Girdhar K. Pandey *Editor*

Elucidation of Abiotic Stress Signaling in Plants

Functional Genomics Perspectives Vol. 1

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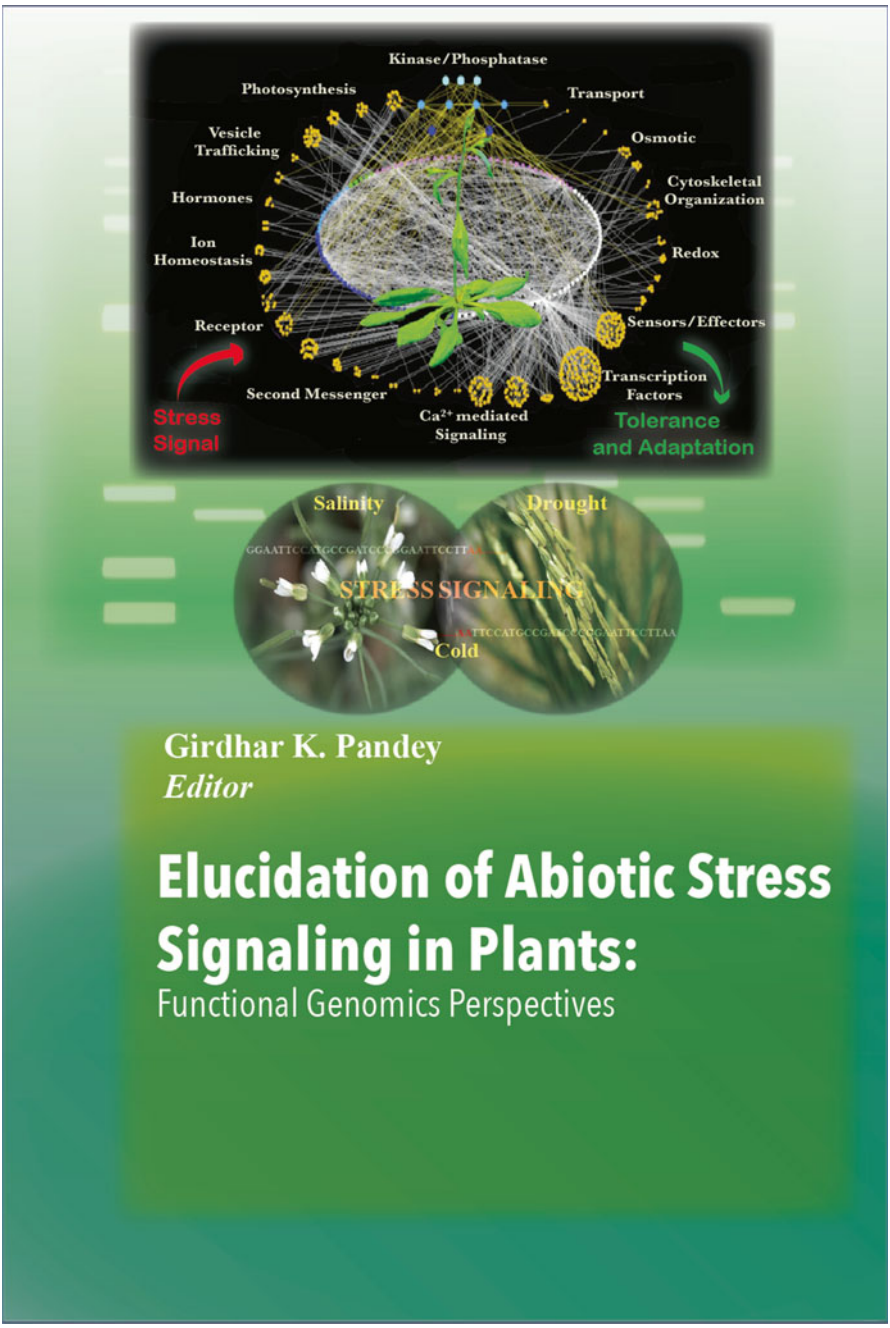

The above image represents a depiction of activation of different signaling pathways by diverse stimuli that converge to activate intricate signaling and interaction networks to counter stress (top panel). Since environmental stresses influence most significantly to the reduction in potential crop yield, progress is now largely anticipated through functional genomics studies in plants through the use of techniques such as large-scale analysis of gene expression pattern in response to stress and construction, analysis and use of plant protein interactome networks maps for effective engineering strategies to generate stress tolerant crops (top panel). The molecular aspects of these signaling pathways are extensively studied in model plant *Arabidopsis thaliana* and crop plant rice (*Oryza sativa*) (below).

