Chapter 2
Molecular Approaches in Deciphering Abiotic Stress Signaling Mechanisms in Plants

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Abstract There has been considerable interest in the area of abiotic stress research, especially in the direction of producing improved crop varieties that can encounter adversities of abiotic stresses such as heat, cold, drought, osmotic, and salinity effectively. These stresses can act alone or in combination cause greater damage to plants. Thus, in order to combat and survive in these extreme environmental conditions, plants have evolved tolerance mechanisms. These mechanisms include interconnected networks of signaling cascade, which involves role of a large number of genes and their products in tolerance mechanism. The clear-cut understanding of these stress tolerance/resistance mechanisms is critical in order to improve the performance of the plant. Deciphering such complex signaling cascades using traditional genomic approach has been difficult, and therefore, high-throughput functional genomics approaches need to be employed using tools like expression profiling, transcriptomics, proteomics, and metabolomics during tolerance response.

Keywords Abiotic stress • Legume • Transcription factor • WRKY family

2.1 Introduction

Dynamic climatic conditions altering the environment play a crucial role in plant performance. These altered environmental conditions act as an environmental stress to plants. Effect of abiotic stresses (temperature, drought, salt, etc.) on plants is drastic, both for vegetative and reproductive potential. These stress conditions lead to change in the resource availability of plants, which are of utmost importance for their optimum performance. One way to mitigate these conditions is environment-induced shift in the phenotype of the plants. The environmental cues that lead to change phenotype of plants are genetically controlled in a hierarchical fashion. A clear-cut understanding of the stress tolerance/resistance mechanisms is critical

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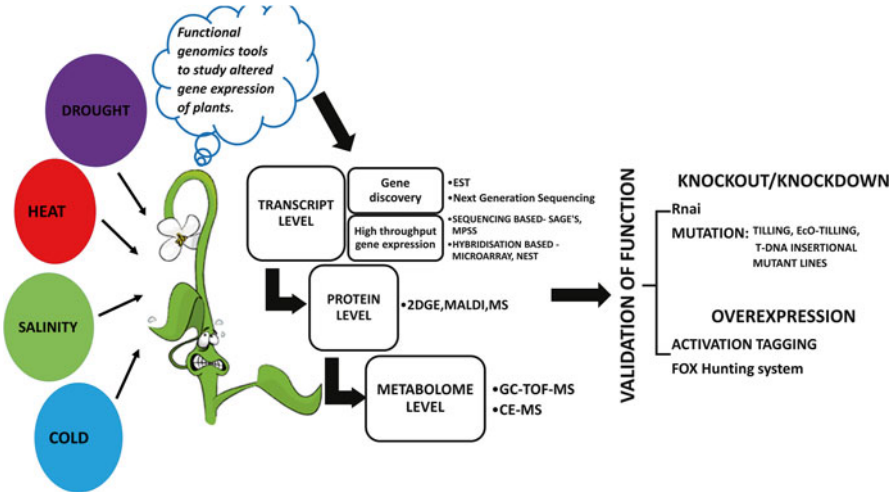

Fig. 2.1 Functional genomics strategies to study altered gene expression of plants under abiotic stress at different molecular levels. At transcript level—*EST* expressed sequence tag, *NGS* next-generation sequencing, *SAGE* serial analysis of gene expression, *MPSS* massively parallel signature sequencing, *microarray*, *NEST* nuclear expressed sequence tag. At protein level—*2DGE* two-dimensional gel electrophoresis, *MALDI* matrix-assisted laser desorption–ionization, *MS* mass spectrometry. At metabolome level—*GC-TOF MS* gas chromatography–time-of-flight mass spectrometry, *CE–MS* capillary electrophoresis–mass spectrometry. Genes identified through the above tools are further validated for their function through knockout/knockdown analysis (*RNAi* RNA interference; mutations—*TILLING* targeting induced local lesions in genomes; T-DNA insertional lines) and overexpression analysis (activation-tagging and FOX hunting system (full-length cDNA Over-eXpressing gene hunting system))

in order to improve plant performance. Thus, a holistic approach through ecological, molecular, and physiological perspectives to understand the behavior of plants is of great importance.

In the twentieth century, an integrated analysis of stress responses on whole plant using functional genomics approaches is being popularized over traditional approaches in understanding complicated plant behavior under stress conditions. The core behind the analyses of functional genomics is how the expression of genome is altered under the unpredictable environmental extremes, using multiple high-throughput screening of stress-treated seedlings at the genome level (next-generation sequencing), at transcriptome level (expressed sequence tag (EST), serial analysis of gene expression (SAGE), and microarray analysis), and at protein level (protein localization and protein–protein interaction). The functional genomics approach brings together physiological and phenotypic characteristics of plants by characterization of the diversity in gene functions under stressed conditions, through random/targeted mutagenesis, complementation analysis, and promoter-trapping strategies (reviewed by Sreenivasulu et al. 2007) (Fig. 2.1).

Although functional genomics approaches provide a gateway to extensive molecular analysis of genes and proteins involved in stress signaling, one of the

major challenges faced by functional genomics biologists is to search the genes, which provide tolerance to plant without compromising with its growth and yield. In this chapter, we are trying to overview the abiotic stress signaling cascades for different stresses with functional genomics perspective.

2.2 Signaling Cascades in Abiotic Stress and Their Effect on Plants

2.2.1 Drought Stress

Drought stress involves multiple cellular and molecular pathways, which lead to change in many biochemical and physiological processes, which adversely affect plant growth, development, and ultimately productivity. Biosynthesis of phytohormone, abscisic acid (ABA), is the most pronounced and primary event, which occurs during drought stress. ABA plays a central role in drought stress by closure of stomata, which in turn reduces water loss and also induces stress-related gene expression (Kim et al. 2010; Pospíšilová 2003).

2.2.1.1 Effect on Plants

During drought stress, the normal bilayer structure of the membrane disrupts and becomes exceptionally porous. In water-deficit condition, due to disruption of membrane, attached proteins/enzymes may displace and lose their activity. Disruption of cellular compartmentalization and high concentration of cellular electrolytes also cause disruption of cellular metabolism. In addition to all this damage, enzymes located in cytosol may also exhibit reduced activity or can even undergo complete denaturation due to dehydration.

2.2.1.2 Signaling Cascade During Drought Stress

In *Arabidopsis*, upon exposure to drought stress, plasma membrane-located His kinase ATHK1 acts as a drought sensor (Wohlbach et al. 2008). GTG1 and GTG2 (G protein-coupled receptor [GPCR]-type G protein) are also hypothesized as ABA receptors (Pandey et al. 2009). ATHK1 senses modification in water status and upregulates transcription of ABA synthesis genes, resulting in an increase in intracellular ABA levels. Precursor of ABA is xanthoxin, which is derived from a direct cleavage of C_{40} carotenoids. This reaction seems to be a key step in ABA biosynthesis in plants. The enzyme, which cleaves C_{40} carotenoids, is 9-*cis*-epoxycarotenoid dioxygenase (NCED), which was originally identified from maize (Schwartz et al. 1997). NCED family has five members in *Arabidopsis*, and among all, AtNCED3 plays a crucial role in drought stress-inducible ABA biosynthesis. Drought stress

induces expression of the gene encoding NCED (Iuchi et al. 2000; Xiong and Zhu 2003). An ATHK1-responsive ABA-independent pathway of stress-responsive gene expression also exists (Wohlbach et al. 2008). Increase in ABA level is perceived by soluble PYR/PYL/RCAR (pyrabactin resistance1/PYR1-like/regulatory component of ABA receptor) receptors, members of START superfamily of ligand-binding proteins (Ma et al. 2009; Park et al. 2009). After binding to its receptor, ABA-receptor complex then binds to type 2C protein phosphatase (PP2C) (Brandt et al. 2012; Fujii et al. 2009; Ma et al. 2009; Nishimura et al. 2009, 2010; Park et al. 2009; Santiago et al. 2009). PP2C is the inhibitor of Snf1-related protein kinase1. However, due to binding of PP2C with ABA-receptor complex, Snf1-related protein kinase1 now gets released from PP2C inhibition (Ma et al. 2009; Melcher et al. 2009). ABI1 and PP2CA are authentic SnRK1 phosphatases, which control SnRK1 activity and signaling during stress (Rodrigues et al. 2013). This activated Snf1-related protein kinase1 now phosphorylates downstream components like ion channels and transcription factors such as basic leucine zipper (bZIP), referred to as ABRE-binding proteins (AREB) or ABRE-binding factors (ABFs) that further regulate expression of concerned genes (Brandt et al. 2012; Fujita et al. 2013; Lopez-Molina et al. 2001). SnRK2 protein kinase (OST1/SRK2E) is activated by ABA and controls stomatal closure (Yoshida et al. 2002; Mustilli et al. 2002). SnRK2s are activated by drought, salinity, and ABA (Yoshida et al. 2002). The PYR/PYL/RCAR–PP2C–SnRK2 signaling module is conserved across land plants, and its key elements mediate ABA responses within the nonvascular plant *Physcomitrella patens* (Chater et al. 2011; Komatsu et al. 2009; Tougane et al. 2010).

Regulation of gene expression in drought stress is broadly classified into two categories, namely, ABA-independent and ABA-dependent regulatory systems (Yamaguchi-Shinozaki and Shinozaki 2005). Genes are dissected at transcriptional level for better understanding of the cascade; *cis*- and *trans*-acting factors have been analyzed critically. The promoter of drought-inducible genes contains two major *cis*-acting elements, ABRE (ABA-responsive element) and DRE (dehydration-responsive element)/CRT (C-repeat), both of which are involved in stress-inducible gene expression (Yamaguchi-Shinozaki and Shinozaki 1994, 2005). ABRE and DRE/CRT are *cis*-acting components that operate in ABA-dependent and ABA-independent gene expression, respectively, in response to abiotic stresses (Shinozaki and Yamaguchi-Shinozaki 2007). ABRE is the major *cis*-acting element involved in ABA-dependent pathways; other *cis* elements beside ABRE are MYC-like basic helix–loop–helix (bHLH) and MYB. MYC and MYB recognition sites are present in *RD22* promoter region and involved in drought- and ABA-induced gene expression of *RD22*. Transgenic plants that overproduce AtMYC2 and AtMYB2 have higher sensitivity towards ABA, thereby showing its role in drought stress (Abe et al. 2003). In ABA-independent gene expression, dehydration-responsive component (DRE) acts as a very important *cis*-acting component. The *RD29* gene induction with and without ABA suggests its involvement in both ABA-dependent and ABA-independent pathways. *RD29* promoter has 9-bp conserved sequence, TACCGACAT, dehydration-responsive element (DRE), and is responsible for regulating *RD29A* induction in the ABA-independent response to dehydration and cold (Yamaguchi-Shinozaki and Shinozaki 1994).

2.2.2 *Temperature Stress*

Temperature acts as a key physical parameter affecting growth and development of plants. Any fluctuation in the optimal level of temperature regime results in altered growth and productivity of plants. Mitigation of these changes in temperature extremes is brought through the evolution of complex mechanisms, which are illustrated by transcriptional changes observed in seedlings, leaves, roots, and pollen (reviewed by Zinn et al. 2010). Temperature stress results in multiple alterations of cellular mechanisms (Wang et al. 2003), and effectiveness of the stress relies upon multiple factors together with intensity, period, and rate of natural action (Wahid et al. 2007; Thakur et al. 2010). Broadly, temperature stress is classified as high-temperature (heat) and low-temperature (cold) stresses.

2.2.2.1 High-Temperature (Heat) Stress

Plants respond differently to varying temperature; regimes above an optimal level of induction result in development of heat stress on plants. Heat stress has been primarily divided into two major categories—the first one involves subjecting a plant growing in its natural temperature (~21 °C for *Arabidopsis thaliana*) to an abrupt rise in temperature (~42 to 45 °C for *Arabidopsis thaliana*) for a period of 0.5–1 h (Larkindale and Vierling 2008; Suzuki et al. 2008). The second type of heat stress is *priming*, during which plants are subjected to moderate heat stress (36–38 °C), for a few hours, and then again subjected to an abrupt episode of severe heat stress (46 °C) (Larkindale and Vierling 2008; Qin et al. 2008).

Effect on Plants

High temperature has an overall stressful effect on plant growth and development (Lobell et al. 2011); however, reproductive tissues are highly sensitive to heat waves. Slight increase in the optimal temperature regime bears drastic effects upon flowering time and total yield of plant (Mittler and Blumwald 2010; Zinn et al. 2010). At subcellular level, heat stress has been reported in fluidizing the membrane (by hampering their lipid content), instability of proteins and cytoskeletal structures, and efficiency of enzymes, thereby causing an overall metabolic imbalance within the cell (McClung and Davis 2010; Ruelland and Zachowski 2010; Suzuki et al. 2012).

