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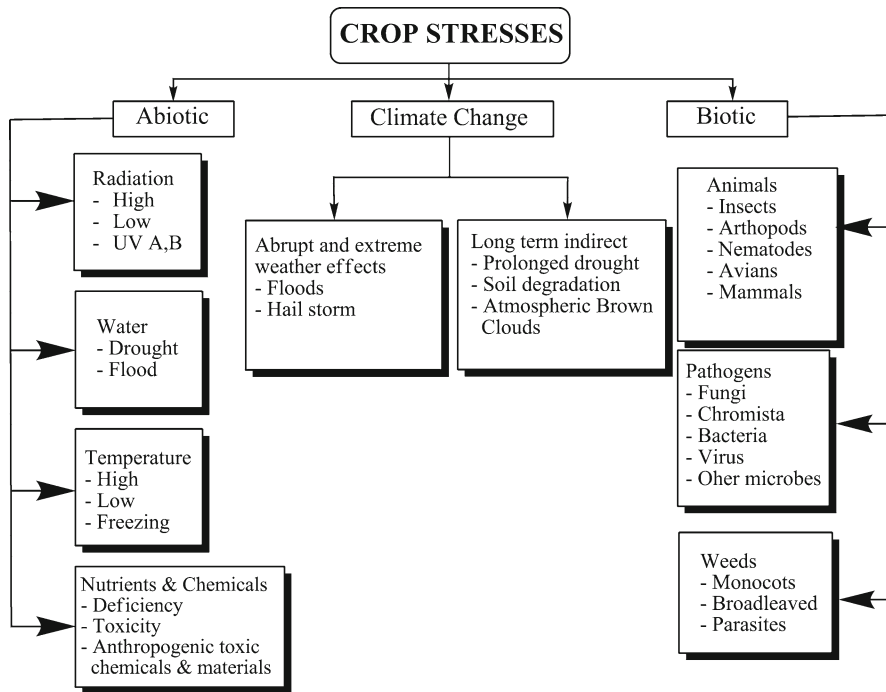
Fig. 1.1 An overview of biotic and abiotic factors that cause crop stress

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 exaspered 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).
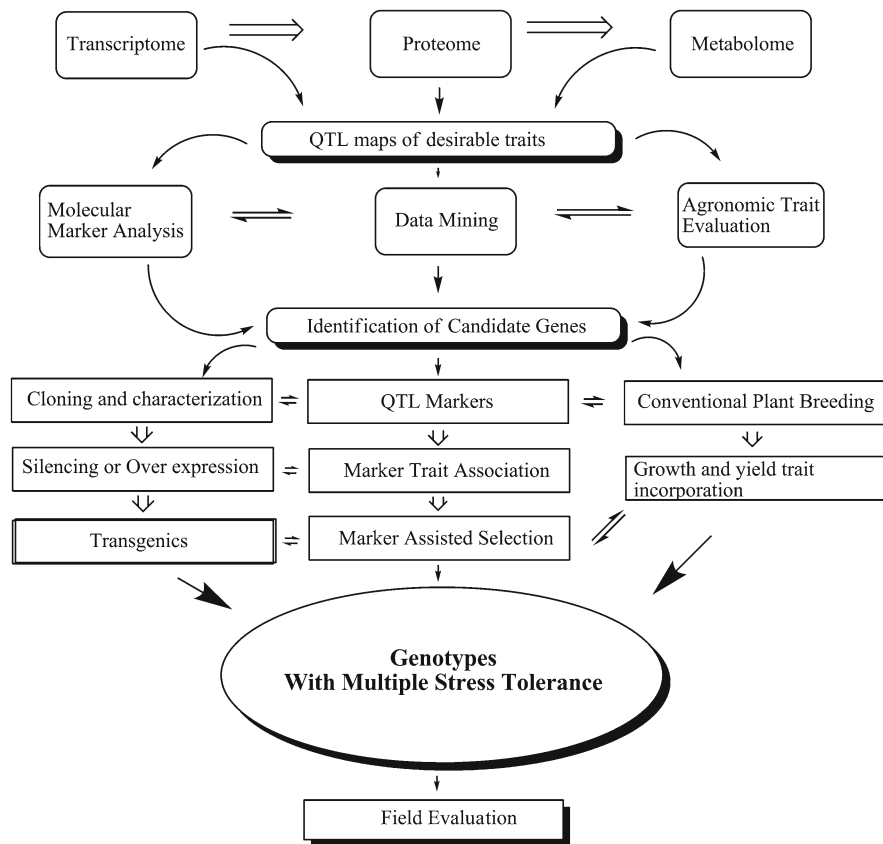
Fig. 1.2 Process network in development of stress tolerant cultivars