Girdhar K. Pandey
Editor

Elucidation of Abiotic Stress Signaling in Plants

Functional Genomics Perspectives, Volume 1

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Preface

Plants are considered the backbone of life on earth. The colorful life on this planet has emerged as a consequence of over 3.5 billion years of unceasing evolution. Life on earth cannot sustain without plants, as they harness solar energy to produce sugars and oxygen, the primary constituents for supporting life. Humans are primarily dependent on plants and have developed a systematic discipline called "agriculture" to cultivate or domesticate plants over a period of time for food, biofuel, and fodder. At present time, crop productivity faces a major challenge from rapidly growing population and diminishing fertile land due to excessive anthropogenic activities. In addition, expanding human population and climate changes due to increased exploitation of natural resources imposes several major unfavorable conditions that reduce the crop productivity. These unfavorable conditions are primarily categorized as physical (or *abiotic*) and biological (or *biotic*) variables hindering normal growth and development in plants. Interestingly, stress perceived by one plant species may not be a stress factor for another plant species due to different growth habits and adaptation acquired during the course of evolution. Because of domestication and cultivation of crop plants by humans over a period of 10,000 years, many of these wild traits responsible for adaptive responses were lost, increasing the vulnerability of crop plants to biotic and abiotic stresses. Under abiotic stresses, limitation of water (drought), extremes of temperature (both high and low temperatures), nutrient deficiency, and soil contaminated with salt and heavy metals or pollutants are the major environmental factors contributing to crop losses worldwide.

In the past, agriculture has relied on breeding approaches to develop high yielding crop varieties which can grow optimally under stress conditions without affecting crop yield and productivity. In an effort to find an alternative tool faster than the traditional breeding approach, the last two decades has seen the advent and development of genetic engineering. This technique involves the identification, transfer, and stable integration of desired genes into genomes of crop plants to generate transgenic plants, exhibiting improved trait for tolerance against one or other stress factors in contained experimental conditions such as green houses.

However, plants are constantly exposed to a multitude of stresses at any given time in the natural environment, and not much has been achieved till now to generate crop varieties that can tolerate these multiple stresses without yield penalty. In order to develop stress-tolerant crop varieties with the ability to withstand multiple stresses in their environmental growth condition, an in-depth and systematic understanding of stress sensing, signal transduction, and generation of response is required.

Evolutionarily, the major distinction between plants and animals in sensing and responding to a plethora of stresses is due to their sessile versus mobile nature, respectively. In the case of animals, the primary response against a particular stress is avoidance of stress, whereas in plants, due to their immobilization, development of stress tolerance is the only escape response. Moreover, plants lack a well-defined brain and nervous system unlike their animal counterpart, leading to development of higher degree of plasticity in their communication skills by numerically expanding their signal transduction machinery. Despite the variances amid plants and animals, many of the signal transduction components can be found to be conserved. These include receptors, second messengers, signal-transducing molecules like kinases, phosphatases, small and large G-protein, and others, which finally affect the activity of either transcription factors to regulate the gene expression or transporters/channels, metabolic enzymes, and cytoskeletal proteins to directly change the physiology of the cell. Additionally, analogous to networking in the nervous systems, the signaling pathways in plants also exhibit scale-free web of networks instead of linear or definite pathways. These scale-free networks constitute extremely connected points called *nodes* and *hubs*, which are responsible for efficient processing, channeling, and integration of multiple signaling pathways at a given time to generate specificity as well as cross talk in the signaling networks.

Plants primarily rely on the complex, intertwined, and dynamic signal transduction pathways for developing a higher order of networks. This involves sophisticated control circuits like the nervous system of animals, where they learn, generate memory, alter behavior, and develop intelligence, which make them ready for future challenges. In nutshell, the complex interplay of signal transduction networks and machinery in plants leads them to sense, process, and integrate the signals they confront in their environment. Plants also develop behavioral changes accordingly or develop cognition and storage of processed information to adapt in rapidly changing or variable environment.

Identification of the role of a single or set of genes involved in signal transduction pathway has enabled researchers to understand and develop linear or complex signaling pathways, or maps in response to particular stimuli. However, because of the complete genome sequencing of many plant species including crop plants, a drift towards understanding the stress-signaling pathways involved in single or multiple stresses using high-throughput approaches has emerged. In the post-genomic era, the development of *-omic*-based approaches such as transcriptomic, proteomic, metabolomic, interactomic, and phenomic in several model organisms have laid the foundation of functional genomics. This area of plant science deals with the

understanding of large network of genes and proteins and integration of transcript data to proteins which then go to metabolite, and the complex and dynamic interaction develops a response or phenotype.

Elucidation of Abiotic stress signaling in Plants: *Functional Genomics Perspectives* comprises 30 chapters divided into two volumes (Volume I and II) in which some of the world's most well-known plant biologists have contributed in the field of stress signaling in plants with a special emphasis on functional genomics aspects. This book provides timely research in the field of stress-mediated signaling to develop a better and holistic understanding of stress perception and its transduction followed by the generation of response. In spite of the advent of different approaches to develop stress-tolerant crops towards multiple stress conditions in the field, the success in achieving this goal is still unsatisfactory. This is because stress tolerance is a very complex process involving plethora of components starting from stress sensing to generation of final adaptive response. As mentioned above, there are several factors, which act as nodes and hub in the signaling pathways, also serving as master-control switches in regulating a myriad of stress-signaling pathways by affecting diverse target genes or gene products to finally bring about a stress tolerance response. Therefore, in-depth understanding of these master-control switches and key components in signal transduction pathway will be highly beneficial for designing crop plants tolerant to multiple stresses in the field.

