Abiotic Stress Responses in Plants: Metabolism to Productivity

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Andrea Furtado Macedo

Abstract

Plants are sessile beings, so the lack of mechanisms to escape from adverse conditions has fostered, through evolution, the development of unique and sophisticated responses to environmental stress. Depending on the degree of plasticity that a plant possesses to deal with a new environmental situation, in response to abiotic stress, morphological, anatomical, and physiological changes may occur. These changes can affect plant growth, productivity in agriculture, metabolic profile, and plant nutritional potential, for example. Therefore, plant abiotic stress has been a matter of concern for the maintenance of human life on earth and especially for the world economy. To meet these challenges, genes, transcripts, proteins, and metabolites that control the architecture and/or stress resistance of crop plants in a wide range of environments will need to be identified, in order to facilitate the biotechnological improvement of crop productivity. The combination of different "omics" tools, which rather than investigating a limited number of substances, enable the large-scale scanning of various substances, offers great potential for postgenomics to elucidate the genotype–phenotype relationships. This chapter is intended to be a synopsis of current knowledge on this regard. It focuses on plant proteome and metabolome affected by abiotic factors. It will include informations on recent advances in methods of omics like proteomics and metabolomics, which should be considered as a new opportunity to understand abiotic responses and identify genes responsible for important crop traits.

Keywords

Abiotic stress • Plant responses • Productivity • Proteome • Metabolome • Tolerance

A.F. Macedo (✉)
Laboratório Integrado de Biologia Vegetal,
Departamento de Botânica, Instituto de Biociências,
CCBS, Universidade Federal do Estado do
Rio de Janeiro, Rio de Janeiro, RJ, Brazil
e-mail: andreafm@unirio.br; andreafm22@yahoo.com.br

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1 Introduction: Abiotic Stress Responses – Importance

Stress is the negative effect that an organism may suffer, and can be classified as internal or external. Internal stress is that derived from mutations or abnormal cell divisions that can lead to metabolic changes. External stress can have a biotic or abiotic origin. Biotic stress may be caused by the attack of herbivores and pathogens. Biotic stress refers to the physical or chemical changes in the environment of the individual (Madlung and Comai 2004). Among the most common abiotic stresses are those related to drought, excess salt in the soil, extremes of temperature, and the presence of toxins contaminating the environment (Bhatnagar-Mathur et al. 2008).

Plants are sessile beings, so the lack of mechanisms to escape from adverse conditions has fostered, through evolution, the development of unique and sophisticated responses to environmental stress. The chain of events that culminate in a response begins with the perception of a specific signal. This signal will generate a specific set of internal responses that lead from the changes in gene expression to changes in metabolism (Hazen et al. 2003; Shao et al. 2007; Agrawal et al. 2010). All these sets of changes represent nothing less than the effort of this sessile organism to overcome the stress situation, maintain homeostasis and adapat (Altman 2003; Hazen et al. 2003).

Survival in hostile environments involves developing mechanisms of tolerance, resistance, or avoidance. Plants that develop tolerance to a given factor can, over time, overcome the effects of this factor without injury. For instance, *Anastatica hierochuntica* and several species in the genus *Selaginella* are called "resurrection plants" because of their ability to withstand and recover from extended periods of internal water deficit. Another tolerance mechanism to avoid dehydration is the accumulation of osmolytes and changes in metabolism (Bouchabke et al. 2008).

To develop resistance means to submit to a given environment by means of counter measures. Acceleration of the plant life cycle to allow flowering before a drought period is a good example of this strategy. Many arid-land grain crops have been improved through breeding programs that allow the crop to avoid seasonal dry periods (Des Marais and Juenger 2010).

Avoidance prevents exposure to the stress (Madlung and Comai 2004). A good example of this strategy is what happens to plants subjected to osmotic stress (drought). Plants can adjust their absorption and water loss by regulating the physiological function of the roots and transpiration, respectively. Stomatal regulation is a strategy to avoid dehydration (Buckley et al. 2003). However, despite this conservative strategy, reductions of photosynthesis and growth can occur.

Plants are often unable to adjust to a certain condition and become sensitive to it (Wang et al. 2003). Depending on the degree of plasticity that a plant possesses to deal with a new environmental situation, in response to abiotic stress, morphological, anatomical, and physiological changes may occur. These changes can affect plant growth, productivity in agriculture, metabolic profile, and plant nutritional potential, for example (Altman 2003). Therefore, plant abiotic stress has been a matter of concern for the maintenance of human life on earth and especially for the world economy.

The main aim of improvements in agricultural production is the eradication of hunger for the ever-increasing human population. It is worrying that 70% of the extremely poor who suffer from hunger live in rural areas (Sanchez and Swaminathan 2005). It is important to improve the nutritional quality of food for 854 million people (about 14% of our population) worldwide who are chronically or acutely malnourished (FAO 2004). It is urgent to increase agricultural productivity with the most nutritious plants; however, the predominant concern is the maintenance of a healthy environment and conservation of local biodiversity, both of which are becoming progressively degraded and subject to accelerated global climate change (Hu et al. 2010).

Specifically in agriculture and the eco-environment, abiotic stresses such as extreme temperatures, salinity, and drought have decreased productivity as much as 50% (Boyer 1982; Bray 1997). Osmotic stress may reduce crop yields to

less than half of their potential (Boyer 1982). Forecasts for the year 2050 indicate that up to 50% of farmland may become saline (Wang et al. 2003). Salinization is a problem today, and presently affects 22% of arable areas (FAO 2004). All these data are tightly linked to plant biology, because plants offer the globe its only renewable resource, not only of food, but also of building material and energy. Knowledge of plant biology is also a powerful tool to use natural resources reasonably (Agrawal et al. 2003; Beer and Tavazoie 2004).

In view of this general situation, a major question arises: how to overcome all these adverse factors? One simple answer is to study the responses to different stresses. A key challenge for plant breeding is to investigate these responses at the genetic level, identifying genes responsible for important crop traits. Studying plant responses to different abiotic stresses can reveal how some plant species overcome these adverse conditions by developing resistance or tolerance, the nature of environmental changes can be explored, and finally, tolerant and/or resistant plants can be developed (Meyerowitz 2002; Gesch et al. 2003).