Signaling Cascade During Heat Stress

Surviving such drastic effects of heat stress is achieved through initialization of a complex signaling cascade after sensing heat stress. Sensing occurs at plasma membrane of *Physcomitrella patens* (Saidi et al. 2009; Mittler et al. 2012). The signaling

cascade, thereafter, results in opening of Ca^{2+} channel, which leads to rise in calcium concentrations within the cell. The increased Ca^{2+} influx thereafter can regulate many signaling pathways in plants (Zhang et al. 2009; Liu et al. 2008). In *Arabidopsis*, this increased calcium concentration could activate AtCaM3 (calmodulin) which may further downstream activate different transcription factors such as WRKY (Li et al. 2010) and HSF (heat-shock transcription factor) directly/indirectly (LIU et al. 2011). The indirect activation of HSF is a consequence of cascading activation of CBK (Ca^{2+}/calmodulin-binding protein kinase), which phosphorylates HSFA1a rendering it active (Liu et al. 2008). The activated HSFs further trigger increased expression of several stress-related transcripts, such as HSP (heat-shock proteins), of which few act as chaperons to stabilize protein (Wang et al. 2004), reviewed by Zinn et al. (2010). The advent of heat stress also has a potent role in increasing plasma membrane fluidity, which might further lead to the activation of phospholipase D (PLD) and phosphatidylinositol 4-phosphate 5-kinase (PIPK) and accumulation of PIP2 (phosphatidylinositol 4,5-bisphosphate) and IP_3 (inositol 1,4,5-trisphosphate)—a key member of lipid signaling (Mishkind et al. 2009). Increased lipid concentration within cell results in opening of channels and thereby influx of a calcium ion within cell, which eventually results in a heat-responsive signaling across cell. Although much have been discovered in downstream high-temperature signaling components, evidences for temperature-induced plasma membrane channel modulation and lipid signaling activation still remain unanswered in plants (Mittler et al. 2012).

2.2.2.2 Low-Temperature (Cold) Stress

Low-temperature stress is one of the most common stresses faced by plants growing in temperate regions of the world. Low-temperature stress has been broadly classified as chilling (0–15 °C) and freezing (<0 °C).

Effect on Plants

Of late, effects of both chilling and freezing stresses have been reported to be deleterious to overall plant growth and development. Symptoms shown in vegetative plant parts in response to chilling treatment include wilting, chlorosis, and reduced leaf expansion eventually leading to necrosis. Not only vegetative but reproductive potential of plants has also been reported to be severely affected leading to sterility in flowers in response to chilling (Wen et al. 2002). Freezing stress on the other hand induces membrane damage due to dehydration, thereby resulting in expansion-induced cell lyses and fracture lesions (Steponkus et al. 1993; Uemura and Steponkus 1997). Other major effects associated with cold stress include ice crystal formation in the apoplastic region of cell, which on further enlargement due to movement of cytosolic content acts as a mechanical strain on plasma membrane and cell wall leading to cell rupturing (McKersie and Bowley 1997).

Signaling Cascade During Cold Stress

The low-temperature signaling across plant cell is a complex phenomenon involving a myriad of transcriptional regulators, thereby affecting the downstream stress-induced gene expression. One of the critical components of this signaling includes a transcriptional activator family CBF (C-repeat-binding factors), comprising of CBF1–4 members, of which CBF1 and CBF3 are reported to be low-temperature-responsive factors (Novillo et al. 2004; Mahajan and Tuteja 2005). In response to cold, unknown sensors on the cell surface get activated, which further downstream activate a transcription factor ICE1 (inducer of cold expression). ICE1 belongs to a family of transcription factors ICE, which are constitutively expressed in all plant tissues, whose overexpression results in increased freezing tolerance in plants. The activation of ICE1 by phosphorylation of its MYC bHLH domain (Chinnusamy et al. 2003) results in binding of ICE1 to MYC recognition sequences present on the CBF3 promoter, thereby activating CBF3 transcription downstream (Chinnusamy et al. 2003). The nascent transcript of CBF3 thus formed binds to promoter region (bearing C-repeat conserved sequences CCGAC (CRT/DRE element)) of COR (cold-regulated) genes, thereby activating target gene and downstream components leading to induction of a low-temperature acclimations in *Arabidopsis*. The complexity of cold acclimatization is further augmented by upstream negative regulation of CBF through HOS1 (high expression of osmotically responsive gene) and a feedback inhibition through CBFs in CBF-dependent signaling cascade. The feedback inhibition of CBFs through knockout analysis of CBFs 1, 2, and 3 have shown a negative regulation of CBF1 and CBF3 by CBF2 and vice versa (Novillo et al. 2004).

2.2.2.3 Salinity Stress

Maintenance of an optimal saline environment within plant cell is an important aspect of growth and development of plant. Fluctuations in extracellular saline conditions result in hyper ionic and hyperosmotic conditions within cells, thereby altering the homeostasis of the cell. The major ions responsible for such fluctuations include Na^+, K^+, Mg^{2+}, and Cl^-, while maintenance of equilibrium during this ionic imbalance is maintained by Na^+, K^+, H^+, and Ca^{2+} signaling across cells (Mahajan and Tuteja 2005).

Effect on Plants

Increased Na^+ concentration within plant cell bears a toxic and inhibitory effect upon cell metabolism and functioning of several enzymes (Niu et al. 1995). The osmotic imbalance caused due to this increased Na^+ concentration also results in membrane disorganization and inhibition of cell division/expansion and growth. Overall, these imbalances result in the reduction of photosynthetic apparatus of plants with an increased formation of reactive oxygen species (ROS) (Mahajan and Tuteja 2005).

Signaling Cascade During Salinity Stress

In order to cope with the increased salt concentration, plants activate their SOS (salt overly sensitive) signaling pathway. The activation of the pathway is preceded by perceiving the altered ionic concentrations by unknown sensors present on the cell, which further downstream result in an increase in cytosolic Ca^{2+} influx (Knight et al. 1997) that acts as an elicitor for activation of SOS3—a myristoylated calcium-binding protein (Ishitani et al. 2000; Liu and Zhu 1998). The activated SOS3 further interacts with an active serine/threonine protein kinase SOS2 (Halfter et al. 2000; Liu et al. 2000) and regulates the expression of SOS1, a salt-tolerant effector gene encoding Na^+/H^+ antiporter (present on plasma membrane) (Halfter et al. 2000), which thereby maintains the sodium ion homeostasis within cell. Although, SOS signaling is an important aspect of maintaining the ionic balance within the cell, its negative regulation is also reported by AtHKT1, inhibiting the activity of SOS2–SOS3 complex (Rus et al. 2001; Uozumi et al. 2000). In addition to SOS1-mediated maintenance of osmotic balance within cell, SOS2-mediated activation of Na^+/H^+ exchange for vacuolar sequestration of increased Na^+ ions (Qiu et al. 2002) also results in maintaining the cellular homeostasis. Acclimation to salt stress at physiological level is achieved by accumulation of compatible solutes within cells, thereby preventing water loss and maintenance of turgor within cell (Bressan et al. 1998; Ford 1984). Accumulation of these osmolytes (sugars—fructose and sucrose, sugar alcohols, trehalose, fructans, glycine betaine, proline, and ectoin) does not configure any change on the metabolic functioning of the cell and helps in overall maintenance of osmotic balance within it (Delauney and Verma 1993; Louis and Galinski 1997; McCue and Hanson 1990).

2.2.2.4 ROS Stress

In order to cope with the stressful conditions, plants have evolved various regulatory pathways. Generation of ROS is one of them. When plants are subjected under environmental stress, several distinct types of ROS are generated within various intracellular compartments, either they can lead an oxidative damage or they might act as a signal.

Plants require light and oxygen for their growth and development. Under unfavorable conditions, there is an increase in the number of oxidizing compound compared with antioxidizing molecules (Shapiguzov et al. 2012). ROS are the reactive forms of molecular oxygen, which include hydroxyl radical (HO^-), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) (Shapiguzov et al. 2012). ROS are accumulated into cells through various mechanisms like electron transport in the chloroplast and mitochondria or with the enzymatic mechanisms like cell wall peroxidase and NADPH oxidase (in plants known as respiratory burst oxidase homologues (RBOHs)) in extracellular space and peroxisome (Wrzaczek et al. 2013). Major contributors for ROS are the mitochondria in mammals, while in plants largest ROS producers are chloroplast and peroxisomes (Foyer et al. 2003;

Rhoads et al. 2006). In plants peroxisomes contribute 50 % of the total extracellular ROS production, and the rest part is contributed by RBOHs and from intracellular sources (O'Brien et al. 2012). In *Arabidopsis* a network of at least 152 genes are involved in managing the ROS level (Mittler et al. 2004). Silencing and knockdown studies have shown that peroxidase 33 (PRX33) and peroxidase 34 (PRX34) are the major contributors for ROS production in *Arabidopsis* (Bindschedler et al. 2006) and RBOHs constitute a multigene family comprised of ten genes (AtRBOHA–AtRBOHJ) (Baxter et al. 2013). During signaling mechanism of ROS, except H_2O_2, other forms of ROS are involved in localized signaling rather than the long distance (Jammes et al. 2009). H_2O_2 operates as a long-distance messenger due to its stability and ability to cross the membranes either through aquaporins or via diffusion mechanism (Bienert et al. 2007). Plant RBOHs are involved in different signaling pathways including root hair growth, stomatal closure, pollen stigma interaction, and plant defense and acclimation to different abiotic stresses (Torres and Dangl 2005; Jammes et al. 2009; Monshausen et al. 2007; Miller et al. 2009; McInnis et al. 2006; Suzuki et al. 2011).

Early signaling events during stress conditions involved increased flux of Ca^{2+} into the cytosol, protein phosphorylation, and activation of mitogen-activated protein kinases (MAPKs) (Benschop et al. 2007). Membrane permeable nature of H_2O_2 makes a key signaling molecule during different stress responses (Sagi et al. 2004). It is found that H_2O_2 accumulation in extracellular space causes initiation and self-propagation of rapid cell-to-cell signal in a RBOHD-dependent manner (Mittler et al. 2011). During signaling mechanism of ROS, it is found that any mechanical stimulus of plant tissue initiates an increase in the cytosolic Ca^{2+} from extracellular space; this increased Ca^{2+} then leads to ROS production through RBOHC-dependent manner, which at last regulates the root hair development (Monshausen et al. 2007; Takeda et al. 2008). Although ROS has been involved in various developmental processes, the specificity of signaling is not clear (Møller and Sweetlove 2010). There are various possibilities regarding the mode of action of ROS like the following: ROS may function as a general signal to activate cellular signaling network of cell to convey their specificity, and a good example of this signaling network is the activation of different MAPK cascades following ROS accumulation (Jammes et al. 2009; Teige et al. 2004), or it may contain any decoded messages, which have a specific pattern during its signaling mechanism (Mittler et al. 2011). The other possibility could be that each cellular compartment or individual cell has the specific ROS receptor, which conveys the message of specific ROS to other networks like Ca^{2+} or protein phosphorylation (Mittler et al. 2011).

Recently, a lot of information is accumulating about ROS signaling, but still there exist large gaps in ROS-mediated signaling mechanism such as whether ROS induce new signaling systems as second messengers or they are only involved in the regulation of already known signaling pathways. There is again no clear answer about the primary sensor of ROS and the primary gene response during ROS signaling. ROS sensors may be either transcription factors or protein kinases, which show activity change in response to various ROS (Fig. 2.2).

