aflatoxin pathway gene cluster was established in *A. parasiticus* and *A. flavus* (Yu et al. 1995). Since then the progress in elucidating this pathway, pathway intermediates, pathway genes, corresponding enzymes, and regulatory mechanisms have progressed steadily (Bennett and Klich 2003; Bennett and Lee 1979; Bennett and Papa 1988; Bhatnagar et al. 1992; Chang 2004; Chang et al. 1993, 1995, 1999; Cleveland and Bhatnagar 1987; Crawford et al. 2008a; Ehrlich 2009; Ehrlich et al. 1999; Ehrlich and Yu 2009; Keller et al. 1993; Yu et al. 2004a). With rapid gene cloning and enzyme characterization, as many as 15 structurally-defined aflatoxin pathway intermediates have been identified (Bhatnagar and Cleveland 1991; Bhatnagar et al. 1989, 1992; Cleveland and Bhatnagar 1987, 1990, Cleveland and Bhatnagar 1991; Crawford et al. 2008b; Keller et al. 2000; Minto and Townsend 1997; Townsend 1997; Trail et al. 1995a; Yu et al. 1995, 1997, 1998, 2004d). At least 27 enzymatic steps have been characterized or proposed in the bioconversion of aflatoxin intermediates to aflatoxins (Ehrlich 2009). In *A. flavus* and *A. parasiticus* the75-kb aflatoxin pathway gene cluster is located on chromosome III near a telomere (Townsend 1997; Trail et al. 1995a, b; Wilson 1989; Yu et al. 1995, 2004b, c). A revision of the gene nomenclature for this pathway in *Aspergillus* has been proposed (Yu et al. 2004b, c).

For the rapid discovery of genes, enzymes, pathways, pathogenicity factors, mechanisms of gene regulation under stressed conditions and crop-fungus interaction, the genomics of *A. flavus* has been pursued vigorously since the sequencing of several *Aspergillus* genomes. These include *A. fumigatus* (Nierman et al. 2005), *Neosartorya ficheri* (anamorph *A. fisheri*), *A. nidulans* (Galagan et al. 2005), *A. oryzae* (Machida et al. 2005), *A. niger* (Baker 2006; Pel et al. 2007), *A. terreus* by the Broad Institute, and *A. clavatus* by the J Craig Venter Institute (JCVI). A large scale of *A. flavus* Expressed Sequence Tags (EST) has been completed by Yu et al. (2004a). The whole genome of *A. flavus* has been sequenced (Payne et al. 2008). With these valuable sequence resources, several types of *A. flavus* microarrays have been constructed and used for gene profiling and gene regulation studies (Fedorova et al. 2009; OBrian et al. 2007; Rokas et al. 2007; Wilkinson et al. 2007a, b; Yu et al. 2008).

11.3 Insect Herbivory Stress

After these previous risk factors in crop aflatoxin contamination, Arthropod damage is the primary biotic risk factor, while drought and heat are the primary abiotic risk factors. Ear-feeding insects contribute aflatoxin contamination of corn (Anderson et al. 1975). Fennell et al. (1975, 1977, 1978) had reported an association between insect damage and aflatoxin contamination in stored samples. These investigations were followed by the confirmation of aflatoxin contamination as a preharvest problem associated with insect feeding damage (Widstrom et al. 1975; LaPrade and Manwiller 1977; McMillian et al. 1978; Lillehoj et al. 1978). McMillian et al. (1985a) demonstrated a consistent association between insect damage and field aflatoxin contamination. Several insects have been found to be associated with contamination of corn kernels. Among these are the corn earworm, *Helicoverpa zea* (McMillian et al. 1978, 1990), the corn weevil, *Sitophilus zeamais* (McMillian et al. 1980; Barry et al. 1985), and the European corn borer, *Ostrinia nubilalis* (Guthrie et al. 1981; McMillian et al. 1988). A number of insects have been identified as vectors or facilitators of mycotoxigenic fungal infections in grain crops (Dowd 2003). Eighteen species of arthropods that use maize as a host have been identified as contributing to aflatoxin contamination worldwide. A multiple-state study using maize weevil [*Sitophilus zeamats* (Motschulsky)] and wheat curl mite [*Eriophyes tultpae* (Kifer)] as vectors for *A. flavus* infection in maize kernels demonstrated that maize weevil could cause high level of contamination in trials conducted in Missouri where *A. flavus* infection is not common, whereas wheat curl mite treated with *A. flavus* spores did cause the increase in aflatoxin contamination, but not as high as maize weevil did in Missouri (Barry et al. 1985). A number of studies have shown a positive association between ear-feeding insects and aflatoxin contaminations in the kernels (Widstrom et al. 2003a). At the same time, the infestation of maize weevil dusted with *A. flavus* spores did not cause the increase of aflatoxin level compared to untreated weevils in either Georgia or Tennessee (Barry et al. 1985) where aflatoxin contamination is a constant problem. Although no difference in aflatoxin levels was detected between *A. flavus*-treated and control weevil treatments, aflatoxin levels in both weevil infestation treatments were significantly greater than the non-vector control in Georgia, but not in Tennessee (Barry et al. 1985). This clearly suggested the maize weevil is a contributing factor for aflatoxin contamination in Georgia where heat and drought conditions prevail through the growing season. Maize weevil damage and aflatoxin accumulation has also been examined at pre-harvest and post-harvest respectively, and Dix and All (1987) reported that maize weevil infestation significantly increased *Aspergillus flavus* growth in stored corn. Ni et al. (2011) reported that, by grid-sampling of a pre-harvest corn field in 2005, aflatoxin levels were positively correlated to the number of weevils, but not correlated to the percentage of stink bug-damaged kernels. In contrast, the grid-sampling data of 2006 showed that aflatoxin levels were positively correlated to both maize weevil numbers, and stink bug-damaged kernels.

Ni et al. (2011) conducted a multiple-year study utilizing high resolution grid-sampling technique throughout a field to assess the spatial patterns of maize weevil infestations and other ear insect damage in relation to aflatoxin contamination. The data from 2006 and 2007 showed that the patterns of maize weevil infestation, percentage of stink bug-discolored kernels, and aflatoxin levels in the sampled corn grains were distributed in clusters throughout a field with a strong edge effect at pre-harvest. The results from the present study confirmed that the previous reports by Barry et al. (1985, 1986) that maize weevil played an important role in aflatoxin contaminations in corn production. The findings also supported the results from the previous report (Scully et al. 2009) that a strong edge effect and clustered distribution was observed in 2005. The separation of tissue-specific insect feeding (i.e., silk and cob feeding versus kernel feeding) in examination of their contributions to aflatoxin contamination indicated that the kernel-feeding insect damage might be critical although all insect damage might influence aflatoxin accumulations. Maize weevil population and stink bug damage were positively correlated to aflatoxin levels in both years, while silk- and cob-feeding damage by the corn earworm and the fall armyworm was positively correlated to aflatoxin level only in 2007. The findings suggested kernel feeding damage at pre-harvest might be more important than the silk- and cob-feeding damage by the corn earworm and fall armyworm post flowering time. The findings provided more details in addition to Widstrom et al. (1975, 1976) reported that the ear-feeding lepidopteran insects are one of the important factors for aflatoxin contamination in corn fields at pre-harvest under warm climatic conditions.

The Ni et al. (2011) report also indicated that, in addition to insect damage, the variation between 2006 and 2007, as well as among the fields in 2007 confirmed the illusive nature of the aflatoxin problem in crop production under warm climate conditions. The variation in aflatoxin levels could be the results of other ecological factors, e.g., temperature, rainfall, crop management, and other disease infections of the corn ears at post-pollination, in addition to the effect of biotic stress factors (e.g., various insect feeding damage, and disease infections).

11.4 Environmental Factors Cause Crop Stress

The major abiotic environmental components were identified in the 70s and 80s. In addition to their identification, each of the environmental components has been critically assessed to determine which have the largest influence on aflatoxin contamination. The regions of the world with the worst aflatoxin contamination provided the ideal sites to identify the major factors. Identification of specific abiotic causes of stress is often achieved with great difficulty due to the confounding interactions that occur among these factors, but ultimately temperature, water stress, humidity and soil type were identified as critical in the management of aflatoxin contamination in crops.

11.4.1 Temperature and Drought

The optimal temperature for production of aflatoxin is approximately 30°C (Sorenson et al. 1967) while that for growth of corn is about 27°C (Aldrich et al. 1975; Shaw 1977), and lower when the plant is subjected to drought conditions (Zuber and Lillehoj 1987). Average daily temperatures during grain fill reach or exceed this value in several southern states (Zuber and Lillehoj 1987). Therefore, during years with even short periods of temperatures above 27°C, the fungus will increases its aflatoxin production activity while the plant reduces its capacity for growth and grain filling, and thus is less able to defend against fungal infection. Drought is usually associated with higher than normal temperatures. Such weather is usually accompanied by insect injury and fungus infection of the corn ear (Taubenhaus 1920). *A. flavus* is an uniquely thermo—tolerant organism and is ideally adapted to this environment (drought and heat).

The establishment of temperature as an important component of infection by *A. flavus* and subsequent aflatoxin contamination has been clearly demonstrated under controlled greenhouse conditions (Payne et al. 1988; Thompson et al. 1980). The concept was corroborated by several field studies in which temperatures were monitored (Jones et al. 1980; Zuber et al. 1983). Some efforts to illustrate a relationship between temperature and aflatoxin contamination were, however, unsuccessful (Stoloff and Lillehoj 1981). The reason for this phenomenon can be traced to a detectable relationship that exists only during years when aflatoxin contamination is high. McMillian et al. (1985b) conducted a six year study in which the 3 years with the highest contamination also had the highest average daily temperatures during the growing season. Similarly, in a five year study, a significant positive correlation between aflatoxin contamination and temperatures was obtained only during the two years with exceptionally high concentrations of aflatoxin (Widstrom et al. 1990). These results clearly indicated that high temperatures do significantly contribute to the fungal infection process and the ultimate amount of aflatoxin produced.

The interrelationship of weather factors such as temperature and precipitation cannot be ignored, but each has its own unique contribution to the aflatoxin contamination problem. It has been suggested that differences in precipitation amounts from region to region contribute to contamination (Lillehoj et al. 1978). If so, this has especially important implications for the producer who grows dryland corn while the effect can, to a large extent, be ignored if irrigation is available. Seasons with very low rainfall produce stress conditions for dryland corn and the high temperatures which usually accompany low rainfall are related to the severity of aflatoxin contamination. When late-season rainfall prevents timely harvest of the corn crop, the grain obtained from the delayed harvest can be expected to have increased aflatoxin (Jones and Duncan 1981).

Water stress or drought is also the most important environmental-limiting factor regulating corn growth and yield. The genetic improvement of water-deficit tolerance in corn is an important part of the solution to stabilizing corn yield and reducing aflatoxin contamination. Limited water availability induces various physiological and biochemical changes resulting in reduced growth of aerial parts and, to a lesser extent, of the root system. The phytohormone abscisic acid (ABA) regulates many important physiological and developmental processes in corn and functions in adaptive responses to imposed water stress as well as other forms of environmental stress. ABA also serves as a stress signal. Another phytohormone, indole-3-acetic acid (IAA), functions in maize responses to imposed water stress. IAA has been shown to be involved in immature kernel development, as well as in numerous drought-related physiological processes including regulation of stomata aperture and regulation of root growth.

11.4.2 Relative Humidity and Net Evaporation

Relative humidity and net evaporation are also intricately interrelated to temperature, heat, drought and moisture, and result from the interaction between water and temperature. Lillehoj (1983) discussed these inter-relationships in terms of water activity and noted that water activity of 90% relative humidity and above are ideal for *A. flavus* growth and aflatoxin synthesis while those less than 85% severely reduce aflatoxin production. Significant amounts of aflatoxin are not generated in inoculated samples of corn when incubated for seven days at relative humidities less than 91% (Guo et al. 1996). The determination and application of environmental limits for fungus growth and elaboration of aflatoxin in laboratory experiments can be misleading if the information is extrapolated directly to the field, but these experiments serve to better isolate the impact of individual factor rather than the highly variable field conditions (Lillehoj 1983). Sisson (1987) monitored field conditions in several corn growing states, and determined that high humidity and high temperatures are both conducive to high concentrations of aflatoxin contamination. The common occurrence of heavy dews in the southern U.S., simulated by ear wetting at least three times each week during grain fill, can also significantly increase aflatoxin concentrations in mature ears (McMillian et al. 1985a). Field measurements of mean temperature and net evaporation are significantly correlated with aflatoxin concentrations of grain samples taken at harvest. These measurements are both judged to be more important than relative humidity or total precipitation in determining contamination (McMillian et al. 1985b).

11.4.3 Soil Type

Soil types exert great influence on aflatoxin contamination of crops. Preharvest samples obtained from corn grown on sandy Coastal Plain soils had higher aflatoxin contamination than those sampled from the crop grown on heavier clay soils (Jones et al. 1981). The difference was attributed to additional plant stresses incurred from reduced water availability from the lighter soils. It has been demonstrated that both soil type and cultivation practices influence spore load and crop contamination by aflatoxin (Angle 1987). The sandy soils of the southeastern U.S. have less than one-half the water holding capacity of most soils in the corn-belt, increasing the probability of drought stress during the growing season (Widstrom 1992). Though conservation tillage reduces the loss of water from soils, conventional wisdom suggests that such practices increase the *A. flavus* spore load available for infection of the crop following the rotation (Angle 1987). Crop history also affects the amount and kinds of plant refuse in the soil profile and on the soil surface does influence the micro-environment for fungal development (Martyniuk and Wagner 1978).

11.5 Crop Management Factors

Crop management has not routinely been considered to be an efficient approach to control preharvest aflatoxin contamination, although the influence of crop environment has been known since aflatoxin was first recognized as a preharvest problem (Anderson et al. 1975). Crop management practices can alter the environmental effects and their influence on preharvest aflatoxin contamination (Lillehoj 1983; Jones 1987). The use of these practices to modify or manipulate toxin formation, however, has not been the primary consideration for control of preharvest aflatoxin contamination. More recently, the use of crop management as a key to preventing or limiting aflatoxin contamination of the preharvest crop has been recommended (Widstrom et al. 1984a, b, c). The relative impact on aflatoxin contamination and ease of manipulation of each management component require additional assessment before an effective plan for control can be initiated. Additionally, the economics of the application of control measures will greatly influence their integration into an overall management system.

11.5.1 Planting Date

The choice of a planting date to avoid plant stress during the critical grain filling period was first suggested by Zuber and Lillehoj (1979). Research supporting this concept were reported shortly thereafter (Jones and Duncan 1981; Jones et al. 1981; Lillehoj et al. 1980). The data were conflicting, however, Lillehoj et al. (1980) reported that an early planting had the highest amount of aflatoxin while Jones et al. (1981) suggested that early plantings had reduced amounts of aflatoxin in North Carolina. Comprehensive research was conducted by Widstrom et al. (1990) on planting dates with data accumulated over a 5-year period. They demonstrated a reduction in aflatoxin contamination for plantings as late as June or July on the coastal plain soils at Tifton, Georgia. Normal planting months are March and April. Unfortunately late plantings are also associated with increasing reductions in yield, but this research confirmed the importance of temperature and net evaporation during the critical grain fill period (McMillian et al. 1985a, b). Significant correlations between aflatoxin contamination and both temperature and net evaporation (Widstrom et al. 1990) were quite similar to those reported earlier (McMillian et al. 1985a, b) and discussed previously. In most years, corn planted in early April in North Carolina was less drought-stressed and as planting dates were delayed past middle of April, yield reductions were observed. Late planting shifted the ear development phase which was accompanied by increased temperatures during ear development (Jones and Duncan 1981; Jones et al. 1981).

11.5.2 Irrigation

Many environmental components are responsible for imposing stress on the crop plant during development and maturation. The most common, and possibly the most significant of these, is drought (Zuber and Lillehoj 1979). The obvious remedy is irrigation, especially in areas where rainfall is limited and/or soils are sandy, providing little water holding capacity (Lillehoj 1983). Irrigation, however, does not always prevent aflatoxin contamination of the corn crop (Fortnum and Manwiller 1985). The amount of contamination is normally reduced to some extent when irrigation is applied to alleviate drought conditions (Jones et al. 1981). Irrigation effects have been described as being similar to adequate rainfall in reducing the incidence and amount of aflatoxin in the grain (Jones 1987). Irrigation not only alleviates the moisture stress in the plant, but also changes the overall environment in the field, creating cooler temperature in the plant canopy. Finally, a net beneficial effect of irrigation has been demonstrated by most research on the subject, as indicated in several published research reports (Payne et al. 1986; Smith and Riley 1992; McMillian et al. 1991). A major concern in many areas, where corn is grown and aflatoxin is a problem, is that irrigation is not available. More than one-half of the corn acreage in the southeastern U.S. is corn grown under dry-land conditions (McMillian et al. 1991), but irrigated acreage is increasing.

11.5.3 Tillage and Fertilization

The recent trends toward conservation tillage and organically grown crops may have some impact on the risk for aflatoxin contamination of corn. However, deep tillage or subsoiling is still regularly practiced on sandy soils of the Coastal Plain in the southeastern U. S. as a means of breaking subsurface hardpan layers that develop (Griffin et al. 1981). This practice probably has its greatest effect in reducing drought stress by promoting good root development and penetration. Burying crop residue has obvious advantages such as covering inoculum sources for disease in high-risk mono-culture rotations (Cole et al. 1982). Individual tillage effects on aflatoxin contamination have not been verifiable, possibly because of interaction confounding with other more significant factors, such as moisture availability.

The first definitive research reporting preharvest aflatoxin contamination of corn also reported that stressed growing conditions, such as low nitrogen level, appeared to increase the incidence of aflatoxin contamination (Anderson et al. 1975). Conflicting results were reported for 1976 and 1977 when interpreting the influence of nitrogen fertilization on the amount of field contamination (Jones 1987; Zuber and Lillehoj 1979). Adequate fertilization has been suggested as a cultural practice that will alleviate plant nutrient stress and reduce aflatoxin contamination (Zuber and Lillehoj 1979; Widstrom et al. 1984a, b, c). Similar recommendations advocating sufficient nitrogen fertilization to minimize aflatoxin contamination have been proposed by several other researchers (Lillehoj 1983; Jones 1987; Glover and Krenzer 1980; Widstrom et al. 1984a, b, c; McMillian et al. 1991). Research specifically addressing the effects of nitrogen has been the basis for most of these recommendations (Jones and Duncan 1981; Payne et al. 1989), however, a recent study in Mexico failed to show any effect on aflatoxin contamination due to fertilization (Bucio-Villalobos et al. 2001). Excessive application of nitrogen can increase plant stress and aflatoxin concentration. This precaution probably only applies to those attempting to maximize, rather than optimize their yields.

11.5.4 Weed Control

Weed infestation of corn fields was also a predisposing factor studied by Anderson et al. (1975) to increase weed population contribution to plant stress. The consideration of weeds as potential contributors to field contamination (Jones 1987) is due to management practice that often requires additional tillage and always involves a crop management decision that will alleviate plant stress through a reduction in competition for water and nutrients (Zuber and Lillehoj 1979). These competition effects have been directly linked to amounts of aflatoxin found in kernels (Cobb 1979). The influence of herbicides on aflatoxin contamination, and the interaction of weed populations, has not been investigated extensively. Research to measure aflatoxin concentration in corn grown under three cultivation rates for weed control was inconclusive (Bilgrami et al. 1992). Since most producers practice effective weed control by chemical or other means, it has not been demonstrated to be a critical consideration in an aflatoxin management program (Widstrom 1996).

11.6 Genetic Control Strategies

11.6.1 Genetic Improvement in Resistance

Anderson et al. (1975) were the first to report that contamination of corn grain by aflatoxin was a preharvest problem. Exploratory genetic research for host-plant resistance to aflatoxin contamination in corn was established shortly after it was identified as a preharvest problem. Phenotypic differences among germplasm were often very inconsistent (Zuber 1977), raising questions as to whether heritable differences existed for aflatoxin accumulation in corn. In the early years, a series of review papers on genetic control of field contamination suggests various approaches for development of hybrids with (1) resistance to insects, (2) resistance to plant stress (adapted hybrids), and (3) resistance based on a relationship to other plant traits (Zuber and Lillehoj 1987; Widstrom et al. 1984a, b, c; Widstrom and Zuber 1983; Widstrom 1987). Breeders screened germplasm in a search for resistance among commercial hybrids at the time (Widstrom et al. 1978; Widstrom 1987; LaPrade and Manwiller 1977), experimental hybrids (LaPrade and Manwiller 1976; Widstrom et al. 1978; King and Scott 1982; Zuber et al. 1978) and varieties (Priyadarshini and Tulpule 1978). Results from early screening were inconclusive and significant differences were not always found among germplasm entries. Zuber (1977) proposed a genetic solution to the aflatoxin problem, which led to the establishment of protocols for identification of resistant genotypes. While initial screening was being conducted, other research was initiated to determine and refine methods for field inoculation and evaluation (King and Scott 1982; Widstrom et al. 1981, 1986; Tucker et al. 1986; Campbell and White 1994). The ensuing research provided convincing evidence of the potential for genetic control of resistance to aflatoxin contamination and a genetic solution to the contamination problem (Naidoo et al. 2002; Zuber et al. 1983; Thompson et al. 1984; Widstrom et al. 1984a, b, c, 1987; Scott and Zummo 1988; Gorman et al. 1992).

The first genetic parameter estimates were made among single-crosses, within which, some inbred lines gave large estimates of general combining ability (GCA), and the crosses provided evidence for resistance being recessively inherited (Zuber and Lillehoj 1979). Some of the earliest evolutions were conducted among adapted southern open-pollinated varieties that were grown widely prior to the transition to hybrid corn production (Zuber et al. 1983). The test failed to reveal exceptional resistance in any of the open-pollinated varieties when compared to popular hybrids being grown in the 1980s. General combining ability effects were determined to be responsible for primary control of aflatoxin contamination among southern dent and sweet corn inbreds when tested as single crosses (Widstrom et al. 1984a, b, c). Controlled environment experiments by Thompson et al. (1984) revealed the importance of replication in detecting differences among genotypes. Gardner et al. (1987), using of the same germplasm tested by Zuber et al. (1978), concluded that experiments with eight replications provided a good compromise between controlling the variance estimates in an experiment and the cost for aflatoxin analyses. The germplasm evaluated by Zuber et al. (1978) and Gardner et al. (1987) was again evaluated in a five-state experiment (Darrah 1987). The tests effectively accentuated the difficulties in repeating results in different environments and under varied inoculation techniques. Evaluations among genetically diverse varieties also illustrated difficulties in identifying germplasm with the most resistance in any given test (Kang et al. 1990).

The most accurate time to sample from field tests was determined to be at physiological maturity, since susceptible genotypes tend to accumulate aflatoxin at a higher rate than resistant genotypes (Widstrom et al. 1986). Widstrom et al. (1987) also demonstrated that two separate populations, generated from kernels collected off the same open-pollinated ear, were different in their ability to inhibit elaboration of aflatoxin by *A. flavus*. Kernel infection percentages have been used to identify resistant germplasm (Scott and Zummo 1988, 1990a). When kernel infection percentages are compared to identifications made by other traits, such as total aflatoxin concentrations, the same germplasm sources are usually identified as resistant (Scott et al. 1991), regardless of the inoculation technique. The same problems of interaction with environments and large sampling variances, however, continue to plague all genetic experiments and germplasm evaluation procedures.

Some of the most recent genetic research efforts on *A. flavus* infection and aflatoxin contamination have been focused on ear rot symptoms and aflatoxin production (Naidoo et al. 2002; Campbell and White 1995; Hamblin and White 2000). In general, these studies have produced conflicting results in that resistance is sometimes attributed to additive effects, sometimes to dominance, and sometimes to both. An encouraging common thread through all of these studies seems to be that the same hybrids and/or inbred lines consistently group into resistant and susceptible categories. Eventually, research found that resistance to infection and aflatoxin biosynthesis are at least partially under genetic control, consequently, efforts have been made to reduce grain contamination by aflatoxin through host-plant resistance. Over the years, genetic improvement has resulted in the release of several resistant lines and germplasm (Scott and Zummo 1990b, 1992; McMillian et al. 1993; Williams and Windham 2001; Guo et al. 2007; Menkir et al. 2008; Guo et al. 2011a, b, c) with incremental improvements in resistance to aflatoxin contamination.

11.6.2 Related Plant Resistance Factors

The report of a "lethal silk" factor in corn by Walter (1957), and subsequent studies to investigate these claims (Wann and Hills 1966; Chambliss and Wann 1971; Widstrom et al. 1977), led to the isolation and identification of maysin, a flavone glycoside in corn silks (Waiss et al. 1979) that has biological activity against the corn earworm (Elliger et al. 1980). Snook et al. (1993) found numerous germplasm sources for this compound, some which have been selected for extremely high maysin concentrations and have been publicly released (Widstrom and Snook 2001a; Widstrom et al. 2003a). The inheritance of silk-maysin concentration is known so that transfer to commercial germplasm will not be difficult (Widstrom and Snook 1994, 1998). Molecular studies have located numerous quantitative trait loci (QTL) that influence maysin concentration in corn silks (Byrne et al. 1996, 1997, 1998), several of which are associated with loci found by conventional methods (Guo et al. 1999a, b, 2001a, b, 2004; Widstrom and Snook 2001b). Butron et al (2000) outlined a program of marker-assisted selection to improve resistance to the corn earworm, however, it must be remembered that insect resistance is only one necessary link in solving the problem of aflatoxin contamination in corn. High silk-maysin concentrations will not protect the ear against corn earworm unless husk coverage is sufficient to force the insect to feed on silks when entering the ear (Rector et al. 2002).

Both the length and tightness of husk coverage around the ear appear to be important in aflatoxin contamination. Research supports the concept that complete and tight husk coverage helps protect the ear against invasion by ear-feeding insects and against *A. flavus* infection with or without the presence of insect damage (Lillehoj et al. 1978). The importance of husk traits to prevent ear damage by insects has been further reviewed (Lillehoj and Zuber 1975; Widstrom et al. 1976), and noted that insecticide treatments reduced but did not eliminate aflatoxin contamination. Wiseman et al. (1977) determined that husk coverage beyond the ear tip was not sufficient to provide resistance, but that husk tightness was a necessary condition to prevent ear damage by corn earworm. Similarly, husk coverage and tightness are necessary to protect the ear from invasion and damage by the corn weevil (McMillian et al. 1980; Barry et al. 1985, 1986). Two loose-husked hybrids were contaminated with more than twice as much aflatoxin as two tight-husked hybrids in an inoculation study (Widstrom et al. 1981). Five hybrids, each with a different level of husk tightness, had significantly reduced aflatoxin contamination concentrations as husk tightness increased (Barry et al. 1986). Widstrom et al. (1993) concluded that many corn hybrids depend heavily on husk protection for their resistance against aflatoxin contamination, although none give complete or consistent protection.

11.6.3 Plant Resistance to Aflatoxin Formation

Plant stress was recognized as a factor that enhances aflatoxin contamination in the field prior to the establishment of hybrid differences (Zuber and Lillehoj 1979). Stress is most often associated with periods of extreme drought which, in turn, have been associated with aflatoxin contamination (Lillehoj 1983). Several of the factors discussed previously are responsible for imposing stress on the plant (Widstrom and Zuber 1983), complicating the interpretation of research data (Widstrom et al. 1984a, b, c, Widstrom 1987). Plant stress is commonly related to adaptation, and hybrids with the ability to buffer and regulate local stresses are recommended to growers (Zuber and Lillehoj 1987). In addition to resistance to insect and aflatoxin contamination, efforts are now being made to identify drought stress resistant germplasm and incorporate that trait into agronomically suitable germplasms (Li et al. 2000; Fountain et al. 2010; Luo et al. 2005, 2008, 2010; Payton et al. 2009; Guo et al. 2011b).

Differences among germplasm sources for aflatoxin accumulation were often inconsistent (Zuber 1977; Widstrom et al. 1984a, b, c) leading to speculation about the existence of genetic differences. In 1980, two distinctly different kernel phenotypes were selected from the same open-pollinated ear on a hybrid plant and used to generate two different breeding populations (Widstrom et al. 1987). These populations were tested extensively in the field and laboratory, and found to differ in their resistance to aflatoxin production. The differential reaction to aflatoxin contamination was consistent when tested in experimental crosses to several southern inbred lines (McMillian et al. 1991). The resistant population that resulted from this research was GT-MAS:gk and released in 1992 (McMillian et al. 1993). As a population, GT-MAS:gk contained genetic variation for many agronomic traits as well as resistance to *A. flavus* (Guo et al. 2001a, b, 2002), and further inbred selections have been made (Guo et al. 2007, Guo et al. 2011a, b, c).

In order to utilize the resistance source of the GT-MAS:gk population associated with resistance, such as pericarp wax (Guo et al. 1995, 1996; Russin et al. 1997) and antifungal proteins (Guo et al. 1997, 1998; Chen et al. 1998, 2009), Guo et al. (2007, 2011a, b, c) released several inbred lines from this population with improved resistance and earliness. Resistance to aflatoxin accumulation has been attributed to the wax and cutin layers on the kernel pericarp (Guo et al. 1995), and this finding was confirmed by Russin et al. (1997). Guo et al. (1996) concluded that an aflatoxin inhibitor was induced during germination of the seed, and later determined that a zeamatin-like kernel protein and at least one ribosome inactivating protein (RIP), present in the kernel, were capable of inhibiting growth of *A. flavus* (Guo et al. 1997). Studies of the protein profiles of kernels revealed that several proteins were found in resistant types in greater concentrations than in susceptible, and that others were present only in susceptible types (Guo et al. 1998). Additional research determined that RIP is primarily in the aleurone layer of the endosperm while zeamatin occurs mainly in the embryo (Guo et al. 1999a, b). Both proteins uniquely protect kernels from pathogens and may provide important aspects of resistance to *A. flavus* and aflatoxin contamination in corn.

11.7 Further Researches

Although significant progress has been made since 1977 aflatoxin epidemic in the US, aflatoxin contamination in corn and other crops grown in hotter region has long been considered one of the recalcitrant agricultural problems. Research has progressed in all aspects of the research related to aflatoxin biosynthesis, stress factors related to prevention and reduction of contamination, and host plant resistance to aflatoxin accumulation; breeding efforts have made significant progresses also. However, understanding of ecological interactions of abiotic and biotic factors is receiving an under researched area (Ni et al. 2011). This spatial correlation study was an initial effort to document the impact that both chewing and piercing/sucking insects have on both ear and kernel feeding and aflatoxin contamination (Ni et al. 2011).

Several agronomic practices can reduce preharvest aflatoxin contamination, including use of pesticides and altered cultural practices and irrigation. However, such procedures have thus far demonstrated only a limited potential for reducing aflatoxin levels in the field, especially in years of drought when environmental conditions favor aflatoxin contamination. The 1977 outbreaks in the US reflected several adverse field production factors, such as a drought stress, severe insect infestations, and high temperatures that favored *A. flavus* growth and aflatoxin production and resulted in a devastating crop loss.

In the arena of plant pathology, host plant resistance is the most effective, efficient and dependable tool to protect crops from the preharvest infection and aflatoxin contamination processes. Earlier efforts were focused on identifying the risk factors for increased aflatoxin contamination such as drought, high temperatures, insect damage and other genetic factors such as husk coverage. Later efforts were focused on the development of screening techniques and identifying sources of resistance to *Aspergillus* colonization and/or aflatoxin contamination. All these research activities laid the foundation for conventional breeding programs that have produced breeding lines and cultivars with reduced *Aspergillus* colonization and/or reduced aflatoxin contamination relative to standard control. More recently, research efforts have focused on the use of molecular genetic and genomic approaches to reduce aflatoxin contamination including genetically engineered crops and biological control using atoxingenic strain (Dorner et al. 1999; Cotty et al. 2007).

Host plant resistance to aflatoxin contamination is a complex trait, and dissecting this trait is a complex task. Genetic research links the genotypic variation among the individuals to its respective phenotypic variation. Soon, the next-generation whole genome sequences of thousands of individuals will be the reality. The value of translation of basic research to better understand and control of plant diseases, in the past few years, numerous genome-wide association (GWA) mapping studies have been published such as in maize (Buckler et al. 2009; McMullen et al. 2009) to dissect the complex trait of flowering time. The last is utilization of the resistance traits in wild relatives such as peanut interspecific hybrids (Simpson 1991). The lack of appropriate genome sequence information is ironic, because for years there has been more information about within species polymorphism in plants than in the relatives. The peanut genetics community is therefore excited that the sequencing of the genome of *Arachis hypogaea* and wild progenitors is underway, which hold promise of providing important information about the domestication, evaluation and development of disease resistance cultivars such as nematode resistant "COAN" peanut (Simpson and Starr 2001).

With the availability of these genomic resources, scientists will have a more powerful tool for profiling genes expressed under particular conditions, for studying the mechanisms of aflatoxin formation under different environment, and for studying the crop-fungus interaction under crop stressed and during fungal infection. It is apparent that the main task for the scientists will quickly be shifted from identifying those genes to characterizing their biological functions. With the technological breakthrough in genomics and next-generation sequencing, our understanding on the *Aspergillus* biology would be greatly enhanced, and the control strategies could be targeting the regulations of aflatoxin biosynthesis mechanisms as interacting with other factors as discussed earlier.

Therefore, preharvest aflatoxin contamination of grain is provoked and aggravated by both abiotic and biotic stress factors that influence infection by *A. flavus*. We have knowledge concerning the contamination process to establish a guideline and management practices that will minimize the probability of contamination under certain environment conditions, but long-term solutions are needed if the problem is to be adequately controlled or resolved. The genetic strategies, conventional or molecular, are vital in the long-term effort to develop crop varieties resistant to infection by *A. flavus* and subsequent aflatoxin contamination. For instance, in the southern US or especially southeastern US, high temperature coupled with drought is favorable to *A. flavus* infection and aflatoxin production. Loose-husked hybrids used in the south are free invitation to ear-feeding insects and fungal spores. When grown in the south, these "corn-belt type" hybrids with loose or open husks accentuate insect damage and aflatoxin contamination. The development and breeding "southern-type" hybrids, with good husk coverage and flint-harder kernel character, are important factors for control preharvest aflatoxin contamination. Further ecological research is imperative to define the variation in aflatoxin contamination in various crops as the result of environmentally (i.e., drought, high temperature, and humidity)-mediated interactions among plants, insect vectors, and fungal infection. The development of genetic resistance lines or cultivars needs a multifaceted endeavor that requires an integrated and combined approach across disciplines (Guo et al. 2009; Scully et al. 2009).

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Chapter 12
Role of Ethylene and Plant Growth-Promoting Rhizobacteria in Stressed Crop Plants

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Abstract Ethylene is a gaseous plant growth hormone produced endogenously by almost all plants and plays a key role in inducing multifarious physiological changes in plants at molecular level. Endogenous production of ethylene is accelerated substantially in response to biotic and abiotic stresses which adversely affects the root growth and consequently the growth of the plant as a whole. Certain plant growth promoting rhizobacteria (PGPR) contain a vital enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which regulates ethylene production by metabolizing ACC into α-ketobutyrate and ammonia. Inoculation with such PGPR could be helpful in sustaining plant growth and development under stress conditions by reducing stress-induced ethylene production. In this chapter, all aspects of such PGPR regarding alleviation of "stresses imposed effects" on plants will be discussed.

12.1 Ethylene

Ethylene is a gaseous hormone produced by all plants under both normal and stressed conditions. Though it is very simple in structure having only two carbons but performs a variety of various physiological functions throughout plant ontogeny (Arshad and Frankenberger 2002; Dugardeyn and Van Der Straeten 2008). In the beginning, ethylene was known as a "ripening hormone" or "stress hormone" because of its obvious role in ripening of plant or its accelerated synthesis during stress. Later on, its several roles in critical phases of plant growth and development

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have been documented including regulation of release of dormancy, seed germination, root hair development, tissue differentiation, adventitious root formation, root nodulation, leaf and fruit abscission, flower and leaf senescence and female induction (Bleecker and Kende 2000; Ortega-Martinez et al. 2007; Gallie et al. 2009). Very likely, the regulatory role of ethylene depends on its concentrations that are produced in plant tissues in response to biotic and abiotic factors. Usually, low levels of ethylene are known to stimulate plant growth while its higher levels inhibit normal plant growth, which imparts a visible dent on plant growth and development (Visser and Pierik 2007; Swarup et al. 2007; Shahzad et al. 2010).

In higher plants, the production of ethylene is tightly regulated by internal signals during development and in response to environmental stimuli from biotic (e.g., pathogen attack) and abiotic stresses. It is established that an amino acid, L-methionine (L-MET) serves as a primary substrate for the synthesis of ethylene in plants, which is first converted into S-adenosylmethionine (SAM). In the presence of enzyme ACC-synthase, SAM is further transformed to l-aminocyclopropane-l-carboxylate (ACC) which is considered critical to determine ethylene levels in plant tissues (Yu and Yang 1980; Yoshii and Imaseki 1981). Finally, ACC is oxidized into ethylene by ACC-oxidase (Chaves and Mello-Farias 2006). The magnitude of ethylene production by a plant tissue is regulated mainly by the availability of the substrate ACC. Both positive and negative feedback regulation of ethylene biosynthesis have been reported in different plant species (Nakatsuka et al. 1997; Barry et al. 2000).

Numerous soil microorganisms have been reported capable of producing ethylene, particularly, from methionine (Arshad and Frankenberger 2002). However, very interestingly, none of the microorganisms has yet been reported capable of deriving ethylene in-vitro from MET via its conversion into ACC. Thus, ethylene is synthesized in microorganisms by a pathway that does not include ACC (Arshad and Frankenberger 1998, 2002; Jia et al. 1999).

12.1.1 Accelerated Production of Ethylene in Plants Exposed to Biotic and Abiotic Stresses

As mentioned earlier, ethylene is also regarded as a “stress hormone” because its production is strongly stimulated when plants are exposed to any kind of stress such as mechanical injuries, salinity, drought, extreme temperatures, and inorganic and organic contaminants etc. (Hussain et al. 1999; Belimov et al. 2001a, b, 2009; Watanabe et al. 2001; Reed and Glick 2005; Roussos et al. 2005). Many steps in ethylene biosynthesis could be altered by environmental stresses. Hogsett et al. (1981) reported that stress-induced ethylene production results from de novo synthesis via the methionine pathway and not just from facilitated diffusion of ethylene already present in the plant tissues (Salveit and Dilley 1978). At molecular level, it has been observed that the induction of ACC-synthase (an ethylene biosynthesis enzyme) in response to stress signals is mainly responsible for increased ethylene levels in plants (Olsen et al. 1995). Since ACC is an immediate precursor of ethylene, its high accumulation in plant tissues results in a burst of ethylene in the early stages of plant response to stress (Kukreja et al. 2005).

It is very likely that stress-induced ethylene could promote stress senescence response in plant that may lead to physiological changes in those cells that are at or near the site of the stress. It has been postulated that many of the plant responses to stress (es) are the result of unusual high levels of stress-induced ethylene (Arshad and Frankenberger 2002). This aspect will be the main focus of this chapter.

12.2 Inhibitors of Ethylene Biosynthesis

By using chemical inhibitors of ethylene biosynthesis, the regulatory role of ethylene in plants has been elucidated (Indiraganghi et al. 2008). Amino-ethoxyvinylglycine (AVG), aminooxyacetic acid (AOA) and 1-methylcyclopropene (1-MCP) are well-known inhibitors of ethylene biosynthesis in higher plants (Abeles et al. 1992; Sisler and Serek 1997; Guinel and Sloetjes 2000; Tirichine et al. 2006; Ding and Oldroyd 2009). It is highly likely that by using these inhibitors, the stress-induced ethylene levels could be monitored to some extent and negative impact of stress ethylene could be suppressed. Although, these chemicals have been proven effective to reduce ethylene concentration in plants and enhance plant growth (Peters and Crist-Estes 1989; Tirichine et al. 2006), but in most cases, they are expensive and potentially harmful to the environment.

On the other hand, advances in biotechnology have led scientists to exploit biological means to lower ethylene biosynthesis. During the last decade, use of unique plant growth promoting rhizobacteria (PGPR) carrying gene for the enzyme ACC deaminase has been found more economical, environmental friendly and feasible mean to regulate ethylene synthesis in plant systems as compared to chemical inhibitors. This trait (ACC deaminase) is common in a number of PGPR species, which are native to the rhizosphere and consequently possess a vast array of survival potential in the rhizosphere and rhizoplane. The presence of other traits like synthesis of auxins, gibberellins, cytokinins and/or polyamines in such species would be the additional benefit for improving plant growth (Frankenberger and Arshad 1995; Patten and Glick 2002; Tabor and Tabor 1985; Zahir et al. 2004).

Interestingly, some species of Bradyrhizobium have shown their ability to produce a chemical, rhizobitoxine [an enol-ether amino acid [2-amino-4-(2-amino-3-hydroxypropoxy)-trans-3-butenoic acid], which inhibits ACC synthase (Sugawara et al. 2006; Tirichine et al. 2008). This trait plus ACC-deaminase ability could be exploited very well for enhancing nodulation in legumes.

12.2.1 Bacterial ACC-Deaminase as an Inhibitor of Ethylene Biosynthesis

Several plant growth promoting bacteria are known to carry a unique enzyme, ACC-deaminase which regulates endogenous ethylene levels in plant roots (Farajzadeh et al. 2010; Nadeem et al. 2010). This enzyme inhibits ethylene biosynthesis by converting its immediate precursor ACC, partially or completely, into other products instead of ethylene. Under stress conditions, ACC levels often mount up in the plant tissues and then are excreted into the rhizosphere (Glick et al. 2007). Through root exudation phenomenon, a significant portion of the ACC from plant tissues is released into the rhizosphere where it is hydrolyzed by bacterial ACC deaminase to ammonia and α-ketobutyric acid resulting in less ethylene biosynthesis in roots (Glick et al. 1994; Shaharoona et al. 2007).

It is well established that higher concentration of ethylene suppresses plant growth and any reduction in ethylene levels in plant tissues as a result of bacterial ACC-deaminase activity could promote plant growth (Andrea et al. 2007; Yang et al. 2009). Lowering of the levels of ACC within the plant leads to the reduction in endogenous ethylene which results in elimination of inhibitory effects of high ethylene concentrations on root growth. In a study, when ACC-deaminase gene together with its regulatory region was transferred into Pseudomonas fluorescens CHA0 (a root colonizing bacteria deficient in ACC-deaminase activity), it increased root length of canola plants under gnotobiotic conditions, whereas strains without this activity had no effect (Wang et al. 2000). For the analytical confirmation of the theory that inoculation of plants with ACC-deaminase bacteria decrease the quantity of ACC in plant roots, Penrose and Glick (2000) used Waters AccQ Tag Amino Acid Analysis methodTM for quantification of ACC. Canola seedlings, grown under gnotobiotic conditions for root/shoot elongation assay, were used for the ACC measurement in seedlings. The results of root/shoot elongation assay indicated that the roots from seeds treated with Enterobacter cloacae CAL 3 or Pseudomonas putida were significantly longer than those grown from seeds treated with $MgSO_4$ or the Pseudomonas strain that lacked ACC-deaminase gene. Whereas, ACC contents in root and shoot of PGPR treated seeds were significantly lower than control. Roots from canola seeds treated with the ethylene inhibitor, AVG, also showed a reduced level of ACC although the reduction was relatively less than that observed in the roots from the seeds treated with ACC deaminase-containing bacteria. Likewise, the plants inoculated with bacteria containing ACC-deaminase have also been found resistant to the harmful effects of stress ethylene, generated under undesirable environments (Bonfante and Anca 2009). This may imply that bacterial ACC-deaminase is not only promote plant growth under normal conditions but has special application in stress agriculture.

12.2.2 Role of PGPR Containing ACC-Deaminase Under Stress Conditions

Accumulation of ACC as well as ethylene production in roots is stimulated by several folds in response to various kinds of stresses (Yu and Yang 1980; Hyodo et al. 1985; Morgan and Drew 1997; Mayak et al. 2004b; Saleem et al. 2007; Arshad et al. 2008; Belimov et al. 2009). It is very likely that most of the effects of stress(es) are mediated through accelerated synthesis of ethylene (Saleem et al. 2007).

Since the enzyme ACC-deaminase can act to modulate the level of ethylene in a plant, this provoked the scientist to study the effects of inoculation with ACC-deaminase containing rhizobacteria on plant growth under stress conditions. A number of researchers have reported decreasing ethylene production by suppressing the expression of some of the enzymes (e.g., ACC-oxidase and ACC-synthase) involved in ethylene synthesis (Ben-Amor et al. 1999). Since the activity of many of these enzymes affects processes other than ethylene synthesis, there seems some advantages of utilizing the bacterial ACC-deaminase enzyme, which cleaves ACC to ammonia and α-ketobutyric acid, establishing a sink for ACC. That is achieved either through the interaction of ACC deaminase-containing PGPR with plant roots or by the development of transgenic plants by expressing this enzyme. In either case, stress induced ACC is hydrolyzed by the ACC-deaminase resulting in reduced ethylene synthesis which consequently leads to relatively healthy plant growth under stress conditions (Grichko and Glick 2001a). In the following sections, the impact of ACC deaminase PGPR on alleviation of stress imposed effects, partially or completely, will be critically discussed.

12.2.2.1 Salt Stress

Salinity stress boosts ethylene production which in most of the cases serves as stress hormone (O'Donell et al. 1996; Feng and Barker 1993; Alvarez et al. 2003; Nandwal et al. 2007). Recent studies have shown the capability of plants inoculated with PGPR containing ACC deaminase to sustain the salinity menace by demonstrating normal growth pattern (Saleem et al. 2007). Mayak et al. (2004a) reported that PGPR Achromobacter piechaudii significantly increased the fresh and dry weights of tomato seedlings grown in the presence of up to 172 mM NaCl salt because of its ACC-deaminase activity. They found that this PGPR slightly increased the uptake of phosphorous and potassium, which might have contributed, in part to activation of processes involved in the alleviation of the salt effects. Bacterium also increased the water use efficiency (WUE) in saline environment and helped in alleviating suppression of photosynthesis due to salts. Similarly, Saravanakumar and Samiyappan (2006) reported that Pseudomonas fluorescens strain TDK1 possessing ACC deaminase activity enhanced the saline resistance in groundnut plants, which in turn resulted in increased yield when compared with the groundnuts treated with Pseudomonas strains not having ACC deaminase activity. Likewise, Cheng et al. (2007) also confirmed that ACC deaminase bacteria conferred salt tolerance onto plants by lowering the synthesis of salt-induced ethylene and promoted the growth of canola in saline environment. This premise is also supported by Ji et al. (2006) who found that four bacterial isolates, especially Pseudomonas sp. S1, alleviated salt stress substantially and significantly promoted the seedling growth of annual ryegrass under either 5 or 10 g/kg NaCl stress in gnotobiotic growth pouch assay. They also observed highly significant positive correlations between ACC deaminase activity of four bacterial isolates and plant growth parameters (root length and shoot length) under given salt stress conditions. Very recently, Jalili et al. (2009) demonstrated that under salinity stress, the rate of seed germination and seedling growth was significantly enhanced in response to inoculation with Pseudomonas putida or Pseudomonas fluorescens carrying ACC-deaminase activity. We have also unequivocally documented the effectiveness of inoculation with ACC deaminase PGPR in eliminating the negative impacts of salinity on growth and yield of different crops, to some extent, under both axenic and natural conditions (Nadeem et al. 2006a, b, 2010: Kausar et al. 2009).