Many studies in this direction have been implemented in attempts to identify stress-regulated genes. Some have shown that plants that are exposed to different stresses, have genes that are regulated in singular ways, but that nevertheless induce similar defense responses (Ozturk et al. 2002; Altman 2003). Probably this is because drought, salinity, extreme temperatures, and oxidative stress are interconnected, and may induce similar effects on plants. Salinity and drought, for example, cause similar responses in plant cells: membrane and protein damage and disruption in the distribution of ions (Vinocur and Altman 2005; Rácz et al. 2008; Hu et al. 2010). Stress inducers from abiotic as well as biotic factors also have some common signal and response pathways in plants (Hodge 2004; Bray 2004; Chinnusamy et al. 2004; Hinsinger et al. 2005; Hongbo et al. 2005; Liu and Li 2005; Munns 2005; Leakey et al. 2006; Humphreys et al. 2006) and thereby have the potential to moderate each other's effects through cross-talking (Shigeoka et al. 2002; Shinozaki et al. 2003; Soltis and Soltis 2003; Hongbo et al. 2005). Investigation of those responses that follow a similar pattern can be useful in developing sustainable agriculture by reducing the need for chemicals (e.g., fertilizers, herbicides, insecticides, fungicides) and preserving/optimizing natural resources (e.g., water, reclaiming wasteland for intensive agriculture) (Wang et al. 2003; Agrawal et al. 2010).

Responses to abiotic stress at the gene level fall into one of three types: (a) genes coding proteins that play an important role in signaling cascades and in transcriptional control (Zhu 2001), (b) genes whose products immediately confer protection on membranes and proteins (Bray 1997), and (c) those that are involved in water and ion uptake and transport, such as aquaporins and ion transporters (Blumwald 2000). Examples of the first option are MyC, MAP kinases and SOS kinase (Zhu 2001), phospholipases (Frank et al. 2000), and many transcription factors (TFs) that regulate transcription by binding, and belong to several gene families including AP2/EREBPs (APETALA2 and ethylene-responsive element-binding proteins), HSF, CBF/DREB (dehydration-responsive element/C-repeat-binding), ABF/ABAE families, bZIP (basic-domain leucine zipper), NAC, MYB/MYC, Cys2/His2 zinc-finger, and WRKY (Umezawa et al. 2006; Hongbo et al. 2005). Transcriptional elements can activate or suppress the transcriptional effect of corresponding genes (Beer and Tavazoie 2004; Bray 2004; Liu and Baird 2004).

Genes that code for products that directly confer protection on membranes and proteins and therefore the function of plant cells to resist environmental stress, are those that synthesize proteins related to the support of the integrity of cellular structures, or destruction of structures damaged by osmotic stress. Late embryogenesis abundant proteins (LEA67), heat shock proteins (HSP68) (Bray 1997), antifreezing proteins, osmotic regulatory proteins, free-radical scavengers (Wang et al. 2003), and various proteinase inhibitors are examples of the latter type of proteins (Des Marais and Juenger 2010). LEA and chaperones often have conservative sequences and polar amino acids, so they are stable and can cooperate in stabilizing the structures of proteins and cell membranes (Fu et al. 2007; Jyothsnakumari et al. 2009).

From investigations on genetic identification and/or molecular responses of stress-related plant responses, modern molecular techniques were developed to breed better crops. The principal objective in plant breeding is to obtain plants that combine higher yields, reliable yield stability, better quality, and obvious stress-resistant characters (abiotic and biotic) over different years and locations (Bray 2004; Chaves and Oliveira 2004). The identification and use of molecular markers and introgression of genomic portions (QTLs) involved in stress tolerance is one good alternative, although undesirable agronomic characteristics from the donor plants may be introduced into the target plant (Roessner and Pettolino 2007).

Techniques that are more accurate than conventional or molecular breeding, such as genetic engineering, allow the selection of genes and their overexpression and/or introduction into the genome. By these methods, new cultivars can be produced more rapidly and efficiently with less chance of failure. Genetic engineering techniques can also overcome barriers to sexual crossing, so that genes of interest arising from taxonomically distant organisms can be selected and introduced (Bhatnagar-Mathur et al. 2008).

According to data collected on the productivity of rice, wheat, and corn during the last three decades, the observed increase is related to breeding and selection of high-yielding genotypes (Wang et al. 2003). However, this improvement in productivity is not followed by an increase in the potential yield of crops. That is, even in optimum environmental conditions, without infection by pathogens and without limitation of resources, both old and new cultivars give the same yield. Therefore, better understanding of the responses of cultivars to abiotic stress, in plant breeding, can implement plant improvement at a very practical level (Wang et al. 2003).

Some molecular responses to abiotic stress, or levels of stress, have been well established and can be used to optimize the production of more resistant individuals. Many of these studies were carried out with *Arabidopsis*. However, apart from specific stress responses at the gene or metabolic level, there is a common signal transduction pathway model for stress, which is shared by many higher plants. This model proceeds through the perception of the environmental signal, and subsequently the production of a secondary messenger (such as inositol phosphates and reactive oxygen species – ROS), which will regulate the endogenous levels of Ca^{2+}. From this point, a chain of events occurs that affects protein phosphorylation, reaching proteins linked to the protection of cellular structures or transcription factors controlling specific sets of stress-regulated genes. These genes are related to the production of regulatory molecules such as the plant hormones abscisic acid (ABA), ethylene, and salicylic acid (SA). Some of these regulatory molecules can, in turn, initiate a second round of circulation (Shao et al. 2007). From the analysis of all the data that can be generated from this model of response, from the standpoint of molecular biology as well as physiological, metabolic, and environmental stress, several questions arise. How to integrate all this available information? How to analyze the data completely? How to establish a relationship among different data sets at different levels and obtain accuracy? These questions can be answered by using techniques of the postgenomic era such as proteomics and metabolomics, which will be discussed in the next section.

2 Metabolic Profile of Plant Reponses to Abiotic Stresses: Proteomic and Metabolomic Approaches

2.1 Context in the Postgenomic Era

To cope with food shortages, classical plant breeding methods alone, such as were intensively employed during the Green Revolution in the 1960s, will not achieve the expected result. To satisfy the expanding food requirements of the rapidly growing world population, production of grain crops needs to increase a further 50% by 2025 (Khush 2003). This increase in production must be accompanied by optimization of growing

conditions, which nowadays are suboptimal for plant growth. About 70% of the potential yield is estimated to be lost because of unfavorable physical and chemical factors, even on farms in developed countries (Boyer 1982). To meet these challenges, genes, transcripts, proteins, and metabolites that control the architecture and/or stress resistance of crop plants in a wide range of environments will need to be identified, in order to facilitate the biotechnological improvement of crop productivity. Furthermore, several questions must be elucidated, such as: Which genes and proteins are up- or downregulated by the different types of abiotic stresses? What are the functions of these stress-responsive genes, proteins, and metabolites? What are the characteristics of the following events: stress perception ⇨ signal transduction ⇨ gene activation ⇨ protein expression ⇨ metabolite production ⇨ whole plant response (Altman 2003)?

