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REVIEW

An insight into the drought stress induced alterations in plants

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Abstract

Plants are subjected to several abiotic stresses that adversely affect growth, metabolism and yield. The dynamic research in plant genetics complemented by genome sequencing has opened up avenues to address multiple problems caused by abiotic stresses. Though many drought-induced genes have been phytoengineered in a wide range of plants, the drought signal transduction pathways, and the alteration of plant sensing and signaling systems to adverse environments still remain an intriguing subject for comprehensive investigation. To impart enhanced drought tolerance in plants, a thorough perception of physiological, biochemical and gene regulatory networks is essential. Recent functional genomics tools have facilitated the progress in our understanding of stress signaling and of the linked molecular regulatory networks. This has revealed several stress-inducible genes and various transcription and signaling factors that regulate the drought stress-inducible systems. Translational genomics of these drought specific genes using model plants have provided encouraging outcomes, but the in-depth knowledge of the specific roles of various metabolites in plant stress tolerance will lead to evolvement of strategies for the phytoengineering of drought tolerance in plants in future.

Additional key words: functional genomics, phytoengineering, signal transduction, transcription factors.

Introduction

Plants are exposed to abiotic and biotic stresses that influence their growth and development. Water deficit is a major abiotic factor affecting global crop yield (Manavalan *et al.* 2009). Drought stress has been known to induce a sequence of morphological, biochemical and molecular alterations that negatively affect plant growth and productivity (Wang *et al.* 2001). The occurrence of drought is widespread across many parts of the world (Ludlow and Muchow 1990). It seems the worldwide losses in crop yields from water deficit probably exceed the cumulative loss of all other stresses (Kramer 1983). With ever increasing water limitation, the development of drought-tolerant cultivars will become increasingly important. Thus, understanding of the genetic and molecular mechanisms controlling drought response has become vital in evolving strategies to enhance drought tolerance in crop plants.

Although the universal effects of drought on plant growth are fairly understood, the primary effects of water deficit at the biochemical and molecular level are not well characterized (Zhu 2002, Chaitanya *et al.* 2003, Chaves *et al.* 2003). The net photosynthetic rate decreases with the reduction in the relative water content (RWC) and leaf water potential (ψ_w) (Lawlor and Cornic 2002).

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Abbreviations: ABA - abscisic acid; ABF - ABRE binding factor; ADC - arginine decarboxylase; ALR - aldose/aldehyde reductase; ATAF1,2 - *Arabidopsis* transcription factor1 or 2 like family; AVP1 - H^+-pyrophosphatase; Chl - chlorophyll; Chl-NADP-ME - chlorophyll-targeting NADP-malic enzyme; COX - choline oxidase; DREB2 - drought responsive element binding protein 2; E - transpiration rate; ERF - ethylene responsive factor; GolS - galactinolsynthase; GR - glutathionereductase; g_s - stomatal conductance; MT - methyltransferase; mtlD - mannitol-1-phosphate dehydrogenase; NCED - 9-*cis*-epoxycarotenoid dioxygenase; OA - osmotic adjustment; P5CS - Δ^1-pyrroline-5-carboxylate synthase; PEPC - phosphoenolpyruvate carboxylase; P_N - net photosynthetic rate; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC - relative water content; TPP - trehalose-6-phosphate phosphatase; ψ_p - pressure potential; ψ_s - osmotic potential; ψ_w - water potential.

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However, the debate continues as to whether drought mainly confines photosynthesis by stomatal closure or through metabolic impairment (Tezara *et al.* 1999, Lawson *et al.* 2003). The stomatal limitation is generally accepted to be the main determinant of reduced photosynthesis under mild drought stress (Cornic 2000). It is also reported that drought and salt tolerant plants maintain their pressure potential at low ψ_w by increasing the quantity of solutes in the cells (Johnson *et al.* 1987). In principle, the increased vacuolar solute accumulation could confer salt and drought tolerance. The sequestration of ions such as Na^+ may decrease the osmotic potential of the plant cell and simultaneously also mitigates the toxicity of this cation. The changes in the electrical potential difference across plasma membrane have been shown to be the most rapid alterations induced by abiotic stresses (Assmann *et al.* 1985, Serrano *et al.* 1988). This suggests that the transport of ions across the plasma membrane may act as a signal. Transport of Ca^{2+} into the cell could mediate the induction of several biochemical pathways and signaling networks leading to plant stress response (Ebel and Cosio 1994). Furthermore, in recent times there has been various transcriptome studies (Markandeya *et al.* 2007, Clement *et al.* 2008, Guo *et al.* 2009, Lata *et al.* 2010, Zheng *et al.* 2010) and proteomic analysis (Hajheidari *et al.* 2005, Urano *et al.* 2009) in plants exposed to drought/dehydration stress which revealed involvement of drought-responsive genes or proteins in cell cycle, translation/post translation modification, transport, redox and oxidative response, glutamate and aspartate family amino acids synthesis, TCA cycle and signal transduction. This suggests the numerous alterations in gene expression and in signaling network along with physiological changes occurring in plants due to drought stress which need extensive understanding.