Towards achieving this goal, this book is divided into two volumes comprising five sections. Volume I consists of two sections with 14 chapters. The first section "Functional Genomics Approaches in Signal transduction" discusses three chapters on various approaches used to understand the signal transduction networks. These chapters will aware the readers on practical aspect of various "Omic"-based approaches such as transcriptomic, proteomic, phosphoproteomic, metabolomic, interactomic, and phenomic to understand the functions of genes and gene networks in signaling under stress.

The next section "Components of Signal Transduction" comprises 11 chapters discussing the different components of signal transduction pathways. The first three chapters focus on calcium signaling by describing the genes encoding for CAX (calcium-H^+-exchanger) involved in sequestration of calcium ions into vacuoles and maintenance of Ca^{2+} homeostasis. Chapters 5 and 6 discuss the role of Ca^{2+} signal decoding components like sensor and effector proteins. Here, CBLs, CIPKs, and CDPKs gene families have been extensively worked out in model plant Arabidopsis under abiotic stress condition and their role in other crop plant is being elucidated. Chapter 7 describes the role of ROS as redox signaling component in regulating multiple stress responses and in manipulation of ROS levels for imparting stress tolerance in crop plants. The role of MAP kinases as crucial signaling components in biotic as well as abiotic stresses has been discussed in Chapter 8. MAP kinases act as converging points for several signaling pathways, involving the phosphorylation-based relay of information to regulate a large number of targets such as transcription factors, other kinases, and cytoskeletal proteins in stress

signaling. The functional role of small and large G-protein acting as molecular switches to regulate both biotic and abiotic stresses has been discussed in Chapter 9. Chapter 10 deals with the molecular analysis of ABA receptor and ABA signaling in both biotic and abiotic stresses and genetic engineering of ABA receptor for developing stress-tolerant crop varieties. Auxin has been very well known as a plant growth regulator for several decades, and its emerging role in regulating stress signaling and responses is covered extensively in Chapter 11. SA (salicylic acid) is majorly involved in regulating biotic stress, but its role is also appreciated well in abiotic stresses as described in Chapter 12. In Chapter 13, the newly emerging role of methyl glyoxal (MG), which is a cytotoxin generated from both enzymatic and nonenzymatic pathways of metabolic reaction, has been discussed during several abiotic stresses. Chapter 14 discusses the role of immunophilins in diverse biological processes including development and stress management.

Volume II is divided into three sections encompassing 16 chapters. The first section of volume II emphasizes the gene expression regulation of stress signaling, with four chapters discussing the role of transcription factors (mediator complex in Chapter 1 and transcription factors of legumes in Chapter 2) and non-coding and small RNA (Chapters 3 and 4) in regulating abiotic stress responses.

Section two of volume II, comprises ten chapters, discusses the functional genomics aspect of heat/high temperature (Chapter 5), cold/freezing (Chapter 6), drought and dehydration (Chapter 7), flooding and submergence (Chapter 8), salinity (Chapter 9), UV-light (Chapter 10), heavy metal (Chapter 11), nitrogen (Chapter 12), and aging/senescence (Chapter 13) stress signaling responses. In this section, a detailed emphasis has been given in elaborating the respective stress-signaling pathway with a goal of potential candidate genes, which could be used for development of tolerant crop varieties by genetic manipulation and molecular breeding approaches. Moreover, cross talk or overlap in execution of several common signaling components open the scope for taming multiple stresses in future biotechnological intervention.

In the last section of volume II, Chapters 14–16 focus on the development of stress-tolerant crops and sustainable agriculture by utilizing the genes of signal transduction pathways. With the in-depth understanding of several signal transduction components and signaling pathways, the ultimate goal is to utilize the mechanistic knowledge and translate into useful tools to generate the crop varieties by either genetic manipulation of these signaling components or utilization of this knowledge for molecular marker-assisted breeding, ultimately augmenting stress tolerance in crop plants without compromising crop productivity.

Despite rigorous attempts, not every aspect of signaling pathways and components could be discussed here. Nevertheless, I strongly believe that two volumes covering signal transduction machinery and their components in stress condition, with a special emphasis to functional genomics, will be enormously useful to students, teachers, and research scientists.

I am indebted to all the contributors of this work, which could not be possibly compiled without their significant contributions. At last, I would like to express my sincere thanks to Dr. M. C. Tyagi and Dr. Amita Pandey for critical reading and help in copy-editing of this book. I also express my thanks to Ms. Manisha Sharma for designing the theme page.

New Delhi, India Girdhar K. Pandey, Ph.D.

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About the Editor

Girdhar K. Pandey, Ph.D.

Girdhar K. Pandey born in Almora, Uttrakhand, India. He received his B.Sc. (Hon.) in Biochemistry from Delhi University in 1992 and M.Sc. in Biotechnology in year 1994 from Banaras Hindu University (BHU). Subsequently, he joined Ph.D. in the School of Life Sciences, Jawaharlal Nehru University (JNU) and worked in the field of calcium signal transduction under abiotic stresses in plants. He was awarded the Ph.D. degree in year 1999 and then pursued postdoctoral career at Department of Plant and Microbial Biology, University of California Berkeley in year 2000. There, he extended his work in the field of calcium-mediated signaling in Arabidopsis by studying CBL-CIPKs, phosphatases, channels/transporters, and transcription factors involved in abiotic stresses. He has been working as Associate Professor in the Department of Plant Molecular Biology, Delhi University South Campus since October 2007.