Another important topic for understanding the relevance of modern technologies is breeding experiments for environmental stresses, specifically abiotic stresses. These experiments are slow and inefficient because of certain limitations, such as: (1) the types of stresses are highly variable in terms of timing during the plant growth cycle, (2) a type of stress that occurs in a specific period affects various tissues and involves multiple responses, making it very difficult to investigate the genetic control of crop tolerance, (3) the phenotyping of plant materials is very sensitive to environmental conditions (e.g., soil chemistry, soil texture, and weather) (Salekdeh and Komatsu 2007).

The identification of genes, transcripts, proteins, and metabolites involves the use of molecular tools. The molecular tools cover genome-wide genetic and physical maps of the chromosome for mapping, isolating, and sequencing of important genes, microarray, proteomics, and metabolomics for high throughput analysis of gene expression, and transformation and marker-aided selection (MAS) for validating candidate genes and utilizing them in molecular breeding. RNA and DNA microarrays can be used to detect gene expression in organisms. Nevertheless, to understand biological systems we must to go further. The set of proteins and mainly metabolites, for example, which are the final products from genes, directly reflecting the surrounding environment, need to be understood through their interactions and modifications (Salekdeh and Komatsu 2007; Ryan and Robards 2006; Bundy et al. 2009).

Thus, in the postgenomic era, the molecular tools to be used are those related to functional genomics such as transcriptomics, proteomics, and also metabolomics, which together, under a holistic view of the plant, called systems biology, will enable a better understanding of the complex regulatory networks associated with stress adaptation and tolerance (Urano et al. 2010). Systems biology is the integration of data from physiology, genomics, transcriptomics, proteomics, and metabolomics into models that might, eventually, represent and simulate the physiology of the organism. All these platforms combined in systems biology represent a new approach to discovering the genes and pathways that are crucial for stress responsiveness and tolerance. The integration of different data sets derived from a single sample will increase our understanding of data through a more holistic overview (May et al. 2011).

Proteomics and metabolomics, specifically, rely on label-free quantitative MS techniques, enabling absolutely essential high throughput analyses. To better exemplify, two phenotypes of *Arabidopsis* were compared in their responses to abiotic temperature stress, in order to better distinguish them. More refined results were obtained when differentiation integrated the results metabolite/protein dataset, than when only protein or only metabolites were examined (Morgenthal et al. 2007). To integrate these postgenomic platforms, bioinformatics, and computational tools are necessary (Kitano 2002).

The combination of different "omics" tools, which rather than investigating a limited number of substances (e.g., Van Dam and Poppy 2008), enable the large-scale scanning of various substances, offers great potential for postgenomics to elucidate the genotype-phenotype relationships (Wienkoop et al. 2010)

2.2 Proteomic Approach

But what is proteomics? Proteomics is the global study of proteins that are expressed in a given organ, tissue, or cell line. This approach provides unique insights into biological systems that cannot be provided by genomic or transcriptomic approaches, simply because there are many more proteins than protein-coding genes (Wienkoop et al. 2010). Proteomics has been used for systematic purposes, qualitative and quantitative profiling, and evaluation of the functions of proteins that are present in plant cells, tissues, or organelles. Therefore, proteomics is also a good tool to elucidate the elements that are involved in stress perception and transduction, and some reviews covering this area have already been published (Thurston et al. 2005; Jorrín et al. 2006).

The process of proteomics research in plant breeding follows a path that begins with the identification of stress-response proteins through comparison between stressed and control plants. Studies of proteome responses to stress generally compare protein profiles among resistant or tolerant organisms such as wild plants, mutants from genetic model species such as *Arabidopsis*, or crop plants, especially rice, wheat, and maize, or transgenics with susceptible or nontolerant individuals (Cooper and Farrant 2002). Following the numerous attempts to improve cultivars through classical crossover, several lines are available that have different degrees of tolerance (Salekdeh and Komatsu 2007). Different proteins, selected from contrasts between resistant/tolerant versus susceptible/nontolerant, or between optimal growth conditions versus stressed growth conditions, are taken as candidates involved in the stress response. The detection of these candidate proteins may allow correlations with the stress and tolerance trait. Plant growth, the level and duration of stress, and plant phenotyping are relevant topics for stress proteome study (Salekdeh and Komatsu 2007). Irrespective of which stress is applied and what plant species is utilized, most of the different proteins identified appear to be either constitutively present (preformed defenses) or are specifically induced in the resistant/tolerant plants (Cooper and Farrant 2002).

The course of a standard proteomics experiment often includes the following procedures: experimental design, sampling, tissue/cell or organelle preparation, protein extraction/fractionation/purification, labeling/modification, separation, Mass spectrometry (MS) analysis, protein identification, and statistical analysis of data and validation (Jorrín-Novo et al. 2009). The extraction of proteins is a crucial step in reaching the later stages of protein detection and identification. At this stage it is necessary to extract and solubilize proteins. Several extraction protocols are available, but two types of protocols are mostly used for plant material: tissue homogenization in buffer-based media, or in organic-solvent media (TCA-acetone, phenol, precipitation protocols). In order to achieve maximum efficiency in the extraction stage, capturing the greatest possible diversity of proteins is necessary, and this is often accomplished by combining different procedures. To be considered an ideal method, the extraction protocol should be reproducible, while at the same time it should reduce the level of contaminants and minimize artifactual protein degradation and modification (Carpentier et al. 2005; Rossignol et al. 2006).

Separation techniques may involve either gel-based or gel-free approaches. For gel-based studies, 1-DE and 2-DE are the preferred techniques used in combination with MS (Lilley and Dupree 2006; Jorrín et al. 2007; Görg et al. 2009). One of the major criticisms of 2-DE is its low precision, with relative standard deviations reported to fall in the range of 15–70%. Major sources of variability for this technique may include the transfer between the first and the second dimension, the analyst's expertise and the detection of separated proteins (Schröder et al. 2008).

Gel-free liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis, called shotgun proteomics (Leitner and Lindner 2009), can increase the number of different proteins that can be identified from complex samples, compared to more traditional gel-based approaches. Shotgun proteomics has become the method of choice for the analysis of complex protein mixtures (Wolters et al. 2001; Gerster et al. 2010). However, the combination of SDS-PAGE, band cutting, trypsin digestion, and LC separation of the resulting

peptides is the most powerful proteomics tool to cover the majority of proteins (de Godoy et al. 2006; Tribl et al. 2008).

