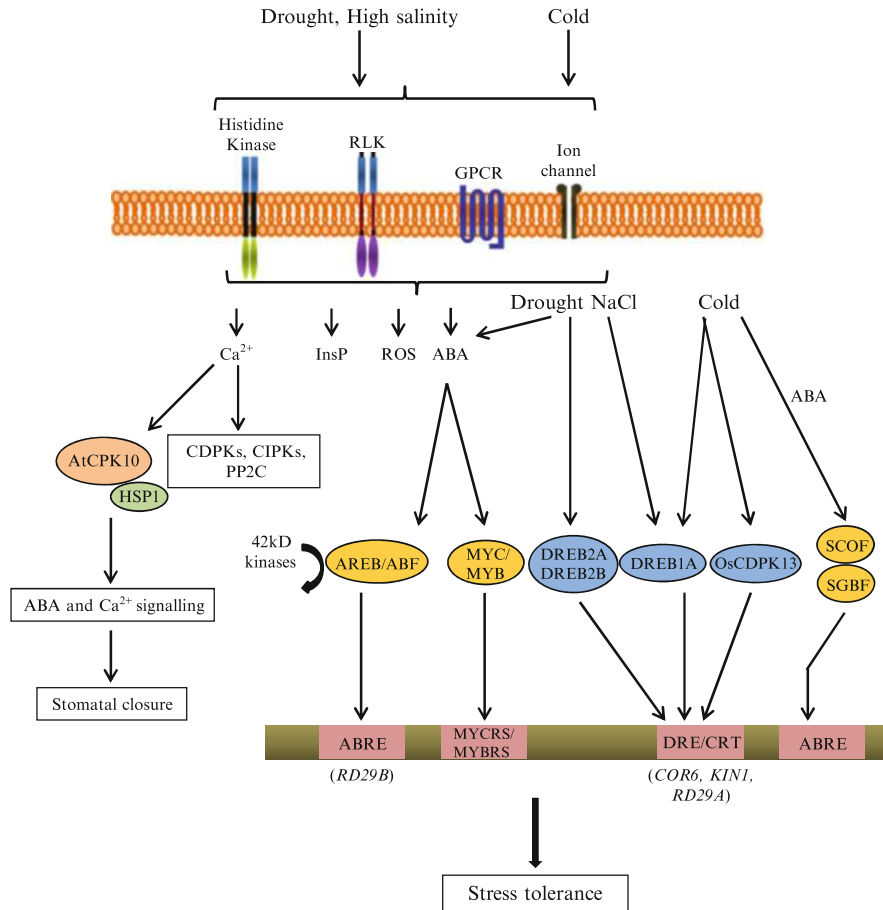


to decode and understand these signaling networks has increased our knowledge regarding signaling pathways. In this review, we have discussed the recent findings in plant signaling pathways during various stresses and the specificity and points of cross-talk between these signaling pathways.

## Abiotic Stresses

### *Drought*

Drought is a condition when there is water deficit in the atmosphere and soil. Plants undergo a series of physiological and biochemical events during drought stress. These responses result in closing of stomata, fall in transpiration rate, suppression of photosynthesis and cell growth, decrease in osmotic potential of plant tissues and activation of respiration. Additionally, there are also adaptations at cellular and molecular levels. Plants accumulate different kinds of osmolytes (viz. mannitol, proline, sorbitol, trehalose, fructans), phytohormone abscisic acid (ABA) and also synthesize new stress tolerating proteins. Studies using microarray analysis identified a number of stress responsive genes in *Arabidopsis* and rice. A total of 299 drought-inducible genes were identified after expression profiling of 1,300 genes using a full-length cDNA microarray in *Arabidopsis* (Seki et al. 2002). More than 50 % of these drought-inducible genes were found induced in response to high salinity and/or ABA treatments. Microarray analysis in rice using 1700 independent cDNAs revealed 73 stress inducible genes (Rabbani et al. 2003). Approximately 40 % of drought-inducible genes were also found induced during cold stress. Moreover, almost all the genes expressed during high salinity and ABA treatment were also induced during drought stress. These results clearly show highly substantial cross-talk between the signaling pathways generated during various stresses, such as, drought, cold, high salinity and ABA treatment. The drought-responsive genes can be divided into two groups on the basis of their biological functions. The first group consists of the genes encoding functional proteins involved in membrane and protein stabilization and cellular homeostasis, such as heat shock proteins (HSPs), late embryogenesis abundant (LEA) proteins and lipid transfer proteins (LTPs). The second group includes Calcineurin-B-Interacting Protein Kinases (CIPK16) and Protein Phosphatases class 2C (PP2C) which function as signal molecules in response to stress (Boominathan et al. 2004; Ok et al. 2005). Therefore, the putative mechanism of drought tolerance can be understood on the basis of the expression patterns of these genes encoding functional and regulatory proteins. Recently, high-throughput Roche 454 sequencing was combined with SuperSAGE and revealed the drought-responsive transcriptome of chickpea roots (Molina et al. 2008). This was quite helpful to overcome the problem of lacking EST and genomic data in a non-model crop plant chickpea. From drought-stressed and