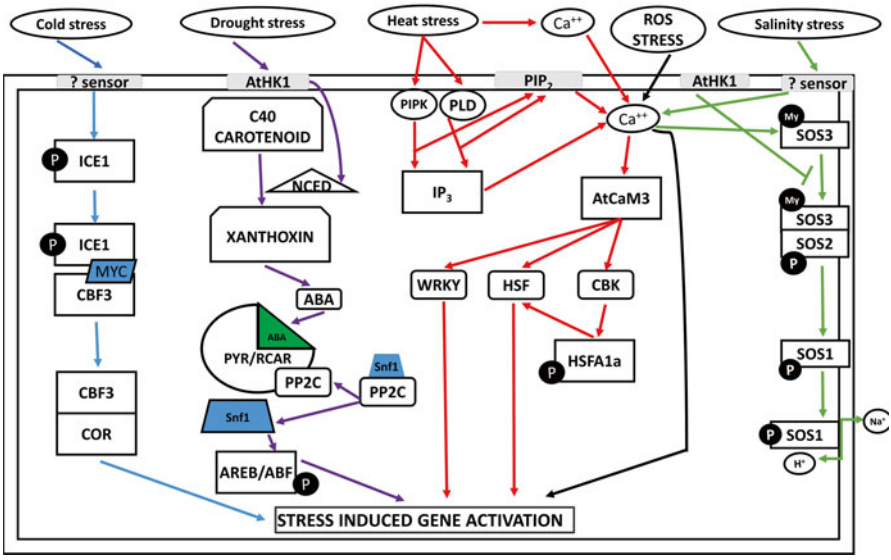

Fig. 2.2 Schematic illustration for abiotic stress signaling in plants. Advent of cold stress is perceived by an unknown sensor at plasma membrane, which further activates a signaling cascade downstream (*blue solid lines*). The activated unknown sensor phosphorylates ICE1 (inducer of cold expression) rendering it activated. This activated ICE1 binds to MYC domain of CBF3 (C-repeat-binding factor) promoter, thereby activating CBF3 transcription. CBF3 thus formed binds to conserved sequence in promoter region of COR (cold regulated) eventually activating target genes. Drought stress signaling (*purple solid lines*) is perceived through a plasma membrane-localized sensor ATHK1, which regulates expression of NCED (9-*cis*-epoxycarotenoid dioxygenase), a key enzyme in ABA (abscisic acid) biosynthetic pathway, regulating C40 carotenoid conversion to xanthoxin. ABA thus formed due to this regulation binds to its receptor PYR/PYL/RCAR (pyrabactin resistance1/PYR1-like/regulatory component of ABA receptor). This complex then binds to PP2C (type 2C protein phosphatase) thereby releasing and activating Snf1-related protein kinase1 (as PP2C acts as an inhibitor of Snf1). The activated Snf1 further activates ABRE-binding factors downstream, which eventually regulate expression of drought stress-inducible target genes in an ABA-dependent/ABA-independent manner. Heat stress signaling (*red solid lines*) is activated through modulation in cellular plasma membrane. This modulation acts as a signal for activating PIPK (phosphatidylinositol 4-phosphate 5-kinase) and PLD (phospholipase D). Both the molecules result in accumulation of second messenger IP_3 (inositol 1,4,5-trisphosphate) and PIP_2 (phosphatidylinositol 4,5-bisphosphate). This increased IP_3 concentration within cell results in an increase in cytosolic Ca^{2+} ion, thereby activating a heat-responsive signaling across cell. Heat stress also results in activation of Ca^{2+} ion channel thereby resulting in an increase in Ca^{2+} ion concentration within cell. The increased ion concentration further activates AtCaM3 (calmodulin) downstream, which in turn triggers activation of transcription factors such as WRKY and HSF (heat-shock protein). HSF is activated in response to heat stress both directly and indirectly, via CBK (Ca^{2+}/calmodulin-binding protein kinase), which phosphorylates Hsfa1, eventually cascading activation of HSF. The activated HSF and WRKY transcription factors finally help in activation of drought stress-induced genes. During salt stress signaling (*green solid lines*), an unknown salt sensor at plasma membrane leads to increase in cytosolic calcium level, which in turn binds to myristoylated SOS3 (salt overly sensitive) protein. This membrane-bound protein further binds to phosphorylated SOS2. This complex formation is however inhibited by AtHK1. The SOS3–SOS2 complex acts as a regulator of SOS1—a salt-tolerant effector gene encoding Na^+/H^+ antiporter, positioned at plasma membrane. The regulation of SOS1 through activation of SOS pathway results in maintaining cellular homeostasis. On the advent of abiotic stress, ROS (reactive oxygen species) accumulation and signaling (*black solid lines*) activates, both in cytosol and in apoplastic region. This increased ROS accumulation leads to an increased calcium ion concentration within the cell, which directly/indirectly helps in the activation of target genes responsive to abiotic stress

2.3 Functional Genomics of Abiotic Stress Signaling

Genetic variations lead to functional variation by changing the structure or function of the proteins. Functional genomics helps to explain functional differences in appearance or performance of altered gene. It enables to explore gene and protein functions and interactions on a global scale. The approach utilizes large-scale assays to study various genes or proteins in metabolic pathway under different environmental conditions. DNA's function is thus discovered by combining information from genes, transcripts, proteins, and metabolome using genomics, transcriptomics, proteomics, and metabolomics, respectively. Genomics involves study of structure, function, evolution, and mapping of genomes; on the other hand, transcriptomics includes structural and functional analyses of coding and noncoding ribonucleic acid. Proteomics deals with protein and posttranslational protein modification in conjunction with their regulatory pathway (Gupta et al. 2013). Posttranslational modification studies have revealed that the molecular mechanisms driving the responses of plants to environmental stresses include histone acetylation, methylation, ubiquitination, and phosphorylation. Combined effects of these modifications play an essential role in the regulation of stress-responsive gene expression (Yuan et al. 2013). Lastly, metabolomics also offers a robust tool to analyze varied metabolites in an integrated method that can act as a powerful tool in distinguishing the complicated network concerned in stress tolerance.

Plant usually modifies their "omics" profiles in order to cope with the environmental stresses. The main objective of the "omics" approaches is to find out the molecular interaction and their relationship with the signaling cascade and to process the information, which in turn connects specific signals with specific molecular responses (Gupta et al. 2013). As rightly said by Edwin Grant Conklin, "Life is not found in atoms or molecules or genes as such, but in organization; not in symbiosis but in synthesis."

2.3.1 Functional Genomics Approaches to Decode Abiotic Stress Signaling

In order to elucidate the role of intermediate components in the cascade of abiotic stress response and to trace out the entire stress response pathway, traditional approach of gene identification has been practiced in the past. Utilization of traditional breeding strategies, involving the use of genetic variation arising from varietal germplasm, interspecific or intergeneric hybridization, induced mutations, and somaclonal variation of cell and tissue cultures, has met with only limited success. These traditional practices have shown limited success in the generation of plant with improved stress resistance under field conditions due to the complex nature of stress tolerance traits, low genetic variance of yield under stress, and lack of economical selection techniques (Flowers and Yeo 1995; Frova et al. 1999; Ribaut et al. 1996, 1997).

In order to improve the existing technique for identification and introgression of genes and metabolite acting in the stress cascade, an updated approach of functional genomics has been implemented by the scientific community to determine the function of individual genes, pathways, and entire genomes. The basis of functional genomics underlies in the evaluation and study of the entire cell or organism as a system and in understanding how different biological processes occur within a system as a whole. The approach has shown quite an improvement and advancement over the last decade with involvement of high-throughput sequencing, proteomics, and metabolomics approaches. The basic outline under which functional genomics functions includes:

(a) Gene discovery
(b) High-throughput gene expression
(c) Validation of function and stress response mechanism

The role of these individual disciplines in crop improvement in response to stress tolerance has been discussed in detail in the following sections:

2.3.1.1 Gene Discovery

Mining of genes associated with abiotic stress mechanism in plants has been one of the primary steps in functional genomics, which leads to elucidation of an intercalating network of abiotic stress tolerance. Availability of genome-wide information in plants acts as a fundamental resource in fishing out candidate stress-related genes. Utilization of large-scale EST plant databases (such as National Center for Biotechnology Information (NCBI) UniGene, http://www.ncbi.nlm.nih.gov/; The Institute for Genomic Research (TIGR) Gene Indices, www.tigr.org; Sputnik, http://mips.gsf.de/proj/sputnik) (Vij and Tyagi 2007) acts as one of the most frequently used tools to explore gene catalogue of a species. ESTs are partial genetic sequences that are generated by single-pass sequencing of cDNA libraries (Bouchez and Höfte 1998), generated from various tissues, developmental stages, or treatments (Yamamoto and Sasaki 1997). Deciphering genes that are related to abiotic stress has been done through a comprehensive analysis of ESTs generated from different cDNA libraries of control and abiotic stress-treated tissues collected at various stages of development. Outcome of such analysis has led to the identification of gene number, gene content, and possible gene families involved in abiotic stress responses (Sreenivasulu et al. 2007). Further functional annotation of mined genes by comparative analysis of BLASTX and SwissProt database provides a possible outline of tolerance mechanism in plants, though ~20 to 30 % of these fished-out genes still remain unannotated.

Over the last decade, attempts have been made to analyze ESTs from salt-treated rice plants that resulted in the identification of genes responsible in cell rescue, transport, defense, and energy metabolism (Bohnert et al. 2001). The identification of abundantly expressed ESTs in libraries of a salt-treated halophyte *Thellungiella halophila* (Wang et al. 2004) as well as from monocots like barley, wheat, and maize

(Zhang et al. 2004) has also taken lead in the characterization of genes involved in stress mechanism. A recent comprehensive analysis of expressed sequence tags in common wheat has revealed that out of 0.68 million ESTs with 37,138 contigs and 215,199 singlets used, 55 % of the total singlets were attributed to stress-related libraries. Functional annotations of the contigs thereafter resulted in 21,125 sequences with molecular functions (redox, binding, catalytic activity, etc.), while 13,354 contig sets showed functions in biological processes such as transport, signaling, and metabolism. An overall annotation also showed that nearly 10.5 % of the gene still remains unannotated, while 3,500 new genes were identified through this approach (Manickavelu et al. 2012).

Thus, an overall analysis of different EST collections helps in the identification of stress-regulated genes, underlying regulatory and metabolic networks (Sreenivasulu et al. 2007). EST sequencing has been employed extensively in the absence of whole-genome sequences, notably in crops with large and repetitive genomes, although the entire transcriptome is unlikely to be resolved (Akpınar et al. 2013). Plant expressed sequence tags (ESTs) help in the well characterization of genes and clustering into nonredundant gene clusters (Vij and Tyagi 2007); however, the use of cDNA libraries as a source of these ESTs may underrepresent rare transcripts or transcripts that are not expressed under certain conditions.

2.3.1.2 High-Throughput Gene Expression

In order to have a broad view on global gene expression of a particular species in response to stress conditions, the use of high-throughput techniques for transcript profiling has been one of the successful approaches. The high-throughput approach utilized for transcript profiling has been broadly classified as hybridization-based, sequence-based, and proteomics-based tools and has been discussed in detail in this section.

Hybridization-Based Method

Evolution of microarray technology in the last decade has revolutionized the analysis of genome-wide gene expression. The technique employs arrays of cDNA libraries on a DNA chip, which are further hybridized to fluorescently labeled cDNA probe pairs prepared from RNA samples of different tissues and growth conditions (Seki et al. 2002). The first report of the use of microarray technology in expression profiling was done by Schena et al. (1995) in model organism *Arabidopsis*, wherein 48 genes were analyzed for differential expression in roots and shoots. Utilization of this technique to elucidate the role of genes of a particular species in response to abiotic stress treatment has been one of the popular approaches for genome profiling. One such attempt has been an extensive microarray expression data for *Arabidopsis* with nearly 7,000 independent full-length cDNA groups, showing a fivefold increase in 53 genes during cold stress, 277 genes during high temperature,

and 194 genes during high-salinity stress, respectively (Seki et al. 2002). Detailed microarray expression data have also been constructed for rice as well as agronomical important plant species such as wheat (Ergen et al. 2009), barley (Close et al. 2004), maize (Luo et al. 2010), cotton (Ranjan et al. 2012), cassava (Utsumi et al. 2012), and tomato (Loukehaich et al. 2012) to unravel stress responses.

In order to analyze the complete repertoire of the drought-inducible genes in *Arabidopsis*, microarray has been performed. A 7,000 full-length complementary DNA microarray was used to spot 299 drought-inducible genes, 54 cold-inducible genes, 213 high-salinity-inducible genes, and 245 ABA-inducible genes (Shinozaki and Yamaguchi-Shinozaki 2007). Microarray analysis again broadly classified drought-inducible gene products into two categories. The first group is comprised of proteins like chaperones, late embryogenesis abundant (LEA) proteins, osmotin, antifreeze proteins, mRNA-binding proteins, key enzymes for osmolyte biosynthesis, water channel proteins, sugar and proline transporters, detoxification enzymes, and various proteases. The second group includes various transcription factors, protein kinases, protein phosphatases, enzymes involved in phospholipid metabolism, and other signaling molecules such as calmodulin-binding protein.