The so-called "second generation" MS technologies for Quantitative Proteomics include difference gel electrophoresis (DIGE), isotope-coded affinity tags (ICAT) (Shiio and Aebersold 2006), isobaric tags for relative and absolute quantitation (iTRAQ) (Wiese et al. 2007; Gan et al. 2007), and stable isotope labeling by amino acids in cell culture (SILAC) (Nelson et al. 2007; Palmblad et al. 2008) are now beginning to be successfully applied to plants for quantitative and large-scale proteomics studies. The gel-free multidimensional protein identification technology (MudPIT) is particularly well suited for the identification of hydrophobic proteins (Tjalsma et al. 2004; Görg et al. 2009) and allows the detection of a much larger number of proteins compared to gel-based methods, its drawback being the lack of quantitative data (Bayer et al. 2006). The gel-based 2-D DIGE technique is adequate for quantitative proteomics, and requires only a small amount of protein (0.025–0.050 mg) compared with 2-DE (ca. 0.7–1.0 mg) and therefore avoids the limitation of the existence of highly abundant proteins in the protein samples (Majeran et al. 2005; Ndimba et al. 2005; Casati et al. 2005; Dunkley et al. 2006).

To investigate highly complicated proteomics, label-free approaches by means of LC–MS, an IT or Fourier transform mass spectrometer have been used (Wang et al. 2006). The simplicity and cost-effectiveness of this technique make its validation with plant extracts desirable (Jorrín et al. 2007).

Although Bottom-up Proteomics (analysis of proteolytic peptide mixtures) remains the predominant platform, top-down strategies (analysis of intact proteins) should allow a more complete characterization of the proteome, including protein isoforms and posttranslational modifications (PTM). All these aspects have been discussed in detail in recent reviews (Aebersold and Mann 2003; Cravatt et al. 2007; Zubarev and Mann 2007; Molina et al. 2007; Good et al. 2007).

Using classical quadrupole and ion trap mass analyzers, intact protein masses can be determined with standard deviations in the range of 2–5 kDa. The use of Fourier transform mass spectrometry ion cyclotron resonance (Meng et al. 2007) can avoid the problems relating to complex mixtures of protein isoforms, which may complicate the determination of protein mass (Katz et al. 2007; Bräutigam et al. 2008). Surprisingly, it has been reported that a set of proteins can only be detected by a specific technology (Komatsu et al. 2006; McDonald et al. 2006; Wu et al. 2006), which is in agreement with the idea that a combination of different methodologies is still needed to characterize entire proteomes.

Some innovations in the field of proteomics have allowed leveraging of resources to better detect and identify proteins. In the past few years, the development of new Orbitrap and dissociation methods such as electron-transfer dissociation, have opened up new possibilities in proteome analysis. The mass spectrometer, despite constant improvement in terms of machines, software, and protocols, has reached the limit of its capacity (Jorrín et al. 2007).

Proteomics platforms have a number of restrictions, such as sensitivity, resolution, and speed of data capture. They also face a number of challenges, such as deeper proteome coverage, proteomics of unsequenced "orphan" organisms (Carpentier et al. 2005), top-down proteomics (Han et al. 2006) and protein quantitation (Cox and Mann 2007). These restrictions and challenges arise from the huge diversity of proteins, with widely differing physical and chemical characteristics, that are present in organisms.

Finally, in silico proteomics, although it is as yet only applicable where the full genomic sequence is available (i.e., *Arabidopsis* and rice), is useful in both predicting and validating experimental data (Heazlewood et al. 2007). Because of the large amounts of data generated by proteomics analyses over the past year, there have been efforts to form a database where proteomics information can be deposited and made available to the scientific community: the PPDB, http://ppdb.tc.cornell.edu (Sun et al. 2009); the PODB, http://proteome.dc.affrc.go.jp/Soybean/; the Organellome, http://podb.nibb.ac.jp/Organellome (Mano et al. 2007); and the knowledge-based UniProt (Jorrín-Novo et al. 2009).

Efforts to form a searchable database of MS/MS reference spectra have been implemented by committees such as the Subcommittee of the

Multinational Arabidopsis Steering Committee, through projects such as the "Green Proteome" (Weckwerth et al. 2008; Hummel et al. 2007), Plant Proteomics in Europe (COST Action FA0603). The database permits authentic protein identification through a genome-independent approach, since newly generated MS/MS spectra can be matched against previous experimental MS/MS spectra. This approach allows semiquantitative analysis at the same time as spectrum matching.

Initiatives have also begun to create a guide for conducting proteomics experiments to achieve more consistent results, because many papers contain errors in the experimental design, the analysis, and the interpretation of the data (Nesvizhskii et al. 2007). More consistent data cannot rely upon speculation, especially when the genome or transcriptome of the species being studied is still unknown. Analysis of the greatest possible number of proteins, rather than only a fraction, also improves the consistency of results. Therefore, the HUPO's Proteomic Standard Initiative has developed guidance modules (Orchard and Hermjakob 2008) that have been translated into Minimal Information about a Proteomic Experiment (MIAPE) documents. The MIAPE documents recommend proteomics techniques that should be considered and followed when conducting a proteomics experiment. Proteomics journals should be, and in fact are, extremely strict in recommending that investigators follow the MIAPE standards for publishing a proteomics experiment (Jorrín-Novo et al. 2009).

What are the protein profiles that are found in plants under abiotic stress?

According to individual studies and reviews, few proteins are specific for the type of stress applied (Bolwell et al. 2001; Cooper and Farrant 2002; Skylas et al. 2002; Hajheidari et al. 2007). For some differential proteins, multiple isoforms or specific PTMs may be detected, each responding differently according to the stress applied (Hammond-Kosack et al. 1998). Proteins that are expressed by the same stressors, clearly confer a physiological advantage under stress conditions, and thus are simultaneously potential targets for marker-assisted selection and rational candidate genes for the identification of quantitative trait loci.

Drought stress, metal toxicity, and salt-osmotic stress are the types of abiotic stress that are most often investigated. In contrast to the intensive study of the influence of water and nutrient status on plant proteomes, studies of plant responses to light and temperature stress are rare. Various sources of plant material were examined in proteome experiments: leaves and cotyledons, roots, fruits, phloem and xylem saps, apoplastic fluid, entire seedlings, shoots, stem segments, seeds, nuclear fractions, gametophores, and meristem tissue.