Pandey's research interests involve detail mechanistic interplay of signal transduction networks in plant under mineral nutrient deficiency (mostly potassium, calcium, and nitrate) and abiotic stresses such as drought, salinity, and oxidative stresses induced by heavy metals. His laboratory is working on the coding and decoding of mineral nutrient deficiency and abiotic stress signals by studying several signaling

components such as phospholipases (PLA, PLC, and PLD), calcium sensors such as calcineurin B-like (CBL) and CBL-interacting protein kinases (CIPK), phosphatases (mainly PP2C and DSP), transcription factors (AP2-domain containing or ERF, WRKY), transporters and channels proteins (potassium and calcium channels/transporters), small GTPases, and Armadillo domain containing proteins in both Arabidopsis and rice. The long-term goal of his research group is to establish the mechanistic interplay and cross talk of mineral nutrient-deficient conditions and different abiotic stress signaling cascades in Arabidopsis and rice model system by using the advance tools of bioinformatics, genetics, cell biology, biochemistry, and physiology with greater emphasis on functional genomics approaches.

He has been awarded with Far Eastern Regional Research Organization (FERRO) fellowship to work at Beltsville Agricultural Research Center (BARC), United States Department of Agriculture, Beltsville, MD (1998). Later, he was awarded with Indian National Science Academy (INSA)-Deutsche Forschungsgemeinschaft (DFG) bilateral exchange visiting scientist fellowship in 2011. Also Department of Biotechnology (DBT), India, has awarded him with prestigious DBT-CREST Award (Cutting-edge Research Enhancement and Scientific Training) in 2011–2012. See Pandey's web page for further information about his lab and research work: https://sites.google.com/site/gkplab/home; http://www.dpmb.ac.in/index.php?page=girdhar-pandey.

Part I
Functional Genomics Approaches in Signal Transduction

Chapter 1
Towards Understanding Abiotic Stress Signaling in Plants: Convergence of Genomic, Transcriptomic, Proteomic, and Metabolomic Approaches

Praveen Soni, Kamlesh Kant Nutan, Neelam Soda, Ramsong C. Nongpiur, Suchismita Roy, Sneh L. Singla-Pareek, and Ashwani Pareek

Abstract All aspects of a plant's life—beginning with the seed germination and ending with the seed formation—are adversely affected by different abiotic stresses such as salinity, flood, drought, heat, cold, etc. Being sessile, plants have developed excellent mechanisms of stress perception and signal transduction. Multiple, complex, and dynamically intertwined interactions among nucleic acids, proteins, and metabolites determine the phenotype and final response of plants towards environmental stresses. In response to these stresses, a multitude of processes are activated which enable the plants to cope with these stresses up to a certain extent. These include alteration of expression of stress-responsive genes, production of stress proteins, alteration of ion transport, activation of various antioxidant systems, and compatible solute accumulation. Our knowledge of abiotic stress signaling has grown in leaps and bounds since the emergence and developments in the *omics* technologies. Genome-scale studies at transcript, protein, and metabolite levels provide information about dynamic changes taking place at these functional levels. For full understanding of signaling networks, it is essentially important to integrate all these aspects. This approach is of remarkable applicability when the aim is to understand how plants react to abiotic stresses. In order to understand molecular basis of stress tolerance along with signaling network under unfavorable environmental situations, recent progress on systematic use of *omics* technologies including genomics, transcriptomics, proteomics, and metabolomics has been summarized in this chapter.

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Furthermore, the integration of all these approaches, which provide systems biology method for understanding stress response in plants, is also discussed.

Keywords Abiotic stresses • Drought • Genomics • Metabolomics • Proteomics • Salinity • Transcriptomics

1.1 Introduction

Plants being sessile face various extreme environmental conditions throughout their life cycle and respond accordingly to maintain their vital metabolic homeostasis by regulating their gene activity. Thus, understanding the intricacies of these plant responses to environmental stress is the first step towards improving crop productivity under these unfavorable conditions. Further, it is also known that these abiotic stresses do not occur alone; rather their combinations contribute at unpredictable amounts to the overall stress perceived by the plant. The signaling pathways are very complex involving different molecules (Punjabi-Sabharwal et al. 2010). Consequently, it is reported that engineering genes related to protection and maintenance of cellular constituents can improve tolerance of plants towards stresses. The most important fact is to identify the candidate gene and characterize it in context of stress (Xianan and Baird 2003). In this regard, a host of genes have been identified and already reported to improve tolerance of plants towards stresses. Some of the genes are listed in Table 1.1. Similarly, analysis of transcriptomes can also pave way towards isolation of "candidate genes" which would be suitable for raising transgenic plants with improved tolerance. In recent past, several publications have reported that using the comparative transcriptomic approach between contrasting genotypes of rice (IR64 and Pokkali), several differentially regulated genes could be isolated, which then served as useful genes in functional genomic studies (Kumari et al. 2009; Karan et al. 2009; Mustafa et al. 2010; Kumar et al 2012; Soda et al. 2013). It is also true that the analysis of changes in proteome of crop plants in response to abiotic stresses can also be used as a starting point to fish out the genes, which may ultimately serve as the "candidate genes" (Ruan et al. 2011)

In this chapter, we have described the various "omics"-based approaches, which have been employed in recent years to understand the response of plants towards abiotic stresses. For brevity sake, we have restricted our discussion to the identification of signaling molecules, which have been studied by employing the particular "omics" approach. Technical details about the technique/approach have also been provided at appropriate places in the text.