The use of a microarray to study global gene expression profiling in response to abiotic stress in rice was first reported by Kawasaki et al. (2001) who compared the gene expression profiles in salt-tolerant (var. Pokkali) and salt-sensitive (var. IR29) rice in response to salt stress. The analysis thus performed included 1,728 cDNA clones prepared from unstressed or salt-stressed roots of Pokkali, showing a delayed response in the expression pattern of IR29 as one of the major probable reasons of sensitiveness as compared to its tolerant variety (Pokkali) (Kawasaki et al. 2001). A rice genome array containing 48,564 japonica and 1,260 indica sequences has also been used to compare the transcriptome of salt-tolerant (FL478) and salt-sensitive (IR29) rice varieties. The response of the two varieties was strikingly different, with a much larger number of genes expressed in IR29 than in FL478 on exposure to salt stress. This difference in expression pattern is mainly attributed to the fact that FL478 is salt tolerant and maintains a low Na^+-to-K^+ ratio (Walia et al. 2005). In legumes, high-density cDNA-based arrays have been employed to study 27,513 unigene sets from various developmental stages and stress-exposed tissues of soybean. The result thus obtained identified a large number of genes involved during different biotic (herbicide and pathogen) and abiotic stresses (drought, heat, flooding) (Vodkin et al. 2004) reviewed by Kudapa et al. (2013). Other legumes such as chickpea have also been subjected to global gene expression analysis by various researchers, and in the year 2010 Mantri et al. (2010) identified differentially expressed genes in varying genotypes, in response to various abiotic stresses by comparing a 768 feature microarray of chickpea cDNA (559), grass pea cDNA (156), lentil resistance gene analogue cDNA (41), and control (12). Although microarray technique acts as a powerful tool in the evaluation of gene expression across species, its targeted mode of action acts as a hindrance in its utilization for species where the entire genome is not sequenced. For species where the entire genome sequence is unrevealed, a prior knowledge of transcript to be analyzed and thereby designing of probe remains out of question (Rensink and Buell 2005).

One of the other drawbacks of the microarray studies remains the posttranscriptional and posttranslational modification of transcript, which remains unsolved in this technique (Akpınar et al. 2013).

Another approach through which transcript profiling at genome level is performed is termed as nuclear expressed sequence tag (NEST) analysis. The technique employs a combination of fluorescence-assisted nucleus sorting and cDNA generation (based on the expression of nucleus-targeted green fluorescent protein [GFP], which is controlled by a cell-specific promoter) from the RNA of isolated nuclei (Macas et al. 1998). The isolated RNA in this technique overlays the posttranscriptional modifications and turnover of transcript in the cytosol, thereby accurately reflecting the total nuclear transcript abundance. The cell-specific cDNAs thus obtained are further characterized through differential-display reverse transcriptase-mediated PCR or by EST analysis. The use of NEST analysis in tobacco has revealed an approximate 25 % of salinity-induced transcripts that showed significant homology to functionally unknown genes (Cushman and Bohnert 2000).

Sequence-Based Approach

SAGE acts as one of the essential tools in the quantification of transcripts at a genome-wide scale. The techniques employs generation of unique short sequences tags of 9–17 base pairs (Velculescu et al. 1995; Saha et al. 2002) that are eventually concatenated and sequenced. Generation and quantification of transcript-specific tags help in estimating the expression level of the particular transcript eventually leading to the identification of novel expressed regions of the genome by providing an overall view of gene expression (Vij and Tyagi 2007). For the first time, Matsumura et al. (1999) successfully used this technique in rice seedlings, wherein 10,122 tags from 5,921 expressed genes from seedlings were analyzed and novel genes were identified. Of the many novel genes identified, 18 genes were found to be anaerobically induced and coding for prolamin, expansin, and glycine-rich cell wall protein, while six genes were found repressed under anaerobic conditions (Matsumura et al. 1999).

Over the last decade, reports of SAGE being utilized to analyze changes in gene expression in leaves of *Arabidopsis* in response to cold stress have come up (Jung et al. 2003). Revelation of SAGE analysis has shown that genes that are involved in anabolic pathway such as photosynthesis remain repressed under cold stress conditions, while expression of genes involved in cell rescue, defense, cell death, aging, protein synthesis, metabolism, and transport facilitation are upregulated. In order to identify genes responsible for the sensitiveness of *Arabidopsis* pollen towards cold stress, a comparison of control and stressed SAGE tags was made by Lee and Lee in the year 2003 (Lee and Lee 2003). The results thus obtained showed low accumulation of cold-responsive genes such as COR (cold responsive), beta amylase, and lipid transfer proteins in cold stress-treated pollens as compared to their control counterparts, suggesting it as one of the probable causes of cold susceptibility of *Arabidopsis* pollen (Chinnusamy et al. 2003).

Transcript profiling through SAGE is one of the popular methods in animal system; however, in plants, the technique still remains not widely applicable. As the SAGE tags are generally 9–17 bp in length, their applicability in tag-to-gene annotation remains limited to model organism where the entire genome sequence is known. In order to cope with these problems, variant of SAGE technology, SuperSAGE, has been of much use in plant species. The technique utilizes the same methodology as that of SAGE with only difference in tag length (26 bp), which remains longer than its precursor. The longer tag length has been a boon for transcript profiling in non-model organism as the tags themselves allow designing of PCR primer for cDNA synthesis of corresponding genes via RACE (Matsumura et al. 2003). The use of SuperSAGE technique in plant has been widely implemented for studying responses of genes under various biotic and abiotic stresses across different species, such as banana, tobacco, capsicum, chickpea, etc. Utilization of this technique for elucidating role of genes in abiotic stress response has helped in the elucidation of 3,000 stress-related transcripts from 360,000 transcripts of salt- and drought-related transcriptome of chickpea and lentil (Kudapa et al. 2013). The amenability of SuperSAGE with integrative transcriptome, SuperSAGE microarray, and next-generation sequencing has also led to an increase in its efficiency towards gene expression profiling (Matsumura et al. 2005). Application of NGS to gene expression analysis has led to the development of techniques like digital gene expression-TAG (DGE-TAG), DeepSAGE (Nielsen et al. 2006), RNA-seq (Marioni et al. 2008; Matsumura et al. 2010; Nagalakshmi et al. 2008), and eventually high-throughput SuperSAGE precisely known as deepSuperSAGE, which provide digital, highly accurate, easy-to-perform, and cost-effective solution to expression profiling over its hybridization counterpart microarray (Matsumura et al. 2010). Application of deepSuperSAGE in drought-treated and control chickpea roots has further identified 80,238 tags representing 17,493 unique transcripts (Molina et al. 2008, 2011).

An alternative approach to genome-wide transcript profiling through sequencing-based approach lies in massively parallel signature sequencing (MPSS). The technique follows similar tag-based approach to ascertain the expression pattern of mRNA within a species. These expressed mRNAs are further related to their corresponding ESTs or whole genome, thereby providing a larger perspective of expression profiling (Brenner et al. 2000; Pollock 2002). The basic idea behind the technique lies in the generation of longer sequence tags, which are ligated to microbeads and sequenced in parallel, thereby enabling analysis of millions of transcripts simultaneously (Brenner et al. 2000) with greater specificity and sensitivity and at a much faster speed. The use of MPSS has been widely popular and beneficial in species that lack a whole-genome sequence (Reinartz et al. 2002). In plants, MPSS has been employed in studying expression response of mRNA transcripts towards abiotic stress conditions. One of the earliest examples of the use of MPSS in gene expression profiling in response to abiotic stress conditions has been in studying ABA-responsive gene expression in ABA-insensitive mutants (*abi1-1*) and wild-type (WT) *Arabidopsis* plants. A study showed that a very few genes were affected

by ABA in the ABA-insensitive mutant (*abi1-1*), whereas the ABA-responsive gene expression of the majority of genes was drastically impaired in the *abi1-1* mutant, thereby postulating existence of two ABA signaling pathways, only one of which is impaired in the *abi1-1* mutant (Hoth et al. 2002). Over the last decade, reports of MPSS analysis in expression profiling of small RNAs (Sunkar et al. 2007) having a distinct role in abiotic stress responses have also come into light. Although MPSS is a quick, reliable, and reproducible tool, the realization of its full potential in the global expression profiling towards abiotic stress response is yet to be achieved (Vij and Tyagi 2007) owing to its cost. As of now, plant MPSS database (http://mpss.udel.edu/) has been a repository and publicly available MPSS expression data of many plant species, some of which include *Arabidopsis*, rice, maize, soybean, and grapes. The resource for rice includes 20 MPSS libraries constructed from different tissues, of which three libraries belong to abiotic stress conditions, namely, cold, salt, and dehydration (Nakano et al. 2006).

2.3.1.3 Proteomics and Metabolomics Approach

Proteins act as one of the prime targets of abiotic stress response when looked up beyond their genetic counterpart. Identification of proteins as target of abiotic stress acts in nullifying the effect of posttranscriptional and translational modifications. Utilization of proteomic tools such as two-dimensional gel electrophoresis (2DGE), MALDI, and mass spectrometry (MS) in the identification of these target proteins has been a popular approach in *Arabidopsis* in response to cold stress. Analysis of *Arabidopsis* nuclear proteome thus revealed that out of 134 protein spots identified, nearly 30 % of the protein spots showed an altered response towards cold stress. Few of these proteins included heat-shock proteins, transcription factors (OBF2 and AtMYB2), DNA-binding proteins (Dr1 and DRT102), catalytic enzymes (phosphoglycerate kinase, serine acetyltransferase, and glyceraldehyde-3-phosphate dehydrogenase), and calmodulin, syntaxin, and germin-like proteins (Bae et al. 2003). A similar proteome analysis of rice plant using 2D gel electrophoresis showed that 42 out of 1,000 protein spots showed an altered response towards drought stress conditions (Salekdeh et al. 2002). Out of these 42 proteins with altered expression in stress conditions, 16 were identified as drought-responsive proteins using MS analysis. Detailed analysis of rice root proteome through 2DGE and MS analysis identified 54 proteins with affected expression in response to salt stress. Out of the 54 identified proteins, 6 proteins were identified and annotated as novel salt-responsive proteins, such as Cox6b-1, UGPase, GS root isozyme, α-NAC, putative splicing factor-like protein, and putative ABP (Yan et al. 2005). Extensive proteome analyses of rice seedling and anthers (Imin et al. 2004) in response to cold stress have also resulted in the identification of a number of cold stress-responsive genes like chaperones, proteases, and detoxifying enzymes (Cui et al. 2005). Other than *Arabidopsis* and rice, proteome approach to find proteins as putative targets of abiotic stress response has also been observed in tobacco apoplastic protein wherein

20 salt stress-responsive proteins were identified, which included chitinases, germin-like protein, lipid transfer proteins, and a few well-characterized stress-associated proteins (Dani et al. 2005).

Integrated metabolome and transcriptome analyses of model plants have also helped in the identification of metabolites in response to various stresses in plants. A comprehensive metabolite profiling of model plants in different abiotic stress (cold, drought, temperature, and salt) conditions also helps in fine-tuning the stress pathway of plants. Metabolite profiling analysis of *Arabidopsis* in response to dehydration stress using an integrative approach of GC-TOF MS, CE–MS, and DNA microarrays has revealed that accumulation of glucose, fructose, and branched-chain amino acid in response to dehydration stress is regulated by ABA accumulation during dehydration, thereby postulating an important role of ABA in stress response (Urano et al. 2010). Metabolic profiling has also revealed that sucrose replaces proline in plants as the major osmoprotectant during the more severe combined dehydration and heat-stress treatment (Rizhsky et al. 2004).

In response to cold stress, metabolite profiling of *Arabidopsis* has indicated that an increased tolerance to freezing stress in transgenic plants overexpressing DREB1A may be a consequence of the accumulation of low-temperature-regulated metabolites, especially raffinose, sucrose, myoinositol, and galactinol (Cook et al. 2004; Maruyama et al. 2009). In response to cold and heat shocks on *Arabidopsis*, using GC–MS (Kaplan et al. 2004) and GC-TOF MS (Wienkoop et al. 2008) has revealed that metabolites such as compatible solutes that are produced during extreme temperature overlap during both heat- and cold-shock treatments, thereby postulating that accumulation of compatible solutes bears an important role in temperature stress condition (Urano et al. 2010). Comparative metabolome analysis of a model halophyte *Thellungiella halophila* with *Arabidopsis* under salt stress conditions through GC–MS and microarrays (Gong et al. 2005) has shown that even in control conditions, *Thellungiella* contains higher levels of various osmolytes, such as fructose, sucrose, complex sugars, malate, and proline, and their respective biosynthetic genes compared with *Arabidopsis* (Gong et al. 2005). The results thus obtained suggest that a constant state of stress-anticipatory preparedness exists in *Thellungiella*.

2.4 Validation of Functions and Stress Response Mechanisms

In order to validate the function of genes mined from genome-wide analysis of a species, in response to abiotic stress mechanism, knockout analysis through RNA interference (RNAi), T-DNA insertional mutants, TILLING, and overexpression experiments has been carried out. These transgenic approaches have helped in validating the role of identified genes during different abiotic stresses faced by plants.