In view of the fact that drought is one of the major global environmental constraints (Boyer 1982), engineering of drought tolerance in plants has gained utmost attention and significance. Although a plethora of approaches have been employed to alleviate the problem of drought, plant breeding in conjunction with genetic engineering seems to be an efficient and durable means of tailoring crops to grow successfully in drought-prone environment (Ashraf 2010). To develop strategies for this purpose, one of the primary research goals is to understand the molecular mechanisms underlying drought induced changes and to identify the functional genes that are directly involved in these events. This review focuses on recent advances in the understanding of the drought induced changes at physiological, biochemical and molecular level and signaling pathways. We emphasize on the recently identified milestones in drought tolerance and genetic engineering approaches.

Drought induced physiological and biochemical responses

Plants respond to drought stress through physiological, biochemical and metabolic adjustments occurring in all plant organs. These cellular alterations include decrease in pressure potential and membrane fluidity, changes in solute concentrations and protein-protein and protein-lipid interactions (Valliyodan and Nguyen 2006). The plants have a lot of typical physiological and biochemical responses to stress such as accumulation of osmolytes, amino acids and some hormones, modulation of sugar metabolism and loss of photosynthetic activity. It has been reported that drought stress inhibits cell division and expansion owing to reduced pressure potential leading to decreased plant growth. Relative water content (RWC) and water potential (ψ_w) and its components, pressure potential (ψ_p) and osmotic potential (ψ_s), are used as indicators of plant water status (Kiani *et al.* 2007). During drought stress, plants follow important physiological mechanisms of maintaining pressure potential by means of increased cell solute concentration (so called osmotic adjustment, OA; Morgan 1984, Ober *et al.* 2005). The lowering ψ_w due to the accumulation of compatible solutes allows rapid water intake from rather dry soil, thus mitigating water stress effects (Kumar *et al.* 2003). There have been reports of proline accumulation during extreme temperatures and drought (*e.g.* Sairam *et al.* 2002). Further, accumulation of proline has been implicated as standard for selection for stress tolerance (Yancy *et al.* 1982). In plants, proline biosynthesis and its accumulation during drought stress is also involved in detoxification of ROS (Gong *et al.* 2002, Hrabak *et al.* 2003). Transgenic tobacco overexpressing proline biosynthesis enzyme, Δ^1-pyrroline-5-carboxylate synthase (P5CS), was found to survive better in response to water deficits (Posas and Saito 1997, Park *et al.* 2003). Furthermore, the transgenic tobacco line with suppression of proline synthesis resulted in increased sensitivity to water deficit (Nakagami *et al.* 2005). It was also reported that transgenic *Petunia* plants that co-overexpressed the *AtP5CS* and *OsP5CS* genes from *Arabidopsis* and rice, respectively, can withstand drought conditions for longer durations than wild type plants (Yamaguchi-Shinozaki and Shinozaki 2005). The increased proline accumulation due to enhanced P5CS activity in tolerant safflower has been considered as an index for water stress tolerance (Thippeswamy *et al.* 2010).