12.2.2.2 Water Deficit Stress/Drought Stress

Water deficit stress is emerging as one of the most critical stress hampering crop yields world over (Hewitt 1997; Kogan 1997). Plants respond to drought stress at cellular and molecular levels (Ingram and Bartels 1996; Bray 1997). Drought, like salinity, also induces accelerated ethylene production in plant tissues which leads to abnormal growth of a plant (Mattoo and Suttle 1991a, b). Mayak et al. (2004b) observed an interested phenomenon that PGPR Achromobacter piechaudii ARV8 significantly increased the fresh and dry weights of both tomato and pepper seedlings exposed to transient water stress. Following water stress, the bacterium also reduced the production of ethylene by tomato seedlings. During water stress, the bacterium did not influence the reduction in relative water content; however, it significantly improved the recovery of plants when watering was resumed. Inoculation of tomato plants with the bacterium resulted in continued plant growth not only during the water stress but even when watering was resumed. Similarly Dodd et al. (2004) documented the effect of inoculation with Variovorax paradoxus 5C-2 containing the enzyme ACC deaminase on growth of peas at two levels of soil moisture. They reported that the bacterium stimulated root biomass by 20–25%, irrespective of soil moisture regime, and whole plant biomass was also increased up to 25% in plants grown in drying soil. They also compared the effect of inoculation with a chemical inhibitor of ethylene, aminoethoxy vinyl glycine (AVG). Both treatments were compared to control pots irrigated with distilled water. Although both AVG and bacterial treatments promoted root growth at both soil moisture regimes, shoot and whole plant biomass was only increased relative to control plants in case of inoculation with bacteria under dry soil conditions. Plant responses to bacteria were qualitatively similar to treatment with AVG, suggesting that bacterial ACC deaminase was involved in the plant-bacterium interaction and the observed effects of V. paradoxus 5C-2 on pea plants were mediated by ethylene. We have also observed that the inoculation with ACC deaminase bacteria partially eliminated the effects of water stress on growth, water use efficiency and ripening of Pisum sativum L. both in pot and field trials (Arshed et al. 2008; Zahir et al. 2008). Very recently, Belimove et al. (2009) reported that inoculation with V. paradoxus 5C-2, but not with a transposome mutant with massively decreased ACC deaminase activity, improved growth, yield and water-use efficiency of droughted peas. This highly positive role of ACC-deaminase PGPR in promoting growth and yield of inoculated plants under water deficit stress conditions could most likely be attributed to prolific root growth resulted from lowering of stress induce ethylene (Dodd et al. 2004; Arshad et al. 2008).

12.2.2.3 Water Logging/Flooding/Submergence Stress

Water logging, flooding and submergence are also known to increase ACC and ethylene and mobilize ACC within plant tissue (Else et al. 1995; Olson et al. 1995; Ahmed et al. 2006). However, very little work has been conducted on modulation of accelerated ethylene synthesis in plant roots under flooding/waterlogging/submergence conditions. Jackson (1997) reported that the accelerated production of ethylene in the shoots of flooded tomato plants is responsible for the abnormal growth phenotype under flooding conditions. Grichko and Glick (2001a) documented the effect of inoculation with rhizobacteria containing ACC-deaminase on growth of tomato under flooded conditions. They inoculated the tomato seeds either with Enterobacter cloacae UW4, E. cloacae CAL2, Pseudomonas putida ATCC17399/PRKACC or P. putida ATCC17399/PRK415, the first three of these bacterial strains expressing the gene for ACC-deaminase. They flooded 55 days old tomato plants for 9 consecutive days, and reported that tomato plants inoculated with organisms expressing ACC-deaminase showed a substantial tolerance to flooding stress. In a number of field trials, we have recorded substantial improvement in paddy yield of lowland (flooded) rice in response to inoculation with Pseudomonas fluorescens and Pseudomonas putida (Arshad et al. 2009). More work is direly needed in this field to cope with waterlogging stress.

12.2.2.4 Temperature Stress

Plants are considered sensitive to changes in temperature, and respond both to seasonal variations and more so to diurnal changes in the season. Like other stresses, accelerated ethylene production under high and chilling temperatures in plant tissues has widely been reported by various researchers (see Arshad and Frankenberger 2002). Only a few studies have indicated highly positive role of bacterial ACC-deaminase in coping the effects of high and low temperature stress on plant growth. Bensalim et al. (1998) found that a PGPR strain Burkholderia phytoWrmans PsJN rescued potato plants from the effects of Temperature stress. Similarly, Barka et al. (2006) reported that inoculation of grapevine (Vitis vinifera L.) cv. Chardonnay explants with the bacterium having ACC-deaminase activity enhanced plant growth and physiological activity at both ambient (26°C) and low (4°C) temperatures. Inoculation also increased root growth (11.8- and 10.7-fold increases at 26°C and 4°C, respectively) and plantlet biomass (6- and 2.2-fold increases at 26°C and 4°C, respectively). Additionally cold tolerance of plantlet was improved in response to inoculation. Similarly, Cheng et al. (2007) has also reported that a psychrotolerant ACC deaminase bacterium P. Putida UW4 promoted canola plant growth at low temperature under salt stress. These few studies clearly demonstrated the potential of ACC deaminase in rescuing the plants exposed to temperature extremes, however, more work needs to be done to adjust the existing agriculture with the global climate change scenario.

12.2.2.5 Heavy Metal Stress

High metal concentrations in the root zone have also been shown to increase ethylene production in plant roots substantially (Pennasio and Roggero 1992; Arteca and Arteca 2007). A number of studies have documented the ability of ACC deaminase PGPR to rescue the plant from negative impacts of excessive amount of heavy metals (Table 12.1). Studies have clearly demonstrated that the presence of specific PGPR containing ACC deaminase in root zone of plants exposed to high concentration of heavy metals improves the tolerance of plants against heavy metals, suppresses toxic effect of metals on plant roots and rescue the plants against negative effects of heavy metals on overall growth of plants. It is very likely that the most of the plant responses to high concentration of heavy metals might be induced by the accelerated production of ethylene which is suppressed by the presence of ACC deaminase PGPR in the root zone. However, further advancement to confirm this premise could be made by observing the growth of transgenic plants with expression of bacterial ACC deaminase in the presence of heavy metals.

12.2.2.6 Biotic Stress

Infection with Rhizobium in legume or with pathogens poses biotic stress and also stimulates ethylene synthesis which may regulate nodulation in legume or pathogenesis in infected plants. It has been generally observed that nodulating legume roots produce more ethylene than non nodulating legume roots (Ligero et al. 1999). The higher production of ethylene during nodulation is most likely a plant response to the infecting bacteria (Zaat et al. 1989). Moreover several authors have reported that ethylene affects nodulation negatively (Oldroyd and Downie 2008; Musarrat et al. 2009). It has been shown that inoculation with PGPR other than rhizobia has been shown to increase nodulation in legumes either by changing root architecture to facilitate root infection with rhizobia or by suppressing ethylene biosynthesis in legume roots. Several authors have reported that co-inoculation with rhizobacteria containing ACC-deaminase promote nodulation of legumes by lowering ethylene concentrations (Shaharoona et al. 2007; Arshad et al. 2010; Shahzad et al. 2010).

Accelerated ethylene synthesis in response to pathogenic infection has also been documented by many researchers (Robison et al. 2001; Czarny et al. 2006) which is considered as signal for defense response during plant-microbe interactions (Indiragandhi et al. 2008a, b). Interestingly, the timing of increased ethylene emission was paralleled with the development of disease symptoms in crop plants (Chen et al. 2003). Therefore, inoculation with PGPR containing ACC-deaminase could be effective in decreasing the detrimental effects of pathogens on infected plant. Several researchers have supported this premise as summarized in Table 12.2.

Table 12.1 Dilution of heavy metal imposed impacts on plant growth by PGPR containing ACC deaminase

Plant species	PGPR containing ACC-deaminase	Comments	References
Brassica juncea L.	Achromobacter xylosoxidans strain Ax10	The bacterial strain not only protected plant from Cu toxicity but also enhances the Cu accumulation in plant tissue with concurrent stimulation of plant growth	Ma et al. (2009a)
Brassica juncea L.	Pseudomonas sp. SRI2	Inoculation significantly increased the growth of plants in Ni contaminated soils	Ma et al. (2009b)
Brassica juncea L.	Enterobacter aerogenes NBRI K24 Rahnella aquatilis NBRI K3	PGPR were capable of stimulating plant biomass and enhance phytoextraction of metals (Ni and Cr) from fly ash	Kumar et al. (2009)
Brassica juncea L.	Kluyvera ascorbata SUD165 Kluyvera ascorbata SUD165/26	Toxic effects of heavy metals (Ni^{2+}, Pb^{2+}, and Zn^{2+}) were not pronounced in inoculated plants.	Burd et al. (2000)
Brassica juncea L.	Variovorax paradoxus, Rhodococcus sp.	Plant growth was improved in Cd^{2+} supplemented media in response to inoculation.	Balimov et al. (2005)
Brassica juncea L.	Pseudomonas brassicacearum Pseudomonas marginalis Pseudomonas oryzihabitans Pseudomonas putida Pseudomonas sp. Alcaligenes xylosoxidans Alcaligenes sp. Variovorax paradoxus Bacillus pumilus, and Rhodococcus sp.	The bacteria were tolerant to Cd^{2+} toxicity and stimulated root elongation of rape seedlings in the presence of 300 mM $CdCl_2$ in the nutrient solution.	Belimov et al. (2001a, b)
Brassica napus	P. tolaasii ACC23	Inoculation protected the plants against the toxic effects of Cd^{2+} and effectively promoted the growth of plants	Dell'Amico et al. (2008)
Brassica napus	Kluyvera ascorbata SUD165	Plant demonstrated normal growth under high levels of Ni^{2+}, Pb^{2+}, Zn^{2+}, and CrO_4^{2-}	Burd et al. (1998)

(continued)

Table 12.1 (continued)

Plant species	PGPR containing ACC-deaminase	Comments	References
Brassica napus	P. fluorescens G10 Microbacterium sp. G16	The bacteria helped plants in protecting against the inhibitory effects of high concentrations of Pb	Sheng et al. (2008)
Brassica oxyrrhina	Pseudomonas sp. SRI2	The bacterial strain significantly increased the growth of plants in Ni contaminated soils	Ma et al. (2009b)
Lycopersicum esculentum Mill.	Kluyvera ascorbata SUD165 Kluyvera ascorbata SUD165/26	Toxic effects of the heavy metals (Ni^{2+}, Pb^{2+}, and Zn^{2+}) were not pronounced in inoculated plants.	Burd et al. (2000)
Lycopersicum esculentum Mill.	Methylobacterium oryzae strain CBMB20 Burkholderia sp. Strain CBMB40	The bacterium not only decreased the Ni or Cd toxicity but also reduced their uptake and translocation to the plants	Madhaiyan et al. (2007)
Phragmites australis	Pseudomonas asplenii ACa	Inoculation resulted in normal plant growth under high levels of Cu^{2+} and creosote.	Reed et al. (2005)
Pisum sativum L.	Pseudomonas brassicacearum Am3 Pseudomonas marginalis Dp1	Inoculation with bacteria counteracted the Cd-induced inhibition of nutrient uptake by roots.	Safronova et al. (2006)
Pisum sativum L.	Pseudomonas brassicacearum, Pseudomonas marginalis Pseudomonas oryzihabitans, Pseudomonas putida, Pseudomonas sp. Alcaligenes xylosoxidans, Alcaligenes sp., Variovorax paradoxus Bacillus pumilus, and Rhodococcus sp.	The bacteria were tolerant to Cd^{2+} toxicity and stimulated root elongation of rape seedlings in the presence of 300 mM $CdCl_2$ in the nutrient solution.	Belimov et al. (2001a, b)

Updated from Saleem et al. (2007)

Table 12.2 Ameliorating impact of bacterial ACC deaminase on pathogenic infection

Plant	Infection	Microorganisms with ACC-deaminase	Pathogen/disease/infection	Comments	References
Cucumber	Pathogenic	Pseudomonas fluorescens CHA0	Pythium damping-off	Protected plants against disease in hermetically sealed containers	Wang et al. (2000)
Ground nut	Pathogenic	Methylobacterium	Aspergillus niger/Sclerotium rolfsii	Stimulated seed germination and induced defence responses	Madhaiyan et al. (2006)
Mung bean	Non-pathogenic	Ps. putida biotype A Ps. fluorescens	Bradyrhizobium		Shaharoona et al. (2006)
Peanut	Non-pathogenic	Pseudomonas fluorescens	collar rot, stem rot	Suppressed disease attack and increased pod yield	Dey et al. (2004)
Peas	Non-pathogenic	Variovorax paradoxus 5C-2	Indeginous rhizobium	Improved nodulation in peas	Belimove et al. (2009)
Potato tuber	Pathogenic	Ps. fluorescens CHA0	Erwinia soft rot	Protected plants against disease in hermetically sealed containers	Wang et al. (2000)
Tomato	pathogenic	Acinetobacter sp.	Phytopathogens	Bacterial strain showed inhibitory effects on phytopathogens which led to growth promotion of tomato plants	Belimov et al. (2007)
Tomato	Pathogenic	Methylobacterium oryzae CBMB20	Pseudomonas syringae pv. tomato	Reduced disease severity	Indiragandhi et al. (2008a, b)
Tomato	Pathogenic	Streptomycete actinomycetes	Fusarium oxysporum f. sp. lycopersici	Decreased incidence and severity of wilt disease	El-Tarabily and McKenna (2008)
Tomato	Pathogenic	Transgenic Tomato	Verticillium dahliae	Reduced symptoms, increased tolerance to Verticilium wilt	Robinson et al. (2001)

12.3 Transgenic Plants with Expression of Bacterial ACC-Deaminase

The plant growth promotion observed in response to inoculation with bacteria containing ACC-deaminase provoked scientists to develop transgenic plants with the expression of ACC-deaminase genes. These transgenic plants produce less ethylene compared to wild-type parental plants, which subsequently affects the physiological processes and growth of plants. As inoculation of plants with ACC deaminase containing bacteria reduce stress ethylene, similarly transgenic plants expressing ACC deaminase genes were less responsive to stress.

Sergeeva et al. (2006) developed genetically engineered canola plants by expressing the bacterial ACC-deaminase and then tested its ability to withstand otherwise inhibitory saline conditions. The growth of transgenic canola plants expressing ACC deaminase under the control of rolD promoter was inhibited to a lesser extent when grown in the presence of high salt concentration than the growth of non-transformed plants. Farwell et al. (2007) reported that transgenic canola and/or canola treated with P. putida UW4 had greater shoot biomass compared to non-transformed canola under low flood-stress conditions. Under high flood-stress conditions, shoot biomass was reduced and Ni accumulation was increased in all instances relative to low flood-stress conditions.

12.4 Future Prospects and Applications

The information narrated in the preceding sections, document unequivocally that ACC-deaminase has multidimensional impact on plant growth under normal as well as stressed conditions. With the passage of time, agriculture is getting confronted more and more by various kinds of stresses, so it is the need of time to develop technologies to sustain agriculture under these stresses. The development of biofertilizers based upon highly effective PGPR containing ACC deaminase could be of great use to improve crop productivity even under biotic or abiotic stress environments. Moreover, this bacterial ACC deaminase trait provides endless opportunities for plant biotechnologists to develop transgenic crop plants with expression of bacterial ACC deaminase to have better crop stand against various kind of stress(es).

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Chapter 13
RNAi: Machinery and Role in Pest and Disease Management

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Abstract RNA interference (RNAi) is a homology-dependent gene silencing technology that is initiated by double stranded RNA (dsRNA). A multitude of small RNAs accumulate in plant tissues, although heterogeneous in size, sequence, genomic distribution, biogenesis, and action, most of these molecules mediate repressive gene regulation through RNA silencing. Micro and small interfering RNAs represent small RNA families that are recognized as critical regulatory species across the eukaryotes. Besides their roles in developmental patterning and maintenance of genome integrity, small RNAs are also integral components of plant responses to adverse environmental conditions, including biotic stress. Recent studies broaden the role of RNAi, and many successful examples have described the application of RNAi for engineering plant resistance against a range of prokaryotic and eukaryotic organisms. Expression of dsRNA directed against suitable pathogen and insect genes in transgenic plants showed protection against pests, opening the way for a new generation of pest and disease resistant crops. Here, current knowledge on the uptake mechanisms of dsRNA in plant pests and the potential of RNAi to control pest and pathogen is described. Concerns regarding further research on dsRNA uptake mechanisms and the promising application possibilities for RNAi in pest and disease management have been discussed. Further, the progress of RNAi-based transgenic plant resistance against eukaryotic pests, as well as future challenges and prospects are addressed.

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13.1 Introduction

RNA interference (RNAi) is a gene silencing phenomenon found in almost all the eukaryotes that inhibits gene expression through homology based sequence-specific interactions. Biochemical and genetic studies have demonstrated that the core mechanisms and machinery of RNA silencing are shared among different organisms. The phenomenon is conserved across kingdoms and is manifested as quelling in fungi, RNAi in animals, and co-suppression or post-transcriptional gene silencing (PTGS) in plants. RNAi is considered as one of the major breakthrough of twenty-first century in molecular biology. It was first discovered in plants and fungi when the over-expression of a gene suppressed the expression of both the endogenous gene and transgene (Napoli et al. 1990). A similar phenomenon in *Neurospora crassa* was studied, wherein introduction of homologous RNA molecules resulted "quelling" of the endogenous gene (Romano and Macino 1992). In animals, the phenomenon was first reported by Guo and Kemphues (1995) where they found that the introduction of sense or antisense RNA to par-1 mRNA resulted in degradation of the par-1 mRNA in *Caenorhabditis elegans*. Anderw Fire and Craig Mello got the Nobel Prize for their landmark observation in *C. elegans* that RNAi affect is mediated by dsRNA (Fire et al. 1998). Evidence supporting a role for 21–25 nt RNA cleavage products as mediators of the RNAi effect in plants was provided thereafter (Hamilton and Baulcombe 1999). Introduction of synthetic 21 nt dsRNA duplexes with complete homology to their target sequences could exert the RNAi effect in mammalian cells and were termed small interfering RNAs (siRNAs) (Elbashir et al. 2001). These siRNAs guide the sequence-specific inactivation of target mRNAs by the RNA-induced silencing complex (RISC). In general, RISC mediates cleavage of target mRNA when there is perfect or near perfect base pairing between mRNA and the siRNA and translation repression when there is partial complementarity.

In plants, there are mainly three RNAi pathways (Baulcombe 2004). The first pathway is transcriptional gene silencing (TGS) which is associated with siRNA directed epigenetic regulation (Chan et al. 2006). The second pathway involves a class of endogenous small RNAs, micro RNAs (miRNAs) associated with the stress and developmental gene regulation. The third pathway, mainly involved in defense functions, comprises 21 nt siRNAs that are processed from dsRNAs. Irrespective of the origin, the presence of dsRNA in the cytoplasm of plant cells induces RNAi pathway. Crops can be modified by engineering novel RNAi pathways that create small RNA molecules to alter gene expression in crops or plant pests. RNAi has the potential to provide protection against insects, nematodes and pathogens without introducing new proteins into food and feed products.

13.2 Biochemical Properties of RNAi Components

Biochemical and genetic experiments have established a general mechanistic model for silencing pathways and identified factors that are required for RNA silencing in a variety of organisms. The components involved in small RNA generation are given in Table 13.1.

13.3 Small RNAs – Big Roles

In RNAi pathway dsRNA precursor are cleaved into small non-coding RNAs. These small RNA molecules are classified into two categories i.e. Dicer dependent and independent. siRNA and miRNA are dicer dependent while piwi interacting RNA (piRNA) and 21-U RNA are dicer independent. siRNA and miRNA are found in animals and plants both while piRNA and 21-U RNA are reported from animals only. Recently, some of the small RNAs are found and reported in fungi only viz., qiRNA (quelling interfering RNA), milRNA (miRNA like RNA) and disiRNA (dicer independent siRNA). These small RNAs are the backbones of RNAi activities and have revolutionized the biology of gene regulation. Two major classes of small RNAs i.e. siRNA and miRNA shared common features but there are some differences in their biogenesis and functions (Fig. 13.1; Table 13.2). The small RNAs are discussed below.

13.3.1 Small Interfering RNA (siRNA)

siRNAs are the product of dsRNA precursor originated from transgenes, endogenous repeat sequences, virus or transposons and act through post transcriptional gene silencing or transcriptional gene silencing pathways. These siRNAs are regulatory molecules of ~21–24 nt in length and protect the cell from intrusions of exogenous nucleic acid like viruses or any other pathogens. Depending on the nature of loci and biogenesis, several siRNA are reported as follows:

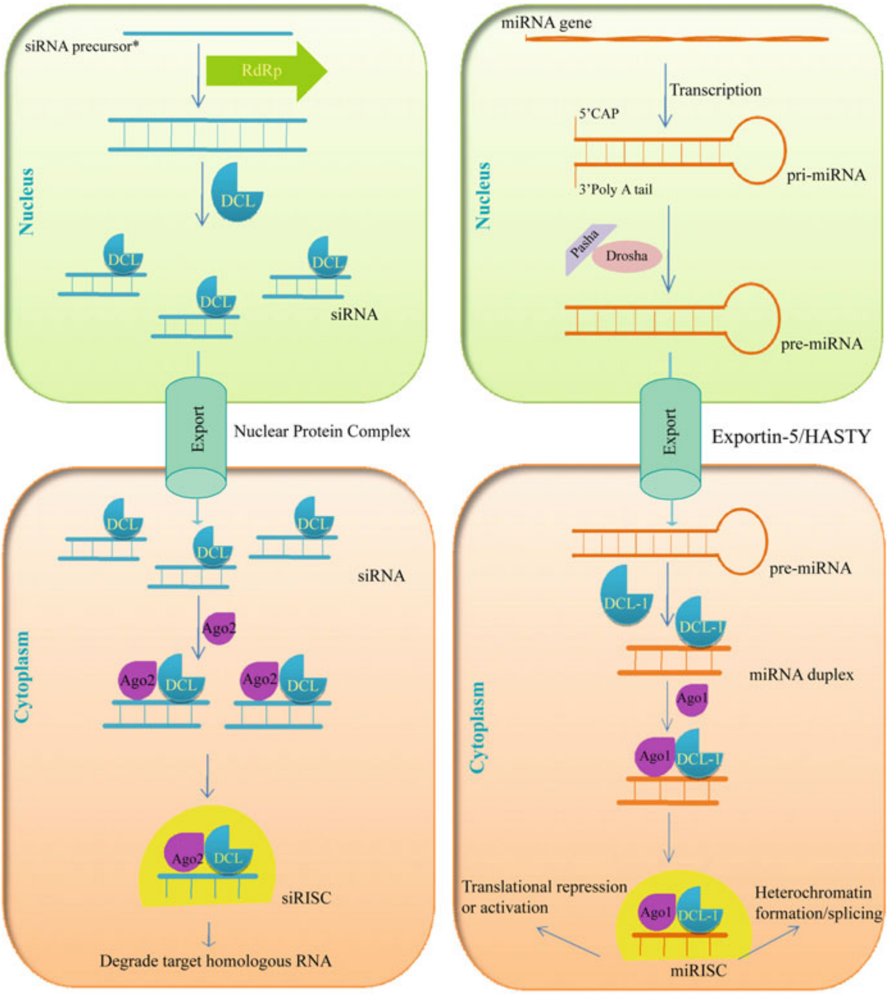
repeat associated siRNA (rasiRNA): are ~24–26 nt long products of dsRNA resulted from DCL3 activity. These dsRNA formed during unchecked transcription event, usually retro-transposon loci and were found in mammalian germline cells. rasi-RNAs participate during gametogenesis in flies, worms and mammals by modulating the chromatin status, and silencing viral transcripts by recruiting histone modifying proteins (Vagin et al. 2006).

trans acting siRNA (tasiRNA): are ~21 nt long small RNAs generated from DCL4 and AGO7 activity on endogenous transcript as template. Humans and flies lacks the RdRP and does not produce tasiRNA. Generation of these small RNA requires the RdRP activity (Montgomery et al. 2008).

Table 13.1 Properties and function of enzymatic machinery involved in RNAi pathway

Protein/Enzyme	Property	Function
Dicer	RNaseIII*	Generation of siRNAs from long dsRNAs, or mature micro RNAs from their primary transcript. The production of siRNAs by Dicer is an ATP-dependent step. There are four Dicer-like (DCL) homologs in Arabidopsis. DCL1 is responsible for miRNA generation while DCL2, DCL3 and DCL4 are required for the siRNA generation.
RNA dependent RNA Polymerase (RdRp)	Polymerase	The initial step of RNAi activation or siRNA pathway is signal amplification. The single stranded RNA is recognized by RNA polymerase which leads to formation of double stranded RNA either in primer dependent or independent manner. The dsRNA formed are later cleaved by dicer, another important component of the RNAi.
Drosha	Class II RNaseIII	This enzyme assumes a pseudo-dimer catalytic core similar to Dicer. The substrate of Drosha, micro RNA primary transcripts, is structurally distinct from Dicer substrates. Drosha does not process from a dsRNA terminus; rather, data suggests that the stem–loop structure is recognized. In particular, the loop size appears to be important for recognition.
rde-4 (RNAi deficient-4)	double stranded RNA binding domain (dsRBD) cofactor	This is the first dsRBD that was identified, arose from a genetic screen in C. elegans
Loquacious	dsRBD cofactor	Associates with Dicer-1
R2D2	dsRBD cofactor	Associates with Dicer-2
Pasha	dsRBD cofactor	Associates with Drosha
RNA-induced silencing complex (RISC)	multi-subunit endonuclease silencing complex	ATP-dependent step in which the siRNAs and miRNAs are denatured and incorporated into RISC. Effector complexes called RISCs are assembled upon loading of one selected small RNA strand into one member of the Argonaute (Ago) protein family. Within the activated RISC, single-stranded siRNAs/miRNAs act as guides to bring the complex into contact with complementary mRNAs and thereby cause their degradation (Bernstein et al. 2001; Elbashir et al. 2001).
Argonaute2	Endonuclease	This is the first identified component of RISC. This protein is a member of a gene family conserved in most eukaryotic and several prokaryotic genomes.
VIG	RNA binding protein	Component of RISC
Helicase proteins		Component of RISC
Tudor-SN		Component of RISC

RNaseIII enzymes fall into three classes (Nicholson 2003). Class I enzymes, found in bacteria and yeast, contain a single RNaseIII domain joined to a double stranded RNA binding domain (dsRBD). Class II and III enzymes contain two RNaseIII catalytic domains. Class III enzymes are further characterized by a helicase domain and a PAZ (Piwi/Argonaute/Zwille) domain. This last domain is also present in Argonaute family proteins, known to be essential for RNAi



**Precursor dsRNA are generated by either RNA dependent RNA polymerase (RdRp) activity on aberrant transcripts or transcripts having full or partial complementarity*

Fig. 13.1 Different RNAi machinery involved in siRNA and miRNA generation

<u>Chromatin-associated siRNAs:</u> are generated from endogenous transcript as template by DCL3/RdR2/RNA polymerase IVa dependent pathway (Kanno et al. 2005) and mainly loaded into AGO4 in *Arabidopsis thaliana*. These siRNAs induce transcriptional silencing through histone methylation, DNA methylation or chromatin remodeling (Matzke and Birchler 2005).

<u>Natural antisense transcript siRNAs (nat-siRNAs):</u> are derived from endogenous overlapping *cis*-natural antisense transcripts (NAT), reported from *Arabidopsis*. nat-siRNA requires DCL1, RDR6 for their biogenesis. nat-siRNA from one transcript could regulate the stability of the other (Wu et al. 2007).

Table 13.2 Comparison of small interfering RNAs and microRNAs

Features	siRNA	miRNA
Origin	siRNAs are processed from long dsRNAs	miRNAs are processed from single RNA molecule that include an imperfect stem loop secondary structure
Function	Small interfering RNAs (siRNAs) are implicated in variety of processes including defense against viruses, establishment of heterochromatin, silencing of transposons and transgenes and post transcriptional regulation of genes	Micro RNAs (miRNAs) are small endogenous RNAs that regulate gene expression in plants and animals. Micro RNAs regulate important biological processes, and hence plants and animals with compromised miRNA functions display severe developmental defects
Conserved ness	most exogenously expressed siRNAs are not	Many miRNAs are conserved between related organisms
Regulation	Many (but not all) siRNAs targets the gene from which they are derived or very closely related genes	miRNA regulates gene unrelated to loci encoding the miRNAs
Enzyme machinery	In the Drosophila, siRNAs are products of Dicer-2 whereas in Arabidopsis DCL2, DCL3, and DCL4 process long dsRNA molecules of various cellular origins into siRNA populations that are 22, 24, and 21 nucleotides in length, respectively	In the fruit fly Drosophila, miRNAs are products of Dicer-1 whereas in Arabidopsis DCL1 primarily synthesizes miRNAs
Loading into RISC	In Drosophila Ago1 preferentially binds microRNAs	In Drosophila Ago2 preferentially binds siRNAs
RdRp	many Arabidopsis siRNAs require RdRP for their biogenesis	miRNAs do not RdRp for their biogenesis

scnRNA (scan RNA): Relatively long siRNAs of ~29 nt reported from protozoan *Tetrahymena thermophila* (Mochizuki and Gorovsky 2004).

Long siRNA (LsiRNA): are 30–40 nt in length induced in response to bacterial infection (Katiyar-Agarwal et al. 2007). Reported first in Arabidopsis, activity of DCL1, DCL4 and AGO7 proteins are necessary for the generation of LsiRNAs.

Dicer-independent siRNAs (disiRNAs): another novel type of dicer independent sRNA reported from Neurospora, are about 22 nt long with a strong 50 U preference, processed from dsRNA made from naturally occurring complementary sense and antisense transcripts (Lee et al. 2010).

13.3.2 *Micro RNA (miRNA)*

Micro RNAs are small non-coding RNAs derived from single stranded RNA precursor containing hairpin like structures. These miRNA are reported from plants and animals. miRNAs are dicer dependent, short, 21–24 nucleotides in length, endogenously nontranslated RNA molecules. miRNA genes are transcribed by RNA polymerase II to yield capped and polyadenylated long primary RNA transcripts (pri-miRNA), which are then processed by at least two Dicer-like proteins called Drosha in association with Pasha to release stem-loop-like RNA precursors (pre-miRNAs) and mature miRNAs. The mature miRNA with Argonaute (AGO1) is incorporated into an RNA-induced silencing complex (RISC), and guides the cleavage or translational repression of the target mRNA by sequence-specific base-pairing. MicroRNA genes constitute ~1% of the total coding genes and form the largest class of regulatory molecules (Bartel 2004).

13.3.3 PIWI Interacting RNA (piRNA)

In addition to dicer dependent small RNAs, dicer independent small RNA from mammals and *C. elegans* were also reported recently called piRNA. piRNAs are 25–30 nt long in length and derived mainly from repetitive elements, transposons and large piRNA clusters. They shows strand bias and thus are the product of long single strand precursor molecules. It was observed that sense piRNAs associate with AGO3 while antisense associate with Piwi/Aub and are complementary till first 10 bases. piRNAs have a specific germline function in repressing transposable elements. This repression is thought to involve heterochromatin formation and transcriptional and post-transcriptional silencing and also involved in germline stem cell maintenance and in maintaining germline DNA integrity (Rouget et al. 2010).

13.3.4 21-U RNA

21U-RNAs are specifically 21 nucleotides long, begin with a uridine 5′-monophosphate but are diverse in their remaining 20 nucleotides, and appear modified at their 3′-terminal ribose. 21U-RNAs originate from genomic loci dispersed in two broad regions of chromosome IV of *C. elegans* and these loci share a large upstream motif that enables accurate prediction of additional 21U-RNAs. 21-U RNA might play some role(s) in chromatin reorganization and genome stability. The presence of 21U-RNAs during L1 and dauer stages suggests their role during worm development and in a variety of processes, including virus counteraction, transposon/transgene silencing (Ruby et al. 2006).

13.3.5 qiRNA

A novel class of ~21-nt-long small RNAs significantly induced by DNA damage has been reported from fungi (Li et al. 2010). Under normal growth conditions the level of these small RNAs were low but induced after the treatment with DNA damaging agents. These small RNAs are shorter than the regular 25-nt siRNAs and were named qiRNAs for their interaction with QDE-2, argonaute protein found in fungi (Lee et al. 2009).

13.4 RNAi: Virus Resistance

Viruses are significant threats to crop plants worldwide and the limited sources of natural resistance warrant the development of novel resistance sources. Several methods of transgenic protection have been successfully deployed, including protein and RNA mediated approaches. RNAi is one of the RNA mediated approach which has been successfully deployed for virus resistance. Infact, the first biological function established for RNA silencing was as an antiviral mechanism in plants (Anandalakshmi et al. 1998). It was thus established that virus infection induces RNA silencing, which then targets the viral RNAs to confer virus resistance. Furthermore, virus specific siRNAs of both positive and negative polarities accumulate in plants infected with viruses, demonstrating that viruses are both inducers and targets of RNA silencing in plants. The fact that RNA silencing is an antiviral mechanism in plants is further supported by two additional lines of evidence. First, mutants carrying loss-of-function mutations in essential silencing pathway genes such as *rdr6*, *ago1*, and *dcl2* show enhanced disease susceptibility to virus infection (Xie et al. 2004). Second, essential virulence factors of many plant RNA and DNA viruses are suppressors of RNA silencing (Praveen and Mangrauthia 2006).

13.4.1 RNA Virus Resistance

RNAi technology for generating virus resistance in plants was first demonstrated in *Potato Virus Y* (PVY) wherein complete immunity to the virus was reported in potato plants on simultaneous expression of both the sense and antisense transcripts of the viral helper-component proteinase (*HC-Pro*) gene (Waterhouse et al. 1998). Since then, it paved way for deploying RNAi mediated resistance against several other viruses. *Barley Yellow Dwarf Virus (*BYDV) has long been considered to be the most significant viral disease agent of small grain cereals, worldwide. BYDV causes particularly severe yield losses in oats due to blasting and low seed set. In barley, BYDV derived hairpin RNAs from polymerase gene sequence conferred immunity to the virus in an effective manner (Wang et al. 2000). Hairpin RNA mediated resistance was successfully achieved against *Plum Pox Virus* (PPV) employing the constructs designed to produce silencing effect against P1 and Hc-Pro genes of the virus (Nicola-Negri et al. 2005). Several other reports have demonstrated that RNAi inducing constructs can effectively trigger and initiate RNA silencing and lead to high resistance frequencies in transgenic plants (Chen et al. 2004). Recently, RNAi based resistance was developed in maize against Maize Dwarf Mosaic Virus by targeting the P1 gene of the virus (Zhang et al. 2010).

13.4.2 DNA Virus Resistance

DNA viruses are also targets of RNA silencing. The initial successful attempt of targeting DNA virus through RNAi was reported against the *Mungbean Yellow Mosaic Virus-vigna* (MYMV-vig). Pooggin et al. (2003) have demonstrated that intron spliced hairpin RNA targeting the intergenic region of bi-directional promoter of this geminivirus resulted in resistance to MYMV-vig. Thus the work unequivocally proved that RNAi can be an effective strategy in combating DNA virus infections in plants. Gafni and colleagues obtained plants resistant to *Tomato Yellow Leaf Curl Virus* (TYLCV) by targeting the CP (coat protein) gene with an IR (Inverted repeat) construct (Zrachya et al. 2007). Development of trait stable transgenics have been successfully deployed for *Tomato Leaf Curl Virus* resistance using RNAi based strategies (Ramesh et al. 2007). Efforts have been made to silence the *Rice Tungro Bacilliform Virus* (RTBV) by expressing DNA encoding ORF IV of RTBV, both in sense as well as in anti-sense orientation, resulting in the formation of RNAi inducing dsRNA, where the transformed rice plants showed tolerance to the virus disease (Tyagi et al. 2008).

13.4.3 Multiple Virus Resistance

Higher plants are frequently subjected to multiple virus infections resulting in intensification of symptoms expression and virus accumulation, a phenomenon known as synergism (Hull 2002). In synergistic viral interaction, co-infection with two independent unrelated viruses results in a much more serious disease than either virus induces in a single infection (Mangrauthia et al. 2009). Importance of transgenic approach for virus resistance can be fully realized if multiple virus resistance can be developed, because crops are frequently infected with more than one viruses under field conditions. Post transcriptional gene silencing is a powerful, specific and intracellular RNA degradation mechanism which can be utilized successfully against multiple virus infections. Transgenic plants with multiple virus resistance was obtained by fusing viral gene segments (a segment of tospovirus N gene to the potyvirus CP gene) under the expression control of single promoter and terminator, leading to transgenic plants resistant to a potyvirus and a tospovirus both having RNA genome (Jan et al. 2000). Bucher et al. (2006) developed hairpin RNAi construct using NP gene sequences of four different tomato infecting tospoviruses and demonstrated that this construct shows resistance for all the four viruses. The same strategy was used to develop double resistance to both *Papaya Ringspot Virus* (PRSV) and *Papaya Leaf Distortion Mosaic Virus* (PLDMV). An untranslatable chimeric construct that contained the truncated PRSV-CP and PLDMV-CP genes was transferred to papaya. Through the PTGS mechanism, transgenic papaya plants carrying this chimeric transgene conferred resistance against both PRSV and PLDMV (Yeh 2005).

13.4.4 RNAi Strategies

In plants variety of approaches have been utilized to express double stranded RNAs cognate to viral transcripts so as to initiate the process of viral gene silencing. Initially this was achieved by separately expressing sense and antisense genes of viral origin in plants and bringing them under a single genetic background by crossing. Various ways of construct design strategies were studied for their relative efficiency by different researchers. Four different forms of hairpin RNA mediated silencing, viz., hairpin RNA, intron spliced hairpin RNA (Ihp RNA), Ihp RNA overhang, and IhpRNA spacer, were evaluated for resistance against PVY. Among these four constructs, IhpRNA was found to be superior as 90% of the plants carrying the construct exhibited viral gene silencing (Wesley et al. 2001). Based on these studies many generic intron spliced hairpin vectors (pHANNIBAL or pKANNIBAL etc.) have been generated for the purpose of RNAi. The strategy of employing short-hairpin RNA (sh-RNA) vectors or constructs to achieve silencing of the genes of viral and other endogenous origin have been widely explored successfully. With the RNAi attaining greater attention in recent years in management of viral diseases of plants, the recent technological innovation added in this direction is artificial microRNA (amiRNA) mediated silencing of the viral gene expression in plants. This innovative approach deploys manipulated host miRNA pathway in achieving virus resistance. The findings that the 21 nt of endogenous microRNA can be altered without disturbing its biogenesis and maturation have raised interests in employing microRNA as a tool to silence transcripts of interest. In the field of virus resistance, pre-miR^{159a} of *Arabidopsis* was modified to generate artificial pre-miRNAs^{159} (pre-amiRNAs^{159}) containing sequences complementary to genomes of two plant viruses, *Turnip Yellow Mosaic Virus* (TYMV) and *Turnip Mosaic Virus* (TuMV). Transgenic lines carrying 35S-pre-amiRNA^{159} showed specific resistance to either TYMV or TuMV, depending on the expression of the cognate amiRNA (Niu et al. 2006). In yet another instance, expression of an artificial miRNA, targeting sequences encoding the silencing suppressor 2b of *Cucumber Mosaic Virus* (CMV), has efficiently inhibited 2b gene in transient expression assays and confers transgenic tobacco plants with effective resistance to virus infection (Qu et al. 2007).

13.4.5 Factors Affecting RNAi Efficiency

RNAi mechanism has been adopted to develop virus-resistant plants through expression of virus-derived dsRNAs or hairpin RNAs which in turn are processed into siRNAs by the host's RNA silencing machinery. In most of the reported viral transgenic studies, the observed virus resistance level was correlated to the abundance of virus-specific siRNAs in plants or level of expression of short RNAs from viral derived sequences. Transgenic tobacco lines containing inverted repeat of CMV cDNA were generated with transgene derived from CMV capable of producing intramolecular dsRNA homologous to the 3′ portion of the RNA3 genome.

When the transgenic plants were analyzed for siRNA production, plant lines that failed to produce siRNAs at a detectable level were susceptible to CMV infection, whereas plants producing siRNAs were resistant to CMV (Kalantidis et al. 2002). Recent development suggests different loci results distinct cytoplasmic and nuclear RNAi activity. One locus was necessary and sufficient for development of a resistant phenotype and produced substantial amount of siRNAs-CMV. A second locus, although transcribed, failed to produce detectable levels of siRNAs-CMV and did not confer resistance (Dalakouras et al. 2011). In contrast, Lopez et al. reported that accumulation of transgene-derived siRNAs is not sufficient for RNAi-mediated protection by transforming Mexican lime plants with the 3′-terminal 549 nucleotides of the *Citrus tristeza virus* (CTV) genome in sense, antisense and intron-hairpin formats. When analyzed in intron-hairpin lines with single transgene integration, CTV resistance was correlated with low accumulation of the transgene-derived transcript rather than with high accumulation of transgene-derived siRNAs (Lopez et al. 2010).

Shimizu et al. (2010), demonstrated the effects of potential target sequences in each of the coding genes in the *Rice Stripe Virus* (RSV) genome using transgenic rice plants that expressed a set of inverted-repeat (IR) constructs. Transgenic plants that harboured IR constructs specific for the gene pC3, which encodes nucleocapsid protein, and for pC4, which encodes a viral movement protein, were immune to infection by RSV and were more resistant to infection than the natural resistant cultivars that have been used to control the disease in the field. By contrast, the IR construct specific for the gene pC2, which encodes a glycoprotein of unknown function, and for p4, which encodes a major non-structural protein of unknown function, did not result in resistance. Factors, such as the integration locus of a transgene as well as inherent characteristics of the transcript, its intermediate processing and interaction with various proteins of competing machineries may be critical in determining the fate of hairpin transcripts and efficient triggering of RNAi.

The degree of sequence similarity of siRNA with the target virus is related to the resistance or resistance-breaking phenomena. Transgenic tobacco plants containing a 597-nt hairpin RNA construct of the PVY replicase sequence were challenged with a variety of virus strains. The transgene-carrying tobacco line was immune to five potato PVY strains with high sequence similarity (88.3–99.5%) to the transgene. Infection with more distant tomato and pepper PVY field strains (86–86.8% sequence similarity) caused delayed symptom appearance in the transgenic tobacco. In the resistance-breaking tomato and pepper strains, there were nucleotide differences in the sequences correlated to siRNA transgene accumulation, indicating the role of siRNA specificity in resistance breaking (Gaba et al. 2010).

13.5 RNAi: Fungal Resistance

RNAi known as Quelling in fungi was discovered first in *Neurospora crassa*, one of the first eukaryotic model system for RNAi studies. Quelling silences both transgene and homologous endogenous gene with the minimum length of ~130 nt.

In *N. crassa* QDE-1 and QDE-3 are necessary for aberrant RNA (aRNA) and dsRNA production in fungi. Expression of an inverted repeat-containing transgene results in the production of dsRNA, induce gene silencing with high efficiency. Neurospora has two partially redundant Dicer proteins: DCL-1 and DCL-2. Both DCLs can process dsRNA into about 25-nt small RNAs in an ATP-dependent manner. Double stranded RNA is processed into siRNA duplexes by Dicer protein(s), which are loaded onto the RISC; the Argonaute protein QDE-2 is the core component of the RISC complex and is associated with siRNA. The passenger strand of the siRNA duplex should be removed to activate the RISC as cleavage and removal of the passenger strand from the siRNA duplex are important steps in RNAi pathways. QDE-2 interacts with another protein called QIP with an exonuclease domain which functions by removing the nicked passenger strand from the siRNA duplex in a QDE-2-dependent manner. The Argonaute protein QDE-2 cleaves the passenger strands of the siRNA duplexes, and the exonuclease QIP removes the nicked passenger strands with the guide strands remaining in the RISC complexes; and the activated RISCs guided by the single-stranded siRNA cleaves homologous mRNAs, resulting in gene silencing. Based on these studies, Maiti et al. (2007) proposed a model for the Neurospora RNAi pathway (Fig. 13.2a).

Another RNAi-related mechanism in *N. crassa* is the meiotic silencing by unpaired DNA (MSUD) and is similar to quelling but occurs during meiosis only (Shiu and Metzenberg 2002). Figure 13.2b shows the various steps involved in the MSUD mechanism: (i) an unpaired DNA is a signal to initiate the transcription of aRNAs from the unpaired DNA region during meiosis; (ii) SAD-1 (suppressor of ascus dominance-1) converts aRNA into dsRNA, SAD-1 shares high identities with cellular RdRPs involved in gene silencing; (iii) this dsRNA is processed by DCL-1 also called SMS-3 (suppressor of meiotic silencing-3) into small RNAs; (iv) small RNAs are then loaded onto a RISC complex with Argonaute proteins SMS-2 core component of RISC, which then results in the post-transcriptional silencing of homologous genes. SAD-2 may function in the MSUD pathway by recruiting SAD-1 to the proper location to perform its activity. The future identification of small RNAs associated with SMS-2 and the understanding of the meiotic sensing mechanism will be critical to the understanding of MSUD. MSUD controls meiotic pairing and is important for the correct gene expression during meiosis.