2.4.1 Knockout/Knockdown Analysis

2.4.1.1 RNA Interference in Gene Validation

RNA-mediated gene silencing, collectively termed as RNAi, has been employed as one of the promising tools in the validation of functional genes. The approach utilizes nearly 21–25-nt-long ds small RNAs, which are a product of small portion of desired gene's coding regions and act as a trigger for direct degradation of mRNA in homology-dependent posttranscriptional silencing (Grishok et al. 2001; Hamilton and Baulcombe 1999; Zamore et al. 2000) reviewed in McGinnis (2010). Not only posttranscriptional but also transcriptional regulation is achieved through RNAi-mediated silencing wherein such a construct is designed that the dsRNA thus formed bears homology to the promoter region of desired gene (Mette et al. 2000). siRNA-mediated DNA methylation and heterochromatin formation through epigenetic modulation are also one of the key approaches of transcriptional inhibition of the desired gene (Verdel et al. 2009) reviewed by McGinnis (2010). The technology finds its relevance in areas where antisense technologies fail to perform (Kudapa et al. 2013). The use of a single copy of transgene-induced RNAi, to efficiently block genes belonging to gene families or expressing during various time and places of developing tissues (Kudapa et al. 2013), helps in simplification of several phenotypic assays (such as several generation crosses for marker-assisted silencing) in a functional genomics project (McGinnis 2010).

However, early attempts of homologous recombination-based gene silencing in rice showed minimal success rate with very low efficiency rate (Hanin and Paszkowski 2003). Later attempts (Miki and Shimamoto 2004) showed 90 % silencing through RNAi vectors against 11 rice genes. In other crop plants such as legume, *Medicago* and soybean have shown the effectiveness of RNAi-induced gene silencing and validation of gene functions by efficient silencing of PIN (auxin efflux proteins) genes responsible for root nodule formation (Huo et al. 2006) and myoinositol-1-phosphate regulating cellular metabolism and growth, respectively (Nunes et al. 2006). Though RNAi-induced gene silencing has well been established in many plant species, application still remains limited due to its inefficient long-term establishment and inability to be utilized for large-scale functional analysis (Kudapa et al. 2013; Vij and Tyagi 2007). Other possible means for employing gene silencing in functional genomics has been the deployment of virus-induced gene silencing (VIGS) that provides long-term persistence during vegetative and in vitro propagation and results in generation of genotypically identical silenced genes (Kudapa et al. 2013; Vij and Tyagi 2007). In legumes, the approach has been well established in soybean and pea against genes responsible in biotic stress resistance (Gronlund et al. 2010; Kachroo and Ghabrial 2012). Implication of VIGS has been in both forward and reverse genetic approach to validate gene functions (Senthil-Kumar and Mysore 2011); however, its application is limited due to the unavailability of appropriate vector and vector delivery system in plants (Kudapa et al. 2013).

2.4.1.2 Gene Validation Through Mutations

There are two main complementary approaches developed for identifying target genes through mutations, namely, TILLING and T-DNA insertional mutant lines. Of the two approaches, T-DNA insertional mutant lines involve insertion of foreign DNA into candidate gene, for disrupting gene function and ascertaining their role through reverse genetics approach. In *Arabidopsis*, the technique involves the use of either transposable elements (Parinov et al. 1999) or T-DNA. The foreign DNA not only disrupts the expression of the gene into which it is inserted but also acts as a marker for subsequent identification of the mutation. Because *Arabidopsis* introns are small and there is very little intergenic material, any insertion of order ranging from 5 to 25 kb in length leads to a dramatic alteration in gene function (Radhamony et al. 2005). Although the approach has wide applications in crop improvement, its success has been limited to only few plant species, owing to their dependency on *Agrobacterium*-mediated T-DNA transmission or on endogenous transposon tagging system (McCallum et al. 2000). However, in a large population of T-DNA-transformed lines, one has a very good chance of finding a plant carrying a T-DNA insert within any gene of interest. Mutations that are homozygous lethal can also be maintained in the population in the form of heterozygous plants through this approach (Radhamony et al. 2005).

The second approach, TILLING (targeting induced local lesions in genomes), acts as an alternative approach to insertional mutagenesis, which was developed a decade ago by a graduate scholar Claire McCallum, who wanted to induce targeted mutations in chromomethylase gene of *Arabidopsis*. The method thus invented was the advancement over the existing reverse genetic tools to study genetic data of an organism. The main advantage of TILLING as a reverse genetics strategy envisages its application to any species plant (since it does not require transgenic or cell culture manipulations), regardless of its genome size and ploidy level. Chemical mutagens such as ethyl methanesulfonate (EMS) are generally used in this technique that provide a high frequency of point mutations distributed randomly in the genome. Initially, TILLING strategy was developed as a discovery platform for functional genomics, but it soon became a valuable tool in crop breeding as an alternative to the transgenic approach. The method combines a standard and efficient technique of mutagenesis in combination of a chemical mutagen with a sensitive DNA screening technique that identifies single-base mutations in a target gene. The approach relies primarily on the formation of DNA heteroduplexes. The general protocol for the formation of a tilling population includes the creation of mutated populations through chemical mutagenesis followed by the detection of mutations in target sequence through polymerase chain reaction, and subsequent phenotype thus obtained is eventually analyzed. One of the significant advantages of this technique includes production of an allelic series of mutations including hypomorphic alleles that are useful for genetic analysis. Success of TILLING approach over its T-DNA insertional counterpart has been demonstrated through its wide application across plant species. For a large number of agronomically important crops, including rice,

barley, wheat, maize, sorghum, soybean, rapeseed, and tomato plants, TILLING has been utilized as a wonder technique for crop improvement (Kurowska et al. 2011).

By using these techniques, putative functions of abiotic stress-responsive genes were followed in *Arabidopsis*, rice, maize, and barley. In *Arabidopsis* (ecotype C24) 43,000 T-DNA insertional lines were generated (http://stress-genomics.org/stress.fls/tools/mutants.html), of which 30,000 lines were screened for stress-related gene regulation mutants. The mutants thus obtained, upon screening for salt-stressed conditions, showed hypersensitivity towards salt stress by demonstrating an impaired growth on increased salt conditions, thereby determining the role of knocked-out genes SOS1, SOS2, and SOS3 in maintaining and balancing ion transport across plant membrane (Kamei et al. 2005; Liu et al. 2008; Halfter et al. 2000).

Large-scale forward genetic screens have been used to identify abiotic stress response determinants in a T-DNA-mutagenized *Arabidopsis* population in the RD29a-LUC background. More than 200 mutants with altered stress/ABA response were identified from 250,000 independent insertional lines. These included mutations in genes coding for transcription factors, syntaxin, ABA biosynthetic enzyme, SUMO E3 ligase, and the sodium transporter HKT1 (Koiwa et al. 2006). T-DNA-tagged transgenic rice lines were evaluated for cold-responsive β-glucuronidase (GUS) expression, and detailed analysis led to the identification of two cold-responsive genes, namely, OsDMKT1 (putative demethyl menaquinone methyltransferase) and OsRLK1 (putative LRR-type receptor-like protein kinase) (Lee et al. 2004).

TILLING enables high-throughput analysis of a large number of mutants (McCallum et al. 2000). TILLING is applicable to virtually all genes in all species where mutations can be induced and have been reported in several crop species, including hexaploid wheat (Chen et al. 2012). TILLING mutants are reported in sorghum (Xin et al., 2008), maize (Till et al. 2004), barley (Caldwell et al. 2004), soybean (Cooper et al. 2008), rice (Cooper et al. 2013), and other crops, where further use of certain TILLING mutants in elucidation of stress responses has been demonstrated. In one such similar study, TILLING mutants for a specific kinase were used to assess salt stress response in legume species (de Lorenzo et al. 2009). Another variant of this strategy, referred to as EcoTILLING, has conjointly been developed to spot natural polymorphisms, similar to TILLING-assisted identification of evoked mutations. Polymorphisms demonstrating natural variation in germplasms are valuable tools in genetic mapping. Similar to TILLING, EcoTILLING is applicable to polyploid species, where it can be utilized to differentiate between alleles of homologous and paralogous genes (Comai et al. 2004). Recently, EcoTILLING not only provided allelic variants of a number of genes involved in salt stress response (Negrão et al. 2013), but transcription factors, diversifying stress responses, have also been targeted, to examine natural rice variants exposed to drought stress (Yu et al. 2012). The availability of comprehensive EST databases is central to the success of the above mentioned approaches to identify genes accurately and unambiguously.

2.4.2 Overexpression Analysis

To identify the function of any gene, insertional mutagenesis is also one of the effective techniques. Although loss-of-function mutations identified the function of genes in many organisms, there is a major limitation attached with it. After *Arabidopsis* genome sequencing, it has been discovered that most of the genes have closely related sequences in the genome that belongs to gene families (The *Arabidopsis* Genome Initiative 2000). So gene redundancy, where a gene family contains several genes that have a similar function, can compensate the loss-of-function phenotype, or there may exist alternative metabolic pathways and regulatory networks, which provide plants with the same gene product. This genetic redundancy may prevent elucidation of gene function by loss-of-function approaches. New advancement in this field came after 1992, named as gain-of-function approach, also known as activation-tagging technology. This was originally developed by Rick Walden and colleagues at the Max Planck Institute (Hayashi et al. 1992), who constructed a T-DNA vector with four copies of an enhancer element from the constitutively active promoter of the cauliflower mosaic virus (*CaMV*) *35S* gene (Odell et al. 1985). These enhancers can cause transcriptional activation of nearby genes, and as activated genes are associated with a T-DNA insertion, this approach came to be known as activation tagging. This technology has made the production of gain-of-function mutants possible. It has added advantage of screening of phenotypes in the T_1 generation, as all activation-tagged mutants are dominant. In the case of loss-of-function mutations, the phenotype is usually not seen until the T_2 generation. The T-DNA having tetramer of the *CaMV 35S* enhancer gets randomly inserted into the plant genome via *Agrobacterium* transformation. The inserted enhancer elements then activate the transcription of nearby genes. T-DNA insertion sites are then determined in the transformed mutants to identify candidate genes. Various techniques like plasmid rescue, inverse PCR, or adapter PCR methods can be used to determine the genomic fragment details near the T-DNA right and left border sequences. TAIL-PCR is also an efficient method to determine T-DNA insertion (Burke 2003; Yamamoto et al. 2003).

The activation-tagging system has been employed in identification of genes involved in biotic and abiotic stress responses. The *enhanced drought tolerance 1* (*edt1*) mutant is identified under drought condition using activation tagging. It has been shown that the enhanced drought tolerance is caused by T-DNA-tagged gene that encodes putative homeodomain-START transcription factor. Transgenic tobacco with overexpressed cDNA of this transcription factor also develops drought tolerance. So, activation tagging provides a key regulator that may be used to improve drought tolerance in plants (Yu et al. 2008). *CDR1-D* (constitutive disease resistance) and *FMO1–3D* (flavin-containing monooxygenase) mutants showed increased resistance during abiotic stress and are also identified through activation tagging (Koch et al. 2006; Xia et al. 2004).

However, just like two sides of a coin, activation-tagged mutagenesis does have some disadvantages. Activation of gene by a transcriptional enhancer is not restricted

to one gene and in some cases may result into a complex phenotype caused by complex gene expression (Ichikawa et al. 2003). Therefore, it becomes difficult to identify, which gene corresponds to the mutant phenotype. To get rid of this problem, an alternative of gain-of-function technique is developed known as Full-length cDNA Over-eXpressing gene hunting system (FOX hunting system). This system can eliminate such complexity since the full-length cDNA is expressed in the correct orientation between the CaMV *35S* promoter and the *NOS* terminator. After identification, genomic PCR is performed to isolate the gene that results in mutant phenotype. During this approach, mutant lines were generated by ectopic expression of full-length cDNAs (Ichikawa et al. 2006). Novel salt stress tolerance genes were isolated from *Thellungiella halophila* using the FOX hunting system (Du et al. 2008).

Ectopic expression of some transcription factors may lead to lethality that makes analysis of gene difficult. In such cases, controlled gene expression using inducible system can work. Several ABA insensitivity and salt-tolerant mutants were isolated using this controlled system of induction by cloning an *Arabidopsis* cDNA library into the estradiol-inducible vector (Papdi et al. 2008).

Conclusion

The effective role of functional genomics in elucidation of complex regulatory pathways and respective signaling molecules has been enhanced by remarkable technical advancement in its sub-platforms, which includes genome, transcriptome, metabolome, fluxome, and ionome analyses, respectively. These analyses on the whole provide an overview of the entire cellular machinery. Implications of this advancement are immense and far reaching in many fields including medicine, agriculture, public health, defense, and more. In plants utilization of functional genomics approach has played a crucial role in understating the processes and molecular networks in response to stress conditions. The use of functional genomics to dissect out the role of individual gene, protein, and metabolite in an intriguing cascade of abiotic stress response provides a hint towards crop improvement against abiotic stress. With the advent of next-generation sequencing and RNA-seq techniques, cost-effective genome and transcriptome analysis has further strengthened the pillars of functional genomics. The use of high-throughput techniques has helped in mining greater details at each subcellular level, which upon integration through bioinformatics approach has revealed many of the signaling molecules intriguing in complex signaling cascades over the past few years. Thus, utilization of transgenic approach, molecular breeding, and map-based cloning together could further help in introgression of desired traits to make plant stress tolerant without compromising on its yield. Once identified, desirable QTLs can be utilized for extensive breeding to restore desirable traits along with the introgressed tolerance trait. Although the functional genomics approach is a notch higher in validating the role of genes in various molecular networks, it still bears challenges such as absence of a

comprehensive bioinformatics database that can mine data from individual "omics" platform and provide a holistic view of cellular networks and machinery on the whole under any perturbed condition faced by plants. Thus, a comprehensive approach including functional, structural, and comparative genomics together is necessary to mitigate the various extremes of abiotic stress, through crop improvement program.