It has been reported that mannitol plays a role of osmolyte and it is being produced as major biochemical product in algae and higher plants during water deficit (Wang *et al.* 2005). The introduction of a mannitol dehydrogenase gene (*mtlD*) into wheat (Davies *et al.* 1999) has resulted in a considerable increase in water stress tolerance. The *E. coli mtlD* gene when transformed in sorghum has resulted in an enhanced tolerance to water deficit and salinity (Maheswari *et al.* 2010). Further in tobacco, there is report of mannitol protecting thioredoxin, ferredoxin, and glutathione from the oxidative stress (Mikolajczyk *et al.* 2000). Another example of the protection of sensitive enzymes and membranes is provided by D-ononitol and myo-inositol in the cytoplasm. The transgenic tobacco plants, overexpressing inositol methyl transferase gene (*Imt1*) from the *Mesembryanthemum crystallinum,* have shown increased accumulation of the methylated form of inositol, D-ononitol, leading to enhanced tolerance to drought and salt stress (Li *et al.* 2000). Galactinol also plays important roles in the desiccation tolerance of plants. *Arabidopsis* plants that overexpressed the *AtGolS1* and/or *AtGolS2* genes show enhanced tolerance to drought stress owing to the accumulation of galactinol and raffinose and a reduced transpiration rate (Taji *et al.* 2002). Hence, galactinol and raffinose act as osmoprotectants rather than as osmoregulants (Kobayashi *et al.* 2004). Further, fructans play a significant role in drought- and cold-stress tolerance in plants (Boudsocq *et al.* 2004). There are reports of tobacco and sugar beet plants engineered with the bacterial fructan gene showing enhanced tolerance to drought stress (Mustilli *et al.* 2002, Yoshida *et al.* 2002). Similarly, trehalose functions as reserve sugar and stress protectant, stabilizing proteins and membranes against denaturation (Yoshida *et al.* 2006). Transgenic plants that expressed the trehalose biosynthesis genes resulted in an elevated drought-stress tolerance even though phenotypic abnormalities were noticed in some cases (Cheong *et al.* 2003, Gong *et al.* 2004, Batistic and Kudla 2004, Kolukisaoglu *et al.* 2004). The *Arabidopsis* trehalose-6-phosphate synthase 1 (AtTPS1) plays a significant role in sugar and ABA signaling and its overexpression resulted in enhanced drought tolerance (Kolukisaoglu *et al.* 2004). The trehalose accumulation in transgenic *Arabidopsis* plants that overexpressed *AtTPS1* was slightly altered without morphological abnormalities. Even the overexpression of this gene in rice has resulted in elevated contents of glucose, fructose, and sucrose and improved drought stress response (Yoshida *et al.* 2006). These facts clearly suggest that modulation of the key enzymes involved in production of crucial osmoprotectants may help in rendering drought tolerance in crop plants.

Apart from osmolytes, there have been reports of stress hormone abscisic acid (ABA) increased concentration and transportation to shoots *via* xylem during drought stress (Zeevaart and Creelman 1988).

Furthermore, there have been reports of decreased gas exchange parameters, transpiration rate (E) and net photosynthetic rate (P_N), both regulated by stomatal conductance (g_s) (Tezara *et al.* 2002). Furthermore, the stomatal opening rather than stomata density seems to play crucial role in water loss during drought stress (Zhang *et al.* 2005). In higher plants, P_N is a function of the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) as well as regeneration of RuBP (Ramachandra Reddy 1996, Chaitanya *et al.* 2002, Parry *et al.* 2002). Under drought, reduction in chloroplast volume due to desiccation may lead to conformational changes in Rubisco (Sengupta and Berkowitz 1988). Recently, increased mesophyll CO_2 diffusion conductance, Chl *a*/*b* ratio, leaf nitrogen partitioning in Rubisco and bioenergetics have been reported in *Incarvillea delavayi* plants as an acclimation to long-term water stress, indicating physiological plasticity of this species (Cai *et al.* 2010). Drought stress is also known to acidify the chloroplast stroma, resulting in inhibited Rubisco activity (Meyer and Genty 1999). However, C_4 plants have better water use efficiency than C_3 plants but even there Rubisco plays a crucial role in achievement of higher photosynthesis rate (Parry *et al.* 2002). In addition, the primary carboxylating enzyme of C_4 plants, phosphoenol pyruvate carboxylase (PEPC), is also inhibited under drought (Boyer *et al.* 1997). Drought generally reduces the biochemical capacity for carbon assimilation and utilization and also seems to alter the ratio of the end products of photosynthesis, starch and sucrose. There are reports that the activities of fructose 1,6 bis-phosphatase and sucrose phosphate synthase decline with reduced ψ_w, indicating that the sucrose synthesis is strongly influenced by drought stress (Haupt-Herting and Fock 2002). Hu *et al.* (2010a) showed reduction not only in P_N but also cytochrome respiratory pathway activity in pepper leaves during drought stress.

In *Camellia sinensis*, reduction of chlorophyll (Chl), carotenoids, ascorbate and glutathione contents and the activities of guaiacol peroxidase (POX) and glutathione reductase (GR) upon imposition of drought stress whereas increase in proline, H_2O_2 and superoxide anion contents, lipid peroxidation and activities of catalase (CAT) and superoxide dismutase (SOD) was observed (Upadhyay and Panda 2004). Furthermore, the comparatively less decrease in antioxidants, higher activities of POX, GR, CAT and higher phenolic contents in tolerant tea clones were found in comparison to susceptible ones (Upadhyay *et al.* 2008).