Table 1.1 List of a few representative genes involved in plant abiotic stress responses

Name of the gene	Responsive to abiotic stress(es)	References
14.3.3 gene family	Salinity and drought	Chen et al. (2006)
Annexin	Salt	Lee et al. (2004)
ATAF	Drought, salinity, cold, and wounding	Christianson et al. (2010)
bZIP family	Drought, temperature, and salinity	Corrêa et al. (2008), Weltmeier et al. (2006), Nieva et al. (2005), Baena-González and Sheen (2008), Satoh et al. (2004), Alonso et al. (2009)
CBF/DREB families	Drought, cold, and salinity	Agarwal and Jha (2010), Trujillo et al. (2008)
Glycerol-3-phosphate acyltransferase gene	Cold	Yan et al. (2008)
H-ATPase	Cold	Hashimoto et al. (2009)
HSC 70	Cold	Folgado et al. (2013)
HVA1	Salinity and drought	Fu et al. (2007)
ICS	UV light	Catinot et al. (2008)
LOX	Drought and wounding	Yang et al. (2012), Andreou and Feussner (2009)
MAPK	Abiotic stresses	Pitzschke et al. (2009)
MEKK1 and ANP1	Oxidative and environmental stresses	Nakagami et al. (2006), Suarez-Rodriguez et al. (2007)
MPK3, MPK4, and MPK6	Abiotic stress and oxidative stress	Nakagami et al. (2006), Qiu et al. (2008)
MYB 4, 6, 7, and 44	Drought and salt	Yanhui et al. (2006)
NAC5	Cold, drought, and salt	Takasaki et al. (2010)
OsRMC	Salt	Guo and Song (2009)
SAMS	Salt	Pacheco et al. (2013)
SCF	Salt	Liu et al. (2013)
Vacuolar H^+-pyrophosphatase	Flooding	Komatsu et al. (2009b)
WRKY family	Salinity, temperature, drought, and oxidative stress	Qiu et al. (2009)

1.2 Genomic Approach

1.2.1 Advances in Plant Genomic Technologies

Functional genomic approaches with the help of high-throughput technology allow large-scale gene function analysis and interaction study of gene products at cellular and organism levels. The data collected from the completed sequencing genome projects provide valuable information about genes to be analyzed (Pérez-Clemente et al. 2013). The availability of this plant genome sequence information currently

facilitates studying the function of genes on a genome-wide level (Feuillet et al. 2010; Chain et al. 2009). However, for many plants, genomes have not been sequenced, or sequencing has not been completed. In such cases, the lack of information is compensated, in part, by the availability of huge collection of cDNA sequences and expressed sequence tags (ESTs) (Marques et al. 2009). In functional genomic projects, various tools like cDNA libraries, microarray, serial analysis of gene expression (SAGE), and ESTs are widely used to analyze global gene expression profiles in plants. Identification of gene function by analyzing mutants generated through chemical and physical mutagenesis has become feasible for large-scale analysis due to knowledge of different markers (Lukowitz et al. 2000). Characterization of mutant is the best way to know the function of a given gene. In this way, large collections of mutants and their characterization can complement large-scale expression studies.

Abiotic stress tolerance is quantitative and complex trait controlled by multiple interacting genes in plants (Punjabi-Sabharwal et al. 2010). Advancement in molecular biology techniques provides the function of genes present in plants. It also dissected out the abiotic stress responses governed by one gene or by multiple genes linked to the particular trait called as quantitative trait loci (QTL). QTL mapping advancements lead to the emergence of better breeding approaches such as breeding by design and marker-assisted selection (Peleman and van der Voort 2003). However, understanding the adaptive processes and complexity of stress signaling is inadequate due to lack of complete knowledge of genes involved in plant stress responses.

1.2.2 Gene Expression and Regulation in Response to Abiotic Stresses

In response to environmental stresses, expression of a number of genes involved in the stress defensive mechanism gets activated. Studies of stress-responsive networks have been revolutionized by the use of latest technologies such as microarray and next-generation sequencing (NGS). Basically, regulation of gene expression in plants can be noticed at transcriptional, posttranscriptional, and posttranslational steps. Various elements and factors are involved in the regulation at each step.

Three major elements are involved in transcriptional regulation: chromatin and its remodeling and modification; *cis*-regulatory elements present in promoters and other regulatory sequences, such as enhancers, present downstream and upstream of the coding region; and transcription factor (TF) which binds on *cis*-regulatory elements. Cifre et al. (2005) reported chromatin remodeling and modification involved in response to plant abiotic stresses. The sensitization of stress responsiveness is called priming. Priming, which is preexposure of stress before the actual stress (Conrath et al. 2006; Zimmerli et al. 2009), activated the defensive mechanism and increases the stress tolerance ability of the plant. It was shown that in case of WRKY transcription factors, priming was associated with chromatin modification of promoter (Jaskiewicz et al. 2011).