References

Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell 15(1):63–78

Akpınar BA, Lucas SJ, Budak H (2013) Genomics approaches for crop improvement against abiotic stress. Scientific World Journal 2013

Bae MS, Cho EJ, Choi EY, Park OK (2003) Analysis of the Arabidopsis nuclear proteome and its response to cold stress. Plant J 36(5):652–663

Baxter A, Mittler R, Suzuki N (2014) ROS as key players in plant stress signalling. J Exp Bot 65(5):1229–1240

Benschop JJ, Mohammed S, O'Flaherty M, Heck AJ, Slijper M, Menke FL (2007) Quantitative phosphoproteomics of early elicitor signaling in Arabidopsis. Mol Cell Proteomics 6(7): 1198–1214

Bienert GP, Møller AL, Kristiansen KA, Schulz A, Møller IM, Schjoerring JK, Jahn TP (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 282(2):1183–1192

Bindschedler LV, Dewdney J, Blee KA, Stone JM, Asai T, Plotnikov J, Denoux C, Hayes T, Gerrish C, Davies DR (2006) Peroxidase-dependent apoplastic oxidative burst in Arabidopsis required for pathogen resistance. Plant J 47(6):851–863

Bohnert HJ, Ayoubi P, Borchert C, Bressan RA, Burnap RL, Cushman JC, Cushman MA, Deyholos M, Fischer R, Galbraith DW (2001) A genomics approach towards salt stress tolerance. Plant Physiol Biochem 39(3):295–311

Bouchez D, Höfte H (1998) Functional genomics in plants. Plant Physiol 118(3):725–732

Brandt B, Brodsky DE, Xue S, Negi J, Iba K, Kangasjärvi J, Ghassemian M, Stephan AB, Hu H, Schroeder JI (2012) Reconstitution of abscisic acid activation of SLAC1 anion channel by CPK6 and OST1 kinases and branched ABI1 PP2C phosphatase action. Proc Natl Acad Sci 109(26):10593–10598

Brenner S, Johnson M, Bridgham J, Golda G, Lloyd DH, Johnson D, Luo S, McCurdy S, Foy M, Ewan M (2000) Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nat Biotechnol 18(6):630–634

Bressan RA, Hasegawa PM, Pardo JM (1998) Plants use calcium to resolve salt stress. Trends Plant Sci 3(11):411–412

Burke E (2003) High-throughput TAIL-PCR as a tool to identify DNA flanking insertions. Plant Functional Genomics. Springer, In, pp 241–271

Caldwell DG, McCallum N, Shaw P, Muehlbauer GJ, Marshall DF, Waugh R (2004) A structured mutant population for forward and reverse genetics in Barley (Hordeum vulgare L.). Plant J 40(1):143–150

Chater C, Kamisugi Y, Movahedi M, Fleming A, Cuming AC, Gray JE, Beerling DJ (2011) Regulatory mechanism controlling stomatal behavior conserved across 400 million years of land plant evolution. Curr Biol 21(12):1025–1029

Chen L, Huang L, Min D, Phillips A, Wang S, Madgwick PJ, Parry MA, Hu Y-G (2012) Development and characterization of a new TILLING population of common bread wheat (Triticum aestivum L.). PLoS One 7(7):e41570

Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, Agarwal M, Zhu JK (2003) ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis. Genes Dev 17(8): 1043–1054. doi:10.1101/gad.1077503

Close TJ, Wanamaker SI, Caldo RA, Turner SM, Ashlock DA, Dickerson JA, Wing RA, Muehlbauer GJ, Kleinhofs A, Wise RP (2004) A new resource for cereal genomics: 22K barley GeneChip comes of age. Plant Physiol 134(3):960–968

Comai L, Young K, Till BJ, Reynolds SH, Greene EA, Codomo CA, Enns LC, Johnson JE, Burtner C, Odden AR (2004) Efficient discovery of DNA polymorphisms in natural populations by ecotilling. Plant J 37(5):778–786

Cook D, Fowler S, Fiehn O, Thomashow MF (2004) A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of Arabidopsis. Proc Natl Acad Sci U S A 101(42):15243–15248

Cooper J, Till B, Laport R, Darlow M, Kleffner J, Jamai A, El-Mellouki T, Liu S, Ritchie R, Nielsen N (2008) TILLING to detect induced mutations in soybean. BMC Plant Biol 8(1):9

Cooper JL, Henikoff S, Comai L, Till BJ (2013) TILLING and ecotilling for rice. In: Rice protocols. Springer, pp 39–56

Cui S, Huang F, Wang J, Ma X, Cheng Y, Liu J (2005) A proteomic analysis of cold stress responses in rice seedlings. Proteomics 5(12):3162–3172

Cushman JC, Bohnert HJ (2000) Genomic approaches to plant stress tolerance. Curr Opin Plant Biol 3(2):117–124

Dani V, Simon WJ, Duranti M, Croy RR (2005) Changes in the tobacco leaf apoplast proteome in response to salt stress. Proteomics 5(3):737–745

de Lorenzo L, Merchan F, Laporte P, Thompson R, Clarke J, Sousa C, Crespi M (2009) A novel plant leucine-rich repeat receptor kinase regulates the response of Medicago truncatula roots to salt stress. Plant Cell 21(2):668–680

Delauney AJ, Verma DPS (1993) Proline biosynthesis and osmoregulation in plants. Plant J 4(2):215–223

Du J, Huang YP, Xi J, Cao MJ, Ni WS, Chen X, Zhu JK, Oliver DJ, Xiang CB (2008) Functional gene-mining for salt-tolerance genes with the power of Arabidopsis. Plant J 56(4):653–664

Ergen NZ, Thimmapuram J, Bohnert HJ, Budak H (2009) Transcriptome pathways unique to dehydration tolerant relatives of modern wheat. Funct Integr Genomics 9(3):377–396

Flowers T, Yeo A (1995) Breeding for salinity resistance in crop plants: where next? Funct Plant Biol 22(6):875–884

Ford CW (1984) Accumulation of low molecular weight solutes in water-stressed tropical legumes. Phytochemistry 23(5):1007–1015

Foyer CH, Parry M, Noctor G (2003) Markers and signals associated with nitrogen assimilation in higher plants. J Exp Bot 54(382):585–593

Frova C, Krajewski P, Di Fonzo N, Villa M, Sari-Gorla M (1999) Genetic analysis of drought tolerance in maize by molecular markers I. Yield components. Theor Appl Genet 99(1–2): 280–288

Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park S-Y, Cutler SR, Sheen J, Rodriguez PL, Zhu J-K (2009) In vitro reconstitution of an abscisic acid signalling pathway. Nature 462(7273):660–664

Fujita Y, Yoshida T, Yamaguchi-Shinozaki K (2013) Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. Physiol Plant 147(1):15–27

Gong Q, Li P, Ma S, Indu Rupassara S, Bohnert HJ (2005) Salinity stress adaptation competence in the extremophile Thellungiella halophila in comparison with its relative Arabidopsis thaliana. Plant J 44(5):826–839

Grishok A, Pasquinelli AE, Conte D, Li N, Parrish S, Ha I, Baillie DL, Fire A, Ruvkun G, Mello CC (2001) Genes and mechanisms related to RNA interference regulate expression of the small temporal RNAs that control *C. elegans* developmental timing. Cell 106(1):23–34

Gronlund M, Olsen A, Johansen E, Jakobsen I (2010) Protocol: using virus-induced gene silencing to study the arbuscular mycorrhizal symbiosis in Pisum sativum. Plant Methods 6(1):28

Gupta B, Saha J, Sengupta A, Gupta K (2013) Plant abiotic stress: 'omics' approach. J Plant Biochem Physiol 1:e108

Halfter U, Ishitani M, Zhu JK (2000) The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc Natl Acad Sci U S A 97(7):3735–3740. doi:10.1073/pnas.040577697

Hamilton AJ, Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. Science 286(5441):950–952

Hanin M, Paszkowski J (2003) Plant genome modification by homologous recombination. Curr Opin Plant Biol 6(2):157–162

Hayashi H, Czaja I, Lubenow H, Schell J, Walden R (1992) Activation of a plant gene by T-DNA tagging: auxin-independent growth in vitro. Science 258(5086):1350–1353

Hoth S, Morgante M, Sanchez J-P, Hanafey MK, Tingey SV, Chua N-H (2002) Genome-wide gene expression profiling in Arabidopsis thaliana reveals new targets of abscisic acid and largely impaired gene regulation in the abi1-1 mutant. J Cell Sci 115(24):4891–4900

Huo X, Schnabel E, Hughes K, Frugoli J (2006) RNAi phenotypes and the localization of a protein: GUS fusion imply a role for Medicago truncatula PIN genes in nodulation. J Plant Growth Regul 25(2):156–165

Ichikawa T, Nakazawa M, Kawashima M, Muto S, Gohda K, Suzuki K, Ishikawa A, Kobayashi H, Yoshizumi T, Tsumoto Y (2003) Sequence database of 1172 T-DNA insertion sites in Arabidopsis activation-tagging lines that showed phenotypes in T1 generation. Plant J 36(3): 421–429

Ichikawa T, Nakazawa M, Kawashima M, Iizumi H, Kuroda H, Kondou Y, Tsuhara Y, Suzuki K, Ishikawa A, Seki M (2006) The FOX hunting system: an alternative gain-of-function gene hunting technique. Plant J 48(6):974–985

Imin N, Kerim T, Rolfe BG, Weinman JJ (2004) Effect of early cold stress on the maturation of rice anthers. Proteomics 4(7):1873–1882

Initiative AG (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408(6814):796

Ishitani M, Liu J, Halfter U, Kim C-S, Shi W, Zhu J-K (2000) SOS3 function in plant salt tolerance requires N-myristoylation and calcium binding. Sci Signal 12(9):1667

Iuchi S, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2000) A stress-inducible gene for 9-cis-epoxycarotenoid dioxygenase involved in abscisic acid biosynthesis under water stress in drought-tolerant cowpea. Plant Physiol 123(2):553–562

Jammes F, Song C, Shin D, Munemasa S, Takeda K, Gu D, Cho D, Lee S, Giordo R, Sritubtim S (2009) MAP kinases MPK9 and MPK12 are preferentially expressed in guard cells and positively regulate ROS-mediated ABA signaling. Proc Natl Acad Sci 106(48):20520–20525

Jung S-H, Lee J-Y, Lee D-H (2003) Use of SAGE technology to reveal changes in gene expression in Arabidopsis leaves undergoing cold stress. Plant Mol Biol 52(3):553–567

Kachroo A, Ghabrial S (2012) Virus-Induced Gene Silencing in Soybean. Antiviral Resistance in Plants. Springer, In, pp 287–297

Kamei A, Seki M, Umezawa T, Ishida J, Satou M, Akiyama K, ZHU JK, Shinozaki K (2005) Analysis of gene expression profiles in Arabidopsis salt overly sensitive mutants sos2–1 and sos3–1. Plant Cell Environ 28(10):1267–1275

Kaplan F, Kopka J, Haskell DW, Zhao W, Schiller KC, Gatzke N, Sung DY, Guy CL (2004) Exploring the temperature-stress metabolome of Arabidopsis. Plant Physiol 136(4): 4159–4168

Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert HJ (2001) Gene expression profiles during the initial phase of salt stress in rice. Plant Cell 13(4):889–905

Kim T-H, Böhmer M, Hu H, Nishimura N, Schroeder JI (2010) Guard cell signal transduction network: advances in understanding abscisic acid, CO2, and Ca2+ signaling. Annu Rev Plant Biol 61:561

Knight H, Trewavas AJ, Knight MR (1997) Calcium signalling in Arabidopsis thaliana responding to drought and salinity. Plant J 12(5):1067–1078

Koch M, Vorwerk S, Masur C, Sharifi-Sirchi G, Olivieri N, Schlaich NL (2006) A role for a flavin-containing mono-oxygenase in resistance against microbial pathogens in Arabidopsis. Plant J 47(4):629–639

Koiwa H, Bressan RA, Hasegawa PM (2006) Identification of plant stress-responsive determinants in Arabidopsis by large-scale forward genetic screens. J Exp Bot 57(5):1119–1128