**Fig. 2.1** Schematic representation of signaling during abiotic stresses. Upon stress signal perception, various signaling cascades get activated leading to differential gene expression for generating appropriate stress tolerance

non-stressed control roots, a total of 17,493 unique transcripts were obtained which included genes involved in stress-perception, signaling and transcription initiation.

The signaling pathways induced during drought are either ABA-dependent or ABA-independent (Fig. 2.1). ABA is a phytohormone that is produced during water scarcity. Under the effect of ABA plants become responsive and tolerant to drought and high salinity. Various genes induced during drought and cold stress also gets induced by application of ABA exogenously, indicating ABA-dependent signal transduction. On the other hand, several genes are there which respond to drought and cold stress but does not get induced by application of ABA exogenously, indicating the existence of ABA-independent signal transduction in *Arabidopsis* (Zhu 2002; Yamaguchi-Shinozaki and Shinozaki 2006).

### ABA-Independent Signal Transduction

Generally, transcription factors are involved in regulating the signaling pathways. Several transcription factors have been identified in *Arabidopsis* that gets activated during drought stress in ABA-independent manner. Recent studies depicted major role of DREB1 (dehydration-responsive element binding factor)/CBF (C-repeat binding factor) and DREB2 transcription factors during drought. Molina et al. (2008) identified 124 UniTags from transcription factor transcripts in chickpea against drought which were classified into 26 transcription factor families. Majority of them belonged to bZIP family of transcription factors along with HDZ, HMG and WRKY families. Moreover, transcripts of DREB2 transcription factors were also highly induced.

There are six *DREB1/CBF* genes and eight *DREB2* genes present in *Arabidopsis* genome (Sakuma et al. 2002). Among these genes, *DREB2A* and *DREB2B* get induced during dehydration and high salinity (Nakashima et al. 2000). However, they do not get induced during cold stress. This indicates the important role being played by DREB2/CBF protein in developing the stress tolerance against drought. Rice genome sequence analysis revealed presence of ten *DREB1/CBF* genes and four *DREB2* genes. When *OsDREB1A* was overexpressed in *Arabidopsis*, it showed a similar function in terms of gene expression and stress tolerance (Dubouzet et al. 2003). Overexpression of *OsDREB1* or *Arabidopsis DREB1* in rice made the transgenic plants more tolerant to drought and cold (Ito et al. 2006). This suggests that the similar transcription factors function in same way to improve the tolerance against the abiotic stress both in dicotyledonous and monocotyledonous plants.

### ABA-Dependent Signal Transduction

There are various drought and high salinity-inducible genes in *Arabidopsis* and rice that get induced in response to ABA (Seki et al. 2002; Rabbani et al. 2003). AREB (ABA-Responsive Element Binding factor) or ABFs (which are basically bZIP transcription factors) regulates transcription by binding to ABA-responsive complex present in the promoter region of most of the ABA-inducible genes. Overexpression of the phosphorylated active form of AREB1 leads to the expression of ABA-inducible genes (like *RD29B*) even in the absence of the ABA treatment (Furhata et al. 2006). This results in improved drought tolerance in transgenic plants suggesting that multisite phosphorylation of AREB1 in an ABA-dependent manner is required for its own activation. Its phosphorylation- and dephosphorylation-directed signal transduction is essential for ABA signaling.