13.5.1 Application of RNAi for Fungal Resistance Development

RNA interference is a powerful and versatile genetic tool that can be applied to filamentous fungi of agricultural importance. Recently, it is shown that gene silencing plays an important role in plant defence against multicellular microbial pathogens; vascular fungi belonging to the *Verticillium* genus. Several components of RNA silencing pathways were tested, of which many were found to affect *Verticillium* defence. It is speculated that the gene silencing mechanisms affect regulation of *Verticillium*-specific defence responses (Ellendorff et al. 2009). An early successful application of the RNAi system using sense and antisense RNA was reported for the pathogenic fungus *Cryptococcus neoformans* (Liu et al. 2002). The efficacy of RNAi was demonstrated in *Magnaporthe oryzae*, *Venturia inaequalis, Phytophtora infestans, Histoplasma capsulatum* and *Blastomyces dermatitidis* by expression of GFP gene in fungus and then silencing by RNAi. The ADE2 gene encodes phosphoribosylaminoimidazole carboxylase, which is required for adenine biosynthesis. Silencing of this gene yields pink colonies due to the accumulation of intermediates from the adenine biosynthetic pathway, and thus, transformants with silenced genes can be recognized by their pink phenotype. This strategy to silence simultaneously a reporter together with a target gene was applied in different yeasts and filamentous

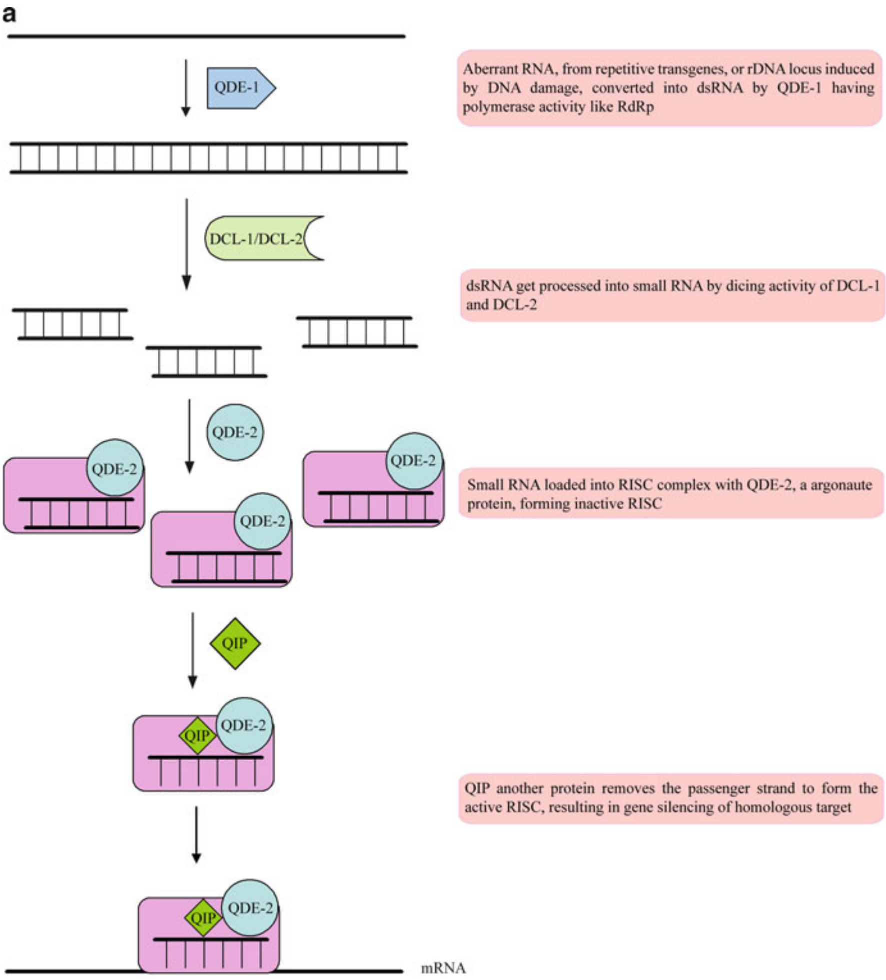


Fig. 13.2 RNAi or quelling pathway in fungi producing qiRNA (**a**) and another pathway called MSUD involved in fungi (**b**)

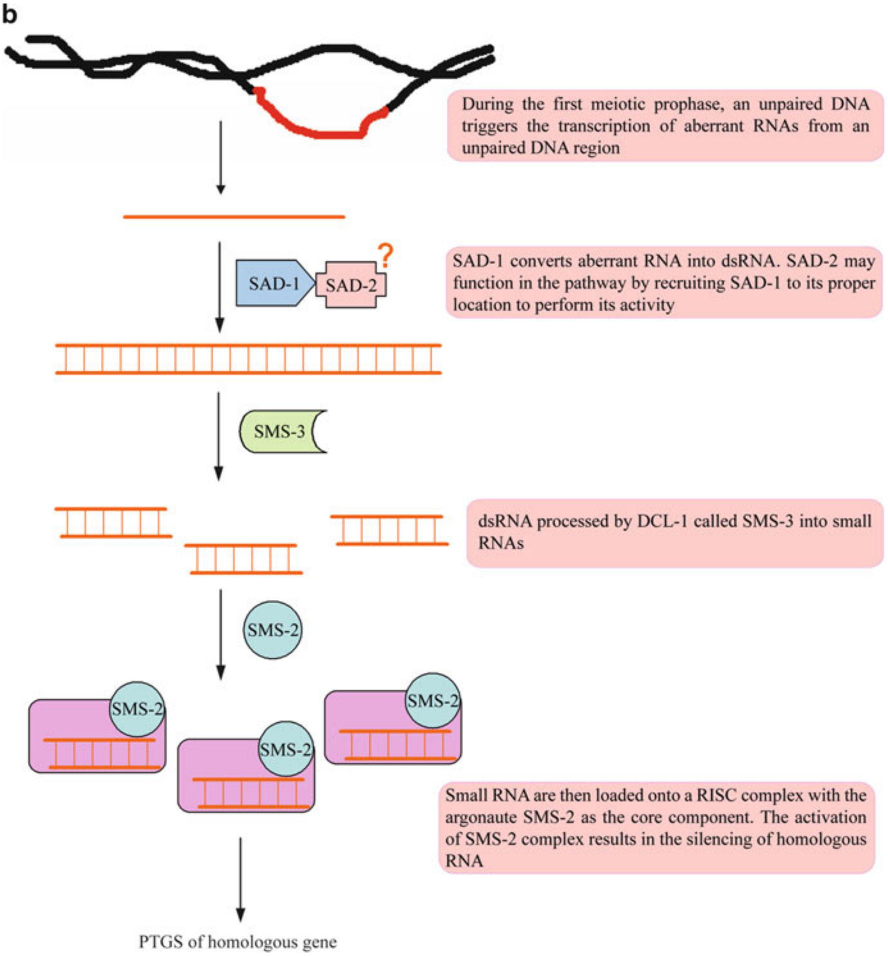


Fig. 13.2 (continued)

fungi (Kuck and Hoff 2010). Rust fungi cause devastating diseases of wheat and other cereal species globally. Gene fragments from the rust fungus, *Puccinia striiformis* f. sp. tritici or *P. graminis* f. sp. tritici, were delivered to plant cells through the *Barley Stripe Mosaic Virus* (BSMV) system and some reduced the expression of the corresponding genes in the rust fungus. The ability to detect suppression was associated with the expression patterns of the fungal genes because reduction was only detected in transcripts with relatively high levels of expression in fungal haustoria. The results indicate that in planta RNAi approach can be used in functional genomics research for rust fungi and that it could potentially be used to engineer durable resistance (Yin et al. 2011). The effectiveness of RNAi is demonstrated in the lignin-degrading fungus *Phanerochaete chrysosporium*. The manganese-containing superoxide dismutase gene (*MnSOD1*) was used as the target for RNAi. RNAi construct was sufficient to decrease the enzymatic activity by as much as 70% relative to control levels (Matityahu et al. 2008). Examples with biotechnical relevance were conducted with *Aspergillus oryzae* where genes for a secreted β-xylosidase or a group of carboxypeptidases were downregulated (Zheng et al. 1998; Kitamoto et al. 1999). Another interesting example is the protein disulfide isomerase, which plays an important role in the folding, assembly, and secretion of homologous as well as heterologous proteins. An antisense approach led to a 50% decrease of the microsomal protein disulfide isomerase in *Aspergillus niger* (Ngiam et al. 2000).

13.6 RNAi: Insects Resistance

Losses due to insect pests are a major limiting factor in increasing food production. Genetically modified plants with endogenous resistance to insect pests have been one of the real successes of transgene technology. Crop plants expressing genes of proteinase inhibitors, lectins, cholesterol oxidase, avidin and various bacterial toxins have shown differing specificities of insecticidal activity toward insect pests (Gatehouse 2008). The transgenic plants expressing *Bacillus thuringiensis* (*Bt*) *cry* genes grown commercially worldwide have revolutionized the insect pest control (James 2010). However all insect pests cannot be targeted by these toxins. Further the development of resistance to these toxins in insects has been reported. Developing viable alternate strategies become necessary for durable pest control.

Expression of dsRNA or siRNA directed against suitable insect target genes in transgenic plants has been shown to give protection against insect pests, opening the way for a new generation of insect-resistant crops. This approach holds great promise for the future because it has the potential to disrupt normal biological functions such as moulting process, reproduction, vitellogenesis, insect behavior, enzyme/hormone biosynthesis, resistance against insecticides, insect immunity (Huvenne and Smagghe 2010). The specificity of RNAi-mediated insecticidal effects is an important consideration for the use of this technology in a practical application; effects on non-target insects could be minimised. Further, the stability of ingested dsRNA or siRNA in the insect gut may be affected both by chemical hydrolysis (which increases with increasing pH) and by enzymes (nucleases) present in the gut contents. Hence, understanding the gut physiology, midgut cells involved in the uptake of dsRNA and systemic spread of silencing signal to other tissues of insects is important (Hakim et al. 2010).

13.6.1 dsRNA Uptake Mechanisms in Insects

Earlier studies suggested that there is no homologue encoding RNA dependent RNA polymerase (RdRP) in insects, which is necessary for the siRNA amplification that leads to persistent and systemic RNAi effects. However, recent studies in *D. melanogaster* have now shown that the D-elp1 gene, a subunit of the cellular

RNA polymerase II, has RdRP activity and is involved in immunity via RNAi (Lipardi et al. 2005). In addition *systemic RNA interference-deficient-1* (*sid-1*) gene in *C. elegans* is essential for the uptake of dsRNA molecules and may also facilitate the release of dsRNA among cells. Many *sid* orthologs have been found in insects, but their precise role in the uptake mechanism of dsRNA often remains to be determined. Another uptake mechanism is based on endocytosis wherein, vacuolar H^+ ATPase plays an important role. In *D. melanogaster* and quite possibly others, the primary mode of dsRNA entry into cells may be by receptor mediated endocytosis. Uptake of dsRNA in these cells appeared to depend on the presence of receptors that bind dsRNA and then stimulate endocytosis (Ulvila et al. 2006). Moreover, other pattern recognition receptors, as well as several vesicle transport proteins and lipid-modifying enzymes, may also play a role in the process of dsRNA uptake in these cells. Over expression of dsRNAs within cells using hairpin RNAs readily triggers RNAi in *D. melanogaster* tissues resistant to systemic RNAi which suggests that poor sensitivity is not related to the RNAi core machinery, but rather to the penetration and transmission of the interfering signal through cells and tissues (Belles 2010). Further all insect species are not equally sensitive to systemic RNAi. Insects from primitive orders are generally more sensitive than the insects from advanced orders. To gain further insight on the dsRNA uptake mechanisms in insects, knowledge on more gene orthologs in different insects is essential to study their role in dsRNA uptake.

13.6.2 Systemic Spread of RNAi

Systemic RNAi requires the following steps: (i) dsRNA uptake by the intestinal cells, (ii) export of either the dsRNA or of dsRNA-derived silencing signals from the intestinal cells, (iii) import of the silencing signals into other tissues (e.g. muscle, epidermis, germline) and (iv) targeted gene silencing via the cell autonomous RNAi machinery. Double stranded RNA must be taken up from the insect gut lumen into the gut cells. If the target gene is expressed in a tissue outside of the gut, the silencing signal will also have to spread via cells and tissues, which is termed as systemic RNAi. RNAi triggered by feeding and soaking has been demonstrated in a variety of insects. However the systemic spread of RNAi signal does not occur uniformly in all insects. Despite the RNA dependent RNA polymerase (RdRP) activity described in Drosophila embryo extracts (Lipardi et al. 2005), the presence of RdRP was not confirmed in insects. This suggests that the spread of dsRNA in insects may be based on another mechanism than that in nematodes.

13.6.3 Delivery Methods

Direct injection of the dsRNA into target tissues or developmental stages is the most common method of delivering dsRNA to insects. Soaking and biolistics have also been shown to be effective at delivering dsRNA into *Drosophila* embryos. Supplying dsRNA via oral feeding that mimics transgenic plant delivery resulted in knockdown of targeted genes in many insect species (Table 13.3). Delivery of the dsRNA into the gut resulted in knockdown of a gene's expression in other tissues, indicating that the RNAi was systemic. Recent studies have demonstrated that transgenic plants can be engineered to produce hairpin dsRNAs *in vivo* that can protect the plants against insect damage (Huvenne and Smagghe 2010).

13.6.4 RNAi and Insect Pest Control in Agriculture

RNAi is a powerful tool for gene function studies and control of insect pests. Several research groups have recently explored the possibility of conducting RNAi in insects through different application methods. There is a wide range of target insects from different insect orders, target genes and feeding methods, demonstrating the richness in application of dsRNA and the potentials of RNAi. Despite having been considered for many years, application of RNAi technology to give resistance to herbivorous insects has only just been realized. The key to the success of this approach would be; (a) Insect species and its life stages (b) Type of exogenous RNA: dsRNA, siRNA, miRNA etc. (c) Dose and method of application (d) Type of target gene and its expression profile (e) Gene function and type of tissue (f) Nucleotide sequence and length of dsRNA (g) Persistence of silencing effect (h) Gut physiology.

Several crop insect pests belonging to different orders were tested for their possible control by RNAi. In these insects, RNAi knockdown has been developed for various genes encoding for developmental proteins, salivary gland proteins, proteins involved in host-insect interaction, hormone receptors and gut enzymes (Table 13.4). Baum et al. (2007) provided evidence for the potential use of RNAi to control insects pest in crop protection and demonstrated the fact that it is possible to silence genes in insects when they consume plant material expressing hairpin dsRNA constructs against well chosen target genes. They reported the reduction of corn root damage in transgenic maize plants producing vacuolar H^+ ATPase dsRNA after infestation of the plant with the western corn rootworm. In another report, the model plants *Nicotiana tabacum* and *Arabidopsis thaliana* were modified with the cytochrome P450 gene of *Helicoverpa armigera*. When the cotton bollworm larvae were fed transgenic leaves, levels of cytochrome P450 mRNA were reduced and larval growth retarded (Mao et al. 2007). Bautista et al. (2009) studied the influence of silencing the cytochrome P450 gene CYP6BG1 that is over expressed in a permethrin-resistant diamondback moth (*Plutella xylostella*) strain. When the gene was silenced after consumption of a droplet of dsRNA solution, the moths became significantly more sensitive to the pyrethroid insecticide. Another significant development employing RNAi is that the susceptibility of insect pests to *Bt* toxins could be enhanced by silencing of the genes involved in Bt resistance development.

Table 13.3 RNA interference through oral feeding in insect pests of agricultural importance

Organism	Target gene	Stage	Method of application	mRNA transcript level	Gene expression evaluation method	References
Diabrotica virgifera virgifera (Chrysomelidae)	Vacuolar ATPase subunit A	Neonates	Transgenic plant	–	N blot, damage to the corn root, LC_{50}	Baum et al. (2007)
	α-Tubulin	Neonates	Transgenic plant	–	Growth	
H. armigera (Noctuidae)	Cytochrome p450 monooxigenase	3rd instar	Transgenic plant	Decreased	N. blot, gossypol tolerance	Mao et al. (2007)
	Glutothione –S-transferase	3rd instar	Transgenic plant	Decreased	N blot and growth	
Plutella xylostella (Plutellidae)	Cytochrome P450, CYP6BG1	Larvae	Droplet feeding	Decreased	RT PCR	Bautista et al. (2009)
H. armigera (Noctuidae)	Acetylcholinesterase	Neonate to pre pupal stages	Artificial diet feeding	Decreased	RT PCR, insect growth and development	Kumar et al. (2009)
Spodoptera exigua (Noctuidae)	Chitin synthase	All larval instars	Ingestion of transformed E. coli	Decreased	N blot, growth and development	Tian et al. (2009)
N. lugens (Delphasidae)	Trehalose phosphate synthase (TPS)	Nymphs	Oral feeding	Decreased	qRT-PCR	Chen et al. (2010)
Helicoverpa armigera (Noctuidae).	Cytochrome P450, CYP6AE14	Larvae	Oral feeding	Decreased	RT PCR	Mao et al. (2011)
Bemisia tabaci (Aleyrodidae)	Actin ortholog, ADP/ATP translocase, α-tubulin, ribosomal protein L9 (RPL9) and V-ATPase A subunit	Adults	Oral feeding	Decreased RPL9 and V-ATPase	RT PCR	Upadhyay et al. (2011)
Bactrocera dorsalis (Tephritidae)	Ribosomal protein Rpl19, V type ATPase D subunit, the fatty acid elongase Noa and a small GTPase Rab11	Adults	Ingestion of transformed E. coli	Decreased m RNA transcripts	qRT-PCR	Li et al. (2011)

Table 13.4 Successful examples of gene function studies employing RNAi in Lepidoptera and other insect pests of agriculture

Insect species	Gene functions studied
Lepidoptera[a]	
Diatraea saccharalis (Crambidae)	Aminopeptidase-N (Bt toxin receptor)
Ostrinia nubilalis (Crambidae)	Role of chitinase genes in regulating chitin content of midgut peritrophic matrix
Spodoptera exigua (Noctuidae)	Ecdysis, chitin synthesis pathway, trehalose synthesis pathway, role of storage hexamerins in development
S. frugiperda (Noctuidae)	Juvenile hormone titer (effect of allatotropins and allatostatins), defense against Bt Cry toxin
S. littoralis (Noctuidae)	Circadian rhythm of sperm release
S. litura (Noctuidae)	Bt toxin receptor (aminopeptidase-N)
Mamestra brassicae (Noctuidae)	Embryonic development (formation of bilateral procephalic lobes)
Helicoverpa armigera (Noctuidae)	Acetylcholinesterase (role in regulation of differentiation and development), Bt toxin receptor (aminopeptidase-N)
Plutella xylostella (Plutellidae)	Insecticide resistance (role of cytochrome P450 in resistance to permethrin), role of cadherin in larval growth and development)
Plodia interpunctella (Pyralidae)	Embryonic development (eye-colour pigmentation)
Manduca sexta (Sphingidae)	Immune system (hemocyte adhesion and encapsulation, phagocytosis and melanotic nodule formation and clearance of E. coli from the hemolymph, protective effect of prior E. coli infection on Photorhabdus luminescens infection, role of plasmatocyte-spreading peptide (PSP) during E. coli and P. luminescens infection, effect of P. luminescens antibiotic on phenoloxidase, protective effect of nitricoxide synthesis on oral Photorhabdus infection), Bt toxin receptor (cadherin), defense against Bt Cry toxins,
Epiphyas postvittana (Tortricidae)	Larval gut carboxylesterase, pheromone binding
Non lepidoptera	
Phyllotreta striolata (Chrysomelidae)	Water specific aquaporin
Leptinotarsa decemlineata (Chrysomelidae)	Vacuolar ATPase subunit A and E
Phyllotreta striolata (Chrysomelidae)	Arginine kinase
Schistocerca Americana (Acrididae)	Eye colour gene vermilion

[a] Refer Terenius et al. (2011) for complete publication list

13.7 Future Perspectives

Most of the RNAi research has been carried out in *Arabidopsis*, *Drosophila*, *C. elegans* and other model systems in green house trials. There are substantial gaps in our knowledge about the RNAi mechanisms in agriculturally important crops and host-pest interactions. Therefore further research is needed to evaluate whether this resistance can be kept in the target crop plant in the field. Suitable target genes of pests for RNAi have not been explored. The technology has not been exploited extensively against insects, pathogens and nematodes having different feeding mechanisms. Understanding the stability and persistence of small RNAs in various pests is essential for the use of this technology to achieve the protection against biotic stress in field crops. Several practical applications in economically important crops as well as research on gene function and expression are possible. RNAi stability in plants is a very important and the development of tissue specific and inducible promoters feature to be accessed in the near future. The use of artificial miRNA to engineer pest resistant plants also shows great potential. With the designing of an RNAi vector aimed at silencing the potent genes of pests, researchers may be able to develop crop plants that are resistant to insect pests and safe to eat.

The RNAi experiments on different insect pests strongly suggest that plant-expressed dsRNA can be delivered into insects and trigger systemic silencing, although some significant challenges remain, there is no doubt that the plant-induced RNAi could be an alternative pest-control strategies. The technology is likely to be taken up for applications where *Bt*-based approaches have proved difficult, for example protection against flies (dipterans), or where no effective *Bt* toxins are known, for example protection against sucking pests such as aphids, leafhoppers and whiteflies. The sequence specificity of dsRNA coupled with its ability to suppress gene function critical for insect survival suggests that dsRNAs could be developed as tailor-made pesticides, for use on insect pest where it is important to target only one or several closely related species, without adversely affecting non-target species.

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Chapter 14
Biological Control, Functional Biodiversity and Ecosystem Services in Insect Pest Management

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Abstract Biological control has come a long way and has been adapting to the changing needs of agricultural pest suppression. Changes in crop management practices, transgenic crops and new pesticide molecules have a profound effect on the natural pest suppression. Biocontrol like any other control technique is not without associated risks and cannot be considered as a panacea for maintaining environmental health. In order to sustain biological control there is a need to study the ecology of crop systems as a whole and design strategies based on the positive and negative interactions. Benefits of biodiversity are contextual and are diluted by trophic interactions which may be positive, negative or neutral. Habitat management strategies are used for enhancing the positive effects of biodiversity on pest suppression and are the basis of conservation biological control. Here we attempt to give an overview of the status of classical and introductory biological control programmes worldwide, risks that need to be addressed and the various principles of the emerging field of conservation biological control which aims at harnessing the *in situ* biodiversity of crop ecosystems.

14.1 Introduction

Insect pests are the most important biotic constraints faced by agriculturists leading to a worldwide pesticide use of 5,211 million pounds of active ingredients in 2007 alone. World pesticide expenditures totaled more than \$35.8 billion in 2006 and more than \$39.4 billion in 2007 (Grube et al. 2011). Pesticide usage has caused serious environmental concerns. Biological control is a pest management system designed by nature to keep populations in equilibrium. Intensive agricultural practices

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and global economy have broken the harmony in this natural equilibrium leading to pest outbreaks, pest invasions and a horde of associated problems. Biological control has been successfully used as a management tool for control of agricultural and forest pests and for protection of natural systems affected by invasive pests (Van Driesche et al. 2010). Applied biological control uses the basic underlying principle of nature and involves the intentional introduction of natural enemies including both predators and parasitoids, for the control of pest organisms such as insects, weeds and diseases. Applied biological control has come a long way from the use of ant, *Oecophylla smaragdina* (Fabr.) for control of citrus pests in China. Early biocontrol studies or observations were on insect predators. Many centuries later, the seventeenth century saw the discovery of insect parasitism. Early phase of biological control was marked by discovery and haphazard use of biocontrol agents without a scientific approach. The classical biological control of cottony cushion scale *Icerya purchasii* Maskell with the coccinellid beetle *Rodolia cardinalis* Mulsant introduced from Australia into the United States paved way for scientific approach to classical biological control. This gave birth to the new field of importation biological control to control invasive pest species. Augmentative biocontrol is a sub set of applied biological control. It differs from introductory biological control in that it requires periodical release of natural enemies over a cropping season as against classical biocontrol, a form of inoculative biological control where agents are released with the aim of establishment and a means of permanent pest control. For augmentative release, native natural enemies whose mass rearing techniques are well established are generally preferred. It can be equated with bombarding the ecosystem with a high number of a natural enemy species to bring about control akin to insecticidal control. More than 100 species of beneficial organisms are commercially available for control of nearly all important insect and mite pests. Some of the natural enemies have been used for more than 20 years for augmentative biocontrol.

Classical biocontrol was flagged for its environment friendliness and it reigned and proliferated into the eighties. The modern era of biocontrol involved more precise assessment of bio-agents and introductions. Howarth (1983, 1991) opened the proverbial Pandora's Box and questioned the eco-friendly bastion of introduced biocontrol through non-indigenous species of natural enemies. His papers challenged the safety of such measures with regard to indigenous natural enemies. The debate leads to the evolution of conserving indigenous natural enemies and the principles of conservation biological control.

Conservation Biological control (CBC) is based on manipulation of habitats to conserve naturally occurring predators, parasitoids and pathogens, thereby leading to natural pest suppression (Barbosa 1998). This approach has received much interest in the last decade due to the fact that (i) no new and exotic organisms are introduced, thereby addressing the major environmental concerns associated with classical biocontrol; and (ii) it would lay the foundation for sustainable, eco-friendly and low cost pest management which does not require the tedious and labour intensive mass rearing of natural enemies as in augmentative biocontrol. In this chapter we discuss the trends in biological control from classical biological control to the emerging field of conservation biological control and harnessing of biodiversity and its ecosystem services for pest suppression.

14.2 Classical Biocontrol – The Good the Bad and the Ugly

Classical biological control is the deliberate introduction of natural enemies to combat invasive pests. The natural enemies were generally sourced from the native region of the pest. Classical biological control has been in practice for more than a century. It is the most touted method to combat invasive species and once successfully established will help reduce pesticide use and provide a long term eco-friendly technique of pest management (Hoddle 2004). According to BIOCAT database, which has records of published introductions, 5,558 species have been introduced until 2006.

Greathead and Greathead (1992) developed and maintained a global database (BIOCAT) of classical biological control introductions of insects to control insects. An analysis of the BIOCAT database was done by Greathead and Greathead 1992. It can be inferred from the database that over 2000 species of parasitoids have been used against 543 species of pests in 200 different countries (Fig. 14.1) until 1992 which increased to 5000 species by the end of the century (Hill and Greathead 2000). The introduced biocontrol agents recorded success against many homopteran pests in contrast to lepidopteran and coleopteran pests. Within USA, in Florida alone around 60 organisms were imported and established until 2003 (Frank and McCoy 2007).

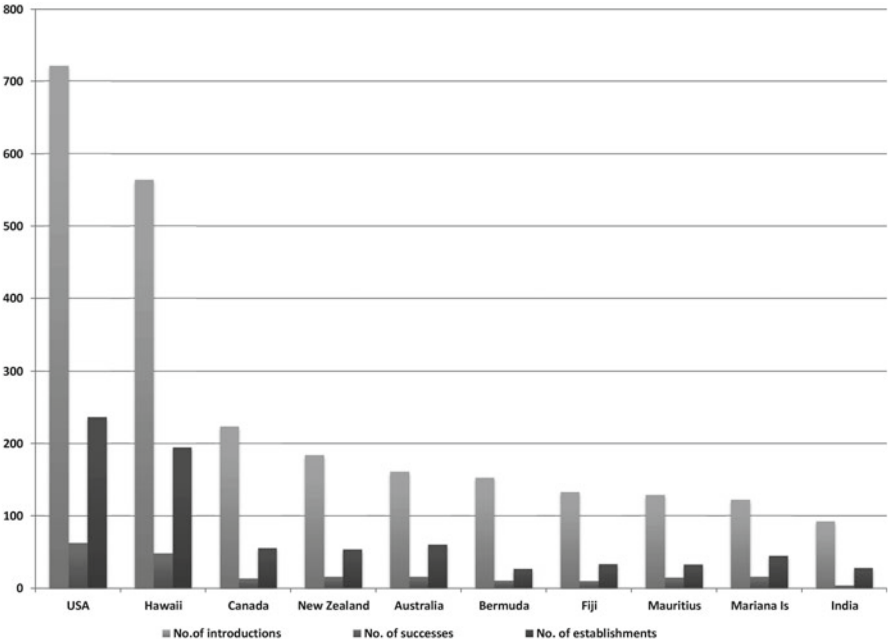

Fig. 14.1 Countries with the largest introductions (Based on Greathead and Greathead 1992)

Classical biological control programs are those that attempt to introduce natural enemies from the pest's home range, and they involve the following steps

1. *Correct identification of pest*: The foremost consideration for success of applied biological control as in field of biology is the correct identification of both the target pest species and their natural enemies and recognition of cryptic species or intra-specific entities. Collaboration with expert taxonomists is of utmost importance in all phases of all biological control programs. Inability to distinguish between closely related species have resulted in failure of many biocontrol introductions. One such example is with the citrus yellow scale *A. citrine* (Coquillett) that was confused with the California red scale *A. aurantii* (Maskell). But in some serendipitous cases, an introduction had effected successful control inspite of wrong identity as in the case of *Pseudococcus citriculus* Green and *P. comstocki* Kuwana which was correctly identified after achieving control of the latter.
2. *Identification of the pest's home range*: If the pest's home range is wrongly identified than it would misdirect the search for natural enemies leading to wastage of time, effort and money.
3. *Importation into a secure quarantine facility*: The host range and host specificity and presence of pathogens, parasitoids, and hyperparasitoids that attack the selected natural enemies must be studied well in order to assess the safety of its introduction into the new geographical location.
4. *Standardisation for mass rearing and release of natural enemies*: The agents must be released in many locations where the pest is present over a period until its establishment. Before release of any natural enemy, the behaviour of the natural enemy needs to be well studied and the factors favouring its buildup and establishment need to be supplemented for favourable establishment.
5. *Monitoring the community structure* and food webs alterations should be carried out to determine the impacts of the introduced natural enemy. There is a great need to study the host range of the natural enemy

In successful biological control programs against invasive pests, in addition to a reduction in pest densities, the community structure is restored to its original pre-invasion status (Bellows 2001; Headrick and Goeden 2001). Following their establishment, successful natural enemies can provide enduring pest control. Once established they can perpetuate without human management, and persist when pest populations are stabilized at very low densities (Bellows and Fisher 1999; Gurr and Wratten 2000).

14.2.1 Successes of Introduction and Augmentation

Classical biological control has many successful introductions that helped establish this field on solid ground. Van Driesche et al. (2010) reviewed 70 cases of classical biological control at ecosystem level and found that of the 21 cases that targeted insect pests 62% achieved complete control. Gurr and Wratten (2000) have comprehensively reviewed the measures of success in biological control. A clear advantage of a successful biological control program is the saving of sometimes huge amounts of pesticides, of which many are known to be harmful for numerous non-target insects, vertebrates and even humans. Worldwide the savings from use of biocontrol was estimated to be US$417 billion annually (Costanza et al. 1997). In the United States alone the economic value for biological control in suppression of pests was estimated at US$4.49 billion annually (Losey and Vaughan 2006). The benefit-cost ratio has been worked out for a few biocontrol programmes. Zeddies et al. (2001) estimated the benefit: cost ratio of biocontrol of the cassava mealy bug *Phenacoccus manihoti* Matile-Ferrero in Africa using the parasitoid *Apoanagyris lopezi* De Santis to be between 200 and 740 – a worthy number in support of biological control programmes.

In this era of increased global trade, invasive alien species that establish in a new range and proliferate due to absence of native natural enemies is of major concern. Introductory biocontrol is the right way to combat these pests. Though biological invasions have been more with weed pests (which has not been included in this chapter), many invasive insect pests have also been combated with introductory biological control. In Hawaii the Erythrina gall wasp *Quadrastichus erythrinae Kim*, has become so invasive and a threat to the endangered tree species. The parasitoid *Eurytoma erythrinae* was introduced from Africa, into Hawaii where observations showed ready establishment, rapid spread, and high impact of *E. erythrinae* (Gates and Delvare 2008).

14.2.2 Importation Biocontrol: Environmental Risks and Adverse Effects

Dramatic successes in importation and introduction of natural enemies and the associated cost savings (Hoddle 2004) have lead to a skewed perspective about its advantages. The impact of introductions was assessed by its success or failure and scarcely addressed the environmental impacts of such introductions (Babendreier 2007). Lynch et al. (2001) listed 80 records out of the 5,279 classical introductions of insects listed in BIOCAT with one or more non target effects.

Many retrospective studies on the effect of non target organisms show a minimal effect (Table 14.1). The more common impacts are low level parasitisation of non target insects by introduced bio-agents, displacement of local natural enemies and in some cases hybridization with local species. However, the genetic impacts from the viewpoint of post-release evaluation remain largely unexplored (Yara et al. 2010). *Torymus sinensis* Gates & Delvare, a parasitoid wasp was introduced from China into Japan to control the invasive Asian chestnut gall wasp, *Dryocosmus kuriphilus Yasumatsu*. Increasing numbers of F1 hybrids were detected every year reaching up to 22% and it was also observed that the indigenous late-spring strain of *T. beneficus Yasumatsu et Kamijo* was rapidly displaced by the introduced parasitoid (Yara et al. 2010).

Some of the introduced biocontrol agents have caused major impacts and are now considered as invasive species. Lynch and Thomas (2001) have classified five introductions to have a score of greater than 5 on a scale of 0–9 with reference to

Table 14.1 Minimum impact non-target effects of introductory Biocontrol

Introduced organism	Target	Non target effect	Reference
Pteromalus puparum L.	*Pieris rapae* (L.)	*Bassaris gonerilla Fabr.*	Barron et al. (2003, 2007)
Cotesia glomerata L. and *C. rubecula (Marshall)*	*Pieris rapae* (L.),	*Pieris virginiensis Edwards*	Benson et al. (2003)
Trichogramma brassicae Bezdenko	*Ostrinia nubilalis* Hubner	*Papilio machaon L.* *Clossiana selene L.* *Maniola jurtina L.* *Melanargia galathea L.* *Hipparchia alcyone (Denis & Schiffermüller)* *Cyaniris semiargus (Rottemburg)*	Babendreier et al. (2003a, b, c) and Orr et al. (2000)

non target effects. Two cases may be cited to emphasise the negative effects of introduced agents. The case of the coccinellid, *Harmonia axyridis (Pallas)*, introduced to control aphids and coccid pests becoming an invasive species (Berkvens et al. 2010) is well documented in North America (Koch et al. 2003; Koch and Galven 2008) and in Europe (Brown et al. 2008; Adriaens et al. 2008). The invasive coccinellid has become a threat to native coccinellids due to its multivoltine nature, high adaptability and resilience, ability to survive and develop on pollen alone (Berkvens et al. 2008), and lesser susceptibility to parasitoid *Dinocampus coccinellae* (Schrank) (Brown et al. 2008). The second case is the extinction of the coconut moth, *Leavuana iridescens Bethune-Baker* in Fiji (Howarth 1991). Therefore, applied biological control should not be viewed as a panacea, but treated with caution, as any control method and detailed studies into host range, biology and impact on non target organisms should be rigorously assessed before introductions and augmentations. Many authors have reviewed risks involved in biological control (Strong and Pemberton 2000; Louda and Stiling 2004; Parry 2009). But in general, assessing risks of biological control is difficult because it is hard to predict community and ecosystem impacts of introduced species which may further disperse (Simberloff and Stiling 1996).

14.2.3 Specialists vs. Generalists

Many studies advocate the use of highly host specific natural enemies in biocontrol programmes (Pearson and Callaway 2005) so that the non target effects may be minimized by the self decimation of the introduced agent when populations of the hosts are low. Nevertheless, generalists have also been indicated to effectively control pest populations which have been well reviewed by Symondson et al. (2002). Successful introductions have been made on the principle of host specificity and classic failures can be pinned to the broader host range of the introduced agent. Stiling and Cornelissen (2005) analysed the qualities that make up a successful bio agent from literature published on biological control. A meta analysis of 145 studies over a 10 year period indicated that multi-species releases of biocontrol agents were more efficient in reducing pest populations than single species releases and that generalist predators have greater potential to control pest abundance (Stiling and Cornelissen 2005).

14.3 Biodiversity and Biological Control in Ephemeral Ecosystems

Conservation biological control is defined as "the practice of enhancing natural enemy efficacy through modification of the environment or of existing pesticide practices" (Eilenberg et al. 2001). The last decade has seen a tremendous increase in this earlier neglected field of biological control. The impacts of conservation biological control are not as profound as classical biological control and to gain true believers is more difficult. The advantages of conservation biological control are many fold. The first and foremost being that it is low cost and requires smaller changes at the field level which even an individual grower can adopt. The concept tries to harness the on farm diversity of flora and fauna for the benefit of pest suppression. If adopted on large scale, temporally and spatially it would lead to sustainable land use. But conservation efforts have generally been directed towards perennial systems than ephemeral annual cropping systems.

14.3.1 Indicators of Biodiversity in Agricultural Ecosystems

Ideally, an indicator for biodiversity is a linear correlate to the entity or aspect of biodiversity under evaluation (Duelli and Obrist 2003). But the indicators we choose will depend upon the ecosystem services we want to harness. In agricultural pest management we are mainly concerned with conserving or enhancing biodiversity of the beneficial organisms through which we can achieve pest suppression. In contrast, in natural ecosystems, conservation of overall biodiversity is targeted regardless of its functions. Indicators of biodiversity are also used for agro ecosystem monitoring to determine the health of the ecosystem. Therefore, in agricultural ecosystems the indicators of biodiversity encompasses all natural enemies in the ecosystem. To maximize the services from biodiversity, species richness should be given as much importance as species abundance. But the approach that is being followed is aimed mostly at well studied taxonomic groups such as carabids, as an indicator of ecosystem health, for which identification keys are readily available. Very few studies focus on multiple species and their interrelationships.

Biodiversity assessment requires an understanding of the complex linkages within an ecosystem and indicators for biodiversity should simplify these linkages and should be reliable and transparent (Feld et al. 2010). Most assessments indicate directly to species richness and abundance but lack in indicating the ecosystem services provided (Feld et al. 2009). We are yet in the process of developing a benchmark or reference value for functional biodiversity.

14.3.2 Ecological Rewards of Biodiversity

The direct impact of biodiversity in agricultural landscape is pest suppression with reduced pesticide use. A sustainable agricultural landscape should have reduced inputs, high biodiversity index, reduced pest problems and ultimately economically viable yields. But in practice, the conservation practitioners are often isolated from agronomists leading to a large gap in translation of ecosystem services into economical yield increase. Multiple enemy-prey assemblages are often encountered in agro-ecosystems. Based on the additive or non-additive effects of the natural enemy assemblages, impacts may be positive (Ives et al. 2005; Duffy et al. 2007), negative or neutral.

Prey biodiversity increases the fitness of the predator guild. Harwood et al. (2009) found mixed species diets were optimal, compared with restricted diets of pests alone on carabid *Pterostichus melanarius Illiger* and the linyphiid spider, *Erigone atra Blackwall*, with respect to life cycle parameters and predator survival. Many earlier reports corroborate this fact (Luck et al. 2003). But with intensive farming practices and loss of plant biodiversity due to use of herbicides and herbicide tolerant crops, the alternate prey availability is lost.

Finke and Snyder (2010) list five effects of predator-prey biodiversity on pest suppression. Of these five, niche complementarity can be attributed for increased pest suppression in relation to predator diversity. The positive effects of natural enemy diversity are achieved through niche complementarity, where the predators and their prey are separated temporally or spatially within a microhabitat. Such resource partitioning is the mainstay of biodiversity impacts on pest suppression. Tylianakis and Romo (2010) evaluated effectiveness of diverse enemy assemblages under six hypothetical situations. They found very few studies that directly assess the various categories. Secondly, in diverse communities, functional facilitation could occur, where hunting by one predator facilitates preying by another. The third effect occurs when a single predator has higher efficiency in an array of predators and can cause greater pest suppression. But in this case aiming at conserving the most efficient predator species will have more impact than conserving overall biodiversity *per se*. In addition, phytophagy in many heteropteran predators can contribute to their resilience in ecosystems (Perdikis et al. 2011; Torres et al. 2010) and enhance the effects on biocontrol.

14.3.3 Ecological Penalties of Biodiversity

In multiple natural enemy assemblages the negative impacts are mainly due to competition between species for resource directly or indirectly (*interference*). Dilution effects of biodiversity are rarely examined for predator communities. *Intraguild predation* is another major factor for negative effects of biodiversity. Predators often feed heavily on one another (Fig. 14.2). Unlike the mechanisms mentioned above, intraguild predation can lead to a weakening of herbivore suppression at higher

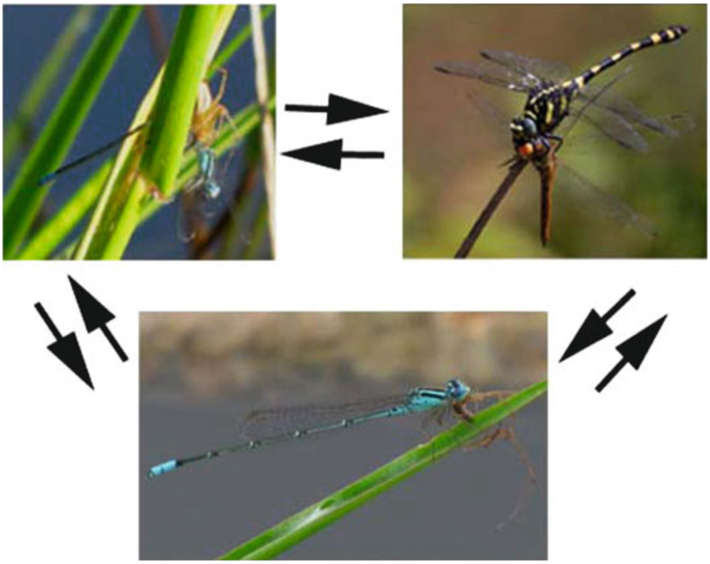

Fig. 14.2 Examples of intraguild predation observed in rice ecosystem

diversity levels (Tylianakis and Romo 2010). But in some cases a dominant invasive predator may increase ecosystem services (positive effect) while causing decline in numbers of native predators (negative effect). This was well demonstrated by Hogg and Daane (2011) with the exotic wandering spider *Cheiracanthium milde* effecting greater control of the grape leafhopper while negatively impacting some native spider species. The third negative impact occurs through cannibalism. Egg predation and cannibalism are common phenomena in predatory ladybirds (Kajita et al. 2010) and many other generalist predators. Many recent studies focus on the natural enemy traits that promote intraguild predation, functional redundancy, and niche complementarity. Such studies would help to identify the "right" kind of diversity and may ultimately improve the practice of conservation biological control (Straub et al. 2008).

14.4 Functional Significance of Biodiversity in Annual Crops – A Case Study of Cotton and Rice

The arthropod community in any arable land can be classified based-on their trophic behaviour. Guild structure of arthropod communities in agro ecosystems mainly consists of primary consumers or herbivores, secondary consumers consisting of predators and parasitoids of the primary consumers and the tertiary consumers or higher order guild predators and hyper parasitoids of natural enemies. The natural enemies are classified into the beneficial guilds in many cases. The herbivores or

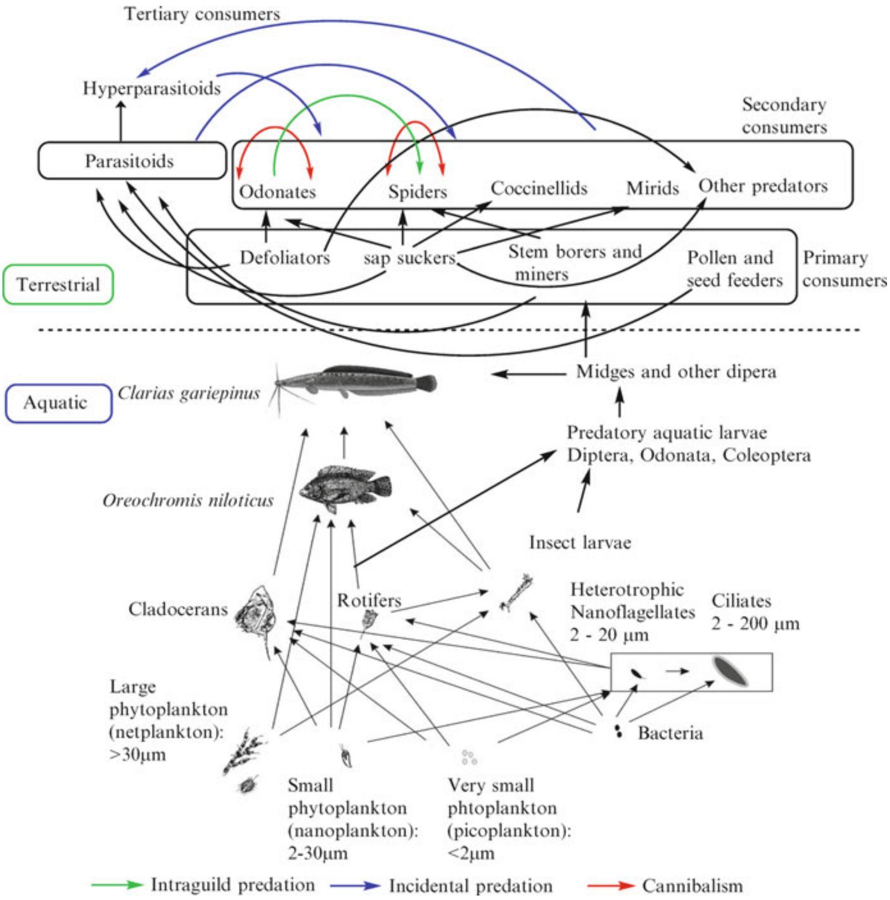

Fig. 14.3 Trophic linkages in irrigated rice ecosystem (Modified from d'Oultremont and Gutierrez 2002)

phytophages may again be classified based on their feeding niche as defoliators, stem tunnelers, sap suckers, pollen and seed feeders. A model functional group classification on rice is given in Fig. 14.3.

In a crop like rice, intensive research has been carried out to study the food web patterns. Schoenly et al. (1996) described 687 taxa consisting of pathogens, nematodes, mites, spiders, insects, snails and vertebrates and 10,000 consumer-resource trophic links. Rice crop is very unique in that it has three distinct ecological niches – the benthic, aquatic and the arboreal communities. Biodiversity assessment studies have mainly concentrated on the above water level diversity and some studies aim at studying the aquatic fauna. An overall biodiversity assessment integrating the effects of the components is required to understand the complex trophic relationships. Bambaradeniya et al. (2004a, b) recorded the total number of biota from the rice field ecosystem in Sri Lanka and found it to consist of 494 species of invertebrates belonging to 10 phyla and 103 species of vertebrates, while the flora included 89 species of macrophytes, 39 genera of microphytes and 3 species of macrofungi. Arthropods were the dominant group of invertebrates (405 species), of which 55 species were rice pest insects, and 200 species were natural enemies of pest insects. It has been established that conserving the natural enemy guild directly helps in pest suppression (Way and Heong 1994; Schoenly et al. 1996; Settle et al. 1996). In addition to invertebrate diversity rice paddies are also home to water birds (Acosta et al. 2010) fishes, frogs, molluscs, rats and snakes. Stocking fishes in rice paddies was found to reduce the dipteran population sampled from the rice fields (Frei et al. 2007a, b). With such diversity pest suppression should theoretically be well below threshold levels. Use of pesticides is the main threat for loss of biodiversity and reduced pesticide use in the early season helps colonization by predators (Way and Heong 1994). The impact of agricultural practices on aquatic invertebrate communities and in turn on the terrestrial colonization of predators has been lesser addressed (Wilson et al. 2008). Early season food linkage of filter feeders such as chironomids and entomobryids with predators was established using stable isotope analysis. It was later in the season that predator herbivore/pest linkages were observed (Park and Lee 2006). Natural enemies accounted for nearly 60% of all the terrestrial arthropod taxa collected in a rice field in Sri Lanka (Bambaradeniya and Edirisinghe 2008). Intraguild predation has been documented by Heong et al. (1990) with *Pardosa pseudoannulata (Boesenberg & Strand)* preying upon brown planthopper and its mirid predator, bug *Cyrtorhinus lividipennis Reuter.* But intraguild predation may also be helpful in sustaining populations of the beneficial guild during low availability of pest insects as prey (Way and Heong 1994). Though an annual crop, the high biodiversity of rice field arthropod fauna confirms the long term stability of the rice agro-ecosystem with respect to pests and natural enemies. The rice field biodiversity can also be taken as a classic example for other ecosystem services excepting pest suppression. Recent studies show that around 100 different aquatic species from rice fields are being collected and utilized by rural people during one season (Halwart 2006).