Drought conditions may induce proteins related to detoxifying reactive oxygen species (ROS) (Hajheidari et al. 2007), but many other abiotic stresses can enhance production of ROS resulting from photosynthesis, respiration, and NADPH oxidase (Hammond-Kosack et al. 1998). This observation makes sense, since most stressors increase production of reactive oxygen species ROS in plants. Cells exposed to high amounts of (ROS) may be damaged. ROS act as secondary messengers involved in the stress-response signal transduction pathway. Therefore, to detoxify the cell, that is, remove excess ROS, plants have two mechanisms. The most important ways to combat ROS are those that involve SOD (Hajheidari et al. 2005), the water–water cycle, the ascorbate–glutathione cycle, glutathione peroxidase, and catalase (del Río et al. 2006). In the early stages of drought stress, many proteins associated with root morphogenesis and carbon/nitrogen metabolism, which may contribute to drought avoidance by enhancing root growth are stimulated (Yoshimura et al. 2008). 2-Cysteine peroxiredoxin is a protein that can be synthesized from drought stress, and belongs to the group that reduces H_2O_2 and alkyl hydroperoxide (Dietz et al. 2002). This protein constitutes an important alternative to detoxification under oxidative stress conditions. Small heat shock proteins (sHSPs) are also induced by heat and drought stresses. HSPs function as chaperones and play an integral role in protein folding and assembly (Sun et al. 2002). Therefore, sHSPs are promising protein markers for marker-assisted breeding programs to increase stress tolerance. The response to drought stress (Hajheidari et al. 2005) also involves the expression of cytosolic Cu–Zn SOD, cyclophilin, nucleoside-diphosphate

kinase, a nascent polypeptide-associated complex a-chain, and the large subunit of Rubisco. Nucleoside diphosphate kinase (NDPKs) is also more strongly expressed after heat and drought stress (Escobar Galvis et al. 2001; Moon et al. 2010). NDPK uses ATP to maintain the cellular levels of CTP, GTP, and UTP (Moon et al. 2010) and cooperates in cellular redox regulation. The overexpression of AtNDPK2 leads to decreased constitutive ROS levels and increased tolerance to multiple environmental stresses.

Actin depolymerizing factor 4 (ADF) is also correlated with responses to drought and salt stress (Salekdeh et al. 2002; Ali and Komatsu 2006; Yan et al. 2010). ADF is related to osmoregulation under osmotic stress. This group of proteins is involved in the regulation of different cellular processes including cytokinesis, remodeling of actin filaments, cytoplasmic streaming, and signal transduction events (Dong et al. 2001). The upregulation of ADF under drought and salt stress indicates that this protein might be associated with dynamic reorganization of the cytoskeleton during drought stress. Redox proteins such as glutathione dehydrogenase (At1g19570) are affected in stress regulation (Morgenthal et al. 2007; Wienkoop et al. 2008).

Mitogen-activated protein kinases (MAPKs) are upstream regulators of many aspects of plant cell signaling. MAPK cascades usually require three components: MAPK kinase kinases (MPKKKs), which phosphorylate MAPK kinases (MPKKs), which phosphorylate MAPKs, which phosphorylate diverse proteins (Chinnusamy et al. 2004; Ren et al. 2008). After MAPK is activated, it further activates transcription factors in the nucleus, or phospholipid-cleaving enzymes in the cytoplasm. This set of enzymes is related to stress response (Cheong et al. 2002; Xu et al. 2003; Chinnusamy et al. 2004; Hu et al. 2006). MPK4 and MPK6 have received considerable attention for their role in abiotic stress signaling. Posttranslational activation of these two kinases is stimulated by cold, low humidity, salt, wounding, reactive oxygen species, and touch (Ichimura et al. 2000; Yuasa et al. 2001).

Salt stress responses involve the substrate-binding proteins of ABC transporters. Products including H1 transporting ATPases, signal transduction-related proteins, transcription/translation-related proteins, detoxifying enzymes, amino acid, and purine biosynthesis-related proteins, proteolytic enzymes, HSPs, and carbohydrate metabolism-associated proteins are also involved in salt stress (Des Marais and Juenger 2010).

Excessive light enhances production of proteins involved in photosynthesis, as well as some known light stress-related proteins, such as HSP, dehydroascorbate reductase, and SOD (Cushman and Bohnert 2000). The cold stress response leads to accumulation of dehydrins and low-temperature-induced protein (Uno et al. 2000).

2.3 Metabolomic Approach

What is metabolomics? Metabolomics is the untargeted analysis of a set of metabolites that are produced by an organism, so the metabolome is the set of metabolites, specifically low-molecular-weight molecules (typically 3,000 m/z), present in a cell, tissue, or organ in a particular physiological or developmental state (Oliver et al. 1998). It is the layer downstream from large-scale analysis of RNA (transcriptomics) and proteins (proteomics) (Weckwerth 2003; Bino et al. 2004).

Understanding the metabolome is important to elucidate the complex network related to abiotic stress. The idea that metabolites are only the final product of gene expression is outmoded (Hollywood et al. 2006). It is increasingly understood that metabolites themselves regulate macromolecular operations through, for example, feedback inhibition and as signaling molecules. The cellular processes are in reality intimately networked, with many feedback loops, and thus should be represented as dynamic protein complexes interacting with neighborhoods of metabolites (Caspi 2006). Metabolomics analyses are therefore destined to provide an integrated perspective of the functional status of an organism (Dixon et al. 2006). More than this perspective, metabolome investigation is complementary to transcriptomics and proteomics, and may have special advantages. While changes in the levels of individual enzymes may be expected to have

little effect on metabolic fluxes, they can and do have significant effects on the concentrations of a variety of individual metabolites. In addition, as the "downstream" result of gene expression, changes in the metabolome are amplified relative to changes in the transcriptome and the proteome, which is likely to allow for increased sensitivity (Dixon et al. 2006). Finally, it is known that metabolic fluxes are regulated not only by gene expression but also by posttranscriptional and posttranslational events, and as such, the metabolome can be considered to be closer to the phenotype (Siritunga and Sayre 2003).

Metabolomics is not intended to identify a particular metabolite or set of metabolites, as is done in traditional phytochemical studies. The broader purpose of this technique allows the evaluation not of only a very small fraction of the metabolism, but of the maximum possible number of metabolites. This is because none of the existing techniques allows the evaluation of all the metabolites that are present in an organism (Ryan and Robards 2006). To capture all of them, different analytical platforms must be combined, considering that plant metabolites have different chemical properties (Fernie et al. 2004; Moco et al. 2007). Their differences are based on the degree of volatility, polarity, and concentration in a given tissue (Weckwerth 2003). Because of this wide variability of physicochemical characteristics, metabolomics studies are usually based on substances with certain chemical affinities.