Komatsu K, Nishikawa Y, Ohtsuka T, Taji T, Quatrano RS, Tanaka S, Sakata Y (2009) Functional analyses of the ABI1-related protein phosphatase type 2C reveal evolutionarily conserved regulation of abscisic acid signaling between Arabidopsis and the moss Physcomitrella patens. Plant Mol Biol 70(3):327–340

Kudapa H, Ramalingam A, Nayakoti S, Chen X, Zhuang W-J, Liang X, Kahl G, Edwards D, Varshney RK (2013) Functional genomics to study stress responses in crop legumes: progress and prospects. Funct Plant Biol 40(12):1221–1233

Kurowska M, Daszkowska-Golec A, Gruszka D, Marzec M, Szurman M, Szarejko I, Maluszynski M (2011) TILLING-a shortcut in functional genomics. J Appl Genet 52(4):371–390

Larkindale J, Vierling E (2008) Core genome responses involved in acclimation to high temperature. Plant Physiol 146(2):748–761

Lee J-Y, Lee D-H (2003) Use of serial analysis of gene expression technology to reveal changes in gene expression in Arabidopsis pollen undergoing cold stress. Plant Physiol 132(2):517–529

Lee S-C, Kim J-Y, Kim S-H, Kim S-J, Lee K, Han S-K, Choi H-S, Jeong D-H, An G, Kim S-R (2004) Trapping and characterization of cold-responsive genes from T-DNA tagging lines in rice. Plant Science 166(1):69–79

Li S, Zhou X, Chen L, Huang W, Yu D (2010) Functional characterization of Arabidopsis thaliana WRKY39 in heat stress. Mol Cells 29(5):475–483

Liu J, Zhu J-K (1998) A calcium sensor homolog required for plant salt tolerance. Science 280(5371):1943–1945

Liu J, Ishitani M, Halfter U, Kim C-S, Zhu J-K (2000) The Arabidopsis thaliana SOS2 gene encodes a protein kinase that is required for salt tolerance. Proc Natl Acad Sci 97(7): 3730–3734

Liu HT, Gao F, Li GL, Han JL, Liu DL, Sun DY, Zhou RG (2008) The calmodulin-binding protein kinase 3 is part of heat-shock signal transduction in Arabidopsis thaliana. Plant J 55(5):760–773

LIU HC, LIAO HT, CHARNG YY (2011) The role of class A1 heat shock factors (HSFA1s) in response to heat and other stresses in Arabidopsis. Plant Cell Environ 34(5):738–751

Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and global crop production since 1980. Science 333(6042):616–620

Lopez-Molina L, Mongrand S, Chua N-H (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. Proc Natl Acad Sci 98(8):4782–4787

Louis P, Galinski EA (1997) Characterization of genes for the biosynthesis of the compatible solute ectoine from Marinococcus halophilus and osmoregulated expression in Escherichia coli. Microbiology 143(4):1141–1149

Loukehaich R, Wang T, Ouyang B, Ziaf K, Li H, Zhang J, Lu Y, Ye Z (2012) SpUSP, an annexin-interacting universal stress protein, enhances drought tolerance in tomato. J Exp Bot 63(15): 5593–5606

Luo M, Liu J, Lee RD, Scully BT, Guo B (2010) Monitoring the expression of maize genes in developing kernels under drought stress using oligo-microarray. J Integr Plant Biol 52(12): 1059–1074

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 324(5930):1064–1068

Macas J, Lambert GM, Dolezel D, Galbraith DW (1998) Nuclear expressed sequence tag(NEST) analysis: A novel means to study transcription through amplification of nuclear RNA. Cytometry 33(4):460–468

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444(2):139–158

Manickavelu A, Kawaura K, Oishi K, Shin T, Kohara Y, Yahiaoui N, Keller B, Abe R, Suzuki A, Nagayama T (2012) Comprehensive functional analyses of expressed sequence tags in common wheat (Triticum aestivum). DNA Res 19(2):165–177

Mantri NL, Ford R, Coram TE, Pang EC (2010) Evidence of unique and shared responses to major biotic and abiotic stresses in chickpea. Environ Exp Bot 69(3):286–292

Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome Res 18(9):1509–1517

Maruyama K, Takeda M, Kidokoro S, Yamada K, Sakuma Y, Urano K, Fujita M, Yoshiwara K, Matsukura S, Morishita Y (2009) Metabolic pathways involved in cold acclimation identified by integrated analysis of metabolites and transcripts regulated by DREB1A and DREB2A. Plant Physiol 150(4):1972–1980

Matsumura H, Nirasawa S, Terauchi R (1999) Transcript profiling in rice (Oryza sativa L.) seedlings using serial analysis of gene expression (SAGE). Plant J 20(6):719–726

Matsumura H, Reich S, Ito A, Saitoh H, Kamoun S, Winter P, Kahl G, Reuter M, Krüger DH, Terauchi R (2003) Gene expression analysis of plant host–pathogen interactions by SuperSAGE. Proc Natl Acad Sci 100(26):15718–15723

Matsumura H, Ito A, Saitoh H, Winter P, Kahl G, Reuter M, Krüger DH, Terauchi R (2005) SuperSAGE. Cell Microbiol 7(1):11–18

Matsumura H, Yoshida K, Luo S, Kimura E, Fujibe T, Albertyn Z, Barrero RA, Krüger DH, Kahl G, Schroth GP (2010) High-throughput SuperSAGE for digital gene expression analysis of multiple samples using next generation sequencing. PLoS One 5(8):e12010

McCallum CM, Comai L, Greene EA, Henikoff S (2000) Targeting induced local lesions in genomes (TILLING) for plant functional genomics. Plant Physiol 123(2):439–442

McClung CR, Davis SJ (2010) Ambient thermometers in plants: from physiological outputs towards mechanisms of thermal sensing. Curr Biol 20(24):R1086–R1092

McCue KF, Hanson AD (1990) Drought and salt tolerance: towards understanding and application. Trends Biotechnol 8:358–362

McGinnis KM (2010) RNAi for functional genomics in plants. Brief Funct Genomics 9(2): 111–117

McInnis SM, Desikan R, Hancock JT, Hiscock SJ (2006) Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signalling crosstalk? New Phytol 172(2):221–228

McKersie BD, Bowley SR (1997) Active oxygen and freezing tolerance in transgenic plants. In: Li PH, Chen THH (eds) Plant cold hardiness. Plenum, New York, pp 203–214

Melcher K, Ng L-M, Zhou XE, Soon F-F, Xu Y, Suino-Powell KM, Park S-Y, Weiner JJ, Fujii H, Chinnusamy V (2009) A gate–latch–lock mechanism for hormone signalling by abscisic acid receptors. Nature 462(7273):602–608

Mette M, Aufsatz W, Van der Winden J, Matzke M, Matzke A (2000) Transcriptional silencing and promoter methylation triggered by double-stranded RNA. EMBO J 19(19):5194–5201

Miki D, Shimamoto K (2004) Simple RNAi vectors for stable and transient suppression of gene function in rice. Plant Cell Physiol 45(4):490–495

Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R (2009) The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. Sci Signal 2(84):ra45

Mishkind M, Vermeer JE, Darwish E, Munnik T (2009) Heat stress activates phospholipase D and triggers PIP2 accumulation at the plasma membrane and nucleus. Plant J 60(1):10–21

Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and perspectives. Annu Rev Plant Biol 61:443–462

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9(10):490–498

Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K, Gollery M, Shulaev V, Van Breusegem F (2011) ROS signaling: the new wave? Trends Plant Sci 16(6):300–309

Mittler R, Finka A, Goloubinoff P (2012) How do plants feel the heat? Trends Biochem Sci 37(3):118–125

Molina C, Rotter B, Horres R, Udupa S, Besser B, Bellarmino L, Baum M, Matsumura H, Terauchi R, Kahl G, Winter P (2008) SuperSAGE: the drought stress-responsive transcriptome of chickpea roots. BMC Genomics 9(1):553

Molina C, Zaman-Allah M, Khan F, Fatnassi N, Horres R, Rotter B, Steinhauer D, Amenc L, Drevon J-J, Winter P, Kahl G (2011) The salt-responsive transcriptome of chickpea roots and nodules via deepSuperSAGE. BMC Plant Biol 11(1):31

Møller IM, Sweetlove LJ (2010) ROS signalling–specificity is required. Trends Plant Sci 15(7):370–374

Monshausen G, Bibikova T, Messerli M, Shi C, Gilroy S (2007) Oscillations in extracellular pH and reactive oxygen species modulate tip growth of Arabidopsis root hairs. Proc Natl Acad Sci 104(52):20996–21001

Mustilli A-C, Merlot S, Vavasseur A, Fenzi F, Giraudat J (2002) Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. Plant Cell 14(12):3089–3099

Nagalakshmi U, Wang Z, Waern K, Shou C, Raha D, Gerstein M, Snyder M (2008) The transcriptional landscape of the yeast genome defined by RNA sequencing. Science 320(5881): 1344–1349

Nakano M, Nobuta K, Vemaraju K, Tej SS, Skogen JW, Meyers BC (2006) Plant MPSS databases: signature-based transcriptional resources for analyses of mRNA and small RNA. Nucleic Acids Res 34(suppl 1):D731–D735

Negrão S, Cecília Almadanim M, Pires IS, Abreu IA, Maroco J, Courtois B, Gregorio GB, McNally KL, Margarida Oliveira M (2013) New allelic variants found in key rice salt-tolerance genes: an association study. Plant Biotechnol J 11(1):87–100

Nielsen KL, Høgh AL, Emmersen J (2006) DeepSAGE—digital transcriptomics with high sensitivity, simple experimental protocol and multiplexing of samples. Nucleic Acids Res 34(19):e133

Nishimura N, Hitomi K, Arvai AS, Rambo RP, Hitomi C, Cutler SR, Schroeder JI, Getzoff ED (2009) Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. Science 326(5958):1373–1379

Nishimura N, Sarkeshik A, Nito K, Park SY, Wang A, Carvalho PC, Lee S, Caddell DF, Cutler SR, Chory J (2010) PYR/PYL/RCAR family members are major in vivo ABI1 protein phosphatase 2C-interacting proteins in Arabidopsis. Plant J 61(2):290–299

Niu X, Bressan RA, Hasegawa PM, Pardo JM (1995) Ion homeostasis in NaCl stress environments. Plant Physiol 109(3):735

Novillo F, Alonso JM, Ecker JR, Salinas J (2004) CBF2/DREB1C is a negative regulator of CBF1/DREB1B and CBF3/DREB1A expression and plays a central role in stress tolerance in Arabidopsis. Proc Natl Acad Sci U S A 101(11):3985–3990

Nunes AS, Vianna G, Cuneo F, Amaya-Farfán J, Capdeville G, Rech E, Aragão FL (2006) RNAi-mediated silencing of the myo-inositol-1-phosphate synthase gene (GmMIPS1) in transgenic soybean inhibited seed development and reduced phytate content. Planta 224(1):125–132. doi:10.1007/s00425-005-0201-0

O'Brien JA, Daudi A, Finch P, Butt VS, Whitelegge JP, Souda P, Ausubel FM, Bolwell GP (2012) A peroxidase-dependent apoplastic oxidative burst in cultured Arabidopsis cells functions in MAMP-elicited defense. Plant Physiol 158(4):2013–2027

Odell JT, Nagy F, Chua N-H (1985) Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature 313(6005):810–812

Pandey S, Nelson DC, Assmann SM (2009) Two novel GPCR-type g proteins are abscisic acid receptors in *Arabidopsis*. Cell 136(1):136–148

Papdi C, Ábrahám E, Joseph MP, Popescu C, Koncz C, Szabados L (2008) Functional identification of Arabidopsis stress regulatory genes using the controlled cDNA overexpression system. Plant Physiol 147(2):528–542

Parinov S, Sevugan M, Ye D, Yang W-C, Kumaran M, Sundaresan V (1999) Analysis of flanking sequences from dissociation insertion lines: a database for reverse genetics in Arabidopsis. Plant Cell 11(12):2263–2270

Park S-Y, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF et al (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science 324(5930):1068–1071

Pollock JD (2002) Gene expression profiling: methodological challenges, results, and prospects for addiction research. Chem Phys Lipids 121(1):241–256

Pospíšilová J (2003) Participation of phytohormones in the stomatal regulation of gas exchange during water stress. Biol Planta 46(4):491–506

Qin D, Wu H, Peng H, Yao Y, Ni Z, Li Z, Zhou C, Sun Q (2008) Heat stress-responsive transcriptome analysis in heat susceptible and tolerant wheat (Triticum aestivum L.) by using Wheat Genome Array. BMC Genomics 9(1):432