Therefore, various studies carried out using transcriptomic approach concludes the 3generation of appropriate response against drought stress which begins with the perception of drought signals by various membrane receptors such as GPCRs, RLKs, histidine kinases and ion channels (Fig. 2.1). This brings changes in the

cytoplasmic  $\text{Ca}^{2+}$  levels and generates secondary signaling molecules (ABA, ROS, inositol phosphates, etc.). These secondary messengers initiate a protein phosphorylation cascade by various kinases like CDPKs, CIPKs, protein kinases and protein phosphatases. They further activate transcription factors such as DREB/CBF, AREB/ABF, bZIP, MYC/MYB, WRKY etc. through phosphorylation and dephosphorylation events. In turn, these transcription factors lead to the activation of numerous stress-responsive genes which includes genes encoding HSPs, LEA proteins, LTPs, antioxidants, osmolytes, ion transporters, etc. Finally, tolerance against drought stress is generated and the damages are repaired.

To investigate the initial response of rice against drought, a proteomic approach was used by Ali and Komatsu (2006). It revealed four novel drought-responsive mechanisms in the rice leaf blade; up-regulation of an S-like RNase homologue, an actin depolymerizing factor and RuBisCO activase, and down-regulation of an isoflavone reductase-like protein. Dehydration-responsive extracellular matrix (ECM) proteins were analysed in chickpea and rice (Bhushan et al. 2007; Pandey et al. 2010). ECM functions as a depot for numerous components of the cell signaling process and acts as a frontline defense. More than 100 differentially expressed ECM proteins were identified which consisted of predicted as well as novel dehydration-responsive proteins. These proteins were involved in various cellular functions such as cell wall modification, metabolism, signal transduction, cell defense and rescue.

Protein analysis is the most direct approach to describe the function of its associated gene. Therefore, proteome analysis linked to genome-sequence information proves to be a very powerful tool in functional genomics. In bread wheat, differential expression of 114 proteins in response to drought stress was revealed by proteomic analysis (Peng et al. 2009). Recent report in alfalfa leaf indicated that drought causes impairment of photosynthetic activity which was evident by down-regulation of Rubisco and proteins involved in Rubisco assembly (Aranjuelo et al. 2011). Recently, first shotgun proteomics study was performed to gain insights into protein responses to drought in wheat (Ford et al. 2011). This identified the largest number of differentially expressed wheat proteins (1,299) in a single study. Significant increase in the expression level of superoxide dismutases and catalases suggested an increase in oxidative stress metabolism and reactive oxygen species (ROS) scavenging capacity. There was decrease in the proteins involved in photosynthesis and Calvin cycle which suggest ROS avoidance by plants in response to drought. Therefore, there is a massive transcriptional and translational reprogramming which results in protecting and repairing the cell wall, modification of metabolism and signal transduction to generate appropriate response in order to make the plants tolerant against stress.

## *Cold*

Another very common environmental stress that unfavorably affects the plant growth and crop yield is cold i.e. low temperature. Although most of the plants are not quite tolerant to freezing temperatures, however, they can increase their tolerance against freezing temperatures by getting exposed to the chilling,

non-freezing temperatures. This process is known as cold acclimation. It restructures the cell membranes physically and biochemically by changing the lipid composition and inducing other non-enzymatic proteins. Recently, substantial transcriptomic studies have been carried out to elucidate the sensing and regulatory mechanisms of plants against cold that enables them to develop cold acclimation response.