Augmentative releases of *Trichogramma* spp against leaf folder and stem borers of rice had limited success and the introductory release of the braconid *Allorhogas pyralophagus Marsh.* from Mexico into India has not been successful for control of the stem borer *Scirpophaga incertlas Walker* (Beevi et al. 2000). Recently the braconid, *Cotesia flavipes Cameron* was assessed as an augmentative biocontrol agent for the *Diatraea saccharalis* (F.), on rice at Texas, USA. It was observed that the parasitoids were unable to provide economic control in temperate-subtropical areas due to high rearing cost, low effective search rate, low parasitization rate and failure of the spring emerging parasitoids to find hosts. While augmentation and introductions have not been very successful, harnessing the high level of biodiversity available in rice fields and their capacity to control pests naturally, offers great scope for conservation biological control.

Cotton is a commercial crop and has been subjected to a maximum of crop management practices to combat its wide range of pests which includes an array of chewing and sucking pest complexes. Widespread use of transgenic crops also impacts the insect community structures in cotton. It has been observed that herbicide tolerant cotton has increased the use of conservation tillage practices which in turn is beneficial for biological control (Lundgren et al. 2009). Further, predatory heteropterans are the dominant group in the predatory guilds (Perdikis et al. 2011) and have been utilized for the natural control of these pests. The most abundant predator was *Orius insidiosus* (Say) on cotton crop (Sansone and Smith 2001) accounting for 69.5% of the predator numbers on non-chemically treated cotton (Greenberg et al. 2009). Other heteropteran predators reported were *Geocoris punctipes* (Say), *G. pallens* (Stal), *O. tristicolor* (White), *Nabis alternatus* Parshley and *Zelus renardii* Kolenati, in California and Arizona (Naranjo and Ellsworth 2002). The use of conventional insecticides on cotton highly reduced the populations of these natural enemies. Delaying the first insecticide application and use of more selective insecticides helped in conservation of natural enemies on cotton (Naranjo et al. 2004). *G. punctipes* acting together with *O. insidiosus* managed to keep the populations of sucking insect pests below economic injury level at all the phenological stages of the cotton plants (Solangi et al. 2008). Insecticidal GM crops had apparently no impact on the egg predators in the system as the egg load received in conventional and Bt cotton regimes were the same (Torres et al. 2006). Similarly, studies indicate comparable predation of non target pests in Bt- and non Bt- cotton (Sisterson et al. 2004; Naranjo 2005) which was corroborated by Romeis et al. (2006).

Beneficial insects comprised 42% of all arthropods collected in cotton and were dominated by predatory Coleoptera in Australia (Mansfield et al. 2006). An index was devised by Hoque et al. (2002) called the Beneficial Disruption Index (BDI) to measure impacts of insecticides on beneficial arthropods. The insecticidal treatments are scored on a scale of 0–9 based on the reduction they cause to beneficial biodiversity. Beneficial insects were more abundant in those fields subjected to a more selective insecticide regime (low BDI) than in fields that received broad-spectrum insecticide treatments (high BDI) but spider abundances were not affected by insecticides. Though the index has been developed for Australian cotton it should be modified and adopted to all cotton growing areas.

The white fly *Bemisia tabaci* (Gennadius) is another major sap sucking pest of cotton crop. In cotton, unlike in rice, most biodiversity assessments are with respect to a particular pest or a predatory group. A large assemblage of predators, parasitoids and fungi are known to attack *B. tabaci* in agricultural systems world-wide consisting of 114 species of predatory arthropods, nearly 50 species of parasitoids, and 11 species of naturally occurring fungi (Faria and Wraight 2001; Gerling 2001). While the above cases exhibited increased pest suppression with increased biodiversity of natural enemies a contrasting situation occurs with the tetranychid mite complex in Californian cotton. The Mites of the genus *Tetranychus* could be suppressed by four major predators – *Galendromus occidentalis* (Nesbitt), *Frankliniella occidentalis* (Pergande), *O. tristicolor*, and *Geocoris* spp., when tested singly (Colfer et al. 2003, 2004). But under field conditions, population increases of the mite occurred in spite of the presence of these predators. The reasons observed were intraguild predation of *Orius* by *Geocoris* and other predators, parsitisation of eggs of *Geocoris* and cannibalism observed in *Geocoris* (Rosenheim 2001, 2005).

Thus instead of having an additive effect, the predator diversity caused a dilution in pest suppression. On the whole, in a system like cotton with insecticide- dominated management systems, enhancing biological control will mainly depend on the use of selective insecticides and the manipulation of biodiversity for its positive effects on pest suppression.

14.5 The Number Game – Does It Really Count?

In assessing biodiversity, we take into consideration two aspects of the natural enemies – the species richness and abundance of each species. We expect theoretically that an increase in predators will be positively correlated with pest suppression. The abundance of each species and the evenness of distribution of the natural enemies would differ in the field. In the field often one species may dominate over the others. Most of the multiple enemy effects studied uses an equal distribution of the natural enemies (Finke and Snyder 2010; Straub and Snyder 2008). This may not apply in all cases. In north-west Europe the green spruce aphid *Elatobium abietinum* (Walker) had a variety of invertebrate predators – coccinellid and cantharid beetles, syrphid larvae, brown lacewings, spiders and harvestmen. Predator densities were most abundant at low altitudes which did not correspond with *E. abietinum* densities, but was correlated to climate and availability of alternate prey (Straw et al. 2009). Intraguild interactions in generalist predator assemblages can significantly affect their effectiveness on pest suppression (Snyder and Ives 2001; Prasad and Snyder 2004; Finke and Denno 2004). Within the guild the number game is important in that the effectiveness of the numerically dominant predator species and its interactions with subdominant species are taken into consideration. Theoretically we expect that the effects are summative (Snyder and Ives 2003) or more than the summed impact. The increased impact may be due to resource complementarity (Wilby et al. 2005; Casula et al. 2006) or functional complementarity (Straub and Snyder 2008). In contrast intraguild predation and mutual interference may also dilute the effects of the guild in pest suppression (Lang 2003; Prasad and Snyder 2004). In some cases though observed parasitisation was lowered by predators it did not negatively impact pest suppression (Snyder and Ives 2003).

Beyond the numerical abundance, the effectiveness of the predator also plays a role (Lundgren et al. 2006; Bologna 2007). The numerically dominant predator may not be the most effective predator in the field. Therefore in addition to the abundance an important criterion to be considered is the taxonomic identity of the pest (Moreno et al. 2010). In many cases the outcome of predator diversity may therefore be context dependant (Cakmak et al. 2009; Tylianakis and Romo 2010). The impact of natural enemy diversity on pest suppression is not merely a direct effect of the richness and abundance of predators, but a matrix of efficiency of predators, trophic interactions and response to prey density. The suppression of *Aphis glycines* on soybean was successfully effected by coccinellids though a complex of predators and parasitoids are reported for this pest (Costamagna et al. 2008) Further the presence of alternate prey or prey diversity also plays an important role (Letourneau et al. 2009). It can be inferred from the various studies that mere species richness or abundance cannot indicate increased pest suppression which will be influenced by the efficiency of dominant predator and its intraguild interactions.

14.6 Enhancing Ecosystem Services

14.6.1 Provision of Refuges

Refuges have long since been advocated for early colonization and conservation of generalist predators. Predator refugia are provided in the form of intercrops, cover crops, field margins, hedgerows, fencerows, windbreaks and the like. Beetle banks have been successfully deployed in the United Kingdom for over a decade to enhance within-field generalist predator diversity and are of value as refuge habitat for predatory invertebrates (Sotherton 1995; Thomas et al. 2002). Refuge provides the natural enemies with nectar sources, pollen and alternate prey in addition to providing shelter habitat.

Nectar resources: Most natural enemies including predators require sugar sources. Nectar feeding can increase the survival of parasitoids 20-fold (Bianchi and Wackers 2008), enhance fecundity, increasing general reproductive fitness (Baggen and Gurr 1998; Schmale et al. 2001; Winkler et al. 2006). Parasitoids were more abundant in habitats in which flowers are in bloom than in nearby habitats without flowers. Selection of flowers is also an important factor. Not all flowers are suitable to parasitoids. Before deployment of these plants in conservation biological control we need to confirm the effects on natural enemies. Vattala et al. (2006) studied the effects of seven flower species on the longevity of *Microctonus hyperodae*, a parasitoid of the Argentine stem weevil. Only two species of the plants studied were able to increase longevity. These factors have a cumulative impact on parasitism efficacy, underscoring the vital role of food availability in biological pest control. Another cautionary evidence to be considered is the effects of floral resources on the fourth trophic level. Buckwheat a commonly used nectar and pollen source was found to increase hyperparasitisation of a parasitoid (Araj et al. 2008) and a predator (Jacometti et al. 2010). It was observed that nectar of buckwheat increased the searching efficiency of both the third and fourth trophic parasitioids (Araj et al. 2011). In such cases the summative effect of third and fourth trophic level on pest suppression should be analysed. The distance from flower source will also affect the movement of parasitoids and in turn the parasitisation level (Lavandero et al. 2005)

Pollen sources: Vandekerkhove and Clercq (2010) observed 80% survival of nymphs of mirid predator *Macrolophus pygmaeus,* and advocated release of mass reared predators on pollen producing plants before pest population build-up on the main crop as a preventative release strategy similar to the one suggested by Cocuzza et al. (1997) for *Orius* spp.

Alternate prey: The widely recognized importance of heteropteran predators on cotton crops led to the evaluation of their potential in CBC using other crop or non-crop host plants. Loya-Ramírez et al. (2003) showed that interplanting canola, alfalfa, vetch and sanfoin supported high populations of heteropteran predators, *Geocoris* spp in particular accompanied by a significant migration toward the intercropped cotton. In comparison, treated cotton attained the lowest number of predators. This approach was further explored by Prasifka et al. (1999). They used mark and recapture methods to record the movements of the predators including *Orius* in adjacent plantings of cotton and grain sorghum. Planting molasses grass, *Melinis minutiflora* Beauv. between the rows of maize significantly increased the parasitism of maize stemborer larvae by *Cotesia sesamiae* (Cameron) (Khan and Pickett 2004).

Shelter: Shelter habitats aim to provide suitable conditions for overwintering, aestivation, reproduction and a general safety from anthropogenic disturbances in farmland (Griffiths et al. 2008). They could be a source of alternate prey and also add to general biodiversity of crop lands. Field margin refuges around arable fields have been shown to enhance the within-field activity and density of polyphagous predators (Holland et al. 2002). In rice fields, spiders which are the most abundant predators, tend to take refuge on field bunds. Studies have shown that addition of straw bundles placed in sorghum fields to attract spiders and then transferred to rice fields significantly reduced stem borer and leaf folder populations (Tanwar et al. 2011).

Banker plant systems are a technique of conservation biological control wherein a non-crop plant is deliberately infested with a non-pest herbivore as an alternate host for a parasitoid or predator of the target crop pest (Van Driesche et al. 2008; Pickett et al. 2004). A specific natural enemy may also be established on the banker plants to target a specific pest (Frank 2010). In contrast to habitat manipulations for conservation biological control that increase diversity and fitness of natural enemies in general, banker plant systems are more specific and help to conserve a single natural enemy species that is selected for its efficacy in controlling target pests with a particular food resource. In situations where a single natural enemy has more impact on pest suppression such a tactic can be used. Kehrli et al. (2005) advocates the use of mass- emergence devices in which the plant material containing the host and its parasitoids can be collected and stored from which the generally smaller parasitoids can emerge through a selective tissue filter of appropriate mesh size.

Avian insectivores could also be used for pest suppression. In organic farming where pesticide use is avoided it would be a boon to harness the power of nature to control pests. Jones and Sieving (2006) reported that intercropping sunflower effectively increased avian insectivore numbers and insect-foraging time in organic vegetables. Tree branches and T- shaped bamboo sticks as inanimate perch and sunflower and sorghum with goose neck as animate perch were evaluated by Gopali et al. (2009) in a chick pea ecosystem. They found that sunflower acted as most suitable live bird perch for major predatory birds in chickpea ecosystem providing rigid support for alighting insectivorous birds right from vegetative stage till maturity of the crop and reducing the larval number in the shortest time. Tremblay et al. (2001) found higher density of insect populations on corn where birds were excluded.

Gunathilagaraj (1996) observed that rice yield from fields with owl perches was two times more than that of the fields without owl perches and a dramatic increase in rat burrows was noted when owl perches were removed.

14.6.2 Provision of Artificial Food

Wade et al. (2008) reviewed literature on artificial sprays spanning more than four decades. They found that the density of natural enemies increased in 87% of cases and pest populations declined in 47%. But the analysis revealed the lack of linkage between the sprays and profit gained. The food sprays consisted mainly of sucrose as carbohydrate source with some protein sources added. The response of different taxa was found to be different for different combinations of food sprays. Artificial food sprays consisted of carbohydrates such as honey, syrups, unrefined sugars, sucrose and other sugars; proteins comprised of hydrolysates or non-hydrolysated yeast, animal products and individual amino acids. Rarely lipids, minerals and vitamins were used as additives (Wade et al. 2008). Many commercial formulations are also available like Envirofeast. A well researched fact is the effect of extrafloral nectar on foraging behaviours of spiders (Taylor and Pfannenstiel 2008; Taylor and Bradley 2009; Patt and Pfannenstiel 2009). Patt et al. (2011) found that binary mixtures of sucrose plus baker's yeast or toasted soy flour were more effective in promoting growth and development in prey-limited spiders. Artificial sprays have great scope in conservation biological control, but require more focused studies on taxa based mixtures and their efficacy vis-à-vis economics

14.6.3 Chemical Ecology Basis for Enhancement

Chemical ecology has been the basis of the Push-Pull strategies advocated for crop pest management. Plants colonized and damaged by herbivorous insects produce plant volatiles some of which may be attractive to the next trophic level *i.e.* the natural enemies of herbivores. The plant volatiles may directly help in defense of the plant or indirectly attract its natural enemies (Kessler and Baldwin 2001, 2004). The plant volatiles emitted differ from plant to plant and even within cultivars. Lou et al. (2006) found that parasitisation by *Anagrus nilaparvatae Pang et Wang* on *Nilaparvata lugens Stal.* eggs was influenced by volatiles emitted by rice varieties. The use of synthetic plant volatiles in conservation biological control has been recently reviewed by Khan et al. (2008). Rapid strides in study of secondary metabolites and the possibility to modify their production metabolism could allow appropriate semiochemical cues to be generated by plants at certain growth stages. The present status in plant molecular techniques can be used to identify plant defense genes associated with external plant signals that induce biochemical pathways which could be 'switched on' prior to insect attack (Khan et al. 2008). Tryptophan, a substance present in the honey

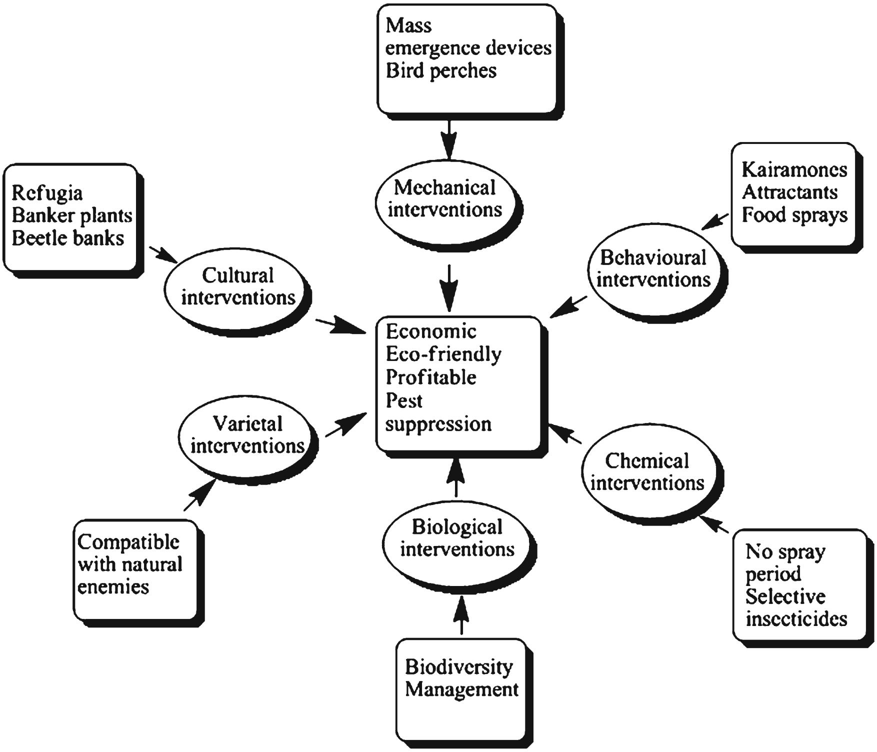

Fig. 14.4 Integrating conservation biological control in the IPM framework

dew of aphids has been effectively used to attract the predator, *Chrysoperla carnea* (Stephens). The use of plant defense chemicals for enhancing biological control is an interesting and upcoming field with enormous possibilities in the present context of rapid strides in plant molecular analysis. The translation of research findings into practical technologies in chemical ecology needs to be focussed on.

14.6.4 CBC and Integrated Pest Management (IPM)

The integration of CBC as an integral part of integrated pest management system will be the stepping stone for its successful extension to farming systems. The agricultural scenario today is plagued by a double pronged attack of invasive species and eruptions in indigenous pests due to the rampant misuse of pesticides resulting in resistance to insecticides, resurgent pest populations and emergence of new key pests. The two important components of IPM *viz.,* chemical and biological control should be integrated with cultural techniques to conserve natural enemies (Fig. 14.4). The best bet approach will be to reduce broad spectrum insecticides pesticides, and judicious use of selective pesticides. The time of pesticide application should be such that early colonization of natural enemies is favoured. Delaying early season application of insecticides in rice helped colonization of natural enemies and in turn execute pest suppression (Settle et al. 1996; Sigsgaard 2007). Pingali and Gerpacio (1997) observed that withholding insecticides early in the crop season up to 40 days reduced further insecticide requirement due to the abundance of predator populations in the paddy fields. When combined with kairomonal interventions, varietal manipulations and habitat management to favour natural pest suppression we would be able to achieve economically viable pest management at the farm level and preserve environmental health of the ecosystem.

14.7 The Road to Take

The future of biological control in all forms should be based on rigorous studies on risks of introductions, economics and cost benefits of biocontrol. Trophic linkages extending to the third and fourth trophic level should be given importance and effect of crop management practices on these linkages should be studied. Integrated pest management taking into consideration the ecological principles for harnessing the in-situ biodiversity for pest suppression will be the future of sustainable management practices. But lacunae exist in assessment of biodiversity, trophic linkages and the methods of conserving and enhancing the beneficial guild. The pest management systems have to be sculpted contextually to suit different ecosystems. Farmers' perceptions need to be changed through on farm field trials which would influence the social learning process. Scientific research alone cannot guarantee the adoption of conservation biological control. Economic incentives to reward farmers for undertaking the transition to conservation biological control can expand implementation of CBC (Cullen et al. 2008). Strengthening extension methods with knowledge management techniques wherein required information can be delivered to the farmers at the appropriate time in accessible ways will go a long way in giving impetus to pest management strategies. Conservation tactics must be extended over larger areas in order to be successful for which regional policy planning plays a crucial role. Lastly as Fiedler et al. (2008) puts it, habitat management should not be considered as a stand-alone practice and should be viewed holistically for the multiple ecosystem services it can afford.

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Chapter 15
Postharvest Biocontrol – New Concepts and Application

Neeta Sharma, Swati Sharma, and Basant Prabha

Abstract Fruits and vegetables are grown and consumed worldwide as they are source of nutrients and fiber in human diet. They are consumed either fresh or after processing and are produced in farms with conventional organic agricultural production methods or under intensively managed environmentally controlled glasshouses. It is not known when cultivation of first plant took place however domestication of plant species provided innumerable plant products required to satisfy the needs of living beings. As the plant species were selected for higher yield potential and better quality, the levels of resistance to biotic as well as abiotic stresses were depleted over long periods. Consequently, plant pathogenic fungi continue to inflict considerable losses in harvested yield and also reduced the aesthetic value and storage life of agricultural crops. Losses in quantity and quality of agricultural produce due to postharvest diseases have been proposed to be as high as that from field crop diseases, though precise estimates have not been made so far. However, considerable economic losses up to an extent of 10% are recorded in developed world which are higher in less industrial regions. Despite recent technological advances in the development of resistant varieties of crop plants using genetic engineering approaches, and with discoveries of novel, site specific fungicides and continually evolving crop protection practices, fungal plant pathogens continue to find opportunities to destroy crop plants. The challenges for producers in managing these diseases are ever increasing as consumer demand for year round production of fresh products with reduced or no pesticides residues continue to grow. Concerns over the potential impact of disease management practices including the use of fungicides on the environment or on consumer health have promoted producers to examine alternatives to combat these postharvest diseases.

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15.1 Initial Step

The concept and use of bioagents was restricted only against soil and foliar pathogens (Cook and Baker 1983) with only one example of the biocontrol of postharvest disease of fruit or vegetable. This was an only example in which *Trichoderma* was used to control Botrytis rot of strawberry (Tronsmo and Dennis 1977). Plant pathogenic fungi attacking and invading perishables range from biotrophs to necrotrophs. Biotrophic fungi, such as powdery mildews, only grow and reproduce on the living host plants, where as *Botrytis cinerea* are opportunistic fungi that grow and reproduce on plant debris or organic matter but can rapidly invade wound/senescing plant tissue. An understanding of events leading from initial infection to colonization and reproduction of the pathogen and life cycles of the pathogen are crucial to the use of successful use of any disease management strategies. Prevention of infection, reduction in colonization of host tissues, or reducing sporulation or survival of the pathogen, can each provide a level of disease control using biological control agents.

The concept of colonizing wounds with an antagonist bacterium is clear, but little interest developed in this area until 1980. Earlier efforts to control postharvest diseases involved the use of *Bacillus subtilis* (Pusey and Wilson 1984) and *Pseudomonas cepecia* (Jainieswicz 1998).This seminal work provided the initial ideas and principles that, over the ensuing 25 years fostered a wealth of research and product development around the world (Wisniewski et al. 2007).The basic rationale underlying these efforts was to reduce the use of synthetic chemicals on harvested commodities which was strengthened by reports from US National Research Council (1987). This report indicated that fungicides constitute 60% of oncogenic risk among all pesticides. Loss of these chemicals would have an adverse economic impact on the production of some crops because of lack of viable alternatives.

The use of biological control agents (BCA's) in a post harvest environment holds special promises in comparison to other diseases. In the post harvest environment parameters such as temperature and humidity are rigidly controlled and can be taken into account when selecting a suitable candidate as BCA. Also, harvested commodities present a more concentrated target for the application of bioagents. Thus, the regulated environment, the ability to target the application of the biocontrol agent and the high value of the harvested commodity together suggest that the use of BCA's would have an excellent chance of success.

Post harvest environment may be well suited and favorable for the development of biocontrol products but certain facts have to be established before their commercial use (Sharma et al. 2007a).

The characteristics of an ideal antagonist for the post harvest environment should be as shown in Fig. 15.1.

15.1.1 First Generation of Yeast Biocontrol Products

In the early years of research, several yeast antagonists were identified that had commercial potential. The first yeast antagonist, strain US-7 of *Candida guilliermondii,*

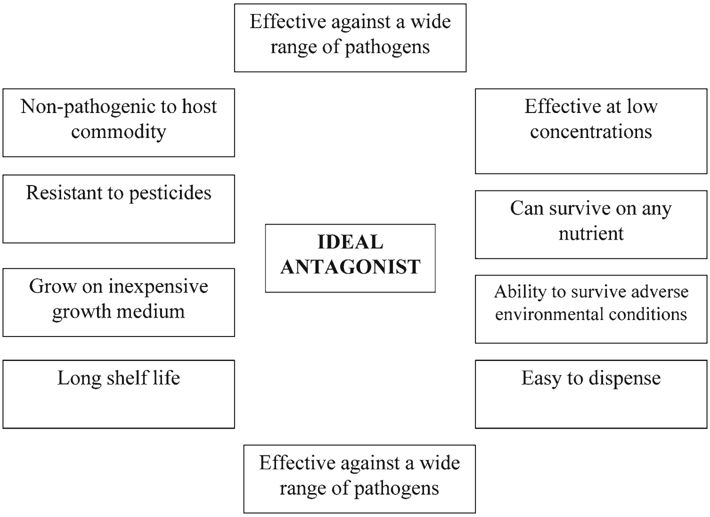


Fig. 15.1 The characteristics of an ideal antagonist for the post harvest environment

was originally misidentified as *Debaryomyces hansenii.* This caused some confusion in the patenting process and emphasized the need to have at least two conforming identifications by reputable yeast taxonomic services. It also emphasized the weakness of using physiological tests as the basis for making taxonomic determinations. Using the criteria outlined in figure (1), the decision was made, however, to abandon the commercialization of US-7 because other isolates of *C. guilliermondii* had been reported in the medical literature as pathogenic to humans. This decision was made despite the fact that US-7 showed excellent biological control activity and did not show any pathogenicity in Level I toxicology studies. Instead, focus on the commercialization of *Candida oleophila* (Strain I-182) was based on its superior biocontrol activity and the fact the species did not grow at 37°C. The use of this organism was also protected by a patent.

Another critical ingredient for achieving the goal of a commercial product was the relationship that was developed with a small venture-capital company, Ecogen. This was a US-based company, with a subsidiary in Israel, interested in biological control products. It was the relationship with Ecogen that provided the bridge between theory and practice. They were able to develop a formulated product, based on growing I-182 on a low-cost substrate of industrial by-products, which had an un-refrigerated shelf-life of over 1 year (Wilson and Wisniewski 1994). Ecogen also provided critical monetary support for conducting semi-commercial pilot tests on apples and citrus in the USA and Israel, respectively. Semi-commercial packing lines, allowed us to conduct large-scale studies and determine the performance of a formulated product under more realistic conditions.

The patent on *C. oleophila* was licensed to Ecogen and thus the first yeast-based postharvest biocontrol product was launched under the trade name of Aspire™ beginning in 1995. After registration, commercial evaluation of Aspire™ continued in order to better understand how to adapt the use of the product to different packing-house environments and to different commodities (Droby et al. 1998). This led to continued research on how to enhance the reliability and efficacy of the product and established the foundation for a second generation of postharvest biocontrol products (Droby et al. 2003b; El Ghaouth et al. 2004). A parallel but completely independent programme on postharvest biocontrol focusing on bacterial antagonists was being conducted in the USDA-ARS laboratory during this time by Dr Janisiewicz. This effort, in collaboration with the US-based company Ecoscience, led to Postharvest Biocontrol development of Bio-Save™, based on an isolate of *Pseudomonas syringae*.

15.1.2 Second Generation of Yeast Biocontrol Products

A broader concept of biological control would be needed if postharvest biocontrol was going to be commercially successful. Plant pathologists have adopted the entomologists definition of biocontrol, which involves the control of one organism with another organism. But, a plant disease is not an organism, it is a process. Therefore, the biological control of a plant disease shall be defined as 'control of a plant disease by a biological process or the product of a biological process'.

Using this broader definition of the biological control of plant diseases, a number of avenues become available for developing effective, commercially successful biological control products and practices: (i) the classical idea of using an antagonist; (ii) innate or induced resistance, which is a biological process; and (iii) natural antimicrobials, which are the product of a biological process. While some of these approaches are being pursued without commitment to a formal paradigm, it is important to conceptualize the paradigms that drive scientific research, in order to overcome limitations and expand possibilities. This new paradigm of biological control was the primary concept that was used to develop a second generation of postharvest, biocontrol products.

The main objective in developing a new product was to address the poor ability of Aspire™, and other postharvest biocontrol products, to control pre-established and latent infections. By using a combination of natural products along with a yeast antagonist this problem was overcome. The focus also shifted in finding new yeast antagonist in order to enhance patent opportunities and attract new industrial partners. These research efforts led to the development of two new products, whose main components consisted of the yeast antagonist *Candida saitoana* and a derivative of either chitosan (Biocoat) or lysozyme (Biocure). Both of the compounds had been tested worldwide and shown to have strong eradicative activity. The two commercial products also contain other additives such as sodium bicarbonate. The additives were found to enhance control efficacy to levels equivalent to that found with available postharvest fungicides. Patents have been issued to cover this technology (El Ghaouth and Wilson 2002; Wilson and El Ghaouth 2002). While this research was initially conducted under a CRADA with American Cyanamid and Postharvest Biocontrol then MicroFlo (a subsidiary of BASF), the technology has now been licensed to Inova Technologies and is awaiting registration by the US Environmental Protection Agency.

A more recent product has taken the approach of preventing postharvest decay by application of a yeast biocontrol agent to flowers and fruit in the field, several times throughout the growing period. This approach also addresses the problems of pre-established and/or latent infections. The product is based on the use of a heat-tolerant strain of *Metschnikowia fructicola* and is marketed under the name ProYeast-ST and ProYeast-ORG in Israel by the company AgroGreen. It has been shown to be effective against rots caused by *Botrytis, Penicillium, Rhizopus* and *Aspergillus* on strawberries (Karabulut et al. 2004), grapes and citrus.

15.2 Mode of Action of BCA's

Research to elucidate whether these organisms could potentially be used as biological control agents to combat diseases has intensified over the past 20 years, and this has led to the commercial development of several registered microbial agents for disease management of perishables (Table 15.1). Following their initial discovery, the development of these biological control agents from a research laboratory to a commercial product is quite a daunting task.

Information needs to be gathered on the efficacy and mode(s) of action of the agent, as well as on the survival and spread and potential toxicity to non-target species (Harman 2000). Furthermore, formulation, product stability and shelf-life studies are also required (Harman 2000). The quest to identify biological control agents for plant diseases has, however, provided tremendous opportunities for plant pathologists, mycologists, geneticists, biochemists and molecular biologists to interact, work together, and gain a better understanding of how these microbes can reduce fungal pathogen development and disease. Here, we summarize recent scientific developments and illustrate how modern research techniques are being applied to better understand the way in which biological control of diseases using fungi and yeasts is achieved.

There is no single unifying feature that can be identified among the biological control agents that would explain how they manifest themselves as antagonists to disease-causing fungi. However, several important features have emerged from scientific investigations concerning their modes of action (Fig. 15.2), and these are discussed later.

15.2.1 Role of Mycoparasitism in Pathogen Biocontrol

Reports dating back to the 1930s first showed that fungal pathogens could be infected or parasitized by other fungi (mycoparasites) (Howell 2003). There are several fungal parasites of plant pathogens, including those that attack sclerotia (e.g. *Coniothyrium minitans*) while others attack living hyphae (e.g. *Pythium oligandrum*) and a

Table 15.1 Commercially available fungi and yeast biological control products to manage diseases

Product trade name	Microorganism(s) contained	Fungal disease target	Manufacturer or distributor
AQ 10	Ampelomyces quisqualis M-10	Powdery mildews	Ecogen, USA
Binab	Trichoderma spp.	Root rot, wilt	Binab, Sweden
Biofox C	Fusarium oxysporum (non-pathogenic)	Wilt	S.I.A.P.A., Italy
Biofungus, Superesivit	Trichoderma spp.	Root rot, wilt	Bioplant, Denmark
Contans WG, Intercept WG	Coniothyrium minitans	Root rot	Prophyta Biologischer, Germany
Fusaclean	Fusarium oxysporum (nonpathogenic)	Wilt	Natural Plant Protection, France
KONI	Coniothyrium minitans	Root rot	Bioved, Hungary
Polyversum	Pythium oligandrum	Root rot	Biopreparaty, Czech Republic
Primastop (Prestop Mix)	Gliocladium catenulatum	Root rot, wilt	Verdera Oy, Finland
Root Pro	Trichoderma harzianum	Root rot	Efal Agri, Israel
Root Shield, Plant Shield, T-22 Planter box	T. harzianum T-22	Root rot	Bioworks, USA
Soil Gard	Trichoderma (Gliocladium) virens GL-21	Root rot	Certis, USA
Sporodex	Pseudozyma flocculosa	Powdery mildews	Plant Products, Canada
Trichodex	T. harzianum	Grey mold	Makhteshim Chemical Works, Israel
Trichopel	Trichoderma spp.	Root rot	Agrimm Technologies, New Zealand
Trieco	Trichoderma viride	Root rot, wilt	Ecosense Laboratories, India

single fungal pathogen can be attacked by multiple hyperparasites. For example, *Acremonium alternatum, Acrodontium crateriforme, Ampe/omyces quisqualis, Cladosporium oxysporum,* and *Gliocladium virens* are just a few of the fungi that have the capacity to parasitize powdery mildew pathogens (Kiss 2003).

The most widely studied fungi in this regard were different species of *Trichoderma* (Fig. 15.3). *Trichoderma* hyphae encircle and coil around the host which results in first lose then dense coiling around host hyphae (Fig. 15.3). In the late 1980s, yeasts were discovered that could reduce the growth and spore production of plant pathogenic fungi (Avis and Belanger 2001). These interactions between plant pathogen and mycoparasite require that they be in close physical proximity to each other. As a result, most observations have been made from Petri dishes or detached plant tissues under controlled conditions. For example, *Ampelomyces quisqualis* (AQ 10) was shown to be parasitic on powdery mildew hyphae and conidia (Rotem 1999),

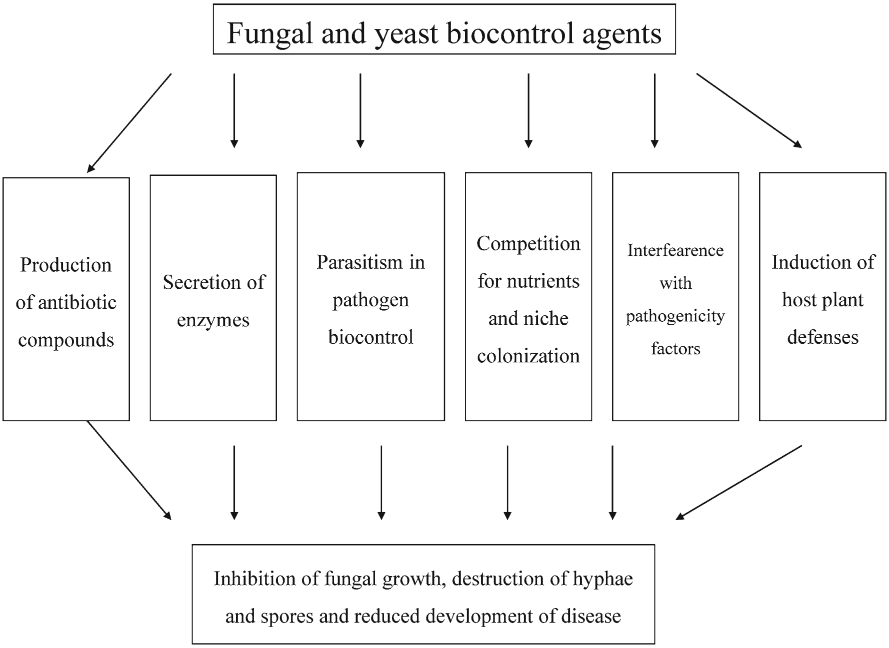
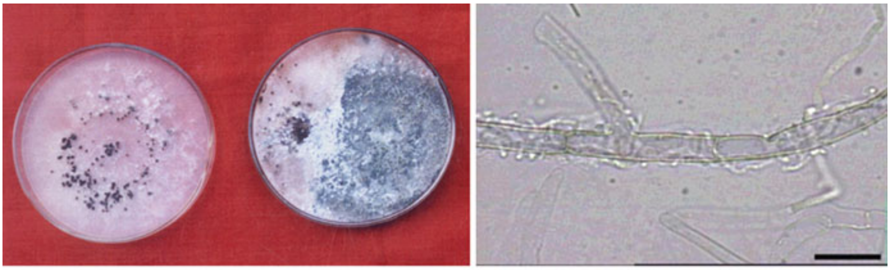


Fig. 15.2 Mode of action of biocontrol agents

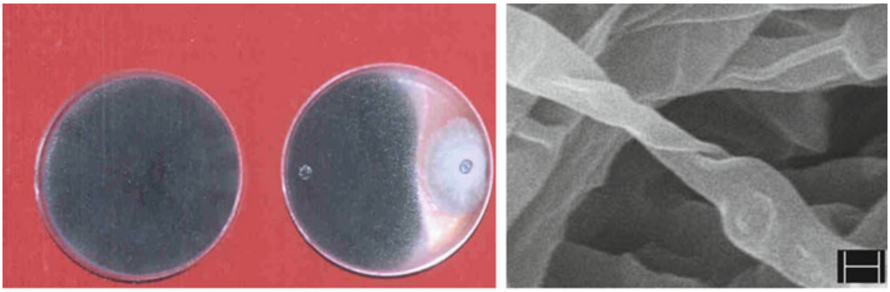

Against *Rhizoctonia solani* Kühn (left)

Hyphae of *Trichoderma* sp. coils around the hyphae of pathogen

Fig. 15.3 Hyperparasitism by BCA

whereas *Coniothyrium minitans* (Contans) is mycoparasitic on sclerotia of *Sclerotinia* spp. (Budge and Whipps 2001) and *Pythium oligandrum* (Polyversum) is mycoparasitic on other *Pythium* spp. and some other fungi (Benhamou 1999). These findings have now been extended outside the confines of the laboratory to vegetable crops grown under glasshouse and field conditions, and these specific microbial agents have been shown to reduce disease development significantly and are now registered products.

In many cases, however, the importance of mycoparasitism is difficult to demonstrate conclusively in situ, even though it is evident under experimental conditions.



Against pathogenic fungi (left)

Scanning Electron Micrographs showing distorted empty hyphae

Fig. 15.4 Antibiosis by BCA

For example, although *Trichoderma* spp. were among the first mycoparasites to be described, their role in reducing infection and colonization of host tissues through mycoparasitism has been difficult to confirm (Howell 2003). There are technical difficulties in making microscopic observations to demonstrate mycoparasitic activity in situ, such as at the soil–root interface, even with the availability of fluorescence imaging and differential staining methods. The recent development of a monoclonal antibody, prepared against a b-1, 3-glucanase enzyme, specific to the genus *Trichoderma* and related species, showed use in staining the cell walls of the mycoparasite but not the fungal pathogen *Rhizoctonia solani*, and could therefore be used to study dual interactions (Thornton 2002). The antibody could also be used in a combined baiting ELISA technique to detect *Trichoderma* spp. in composts. Such techniques should significantly facilitate an understanding of the role of mycoparasitism in biological control studies.

15.2.2 Production of Antibiotic Compounds by Fungi and Yeasts

The production of antibiotic compounds is characteristic of many effective fungal and yeast biocontrol agents, and can be shown both *in vitro* (Fig. 15.4) and *in vivo* (Sharma and Awasthi 2007). Species of *Trichoderma* and *Gliocladium*, as well as the yeast *Pseudozyma*, which are currently all registered biological control products, are known to produce several secondary metabolites with broad-spectrum antimicrobial activity. For example, gliotoxin and gliovirin are well-described antibiotics (among others) produced by *Trichoderma* (*Gliocladium*) *virens* (Howell 2003). The antibiotics produced by *Pseudozyma flocculosa* are a mixture of fatty acid-containing derivatives that affect membrane permeability of the target organisms, thereby inhibiting their growth (Avis and Belanger 2001).

Conclusive evidence for the role of antibiotics in biocontrol-mediated disease suppression has required the development of antibiotic-minus mutant strains with a subsequent evaluation of their efficacy. Mutants of *T. virens* unable to synthesize gliotoxin and gliovirin were shown to have lost their capacity to control root-infecting fungi such as *Pythium* (Howell 2003). In some instances, disease-suppressive activity was directly correlated with the timing and amount of antibiotic produced (Wilhite and Straney 1996). However, not all studies aimed at generating antibiotic-minus mutants have provided clear results, as there are reports of *T. virens* mutants lacking gliotoxin production, which provided significant control of *Rhizoctonia* root rot (Howell 2003). Therefore, other attributes of the biological control agent *Trichoderma* clearly also play a significant role.

15.2.3 Role of Extracellular Enzyme Production by Fungi and Yeasts

Secretion of hydrolytic enzymes, particularly chitinases and glucanases, is a feature common to many effective biological control agents (Pal and Gardener 2006; Sharma 2007). Strains of *Trichoderma*, for example, are efficient producers of lytic enzymes, and many are used in commercial enzyme manufacturing.

Using molecular biology techniques, the genes encoding chitinases, glucanases and proteinases have been cloned and sequenced from *Trichoderma* species. Mutant strains with disrupted activity of ech 42, a chitinase-encoding gene, were shown to be less effective as biocontrol agents against *Rhizoctonia solani* and *Botrytis cinerea* compared with wild-type strains (Punja and Utkhede 2003). Conversely, the over expression of several genes encoding enzymes such as chitinase (ech 42, chit 33), endoglucanase (egl1), and proteinase (prb1), in transformed *Trichoderma* spp. improved the antagonistic potential of the agent against pathogens such as *Rhizoctonia* and *Pythium*, both *in vitro* and *in vivo* (Punja and Utkhede 2003). A mutant strain of *T. harzianum* with an enhanced ability to hydrolyze pustulan (a polymer of b-1, 6-glucans) showed enhanced production of chitinase and b-1, 3- and b-1, 6-glucanases, and produced more extracellular proteins and other compounds (Punja and Utkhede 2003). This strain provided a significantly increased inhibitory activity against *Botrytis cinerea in vitro*. There is probably a complex sequential time-course of induction of these enzymes, and interactions with other compounds are possible (e.g. with antibiotics to achieve optimal biological activity). In *T. harzianum*, differential expression of a series of chitin-degrading enzymes was reported during mycoparasitism, which varied with time and the fungal pathogen host (Harran 1996).

The importance of hydrolytic enzyme production in the biocontrol activity of yeasts can vary with the organism, with *Candida* producing biologically relevant levels for Botrytis control (Punja and Utkhede 2003) and *Tilletiopsis* spp. and also producing insignificant enzyme activity against powdery mildews (Urquhart and Punja 2002; Bar-Shimon et al. 2004). In situ localization of various lytic enzymes during interactions between a specific biocontrol agent and pathogen remains a challenge, but microscopic observations have revealed that pathogen hyphae in addition to other pathogen structures were disintegrated and collapsed (Benhaamou and Chet 1997).

The attachment of yeasts to pathogen hyphae is an important factor for biocontrol activity, enabling cell wall degrading enzymes to have an effect. Taken together, recent research on extracellular enzyme production by fungal and yeast biocontrol agents convincingly demonstrate their involvement in reducing pathogen growth and infection. Predictably, synergies between these enzymes and antibiotic compounds have also been reported. For example, hydrolytic enzymes in the presence of gliotoxin, peptaibols or other antifungal compounds significantly suppressed the growth of *B. cinerea* and *Fusarium oxysporum* compared with either one alone (Punja and Utkhede 2003).

15.3 Biocontrol Agents Can Affect Pathogenicity of Fungal Pathogens

There are several pathogenicity factors that influence the degree and extent to which a fungal organism can invade host plant tissues (Idnurm and Howlett 2001). Disruption of any of these through site-directed mutagenesis, for example, can render a plant pathogen unable to infect. One interesting aspect of biocontrol agent-induced suppression of disease is the reported affect of *T. harzianum* on development of gray mold disease caused by *B. cinerea* through a reduction in its pathogenicity (Elad and Kapat 1999). Production of cystein protease enzymes by *Trichoderma* was reported to inhibit the activities of hydrolytic enzymes – especially polygalacturonases – in the pathogen, which are important pathogenicity factors in *Botrytis* and many other fungi (Elad and Kapat 1999). The proteases inactivated the pathogen enzyme by cleaving the molecule. Reduction in disease was also demonstrated with extracts containing proteases from *Trichoderma* culture filtrates and from infected bean leaves and was reversed by adding protease inhibitors (Elad and Kapat 1999). For such an interaction to be evoked on the plant leaf surface infected with *B. cinerea*, the interacting organisms must spatially and temporally occupy the same niche and be in close proximity to one another, providing localized protection.

15.3.1 Induction of Host Plant Defenses by Fungi and Yeasts

Higher plants are able to defend themselves against potential pathogens through the induction of a diverse array of chemical compounds whose production is triggered by elicitor molecules or inducing agents (Benhamou 1996). An interesting area of biocontrol agent-induced suppression of plant diseases is the induction of host defense responses. Following their application to plants, *Trichoderma* spp., *Pythium oligandrum* and nonpathogenic *Fusarium oxysporum* (Table 15.1) have all been reported to enhance structural and biochemical changes, which increased resistance to disease. These changes included enhanced deposition of plant cell wall materials (e.g. callose) at the infection site, as well as increased activity of several enzymes (e.g. peroxidase and chitinases) and other pathogenesis-related proteins (Bao and Lazarovits 2001). In some of these reports, however, the biochemical changes might provide only correlative and not confirmatory evidence for their role in induced resistance. In one report, biocontrol activity of *T. virens* was highly correlated with induction of terpenoid synthesis and peroxidase activity in plant roots, which were shown to be inhibitory to *R. solani* (Punja and Utkhede 2003). Yeast biocontrol agents have also been reported to induce host defense responses following their application to plant tissues (Droby et al. 2002; Sharma 2007).