The most widely used model for studying this platform is *Arabidopsis thaliana*, but other species including food plants such as tomato and potato have been investigated by means of this approach (Catchpole et al. 2005; Kristensen et al. 2005; Keurentjes et al. 2006; Moco et al. 2006; Leiss et al. 2009; Kunin et al. 2009).

Metabolomics investigations are based on techniques that include nuclear magnetic resonance (NMR), Fourier transform ion cyclotron resonance coupled with mass spectrometry (FT-ICR-MS), and separation-based techniques such as gas chromatography and liquid chromatography coupled with mass spectrometry (GC–MS and LC–MS). These analytical tools can profile the impact of time, stress, nutritional status, and environmental perturbation on hundreds of metabolites simultaneously, resulting in massive, complex data sets. This information, in association with transcriptomics and proteomics, has the capacity to produce a more holistic view of the composition of food and feed products, to optimize crop trait development, and to enhance diet and health (Dixon et al. 2006).

Samples intended for this approach are prepared using rapid freezing that stops enzyme activity. Subsequently, metabolites are extracted by different methods, for example, with methanol to extract semipolar metabolites. The extract can then be analyzed by many different methods and approaches (Hollywood et al. 2006).

Because of the unique structural composition and three-dimensional configuration of each compound, NMR yields a specific spectrum for each substance. The advantage of this method is that it is highly reproducible and nondestructive, and can also quantify the metabolites (Verpoorte et al. 2007). Despite this, metabolites that are present in smaller quantities will not be detected. While NMR uses magnetic resonance, all the other metabolomics platforms use mass spectrometry (MS) for identification (Macel et al. 2010).

The most widely used metabolomics platforms are MS combined with chromatographic separations, because of the availability and relatively low cost of these techniques. With MS, metabolites are ionized (charged) and their mass-to-charge ratios (m/z) are measured using electric and magnetic fields in a mass analyzer. These mass-to-charge ratios are specific for each metabolite. The disadvantage of the MS platform is that quantification of the substances is difficult and can generally only be measured in relative terms. The reproducibility is lower compared to NMR, although the MS method is much more sensitive. "Hyphenated" techniques of LC–MS (Yamazaki et al. 2003a, b) combine retention times (the time needed to pass through the column that separates the compounds) with MS for identification, normally, of the nonvolatile metabolites, particularly the semipolar secondary metabolites such as flavonoids, alkaloids, and glucosinolates, but also sugars and amino acids (Macel et al. 2010). GC–MS is widely used to analyze low-molecular-weight volatiles

(Fiehn et al. 2000; Roessner et al. 2001). Analytical methods for metabolic fingerprinting analyses of crude extracts with no previous separation steps, involve Fourier-transform ion cyclotron resonance (FTICR) mass spectrometry and time-of-flight (TOF) mass spectrometry (Aharoni et al. 2002; Brown et al. 2005), which cover a broad range of substances (Hagel and Facchini 2007). The mass-to-charge ratios are established from the cyclotronic frequency of the ions in a fixed magnetic field (Macel et al. 2010). Although FT-ICR-MS has a higher sensitivity and resolution compared to NMR, it mainly gives the elemental composition of a metabolite (through MS) without providing much extra information about the chemical structures of the molecules (Macel et al. 2010).

In contrast to transcriptome studies (but in common with protein analysis), no tools are available for amplification of metabolites, and consequently sensitivity is a major issue. Metabolites have huge chemical differences, and are often present in a wide dynamic range. All of these challenges need to be adequately addressed by the analysis strategy employed.

Because of the huge amount of data that can be generated by the techniques mentioned above, as also occurs in proteomics, data processing is required (Lommen 2009). For data analysis, knowledge of bioinformatics is required (Smilde et al. 2005; Sumner et al. 2007). Data can be examined by multivariate statistics such as principal components analysis (PCA), nonmetric multidimensional scaling (NMDS), and partial least squares discriminant analysis (PLS-DA) (Westerhuis et al. 2008). These multivariate methods will show whether the metabolome, and to a certain extent also which metabolites, differ between treatments or species. To investigate the behavior of individual metabolites, Student's *t*-tests or univariate analyses of variance (ANOVA) can be used in combination with correction for false discovery rates (FDR) (Macel et al. 2010).

With the intention of gathering the largest possible amount of metabolomics data, a World Wide Web-access system was created. The PlantMetabolomics.org (PM) website allows public consultation of the MS-based plant metabolomics experimental results from multiple analytical and separation techniques. PM has extensive annotation links between the identified metabolites and metabolic pathways in AraCyc (Mueller et al. 2003) at The Arabidopsis Information Resource (Rhee et al. 2003) and the Plant Metabolic Network (www.plantcyc.org), the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa 2004), and MetNetDB (Wurtele et al. 2007). The rationale for the development of PM as an information portal is to provide free public access to experimental data, along with cross-references to related genetic, chemical, and pathway information. The portal also serves as an information resource for the field of metabolomics by providing tutorials on how to conduct metabolomics experiments. It describes minimum reporting standards (Fiehn et al. 2007; Sumner et al. 2007) for plant metabolomics experiments, based on the recommendations of the MSI. In addition, PM contains background information about the experimental design and tools that can be used to analyze the collected data (Bais et al. 2010).

Using the appropriate technology, there are different strategies to investigate the metabolome. The strategy most often used to study abiotic stress is metabolite target analysis, which is an approach that is restricted to metabolites of, for example, a particular enzyme system that would be directly affected by abiotic or biotic perturbation (Hollywood et al. 2006).

What are the metabolic profiles found in plants under abiotic stress?

Dehydration–stress response metabolome studies have shown that both ABA-dependent and ABA-independent pathways are involved in this kind of stress (Yamaguchi-Shinozaki and Shinozaki 2006). The endogenous ABA level rises in response to water-deficit stress, to modulate physiological stress responses and gene expression. ABA produced during dehydration affects the accumulation of various amino acids and sugars such as glucose and fructose. In particular, the dehydration-inducible accumulations of BCAAs (branch-chain amino acids), saccharopine, proline, and agmatine are correlated with the dehydration-inducible expression of their key biosynthetic genes (BCAT2, LKR/SDH, P5CS1, and ADC2, respectively), which are regulated by

endogenous ABA (Urano et al. 2009). On the other hand, the levels of raffinose and galactinol are not regulated by ABA during dehydration stress. Thus, it seems that ABA has an important role in regulating metabolism during water stress (Urano et al. 2010).