Transcription factor (TF) plays a very crucial role on gene expression which is initiated by binding of transcription initiation factor IID (TFIID) on the regulatory region of promoter which subsequently forms the transcription initiation complex along with other components. Formation of transcription initiation complex initiates the transcription process by recruiting the RNA polymerase II (Juven-Gershon et al. 2008). Apart from transcription factors (TFs) involved in the normal process of transcription, many stress-responsive TFs such as members of basic leucine zipper (bZIP), zinc finger families, MYB, and dehydration-responsive element-binding (DREB) or C-repeat binding factor (CBF) have been reported to be involved in the regulation of gene expression in plants under stress responses. Most of these transcription factors (TFs) bind to the *cis*-acting element present on the promoter of their targeted stress-inducible gene and regulate its expression under stress (Hu et al. 2006). Recently, the role of WRKY transcription factors in plant salinity and drought stress responses were reported in plants (Chen et al. 2012; Golldack et al. 2011). Overexpression of various members of WRKY TFs were leads to increase in abiotic stress tolerance by regulating stress-related genes in rice (Song et al. 2010; Wu et al. 2009).

Plant-specific NAC TFs are reported to be involved in abiotic stress response such as drought and salinity in plants (Nakashima et al. 2012; Yamaguchi-Shinozaki et al. 1992). It was reported that a rice OsNAC6 TF expression, which shows higher homology with *Arabidopsis* abiotic stress-responsive NAC TFs (ANAC019, ANAC055, and ANAC072), is induced by drought, salinity, ABA, and cold (Ooka et al. 2003).

Members of the 14.3.3 gene family G-box factor 14-3-3b protein (GF14b) and G-box factor 14-3-3c protein (GF14c), induced by abiotic stresses such as salinity, drought, and ABA, have been reported in rice (Chen et al. 2006). Members of this family of protein are also regulated by stress-responsive TFs (Chen et al. 2006). Pulla et al. (2009) reported that *S*-adenosyl-L-methionine synthetase (SAMS) gene of *Panax ginseng* (*PgSAM*) expressed under various abiotic stresses in *Panax ginseng* and might be providing protection against environmental stresses.

Some of the drought-responsive genes like RD20, RD22, RD29B, COR47, ERD14, VSP2, and RHL41 are also responsive to other abiotic stresses, which show the role of one gene in multiple stresses (Debnath et al. 2011). In *Arabidopsis*, 67 genes were identified to be responsive for multiple abiotic stresses (Swindell 2006). Similarly, transcriptome analysis in rice under salinity, drought, cold, and ABA stress showed the induction of 73 genes. Among 73 stress-responsive genes, 57, 62, 36, and 43 were induced by salinity, drought, cold, and ABA, respectively (Rabbani et al. 2003). It was also observed that out of 73 stress-inducible genes identified in rice, 51 are common between rice and *Arabidopsis*. However, some of the genes are specific to rice only, suggesting the different stress responsiveness between rice and *Arabidopsis* (Rabbani et al. 2003).

Gene expression regulation occurs at transcription level but also at posttranscriptional level which includes mRNA processing (capping, splicing, and polyadenylation), mRNA nucleocytoplasmic trafficking, mRNA turnover and stability, and mRNA translation (Floris et al. 2009).

Posttranslational regulation is the third level of regulation which includes phosphorylation, sumoylation, and ubiquitination of proteins. Under abiotic stresses, posttranslational regulation like phosphorylation and dephosphorylation plays an important role in signaling which activates the defense mechanism of plants. Under drought and osmotic stress, various signal transduction cascades formed by SNF1-related protein kinases (SnRKs) and mitogen-activated protein kinases (MAPKs) activated the phosphorylation of specific molecules (Zhu 2002). In *Arabidopsis*, ABA-dependent responses to water deficit, like stomata closure, are known to be regulated by SnRK2 proteins (Yoshida et al. 2006).

1.2.3 Transgenic Approach to Understand Gene Functions

Elucidating the mechanisms of stress tolerance by raising transgenic plant by manipulating stress-specific genes through genetic engineering is gaining popularity. Nowadays, success has been achieved in genetic improvement of plants for better abiotic stress tolerance by manipulating the gene-encoding enzymes involved in the regulatory pathways (Kumar et al. 2012).

Abscisic acid (ABA), which is also called as stress hormone, is involved in the regulation of various adaptive mechanisms in plants under different environmental stresses (Arbona and Gómez-Cadenas 2008). Therefore, with an aim to increase tolerance against abiotic stresses, many transgenic plants have been raised by manipulating key enzymes involved in the ABA biosynthetic pathway (Ji et al. 2011).

Compatible solutes and chaperoning protect the plant by various abiotic stresses by protecting biomolecules and membranes (Zhang et al. 2008). Transgenic plants overexpressing the genes involved in the biosynthesis of these solutes enhance the drought and osmotic stress ability of plants (Zhang et al. 2008). Transgenic plants overexpressing many genes encoding stress-related biomolecules such as proline (Hmida-Sayari et al. 2005), LEA (Rohila et al. 2002), chloroplast glycerol-3-phosphate acyltransferase (Sui et al. 2007), etc., have shown higher tolerance to abiotic stresses.