Induction of plant defense responses through signaling pathways is well-described for plant growth-promoting rhizobacteria, which use several mechanisms to achieve induced systemic resistance in the plant. However, it is unclear whether specific or general elicitors are produced by the fungal and yeast biocontrol agents, and whether a host recognition system is triggered to initiate the defense mechanism similar to that occurring in incompatible pathogen-host interactions (Punja 2001). The release of fungal pathogen elicitor molecules (e.g. oligosaccharides) is known to trigger host defense responses against pathogens and biocontrol agents could potentially act in the same way. In addition, *T. harzianum* has been reported to produce growth-promoting substances that enhanced root and shoot growth following application in the absence of pathogens (Bailey and Lumsden 1998). A number of chemical elicitors of SAR and ISR may be produced by the BCA upon inoculation, including salicylic acid, siderophore, lipopolysaccharides, and 2,3-butanediol, and other volatile substances (Van Loon et al. 1998; Ongena et al. 2004; Ryu et al. 2004). Again, there may be multiple functions to such molecules blurring the lines between direct and indirect antagonisms. More generally, a substantial number of microbial products have been identified as elicitors of host defenses, indicating that host defenses are likely stimulated continually over the course of a plant's lifecycle. Excluding the components directly related to pathogenesis, these inducers include transglutaminase, elicitins, and p-glucans in Oomycetes; invertase in yeast; chitin and ergosterol in all fungi; andxylanase in *Trichoderma* (Nurnberger et al. 2004) these data suggest that plants would detect the composition of their plant-associated microbial communities and respond to changes in the abundance, types, and localization of many different signals.

The induced systemic response in citrus fruits treated with yeast cells involved an increase in ethylene, phenylalanine ammonia lyase, Phytoalexin (scoparone and scopoletin) (Arras 1996), 1,3-ß-glucanase (Yehuda et al. 2003a, b) and in apple fruit glucanase, chitinase and peroxidase activities (Ippolito et al. 2000; Castoria et al. 2001).

15.3.2 Competition for Nutrients and Niche Colonization by Fungi and Yeasts

Fungal pathogens require entry points to gain access into plant tissues. For obligate parasites, this is usually achieved by direct penetration of hyphae through the cuticle and epidermis of the plant. For facultative parasites that are mostly saprophytic,

Fig. 15.5 Biocontrol as shown by yeasts in different fruits. Treated fruits with bioagents are in the *upper line* while infected fruits can be seen on the *bottom side*

entry can be through wounds, senescing host tissues, or natural openings such as stomata and lenticels. These areas are generally nutrient-rich owing to exudation of sugars and amino acids. Biological control agents that can compete effectively with the pathogen to occupy these infection sites and use the nutrients can effectively displace the pathogen by preventing germination of propagules or infection (Fig. 15.2). Biocontrol agents such as *Trichoderma* and *Gliocladium* spp. can outgrow and outcompete the pathogen, especially if applied before pathogen arrival. Colonization of roots by *Trichoderma* (rhizosphere competence) is an important aspect contributing to its biocontrol efficacy (Harman 2000). Nonpathogenic *F. oxysporum* strains can also compete with pathogenic strains for infection sites and nutrients (e.g. carbon on the roots) (Eparvier and Alabouvette 1994). Competition for nutrients is also a mechanism by which many yeast biocontrol agents that colonize wounds or senescing tissues can prevent infection by pathogens (Filonow 1998). Treatment of wounded fruits (Fig. 15.5), germinating seeds, flower blossoms can therefore provide opportunities to establish biocontrol agents that can then outcompete pathogens.

Other selected microorganisms, particularly yeast, act mainly competing for space or for the utilization of some nutrients with the pathogen as shown by yeast in different fruits. In a study washed yeast cell suspensions gave the best control and autoclaved cell cultures and culture filtrates were completely ineffective in inhibiting the pathogen in fruits (Sharma et al. 1997). Competition for nutrients was demonstrated for *Pichia guilliermondii* against *Penicillium digitatum*, *Debaryomyces hansenii* against *Penicillium chrysogenum, P. italicum, P. digitatum, Geotrichum candidum* and *Aspergillus niger* co-cultivated on synthetic media (Sharma 1992, 1998; Mehrotra et al. 1996). It was noted that addition of exogenous nutrients resulted in a reduced efficacy because the antagonist offered better results when nutrients were scarce. A rapid multiplication and colonization of the wounds by antagonist was also elucidated. Novel discovery is the ability of the bioagents such as yeast to form a biofilm and prevent germination cues and/or nutrients from reaching the pathogen (Fig. 15.6). Janisiewiez et al. (2000) have developed a non-destructive method using tissue culture plates having a defusing membrane at the lower end of cylindrical inserts to study competition for nutrients separated from the competition for space.

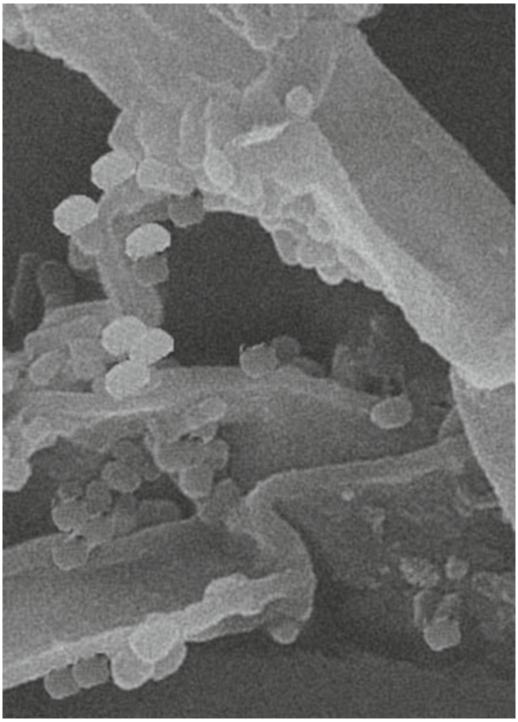


Fig. 15.6 Yeast cells growing as biofilm on host cells

15.3.3 *Other Fungi and Yeasts Show Biocontrol Potential*

Many reports have described the biocontrol potential of other endophytic as *Chaetomium* (Sharma et al. 2007b) and *Trichoderma* (Rai et al. 2006); saprophytic fungi and yeasts to reduce diseases on vegetable crops. The mechanisms of action of these agents fall into the same categories as those described here. Further research on these organisms and their advancement through the microbial registration process should increase their chances of being used as biocontrol agents in the future, thereby adding to the list of current products.

15.4 Applications of Molecular Methods to Biocontrol Fungi and Yeasts

Several fungi and yeasts that have the potential to reduce the development of vegetable crop diseases have recently been characterized using molecular methods. Mitochondrial DNA, internal transcribed spacers of rDNA (ITS1 and ITS2 regions), and nuclear DNA have been evaluated using techniques such as restriction fragment length polymorphisms (RFLP), arbitrarily primed polymerase chain reaction

(AP-PCR), random amplified polymorphic DNA (RAPD), sequence-characterized amplified region (SCAR) and other molecular approaches. These studies were used to distinguish between species and among strains of the biocontrol fungi *Trichoderma* (Gams and Meyer 1998), *Gliocladium*, *Ampelomyces*, and the yeasts *Pseudozyma* and *Tilletiopsis* (Punja and Utkhede 2003). In addition to resolving taxonomic affinities, the molecular tools provided strain-specific markers to track the movement of strains and to test the genetic stability over successive generations of propagation (Punja and Utkhede 2003). Molecular tools will prove to be valuable in monitoring the survival and environmental fate following release of these biocontrol agents as well as in ensuring product quality and stability during large-scale production of inoculum.

Genetic transformation techniques have been applied to *Trichoderma* and nonpathogenic *F. oxysporum*. Transformation of *T. harzianum* with the b-glucuronidase (uid A, gus) gene and the hygromycin B (hyg B) gene provided markers for use in population dynamic studies (Green and Jensen 1995). Co-transformation with genes encoding b-glucuronidase and green fluorescent protein (GFP) has also been described (Bae and Knudsen 2000). Similar markers (gus, hyg B) were developed for nonpathogenic *F. oxysporum* strains (Bao and Lazarovits 2001) and used to study the role of competition for infection sites on plant roots and to estimate fungal biomass on roots in relation to the pathogenic strains. Techniques of protoplast fusion were used to create strain T-22 of *T. harzianum* by fusing a mutant strain capable of colonizing plant roots with a strain able to compete with bacteria under iron-limiting conditions (Harman 2000). This new strain had the enhanced ability to colonize the root system of host plants, resulting in greater efficacy as a biological control agent for long-term root protection (Harman 2000). Yeasts with biocontrol potential have also been transformed with the GFP, which was used to track movement. Transformation of *Saccharomyces* to express a cecropin-A based peptide with antifungal activity was recently described (Jones and Prusky 2002) that enhanced the ability of the strain to reduce decay when applied to tomato fruits. Expression of novel antimicrobial compounds in biological control agents through genetic transformation provides a new approach for disease control. Finally, the use of antimicrobial genes from biocontrol fungi, such as Trichoderma, when expressed in transgenic plants, has also opened up new opportunities to enhance resistance to pathogens (Lorito and Hcala 1995). Several crop species have now been engineered with genes expressing chitinases; these transgenic plants were shown to express resistance to many different fungal pathogens (Punja 2001).

15.5 Where We Stand and Where to Go

The past 25 years have seen tremendous growth in the science and practical application of biological control of postharvest diseases. The available literature has expanded from a few publications in the early 1980s to hundreds, if not thousands, by 2005. The number of labs that conduct research in this area has also changed from 2 to 3 located in the USA and Israel to dozens located throughout the industrial and developing world, and several products have been made available. Our own success and influence in this field of research was a direct result of having a timely idea (i.e. being at the right place at the right time). As indicated, the use of the available postharvest biocontrol products thus far has been rather limited, given the potential market. Some of the reasons for the lack of adoption of these products have been overcome in the 'second generation' products that are, or will soon be, available. The future success of these products will depend on market conditions. Synthetic fungicides have a long history of use, are generally easy to apply, and continue to be highly effective. Growers will only replace chemical pesticides with biologicals if there is a continued demand by consumers for pesticide-free food products. Organically grown fruit represents a large potential market for use of biological agents, since the use of synthetic fungicides is strictly prohibited. The demand for such produce has seen tremendous growth in the last decade and this does not seem to be slowing down. Importantly, new biological postharvest products must be adaptable and effective as stand-alone products, without the need for additional inputs if they are to be competitive with synthetic fungicides. Postharvest biologicals must also begin to address problems of decay management in commodities where postharvest disease is harder to control, such as stone fruits and berries.

Lastly, the huge potential of providing extended decay control to the consumer, prior to and after commodity purchase, through the use of antimicrobials in modified and intelligent packaging should be recognized. The greatest hope for a biological approach (using a broad definition of biological control) lies in a further understanding of the mechanism(s) of action of microbial antagonists and natural products, innate and induced resistance in the host, and the biology of decay pathogens. It is expected that this knowledge will lead to new, innovative approaches for controlling decay in harvested commodities and presents the best hope for the future of the biological control of postharvest disease.

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Chapter 16
Remote Sensing of Biotic Stress in Crop Plants and Its Applications for Pest Management

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Abstract Remote sensing (RS) of biotic stress is based on the assumption that stress interferes with photosynthesis and physical structure of the plant at tissue and canopy level, and thus affects the absorption of light energy and alters the reflectance spectrum. Research into vegetative spectral reflectance can help us gain a better understanding of the physical, physiological and chemical processes in plants due to pest and disease attack and to detect the resulting biotic stress. This has important implications to effective pest management. This review provides an overview of detection of various biotic stresses in different crops using various RS platforms. Previous work pertaining to the use of RS technique for assessing pest and disease severity using different RS techniques is briefly summerized. The available sources of ground based, airborne and satellite sensors are presented along with various narrow band vegetation indices that could be used for characterizing biotic stress. Using relevant examples, the merits and demerits of various RS sensors and platforms for detection of pests and diseases are discussed. Pest surveillance programs such as field scoutings are often expensive, time consuming, laborious and prone to error. As remote sensing gives a synoptic view of the area in a non-destructive and non-invasive way, this technology could be effective and provide timely information on spatial variability of pest damage over a large area. Thus remote sensing can guide scouting efforts and crop protection advisory in a more precise and effective manner. With the recent advancements in the communication, aviation and space technology, there is a lot of potential for application of remote sensing technology in the field of pest management.

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16.1 Introduction

Plant stress is defined as a significant deviation from the optimal conditions for plant growth that could cause harmful effects when the limit of plants' ability to adjust is reached (Larcher 1995). Plant stress can affect almost every part of a plant, although typically one or few plant structures are influenced depending on the age and the source of stress. In case of biotic stress, the mechanism of damage by the pest largely influences the physiological response of plants, which is in turn gets manifested into typical symptoms. Plants may respond to pest and disease stress in a number of ways, including leaf curling, wilting, chlorosis or necrosis of photosynthetic plant parts, stunted growth, or in some cases reduction in leaf area due to severe defoliation. Major types of pest damage mechanisms are classified as germination reduction, stand reduction, light stealing, assimilation rate reduction, assimilation sapping, tissue consumption and turgor reduction (Boote et al. 1983; Aggarwal et al. 2006). While many of these responses are difficult to visually quantify with acceptable levels of accuracy, precision, and speed, these same plant responses will also affect the amount and quality of electromagnetic radiation reflected from plant canopies. Thus, remote sensing instruments that measure and record changes in electromagnetic radiation may provide a better means to objectively quantify disease stress than visual assessment methods. Furthermore, the effects of many pest/disease infestations are often not noticeable to the human eye, until it reaches an advanced stage when it becomes too late to control the outbreak. Remote sensing provides an alternative cost effective method to obtain detailed spatial information for entire crop fields at frequent intervals during the cropping season (Datt et al. 2006). Additionally, remote sensing can be used repeatedly to collect sample measurements non-destructively and non-invasively (Nilsson 1995; Nutter et al. 1990; Nutter and Litterell 1996).

Pests and diseases cause serious economic losses in yield and quality of many cultivated crops, which is estimated at 14% of the total agricultural production (Oerke et al. 1994). Timely detection and assessment of their damage symptoms is very crucial. Traditionally, pest and disease assessment of crop plants is being done by a visual approach i.e., relying up on human eye and brain to assess their incidence. However the problem with the traditional approach is that they are often time consuming and labour intensive. Recent advances in the field of radiometry and other remote sensing technologies offer ample scope for exploiting these technologies towards developing an alternate means that can enhance or supplement the traditional approaches. Precise knowledge of areas where pest or disease activity has started would enable the farmer to apply just the right amounts of pesticides to the affected areas, thereby yielding both economic and environmental benefits (Datt et al. 2006).Though there are several distinct regions in the electromagnetic spectrum. In this review we restricted to the 'optical region' as this is the most extensively studied and widely used for remote sensing of biotic stresses in crop plants. Optical remote sensing makes use regions of visible, near infrared and short-wave infrared sensors. Several reviews were published from time to time on remote sensing of biotic stress (Jackson 1986; Riley 1989; Hatfield and Pinter 1993; Nilsson 1995;

Everitt et al. 2003; West et al. 2003; Yang et al. 2004; Kelly and Guo 2007). This review is an attempt to provide updated and comprehensive, if not exhaustive information on this topic.

16.2 Principle of Operation

Remote sensing is the science and art of obtaining information about an object, area, or phenomenon through the analysis of data acquired by a device that is not in contact with the object under investigation. When electromagnetic energy is incident on any feature on the earth surface, three energy reactions with the feature are possible: reflection, absorption and/or transmission (Lillesand et al. 2004). The portion of energy reflected, absorbed or transmitted will vary for different earth features depending on their material type and condition. Even within a given feature type, the portion of reflected, absorbed and transmitted energy will vary at different wavelengths. Thus, two features may be distinguishable in one spectral range and be very different in another wavelength band. Because many remote sensing systems operate in the wavelength regions in which reflected energy predominates, the reflectance properties of earth surface are very important. The reflectance characteristics of earth surface features may be quantified by measuring the portion of incident energy that is reflected (Panda 2005). Reflectance is measured as a function of wavelength and is called spectral reflectance. A graph of the spectral reflectance of an object as a function of wavelength is termed as 'spectral reflectance curve' (Fig. 16.1).

The configuration of spectral reflectance curve gives us the insights into the spectral characteristics of an object and has a strong influence on the choice of

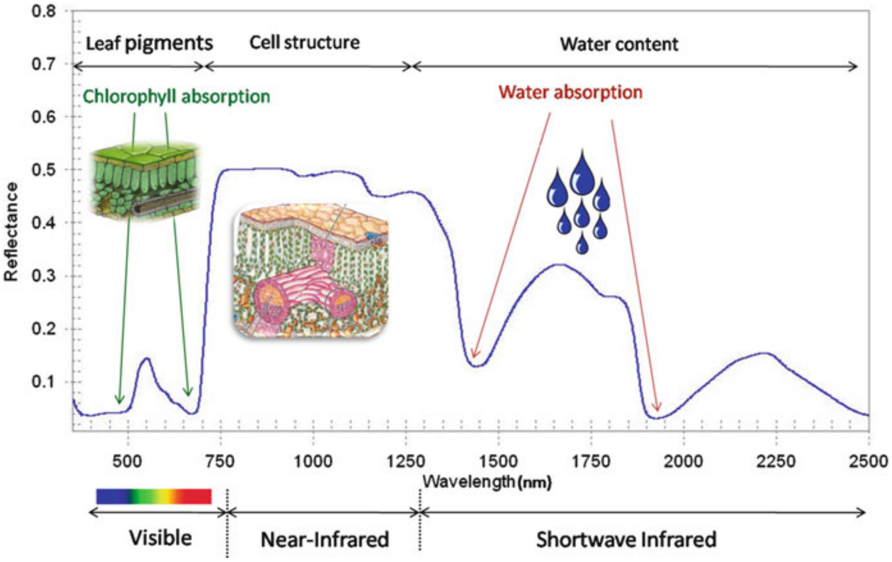

Fig. 16.1 Typical spectral reflectance curve of healthy vegetation depicting different absorption peaks

wavelength regions in which remote sensing data need to be acquired for particular application. Physical and physiological basis for the reflectance of visible and near infrared radiation from vegetation has been extensively studied (Knipling 1970; Zhang et al. 2003). Reflectance spectra of crop canopies are known to be a function of canopy optical properties with contributions from biophysical and biochemical attributes of vegetation, viewing geometry of detector, illumination conditions of the surroundings, and background effects (Asner 1998; Barrett and Curtis 1992; Goel 1998; Myneni et al. 1989). The three dimensional orientation of biophysical attributes of vegetation provides a better architecture for photon reception from incident radiation, yet creates variation in the spectral characteristics. The biochemical components of the plant parts also influence plant reflectance spectra (Buschman and Nagel 1993; Baret et al. 1994; Kupiec and Curran 1995).

Rapid and accurate quantification of early symptoms are important from a pest management point of view, and efforts at remotely detecting plant stress due to disease or insect activity utilize principles of biophysical remote sensing (Jensen 1983). Plant stress usually results in an increase in visible reflectance (due to a decrease in chlorophyll and a resulting decrease in absorption of visible light), and a decrease in NIR reflectance from changes in the internal leaf structure (Hatfield and Pinter 1993). Use of remote sensing techniques for detection crop pests and diseases is based on the assumption that stress induced by them interferes with photosynthesis and physical structure of the plant and affects the absorption of light energy and thus alters the reflectance spectrum of the plants (Riley 1989; Hatfield and Pinter 1993; Moran et al. 1997). Natural growth processes (e.g. increase of biomass, development, maturation, senescence, plant architecture and natural fluctuations in hydraulic properties) and the related biochemical changes, such as concentration of chlorophyll and other pigments, also have an impact on the amount of solar energy that is reflected, absorbed, and transmitted by plants (Carter 1993; Lillesand et al. 2004; Ustin et al. 2002). Thus, research into vegetative spectral reflectance can help better understand the physiological, chemical and physical processes in plants and to detect plant stress when remedial action may still be effective.

16.3 Types of Remote Sensing Platforms

Remote sensing platforms can be field-based (ground based), or mounted on aircraft (airborne) or satellites (space borne). Ground-based platform, such as handheld spectroradiometer, is typically used for ground truth study. Airborne RS is flexible and able to achieve different spatial resolutions with different flight altitudes. Satellite RS is generally for small scale (large area) study but it often times cannot meet the requirement of spatial resolution in applications. However, with recent advent of high resolution sensors, there is lot of potential for large scale (small area) field applications. Depending on the band width, number of bands and contiguous nature of recording spectral scanner scan be of two types viz., multispectral or broad band and hyperspectral or narrow band (Table 16.1). Multispectral scanners

Table 16.1 Spatial and spectral characteristics different satellite sensors

Satellite/Sensor	No of bands	Band width (μm)	Spatial resolution (m)	Temporal resolution (days)
Multispectral				
Landsat-1,2,3 MSS	4	0.5–0.6, 0.6–0.7, 0.7–0.8, 0.8–1.1	56 × 79	16
Landsat-4,5 TM	7	0.45–0.52, 0.52–0.60, 0.63–0.69, 0.76–0.90, 1.55–1.75, 10.4–12.5, 2.08–2.35	30	16
Landsat-7 ETM+	8	0.52–0.90 (p)	15	16
		0.45–0.52, 0.52–0.60, 0.63–0.69, 0.76–0.90, 1.55–1.75, 10.4–12.5, 2.08–2.35	30	
ASTER	14	VNIR: 3 bands (0.52–0.86)	15	16
		SWIR: 6 bands (1.6–2.43)	30	
		TIR: 5 bands (8.125–11.65)	90	
ALI	10	0.48–0.69 (p)	10	16
		VIS: 4 bands (0.433–0.69)	30	
		NIR: 3 bands (0.775–1.30)		
		SWIR: 2 bands (1.55–2.35)		
SPOT-4	5	0.43–0.47, 0.50–0.59, 0.61–0.68, 0.79–0.89, 1.58–1.75	2.5–20	26
SPOT- 5				
-HRG	6	0.48–0.71 (p)	2.5–5	26
		0.43–0.47, 0.50–0.59, 0.61–0.68, 0.79–0.89, 1.58–1.75	10–20	
-HRS	5	0.49–0.69 (p)	5–10	26
		0.45–0.52, 0.61–0.68, 0.78–0.89, 1.58–1.75	1,000	
RESOURCESAT-1 (IRS-P6)				
-AWiFS	4	0.52–0.59, 0.62–0.68, 0.77–0.86, 1.55–1.70	56	5
-LISS III	4	0.52–0.59, 0.62–0.68, 0.77–0.86, 1.55–1.70	23	24
CBERS -2				
- CCD	5	0.51–0.73 (p), 0.45–0.52. 0.52–0.59, 0.63–0.69, 0.77–0.89	20	26
-IR MSS	4	0.50–1.10 (p), 1.65,2.22, 11.45	80–160	26
-WFI	2	0.66, 0.83	260	5
NOAA-14-AVHRR	5	0.58–0.68, 0.72–1.1, 3.55–3.93, 10.5–10.5, 11.5–12.5	1,000	Daily
MODIS-TERRA	36	0.62–14.385	250–1,000	Daily

(continued)

Table 16.1 (continued)

Satellite/Sensor	No of bands	Band width (μm)	Spatial resolution (m)	Temporal resolution (days)
Hyperspectral				
EOS-Hyperion	196	VNIR – 427.55–925.85 nm (band 8–57) SWIR 932.72–2395.53 nm (band 79–224)	30	16
Hyperspatial				
IKONOS	4	0.45–0.90 (p)	1	5
		0.45–0.52, 0.52–0.60, 0.63–0.69, 0.76–0.90	4	
QUICKBIRD	4	0.45–0.90 (p)	0.61	5
		0.45–0.52, 0.52–0.60, 0.63–0.69, 0.76–0.90	2.40	
RESOURCESAT-1 LISS IV	3	0.52–0.59, 0.62–0.68, 0.77–0.86	5.8	5
Rapid Eye	5	0.44–0.51, 0.52–0.59, 0.63–0.68, 0.69–0.73, 0.76–0.85	6.5	1–5
WorldView-2	8	0.45– 0.80 (p)	0.46	2–5
		VIS: 6 bands (0.45–0.745)	1.85–2.07	
		NIR: 2 bands (0.77–1.04)		
CARTOSAT-2	1	0.52– 0.85 (p)	1	5
FORMOSAT-2	5	0.45–0.90 (p)	2	Daily
		0.45–0.52, 0.52–0.60, 0.63–0.69, 0.76–0.90	8	
KOMPSAT-2	5	0.5–0.9 (p)	1	14
		0.45–0.52, 0.52–0.6, 0.63–0.59, 0.76–0.90	4	
ALOS-AVNIR-2	5	0.52–0.77 (p)	2.5	2
		0.42–0.50, 0.52–0.60, 0.61–0.69, 0.76–0.89	10	

(p): panchromatic

sense several wavebands in a wider range of discrete wavelengths while hyperspectral scanners provide the opportunity to sense many very narrow wavebands over a wide range of wavelengths with much greater number of sensors. Multispectral systems measure energy in specific, strategically restricted portions of the electromagnetic spectrum while hyperspectral systems measure several consecutive wavebands across a specified region of the electromagnetic spectrum. However, a major limitation of broadband RS products is that they use average spectral information over broadband widths resulting in loss of critical information available in specific narrow bands (Blackburn 1998a, b; Thenkabail et al. 2002). Most hyperspectral sensors acquire radiance information in less than 10 nm bandwidths from the visible to the SWIR (400–2,500 nm) (Asner 1998). For example, the spectral shift of the red-edge (670–780 nm) slope associated with leaf chlorophyll content, phenological state and vegetation stress, is not accessible with broadband sensors (Collins 1978; Horler et al. 1983). Recent developments in hyperspectral RS or imaging spectrometry have provided additional bands within visible, NIR and shortwave infrared (SWIR) which are useful for biotic stress detection (Pena and Altman 2009; Yang 2010; Jones et al. 2010).

The data the RS sensors capture is often characterized by four kinds of resolutions viz., (i) spatial (the smallest resolvable unit on the ground, also called the pixel), (ii) spectral (how sensitive is the sampled spectra), (iii) temporal (how often the data can be captured) and (iv) radiometric (the ability to discriminate very slight differences in reflected or emitted energy) (Kelly and Guo 2007). The common pixel sizes (spatial resolution) are wide-ranging across different satellites (Table 16.1). Weather satellites have pixel resolutions larger than 1 km; the AVHRR sensor, an early multispectral sensor still in use has a 1 km pixel size; the series of Landsat sensor have 30 m pixels, and there are a range of newer commercial satellites (e.g. Quickbird and IKONOS) that have near and under 1 m spatial resolution. Sub-meter resolution imagery is increasingly common, especially with the use of aircraft-borne sensors. The spectral information contained in imagery can include multispectral (<10 bands of spectra, covering the visible and NIR portion of the spectrum), hyperspectral (10s to 100s of bands, covering a wider range of the spectrum) and thermal spectra (covering longer wave infrared emittance spectra) (Kelly and Guo 2007). A new RS product, RapidEye, is now available with 5-m spatial resolution with a red-edge band. This red-edge band, which is the region of rapid change in reflectance of chlorophyll in the near infrared range, can improve the accuracy for mapping plant diseases (Santoso et al. 2011).

16.4 Concept of Spectral Vegetation Index

A vegetation index (VI) can be defined as a dimensionless, radiation based measurement computed from the spectral combination of remotely sensed data. Numerous vegetation indices, broadband as well as narrowband, have been developed to detect plant stress (Carter 1994). Single wavebands are often good indicators of biochemical constituents, but are subject to variability caused by environmental factors such as illumination differences including solar angle and background scattering. Vegetation indices also lead to data dimensionality reduction and therefore might be helpful in terms of data processing and analysis. Such indices are also able to overcome the limitations of single band applications by minimizing external factors, and therefore correlate more closely with vegetative biochemical constituents (Delalieux et al. 2009). VIs also enhances sensitivities to green vegetation spectral signals and reduces external effects such as noise related to soil and atmospheric influences (Zhao et al. 2005). Ratios can be simple two band ratios or can include a combination of bands. Several researchers have proposed several ratios for different applications (Tables 16.2 and 16.3). These VIs can be divided into four broad groups (Mirik 2001). (i) Ratio-based VIs: They are based on the ratio between red and NIR reflectance.

Table 16.2 Sensitive bands and spectral indices for different kinds of biotic stresses in different crops

S.No.	Crop	Pest	Platform	Spectral resolution	Optimum bands (in nm)/ indices/technique used	Reference
1.	Wheat	Leaf rust, yellow rust	Space borne	Multispectral (Landsat)	NDVI	Nagarajan et al. (1984)
2.	Peanut	Leaf spot	Ground based	Multispectral	800 nm	Nutter (1989), Aquino et al. (1992)
3.	Bentgrass	Blight	Ground based	Multispectral	760,810 nm	Raikes and Burpee (1998)
4.	Wheat	Cereal aphid	Ground based	Hyperspectral	NPCI	Riedell and Blackmer (1999)
5.	Apple	Scald	Ground based	Hyperspectral	550 & 700 nm; BRI	Chivkunova et al. (2001)
6.	Rice	Panicle blast	Ground based & Airborne	Hyperspectral	485,675 nm; (R470/R570);(R520/R675);(R570/R675);(R550/R970); (R725/R900)	Kobayashi et al. (2001)
7.	Alfalfa	Leaf spot	Ground based	Multispectral	810 nm	Guan and Nutter (2002)
8.	Wheat	Yellow rust	Ground based	Hyperspectral	543,630,750, 861 ± 10 nm	Bravo et al. (2003)
9.	Rice	Leaf blast	Ground based	Multispectral	(R550/R675), (R570/R675)	Kobayashi et al. (2003)
10.	Cotton	Armyworm	Ground based & Airborne	Multispectral	NDVI	Sudbrink et al. (2003)
11.	Sugarcane	Orange rust	Space borne	Hyperspectral (Hyperion)	DWSI	Apan et al. (2004)
12.	Rubber	Corynespora	Space borne	Multispectral (IRS 1 C)	NDVI	Ranganathan et al., 2004
13.	Cotton	Spider mite	Airborne	Hyperspectral (AVIRIS)	SMA	Fitzgerald et al. (2004)
14.	Tomato	Late blight	Ground based Airborne	Hyperspectral Multispectral (ADAR)	5-index feature vector method	Zhang et al. (2005)
15.	Wheat	Greenbug	Ground based	Multispectral	694, 800 nm	Yang et al. (2005), Yang et al. (2009)
16.	Rice	Sheath blight	Airborne	Multispectral (ADAR)	RI, SDI	Qin and Zhang (2005).
17.	Mustard	Alternaria	Space borne	Hyperspectral (Hyperion)	DWSI	Datta et al. (2006)
18.	Wheat	Powdery mildew and take-all disease	Ground based	Hyperspectral	490_{780}, 510_{780}, 516_{1300} and 540_{1300} nm	Graeff et al. (2006)

19.	Vegetables	Silver leaf whitefly, soft rot, leaf spot, caterpillar damage	Ground based	Hyperspectral	Silverleaf index,Broccoli soft rot index, Bacterial leaf spot index, Sunburn index	Datt et al. (2006)
20.	Cotton	Aphid, spider mite	Airborne, Space borne	Multispectral (SAMRSS, QuickBird); Hyperspectral (AV-NIR)	NIR are more sensitive	Reisig and Godfrey (2006)
21.	Wheat	Green bug	Ground based	Hyperspectral	AI	Mirik et al. (2006a)
22.	Wheat	Green bug	Ground based	Hyperspectral	DSSI	Mirik et al. (2006b)
23.	Peanut	Late leaf spot	Ground based	Multispectral	700–850 nm	Prabhakar et al. (2006)
24.	Wheat	Aphid	Airborne	Multispectral	NDVI	Elliott et al. (2007)
25.	Wheat	Powdery mildew and leaf rust	Airborne and Space borne	Hyperspectral (HyMap) Multispectral (QuickBird);	SMA, MTMF, NDVI	Franke and Menz (2007)
26.	Tomato	Leaf miner	Ground based	Hyperspectral	800 to1100 nm, 1450 and 1900 nm	Xu et al. (2007)
27.	Rice	Brown plant hopper, leaf folder	Ground based	Hyperspectral	426,1450 nm, linear correlation intensity analysis	Yang et al. (2007)
28.	Rice	Leaf folder	Ground based	Hyperspectral	757,445 nm	Yang et al. (2007)
29.	Forest trees	Gypsy moth	Space borne	Multispectral (MODIS)	NDVI, EVI, NDWI, NDII	Beurs and Townsend (2008)
30.	Forest tress	Pine beetle	Space borne	Multispectral (Landsat)	NDMI	Goodwin et al. (2008)
31	Rice	Brown spot	Ground based	Hyperspectral	(R702/R718), (R692/R530), (R692/R732)	Liu et al. (2008)
32.	Wheat	Sunn pest	Ground based	Hyperspectral	NDVI, SIPI	Genc et al. (2008)
33.	Pine	Bark beetle	Space borne	Multispectral (Landsat)	LAI	Coops et al. (2009)
34.	Conifer	Aphid	Space borne	Hyperspectral (Hyperion)	ARI	Pena and Altman (2009)

(continued)

Table 16.2 (continued)

S.No.	Crop	Pest	Platform	Spectral resolution	Optimum bands (in nm)/ indices/technique used	Reference
35.	Wheat	Aphid	Airborne	Multispectral	Unsupervised classification, spatial pattern analysis	Backouloua et al. (2010)
36.	Rice	Bacterial leaf blight	Ground based	Hyperspectral	943 and1039 nm, MLR	Yang (2010)
37.	Tomato	Bacterial leaf spot	Ground based	Hyperspectral	Partial least squares (PLS) regression, stepwise multiple linear regression (SMLR)	Jones et al. (2010)
38.	Wheat	Streak mosaic	Space borne	Multispectral(Landsat TM)	Maximum likelihood classifier	Mirik et al. (2011)
39.	Oil palm	Stem rot	Space borne	Multispectral (QuickBird)	ARVI, GNDVI, GBNDVI, NDVI, SAVI, SR	Santoso et al. (2011)

R: Reflectance

Table 16.3 Narrow band vegetation indices for use in biotic stresses detection

S. No.	Index	Formula	Reference
1.	Normalized Difference Vegetation Index (NDVI)	(R800 – R670)/(R800 + R670)	Rouse et al. (1974)
2.	Red Edge Position (REP)	700 + 40(RRE – R700)/(R740 – R700) RRE = (R670 + R780)/2	Guyot and Baret (1988)
3.	Chlorophyll Index (CI)	(R415 – R435)/(R415 + R435)	Barnes (1992)
4.	Photochemical Reflectance Index (PRI)	(R531 – R570)/(R531 + R570)	Gamon et al. (1997)
5.	Simple Ratio (SR)	R695/R420	Carter 1994
6.	Normalized Pigment Chlorophyll Index (NPCI)	(R680 – R430)/(R680 + R430)	Penuelas et al. (1995a)
7.	Structure Insensitive Vegetation Index (SIPI)	(R800 – R445)/(R800 + R680)	Penuelas et al. (1995b)
8.	Green Normalized Difference Vegetation Index (GNDVI)	(R750 – R550)/(R750 + R550)	Gitelson et al. (1996)
9.	Optimized Soil-Adjusted Vegetation Index (OSAVI)	(1 + 0.16) (R800 – R670)/(R800 + R670 + 0.16)	Rondeaux et al. (1996)
10.	Water Index (WI)	R900 nm/R970 nm	Penuelas et al. (1997)
11.	Red-edge Vegetation Stress Index (RVSI)	(R714 nm + R752 nm)/2 – R733 nm	Merton and Huntington (1999)
12.	Modified Chlorophyll Absorption Reflectance Index (MCARI)	[(R700 – R670) – 0.2 (R700 – R550)] (R700/R670)	Daughtry et al. (2000)
13.	Transformed Chlorophyll Absorption Reflectance Index (TCARI)	3 [(R700 – R670) – 0.2 (R700 – R550)(R700/R670)]	Haboudane et al. (2002)
14.	Ratio of TCARI and OSAVI	TCARI/OSAVI	Haboudane et al. (2002)
15.	Browning Reflectance Index (BRI)	(1/R550 – 1/R700)/(R750)	Chivkunova et al. (2001)
16.	Anthocayanin Reflectance Index (ARI)	$(R550)^{-1} - (R700)^{-1}$	Gitelson et al. (2001)
17.	Zarco Tejada and Miller (ZTM)	R750/R710	Tejada et al. (2001)
18.	Modified red edge Normalized Difference Vegetation Index (mNDVI705)	(R750 – R705)/(R750 + R705 – 2*R445)	Sims and Gamon (2002)
19.	Disease Water Stress Index 2 (DWSI-2)	R1660/R550	Apan et al. (2004)
20.	Damage Sensitive Spectral Index-2 (DSSI 2)	(R747 – R901 – R537 – R572)/(R747 – R901) + (R537 – R 572)	Mirik et al. (2006a)
21.	Aphid Index (AI)	(R761 – R908)/(R712 – R719)	Mirik et al. (2006b)
22.	Broccoli soft rot index	(D725 – D700)/(D725 + D700)	Datt et al. (2006)
23.	Bacterial lea spot index	(R550 – R640)/(R550 + R640)	Datt et al. (2006)
24.	Sunburn Index	(R450 – R680)/(R450 + R680)	Datt et al. (2006)

R: Reflectance at corresponding wavelength (nm); D: First order derivative at corresponding wavelength (nm)

The normalized difference vegetation index (NDVI) (Rouse et al. 1974) and ratio vegetation index (RVI) (Pearson and Miller 1972) are the most commonly used ratio-based VIs. (ii) Orthogonal VIs: Defined by a line in spectral space for identification at bare soils. The transformed soil-adjusted vegetation index (TSAVI) (Baret et al. 1989), second soil adjusted vegetation index (SAVI2) (Major et al. 1990) and modified second soil-adjusted vegetation index (MSAVI2) (Qi et al. 1994) are examples of orthogonal VIs. (iii) Derivative VIs: First and second-order derivative greenVIs introduced by Elvidge and Chen (1995). (iv) Atmospheric corrected indices: Such as the visible atmospherically resistant index (VARI) (Gitelson et al. 2002).

These VIs have been shown to be quantitatively and functionally related with canopy parameters such as the leaf area index (LAI), aboveground biomass, chlorophyll and other leaf pigment content and vegetation fraction. Research results indicate these VIs have potential applications in agriculture in general and for monitoring pests and diseases in particular (Riedell and Blackmer 1999; Elliott et al. 2007; Franke and Menz 2007; Coops et al. 2009; Pena and Altman 2009). Sensitive bands identified by various workers and VIs used to detect biotic stresses caused due to specific pests and diseases are summarized in Table 16.2. It can be inferred that damage by different pests on the same host requires different band combinations for their detection. Sensitivity of the spectra to the stress are crop and pest specific as it varies depending on the nature of damage induced by the pest.

16.5 Ground Based Remote Sensing of Biotic Stress

Spectroradiometry is the technique of measuring the spectrum of radiation emitted by a source. In order to do this the radiation must be separated into its component wavebands and each band measured separately. It is achieved by diffraction grating technique within the spectroradiometers to split the radiation entering the system into its constituent wavebands. A suitable detector is then used to quantify the radiation of each wavelength (ASD 1999). The field spectroscopy concerns *in situ* measurement of the reflectance of composite surfaces. Increasingly, spectral data are being incorporated into process-based models of the Earth's surface and atmosphere, and it is therefore necessary to acquire data from terrain surfaces, both to provide the data to parameterise models and to assist in scaling-up data from the leaf scale to that of the pixel (Milton et al. 2009). In most cases, the reflectance of a vegetation canopy or a soil surface is presented as a 'reflectance factor'. Nicodemus et al. (1977) introduced the concept of a reflectance factor, being the ratio of the radiant flux actually reflected by a sample surface to that which would be reflected into the same reflected-beam geometry by an ideal (lossless) perfectly diffuse (Lambertian) standard surface irradiated in exactly the same way as the sample. Spectralon has become established material of choice for recording reference spectra (U.S. National Institute of Standards and Technology, NIST). Milton et al. (2009) provided an exhaustive review of developments in the field of field spectroscopy and its applications.

Evidently, a limiting factor of ground based remote sensing is that their applicability is for small areas when compared with aircraft and satellite sensors. However, using hand-held spectrometers to quantify the unknown spectral characteristics of un-infested and infested plant canopies due to insect feeding at a small-scale is needed because hand-held remote sensing devices have better temporal, spectral, and spatial resolutions, as well as the accuracy of collecting reflectance data over per unit area. Reflectance data obtained by hand-held instruments over small-areas provide information to understand spectral interactions between insect pests and their host plants, as well as fundamental ground-truth for interpretation of RS data measured from satellite and aircraft. Therefore, a logical initial step is to use a field spectrometer for understanding the spectral response of crop stress (Mirik et al. 2006b). Over the years several other studies have characterized stress in crop plants using multi and hyperspectral hand held radiometers.

16.5.1 Ground Based – Multispectral

There are number of studies on use of multispectral radiometers for pest and disease detection. Maize leaves infected with dwarf mosaic virus showed significantly lower reflection even before visible symptoms could be noted, when compared to healthy leaves (Ausmus and Hilty 1971). Such a change in reflectance characteristics was used to make an early diagnosis of disease symptoms. Changes in citrus soft scale infestation levels were detectable because the honeydew excreted by the scale insects was an excellent growth medium for a sooty mold fungus that showed very low reflectance in both the visible and NIR wavelength regions and tended to accumulate as the season progressed (Gausman and Hart 1974). Infection of *Sclerotina* stem rot in oil seed rape, net blotch disease in barley and barley stripe disease (Nilsson 1991) were studied using ground based radiometry. The highest correlations with disease severity were found in the NIR bands, while some effects were traced to the visible bands. High correlations were also reported between disease incidence and the NIR to red and the green-to-red reflectance ratios.

Sharp et al. (1985) monitored the onset of stripe and stem rust using a radiometer and four VIs based on broad Multi Spectral Scanner (MSS) channels. Yellow rust infested winter wheat was successfully detected (classification error of 2.1 per cent) in field using four wave band spectrograph (Bravo et al. 2003). Ibragimov et al. (1994) reported that changes in the spectral characteristics in the visible and near-infrared region were related to rust infections and they were registered under laboratory and field conditions. Correlations were found between quantitative and qualitative changes of spectral characteristics, the type of rust infection and disease intensity in wheat. There have also been reports of field experiments using ground-based radiometers to assess the severity of watermelon disease (Blazquez and Edwards 1986), various leaf and root diseases (Nilsson 1991), anther smut disease in *Silene dioica* (Nilsson and Carlsson 1994), barley stripe disease (Nilsson and Johnsson 1996) and Rhizoctonia blight in creeping bentgrass (Raikes and Burpee 1998a, b), early blight of tomato (Lathrop and Pennypacker 1980) and aphid damage in broccoli (Costello 1995).

Kobayashi et al. (2001) used multi spectrometer reading to examine the progression of rice panicle blast disease, *Magnaporthe grisea* Barr and found that early stages of disease infestation, changes in visible reflectance are most indicative of the disease, and as the disease progresses, changes in NIR reflectance are more useful. Remote sensing provided a more precise method to estimate impacts of foliar pathogens on alfalfa and it was conclusively demonstrated that percent reflectance had better relationship than the destructive and more labour intensive visual estimation of defoliation caused by the pathogen (Guan and Nutter 2002). A strong relationship between defoliation and canopy reflectance and pod yield in the peanut late leaf spot pathosystem was found (Nutter and Litterell 1996) and subsequent intensity of peanut leaf spot disease was successfully assessed using a ground based multispectral radiometry (Nutter et al. 1990; Aquino et al. 1992).

Riedell and Blackmer (1999) investigated the effects that sucking insects have on leaf reflectance by infesting wheat seedlings with aphids (*Diuraphis noxia* Mordvilko) or greenbugs (*Schizaphis graminum* Rondani). Compared with healthy plants, the leaves from infested plants had lower chlorophyll concentrations and displayed significant changes in reflectance spectra at certain wavelengths viz., 500–525, 625–635, and 680–695 nm. The band centered at 694 nm and the vegetation indices derived from bands centered at 800 and 694 nm were identified as most sensitive to damage due to greenbug (*Schizaphis graminum*) infestation in wheat and broad Landsat TM bands and derived vegetation indices also showed potential for detecting the stress (Yang et al. 2005). It was found that infrared spectral region between 700–850 nm was found to be sensitive to leaf spot disease in peanut (Prabhakar et al. 2006). They observed the percent reflectance in 700–800 nm range of spectrum was higher for healthy plants when compared to diseased plants. Additionally many broad band indices were tested for their ability to differentiate disease severity. However, low level of disease intensity could not be detected using multispectral reflectance. Use of multispectral radiometry for assessment of Rhizoctonia blight in creeping bentgrass (Raikes and Burpee 1998a, b) and insect infestation in soybean have been proved (Board et al. 2007). It was also demonstrated that using multispectral radiometry it was possible to differentiate stress caused by Russian aphid and greenbugs in wheat (Yang et al. 2009).

16.5.2 Ground Based – Hyperspectral

Hyperspectral imaging spectrometers measure crop canopy reflectance in hundreds of narrow bands across the solar spectrum. Such high-resolution data or “spectral signatures” are able to detect subtle changes in plant chemistry and physiology caused by disease development or other stress factors (Datt et al. 2006). Malthus and Madeira (1993) investigated spectral reflectance properties of faba bean infected by *Botrytis* disease over the wavelengths range of 400–1,100 nm. The most significant changes in the spectral reflectance associated with the disease were a flattening of the response in the visible region and a decrease in the near-infrared reflectance shoulder at 800 nm. Both responses were attributed to collapse of leaf cell structure as the fungus spread. Riedell and Blackmer (1999) reported that feeding due to Russian wheat aphid resulted in a reduction in the leaf dry weight and area in the third and fourth leaves, and a reduction in total chlorophyll concentration in all leaves. Leaf reflectance in the 625–635 nm and the 680–695 nm ranges, as well as the normalized total pigment to chlorophyll-a ratio index (NPCI) were significantly correlated with total chlorophyll concentrations in plants damaged due to green bug and Russian wheat aphids. Yang and Cheng (2001) measured spectral characteristics of rice plants at various levels of infestation by the brown plant hopper, *Nilaparvata lugens*. There were significant differences in reflectance among infestations at wavelengths of 755 and 890 nm. Particularly spectral parameters such as the NDVI and cumulative reflectance may also be used to discriminate levels of hopper infestation. Later Yang et al. (2007) identified sensitive narrow bands for damage caused by rice brown plant hopper and leaf folder. They studied the damage due to these pests using both narrow and broad-band spectral indices.