Some studies indicate that more glucose, malate, and proline tend to be produced in plants under dehydration stress than under salt stress. Probably this difference results from the need for plants subjected to salt stress to make a greater osmotic adjustment, detoxify ROS, and ameliorate photoinhibition (Cramer et al. 2006). When the plant treatment occurs under more severe conditions such as dehydration and heat-stress treatment, sucrose replaces proline as the major osmoprotectant (Rizhsky et al. 2004).

The temperature stress response such as to cold and other stresses, involves the DREB1/CBF (dehydration-responsive element-binding factor/C-repeat) transcriptional network. A correlation between the metabolome (monosaccharides, disaccharides, oligosaccharides, and sugar alcohols) and the DREB1A/CBF3 transcription factor under low temperature was also observed. The low-temperature-inducible accumulation of galactinol and raffinose is correlated with the expression of the GolS3 gene, which is a direct target of DREB1A/CBF3. Some studies indicate that the expression of DREB1A affects the accumulation of low-temperature regulated metabolites, especially sucrose, raffinose, galactinol, and myoinositol (Maruyama et al. 2009).

According to some metabolome studies the majority of metabolites produced in response to heat shock overlapped with those produced in response to cold shock. Furthermore, these results indicate that a metabolic network of compatible solutes including proline, monosaccharides (glucose and fructose), galactinol and raffinose has an important function in tolerance to temperature stress (Kaplan et al. 2004).

Salt stress responses are correlated with higher levels of various osmolytes, such as fructose, sucrose, complex sugars, malate, and proline (Gong et al. 2005). Short-term response to salt stress seems to involve the simultaneous induction of several pathways: the methylation cycle for the supply of methyl groups, the phenylpropanoid pathway for lignin production, and glycine betaine (GB) biosynthesis (Kim et al. 2007). In the long-term response to salt stress, however, glycolysis and sucrose metabolism were coinduced, and then the methylation cycle was coreduced. As observed in experiments with drought stress, under salt stress ABA was also shown to have an important role in establishing metabolite profiles (Kempa et al. 2008). Complex readjustment of carbohydrate metabolism occurs throughout the period of salt stress, and ABA triggers the initial stages of carbon mobilization.

To integrate multiple datasets from metabolite, transcript, and protein information, some initiatives have been developed, such as the creation of DOME (database for OMEs). This platform allows the storage of DNA microarray data, protein fragment mass spectral data from two-dimensional gel separations, and metabolite MS data after separation by GC, LC, or CE. Additional databases and programs that allow integration of metabolite with transcript data are AraCyc (http://arabidopsis.org/tools/aracyc/) as mentioned above, MAPMAN, and KaPPA-View (http://kpv.kazusa.or.jp/kappa-view/) (Dixon et al. 2006).

3 Plant Stress Tolerance or Resistance: Productivity and Prospects

A single cross between two plants, as used in conventional breeding, joins two sets of about 15,000–25,000 genes. This is the assumption that guides classical agriculture. Plant breeding has been performed since the start of agricultural practices thousands of years ago. Classical breeding relies on the process of homologous recombination between two genomes, creating novel genetic diversity. In contrast, modern biotechnological methods allow only a few genes to be modified at the same time, leaving the rest of the genome unaltered (Akhond and Machray 2008). Moreover, genes from any source can be introduced into a crop plant, genes, and their products can be tested to evaluate their safety, and genes can be altered and assessed under laboratory conditions to change their properties before being introduced into a plant.

Combining the resources of classical breeding with modern biotechnology, a novel variety of genotypes and phenotypes can be created, agricultural productivity can be increased, and human survival in the face of population growth and climate change can be achieved (Altman 2003). This is an important subject for agricultural research, as increased competition with other land uses pushes farms into harsher environments, fresh water becomes scarcer, and the climate change anticipated by some scientists increases environmental stress. Therefore, to increase productivity by engineering plants that are more resistant or tolerant to abiotic stress, genes, and their products are the target of this initiative. However, this task seems more daunting than engineering plants that are resistant to pests and herbicides. Biotic stress is largely dependent on monogenic traits, while abiotic stresses are multigenic and thus more difficult to control and engineer (Vinocur and Altman 2005).

According to James, "In 2007, the global area of biotech crops increased for the twelfth consecutive year at an annual growth rate of 12%. While the technology was initially applied in developed countries, 12 million farmers in farmers were in 2007 biotech crops were grown by 23 countries covering 114.3 million hectares and over 90% of the beneficiary resource-poor in developing countries, with increased incomes from biotech crops contributing to the alleviation of poverty" (Akhond and Machray 2008).

The sequence of breeding for plant tolerance to abiotic stress consists of several stages: (1) conventional breeding and germ plasm selection; (2) clarification of the specific molecular control process in tolerant and sensitive genotypes; (3) biotechnology-oriented improvement of selection and breeding operations by functional genomics investigations, use of molecular probes and markers for selection among natural and bred populations, and transformation with specific genes; (4) large-scale propagation (seed or vegetative) of the engineered and selected genotypes; and (5) improvement and adaptation of current agricultural practices (Altman 2003).

Stress-induced gene expression can be broadly categorized into three groups: (1) genes encoding proteins with known enzymatic or structural functions, (2) proteins with as yet unknown functions, and (3) regulatory proteins. Transgenic plants tolerant to certain types of abiotic stresses were developed based on the manipulation of genes that protect and maintain the function and structure of cellular components. Initially, some studies have focused on identifying genes responsible for the synthesis of a single metabolite. In the case of abiotic stress related to salinity and drought, studies have evaluated proteins that are involved in water channels, the synthesis of osmolytes (proline, betaine, sugars such as trehalose), or transport to the work of transformation. Metabolic traits, especially pathways with relatively few enzymes, have been characterized genetically and appear more amenable to manipulation than do structural and developmental traits (Bhatnagar-Mathur et al. 2008). However, this perspective neglects the likelihood that abiotic stress tolerance involves many genes at once, and that single-gene tolerance is unlikely to be sustainable. Given this limitation, new prospects have arisen for the development of transformed plants that have resistance or tolerance to abiotic stress. One possibility involves the manipulation of genes that belong to the third category mentioned above, genes that express regulatory proteins. Through these proteins, many genes associated with stress responses can be simultaneously regulated by a single-gene encoding the stress-inducible transcription factor (Kasuga et al. 1999), thus providing conditions to enhance tolerance to multiple stresses including drought, salinity, and freezing. This new ability to engineer more resistant or tolerant plants, recognized as the "second wave", coincides with a better combination of genetic engineering with plant physiology. Gene cassettes driven by stress-induced promoters are being used to generate transgenics, since stress-induced promoters (particularly those induced by anaerobic conditions, low or high temperatures, and salt stresses) have now been characterized (Bhatnagar-Mathur et al. 2008).