Reactive oxygen species (ROS) production is a common factor among most stresses (Hirayama and Shinozaki 2010). ROS performs dual roles. ROS is not only toxic to cells, but it also plays an important role as a signaling molecule. Scavenging the highly active ROS is a very important strategy for plant defense in which a series of interlinked enzymes are involved. Overexpression of the ROS-scavenging enzymes such as superoxide dismutase, glutathione reductase, glutathione peroxidase, and ascorbate peroxidase helps the plants in stress tolerance (Tang et al. 2006).

But the main hurdle in plant transgenic technology is the lack of effective single-copy gene transfer in plants. Secondly, a well-standardized tissue culture protocol is lacking for many plant species. Although plant transformation without tissue culture is known in *Arabidopsis*, it can be used in other crops. Therefore, effective transformation methods and easy tissue culture protocol need to be discovered.

1.3 Transcriptomic Approach

The transcriptome is the sum total of entire RNA molecules (mRNA, tRNA, rRNA, miRNA, long noncoding RNA, and only recently circular RNA) present within one or a population of cells at a particular time point. Thus, at different conditions or at different time points, the transcriptome is subject to variation. Transcriptomics, in short, can be defined as that field of functional genomics which concerns the study of transcriptomes. Transcriptomics, however, mainly focuses on gene expression, i.e., mRNA transcripts, as differential gene expression has been known to alter phenotypes of cells or a population of cells or entire organisms. Ultimately, a cell's transcriptome determines its phenotype in terms of development, differentiation, and ability to respond to environmental stimuli.

1.3.1 Understanding Abiotic Stress Tolerance in Plants Through Transcriptomics: The General Approach

As has been stated, differential gene expression determines phenotype. In fact, difference in expression of even a single gene can lead to highly altered phenotype. For example, *Arabidopsis* mutants, in which the CBF2/DREB2C gene was disrupted, displayed higher capacity to tolerate freezing, salinity, and dehydration stress (Novillo et al. 2004). One can only imagine that if differential expression of one gene can alter multiple phenotypes, then differential expression of a set of genes would definitely lead to a higher degree of phenotypic variations. Furthermore, it is known that the response of plants to external stimuli involves the following sequence of events:

1. *Perception:* usually involves membrane-localized sensor or receptor proteins.
2. *Signaling*: upon perception of external stimuli, downstream signaling proceeds either through protein–protein interactions or via the application of secondary messengers.
3. *Altered gene expression*: usually involves gene expression regulation through transcription factors.

Plants need to constantly alter their transcriptome to adjust to any abiotic stress, and this ability to adjust forms the basis of stress tolerance. These aspects were highly considered when researchers first started to envisage the basis of abiotic stress tolerance in plants. Transcriptomic studies were carried out on a large scale with the intent to decipher the signaling components of the abiotic stress response of plants. The overall aim was to decipher how a stressful environment affects gene expression in plants. The general experimental plan involved:

1. The comparison of transcriptomes of contrasting genotypes of a particular organism or phenotypically contrasting organisms in terms of abiotic stress tolerance for the identification of transcripts responsible for stress tolerance

2. Comparisons of transcriptomes of untreated and stressed samples of the same genotype to identify stress-responsive genes

1.3.2 Transcriptomics: Tools and Technologies and Their Contributions

Various approaches have been used for plant abiotic stress-related transcriptomic studies. A few of them, which have been highly useful towards the understanding of abiotic stress response in plants, are briefly described below.

1.3.2.1 Suppression Subtractive Hybridization

This is a PCR-based technique, which involves the amplification of cDNA obtained from control (driver) and experimental (tester) transcriptomes. This technique, as the name suggests, involves the hybridization of driver and tester molecules, which eventually leads either to their amplification or elimination from amplification based on the hybrids formed. Further, there is an equalization of amplification where low differentially expressed target molecules are amplified exponentially, whereas the high differentially expressed transcripts are subjected to a PCR-suppression effect and hence exhibit suppressed amplification. Thus, suppression subtractive hybridization (SSH) is a powerful tool to identify differentially expressed transcripts as even the low differences in transcript abundance between control and test samples can be detected. Various stress-responsive genes have been identified using SSH. Using SSH, a total of 1,058 genes were identified to be differentially expressed from eight stress cDNA libraries of *Arabidopsis*, and out of which 55 % of the stress-induced transcripts were rarely expressed in unstressed plants, and 17 % of them were completely absent in *Arabidopsis* EST databases present at the time (Mahalingam et al. 2003). Using SSH, Gulyani and Khurana (2011) obtained 1920 clones representing 208 contigs and 151 singletons, which were drought regulated in two contrasting cultivars of mulberry. The greatest advantage of this technique is that it does have a prerequisite such as whole genome sequence of the organism. SSH can be performed for any organism subjected to any condition even one which has never been tested before. SSH is only one of the techniques, which involve large-scale Sanger sequencing of ESTs, but the experiments are low throughput. Other techniques that use Sanger sequencing for identification of genes include the tag-based methods such as massively parallel signature sequencing (MPSS) (Brenner et al. 2000), SAGE (Velculescu et al. 1995; Harbers and Carninci 2005), and cap analysis of gene expression (CAGE) (Kodzius et al. 2006; Shiraki et al. 2003). These tag-based methods are high throughput and have also contributed significantly to the understanding of the transcriptomic changes that occur in plants in response to stress.