Hyperspectral images of healthy and yellow rust infected wheat plants taken with an imaging spectrograph under ambient lighting conditions were found to classify diseased and healthy plants in the field using a quadratic discriminating model based on the reflectance of four wavebands (543 ± 10 nm, 630 ± 10 nm, 750 ± 10 nm and 861 ± 10 nm) with high coefficient of determination (Bravo et al. 2003). Several studies have demonstrated the utility of hyperspectral in diagnosing the pest and disease infestations in vegetable crops (Apan et al. 2005; Datt et al. 2006), rice and castor (Prabhakar et al. 2008) and citrus canker disease (Burks et al. 2009). By characterization of the spectral properties of wheat plants infested by green bug (Mirik et al. 2006a, 2006b) and Russian aphid *D. noxia* (Mirik et al. 2007) using a ground based hyper spectral spectrometry new indices and algorithms for estimating these pests on wheat has been suggested. By comparing leaf reflectance measurements in the visible and near-infrared, a reduction in chlorophyll was detected in the early stages of disease development in *Nicotiana debneyi* plants infected with tomato mosaic virus, even though visible symptoms were not apparent until several days later (Polischuk et al. 1997). While Delalieux et al. (2007) differentiated leaves of plants infected with apple scab (*Venturia inaequalis*) based on leaf reflectance in narrow wavebands. These results indicated that the specific wavelengths that yielded the best discrimination power were dependent upon the number of days following infection, as well as the plant cultivar (resistant or not). Discern differences in hyperspectral signatures were used to differentiate healthy palm oil leaves from Ganoderma infected leaves (Shafri and Anuar 2008).

Muhammed (2005) characterized hyperspectral crop reflectance data for estimating fungal disease severity in wheat and established a procedure with low computational load that were suitable for real-time applications. Specific differences in vegetation indices and wavelength intervals were observed between leaf roll virus-infected grape leaves and uninfected leaves in the green peak (near 550 nm), the near infrared (near 900 nm) and in the mid-infrared (near 1,600 and 2,200 nm).

Further analysis suggest that different vegetation indices and/or individual wavelength bands may differ in their ability to detect leaf roll disease in grapes depending on whether there are visible symptoms in the virus-infected leaves (Naidu et al. 2009). Using ground based hyperspectral radiometry Datt et al. (2006) developed narrow-band indices specific to pest and disease damage vegetable crops like silver leaf white fly in pumpkin, bacterial rot in broccoli, bacterial leaf spot in lettuce and caterpillar damage in cabbage

Devadas et al. (2008) tested different narrow band indices to discriminate three rust diseases on wheat and found no single index was capable of discriminating all three rust species on, but sequential application of selected indices would provide for the required species discrimination under laboratory conditions and thus, could form the basis for discrimination of rust species in wheat under field conditions. Genc et al. (2008) tested different hyperspectral indices for detection of sunnpest (*Eurygaster integriceps*) on wheat and found NDVI and SIPI as more suitable for assessing their damage levels. Ray et al. (2010) demonstrated the use of hyperspectral indices based on narrow bands to differentiate healthy and blight infested potato plants. Ultraviolet, visible, and near infrared reflectance spectroscopy was used to determine the disease severity of tomato leaves infected with bacterial leaf spot, *Xanthomonas perforans* and identified wavelengths around 750–760 nm as significant and seem highly related to the disease (Jones et al. 2010). While Liu et al. (2008) estimated brown spot fungal disease of rice using hyperspectral reflectance data and identified sensitive bands specific to this disease. Jusoff et al. (2010) developed a signature library profile of leaf fall disease affecting rubber trees and opined that such studies certainly assists in the development of an early disease warning system using an airborne hyperspectral imaging system. Recently Sindhuja et al. (2010) reviewed the possibility of using spectral reflectance for detection of several plant diseases. Characterization of reflectance spectra of several biotic stresses on various crops during ground- truth studies so far provide crucial information required for interpretation of remote sensing data obtained from airborne and space borne platforms. Hence it is important that this kind of basic studies should be carried out using hand-held radiometry to all the important biotic stresses on which such information is not available so far. The narrow band hyperspectral radiometers provide more useful and meaningful results compared to multispectral radiometers, specially for biotic stress studies.

16.6 Airborne Remote Sensing of Biotic Stress

Studies on the use of airborne remote sensing for crop disease assessment started long time ago. For example, in the late 1920s, aerial photography was used in detecting cotton root rot (Taubenhaus et al. 1929). The use of infrared photographs was first reported in determining the prevalence of certain cereal crop diseases (Colwell 1956). William Collins and Sheng-Huei Chang, along with Hong Yee Chiu developed the first airborne spectrometer for vegetation stress applications based on variations in the wavelength position of the red-edge (Chiu and Collins 1978). In the early 1980s, Toler et al. (1981) used aerial colour infrared photography to detect root rot of cotton and wheat stem rust. In these studies, airborne cameras were used to record the reflected electromagnetic energy on analogue films covering broad spectral bands. Since then, RS technology has changed significantly. Everitt et al. (2003) provided an overview of aircraft remote sensing in integrated pest management with four exemplary examples viz., blackfly in citrus, silver whitefly in cotton, harvest ant infestations in rangelands and western pine beetle infestations in a forested area. They concluded that integration of remote sensing, GPS and GIS provide valuable tools that can enable resource managers to develop maps showing distribution of insect infestations over large areas. The digital imagery can serve as permanent data base for monitoring future contraction or spread of insect infestation over time. However, aircraft RS data may suffer from mismatching of the image pixels to the ground features, and also from the problem of spectral pixel mixing, which is the mixture of the signals from different objects such as soil, healthy and infested plants or vegetation, different species, and varying cover levels (Mirik et al. 2005). Nevertheless, airborne multi-spectral imaging system has a great potential for use in area wide pest management systems (Lan et al. 2007; Huang et al. 2008).

16.6.1 Airborne – Multispectral

Hart and Meyers (1968) used colour-infrared (CIR) photography and hyperspectral reflectance data to identify citrus trees infected with brown soft scale insects (*Coccus hesperidum*). Airborne RS technology has been employed for detecting crop disease and assessing its impact on productivity (Heald et al. 1972; Henneberry et al. 1979; Schneider and Safir 1975). Wheat disease severity assessment has been advocated using airborne remote sensing (Kanemasu et al. 1974). Detection of coconut wilt disease is one of the earliest applications on use of airborne RS for pest detection from India (Dakshinamurti 1971). The recent advent of high spatial resolution aircraft-borne imaging instrumentation has demonstrated several applications in pest management. Such multispectral instruments typically capture reflectance in three visible and the NIR band, and thus their imagery is often used to map vegetation. One increasingly commonly used system is the high spatial resolution multispectral imaging system called Airborne Data Acquisition and Registration (ADAR), which has been used with some success to map and monitor crop health. The ADAR camera is digital, and captures reflectance in blue, green, red and near infrared. Because it can be mounted on aircraft, flight altitude and spatial resolution can be controlled. Qin and Zhang (2005) used multispectral ADAR imagery to detect rice sheath blight disease in Arkansas, USA. They had better success in discriminating severe infestation levels, and had more trouble discerning early stages of the disease. Broad band airborne multispectral imageries were successfully used to identify the differences in growth pattern induced by tarnished bug infestation (Willers et al. 1999, 2005), beet army worm and cabbage looper damage in cotton (Sudbrink et al. 2003) and citrus greasy spot disease in citrus (Du et al. 2008).

Study by Zhang et al. (2005) provided a method of identifying late blight infection on tomato fields in California and demonstrated the capability of utilizing multispectral images in monitoring crop growth and precisely managing diseases in fields. However they found that earlier detection of this disease can be difficult due to its similar spectral response to that of healthy plants, disease was identified when infection reached stage 3 or above. Elliott et al. (2007) used a three band multispectral imaging system, SSTCRIS on board a Cessna 17 air craft was used to differentiate varying levels of injury caused by Russian wheat aphid, *Diuraphis noxia.* They demonstrated that the multispectral RS data acquired by a relatively inexpensive and easy to use multispectral imaging system could detect aphid-induced stress in production winter wheat in the presence of other stress inducing variables (Elliott et al. 2007). Further, Backoulou et al. (2010) showed the potential of combining multispectral airborne imagery with spatial pattern recognition to identify and spatially differentiate the aphid (*Diuraphis noxia*) infestation in wheat fields, and also showed that patches of wheat stressed by *D. noxia*, drought, and agronomic conditions differed spatially with respect to size, shape, and spatial arrangement within a wheat field.

16.6.2 Airborne – Hyperspectral

Hyperspectral data recorded from low attitude flights usually have high spectral and spatial resolution, which can be very useful in detecting stress in green vegetation. An airborne visible infrared imaging spectrometer (AVRIS) image with 224 bands with the wavelength range of 0.4–2.5 μm was used to detect stress in tomatoes induced by late blight disease in California, USA (Zhang et al. 2003) and strawberry spider mite (*Tetranychus turkesni* U.N.) in cotton (Fitzgerald et al. 2004). Williams et al. (2004) developed hyper spectral signatures using airborne data that characterised individual tree species and health class which in turn may be used to classify hyperspectral images to produce maps of emerald ash borer host trees.

With the rapid developments in RS technology in recent decades, hyperspectral remote sensors, such as airborne visible infrared imaging spectrometer (AVIRIS), compact airborne spectrographic imager (CASI), multispectral infrared and visible imaging spectrometer (MIVIS), and hyperspectral mapping (HyMap) system, are now available to agricultural applications (Zhang et al. 2003). These sensors can provide quality images with high spatial and spectral resolutions required for precision agriculture (Fraser 1998; Treitz and Howarth 1999). Because of the high spectral resolution with a narrow band range of about 10 nm or finer, hyperspectral remote sensing images produce a complete spectrum for each pixel within the scene. These characteristics combined with high signal-to-noise ratio enable us to differentiate various vegetation stresses based upon spectra of small patches of ground surface (Rush 2002; Christ et al. 2000; Lelong et al. 1998). Muhammed (2005) developed a technique for rapid analysis of hyperspectral data to identify stress caused by fungal disease severity in wheat. Hence, cost effective air-borne hyperspectral remote sensing data, if made available to the users could play a key role in detection of biotic stress in several crops with a better temporal, spectral and spatial resolution compared to the available multispectral data from several satellite platforms.

16.7 Space Borne Remote Sensing of Biotic Stress

A large number of satellite remote sensing products are available at present. Each satellite has different spectral, spatial, temporal and radiometric resolutions (Table 16.1) and the choice of product depends on application. Some of the recent satellites with multispectral and hyperspectral sensors on board rapidly generate vast amounts of data in a cost effective manner and at higher spatial and spectral resolutions. However the use of these RS from satellite platform for detection of pests and diseases is limited owing to high spatial and temporal resolution of data required for this purpose (Table 16.2). More so, availability of cloud free data during the crop season is another issue that limits use of satellite RS for crop protection. Additionally, most of the successful applications for pest detection using space data are for forestry and some plantation crops where the spatial spread of the pest damage is large.

16.7.1 Space Borne – Multispectral

Moderate resolution imaging applications, beginning with the launch of the Landsat sensors in the 1970s, provided support for large scale plant disease and insect damage mapping and monitoring. Landsat 2 imagery was used to discriminate between cabbage and potato fields for subsequent evaluation of club root disease (Torigoe et al. 1992) and later Landsat MSS data was used to detect leaf rust disease (*Puccinia recondita* f. sp. *tritici*) and yellow rust (*Puccinia striiformis*) over large areas of wheat in Pakistan (Nagarajan et al. 1984). Fitzgerald et al. (1999) demonstrated that multispectral RS (MRS) would allow farmers to detect early infestation of mites in large scale cotton fields due to colour shifts and changes in canopy appearance over time. Areas identified on the map could be located with the help of portable GPS equipment by field scout, verify mite population in these areas and recommend regions in the field that require pesticide application. Preliminary analysis using IRS LISS III data showed the feasibility of discriminating fully damaged late blight potato fields from healthy ones (Arora et al. 2004). Similarly, successful evaluation of remotely sensed data (IRS-1-C) was conducted for detection, mapping and monitoring of rubber plantations affected by *Corynespora* and *Gloeosporium* fungi which causes leaf spot and leaf fall disease (Ranganath et al. 2004).

Nutter et al. (2002) used a combination of Landsat 7 and high spatial resolution multispectral imagery to map damage caused by soybean cyst nematode (*Heterodera glycines*). While other researchers have used Landsat (Nelson 1983; Vogelmann and Rock 1989; Goodwin et al. 2008) and SPOT (Buchheim et al. 1984; Ciesla et al. 1989; Sirois and Ahern 1989) satellite imagery with coarse spatial resolutions to detect and assess insect damage to forests. It has been demonstrated that by using Landsat TM data it is possible to assess mountain pine beetle (*Dendroctonus ponderosae*) in western Canada (Goodwin et al. 2008) bark beetle damage in pine forests (Coops et al. 2009). A spatial model has been developed using Landsat imagery and field observations based on environmental factors such as topography and soil types to predict densities of wheat aphid, *Diuraphis noxia* (Merrill et al. 2009). Recently using Landsat TM data, Mirik et al. (2011) separated healthy and diseases (streak mosaic) wheat fields by maximum likelihood classifier method with an overall classification accuracies of 89.47–99.07%. Currently, this method appears to be one of the best for identifying and mapping disease incidence over large and remote areas by offering a repeatable, inexpensive, and synoptic strategy during the course of a growing season.

Ji et al. (2004) evaluated the potential of hyper spatial data from MODIS to monitor locust outbreaks in China and showed that the NDVI reliably distinguished between before and peak damage situations for each category of damage. Areas where NDVI decreased could be clearly mapped and classified into light, moderate, and heavy damage categories. High resolution multi-spectral data from QuickBird were generally used to detect in-field heterogeneities of crop vigour but are only moderately suitable for early detection of crop infections by diseases. However QuickBird imagery was used for detecting citrus orchards affected by sooty mould (Fletcher 2005) and wheat diseases caused by powdery mildew (*Blumeria graminis*) and leaf rust (*Puccinia recondita*) (Franke and Menz 2007). A regional level spatial distribution model of aphid (*Lipaphis erysimi*) growth in Indian mustard using satellite based remote sensing data has been developed (Bhattachrya et al. 2007 and Datta et al. 2008). They employed near surface meteorological parameters derived from National Oceanic and Atmospheric Administration (NOAA) Television and Infrared Operational Satellites (TIROS) Operational Vertical Sounder (TOVS) data and field observations of pest infestation. Second order polynomials fits between peak aphid count and TOVS cumulative air temperature were produced in Northern India i.e., Bharatpur and Kalyani.

It was shown that daily MODIS data on an annual time can be used to monitor insect defoliation due to gypsy moth in North American forests for patches larger than 0.63 sq km (Beurs and Townsend 2008). A recent study by Santoso et al., (2011) investigated the potential of high resolution QuickBird satellite for detecting and mapping basal stem rot disease (*Ganoderma boninense*) in oil palms. Six vegetation indices derived from VIS and NIR were used for to identify palms infected by the disease and image segmentation effectively delineated areas infected by the disease with a mapping accuracy of 84%.

16.7.2 Space Borne – Hyperspectral

Satellite based imaging sensors, equipped with improved spatial, spectral and radiometric resolutions offer enhanced capabilities over those of previous systems. The Hyperion sensor, on board the EO-1 satellite provides continuation of broad spatial coverage with increased spectral sensitivity (over 200 bands from 0.4 – 2.5 nm) that can help in plant disease or pest damage discrimination. Inclusion of the moisture sensitive bands in the longer wavelength region (1,660 nm) from the Hyperion data increased the ability to map orange rust disease (*Puccinia kuehnii*) on sugarcane in Australia, when compared to the use of visible and NIR reflectance bands alone (Apan et al. 2004). They proved that substantial change in leaf water content or pigmentation of stressed crop can be detected due to decrease in reflectance in middle infrared bands (1,200–2,500 nm) and due to shift in red edge in near infrared red bands (600–750 nm). They further formulated new indices viz., 'Disease Water Stress Indices' (DWSI) which produced the largest correlations with the stress, indicating their superior ability to discriminate sugarcane areas affected by orange rust disease. Later, using the same central wave lengths at 550, 680, 800 and 1,600 nm and also the same vegetation index (DWSI), Datta et al. (2006) successfully detected disease severity in mustard crop in India using Hyperion data. Pena and Altman (2009) explored the suitability of vegetation indices derived from satellite hyperspectral data for identifying stress symptoms induced by the invasion of cypress aphid (*Cinara cupressi* Buckton) in central Chile. In their studies strongest correlations were recorded for two anthocyanin reflectance indices and the photochemical reflectance index. Hence vegetation indices derived from hyperspectral images are potentially very useful in the detection, assessment and monitoring of the damage caused by the aphid. Satellites provide synoptic view of large areas with valuable data for land use classification. Application of space borne satellite data for biotic stress detection so far is mostly for forest pests and diseases and to a limited extent in field and plantation crops. The major constraints for its wide use in biotic stress of agricultural crops appear to be spatial and spectral resolution of the satellite sensors and availability of cloud free optical RS data during the season. Hopefully the next generation satellites would address some of this issues.

16.8 Conclusions

Over the years lot of information has been generated on characterizing biotic stress using hand held multi spectral radiometry. With the advent of hyperspectral radiometry, it has been possible to have insights into more details and better understanding of the crop stress induced by insect pests and diseases. It was also feasible to differentiate between biotic and abiotic stresses with reasonable accuracy using hyperspectral radiometry. Reflectance data obtained by ground based remote RS provides vital information to understand spectral interactions between pests damage on the host plants and also to collect fundamental ground-truth information required for interpretation of remote sensing data obtained from space borne and airborne platforms. Satellite remote sensing provides sufficient data for large scale studies, but it has limitations such as temporal and spatial resolution, and more importantly, availability of cloud free data. On the other hand, airborne systems have a higher resolution and time flexibility and provide sufficient lead time for dissemination of crop protection advisory. Though application of airborne RS for biotic stress has been in vogue in several developed countries, it is yet to find wide usage in many of the developing countries. One of the main reasons could be the high cost involved, availability of suitable sensors, small farm holdings and diverse cropping systems.

Hence it is challenging to make airborne hyperspectral remote sensing a reality in these countries.

Limited availability of fine spatial resolution, near real-time data has slowed the application of satellite RS in the past, but now with the launch of new generation satellites this might not be a limiting factor. The narrow bands in the hyperspectral sensors are able to measure the characteristic absorption peaks of plant pigments and other related parameters more precisely and thereby provide better information related to plant health. But availability of hyperspectral data from satellite platforms is still in its infancy. Nevertheless, airborne and space borne remote sensing can provide spatial variability of biotic stress and a synoptic view of the large area in a non-destructive and non- invasive way. Hence it can supplement many of the on-going field surveillance programs, which is often expensive, time consuming, laborious and many at times error-prone. It has been proved that RS technology can provide accurate and reliable information to guide decision-making in crop protection and hence have great potential for use in pest management.

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Chapter 17
Plant – Nematode Interactions: Consequences of Climate Change

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Abstract Plant pathogenic nematodes are one of the important biotic constraints in crop production. Climate change due to increased emission of greenhouse gases is posing a serious challenge to sustainability of crop production by interfering with biotic and abiotic components and their interactions with each other. Global warming resulting in elevated carbon dioxide (CO_2) and temperature in the atmosphere may influence plant pathogenic nematodes directly by interfering with their developmental rate and survival strategies and indirectly by altering host plant physiology. Available information on effect of global warming on plant pathogenic nematodes though limited, indicate that nematodes show a neutral or positive response to CO_2 enrichment effects with some species showing the potential to build up rapidly and interfere with plant's response to global warming. Studies have also demonstrated that the geographical distribution range of plant pathogenic nematodes may expand with global warming spreading nematode problems to newer areas. Besides plant parasites, other trophic groups (microbial feeders, predators and insect parasites) of soil nematodes also shown to influence the plant productivity indirectly by regulating the key ecosystem processes including decomposition, nutrient mineralization, biological pest suppression and energy transfer in food webs. These findings underline the importance of understanding the impact of climate change on soil nematodes and its implications to crop production while developing mitigation and adaptation strategies to address impact of climate change on agriculture.

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17.1 Introduction

Plant pathogenic nematodes are one of the important biotic constraints in crop production worldwide. Nematodes are microscopic round worms that feed on plants with the aid of characteristic syringe like mouthparts called 'stylet'. Nematodes inflict crop losses directly by feeding on plant parts and indirectly by acting as vectors of plant pathogenic virus and bacteria, providing ports of entry to secondary and weak pathogens, forming disease complexes with other pathogens like bacteria, fungi and virus, and breaking down plant resistance against other pathogens. Nematode infection also makes the plants vulnerable to abiotic stresses resulting in disease syndromes. It is estimated that nematodes cause crop losses worth US$ 125 billion annually in global agriculture production (Chitwood 2003) Table 17.1. In India alone, plant pathogenic nematodes were estimated to cause a monetary loss of about 21 billion rupees annually (Jain et al. 2007). Yield losses due to plant pathogens, including plant-pathogenic nematodes are generally more in tropical than in temperate regions because of congenial environmental conditions for pathogen development, reproduction and dissemination, greater pathogen diversity and lack of awareness

Table 17.1 Estimated annual yield losses due to damage by plant parasitic nematodes – world basis (Source: Sasser and Freckman 1987)

Life sustaining crops	Loss (%)	Economically important crops	Loss (%)
Banana	19.7	Cacao	10.5
Barley	6.3	Citrus	14.2
Cassava	8.4	Coffee	15.0
Chickpea	13.7	Cotton	10.7
Coconut	17.1	Cowpea	15.1
Corn	10.2	Eggplant	16.9
Field bean	10.9	Forages	8.2
Millet	11.8	Grape	12.5
Oat	4.2	Guava	10.8
Peanut	12.0	Melons	13.8
Pigeon pea	13.2	Misc. other	17.3
Potato	12.2	Okra	20.4
Rice	10.0	Ornamentals	11.1
Rye	3.3	Papaya	15.1
Sorghum	6.9	Pepper	12.2
Soybean	10.6	Pineapple	14.9
Sugar beet	10.9	Tea	8.2
Sugarcane	15.3	Tobacco	14.7
Sweet potato	10.2	Tomato	20.6
Wheat	7.0	Yam	17.7
Average	10.7%	Average	14.0%
	Overall average 12.3%		

and resources (human, technical and financial) to control pathogens (De Waele and Elsen 2007). Plant pathogenic nematodes are obligate parasites which feed mostly on roots while some species feed on aerial parts of plants. Nematodes feed either on plant parts (ectoparasites) or by entering completely (endoparasites) or partially (semi-endoparasites) inside the roots. Economically important genera of plant pathogenic nematodes in world agriculture include root-knot nematodes (*Meloidogyne*), cyst nematodes (*Heterodera* & *Globodera*), root lesion nematodes (*Pratylenchus*), burrowing nematodes (*Radopholus*), reniform nematode (*Rotylenchulus*), stem and bulb nematode (*Ditylenchus*) and virus-vector nematodes (*Xiphinema* and *Longidorus*).

In contrast to the plant pathogenic nematodes, other functional groups of nematodes including bacterivores, fungivores, predatory and insect parasitic nematodes play a positive role in improving plant health (Ferris et al. 2001). Bacterial and fungal feeding nematodes accelerate the decomposition of organic matter by dispersing relatively immobile microflora to new sites and by their feeding, which regulates bacterial growth and decomposition rates (Ruess 2003). Predatory nematodes, particularly those belong to mononchid and dorylaimid groups predate on plant parasitic nematodes thus have potential for biocontrol (Ahmed and Jairajpuri 2010). Insect parasitic or entomopathogenic nematodes are beneficial to crop production as they help in biological control of the insect pests of crop plants and reducing the consumption of chemical pesticides (Kaya et al. 2006). Knowledge on the role of different functional groups of nematodes and their impact on crop production under changing climate is essential for enhancing productivity of agroecosystems.

Climate change is a natural phenomenon that has been constantly occurring since the formation of earth. However, rapid increase in its pace and magnitude due to anthropogenic activities over the past few decades is causing concerns for future of the mankind. The growth rate in CO_2 concentration is increasing more rapidly since 2000 than in the previous decades. The atmospheric CO_2 has increased from about 280 μmol mol^{-1} between the end of the last glaciation and 1750, to about 380 μmol mol^{-1} today, and it is expected to reach a level of about 550 μmol mol^{-1} in 2050 (Raven and Karley 2006). This has resulted in several changes in the climate including the increase in the average global surface temperature by 0.2°C per decade in the past 30 years (Hansen et al. 2006). The intergovernmental panel on climate change predicts that with the current emission scenario, global mean temperature would rise between 0.9°C and 3.5°C by the year 2100 (IPCC 2007). Global warming, one of the important manifestations of climate change resulting from increased emission of greenhouse gases into the atmosphere is posing a serious challenge to sustainable development. Global warming influences the productivity of natural and managed ecosystems by interfering with biotic and abiotic components and their interactions with each other. Efforts to increase our understanding of impact of global warming on agriculture, healthcare and livelihood security sectors and identifying appropriate mitigation and adaptation mechanisms are receiving increased attention globally in recent years. Climate change and its broad spectrum of effects on managed and natural systems have become a central focus in recent years.

Climate change will have significant impacts on the productivity of agroecosystems by interfering with biotic and abiotic components and their interactions with each other (IPCC 2007). Global warming, one of the important manifestations of climate change resulting from increase in atmospheric CO_2 and temperature levels is posing a serious challenge to sustainable agriculture. Incidentally, temperature and carbon dioxide are the two important factors influencing the biology of nematodes. Therefore, there is a need to assess the impact of climate change on nematode functional groups and its consequences to crop production for developing appropriate strategies for addressing impact of climate change. This review aims to report and discuss potential and observed impacts of climate change on nematodes and its consequences for sustainable crop production in the light of available information.

17.2 Impact of Climate Change on Plant Pathogenic Nematodes

17.2.1 Nematode Abundance in Soil

Plant growth and the nutritional quality of most plant species is influenced by elevated atmospheric carbon dioxide (CO_2) with potential bottom up effects. Plant parasitic nematodes are major controllers of plant production in natural and agroecosystems. Due to the direct trophic link between herbivorous nematodes and their host plants and the observations that elevated CO_2 often induces increased root production (Rogers et al. 1994), it can be presumed that herbivorous nematode communities will be relatively more affected by increases in atmospheric CO_2 concentration. Positive effects of CO_2 enrichment on the abundance of plant-parasitic nematodes have been reported in some studies (Yeates et al. 1997, 1999, 2003). However, most studies report neutral effects wherein no change in abundance of plant parasitic nematodes was observed, despite increases in root biomass (Hungate et al. 2000; Niklaus et al. 2003; Sonnemann and Wolters 2005).

Nematode genera vary in their response to CO_2 enrichment. The abundances of *Tylenchus* and *Longidorus* increased after 5 years of CO_2 enrichment, but there was no effect of CO_2 enrichment on the abundance of *Paratylenchus, Trichodorus* and members of Hoplolaimidae in pasture plots (Yeates et al. 2003). On the other hand, Yeates et al. (1997) reported increase in abundance of *Meloidogyne* in response to CO_2 enrichment in grassland turfs while seven other herbivorous nematode taxa were not affected by elevated CO_2. The response of nematode taxa to CO_2 may be influenced by the site specific factors. For example, the abundance of *Pratylenchus* was positively associated with CO_2 concentration in gley soil, but not organic soil around a natural CO_2 vent in New Zealand, while none of the other eight herbivorous nematode taxa exhibited a significant relationship to CO_2 concentration (Yeates et al. 1999).

Ayres et al. (2008) assessed the responses of belowground nematode herbivores to atmospheric CO_2 enrichment (approximately 350 ppm above ambient) for 5–6 years in experiments conducted in grassland ecosystem at three locations, Colorado, California (USA) and Montpellier, France. It was observed that elevated CO_2 did not affect the abundance of nematode families in the majority of cases. Only two nematode families were significantly influenced by CO_2 enrichment (Anguinidae increased in one case and Hoplolaimidae decreased in another). Similarly, elevated CO_2 did not influence the total abundance, family richness, diversity or plant parasitic index of the herbivorous nematode community. These findings do not support the hypothesis that specialist nematode herbivores are more responsive to elevated CO_2 than generalists, since Anguinidae was the only specialist nematode family to exhibit a significant effect in this study while Hoplolaimidae is an intermediate in terms of specialization.

It was observed that the addition of crop residues stimulates the structure index and inhibits the plant-parasitic nematode response to the elevated CO_2 in a wheat field (Li et al. 2009). Elevated CO_2 also increased the abundance of omnivores-predators, the values of maturity index (MI) and structural index (SI) of nematode assemblage at the jointing stage of wheat, but the abundance of plant-parasites was not affected by concentration of CO_2 in a rice- wheat rotation system in China (Li et al. 2007). Further, the interaction of elevated CO_2 with Nitrogen fertilization or residue addition significantly affected the soil nematode community indices (Li et al. 2009).

The sensitivity of nematode feeding types in arable soil to CO_2 enrichment is observed to be crop specific. Sticht et al. (2009) analyzed the impacts of elevated atmospheric CO_2 concentration (550 ppm) on nematode abundance, feeding type composition and the stable C-isotopic signatures ($\delta^{13}C$) of feeding types in an arable soil under cultivation of winter wheat and sugar beet in rotation. In this study, the total nematode abundance increased under CO_2-enriched conditions in both crops though the absolute values for nematode abundance were higher for winter wheat compared to sugar beat at ambient and CO_2-enriched conditions. The number of plant parasitic nematodes was significantly higher at crop maturity in both wheat and sugar beet grown under elevated CO_2. However, the total numbers of plant parasitic nematodes were higher under elevated CO_2 wheat than under elevated CO_2 sugar beet. This may be due to the very different root system of both plants. These findings emphasize the need to introduce more than one plant type and sampling date when CO_2 enrichment effects on soil faunal communities are studied. Investigations on effect of climate change on soil nematodes are limited in India. Experiments carried out recently by Directorate of Rice Research, Hyderabad, showed no adverse effect of elevated CO_2 levels up to 700 ppm on the abundance of soil nematodes, and penetration of rice root-knot nematode *Meloidogyne graminicola,* a serious pest of rice (Somasekhar and Prasad 2010). Critical assessment of available information on responses of plant parasitic nematodes to CO_2 enrichment reported from different ecosystems so far indicates that the responses are either neutral or positive but not negative (Somasekhar et al. 2011) (Table 17.2).

Table 17.2 Response of plant parasitic nematodes to CO_2 enrichment

Cropping system	Experimental arena[a]	Nematode[b] response	Location	Reference
Grassland	CER	+/N	New Zealand	Yeates et al. (1997)
Grassland	Vent	N	New Zealand	Yeates et al. (1999)
Grassland	FACE	+/N	New Zealand	Yeates et al. (2003)
Grassland	OTC	+/N	California, USA	Hungate et al. (2000)
Grassland	OTC	N	Colorado, USA	Ayres et al. 2008
Grassland	OTC	N	California, USA	Ayres et al. 2008
Grassland	FACE	+/N	Germany	Sonnemann and Wolters (2005)
Sugar beet & wheat rotation	FACE	+	Germany	Sticht et al. (2009)
Grassland	SACC	N	Switzerland	Niklaus et al. (2003)
Grassland	CER	N	Montpellier, France	Ayres et al. 2008
Rice-wheat rotation	FACE	+/N	China	Li et al. (2007, 2009)
Rice	OTC	N	India	Somasekhar and Prasad (2010)

Source: Somasekhar et al. (2011)

[a] *OTC* Open-top chambers, *SACC* Screen-aided CO_2 control, *FACE* Free air CO_2 enrichment, *CER* Controlled environment room, *Vent* Natural CO_2 vent

[b] + Positive or increase in abundance, *N* Neutral or not affected

17.2.2 Plant-Nematode Interactions

The classic disease triangle establishes the conditions for disease development, i.e. the interaction of a susceptible host, a virulent pathogen and a favorable environment (Ghini et al. 2008). Climate change is likely to affect ecological interactions, including host-parasite interactions and herbivory. Interactions between herbivores and plants are also likely to change as a result of climate influence. For example, although root biomass increased under elevated $CO_{2,}$ the damage due to root-feeding nematodes was more under elevated CO_2 compared to the ambient levels in a grass species (Wilsey 2001). Similarly, neutral responses of herbivorous nematodes to CO_2 enrichment were observed despite increase in root production by 3–32% in different locations (Ayres et al. 2008). This may be due to decrease in root quality (low nitrogen content) or increase in nematode antagonists. It is possible that under elevated CO_2 conditions, nematodes may have to consume more plant material to maintain the same population levels due to low nitrogen content of plant which in turn may result in more plant damage and reduced yields (Fig. 17.1). Another consequence of increased root production, without changes in belowground herbivore populations, might be greater plant inputs to soil, which may lead to greater soil organic matter pools in grassland ecosystems, potentially enhancing soil carbon sequestration (Ayres et al. 2008). CO_2 enrichment effects on soil nematode communities vary depending on type of crop and its growth stage. Under elevated CO_2 the total numbers of herbivore, bacterivore and fungivore nematodes were higher in

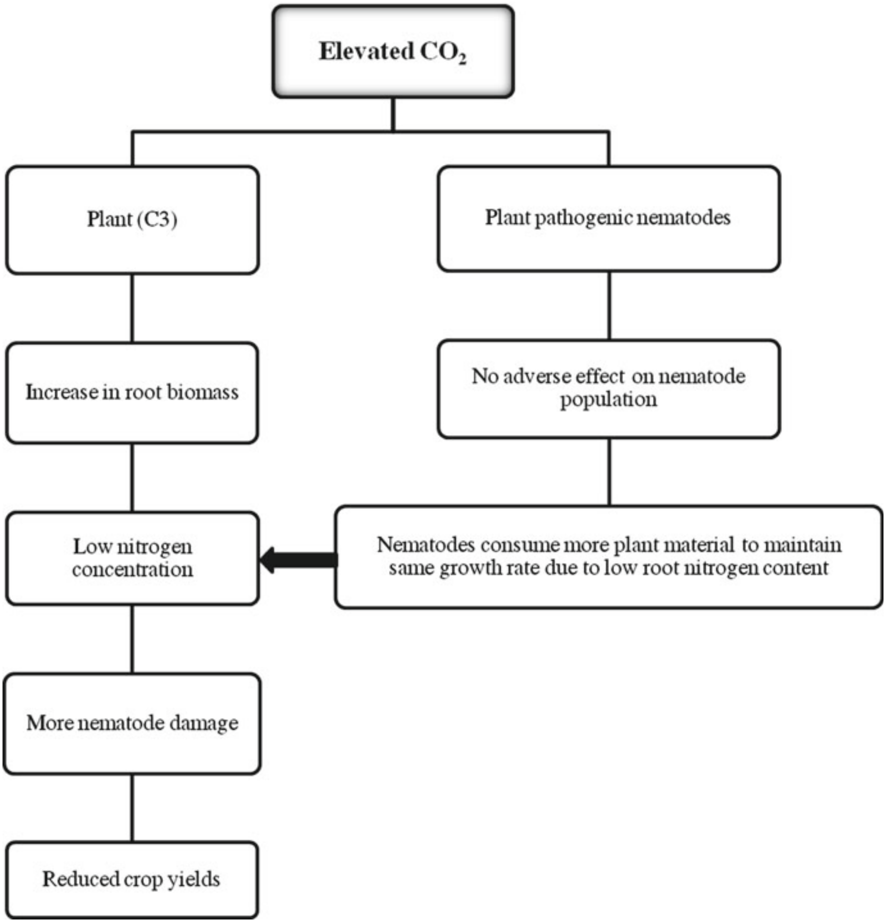

Fig. 17.1 Consequences of elevated CO_2 levels on plant-nematode interaction and crop productivity

wheat than under sugar beet. This is likely due to the very different root systems of both plants (Sticht et al. 2009). Similar results were reported by Drigo et al. (2007) and Li et al. (2007).

High temperatures are also expected to increase symptoms of water stress in plants infected with nematodes thus affecting their nutrition. Rebetez and Dobbertin (2004) demonstrated that strong climatic warming occurring in recent years was the indirect cause for the tree mortality observed in the pine forests in Switzerland based on the analyses of climatic parameters and tree mortality rates over a period of time. They observed that higher temperatures favored pine wood nematodes (*Bursaphelenchus mucronatus*) and bark beetles and increased drought stress reduced tree resistance against pathogens. Climate change may also influence the plant nematode interactions by interfering with host defense mechanisms (Sun et al. 2011).

One of the best characterized and commercially used nematode resistance genes is *Mi*, found in the wild tomato species *Lycopersicon peruvianum. Mi* suppresses nematode development and reproduction and has been used to confer resistance to several root-knot nematode species in tomato (Williamson and Kumar 2006). However, resistance mediated by the *Mi* gene is lost at elevated temperatures (Jablonska et al. 2007). Increased water stress due to climate change diminishes plant vigor and alters C:N ratios, lowering plant resistance to nematodes (Rebetez and Dobbertin 2004). Therefore, identification of a resistance source that would be stable under elevated temperature and CO_2 levels is necessary to perform under projected climate change conditions.

Plant pathogenic nematodes are known to have different optimum temperatures for feeding, hatching, reproduction and survival (Evans and Perry 2009). Small changes in temperature (warming or cooling) can have significant influences over nematode life cycle and majority of soil nematodes complete their life cycle within 2–4 weeks under optimum environmental conditions. Nematode developmental rate is directly influenced by the temperature with slower development at cooler and faster growth rate at warmer soil temperatures (Tzortzakakis and Trudgill 2005). Therefore, increase in atmospheric temperature due to global warming is expected to accelerate the nematode development and shorten the duration of life cycle resulting in more number of generations per season and expansion of their geographical distribution range. Temperature also plays a role in sex determination in some parasitic nematodes with higher temperatures favoring formation of more males which are less or not pathogenic to plants (Papadopoulou and Triantaphyllou 1982). Elevated temperatures may also interfere with the nematode survival strategies like anhydrobiosis, overwintering and diapause of eggs, etc. against extreme environmental conditions (Evans and Perry 2009). These temperature induced effects will be more pronounced in nematode species of temperate and high latitude regions than in tropics due to differences in magnitude of impact of climate change on local environmental variables and composition and response of host plants in temperate and tropical region (Rosenzweig and Liverman 1992).

17.2.3 Geographical Distribution Nematode Diseases

Since nematodes do not have the ability for active dispersal to long distances, it should be possible to forecast changes in their geographical distribution due to long-term climatic change with more certainty than most other pests. There has been considerable concern over the role of climate change in promoting the emergence of new nematode diseases and in changing the distribution of existing ones. As the global warming increases, pests and diseases from low latitude regions, where they are much more prevalent may be introduced at higher latitudes (Rosenzweig and Liverman 1992). Recent developments in experimental and modeling techniques offer considerable promise for developing an improved capability for climate change impact assessment on nematode diseases. There is evidence that climate change may play a role in expanding the distribution range of plant and animal parasitic nematodes.

The predicted effects of climate change on diseases of selected major agricultural and forestry species in Ontario showed that the cyst nematode *Heterodera glycines,* root lesion nematode *Pratylenchus* spp., and pine wood nematode *Bursaphelenchus xylophilus* severity increases due to increase in rate of disease development and potential duration of epidemic due to climate change (Boland et al. 2004). Using simulated climate change, Carter et al. (1996) predicted that warming will expand distribution of the potato cyst nematode (*Globodera rostochiensis*) and also increase number of generations per year by 2050 in Finland.

Ghini et al. (2008) carried out the risk analysis of climate change on coffee nematode *Meloidogyne incognita.* They used the Geographic Information System and compared climatological normal from 1961 to 1990 with future scenarios of the decades of the 2020s, 2050s, and 2080s (scenarios A2 and B2) from five General Circulation Models (IPCC 2001) to predict the changes in spatial distribution of nematode infestation levels based on number of generations per month in Brazil. They predicted that the infestation of *Meloidogyne incognita* races 1, 2 and 4, will increase in future due to greater number of generations per month, than occurred under the climatological normal from 1961 to 1990. The number of generations of nematodes will increase in both scenarios (A2 and B2), but for B2, it will be lower than in the A2 scenario (Fig. 17.2). The distribution range of soybean cyst nematode *Heterodera glycines* has been expanding in the U.S.A., since the early 1950s, but the increase has been more rapid since the early 1970s (Niblack 2005). Before 1970, the soybean cyst nematode was mainly distributed in the Mississippi River Delta area, northern Arkansas, southern Missouri, southern Illinois, and western Kentucky. It is now distributed throughout the main soybean production area and has become the number one soybean pest in the U.S.A. (Rosenzweig et al. 2001; Niblack 2005) (Fig. 17.3).

Neilson and Boag (1996) assessed the possible effect of climatic change on the distributions of some common virus-transmitting longidorid and trichodorid nematode species within Great Britain. They observed that theoretically an increase of 1°C in mean temperature would result in the northward extension of these nematode species by about 160–200 km. This means 1°C rise in temperature would permit populations of *X. diversicaudatum* (vector of both arabis mosaic (AMV) and strawberry latent ringspot (SLRV) viruses), *L. macrosoma* (vector of raspberry ringspot virus (RRV)), and *L. attenuatus* (vector of tomato black ring virus (TBRV)) to extend northwards into north-east Scotland and southern Scotland respectively. Colonization of new areas by virus-vector nematodes has serious implications for agriculture.

Studies addressing impact of climate change on nematode pests of crops are meager in India. Nevertheless, observations on changing infestation patterns of some important nematode pests over the years show that climate change is influencing nematode pest scenario in the country. Root-knot nematode *Meloidogyne graminicola,* once considered to be serious pest only in upland rice, has made its importance felt in all most all the rice growing areas including hill ecosystems in recent years (Prasad and Somasekhar 2009; Somasekhar and Prasad 2009).

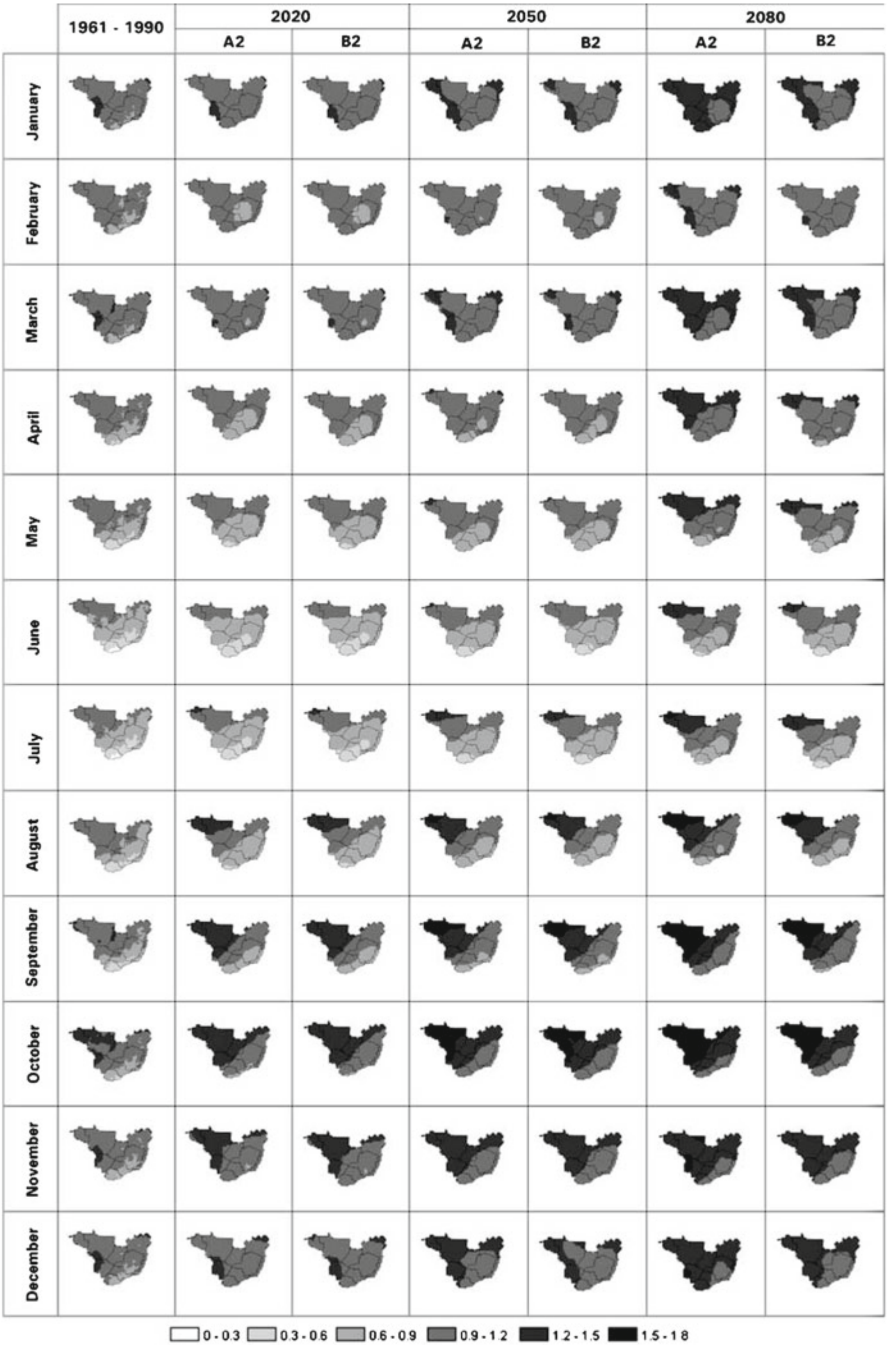

Fig. 17.2 Maps of probable number of generations of *Meloidogyne incognita* races 1 and 2 on coffee plants, from January to December, for the climatological normal from 1961 to 1990 and future climates (2020, 2050, and 2080) in scenarios A2 and B2 (Source: Ghini et al. 2008)

Fig. 17.3 Range expansion of soybean cyst nematode (*Heterodera glycines*) from 1971 to 1998 (Source: Rosenzweig et al. 2001)

Experiments have shown that survival and infectivity of this nematode was not affected by elevated CO_2 (Somasekhar and Prasad 2010). These observations indicate that climate change resulting in reduced water availability, higher temperatures, high frequency of droughts together with introduction of new water saving methods of rice cultivation such as System of Rice Intensification (SRI) and aerobic systems may further aggravate nematode problems in rice.

17.2.4 Management of Nematode Pests Under Changing Climate

Nematode pests of crops are managed by using a combination of physical, chemical, cultural and biological pest suppression methods (van der Putten et al. 2006). However, we cannot continue to rely on currently available nematode management strategies as global environment is changing. Climate change will cause alterations in the spatial and temporal distributions of nematodes and consequently the control methods will have to be altered to suit these new situations. Assessment of the impacts of climate change on pest infestations and disease epidemics in crops provide a basis for revising management practices to minimize crop losses as climatic conditions change (Ghini et al. 2008).

Changes in temperature and precipitation can alter persistence and availability of chemical nematicides in soil which may influence their efficacy. Information on impact of climate change on fate of nematicides in soil is almost negligible. Recent observations suggest that nematode pressures on plants may increase with climate change (Rosenzweig et al. 2000; Boland et al. 2004; Ghini et al. 2008).

As a consequence of this there may be a substantial rise in the use of nematicides in both temperate and tropical regions to control them. Non-chemical nematode management methods (such as green manuring, crop rotation, mulching and application of organic manures) assume great significance under changing climate scenario. Because, these approaches not only help in reducing use of chemical pesticides but also substantially mitigate global warming impacts by promoting carbon sequestration in agroecosystems (Lal 2004).