Cold stress triggers multiple transcriptional cascades because many early cold-responsive genes encode transcription factors which subsequently activate the genes induced late in the cold response. There is a differential regulation of genes involved in biosynthesis or signaling of plant hormones such as ABA, gibberellic acid and auxin. The DNA replication, spliceosome and mismatch repair pathways were also found to be associated with cold stress (Rong et al. 2011). Transcriptome analysis of wheat suggests that there is a massive transcriptome reprogramming during the course of cold acclimation and gaining the freezing tolerance. It requires coordination of many different physiological and biochemical changes and is regulated through the differential expression of many genes (Winfield et al. 2010). Microarray analysis of the mitochondrial transcriptome in germinating wheat embryos and seedlings revealed differential regulation of an array of genes which results in increased transpiration rate both through cytochrome and alternative pathways. Few nuclear-encoded mitochondria-targeted genes suppressed while transcripts of *MnSOD* and *AOX* induced (Naydenov et al. 2010). This suggests involvement of both nuclear and mitochondrial genes in response to cold stress. In rice, microarray analyses revealed that few microRNA are derived from transposable element sequence. They get clustered within an intron and are co-transcribed with the host gene only under cold conditions. In the upstream regions of the cold-responsive microRNAs, hormone-responsive elements are present which indicates the importance of hormones in this microRNA mediated defense system (Lv et al. 2010). Therefore, in response to cold conditions the stress signals are transmitted to transcription factors which are encoded by early cold-responsive genes (Fig. 2.1). This activates various signaling cascades mediated by hormones and  $Ca^{2+}$ . The MAPK signal transduction also comes into action to relay the signals further. It leads to massive nuclear, mitochondrial and chloroplast transcriptome reprogramming which finally results in cold acclimation by bringing different physiological and biochemical changes. In signal transduction, CDPKs also play an important role. Studies shows that *OsCDPK13* (*Oryza sativa* CDPK 13) gets induced at gene and protein level in response to cold and gibberellin (GA) but not in response to drought, high salinity and ABA treatment (Abbasi et al. 2004). In low temperature signal transduction pathways, calcium plays a role as an important messenger. In response to low temperature there is rapid increase in cytoplasmic calcium levels. This is mainly due to an influx of calcium from extracellular reservoirs. It has been shown that calcium is necessary for the complete expression of some of the cold induced genes like the *CRT/DRE* controlled *COR6* and *KINI* genes in *Arabidopsis*.

Proteomic analysis in rice against cold stress showed high expression of molecular chaperones, proteases, energy pathway-linked proteins, enzymes

involved in protein biosynthesis, cell wall biosynthesis, detoxification and signal transduction components. Chaperones and proteases control the protein quality and enhance cell wall components which play important roles in developing the tolerance to cold. Two-dimensional difference gel electrophoresis (2-D DIGE) analysis of sub-cellular fractions of *Arabidopsis* proteome identified a number of early cold-responsive proteins such as HSPs, enzymes involved in starch degradation and sucrose metabolism (Li et al. 2011). During cold stress, some proteins show enhanced degradation especially the photosynthetic proteins such as large subunit of Rubisco (Yan et al. 2006). Similarly, there is down-regulation of the enzymes involved in Krebs cycle and Calvin cycle while ascorbate recycling gets increased (Rinalducci et al. 2011). Cold brings rapid metabolic changes which develop instant protection before temperature drops below freezing point. In sunflower, a total of 243 differentially expressed, non-redundant proteins were identified during cold acclimation (Balbuena et al. 2011). Therefore, in response to cold stress a large set of cellular proteins get differentially expressed and establish cold acclimation in plant.

### ***High Salinity***

Salinity affects a large area of the land every year globally and the affected area is increasing day by day. This makes salinity a major environmental stress that highly limits the crop production. Due to high salinity plants becomes unable to take up water. It rapidly leads to reductions in growth rate, together with a set of metabolic changes. These changes are identical to those caused by water stress. Salt specific impact can be seen when excessive salt enters inside the plant. The amount of salt rises above the toxic levels in the older transpiring leaves. This results in premature senescence and also reduces the photosynthetic leaf area of the plant, as a result of which it cannot sustain growth.

Barley is a well known salt-tolerant crop species. Transcript profiling during salinity stress was performed using a microarray containing ~22,750 probe sets (Walia et al. 2006). One of the prominent features of salinity stress response was the induction of jasmonic acid (JA) pathway genes and genes responsive to JA and ABA. Also, a large number of abiotic stress (drought, heat and cold) related genes were induced which support the existence of cross talk among certain components of abiotic stress responses. Suppression subtractive hybridization (SSH) and microarray approaches were also combined to identify the early salt stress response genes in tomato root (Ouyang et al. 2007). A total of 201 non-redundant genes were found differentially expressed upon 30 min of rigorous salt stress. These genes included the stress sensors, inducible transcriptional activators, upstream signal pathway components and HSPs. Several key components, such as PP2C and MAPKKK were also up-regulated. Recently, a novel CBF transcription factor MtCBF4 has been found highly induced during drought in *Medicago truncatula* (Li et al. 2011). In *Arabidopsis*, MEKK1 (a MAPKKK) mRNA gets highly