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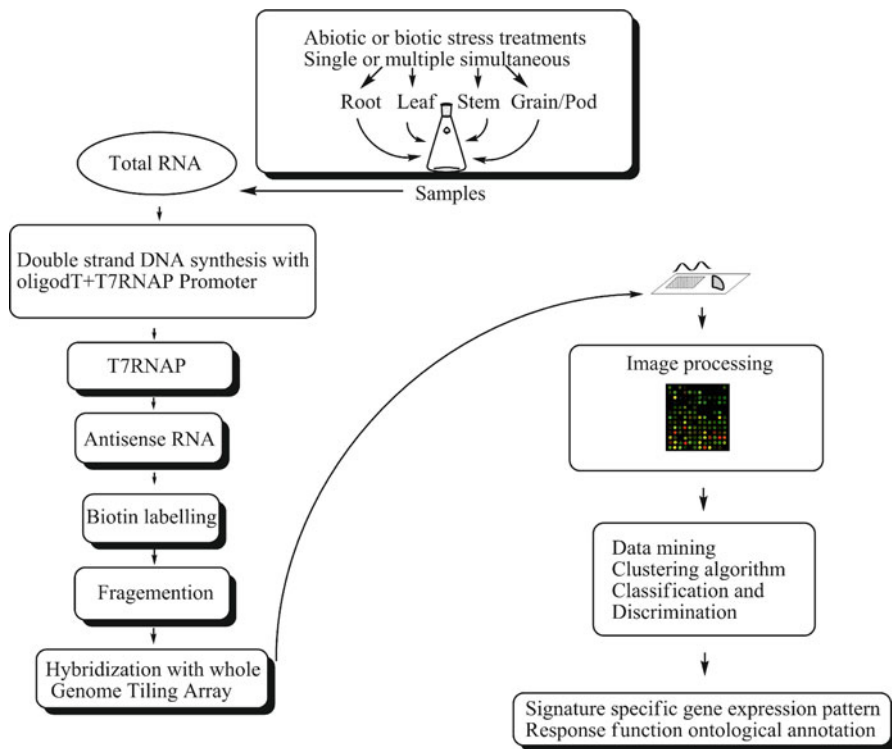
Fig. 1.3 Schematic representation of the transcriptome exemplified for analysis of expression of the whole genome as influenced by biotic and abiotic stress treatments imposed singly or multiple simultaneous

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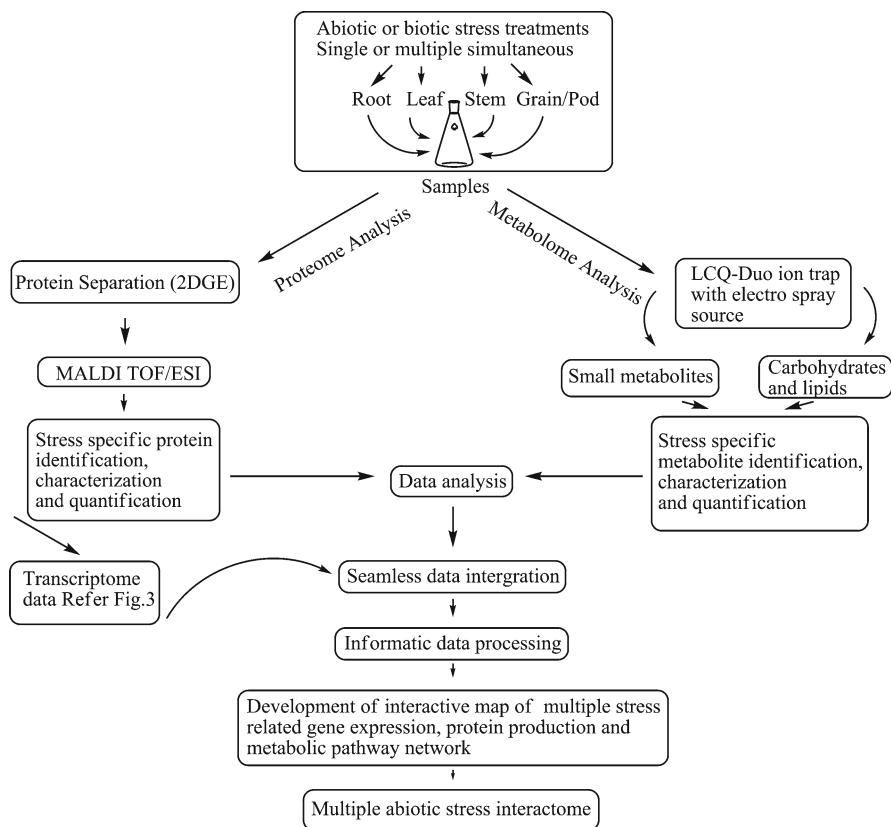
Fig. 1.4 Schematic representation of proteomic and metabolomic data acquisition and integration with transcriptomic data leading to stress interactome

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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