Climate change mediated changes in physiology can alter the expression of resistance genes or mechanisms of cultivars obtained through both traditional and genetic engineering methods (Sun et al. 2011). However, the most serious threat to genetic resistance to nematodes may be posed by the increased selection pressure resulting from acceleration of nematode developmental rate and increase in number of generations per season due to global warming (Boland et al. 2004; Ghini et al. 2008). Similarly, very little is known about impacts of climate change on botanicals and biological control agents. Rezacova et al. (2005) observed that *Chlonostachys rosea*, an important biological control agent of *Botrytis* and other pathogens, and *Metarrhizium anisopliae*, one of the most important entomopathogens for insect pest control, were strongly associated with the cover crop in enriched CO_2 environment. In general, botanicals, entomopathogenic nematodes, bacterial and fungal based biopesticides are highly vulnerable to environmental stresses. Increase in temperature and UV radiation, and a decrease in relative humidity may reduce the efficacy of these control tactics. Therefore, there is a need to develop appropriate strategies for nematode management that will be effective under situations of global warming in future.

17.3 Impact of Climate Change on Microbial Feeding and Predatory Nematodes

Microbial feeding and Predatory Nematodes form an important energy pathway from primary production and detritus to higher trophic groups (Ferris et al. 2001). Bacterial and fungal feeding nematodes are beneficial to crop growth because they help in nutrient cycling in soil. Global warming resulting in enhanced CO_2 can indirectly affect these nematodes through shifts in the quantity and quality of plant litter returned to soil, and complex interactions among the various functional groups of below-ground microfauna (Wardle et al. 1995). Studies on CO_2-induced changes in non-herbivorous nematode functional groups reflect highly variable and contradictory results, ranging from increased to decreased individual densities. Increase in numbers of carnivorous and omnivorous nematodes under enriched CO_2 condition was observed in several studies (Yeates et al. 1997; Hoeksema et al. 2000; Yeates et al. 2003; Li et al. 2007). In contrast to this, Niklaus et al. (2003) and Sonnemann and Wolters (2005) observed a decrease in abundance of carnivorous and omnivorous nematodes due to atmospheric CO_2 enrichment. Similar results including increased (Yeates and Orchard 1993), decreased (Yeates et al. 1997; Neher et al. 2004) or unchanged (Hungate et al. 2000; Niklaus et al. 2003; Li et al. 2007) abundance were reported for bacterivores and fungivores under atmospheric CO_2 enrichment. The type of crop cover may influence the response of nematode functional groups to CO_2 enrichment in agroecosystems. The feeding type composition of nematodes significantly differed between winter wheat and sugar beet grown under elevated CO_2. CO_2 enrichment increased the relative abundance of fungivorous nematodes under winter wheat, and the relative abundance of bacterivores under sugar beet cultivation. This indicates that under CO_2 enrichment the decomposition pathway as indicated by channel ratio shifts more towards fungal based pathway in winter wheat and towards bacterial based pathway in sugar beet (Sticht et al. 2009). Atoninka et al. (2009) observed that the overall community structure and abundance of bacterial feeders, fungal feeders, plant feeders or omnivores was not affected by CO_2 or N enrichment. However, there were 50% fewer predators in the elevated CO_2 treatment. This unexpected result suggests that either the abundance or quality of preferred prey is impacted by CO_2 enrichment.

Frederickson et al. (2001) found that the elevated CO_2 induced decrease in microfauna after 5 months of decomposition, resulting in lower numbers of nematodes, which suggests reduced turnover of the microbial biomass as a consequence of the CO_2 treatment. Nitrogen fertilization negatively influences the abundance of total nematodes, bacterivores, omnivores and carnivores which were more abundant under elevated CO_2 only under the low nitrogen conditions. The interactions among N fertilization, residue addition and elevated CO_2 were observed in the abundance of omnivores and carnivores which were low in numbers in high nitrogen and high residue plots under elevated CO_2 (Li et al. 2009). It is hypothesized that fast-growing r-strategist adapted to easily utilizable substrates are favored under elevated CO_2, and the growth and activity of the slow growing K-strategists might be disadvantaged (Tarnawski and Aragno 2006). Since the omnivores and carnivores belong to the K strategists, which are sensitive to the disturbance and at occupy relatively higher trophic levels in the soil food web (Bongers and Ferris 1999), changes in their abundance might trigger top-down effects on the soil food web (Niklaus et al. 2003). Based on the available information, it can be concluded that the effects of elevated CO_2 on soil nematode communities may not necessarily have a simple functional relationship with rhizosphere carbon and elevated CO_2 can lead to changes in nematode community structure by altering decomposition pathways (Yeates et al. 2003; Neher et al. 2004).

17.4 Impact of Climate Change on Insect Parasitic Nematodes

Insect parasitic nematodes *Heterorhabditis and Steinernema* species have a mutualistic association with the bacteria *Photorhabdus or Xenorhabdus* species, respectively. In this group of nematodes, the symbiotic bacteria produce a food signal to induce dauer juveniles (DJ) to initiate development. Laboratory experiments have showed that increase in concentration of CO_2 enhanced DJ recovery

(Jenssen et al. 2000). Elevated CO_2 up to 700 ppm showed no adverse affect on infectivity of entomopathogenic nematodes (Somasekhar and Prasad 2010). Elevated CO_2 may also influence the entomopathogenic nematodes indirectly due to change in nutritional quality of host insects due to feeding on plants grown under enriched CO_2 condition. Host plant mediated changes in susceptibility of insects to entomopathogenic nematodes have already been documented (Richmond et al. 2004). Since entomopathogenic nematodes are very sensitive to temperature and UV radiation, global warming may have adverse affect on these nematodes.

17.5 Climate Change, Ecosystem Disturbance and Nematode Model Systems

Individual nematode species may react quite differently to climate change. The dominant nematode species in Antarctica *Scottnema lindsayae* prefers drier soils while another species, *Eudorylaimus*, prefers wet conditions. In 2001, a slight warming after a prolonged cooling period caused a flood in Taylor Valley. Following this the population of *Scottnema lindsayae* decreased and that of *Eudorylaimus* increased (Mayer 2008). Documenting this type of changes help to correlate an environmental condition with response of particular species and identification of indicator species Significant elevated CO_2 effects were only found in the numbers of *Psilenchus sp.,* under rice wheat rotation system, with higher values observed in the elevated CO_2 in comparison with the ambient levels suggesting that *Psilenchus sp.,* could be regarded as a potential 'key' genus in showing the effect of elevated CO_2 on the nematode fauna (Li et al. 2007). Nematodes, the marvel of evolution possess incredible ability to survive extreme weather events and hostile environments with a simple body organization (Wharton 2002). Genome sequences of model nematodes have revealed that many genes and physiological processes in these worms are common to humans, higher animals and plants. Understanding the molecular mechanisms and genes underlying their survival under adverse environments may provide new opportunities in developing novel strategies to mitigate the stress imposed by global warming.

Nematode communities in soil are composed of a variety of trophic and ecological groups which can be directly linked to status of key ecosystem functions like primary production, primary consumption, secondary consumption, decomposition, etc. (Ferris et al. 2001). Therefore, soil nematode communities serve as an excellent model system for studying impacts of climate change on the belowground food webs and its implications to aboveground productivity. Nematodes are being extensively investigated in most studies analyzing response of terrestrial ecosystems to climate change. In less diverse Antarctic ecosystems where species diversity and thus functional redundancy is low, ecosystem functioning is dependent on a few 'keystone' species (Barret et al. 2008). Loss of these species will have a major effect on food web dynamics and the carbon cycle. In the Taylor Valley of Antarctica, a single species of bacterial feeding nematode *Scottnema lindsayae,* plays a significant role in carbon cycling. It was observed that there was a 65% reduction in the abundance of *S. lindsayae* between 1993 and 2005, which is ascribed to regional climate change i.e. sudden cooling in the Taylor Valley. Cooling resulted in fewer days of above 0°C, which limited reproduction of this nematode. The 65% reduction in nematode population was observed to be associated with a 32% loss of function in carbon cycling suggesting that changes in abundance of a single key species may have major repercussions particularly in low diversity ecosystems (Barret et al. 2008).

17.6 Conclusions

Studies addressing impacts of climate change on plant pathogenic nematodes have been limited. Nevertheless, findings of these studies give an insight into the response of nematode pathogens to climate change and its consequences to crop production. Analysis of available information on responses of plant pathogenic nematodes to CO_2 enrichment indicates that the responses are either neutral or positive but not negative. CO_2-induced changes in non-plant parasitic nematode functional groups are highly variable. Further, studies predicting the potential changes in development and geographical distribution of plant parasitic nematodes using predicted climate change scenarios give a fairly good idea about future scenarios of nematode diseases of plants. Available information clearly indicates that climate change could alter rates of development of nematodes, modify host resistance, and result in changes in the physiology of plant-nematode interactions. There is ample evidence that global warming will alter the patterns of nematode diseases of crop plants. The most likely consequences are expansion in the geographical distribution range of nematode disease and increased crop losses. Investigations being carried out on Antarctic nematodes are also helping in demonstrating the correlation between nematode biodiversity and ecosystem functions under changing climate. More long-term studies in varied ecosystems under different cropping systems of particularly tropical regions are needed to critically assess the impact of climate change on soil nematodes. This knowledge is vital for developing appropriate mitigation and adaptation strategies to minimize impact of climate change on agriculture.

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Chapter 18
Socio Economic and Policy Issues in Abiotic Stress Management

K. Kareemulla and C.A. Rama Rao

Abstract Abiotic stress and biotic stress are the two broad factors that constrain the growth in agricultural productivity needed for economic development of developing countries such as India. The importance of abiotic stress is likely to increase with wide spread land degradation because of various physical, biological and chemical processes and the projected impacts of climate change will further accentuate the ill effects of abiotic stress. A number of demographic and economic factors have been responsible for degradation of natural resources that predisposes crop plants to abiotic stress. Abiotic stress is different from biotic stress in that the two principal factors, soil and climate, involved are difficult to be manipulated and in that the incidence is more covariate. These two dimensions make abiotic stress more difficult to be managed. While the governments are now better placed in terms of having more resources and better technical and scientific capabilities to deal with impacts of abiotic stress, the households and regions within countries continue to be more vulnerable. What is required is a strategy that coordinates technical and institutional measures to be taken up by different stake holders at varying scales.

18.1 Introduction

Agricultural production in India and in many developing countries has increased considerably, particularly since the mid 1960s. The production of food grains, oilseeds, fibre crops, fruits and vegetables increased by several folds at least in the

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Indian context. However, projections of future requirements show that the productivity is to be improved considerably, at rates higher than the historical rates, if India and similar developing countries have to be self-sufficient and agriculture is to contribute positively to the economic growth.

The growth in agricultural production in India has been driven by expansion of cultivated area and increase in productivity. Given the demographic and economic scenario, the scope for expansion of cultivated area is very limited. There is in fact a possibility of transfer of land from agricultural to non-agricultural uses. The future growth in production has therefore to be driven by productivity growth alone. The challenge facing the agricultural systems worldwide is to raise productivity in a fast changing economic and environmental setting.

With the growth in Indian economy, a country with wide agro-climatic variations, the share of agriculture in the gross domestic product (GDP) fell considerably from more than 50% during 1950s to about 17% during 2009–2010. However, the proportion of population that is dependent on agriculture is still at about 65%. What this implies is that agriculture is still a source of livelihood to millions of people and that the incomes of people dependent on agriculture are much lower than the incomes of those dependent on non-agricultural sectors. In fact these disparities have widened over time. Therefore any stress experienced by the agriculture sector will have wide ramifications on a huge population.

Among various factors that influence agricultural production, biotic and abiotic stresses are two important generic causes that are to be addressed for enhancing productivity levels. The relative importance of abiotic stress is likely to increase in future decades considering the land degradation associated with erosion and unsustainable soil management practices. The projected impacts of climate change, if realized, may further aggravate the abiotic stress. With the projected 15–50% loss in agricultural productivity by 2080 due to climate change, the Asia – Pacific region faces severe threat of food insufficiency and hunger (Nellemann et al. 2009).

18.2 Types and Nature of Abiotic Stress

Scarcity of water and high temperatures are the major components of stress and these two are highly interlinked. Scarcity of water is an important yield reducer especially when there is no access to irrigation. This is a major problem in countries like India where the rainfall is monsoon dependent and the course of monsoon is highly variable both within year and over years. The problem is often manifested in the form of droughts of various intensities. The abiotic stress caused by water scarcity is therefore a critical threat to rainfed agriculture. Quality of soils, in terms of texture, depth, fertility status, can either aggravate or moderate the adverse effects of abiotic stress caused by inadequate amounts of rainfall. The occurrence of repeated droughts, where the rainfall is less than normal, can potentially pose threat to irrigated agriculture too as the irrigation sources are also dependent on annual rainfall. The other important causes of abiotic stress are inadequate supply of plant nutrients resulting from poor quantities of nutrients available in the soil or from the imbalances in the chemical composition affecting the plant's ability to absorb nutrients. Stress due to soil salinity reflects the latter. Degradation of land can cause or aggravate different types of abiotic stress. Commercialization of agriculture and certain government policies also have paved conditions for incidence of abiotic stress. For example, the High Yielding Varieties (HYVs) are highly responsive to added inputs, especially fertilizers and water. The government policies of subsidized water and fertilizers have led to over use of these resources having a deleterious effect on the resource base.

18.2.1 Soil Fertility Stress

Declining soil fertility and nutrient imbalance is another major issue affecting agricultural productivity. Organic matter levels have declined sharply in intensively cropped Indo-Gangetic plains leading to stagnant yields of rice-wheat cropping system. In addition to universal deficiency of nitrogen, deficiencies of potassium, sulphur and micro nutrients are increasing. Zinc deficiency has become most acute followed by sulphur and boron (Motsara 2002). It is estimated that 29.4 m ha of soils in India are experiencing decline in fertility with a net negative balance of 8–10 mt of nutrients per annum (Lal 2004). Poor nutrient use efficiency is another cause of concern.

18.2.2 Salinity and Water Logging

Soil salinity is another constraint limiting productivity on large areas. Salt-affected soils occur extensively in different agro-ecological and soil zones, particularly the arid, semi-arid and the sub-humid regions. The most affected states are Uttar Pradesh, Gujarat, Rajasthan, West Bengal and Andhra Pradesh. The soils are categorized as alkali or saline soils depending upon the nature of soluble salts. The development of the major and medium irrigation projects has led to the occurrence of water logging and secondary salinity in irrigation commands. The area under salinity has been increasing at the rate of 3,000–4,000 ha per annum. As per an estimate (Anonymous 2008), about 5.6 M ha of the salt-affected soils out of the total of 8.5 m ha, are in the irrigation commands. Water-logging of fertile alluviams in eastern India (3.28 m ha) and acidity in about 25 m ha of arable lands in east and north east are other critical constraints affecting productivity.

Excess water or inundation that is experienced during floods is yet another factor of abiotic stress and can be detrimental to survival, growth and yield of a crop. The projected impacts of climate change are likely to increase the frequencies of droughts, floods and an increase in temperature and therefore, the relative importance of abiotic stress will only increase in the coming decades.

18.2.3 Abiotic vs. Biotic Stress

The abiotic stress is different from biotic stress in two respects. First, the two most important factors involved in abiotic stress are soil and climate. It is very difficult to bring about changes in both of these factors. Though the soil quality is amenable to some extent through certain management practices, it takes a long time before any appreciable improvement is observed. Secondly, the incidence of abiotic stress is relatively more covariate in the sense that the 'risk' will occur in a given 'area' rather than to a given individual plot or piece of land. When land holdings are small as is the case in India, it implies that a large number of households are affected by any particular incidence of stress. This makes the risk coping instruments like insurance very uneconomical.

18.2.4 Tackling Abiotic Stress

Crop stress can be broadly described as symptomatic and a combined result. Often, the farmers and the researchers are puzzled at one truth of the nature that is the irregularity of climate variables like rainfall, temperature, etc. For the crop, occurrence of drought is of paramount importance. Simelton et al. (2009) identified socio-economic indicators associated with sensitivity and resilience to drought for crops like rice, wheat and corn under Chinese conditions. Provincial harvest and rainfall data (1961–2001) are used to calculate an annual 'crop-drought vulnerability index'. Similarly, many parameters could be used to understand the socio-economic implications of crop stress.

Crop stress is on account of biotic and abiotic forces. While biotic forces are tackled mechanically/chemically or biologically, the abiotic stress is to be either managed through mitigation or abatement strategies. In view of the imminent climate change impact, the major abiotic stress which is likely to be faced and the possible interventions are illustrated in Table 18.1 (Bennet et al. 2003).

In the five most important crops of the semi-arid tropics (sorghum, pearl millet, pigeonpea, chickpea and groundnut), the biotic and abiotic stress factors have been estimated to cause an estimated loss of US$ 15.74 billion (Sharma et al. 2001).

Technical and policy instruments complement each other for reduction of harmful impacts and thereby build climate change resilience among crops. Therefore, it is imperative that various dimensions of socio-economic issues are taken into account in evolving and implementing techno-economic interventions. Hence, the following issues need to be considered in the process.

- Land degradation and implications – socio economic medium and long term
- Relationship of abiotic stress on poverty and resource poor farmers

Table 18.1 Abiotic stresses, possible interventions and the likelihood of major progress within 5 years

Abiotic stress	Possible interventions	Likelihood of progress within 5 years
Saline soils and water (presence of salts and of sodium salts)	Increase salt tolerance of rice	High
	Improve leaching; apply gypsum, improve various agronomic practices	High
	Improve water and salt management	High
Water logging	Increase tolerance for water logging	Medium
	Improve water management	Medium
Water pollution (agro-chemicals; industrial waste products)	Improve dosage of agro-chemicals and waste water treatment	Low
Acid sulfate soils (low pH, toxic anaerobic conditions in root zone)	Short duration varieties and seedling vigor	High
	Improve water management	Medium
High and low temperatures	Increase heat and cold tolerance at flowering	Medium
	Improve cooling mechanism for leaves	High
Dry periods and droughts	Improve irrigation infrastructure	Medium
	Increase drought tolerance	High
	Short duration varieties and seedling vigor	High
Floods	Increase tolerance for submergence	High
	Improve water management at river basin level	Low
Air pollution	Improve environmental quality (industrial and urban waste gases)	Low

Source: Bennett (2003)

- Community actions for mitigation and coping mechanisms
- Public policies for communities and regions affected by abiotic stress

18.3 Land Degradation and Implications

Land being the basic resource, on which crops are cultivated, any deficiency in this resource certainly adds to the stress experienced by the crop. The land based stress can be on account of the physico-chemical properties like slope, structure, texture, nutrients status, etc. One of the broad indicators of nature of land is the extent of degradation. Crops grown in degraded lands are more likely to be experiencing stress.

To get a glimpse of the land degradation in a highly populous country like India, the state (province) wise distribution degraded land is presented below:

At the country level, the share of degraded land is to the tune of 47%. Across the provinces, this level ranged between 11% and 94% with the least being in Punjab and the highest in Jammu & Kashmir and Nagaland. In the Indo-Gangetic belt which is mainly represented by provinces like Uttar Pradesh, the extent of land degradation is 63%. The Deccan Plateau states like Andhra Pradesh and Karnataka have a degraded land to the extent of 37% and 46%, respectively.

It has been widely propounded that continuous land degradation ultimately leads to desertification which causes extreme stress for crops. Jesu et al. (2000) developed a historical analysis of relationships between human activities and the environment for a period of five centuries, i.e. sixteenth to twentieth century, focusing on agriculture, mining and demography. They have opined that enormous socio-economic changes which began in sixteenth century contributed to the formation of the present day deserts. Similarly, the present day's semi-desert landscapes are a result of anthropogenic processes during the nineteenth century. Particularly, interventions like mining along with large scale consumption of wood, expansion of agriculture and demographic explosion destroyed the forests and significantly altered the ecosystems.

Another significant ecosystem changes have come in the coastal zones, which are home to about 60% of global population. Historically, concentration of urbanizations on the coasts is primarily due to use of seas as the trade route. Consequently, problems associated with urbanization and industrialization resulted in harmful effect on coastal ecologies and on the communities living over there (Lakshmi and Rajagopalan 2000).

The major causes of land use and land-cover change are obviously understood by both scientists and the farmers, which have resulted in environment-development policies. Peoples' responses to economic opportunities, as mediated by institutional factors, drive land-cover changes. Opportunities and constraints for new land uses are created by local as well as national markets and policies. Global forces become the main determinants of land-use change, as they amplify or attenuate local factors (Lambin et al. 2001).

Ye and Ranst (2009) simulated the effect of soil degradation on long term food security in China. They have predicted that by 2030 the loss of productivity will be 9% if the soil continues to be degrading at the current rate. Further, this loss is likely to increase upto a level of 30% by 2050. Technical counter measures and policy interventions need to be implemented to avoid this catastrophe.

18.3.1 Abiotic Stress vs. Poverty

Generally, the degraded areas including deserts and arid areas are endowed with poor resource status including crops. However, the human habitation in these areas will continue to exist for various reasons. Therefore, the stress experienced by crops grown in these areas will have a direct bearing on the populace.

The incidence of drought, the dominant from of abiotic stress, will have many socio-economic consequences. At the individual household or farm level, incidence of drought will cause a reduction in yield and also incomes of the farmers. When the incidence is widespread, it will lead to fall in supply of commodities pushing the prices up. As a result, farmer will suffer both as a producer as well as a consumer. When the purchasing power (incomes) of farmers falls, it will also affect the demand for industrial goods and services and thus lead to overall slow down of economic growth.

It was observed that rural poverty is more concentrated in the regions dominated by rainfed agriculture. Since agriculture is the principal livelihood in rural areas, the abiotic (and biotic stress) will lead to underperformance of the systems adversely affecting the livelihood outcomes. When the resource base, biophysical as well as socioeconomic, is so poor as in rainfed agriculture dominated areas, the farmers are trapped in a vicious cycle of poverty. To get these communities out of poverty, it is essential that farmers' capital – natural, physical, human and social – is strengthened. Recurring crop stress not only affects the immediate livelihood outcomes but also affects the ability of people to cope with the stress and pursue more sustainable livelihood activities.

Wurtenberger et al. (2006) studied the impact of liberalization on environmental and social consequences which are rarely included in the agricultural policies, both at national and international level. They used a method for quantifying and assessing the land use hidden in the trade of agricultural goods for Switzerland. The reason for this might be due to lack of concepts and tools to assess such impacts. Farmers' cropping decisions influenced more by market considerations can prove to be unsustainable. Further research is required for refining the utility functions, including weightage procedures in the aggregation models. Besides this, important aspects like water shortage should enlarge the indicators to be included in such model.

Long-term ecosystem changes in the Benguela region include species alternations and regime shifts, which are sometimes obscured by large intra- and inter-annual variability in the ecosystem (Jarre et al. 2006). No single model or approach can resolve this variability and effectively detect and predict long-term ecosystem changes; a coherent, robust, transparent and reproducible synthesis framework is required. Indicators and models are described that can be used to identify some aspects of the current state of ecosystem structure and to detect and monitor long-term change. A short-term challenge is to synthesize these varied sources of multi-disciplinary (and sometimes contradictory) information in a logical and consistent fashion. An expert system approach is proposed to do this, consolidating results of different indicators and models within a dynamic process that uses feedbacks to validate predictions of the expert system, and to improve it. It is suggested that such an approach should be initiated in the short term, even as models and indicators are being developed further. In parallel, multivariate statistical tools should be refined and applied to existing time series, to identify past periods of ecosystem change. Current data gaps should be filled, including time series of primary production and the abundance of gelatinous zooplankton. In the medium term, the expert system model should evolve to a point where its results can be used to inform various management groups about the state of the ecosystem. Part of this evolution requires that ecosystem indicators be presented with error estimates or formal assessments of quality.

Simelton et al. (2009) raised some fundamental issues pertaining abiotic stress. These are – why is it that sometimes small droughts trigger serious crop losses while in other cases even large droughts do not have such a major effect? They identified socio-economic indicators associated with sensitivity and resilience to drought for each of China's main grain crops (rice, wheat and corn). Provincial harvest and rainfall data (1961–2001) are used to calculate an annual "crop-drought vulnerability index". Sensitive cases (where significant harvest losses occurred in years with only minor droughts) and resilient cases (where harvest losses were minimal despite there being a major drought) were separated and they explored the socio-economic characteristics of these different situations. Results show that sensitive cases were particularly common in economically poor landlocked provinces *and* in wealthy coastal areas that have a limited land base. In such sensitive cases, the size of the rural population and the quantity of agricultural inputs were negatively correlated with drought vulnerability, while for resilient cases, vulnerability was negatively correlated with the abundance of land. This led them to propose a series of drought-vulnerability typologies based on the extent to which land, labour, capital, agricultural technology, and infrastructure buffer or exacerbate the effect of a drought event.

A review of soil erosion research in the West African Sahel finds that there are insufficient data on which to base policy (Warren et al. 2001). This is largely because of the difficulties in measuring erosion and the other components of soil life, and because in the highly spatially and temporarily variable natural and social environment of the Sahel. However, a local political ecology of soil erosion and new methodologies offer some hope of overcoming these problems. Nonetheless, a major knowledge gap will remain, about how rates of erosion are accommodated and appraised within very variable social and economic conditions. An example from recent field work in Niger shows that erosion is correlated with factors such as male migration, suggesting, in this case, that households with access to non-farm income adopt a risk-avoidance strategy in which soil erosion is accelerated incidentally. It is concluded that there needs to be more research into the relations between erosion and socio-economic factors, and clearer thinking about the meaning of sustainability as it refers to soil erosion in the Sahel.

Approaches for targeting agricultural research to benefit poor farmers were explored (Bellon et al. 2005). Using small area estimation methods and spatial analysis, high-resolution poverty maps were generated and superimposed with geo-referenced biophysical data relevant to maize-based agriculture in Mexico. Multivariate classification and cluster analysis can be used to synthesize biophysical data relevant for crop performance with rural poverty data. Results show that the rural poor are concentrated in particular regions and under particular circumstances. Formal maize germplasm improvement trials were largely outside the core areas of rural poverty and there was little evidence for direct spillover of improved germplasm. Agro-climatic classification used for targeting breeding is useful but often ignores some important factors identified as relevant for the poor. Combining this method with poverty mapping improves stratifying and targeting crop breeding efforts to meet the demands of resource-poor farmers. This integrated approach will help increase benefits from agricultural research to poor rural communities.

Water and crop are inseparable. The major abiotic stress experienced by crops is related to water availability. Water is critically important to the livelihoods of more than one billion people living on less than $1 a day, particularly for the 850 million rural poor primarily engaged in agriculture (Namara et al. 2010). In many developing countries, water is a major factor constraining agricultural output, and income of the world's rural poor. Improved agricultural water management can contribute to poverty reduction through several pathways. First, access to reliable water improves production and productivity, enhances employment opportunities and stabilizes income and consumption. Secondly, it encourages the utilization of other yield-enhancing inputs and allows diversification into high-value products, enhances non-farm outputs and employment, and fulfils multiple needs of households. Third, it may contribute either negatively or positively to nutritional status, health, societal equity and environment. The net impact of agricultural water management interventions on poverty may depend individually and/or synergistically on the working of these pathways. Improved access to water is essential, but not sufficient for sustained poverty reduction. Investments are needed in agricultural science and technology, policies and institutions, economic reform, addressing global agricultural trade inequities, etc. But how best to match the agricultural water management technologies, institutions and policies to the needs of the heterogeneous poor living in diverse agro-ecological settings remains unclear.

Pollution of water resources, both surface water and groundwater, and of the air by industrial and urban emissions presents increasingly severe problems for rice production near urban and industrial centers. Its mitigation requires large-scale improvements in industrial and urban waste management. In many developing countries, the impact of air and water pollution in terms of reduced production and health hazards has hardly been assessed and it is only recently that environmental protection measures have been introduced and enforced. The probability appears to be small that much progress will be made in this respect within the next 5 years.

18.4 Community Actions for Mitigation and Coping Mechanisms

For centuries, traditional farmers have developed diverse and locally adapted agricultural systems, managing them with ingenious practices that often result in both community food security and the conservation of agro-biodiversity. This strategy of minimizing risk stabilizes yields, promotes dietary diversity, and maximizes returns using low levels of technology and limited resources. These microcosms of agricultural heritage still cover no less than ten million ha worldwide, providing cultural and ecological services not only to rural inhabitants, but to mankind generally. These services include the preservation of traditional farming knowledge, local crop and animal varieties, and native forms of socio-cultural organization. By studying these systems, ecologists can enhance their knowledge of the dynamics of complex systems, especially the relationship between biodiversity and ecosystem function and practical principles for the design of more sustainable agro-ecosystems appropriate to small farmers. Novel agro-ecosystem designs have already been modeled on successful traditional farming systems.

Water scarcity in northern China has been a topic of concern in China for many years, but the increased frequency and duration of no-flow events in the Yellow River in the 1990s created a flurry of recent activity in the academic and policy arenas (Liu et al. 2008). These low-flow events severely disrupted the supply of irrigation water for agriculture in the lower reaches of the Yellow River and posed a substantial threat to farmers' livelihoods. Within a broader effort to assess farmers' vulnerability to water shortages, this qualitative research focused on the coping mechanisms and adaptive strategies adopted by farming households in three villages in Shandong Province (Ma, Ding, and Xing). With increasing water stress and other stresses from land degradation and lack of market access, farmers' coping mechanisms have evolved, expanding from one-time adjustments to long-term adaptations, and switching focus from securing reliable water sources to improving irrigation efficiency and diversifying both on-farm and off-farm production.

Paavola (2008) examined farmers' livelihood responses and vulnerability to climate variability and other stressors in Morogoro, Tanzania, to understand their implications for adaptation to climate change by agricultural households in developing world more generally. In Morogoro, agricultural households have extended cultivation, intensified agriculture, diversified livelihoods and migrated to gain access to land, markets and employment as a response to climatic and other stressors. Some of these responses have depleted and degraded natural resources such as forest, soil and water resources, which will complicate their living with climate change in the future. This will be particularly problematic to vulnerable groups such as women, children and pastoralists who have limited access to employment, markets and public services. In this light, fair adaptation to climate change by agricultural households in Morogoro and elsewhere in developing countries requires several complementary responses. Adaptation efforts should involve effective governance of natural resources because they function as safety nets to vulnerable groups. In addition, strengthening of national markets by infrastructure investments and institutional reforms is needed to give incentives to intensification and diversification in agriculture. Market participation also demands enhancement of human capital by public programs on health, education and wellbeing.

18.5 Dealing with Abiotic Stress – Socio-Economic Dimensions

As mentioned earlier, improvement of natural resources is critical to dealing with abiotic stress. There are a number of technologies proven to improve the quality of land and water resources. These range from fertilizer application (to address nutrient stress) to *in situ* and *ex situ* soil and water conservation methods, better drainage and to treatments at watershed, catchment and river basin scales. But adoption of these technologies is governed by a wide array of factors. Strengthening human capital of farmers is a necessary condition to enable them to be aware of these technologies. It is also important to recognize the time scale over which the benefits of some conservation technologies can be appreciated by the farmers. For example, the benefits of adoption of technologies for improving the content of organic matter, and for conserving soil and water resources will be visible in terms of protecting the crops from abiotic stress only over a period of time. These technologies also involve higher capital investments which need to be supported by the governments. Another issue is that the technologies are also to be complemented by appropriate management and institutional interventions for being effective which means that the members of communities have to be mobilized and brought together both individually and collectively. The centrality of community participation observed in the successful watershed development programmes is a case in point.

Communities in the drought-prone areas have evolved some typical coping measures to mitigate the effects of drought. These primarily include reduction in consumption, participation in labour market, asset liquidation, migration and many forms of informal resource sharing. But, many of the informal resource sharing have now disappeared as agriculture become more commercialized and market dependent. To the extent that the modern agriculture is dependent on adequate supply of inputs and services, it also became more vulnerable to changes therein. The market risk is further aggravating the climatic risk.

At the macro level, countries and regions are now better placed to deal with climatic risk, particularly droughts and floods than they were a few decades ago. With the economic growth, we now have more resources that can be deployed to manage risk, and have abundant stocks of food grains. Science and technology has so developed significantly that we are in a position to forecast the major weather aberrations and be prepared for minimizing the losses. However, at microlevel, households continue to suffer from the vagaries of weather that inflict production, consumption and welfare losses. It takes a very highly coordinated effort from various channels of governments, communities and civil society organizations to cope with abiotic stress with least strain at individual, household, regional and country levels.

18.6 Public Policies for Communities and Regions Affected by Abiotic Stress

Natural Resource Management, community actions and public policies generally work in different directions. However, from the sustainability view point, these three have to converge in order to bring in resilience in agriculture, especially, in crop abiotic stress management. One of the major areas of such convergence for effective abiotic stress management is in soil nutrient management.

At global scale nutrient flows are unbalanced, resulting in huge surpluses in Western Europe in contrast to the negative balances in Africa (Jager 2005). Existing policy and socio-economic environments in different parts of the world are the major cause of this situation. At lower spatial scales, a much more complex and diverse picture emerges. In Sub-Saharan Africa, different levels and causes of soil fertility decline are found among agro-ecological zones, soil types, farm systems, wealth groups, urban–rural households, cash and food crops, home fields and bush fields. Technology development has been the traditional response to address nutrient imbalances in general, and soil fertility decline in Africa in particular. Farm households have continued to develop and adapt existing technology to situations. National and international research institutions have followed a variety of approaches of which the recently developed participatory approaches have yielded some impressive results in isolated cases. These efforts have, however, not led to the necessary massive reversion of the trend in soil fertility decline. The Dutch policies on nutrient use and the Indonesian policy to adopt Integrated Pest Management are two examples, associated with such major trend reversions. This suggests that promoting and supporting participatory technologies have limited impact when no attention is paid to participatory policy development and implementation. In order to mobilise farm households in a trend reversion, a number of conditions should be met such as stable prices for agricultural outputs, better input/output prices ratios, influence of land users on the research agenda and private-public initiatives focused on smallholders. This observation calls for the establishment of interactive land users-science-policy triangles at various scales (local, national and international) in which joint learning and mediating may lead to more informed decision making, more focused design of an agricultural sector policy, implementation of policies by effective institutions, and appropriate technology development and implementation. Interventions need to be reoriented: less technology development, more policy influence and institution building.

Water scarcity is usually portrayed in absolute or volumetric terms. But do most analyses of scarcity focus on how the ‘problem’ of scarcity is constructed, the need to disaggregate users and their entitlements and the imperative to look at the politics of distribution and technology choice within a frame of political economy? By taking the case of water scarcity in Kutch, western India which is supposed to benefit from the controversial Sardar Sarovar Narmada Project (SSP), it was argued that how scarcity has emerged as a ‘metanarrative’ that justifies controversial schemes such as large dams, allows for simplistic portrayals of property rights and resource conflicts and also ignores the cultural and symbolic dimensions of resources such as water (Mehta 2006). Moreover, water scarcity tends to be naturalised and its anthropogenic dimensions are whitewashed. It is thus necessary to distinguish between the biophysical aspects of scarcity that are lived and experienced differently by different people and its ‘constructed’ aspects. Conceptual approaches such as political ecology, common property resource theory and post-institutional approaches to highlight that scarcity need to be evaluated in this context. Instead, it is usually socially mediated and the result of socio-political and institutional processes. While institutional perspectives have played a key role in moving away from alarmist portrayals of scarcity and property rights by demonstrating how local people can manage and live with scarcity, they need to be complemented by analyses that locate property rights within wider historical, cultural and socio-political processes that combine both discursive and materialist analyses.

Falloon and Betts (2009) reviewed and qualitatively assessed the importance of interactions and feedbacks in assessing climate change impacts on water and agriculture in Europe. The focus was particularly on the impact of future hydrological changes on agricultural greenhouse gas (GHG) mitigation and adaptation options. Future projected trends in European agriculture include northward movement of crop suitability zones and increasing crop productivity in Northern Europe, but declining productivity and suitability in Southern Europe. This may be accompanied by a widening of water resource differences between the North and South, and an increase in extreme rainfall events and droughts. Changes in future hydrology and water management practices will influence agricultural adaptation measures and alter the effectiveness of agricultural mitigation strategies. These interactions are often highly complex and influenced by a number of factors which are themselves influenced by climate. Mainly positive impacts may be anticipated for Northern Europe, where agricultural adaptation may be shaped by reduced vulnerability of production, increased water supply and reduced water demand. However, increasing flood hazards may present challenges for agriculture, and summer irrigation shortages may result from earlier spring runoff peaks in some regions. Conversely, the need for effective adaptation will be greatest in Southern Europe as a result of increased production vulnerability, reduced water supply and increased demands for irrigation. Increasing flood and drought risks will further contribute to the need for robust management practices.

The impacts of future hydrological changes on agricultural mitigation in Europe will depend on the balance between changes in productivity and rates of decomposition and GHG emission, both of which depend on climatic, land and management factors. Small increases in European soil organic carbon (SOC) stocks per unit land area are anticipated considering changes in climate, management and land use, although an overall reduction in the total stock may result from a smaller agricultural land area. Adaptation in the water sector could potentially provide additional benefits to agricultural production such as reduced flood risk and increased drought resilience.

The two main sources of uncertainty in climate impacts on European agriculture and water management are projections of future climate and their resulting impacts on water and agriculture. Since changes in climate, agricultural ecosystems and hydrometeorology depend on complex interactions between the atmosphere, biosphere and hydrological cycle there is a need for more integrated approaches to climate impacts assessments. Methods for assessing options which “moderate” the impact of agriculture in the wider sense will also need to consider cross-sectoral impacts and socio-economic aspects.

The Indo-Gangetic plain (IGP; including regions of Pakistan, India, Nepal, and Bangladesh) is generally characterised by fertile soils, favourable climate and an abundant supply of water. Nevertheless, the challenge of increasing food production in the IGP in line with demand grows ever greater; any perturbation in agriculture will considerably affect the food systems of the region and increase the vulnerability of the resource-poor population. Increasing regional production is already complicated by increasing competition for land resources by non-agricultural sectors and by the deterioration of agri-environments and water resources. Global environmental change (GEC), especially changes in climate mean values and variability, will further complicate the agricultural situation and will therefore, have serious implications for food systems of the region (Aggarwal et al. 2004). Strategies to reduce the vulnerability of the region's food systems to GEC need to be based on a combination of technical and policy options, and developed in recognition of the concurrent changes in socioeconomic stresses. Adaptation options need to be assessed with regard to their socioeconomic and environmental efficacy, but a greater understanding of the interactions of food systems with GEC is needed to be able to do this with confidence. Further information is needed to develop a range of adaptation strategies including augmenting production and its sustainability, increasing income from agricultural enterprises, diversification from rice–wheat systems, improving land use and natural resource management, and instigating more flexible policies and institutions.

Throughout the developing world, resource-poor farmers (about 1.4 billion people) located in risk-prone, marginal environments, remain untouched by modern agricultural technology. A new approach to natural resource management must be developed so that new management systems can be tailored and adapted in a site-specific way to highly variable and diverse farm conditions typical of resource-poor farmers (Altieri 2002). Agroecology provides the scientific basis to address the production by a biodiverse agroecosystem able to sponsor its own functioning. Obviously, a relevant research agenda setting should involve the full participation of farmers with other institutions serving a facilitating role. The implementation of the agenda will also imply major institutional and policy changes.

Irrigated crops are increasingly facing water scarcity and other forms of abiotic stress, including the presence of salts and other pollutants in soil and irrigation water, water logging and flooding of soils, low pH in acid sulfate soils, and anaerobic and toxic conditions in the root zone (Kijne 2006). More progress has been made with the alleviation of some of these stresses than with others. It would be interesting to examine why salinity of soil and irrigation water is common and nearly inevitable in the irrigated lands of the semi-arid tropics and subtropics. Opportunities for and constraints to making management changes that would increase the productivity of water (i.e., the yield per unit of water diverted from the source or consumed in evapotranspiration) at system and basin level were assessed. Success of water management interventions in reducing the impact of abiotic stresses on rice production under saline conditions and on acid soils depends on improved control over the components of the water and ion balances at field and basin level. One of these measures is the installation of drainage systems. The benefits and costs of sub-surface drainage are illustrated by an example from Egypt's Nile Delta. An integrated approach to water resource management is an essential but not sufficient condition to prevent conflicts between different users and consumers of the scare resource.

The world's irrigated area per capita has decreased from a peak of 48 ha/1,000 people in the late 1970s (Gleick 1993) to about 42 ha/1,000 people in 2002. A further decrease will occur so long as population growth continues to exceed the rate of growth of the irrigated area. Population growth and water scarcity combined mean that there is no alternative but to increase the output of existing arable land through technological innovations in order to feed the world's growing population. Past technological innovations that made it possible to provide enough food included the introduction of high yielding varieties of seeds, and high inputs of water, fertilizers and pesticides. However, there is concern that for a variety of reasons the rate of yield increase on irrigated lands in the tropics has leveled off. The case of stagnating yields from the rice-wheat production system in the Indian subcontinent is a classic case.

Reece (2007) explored the possible impact of genomics on the relationship between resource-poor farmers and 'formal' agricultural research. Regions of low-resource agriculture serve as reservoirs of genetic diversity and then outlines some ways in which advances in genomics are enabling agricultural researchers to make increased use of this kind of genetic material. The adoption of genomics-based tools by agricultural research programmes will, it suggests, increase the benefits they can obtain from initiatives that elicit germplasm selected by farmers. The interactions between these two processes are examined in two distinct (and contrasting) contexts: maize in China and rice in Vietnam. Both of these case studies confirm that indigenous genetic resources take on greater significance to research systems that are equipped with genomics-based tools. Ability of resource-poor farmers to demand the provision of research services relevant to their situation may increase as the resources that they control take on greater value to agricultural research.

The world's drylands will face not only increasing temperatures with climate change but more importantly also disruptions to their hydrological cycles resulting in less and more erratic rainfall that will exacerbate the already critical state of water scarcity and conflicts over water allocation (Thomas 2008). The rural poor in dry areas will suffer the most from these changes and will require a range of coping strategies to help them adapt to changing climates. Strategies will include changing of cropping systems and patterns, switching from cereal-based systems to cereal–legumes and diversifying production systems into higher value and greater water use efficient options. The latter include judicious use of water using supplementary irrigation systems, more efficient irrigation practices and the adaptation and adoption of existing and new water harvesting technologies. Scope for the application of conservation agriculture in dry areas is thought to be limited by low biomass production but current evidence suggests that even small amounts of residue retention can significantly decrease soil erosion losses. These options will be supplemented by the development of more drought and heat tolerant germplasm using traditional and participatory plant breeding methodologies and better predictions of extreme climatic events. The majority of drylands are occupied by rangelands with some 828 Mha in West Asia and North Africa alone. These vast areas provide environmental services such as the regulation of water quantity and quality, biodiversity and carbon sequestration. Rangelands have been neglected in the past partly because of problems of ownership, access and governmental policies that discourage investments in rangelands. The idea of payment for environmental services in rangelands is in its infancy but is discussed here as a potential option for better use and management of rangelands and as a safety net to reduce the vulnerability of rangeland inhabitants to climate change.

Most poor people presently reside in rural areas. The rural poor are exposed to many risks while often lacking instruments to manage them adequately, and so are highly vulnerable. Providing appropriate risk-management instruments and supporting the critically vulnerable is thus one key pillar in an effective and sustainable rural poverty-reduction strategy (Anderson 2003). Such provision better allows the able-bodied to engage in high risk/higher return activities and thus with good fortune to move out of poverty. A framework must, to be adequate, involve multiple strategies (prevention, mitigation, coping) and arrangements (informal, market-based, public) for dealing with risk, and instruments that take account of the sources and characteristics of rural risk. It must be seen that rural people as managers, and of policy makers who should be setting the conditions to make the lives of the poor easier and to help them escape from poverty.

In general, the likelihood of progress with the development of an intervention and probably also its implementation in farmers' fields appears to be greater with respect to the plant breeding and agronomic interventions than with the water management interventions (Kijne 2006). There are at least two reasons for this difference. First, the time required for planning, design and implementation of water management interventions generally exceeds those for agronomic interventions. And second, public confidence (i.e., as expressed by funding agencies) in the success of water management changes appears to be less than the confidence in changing agronomic practices and in plant breeding (with the exception in some countries of studies that involve genetic engineering). A possible third reason might be the high cost of most water management interventions. Extreme natural hazards, particularly the hydro-meteorological disasters, are emerging as a cause of major concern in the coastal regions of India and a few other developing countries. These have become more frequent in the recent past, and are taking a heavy toll of life and livelihoods. Low level of technology development in the rural areas together with social, economic and gender inequities enhance the vulnerability of the largely illiterate, unskilled, and resource-poor fishing, farming and landless labour communities. Their resilience to bounce back to pre-disaster level of normality is highly limited. For the planet Earth at crossroads, the imminent threat, however, is from a vicious spiral among environmental degradation, poverty and climate change-related natural disasters interacting in a mutually reinforcing manner. The use of recombinant DNA technology for genetic shielding of agricultural crops for coastal regions against abiotic stress (induced by the water- and weather-related natural disasters), strengthens the foundations of sustainable agriculture undertaken by the resource-poor small farm families (Kesavan and Swaminathan 2006).

Table 18.2 Land degradation in provinces of India

State/provinces	Degraded land (ha)	Geographical area (ha)	Degraded land (%)
Andhra Pradesh	10,306,989	27,506,544	37
Arunachal Pradesh	3,391,291	8,374,215	40
Assam	4,642,071	7,843,860	59
Bihar	1,659,322	9,416,395	18
Chattisgarh	4,947,419	13,518,883	37
Delhi	31,161	148,260	21
Goa	132,131	370,290	36
Gujarat	3,350,636	19,612,517	17
Haryana	582,307	4,421,858	13
Himachal Pradesh	2,946,569	5,567,137	53
Jammu & Kashmir	20,856,943	22,223,695	94
Jharkahand	4,290,431	7,971,624	54
Karnataka	8,740,358	19,179,155	46
Kerala	2,599,450	3,886,749	67
Madhya Pradesh	15,338,850	30,824,985	50
Maharashtra	10,837,506	30,758,377	35
Manipur	1,863,193	2,232,797	83
Meghalaya	1,983,565	2,243,182	88
Mizoram	1,888,687	2,108,318	90
Nagaland	1,561,638	1,658,090	94
Orissa	5,024,846	15,570,900	32
Punjab	552,733	5,036,513	11
Rajasthan	21,013,097	34,224,212	61
Sikkim	531,843	709,918	75
Tamil Nadu	3,850,948	13,005,861	30
Tripura	888,822	1,048,601	85
Uttar Pradesh	15,270,634	24,092,803	63
Uttarakhand	3,215,069	5,348,204	60
West Bengal	2,237,615	8,875,874	25
Other states	16,997	89,674	19
India	154,553,121	327,869,491	47

Where high or low temperatures cause stress and reduce growth, plant breeding for higher tolerance for those conditions appears to be the best remedial action. To some extent, the same is true for dry periods and drought conditions. Of course, since antiquity, irrigation has been introduced to grow crops where water shortages always occur. Increasingly, irrigation has been extended into areas where because of periodic water shortages high yields can only be obtained with additional water applications through supplementary irrigation. Where it was relatively easy to introduce irrigation, it has been done already. Now it becomes more and more expensive to develop irrigation facilities in the more difficult locations. As indicated in Table 18.2, within the short duration of 5 years progress is expected through enhancing drought tolerance and the development of short duration cultivars, where this is appropriate. However, plant breeding for increased drought tolerance is especially complicated when the period that plant-available moisture is in short supply is unpredictable and varies from one growing season to the next. A key objective of breeding is to achieve an enhanced yield under stress without causing a yield penalty when the stress is absent. If stress occurs annually, farmers would be happy to plant varieties with a slightly lower yield in good years because, over a decade, their production would be enhanced. When stress seasons are less frequent and unpredictable, farmers seem to prefer to risk using a high-yielding stress-sensitive variety rather than a stress-tolerant variety with a lower yield potential. The solution lies in either better weather-forecasting or varieties combining tolerance and low yield penalty. For abiotic stress mitigation instruments like insurance – either generic or specific products can be very effective. Governments and corporates are already using these instruments for the purpose.