accumulated in response to stresses including salinity stress (Mizoguchi et al. 1996). There is protein-protein interactions between MEKK1 and MKK2/MEK1 (MAPKKs), between MKK2/MEK1 and MPK4 (a MAPK), and between MPK4 and MEKK1 (Ichimura et al. 1998). In response to environmental stress the signals are conveyed to at least two MAPK cascades. One is the MPK4 cascade (MEKK1-MEK1/MKK2-MPK4) and the other comprises MPK6 and p44MAPK (Ichimura et al. 2000). During salt or cold stress, MAPK pathway involves MEKK1 as an upstream activator of MKK2, however, MAPKs-MPK4 and MPK6 acts downstream (Teige et al. 2004). MAP kinase phosphatases (MKP1) play a negative role through MAPKs-MPK6 and MPK4 (Ulm et al. 2002). Therefore, the JA pathway gets induced as soon as the plant senses the salt stress. The cytosolic  $\text{Ca}^{2+}$  levels also get elevated which activates various signal transduction pathways including SOS (Salt Overly Sensitive) pathway. The transcription factors viz. DREB/CBF, AREB/ABF, bZIP, MYC/MYB also gets activated resulting in further downstream signaling cascade (Fig. 2.1). They help in maintaining the ion homeostasis and improve the tolerance of plants against salt stress.

Proteomic analysis of potato under salt stress revealed 47 differentially expressed proteins in shoot (Aghaei et al. 2008). Photosynthetic genes and protein synthesis-related proteins showed drastic down-regulation. In contrast, there was up-regulation in levels of osmotine-like proteins, TSI-1 protein, HSPs, protein inhibitors, calreticulin, and five novel proteins suggesting that induction of defense associated proteins provide relative salt tolerance to potato plants. In rice roots, changes in plasma-membrane-associated proteins were investigated (Cheng et al. 2009). Change of more than 1.5-fold in the expression of 18 proteins was also reported. The nine up-regulated proteins were found to be involved in membrane stabilization, ion homeostasis and signal transduction. Alterations in the protein phosphorylation patterns have also been detected (Chitteti and Peng 2007). Recently, a salt stress-responsive protein with a putative function in stress signaling has been located in the apoplast of the rice root (Zhang et al. 2009). It indicates the importance of ion uptake, transportation and regulation of signal transduction in the root. The proteome of the root differs for the tolerant and susceptible genotypes of a plant (Manaa et al. 2011). The metabolism-related proteins also play an important role in each organ (e.g. leaves, hypocotyls and roots) in order to achieve adaptation to salinity stress (Sobhanian et al. 2010). Even during the course of seed germination, salt stress induces an array of proteins which generate adequate tolerance (Xu et al. 2011). Therefore, the plants improve tolerance against salinity stress by developing a more efficient osmotic and ionic homeostasis, superior capacity to remove toxic byproducts, and finally a better potential for growth recovery. All these processes involve proteins regulating energy metabolism, ROS scavenging, cytoskeleton stability, protein processing and folding, photosynthesis, photorespiration and signal transduction.

### ***Other Abiotic Stresses***

Together with above mentioned stresses, other stresses encountered by plants include high temperature, flood and presence of heavy metals. Heat stress has detrimental effects on plant physiology and development by affecting cellular components and metabolism. A number of transcriptomic and proteomic studies have been carried out in order to gain better insights. GeneChip Wheat Genome Array of heat susceptible and tolerant variety showed differential expression of large number genes which includes HSPs, transcription factors and other stress related genes (Qin et al. 2008). Involvement of these genes suggests that multiple processes and mechanisms are responsible for developing heat tolerance in a plant. Genomic organization and transcript profiling of heat shock factors (HSFs) were studied in rice in response to high temperature (Mittal et al. 2009). Several *OsHsf* genes were highly induced suggesting that they are critical for the transcriptional induction of heat shock genes. Another approach using cDNA-AFLP and microarray analysis in tomato plants showed different constitutive gene expression profile in heat-tolerant genotype as compared to the heat-sensitive genotype (Bita et al. 2011). These genes consist of genes related to heat shock, metabolism, antioxidant and development pathways indicating substantial genetic differences in adaptation in response to high temperatures. Proteomic analysis of wheat grain revealed up-regulation of various enzymes involved in starch synthesis pathway, carbohydrate metabolism, small HSPs and translation initiation factors (Majoul et al. 2004). Similarly, proteomic approach in rice detected several low molecular weight small HSPs and antioxidant enzymes with high induction (Lee et al. 2007). Heat stress also induces the dephosphorylation of RuBisCo and the phosphorylation of ATP- $\beta$  in rice resulting in decreased activities of RuBisCo and ATP synthase (Chen et al. 2011). This shows involvement of phosphoproteins in the transduction of heat-stress signaling.