1.3.2.2 Microarray

Microarray is probably the transcriptomic method, which has contributed the most towards understanding abiotic stress tolerance in plants. It is based on the hybridization of a nucleic acid sample (target) to an enormous set of oligonucleotide or, occasionally, full-length cDNA probes which are attached to a solid matrix surface. For gene expression profiling cDNA obtained from mRNA is used as the target sample. In microarray oligonucleotides, cDNA sequences, ESTs, or even genomic DNA segments are arrayed on a glass slide to a density of about 1,000 cm^{-2}, which are then hybridized with differently fluorescent-labeled cDNA obtained from control and test mRNA samples, respectively. The difference in the fluorescence intensity of the two fluors provides the parameter for measuring difference in gene expression between control and test samples. Differences in gene expression are represented usually as fold change between control and test samples or also by using a statistical method like ANOVA where the null hypothesis is kept that a gene is equally expressed in both control and test. Microarray technology was first demonstrated in 1995 where ESTs were used to analyze the differential expression of 48 *Arabidopsis* genes in roots and shoots (Schena et al. 1995). Since then the technology has grown in leaps and bounds to the scale of whole genome-scale transcriptome profiling. Stress-responsive genes from many species such as *Arabidopsis*, rice, maize, etc., have been identified using microarrays (Kreps et al. 2002; Seki et al. 2001; Rabbani et al. 2003; Kawaura et al. 2008). In fact, if looked at it from one perspective, microarray provides the platform to understand plants' abiotic stress response from a whole genome point of view. It provided the necessary knowledge to understand the various stress signaling pathways and how different stresses are connected with each other in terms of the response that they evoke.

Microarray has a lot of advantages over other transcriptomic techniques such as EST sequencing or SSH. Microarray allows for studies to be made on the whole genome level. It is a rapid, easy to use technique, which can be easily replicated. Initially, microarray technology had a lot of drawbacks with background noise especially with lowly expressed differentially regulated genes, but a lot of progress has been made on this front, and nowadays the data obtained is much more accurate and reliable. It must be said that microarrays, like all transcriptomic methods, form only the initial high-throughput screening of differential gene expression. The data obtained has to be verified through other, more accurate, methods such as qRT-PCR or Northern blots. Nevertheless, microarray does provide a researcher with a good starting point for his/her experiments. Despite the endless possibilities, microarray technology does have its drawbacks. First and foremost, oligoarrays of an organism's genomic sequence is a prerequisite for oligonucleotide probe design. On the other hand, cDNA arrays do not require genomic sequence to be known, but the cDNA probes do not represent the entire repertoire of genes present in the organism, and hence the data obtained can be incomplete. cDNA arrays are cheap, but they are inaccurate and cannot measure individual samples. Oligoarrays, in contrast, are much more accurate than cDNA arrays, but they are expensive and limited only to certain model species.

1.3.2.3 RNA Sequencing

In the last two decades, there has been tremendous progress in the field of nucleic acid sequencing. Whole genomes of many organisms have been sequenced, which provide a great deal of information towards understanding the abiotic stress response of plants at the molecular level. Apart from genome sequencing, RNA sequencing has also made a huge impact towards understanding transcriptomic networks in general. Already used for a wide taxonomic range from yeast to *Arabidopsis* to humans, this technology employs the sequencing or cDNA generated from the total RNA population of test samples. Perhaps the biggest advantage of RNA sequencing is that an organism's genome sequence or even an EST database is not a prerequisite for the data obtained to be interpreted and analyzed. Furthermore, data acquired from large-scale Sanger sequencing of ESTs are biased against low-abundance transcripts, time-consuming, and expensive (Filichkin et al. 2010). Moreover, de novo sequencing has enabled whole genome level transcriptomic studies to be carried out even on non-model species. Examples of the use of RNA (de novo) sequencing on non-model species include olive (Alagna et al. 2009), chickpea (Garg and Jain 2011), barley (Thiel et al. 2012), and garlic (Sun et al. 2012) besides many others. Although few studies connected with plant abiotic stress have been carried out using RNA sequencing, the potential of this technology in this regard is almost limitless. Through RNA sequencing, one can not only identify stress-responsive mRNA transcripts but also identify siRNAs, miRNAs, as well as long noncoding RNAs, all of which have been shown to regulate the stress response in the model plant *Arabidopsis* (Sunkar et al. 2007; Amor et al. 2009).

1.4 Proteomic Approach

1.4.1 The Rapid Rise of Proteomics in the Post-Genomic Era

Proteomics is the next step in the study of biological systems. It is more complicated than genomics because an organism's genome is comparably constant, whereas the proteome differs in both spatial and temporal manners. A certain set of genes are expressed in different cell types, which in turn lead to cell differentiation and myriad of cellular responses under different conditions. A decade before to specify cellular responses, transcriptome analysis was used to be a preferred technique, but cell transcriptome does not correlate with its protein content (Rogers et al. 2008). It is now known that the complete set of mRNA is not always translated into protein. Marc Wilkins and his colleagues in the early 1990s coined the word proteomics mirroring the word genomics. Proteomics is complimentary to genomics as it describes the whole compliment of a protein component encoded by its genome. It is the study of multiprotein system focusing on their interplay with multiple, distinct proteins in their roles as part of larger system or network.