At plant breeding level, increased tolerance for submergence may help to alleviate the impact under some conditions, e.g., on acid sulfate soils and coastal areas as will be discussed below. However, unpredicted and prolonged flooding requires both reliable climate prediction and costly flood protection measures. The latter become even more necessary and costly when the floodplains and wetlands that serve as buffer zones under high discharge conditions become developed and are used for agricultural production. Schultz (2001) describes why it is necessary to increase the design frequency of flood protection measures when the value of the protected public and private property (including houses, buildings, infrastructure and agricultural land) has increased significantly since the construction of the flood protection works. In his example, the design frequency of 1 in 50 years was economically optimal to keep the combined costs of construction and expected damages to a minimal. Fifty years later, because of the increased value of the protected properties it would be necessary to rebuild the structures at greater cost to protect for a 1 in 1,000-year flood. Not much progress is expected on abating abiotic stress from flooding through water management interventions within the next 5 years.

Analogous risk-reduction effects arose from plant breeding targeted at such attributes as resistance to pests and diseases and to abiotic stresses associated with such natural phenomena as droughts and floods. As Anderson and Hazell (1994) have argued, this process has not been uniformly successful in spite of some notable achievements, and there is a continuing need for further improvements. Filling the need faced is not easy because of the contemporary crisis in funding of public R&D, in countries rich and poor. The private sector surely has a role to play in providing at least some of these needed risk-reducing agricultural services, and indeed is already doing so in crops that lend themselves to efficient production of hybrids, such as of maize, where the property rights surrounding the more productive germplasm can be protected at low cost.

18.7 Conclusion

Abiotic stress which directly affects the production systems has its effect on the livelihoods of vulnerable sections of the population. The people living in degraded lands will be the first and the worst to be affected due to such stress. Although, there are measures to handle such stress by agronomic and engineering technologies, the role of affected communities and the state is critical. The communities have sporadically proven their resilience in some parts on coping strategies. There is a need to build capacity among the vulnerable communities. Insurance and enterprise portfolios diversity are two prudent measures to cope up with abiotic stress management. Innovative policies for promoting medium to long run resilience in production systems through natural resource management interventions, balancing system components, incentives for good management practices, etc. are also equally important. The role of civil society and community based organizations in evolving institutions for abiotic and other stress management will be critical and would be of great impact than the role of state as such.

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Chapter 19
Changing Roles of Agricultural Extension: Harnessing Information and Communication Technology (ICT) for Adapting to Stresses Envisaged Under Climate Change

Shaik N. Meera, V. Balaji, P. Muthuraman, B. Sailaja, and S. Dixit

Abstract The linkages between agriculture and climate are pronounced and often complex. Agricultural systems are most sensitive to extreme climatic events such as droughts, floods and hailstorms, and to seasonal variability and changing rainfall patterns. The role of inadequate institutional support is frequently cited in the literature as a hindrance to adaptation. In context of climate change, extension will increasingly face challenges of addressing vulnerability. The effectiveness of extension will be influenced by the factors such as identifying vulnerable regions, vulnerable groups, farmers having multiple stressors, areas which will be doubly exposed, for assessing and strengthening the coping strategies among vulnerable regions/groups and improve the ability for adaptive measures. Effective and timely provision of information will play a crucial role in future extension. Despite the need for timely and well-targeted information on climatic risks, there are currently a number of gaps and challenges in providing climate information to the farmers. Of late, organisations in agriculture realised the importance of managing the Knowledge (Implicit & Explicit; Internal & External) for the dissemination purposes. In this chapter, we have discussed at length the cases for developing insights into the contemporary initiatives of integrating ICTs and climate information in extension. In order to promote the development of appropriate community-based ICT endeavors for

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sharing climate change information and technology options at grassroots level, it is important that we also understand the attributes that must be considered for building more practical and broad based approaches. For the benefit of the readers, a comprehensive framework involving various components of climate change vis a vis Extension is provided. In the context of climate change, information needs assessment and strategies for strengthening Research-Extension-Farmers linkages are discussed in this chapter. Further looking at the complexities of climate change scenario, it is proposed that ICT enabled extension framework may be tested, refined and adapted in future extension endeavours.

19.1 Introduction

Need for improved agricultural extension throughout the developing world has never been greater. Agricultural and rural development and hence rural extension continue to be in transition in the developing world. These transitions are happening because of the forces that are driving the world agriculture today. Vulnerability of the farming in the developing world is quite evident due to forces like climate change, changes in natural resources quality (including desertification over large tracts), lack of coping strategies at micro and macro levels of decision making, coupled with globalization, emerging market forces like commodity markets, sustainability constraints etc., The challenges for rural extension are never more acute than at present (Fig. 19.1).

Agricultural Extension, in the current scenario of rapidly changing world has been recognized as an essential mechanism for delivering knowledge (information) and advises as an input into modern farming (Jones 1997). Agricultural Extension has to reorient itself beyond the narrow mindset of transfer of technology packages. Instead, it has to rejuvenate its vigour for "transferring knowledge (or) information packages" as the input for modern farming. In such case, extension will become more diversified, knowledge intensive, and demand driven. This requires extension system to be at cutting edge level and master many trades for which the ICTs can help. It is in this context, there are many possibilities for potential application of the ICTs in Agricultural Extension (Zijp 1994).

In this chapter, "agriculture" is taken to mean both the traditional activities of agriculture (e.g., planting, harvesting, marketing, animal husbandry) and the natural resource management activities associated with agricultural work (e.g., water management, soil fertility, agro-forestry, fishery management).

Information and Communications Technology (ICT) is an umbrella term that includes computer hardware and software; digital broadcast and telecommunication technologies as well as electronic information repositories such as the World Wide Web or those found on CD-ROMs. It represents a broad and continually evolving range of elements that further includes television (TV), radio, mobile phones, and the policies and laws that govern these media and devices. ICTs are often used in plural sense (ICTs) to mean a range of technologies instead of a single technology.

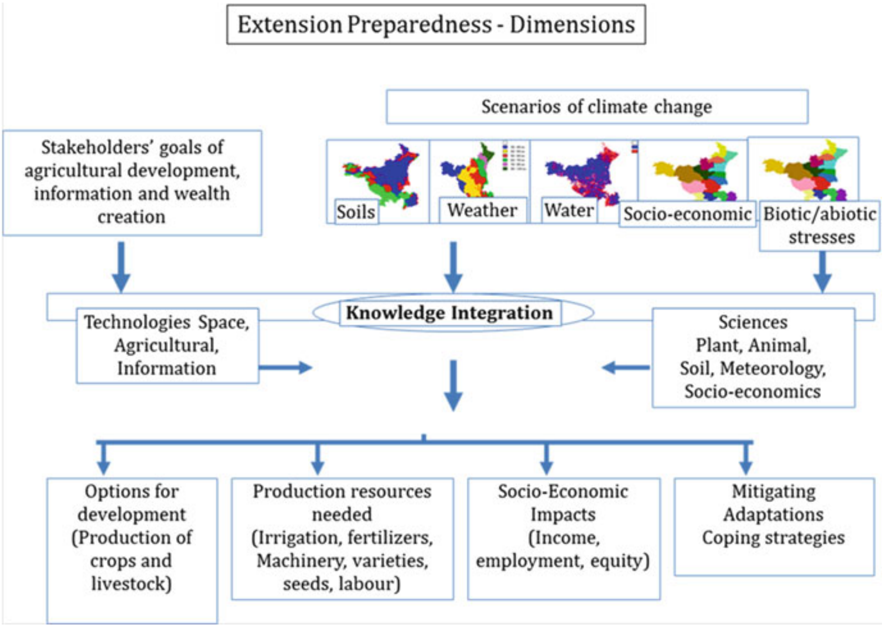

Fig. 19.1 Extension preparedness: dimensions

ICT will bring new information services to rural areas on which, farmers, as users, will have much greater control than ever over current information channels. Access to such new information sources is a crucial requirement for the sustainable development of the farming systems. ICT when applied to the rural and agricultural conditions can improve the linkages between research and farmer sub-systems. For extension sub-system it is time of reckoning to build strong linkages between various other sub-systems of Agricultural Knowledge Information System (AKIS). ICT can help by enabling extension workers to gather, store, retrieve, adapt, localise and disseminate a broad range of information needed by farmers, thus transforming them from extension workers into knowledge workers. Emergence of such knowledge workers will result in the realisation of the much talked about bottom-up, demand driven technology generation, assessment, refinement and transfer (Meera et al. 2004).

19.2 ICTs and Extension in the Context of Climate Change

The linkages between agriculture and climate are pronounced and often complex. Crops and livestock are sensitive to climate change in both positive and negative ways. Agricultural systems are most sensitive to extreme climatic events such as droughts, floods and hailstorms, and to seasonal variability and changing rainfall patterns. Against this backdrop, farmer adaptations are influenced by many factors, including agricultural policy, prices, technology research and development, and agricultural extension services (Kajfez-Bogataj 2005). The poor often bear a disproportionate burden of direct damage from catastrophes and climate change as concluded by most studies in developing countries (IPCC 2001).

The role of inadequate institutional support is frequently cited in the literature as a hindrance to adaptation. For example, Adger and Kelly (1999) and Huq et al. (1999) show how institutional constraints and deficiencies affected managerial capacities to cope with anticipated natural events.

Many observers of rural development in recent times have commented on the frequent manifestations of unsatisfactory extension performance (e.g., Rivera et al. 2001). Feder et al. (2001) have suggested interrelated characteristics of extension systems in the developing world that jointly result in deficient performance, namely low staff morale, reduced efficiency and financial stress etc. One more such key factor is the number of clients and the vast spectrum of information/services needed to be covered by extension systems. Policy makers in the developing world have reacted to this with the deployment of more extension personnel which has continued the emphasis on a more centralized, hierarchical and top-down management systems. The requirement for combining a bottom up approach with the conventional extension process is yet to be fulfilled and the limitations on the extension process to influence issues such as credit availability, input supplies, market linkages and logistics facilitation continue without change. In effect, there has been no visible impact due to such changes within the extension system in many parts of the developing world.

Sulaiman and Hall (2006) have described a range of extension initiatives from the public and private sectors that explain the way extension agenda is expanding as embodied in the concept of “extension plus” and have pleaded for new experiments in extension. Pluralistic institutional arrangements are emerging and are finding wider acceptance and this is mainly because developing countries have realized the need for extension to engage in a wider range of issues beyond merely disseminating production-oriented technologies. Extension pluralism is at the core of farmer adaptation strategies and ICT’s can offer new advantages in enabling reliable and rapid access to expert information support which is much needed in the realization of adaptation strategies on a large scale.

19.3 Climate Change Stresses: Information Needs

Information need assessment is the simplest issue that gets little attention in modern extension management. This section describes in detail about the demand that exists amongst various stakeholders for climate change information.

Analysis of information needs of farmers in the context of climate change can be described in the following three headings.

19.3.1 Farmers

Socio-economic and livelihood variables such as age, caste, education, family type & size, operational holding, market access, migration pattern, technology utilization pattern, cosmopolitans, mass media exposure, extension contacts were etc., along with their perception about the change in climate influence future extension efforts. During a survey conducted by the authors in South Indian province, all the respondents' perceive that there is a change in the temperature in last 20 years. Overall perception of climate change was found to be low (17%) to medium (77%) envisaging the need for generating the awareness on these issues.

The information requirement at individual level is pertaining to household adaptation measures they may undertake. Among the household adaptation measures, change of variety for the current season, operating from multiple & spatially separated farm plots, matching method and time of cultivation practices to seasonal climate, small scale irrigation, watersheds and embankments etc., were preferred by them. Other adaptation methods identified from the farmers were growing alternate crops (31%), growing resilient crops (72%), special care at critical stage of production (91%), soil & water conservation practices (77%), natural retention & flood control (45%) etc.

At societal level, the information needs are something to do with Community level adaptations. To some extent community level adaptation measures were also perceived as good adaptation against climate change. Lack of community level adaptation efforts is understandable and provides various community based organizations (both governmental and non-governmental) opportunities for developing location specific and need based adaptation measures exploiting the cooperation of the farmers as reflected in the results.

19.3.2 Extension

Information required for the extension decision makers and workers is related to projected changes in land use, resource extraction, spread of non-indigenous species, pollution and pollutants, and climate in order to anticipate changes in regional vulnerability. Insights into these changes will result in better-informed decision-making by allowing evaluation of risk management options with regard to possible cumulative and aggregate impacts from multiple stressors. Methods to assess anticipated changes are critical to timely, responsive, and proactive decision-making as well as more effective response measures.

In context of climate change, extension will increasingly face challenges of addressing vulnerability. The effectiveness of extension is going to be influenced by the factors such as identifying vulnerable regions, vulnerable groups, farmers having multiple stressors, areas which will be doubly exposed, for assessing and strengthening the coping strategies among vulnerable regions/groups and improve the ability for adaptive measures. Mapping vulnerability profiles with different stressors (globalization, climate change, marginalization etc.,) for effective extension decision-making will be helpful in this regard. The existing set up of extension decision-making and the strategies are not oriented towards this. The futuristic extension may need to be supported with the ICT enabled tools for facilitating effective decision systems. The following case illustrates application of Geographic Information Systems (GIS) for addressing these needs.

A study on Regional Vulnerability Assessment of Future Scenarios in the Mid-Atlantic Region (GPRA 2003) looks at projected cumulative impacts from multiple drivers of change across a region 20 years into the future. These results can be used to identify probable future vulnerabilities of both human and ecological populations. By identifying areas that are vulnerable to change, resource managers will be able to better protect sensitive resources and anticipate the effectiveness of risk reduction activities given changes in the cumulative stresses that are likely to occur.

Recent studies undertaken by Tata Energy Research Institute (TERI 2003), India strongly advocate that non-ICT interventions need to be integrated with GIS tools for enabling the vulnerable communities to cope with disasters.

19.3.3 Research

Scientific organisations require climate change data pertaining to direct damage to crops and animals, higher temperatures due to climate change. These biotic stresses – including cassava mosaic disease, potato blight, rice blast, wheat stem rust, whiteflies and many others – already take a heavy toll on developing world agriculture. To anticipate and prepare for a worsening of these problems, scientists in various CGIAR Centers are examining the likely effects of climate change on major biotic stresses in agriculture.

The International Potato Center (CIP), for example, has already developed a simulation model for potato late blight, the most destructive disease of the crop worldwide. The model can be used with GIS to predict disease severity under the changes in temperature and rainfall that are likely to result from climate change. Equally important are simulation models for forecasting the expected distribution of insects transmitting viruses, such as aphids and whiteflies. Coupled with the CGIAR's large store of knowledge about disease epidemiology, these models will be critical for anticipating the effects of climate change on the spread of dangerous plant viruses worldwide.

19.3.4 Multi-stakeholder

Information requirements on climate risks are different for different stakeholders. For all aspects, there may not be a rigid set of information requirements for each of these stakeholders. Certain stakeholders are supposed to generate information and certain other making use of it. For example, short-term weather forecasts, including cyclones and flooding prediction, will help communities save lives and property.

But this information may not essentially be coming from agricultural scientists. In such cases the information flow should be across the sectors and to that extent we require to have coordination across the sectors.[1]

Medium-term, seasonal weather predictions can assist farmers, extension officers and development officers in preparing for famine or disease outbreaks. Long-term climate change scenarios of changing rainfall patterns, temperature and sea level rise can inform more strategic decisions about the approach and location of development.

While farmers may use short-term forecasts to decide when to plant or harvest crops, they can use seasonal climate predictions to decide which crops or cultivars to grow, and use longer term climate scenarios to plan migration, livelihood diversification or alternative land-uses. There is a also a need for clearer messages concerning short and long term climate risks, including numbers of people affected and estimated economic impacts, that enable governments' planning and finance departments to act. The quantification of financial and economic impacts is difficult, but is important in highlighting the cost-effectiveness of integrating climate risks in comparison to a business-as-usual approach.

19.4 What Ails Information Provision?[2]

Effective and timely provision of information is going to play crucial role in future extension. At the same time documenting critical contributions of communities to understand the wider context of shocks and stresses they experience, and by specifying the type and form of climate information that could help them to manage climate shocks and variability. Communities can also provide vital information in 'ground-truthing' forecasts and providing surveillance data. There needs to be improved dialogue between information providers and policy-makers so that climate information can be demand-driven and correctly interpreted.

19.4.1 Preparedness of Extension

Despite the need for timely and well-targeted information on climatic risks, there are currently a number of gaps and challenges in providing climate information to the farmers. First amongst them is non-preparedness of extension organisations in terms of climate change. Most of the extension organisations in the developing world are completely unaware of climate change impacts on agriculture. Preparedness in terms of documenting climate change scenarios at grassroots level, extent of adaptation (individual/community level), mapping vulnerable regions, sustainable indicators, access to real time data, effective synthesis & interpreting, better decision

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[1] Adapted for extension context from (DFID 2004).

[2] Adapted for extension context from (DFID 2004).

making for a climate change scenario etc., are missing at present. For most of the extension organisations, climate change means seasonal aberrations. Integrating the multi-disciplinary and multi-sectoral information into a meaningful extension material is beyond the expectations of traditional extension systems.

19.4.2 Data Interpretation

In most of the developing countries, data pertaining to climate variability and its impact on biotic and abiotic stresses is lacking. Even in some cases, data & information on climate variability is available, but the process and the capacity for integrating this information into vulnerability and capacity assessments, to feed into poverty reduction strategies and sectoral planning, is lacking.

19.4.3 Information Targeting

An increasing amount of climate information (in the form of weather forecasts, seasonal forecasts and climate change scenarios) is available and could be used for a range of stakeholders and timeframes. However, information is not always delivered to potential end-users (strategic decision-makers, communities or individuals) in an accessible way so that they can interpret its relevance or be aware of its use.

19.4.4 Risk Assessments

Adapting to current climate variability is an essential first step in adapting to future climate change. However, rather than focusing on the near future (i.e. the 5–10 year period favoured for strategic and financial planning cycles in government), most climate-risk assessments look to the distant future (i.e. 20, 50 or 80 years ahead). Most of the studies in agricultural research institute’s focus on long term effects of climate change rather than on short term adaptations (such as developing a variety that thrives well, if monsoons get delayed by 10 days). It is this kind of lack of information/ technology that makes extension efforts more complicated. (In case of climate change scenarios, there are no low hanging fruits for extension).

19.5 Driving the ICT Use: Tech Driven Need or Need Driven Tech?

The variety of new ICT tools for agriculture is impressive, but the tools need to be placed in an overall context of agricultural information and service needs. By looking at the critical information needs of agriculture and farming communities, the focus can move away from a compendium of "neat gadgets" and their individual applications towards understanding of their overall role in promoting productive, equitable, and sustainable agriculture.

The key framework for this is the *Agricultural Knowledge System* (AKS), consisting of the organizations, sources of knowledge, methods of communication, and behaviors surrounding an agricultural process. Knowledge is not the same as information: knowledge includes information, understanding, insights, and other information that has been processed by individuals through learning and thought. Conventional extension (TOT models) concentrated mainly on providing advisories without coordinating the services required to implement the advisories in the farmer's fields.

As farmers make critical decisions throughout the year (e.g., credit applications, crop selection, tillage methods, pest control, harvesting, post-processing, marketing), a typical household will rely on its own accumulated experience and the support of local organizations (e.g., producer associations, input suppliers, rural credit agencies, extension services, NGOs, schools and others). The household may also receive radio and television broadcasts from more distant sources. Together, these form the local knowledge system accessible to a small farmer.

Information exchange in the local knowledge system is generally by non-digital means: face-to face discussions, printed pamphlets, videocassettes, radio broadcasts, etc. Local communities may lack affordable power and communication systems to drive ICTs, or they may need investments in human capacity to maintain them. The complexities involved in decision making, multitude of key players and service coordination required at the field level demand a tools like ICTs that can handle the requirements in a multi-modal delivery system.

19.6 ICTs in Work – Process of Combating Stresses: A Case of VASAT

Virtual Academy for the Semi-Arid Tropics (VASAT) was implemented across South Asia and Sub Saharan Africa to cover a few of the most drought-prone areas of the Semi-Arid Tropics. We discuss the process to bring out learnings gathered from the South Asia information hub, keeping in mind the contemporary opportunities and challenges of extension (Fig. 19.2).

VASAT was positioned as a technology-mediated extension and knowledge-sharing program partnering with a wide range of organizations including community organizations in rural areas to supplement the organized inter-institutional learning projects of ICRISAT.

From 2002 onwards ICRISAT got involved in a major development programme in Addakal block, supported by the Andhra Pradesh Rural Livelihood Project (APRLP) of Government of Andhra Pradesh in South India. A major community based organization called *Adarsha Mahila Samaikya* (AMS), a federation of women Self-Help Groups (SHGs) in the block became an important stakeholder in the project.

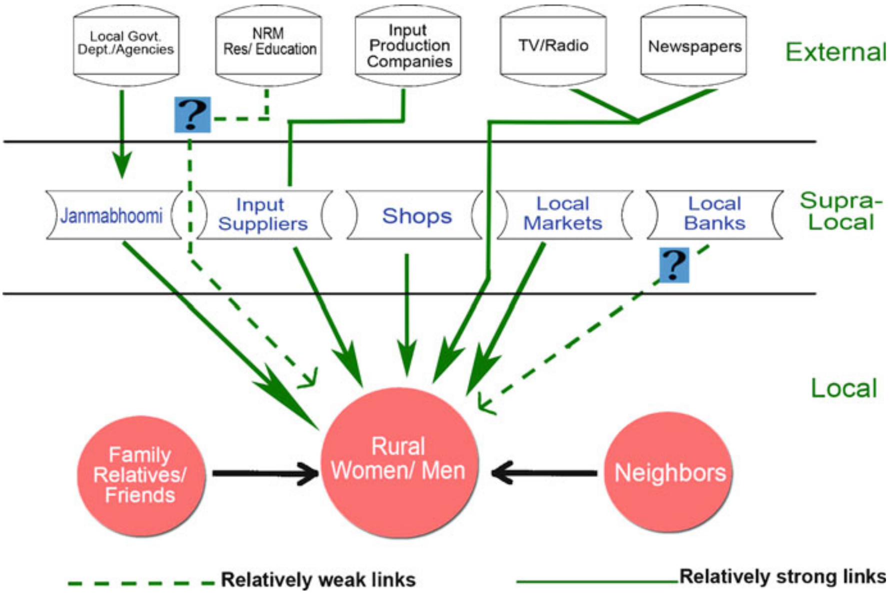

Fig. 19.2 Information flow and linkages in VASAT

ICRISAT in consultation with AMS created certain basic ICT infrastructure facilities at AMS such as a PC-based computer network, low-cost satellite access to the internet, printer etc and later with the help of Indian Space Research Organization, video conferencing facilities. A *hub and spokes* model was planned with AMS being the hub and certain number of villages in the block as *spokes*. On the request of villagers, ICRISAT extended the services to three nearest villages (within 5 km radius). In the initial stage, ICRISAT did not install the computers. AMS moved the available extra computers in the hub center to these three villages. ICRISAT conducted a preliminary study to find out the information linkages operating in the area of agriculture. This study yielded very interesting results, which showed that macro and meso level knowledge development organizations have limited reach with the village community. Televisions and radios, local government agencies, agricultural input organizations etc showed strong linkages. Natural resource management based education and research institutions and local banks have weaker linkages. However the strongest linkage was between villager to villager indicating the role of horizontal transfer of knowledge. Input suppliers and other agricultural traders are the other important source of information. Market, climate, employment and wages are some the important information needs of the community. Each village under AMS has certain number of SHGs and in order to coordinate between the SHGs and federation, AMS has created a cadre of Village Network Assistant (VNA). ICRISAT felt that VNAs in addition to coordinating the micro finance at the village could also play a role in extension and knowledge management. AMS & Villagers requested the VNAs, who are handling the SHGs and account books since from the inception of AMS, to act as kiosk operators. As the ICT based initiatives progressed, these VNAs evolved as knowledge intermediaries. It was then realized that ICT mediated approaches are more effective in rural areas if mediated through trained knowledge intermediaries.

AMS has a paid-coordinator at the headquarters who is now involved in managing the IT in hub. This person is viewed as a Para Extension Worker (PEW) who could help in converting the generic information into locale specific knowledge and act as a bridge between ICRISAT and AMS. The para extension worker and three VNAs were trained by ICRISAT in various types of ICT management. ICRISAT mobilized its expertise and made attempt to reach the villages of Addakal through AMS and the ICT network.

19.6.1 Usage Patterns, User Profile and Absorption Pattern

AMS has emerged as a self-sustaining, self-generating grassroot organization. Its saving and credit turnover is around Rs. 10 million per annum. Over 5,000 women members from 23 villages are actively involved in saving and credit management. In addition to micro finance, AMS is also involved in income generating activities such as dairy industry, Highway Restaurant, Super Market etc and it is encouraging entrepreneurship among its members. It has been able to build infrastructure facilities using various government programs.

Initially AMS and ICRISAT did not have well defined strategy for interacting with the community on ICT platform. The visitors to the AMS who come there for the purpose of micro finance were attracted towards the ICT activities. Gradually ICRISAT started evolving structured approach for interaction through PEW and VNA. The establishment of *spokes* in three villages has helped to strengthen the structured interactions. Initially VASAT platform and emails were used for interaction between ICRISAT and Addakal. Later with the introduction of the video conferencing, the pattern of interaction changed substantially. The three VNAs and the Para Extension Staff regularly collect the queries and issues from the villagers and interact with the experts at ICRISAT using video conferencing facilities. The feedback from ICRISAT is communicated back to the villagers. In addition, ICRISAT also organized structured training programmes using *Technology Mediated Open and Distance Learning* (TechMODE) approach in subjects such as coping with drought since drought is the most serious problem in the area. The role played by the VNAs and the PEW was recognized by MSSRF-Tata National Virtual Academy for Rural Prosperity when some of them were selected as National Virtual Academy (NVA) Fellows.

The three VNAs pass on the queries to PEW, and PEW process the query further if required and send it to ICRISAT either on mail or through video conferencing. The replies are collected and passed on to the villagers. The PEW converts the responses in a multi-media format in local language, Telugu and stores them in the Content Management System developed by ICRISAT. They use the CMS for building a question and answer repository for long-term usage. Initially the questions from

Table 19.1 Analysis of the (questions) data collected during ICT-mediated agro-advisory process

Date	Number of questions received	Repeated questions	New questions	Un-answered	Date of answers provided	Process duration
1st October	8	3	–	0	7th October	6 days
2nd October	6	4	–	0	7th October	6 days
14th October	17	14	3	0	18th October	4 days
After training						
24th October	2	0	2	0	24th October	8 h
4th November	17	12	5	0	5th November	31 h
14th November	24	16	8	0	15th November	26 h

the villagers were not clear to the experts at ICRISAT, which resulted in delays in response. Hence, ICRISAT trained the VNAs and PEW on agro-advisory in distant mode which focused on "appropriately reframing the questions of the villagers" which would help the experts to quickly respond. According to a paper (Dileepkumar et al. 2005) a typical question before the training was like this:

I observe flower dropping in my castor field, please advise me

After the training the VNAs and PEW were able to rephrase the question;

In the 3-month old castor crop in my 4- acres land, I have observed two kinds of flowers, red and green; only the red ones turned into fruit and the green flowers dropped down, please advise me'

The evolution of village level intermediaries has helped to reduce the time lag between the questions from the villages and the answers from the experts. The paper from ICRISAT (Dileepkumar et al. 2005) points out, through a table the impact of the training the village level intermediaries.

Within a short span of time, ICRISAT's initiatives have created continuous learning cycles among villagers particularly among women. The hub and spokes model has also initiated "IT Literacy Training Programme" and in each of the three villages 15–45 persons have attended the program. This program, according to the VNAs, is attracting students and youths in the villages (Table 19.1).

19.6.2 Insights for Extension

VASAT was conceived as an ICT project that would provide a holistic solution to the poor farmers of Semi Arid Tropics. It adopted a systematic approach to content and capacity building at grass-roots level. It recognized the need for multi-modal delivery and adapted cost-effective approaches such as hub and spokes model. It allowed sufficient time for the community to evolve a convenient and reliable source of information. VASAT encouraged rural youth and women to emerge as information volunteers and recognized their role as credible local source of information.

19.7 ICT Enabled Extension for Climate Change: Towards a Comprehensive Framework

Based on the review and analysis of existing ICT initiatives to address climate change challenges, a comprehensive framework can be developed.

19.7.1 ICTs in Local Decision Making[3]

Angelica (2011) explained the factors that play a role in adaptive decision making, and provides a good basis to reflect on the potential of ICT tools -and innovative approaches- within farmer's adaptive decisions. Based on the findings of the report, the following areas of ICT potential in decision-making can be identified:

19.7.1.1 ICTs Helping Farmers Transition from Short-Term to Long-Term Planning

By facilitating the production and access to climate models and projections, ICTs can contribute to the identification of future and emerging risks and opportunities associated with climate change. Local decision-making can be informed by alternative scenarios, and the diversification of livelihoods, farming practices, or skill sets required to deal with change can be considered as part of long-term planning.

19.7.1.2 ICTs Helping to Bridge the Gap Between Researchers, Advisers and Farmers

By making climate change-related information more accessible and relevant to the local actors (e.g. through Web-based materials designed in the local language and addressing local priorities, or through text messages with simple, strategic content delivered to farmers' cell phones) ICTs can contribute to improve the information and knowledge sharing between key stakeholders.

19.7.1.3 ICTs Helping to Strengthen the Links Between Scientific and Traditional Knowledge

By providing a platform to document and share both scientific and traditional knowledge through blogs, audio-files or community videos, among others, ICTs can help to strengthen adaptive practices, learning and social identity.

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[3] *Angelica (*2011*).*

19.7.1.4 ICTs Helping to Foster Inclusion and Connectedness

By enhancing participation, monitoring and exchange between community members and broader networks, the use of ICTs can help to 'give a voice' to groups and individuals that could be, otherwise, excluded. The use of tools such as mobile phones and the Internet can contribute to community-based environmental monitoring, while ICT-capacity building can strengthen local-empowerment and the ability to self-organise in response to external climatic disturbances.

In sum, providing relevant information for long-term planning, building on multi-level and multi-sectorial synergies, linking both new and traditional knowledge, and facilitating more inclusive processes, are some of the areas in which ICT tools can contribute to local decision-making, helping vulnerable groups – such as farmers – to adapt more effectively to the impacts of climate change.

19.7.2 ICTs in Regional Planning

ICTs can strengthen the capacity of national organisations working on climate change by enabling better informed and more participative decision making processes. The use of ICT tools can help Ministries and development departments to coordinate actions and implement regional

Campaigns, and facilitate the provision of locally appropriate mechanisms of prevention and response. For instance, Crop Pest Surveillance Advisory Programme (CROPSAP) is developed by National Centre for Integrated Pest Management (ICAR) that aims at On-Line Monitoring System for major pests in Soybean & Cotton throughout Maharashtra, India. This kind of initiatives require huge amount of datasets both primary and secondary. ICT tools will help in effective regional extension planning.

Further, ICT applications (e.g. geographic information systems) are increasingly embedded in mapping different climate change scenarios and contingency planning for appropriate input supplies.

19.7.3 ICTs on Personalised Interventions[4]

In near future, it is expected that personalised interventions/decision making is possible using wireless sensor technology. As the transformation from web 2.0 standards to 3.0 standards, data servers catering to the mobile applications/ sensor applications may go up. For example, a preliminary study carried out under shed-net condition to observe climate change scenarios on tomato crop. Wireless Sensor Network

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[4] *Arun et al. (*2010*).*

(WSN) technology was used in the studies to monitor climate parameters (temperature, humidity and CO_2 concentration) continuously under micro-climatic conditions. WSN Devices used for the studies were: FieldServer and Agrisens. DSSAT (Decision support for Agrotechnology Transfer) simulation software tool was used to determine scenarios of crop yield under different CO_2 and Temperature conditions. In addition, coping strategies were also studied to combat the change scenarios. Open Source/Free Geographical Information and Communication Techniques (Geo-ICT) tool was used for sensor web enablement and for dissemination to the rural community.

19.7.4 Integrated ICT Approaches

We may not have exclusive ICT projects/ interventions for climate change information. Whatever is discussed in this chapter will have to be a part of bigger picture of ICT enabled extension approaches providing a cafeteria of services. There is no single optimal or best model for providing need specific, purpose-specific and target-specific extension services. This holds good for the ICT enabled extension approaches as well. The ultimate choice of the ICT Enabled agricultural extension approach depends on (1) the ICT policy environment, (2) the capacity of potential ICT service providers, (3) the type of stakeholders ICT approaches wish to target, and (4) the nature of the local communities, including their ability to cooperate and various e-readiness parameters.

Different agricultural extension approaches can work well for different sets of frame conditions. In order to use extension approaches that best fit a particular situation, the agricultural extension system has to be sufficiently flexible to accommodate the different options. To this end, the recent agricultural-sector reforms have been geared toward creating a demand driven, broad-based, and holistic agricultural extension system.

19.7.5 Knowledge Management (KM)

Of late, organisations in agriculture realised the importance of managing the Knowledge (Implicit & Explicit; Internal & External) for the dissemination purposes. Substantial review has been made on opportunities provided by ICTs, which have formed the basis for the recent knowledge management strategies.[5]

There are several issues related to implementation of KM strategies in agriculture with special reference to climate change. We are of the opinion that these issues, if addressed properly would make extension system effective in tacking the climate change challenges.

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[5] Shaik N. Meera (2008).

19.7.5.1 Communication Issues

Communities of Practice – bring people together, often from different departments, to share ideas. This methodology involves the process of sharing tacit knowledge and development of informal networking.

Question and Answer Forums – bring people together, often geographically dispersed, but with similar jobs, usually through email or chat rooms, to solve problems. This methodology involves the sharing of tacit knowledge and also storage of knowledge as the exchanges are usually archived for future use.

19.7.5.2 Storage and Retrieval Issues

Knowledge Mapping – performing an audit to discover the knowledge resources within/ outside an extension organizations and providing location information for these knowledge resources. This methodology involves the discovery of tacit knowledge in order to facilitate eventual sharing

Expert Databases – similar to mapping of knowledge, these maps experts by identifying knowledge of each expert and providing a guide map to help employees find those experts. This methodology may involve discovery if performed by others and may just facilitate the sharing of tacit knowledge if, as in many cases, it is up to the employees to provide his of her own expert profile.

Knowledge Databases – explicit knowledge is stored in databases similar to standard document databases. This methodology facilitates the storage and sharing of explicit knowledge.

19.7.5.3 Dissemination Issues

News Information Alerts – provide for the distribution of selected information and explicit knowledge

Organizational Learning – acquisition of new knowledge by individuals through training, continuing education

19.7.6 Extension Policies – Reorientation

If scientific research related to climate change is to achieve a real impact on farm productivity and livelihoods, new methodologies for dissemination of information have to be developed or adapted. The main direction of reform in agricultural extension should be towards learning rather than teaching paradigm. This learning approach should incorporate new methodologies and approaches of climate change information that are demand-driven and increase the real, interactive participation of local people at all levels of decision making in an extension delivery network. These methods require that the roles and responsibilities of researchers, extensionists, and local people be re-defined and shared.

However, it is imperative that individual countries make situational analyses of the social, political, technical, economic and natural conditions prevalent in their areas before adapting any method, approach, or strategy. An integrated approach (comprising of different strategies) is recommended in diverse socio-cultural, economic and political situations in order to achieve the desired goals. Generally, a sound agricultural extension policy is indispensable to achieve success in transferring knowledge to farmers. What exists now in most developing countries like India does not meet the climate change preparedness. We need extension policies that are cognizant of climate change scenarios and give way to innovative extension strategies.

19.8 From Tactical Methods to Practical Approaches

We have discussed at length the cases providing us with insights into the contemporary initiatives of integrating ICTs and climate information in extension. In order to promote the development of appropriate community-based ICT endeavors for sharing climate change information and technology options at grassroots level, it is important that we also understand the attributes that must be considered for building more practical and broad based approaches. They could be:

- Engage in ongoing dialogue with local people about the role and impact of ICTs in terms of their climate change information needs, attitudes towards the technologies, applications and products, and possible impacts, both positive and negative.
- Local people and their information needs should be the driving force behind ICT initiatives and not the technology.
- Promote dialogue and debate on who has control of information and their possible effects on community power dynamics.
- Engage local people in the validation of the various communication tools and let them identify the most useful medium to meet their needs. Same is the case with climate change information as well.
- Climate change extension programme design should reflect an understanding of the different ways in which individuals and groups learn, communicate and use information; without incorporating this understanding, programmes are likely to fail.
- Shift from technology driven approaches to a more holistic approach in which the wider systemic economic, social and communication aspects of communities are central concerns. In rural areas, ICT efforts should cover all the multi-faceted aspects of rural livelihoods, including agriculture, non-farm employment, environment, health, sanitation, family planning, education and literacy.
- Provide need-based ICT training at all levels, but especially to youth, women and marginal groups.

19.9 Research-Extension-Farmers Linkages in Climate Change Scenario

For the benefit of the readers, let us have a comprehensive model involving various components of climate change vis a vis Extension. The model describes various dimensions of formulating extension strategies with respect to the climate change based innovations. It is generally assumed that farmers will adapt to climate change, with climate resilient technologies generated by Research subsystem. But there are complex issues related to adoption of such technologies. Seldom, these issues are taken into consideration. The linkage matrix given below describes some of steps for formulating effective extension plans.

From the research sub system, the research material needs to be synthesised into extension material. As in case of any other technology research data while transforming into extension information, message distortions take place. As we all know- climate change data, information and knowledge differ from one region to other. Knowledge varies between contexts and emerges from the flows of information around the system. Knowledge includes data and information within a particular context. When a scientists' knowledge moves into an extensionist's context, it is relegated to information and it is the practitioner that has to do the work of making this information into new knowledge. Agricultural Research knowledge is just another piece of information to be incorporated into the knowledge of an extensionist. Of course this works the other way. When we think of multiple stakeholders in the agricultural value chain, the interaction between the data, information and knowledge gets much complicated. Using the traditional knowledge flow mechanisms, it is not possible to facilitate different processes (from collection to decision making) at various hierarchical levels. The KM practices using ICTs are emerging fast as viable solutions.

This is coupled with technology inventory and analysis of alternative technologies for different regions. From this cafeteria of technologies, extension system has to choose three paths. These paths are based on adaptation cost- or risk factors associated with it.

No cost to low cost/ risk (like change planting, harvest dates): This is an effective, low-cost option. Major risk is that there is no inventory of technologies available among extension organisations. If technological options are available, this information may go directly to farmers' subsystem.

Low Cost to Moderate Cost/Risk: (like change varieties grown) Usually a low cost option if R&D Organisations support initially. In some cases seed for new varieties is more expensive, adjustment in cultural practices. In most cases there are no suitable new varieties available. This kind of information can go to six step model for technology assessment and refinement.

Medium Cost/Risk and High Cost/Risk: (like change crop species or livestock produced or diversification). This could bring new profits, but also a risky option because there are no guarantees that there will be the necessary infrastructure and a market for the new crops or livestock products.

This kind of complex information has to harness ICTs and knowledge management strategies. At the end of the ICT interventions, we need to follow conventional six step model before such information is released to farmers' subsystem. The framework of ICTs and KM are shown in Fig. 19.3 and Fig. 19.4.

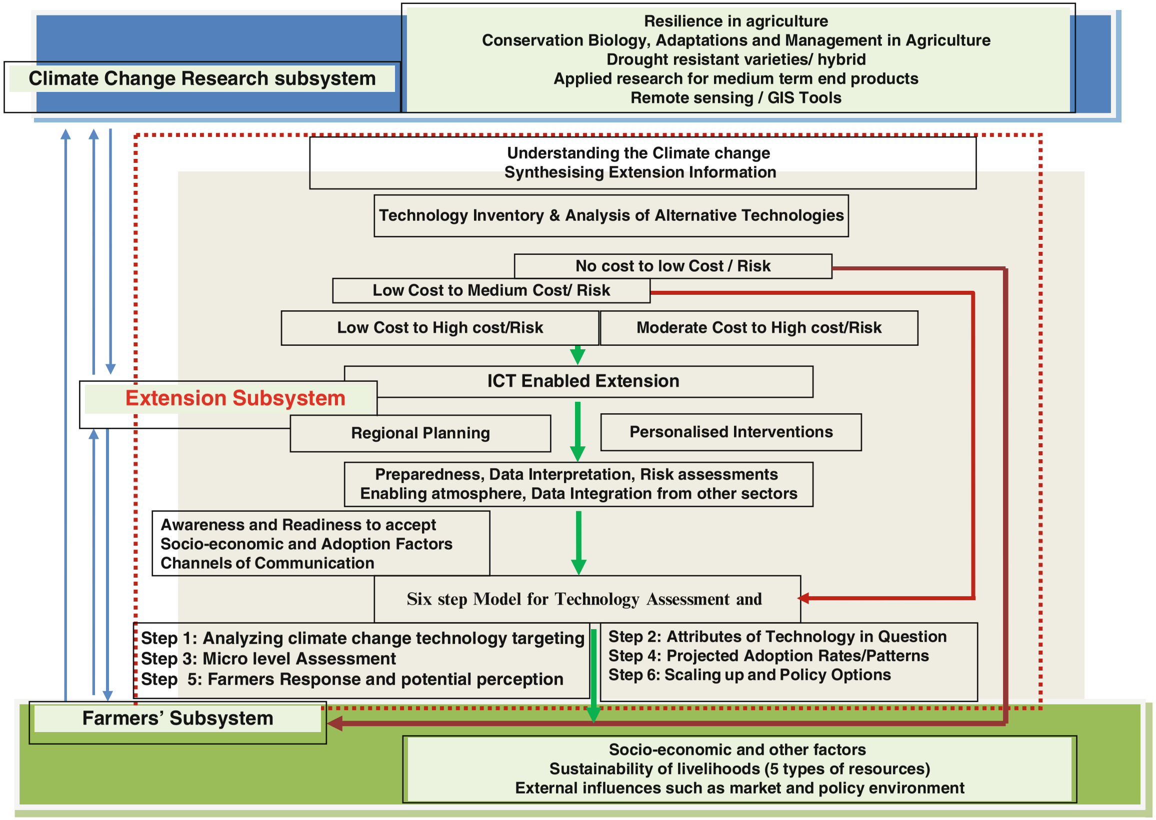

Fig. 19.3 Research-Extension-Farmers Linkages in Climate Change Scenario

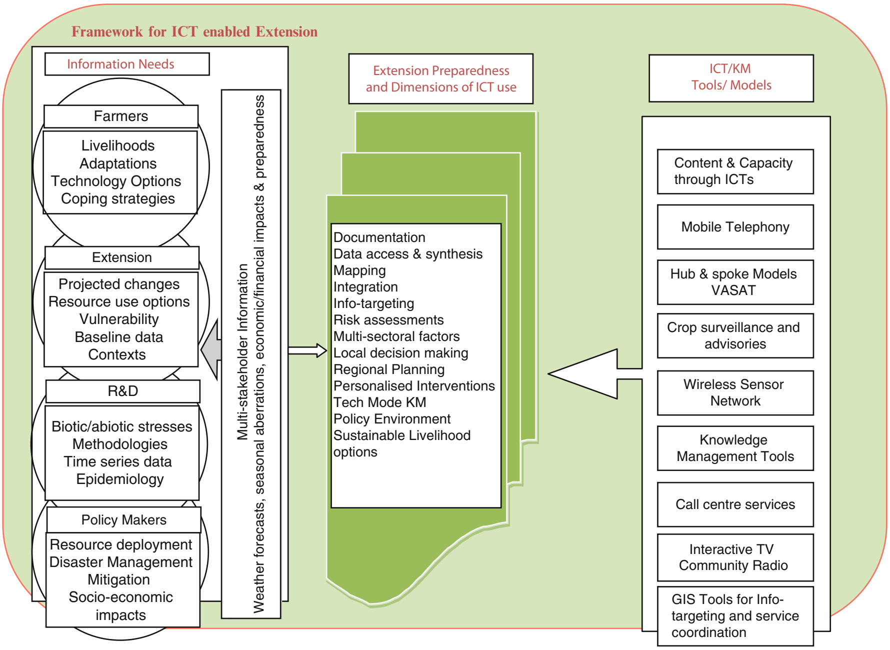

Fig. 19.4 Framework for ICT enabled Extension for adapting to various stresses

19.10 Conclusion

The vulnerability of agriculture and rural life in the developing world is compelling extension to think beyond transfer of technology and perform better under pressure. Access to ICTs implies access to channels and new modes of communication that are not bound by barriers. In this context a paradigm shift is required from ‘technology’ to ‘knowledge’; ‘research centric’ to ‘farmer centric’ and ‘top-down’ to ‘bottom-up’ solutions and finally ‘ICT-centric extension’ to ‘extension centric- ICTs’. In order to revitalize agriculture in the developing world it is time that we started engaging ourselves in evolving ICT enabled extension pluralism. By knowledge empowerment of key players in extension and rural communities, many of the agrarian challenges can be addressed better in the developing world.

In the context of climate change, information needs assessment and strategies for strengthening Research-Extension-Farmers linkages are discussed in this chapter. Further looking at the complexities of climate change scenario, it is proposed that ICT enabled extension framework given by the authors may be tested for refining the framework. While we have attempted to review certain cases where in ICTs are effectively harnessed in addressing climate change challenges, empirical evidences need to be gathered on harnessing ICT/KM strategies in near future.

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