Flooding is another recurrent natural disaster causing plant hypoxia which limits the plant yield worldwide. Transcriptome analysis of maize roots showed up-regulation of a large number of genes in response to waterlogging which were related to signal transduction, protein degradation, carbon and amino acid metabolism, ion transport, and transcriptional and translational regulation (Zou et al. 2010). These genes were grouped into two response processes i.e. defense at the early stage and adaption at the late stage. Crosstalk between carbon and amino acid metabolism provides evidence for the two main roles of amino acid metabolism at the late stage. These are the regulations of cytoplasmic pH and energy supply. During prolonged waterlogging, tolerance-related genes are activated for survival via signal transduction. Another study using microarray analysis of roots of soybean seedlings revealed more than 6,000 flooding-responsive genes which were related to photosynthesis, glycolysis, amino acid synthesis, transcriptional regulation, ubiquitin-mediated protein degradation and cell death (Nanjo et al. 2011). On the other hand, there was down-regulation of genes involved in cell wall synthesis, secondary metabolism, metabolite transport, cell organization and chromatin structure synthesis.



These findings suggest that transcriptional and post-transcriptional regulations resulting in enhanced acclimative responses against flooding protect the soybean seedlings from flooding injury. Proteomic analysis of soybean roots revealed up-regulation of storage proteins and energy producing proteins (Komatsu et al. 2010b). Comparative proteomics analysis of soybean cell wall showed differential expression of many proteins. It concludes that the lignification in the soybean roots and hypocotyls gets suppressed by down-regulation of reactive oxygen species and JA biosynthesis during flooding (Komatsu et al. 2010a). Several proteomics studies have been carried out on specific cellular organelles in plants in response to various abiotic stresses, such as mitochondria, nucleus, chloroplasts, cell wall and the plasma membrane. Hossain et al. (2012) has provided an excellent detailed review in this regard.

Plants require heavy metals, such as iron (Fe), copper (Cu), manganese (Mn), molybdenum (Mo), cobalt (Co) and zinc (Zn), as essential micronutrients in small quantities. They are involved in essential biological functions like redox reactions and acts as enzyme cofactors. However, higher concentrations of these metals can be toxic. Some other heavy metals have no function as nutrients and are very toxic. For example cadmium (Cd), mercury (Hg), lead (Pb), aluminum (Al) and arsenic (As) lead to ROS production and inactivation of enzymes. Microarray analysis in rice against cadmium stress revealed differential expression of several genes encoding cytochrome P450 family proteins, HSPs, glutathione S-transferase, protein kinases, ion transporters and transcription factors such as DREB and NAC. This suggests the role of transporters in Cd detoxification by exporting Cd from the cytoplasm (Ogawa et al. 2009). Similarly, microarray analysis of *B. carinata* seedlings after lithium chloride exposure revealed differential expression of genes involved in defense, primary metabolism, transcription, transportation and secondary metabolism (Li et al. 2009). Various proteomics studies carried out in different plants against cadmium stress revealed high induction of proteins involved in photosynthetic pathways, transcription, translation and encoding molecular chaperones (Zhao et al. 2011).

### ***Biotic Stresses***

Plants are constantly exposed to a variety of potential microbial pathogens such as fungi, bacteria, oomycetes, nematodes and herbivores. In order to defend themselves plants have developed a variety of defense responses many of which are induced by pathogen attack. Penetration of the cell wall exposes the microbes to the plant plasma membrane, where they encounter extracellular surface receptors that recognize pathogen-associated molecular patterns (PAMPs). Recognition a microbe at the cell surface initiates PAMP-triggered immunity (PTI), which usually halts infection before the pathogen gains a hold in the plant. However, pathogenic microbes have evolved the means to suppress PTI by secreting specialized proteins, called as effectors, into the plant cell cytosol that alter resistance signaling or