Qiu Q-S, Guo Y, Dietrich MA, Schumaker KS, Zhu J-K (2002) Regulation of SOS1, a plasma membrane Na+/H+ exchanger in Arabidopsis thaliana, by SOS2 and SOS3. Proc Natl Acad Sci 99(12):8436–8441

Radhamony RN, Mohan Prasad A, Srinivasan R (2005) T-DNA insertional mutagenesis in Arabidopsis: a tool for functional genomics. Electron J Biotechnol 8(1):82–106

Ranjan A, Pandey N, Lakhwani D, Dubey NK, Pathre UV, Sawant SV (2012) Comparative transcriptomic analysis of roots of contrasting Gossypium herbaceum genotypes revealing adaptation to drought. BMC Genomics 13(1):680

Reinartz J, Bruyns E, Lin J-Z, Burcham T, Brenner S, Bowen B, Kramer M, Woychik R (2002) Massively parallel signature sequencing (MPSS) as a tool for in-depth quantitative gene expression profiling in all organisms. Brief Funct Genomic Proteomic 1(1):95–104

Rensink WA, Buell CR (2005) Microarray expression profiling resources for plant genomics. Trends Plant Sci 10(12):603–609

Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol 141(2): 357–366

Ribaut J-M, Hoisington D, Deutsch J, Jiang C, Gonzalez-de-Leon D (1996) Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. Theor Appl Genet 92(7):905–914

Ribaut J-M, Jiang C, Gonzalez-de-Leon D, Edmeades G, Hoisington D (1997) Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor Appl Genet 94(6–7):887–896

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R (2004) When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiol 134(4):1683–1696

Rodrigues A, Adamo M, Crozet P, Margalha L, Confraria A, Martinho C, Elias A, Rabissi A, Lumbreras V, González-Guzmán M (2013) ABI1 and PP2CA phosphatases are negative regulators of Snf1-related protein kinase1 signaling in Arabidopsis. Plant Cell 25(10):3871–3884

Ruelland E, Zachowski A (2010) How plants sense temperature. Environ Exp Bot 69(3):225–232

Rus A, Yokoi S, Sharkhuu A, Reddy M, B-h L, Matsumoto TK, Koiwa H, Zhu J-K, Bressan RA, Hasegawa PM (2001) AtHKT1 is a salt tolerance determinant that controls Na+ entry into plant roots. Proc Natl Acad Sci 98(24):14150–14155

Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratmann JW, Fluhr R (2004) Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in Lycopersicon esculentum. Plant Cell 16(3):616–628

Saha S, Sparks AB, Rago C, Akmaev V, Wang CJ, Vogelstein B, Kinzler KW, Velculescu VE (2002) Using the transcriptome to annotate the genome. Nat Biotechnol 20(5):508–512

Saidi Y, Finka A, Muriset M, Bromberg Z, Weiss YG, Maathuis FJ, Goloubinoff P (2009) The heat shock response in moss plants is regulated by specific calcium-permeable channels in the plasma membrane. Plant Cell 21(9):2829–2843

Salekdeh G, Siopongco J, Wade LJ, Ghareyazie B, Bennett J (2002) Proteomic analysis of rice leaves during drought stress and recovery. Proteomics 2(9):1131–1145

Santiago J, Dupeux F, Round A, Antoni R, Park S-Y, Jamin M, Cutler SR, Rodriguez PL, Márquez JA (2009) The abscisic acid receptor PYR1 in complex with abscisic acid. Nature 462(7273):665–668

Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270(5235):467–470
Schwartz SH, Tan BC, Gage DA, Zeevaart JA, McCarty DR (1997) Specific oxidative cleavage of carotenoids by VP14 of maize. Science 276(5320):1872–1874
Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T (2002) Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J 31(3):279–292
Senthil-Kumar M, Mysore KS (2011) New dimensions for VIGS in plant functional genomics. Trends Plant Sci 16(12):656–665. doi:http://dx.doi.org/10.1016/j.tplants.2011.08.006
Shapiguzov A, Vainonen JP, Wrzaczek M, Kangasjärvi J (2012) ROS-talk–how the apoplast, the chloroplast, and the nucleus get the message through. Front Plant Sci 3
Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. J Exp Bot 58(2):221–227
Sreenivasulu N, Sopory S, Kavi Kishor P (2007) Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. Gene 388(1):1–13
Steponkus PL, Uemura M, Webb MS (1993) A contrast of the cryostability of the plasma membrane of winter rye and spring oat-two species that widely differ in their freezing tolerance and plasma membrane lipid composition. In: Steponkus PL (ed) Advances in low-temperature biology, vol 2. JAI, London, pp 211–312
Sunkar R, Chinnusamy V, Zhu J, Zhu J-K (2007) Small RNAs as big players in plant abiotic stress responses and nutrient deprivation. Trends Plant Sci 12(7):301–309
Suzuki N, Bajad S, Shuman J, Shulaev V, Mittler R (2008) The transcriptional co-activator MBF1c is a key regulator of thermotolerance in Arabidopsis thaliana. J Biol Chem 283(14): 9269–9275
Suzuki N, Miller G, Morales J, Shulaev V, Torres MA, Mittler R (2011) Respiratory burst oxidases: the engines of ROS signaling. Curr Opin Plant Biol 14(6):691–699
Suzuki N, Koussevitzky S, Mittler R, Miller G (2012) ROS and redox signalling in the response of plants to abiotic stress. Plant Cell Environ 35(2):259–270
Takeda S, Gapper C, Kaya H, Bell E, Kuchitsu K, Dolan L (2008) Local positive feedback regulation determines cell shape in root hair cells. Science 319(5867):1241–1244
Teige M, Scheikl E, Eulgem T, Dóczi R, Ichimura K, Shinozaki K, Dangl JL, Hirt H (2004) The MKK2 pathway mediates cold and salt stress signaling in *Arabidopsis*. Mol Cell 15(1): 141–152
Thakur P, Kumar S, Malik JA, Berger JD, Nayyar H (2010) Cold stress effects on reproductive development in grain crops: an overview. Environ Exp Bot 67(3):429–443
Till BJ, Reynolds SH, Weil C, Springer N, Burtner C, Young K, Bowers E, Codomo CA, Enns LC, Odden AR (2004) Discovery of induced point mutations in maize genes by TILLING. BMC Plant Biol 4(1):12
Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol 8(4):397–403
Tougane K, Komatsu K, Bhyan SB, Sakata Y, Ishizaki K, Yamato KT, Kohchi T, Takezawa D (2010) Evolutionarily conserved regulatory mechanisms of abscisic acid signaling in land plants: characterization of ABSCISIC ACID INSENSITIVE1-like type 2C protein phosphatase in the liverwort Marchantia polymorpha. Plant Physiol 152(3):1529–1543
Uemura M, Steponkus PL (1997) Effect of cold acclimation on membrane lipid composition and freeze-induced membrane destabilization. In: Li PH, Chen THH (eds) Plant cold hardiness. Plenum, New York, pp 171–179
Uozumi N, Kim EJ, Rubio F, Yamaguchi T, Muto S, Tsuboi A, Bakker EP, Nakamura T, Schroeder JI (2000) The Arabidopsis HKT1 gene homolog mediates inward Na+ currents in Xenopus laevis oocytes and Na+ uptake in Saccharomyces cerevisiae. Plant Physiol 122(4):1249–1260
Urano K, Kurihara Y, Seki M, Shinozaki K (2010) 'Omics' analyses of regulatory networks in plant abiotic stress responses. Curr Opin Plant Biol 13(2):132–138
Utsumi Y, Tanaka M, Morosawa T, Kurotani A, Yoshida T, Mochida K, Matsui A, Umemura Y, Ishitani M, Shinozaki K (2012) Transcriptome analysis using a high-density oligomicroarray

under drought stress in various genotypes of cassava: an important tropical crop. DNA Res 19(4):335–345
Velculescu VE, Zhang L, Vogelstein B, Kinzler KW (1995) Serial analysis of gene expression. Science 270(5235):484–486
Verdel A, Vavasseur A, Le Gorrec M, Touat-Todeschini L (2009) Common themes in siRNA-mediated epigenetic silencing pathways. Int J Dev Biol 53(2):245–257
Vij S, Tyagi AK (2007) Emerging trends in the functional genomics of the abiotic stress response in crop plants. Plant Biotechnol J 5(3):361–380
Vodkin LO, Khanna A, Shealy R, Clough SJ, Gonzalez DO, Philip R, Zabala G, Thibaud-Nissen F, Sidarous M, Strömvik MV (2004) Microarrays for global expression constructed with a low redundancy set of 27,500 sequenced cDNAs representing an array of developmental stages and physiological conditions of the soybean plant. BMC Genomics 5(1):73
Wahid A, Gelani S, Ashraf M, Foolad M (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61(3):199–223
Walia H, Wilson C, Condamine P, Liu X, Ismail AM, Zeng L, Wanamaker SI, Mandal J, Xu J, Cui X (2005) Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. Plant Physiol 139(2):822–835
Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218(1):1–14
Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9(5):244–252
Wen J-Q, Oono K, Imai R (2002) Two novel mitogen-activated protein signaling components, OsMEK1 and OsMAP1, are involved in a moderate low-temperature signaling pathway in rice. Plant Physiol 129(4):1880–1891
Wienkoop S, Morgenthal K, Wolschin F, Scholz M, Selbig J, Weckwerth W (2008) Integration of metabolomic and proteomic phenotypes analysis of data covariance dissects starch and RFO metabolism from low and high temperature compensation response in Arabidopsis thaliana. Mol Cell Proteomics 7(9):1725–1736
Wohlbach DJ, Quirino BF, Sussman MR (2008) Analysis of the Arabidopsis histidine kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. Plant Cell 20(4):1101–1117
Wrzaczek M, Brosché M, Kangasjärvi J (2013) ROS signaling loops—production, perception, regulation. Curr Opin Plant Biol 16(5):575–582
Xia Y, Suzuki H, Borevitz J, Blount J, Guo Z, Patel K, Dixon RA, Lamb C (2004) An extracellular aspartic protease functions in Arabidopsis disease resistance signaling. EMBO J 23(4): 980–988
Xiong L, Zhu J-K (2003) Regulation of abscisic acid biosynthesis. Plant Physiol 133(1):29–36
Xin Z, Wang ML, Barkley NA, Burow G, Franks C, Pederson G, Burke J (2008) Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically induced sorghum mutant population. BMC Plant Biology 8 (1):103
Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6(2):251–264
Yamaguchi-Shinozaki K, Shinozaki K (2005) Organization of *cis*-acting regulatory elements in osmotic-and cold-stress-responsive promoters. Trends Plant Sci 10(2):88–94
Yamamoto K, Sasaki T (1997) Large-scale EST sequencing in rice. Plant Mol Biol 35(1–2): 135–144
Yamamoto YY, Tsuhara Y, Gohda K, Suzuki K, Matsui M (2003) Gene trapping of the Arabidopsis genome with a firefly luciferase reporter. Plant J 35(2):273–283
Yan S, Tang Z, Su W, Sun W (2005) Proteomic analysis of salt stress-responsive proteins in rice root. Proteomics 5(1):235–244
Yoshida R, Hobo T, Ichimura K, Mizoguchi T, Takahashi F, Aronso J, Ecker JR, Shinozaki K (2002) ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in Arabidopsis. Plant Cell Physiol 43(12):1473–1483

Yu H, Chen X, Hong Y-Y, Wang Y, Xu P, Ke S-D, Liu H-Y, Zhu J-K, Oliver DJ, Xiang C-B (2008) Activated expression of an Arabidopsis HD-START protein confers drought tolerance with improved root system and reduced stomatal density. Plant Cell 20(4):1134–1151

Yu S, Liao F, Wang F, Wen W, Li J, Mei H, Luo L (2012) Identification of rice transcription factors associated with drought tolerance using the ecotilling method. PLoS One 7(2):e30765

Yuan L, Liu X, Luo M, Yang S, Wu K (2013) Involvement of histone modifications in plant abiotic stress responses. J Integr Plant Biol 55(10):892–901

Zamore PD, Tuschl T, Sharp PA, Bartel DP (2000) RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell 101(1):25–33

Zhang H, Sreenivasulu N, Weschke W, Stein N, Rudd S, Radchuk V, Potokina E, Scholz U, Schweizer P, Zierold U (2004) Large-scale analysis of the barley transcriptome based on expressed sequence tags. Plant J 40(2):276–290

Zhang W, Zhou R-G, Gao Y-J, Zheng S-Z, Xu P, Zhang S-Q, Sun D-Y (2009) Molecular and genetic evidence for the key role of AtCaM3 in heat-shock signal transduction in Arabidopsis. Plant Physiol 149(4):1773–1784

Zinn KE, Tunc-Ozdemir M, Harper JF (2010) Temperature stress and plant sexual reproduction: uncovering the weakest links. J Exp Bot 61(7):1959–1968