Some transformation experiments showing promising results for abiotic tolerance have been implemented (Wang et al. 2003). Many reviews elucidating the process of abiotic stress tolerance and on engineering tolerance to stress have been published in the last few years (Akhond and Machray 2008).

As mentioned before, plants subjected to water stress usually tend to produce sugars and similar compounds that act as osmoprotectants. One such substance is trehalose. Trehalose levels have been increased in GM rice by overexpressing genes encoding trehalose biosynthetic enzymes from the bacterium *Escherichia coli* (Garg et al. 2002). Rice plants subjected to this type of experiment showed better performance under salt, drought, and low-temperature stress conditions. On the other hand, some results showed that transformed plants that produced high levels of osmoprotectants suffer from deleterious pleiotropic effects, such as dwarfing (Hazen et al. 2003).

Some genes have been found to be multifunctional. Examples include *BADH*, *P5CS*, and *HAV*, which are involved in preventing drought, salt, osmotic, and heat stress (Hu et al. 2010). Specifically, *BADH* is responsible for the production of betain aldehyde dehydrogenase, and is involved in the biosynthesis of GB, which acts as a compatible solute in plants. Transformed plants with *BADH* have osmoregulation ability, but also improved salt and heat tolerance (Hu et al. 2010).

Accumulation of osmotically active compounds can prevent osmotic damage to cell structures, protein destabilization, and the negative effects of ROS (Wang et al. 2003). The accumulation of proline, betaine, free amino acids, sugars, sugar alcohols, alkaloids, etc. can be achieved by overexpression of enzymes associated with their biosynthesis, or by suppression of enzymes that induce their destruction (Chen and Murata 2002). Transgenic rice, soybean, tobacco, and wheat overexpressing pyrroline-5-carboxylate synthetase (P5CS), which induces the biosynthesis of the above-mentioned enzymes, showed, in specific cases, salinity, drought, salt, and heat resistance, and increased biomass under water stress (Sokhansanj et al. 2006; Vendruscolo et al. 2007).

Other alternatives have been explored to solve the problem of excess salt in the soil. This problem, usually caused by irrigation, now affects vast cultivable areas. An important strategy for achieving greater tolerance to abiotic stress is to help plants to reestablish homeostasis under stressful environments, restoring both ionic and osmotic homeostasis. The target is to achieve Na+ excretion from the root, or its storage in the vacuole, so overexpression of a gene that encodes a vacuolar Na+/H+ antiport pump could be one solution (Apse and Blumwald 2002). Such a pump would allow for more effective removal of salt from the cytoplasm and its transfer to the vacuole. These results have been obtained in GM tomato plants, which showed a higher tolerance to salt concentrations than did nontransformed individuals, and survived better in areas that were previously considered useless for agriculture. Furthermore, the fruit does not accumulate salt, and is edible. This effort to produce food in large areas, that are presently impractical for farming, has also been extended to problems of soil contamination by heavy metals (Shewry et al. 2008).

The transcription factors activate cascades of genes that act together in enhancing tolerance towards multiple stresses. Transcriptional activation of stress-induced genes has been possible in transgenic plants with overexpression of TFs, which also belong to the multifunctional gene family, recognize promoter regulatory elements of these genes, and can induce stress-responsive gene expression and increase tolerance to abiotic stress. DREB can increase the drought, salt, and cold tolerance of many species, as confirmed by transgenic researches (Ito et al. 2006; Sakuma et al. 2006).

Overexpression of 9-*cis*-epoxycarotenoid dioxygenase (NCED) is connected to ABA biosynthesis, results in a relaxation of stress symptoms related to cold, drought, and salt (Jung et al. 2008).

Abiotic stress signaling in plants involves receptor-coupled phospho-relay, phosphoionositol-induced Ca^{2+} changes, the MAPK cascade, and transcriptional activation of stress-response genes. Plant acclimatization to environmental stress involves activation of various kinases, and in turn, a single kinase gene can affect various kinds of stress resistance. From this principle, it has been shown that maize transformed with the tobacco MAPKKK/NPK has an oxidative signal cascade activated, improving cold, heat, and salt tolerance (Shou et al. 2004).

Overexpression of sensors that can perceive stress signals through the combination reactions of signals (Wang et al. 2007), such as calcium-dependent protein kinases (CDPKs), was implemented in barley. CDPKs are unique Ca^{2+} sensors in plants, and transgenic barley with this improvement responds better to cold and salt stress. Other kinds of sensors are salt sensors (Qiu et al. 2004) and osmosensors (Hu et al. 2010).

To avoid stress caused by oxygen radicals, transgenic plants designed to overexpress enzymes involved in oxidative protection, such as glutathione peroxidase, superoxide dismutase, ascorbate peroxidases, and glutathione reductases (Hazen et al. 2003). Overexpression of antioxidative enzymes (such as SOD) in transgenic alfalfa, rice, *Arabidopsis,* and cabbage (Serrot et al. 2008) induces higher cold, drought, and salinity resistance than in wild plants (Samis et al. 2002; Tseng et al. 2007).

Transgenic plants overexpressing genes encoding LEA proteins and molecular chaperones such as HAV1, a group 3 LEA protein gene, can enhance resistance to drought, salt, cold, and other stresses (Jyothsnakumari et al. 2009). For example, transgenic wheat, oats, and rice with overexpression of the HAV1 gene have drought and salt resistance, and show improved growth (Fu et al. 2007). Genetic engineering for increased thermotolerance by enhancing heat shock protein synthesis in plants has been achieved in a number of plant species (Katiyar-Agarwal et al. 2003).

4 Conclusion and Future Perspective

In conclusion, huge efforts have been made in identifying plant abiotic stress responses and proteomics and metabolomics has been identified as the tools of choice for comprehensive analysis of genes functions, protein and metabolites interactions, and modifications. As the gene sequences of new species are being elucidated, comparative studies with *Arabidopsis* may contribute to the elucidation of certain answers.

Plants grown on the stress conditions can be compared to plants with tolerance or resistance to a particular factor. In addition, this study can be conducted in field conditions or in tissue culture. Although potentially expensive, plant tissue culture can offer some advantages over traditional field growing practices. These advantages include manipulation of culture system to occlude the influence of variables that are not desirable such as some environmental factors: climate, nutrient availability, and disease. Functional genomic approaches, including metabolomics, will certainly allow description of biosynthetic and regulatory pathways. These approaches will enable rational plant engineering to produce transgenics of interest on demand.

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