Drought stress responsive proteins

Plants survive different stresses with the help of multifunctional genes which enable the cross-talk among the various abiotic stress resistance systems (Hu *et al.* 2010b). The plants behave differentially and specifically in response to various abiotic stresses with the help of stress responsive regulatory proteins. The gene expression and metabolite analysis of drought-tolerant native Andean potato clones pinpointed upregulation of genes involved in osmotic adjustment, changes in sugar metabolism, membrane modifications, detoxification of oxygen radicals and protein stabilization (Schafleitner *et al.* 2007). It has been reported that stress-responsive proteins, *e.g.*, the late-embryogenesis-abundant (LEA) proteins were involved in the alleviation of cell damage during dehydration although the precise mechanism is still to be explored (Bartels and Sunkar 2005, Vinocur and Altman 2005). The biochemical analysis has further demonstrated that LEA proteins can prevent protein aggregation induced by desiccation as well as by freezing (Goyal *et al*. 2005). The recent studies (Wise 2003, Wise and Tunnacliffe 2004) proposed that LEA proteins function as chaperone-like protective molecules and act against cellular damage. There has been a report of oat acclimation during drought stress by enhanced phosphatidylserine decarboxylase activity in root cells altering membrane phospholipid composition (Larsson *et al.* 2006). Thus, unraveling the mechanisms underlying the action of metabolites and proteins involved in drought tolerance could facilitate the development of stress-tolerant plants.

Numerous genes induced by water deficit stress have been identified in drought tolerant rice cultivar and most of them were related to metabolism, energy, protein biosynthesis, cell defense, signal transduction, and transport (Rodriguez *et al.* 2006). A novel gene *W89* encoding dehydration-responsive protein was isolated from wheat. This protein showed homology with methyltransferase and contained sterile alpha motif (SAM)-binding protein, suggesting its role in network with other proteins or transcription factors (TFs) to regulate the expression of stress-responsive genes during an early stage of drought stress (Zhang *et al.* 2007). Recently, the transcriptional analysis of drought tolerant and sensitive tomato cultivars revealed that drought stress specifically affects the key enzymes of gluconeogenesis (fructose-bisphosphate aldolase), purine and pyrimidine nucleotide biosynthesis (adenylate kinase), tryptophan degradation (aldehyde oxidase), starch degradation (β-amylase), methionine biosynthesis (cystathionine-β-lyase), and the removal of superoxide radicals (catalase) (Gong *et al.* 2010). This suggests a different mechanisms of plant adaptation to drought conditions, *e.g.*, by lessening energy dissipation, conserving ATP and reducing oxidative damage. In addition, the recent progress of metabolome analysis should also contribute to the discovery of novel stress resistance systems that can be utilized for stress tolerance engineering (Oksman-Caldentey and Saito 2005).

Therefore, a detailed understanding of the various alterations in physiological parameters during drought stress in plants will provide a way how to coordinate abiotic stress response networks at biochemical, molecular and signaling level to formulate strategies to impart drought tolerance in plants. Although a large number of drought-induced genes have been identified, a molecular basis for plant tolerance to water stress remains far from being completely understood (Ingram and Bartels 1996).

Drought responsive transcription factors

The integration of advanced molecular biology techniques with functional genomics, aided by the availability of genome sequence information, has unraveled the transcriptional regulation of drought response. Transcriptome analyses using microarray technology, together with conventional approaches, have revealed that numerous transcription factors (TFs) are involved in the plant response to drought stress (Bartels and Sunkar 2005, Vinocur and Altman 2005). Most of these TFs fall into several large TF families, such as APETALA type 2 / ethylene responsive factors (AP2/ ERF), basic region/leucine zipper motif (bZIP), NAM/ATAF/CUC transcription factor (NAC), myeloblastosis (MYB), myelocytomatosis (MYC), Cys2His2 zinc-finger proteins (ZFP) and WRKY domain binding transcription factors (WRKY) (Umezawa *et al.* 2006). The ABA-dependent and -independent stress signaling pathway leading to gene expression involve different TFs such as drought responsive element binding proteins (DREB), MYC/MYB, ABA responsive element binding protein (AREB)/ ABA responsive element binding factor (ABF), (no apical meristem)-like protein (NAM), *Arabidopsis* transcription factor 1 or 2 like family (ATAF1 and ATAF2), cup-shaped cotyledon transcription factor (CUC), and their respective *cis*-elements DRE, MYCRS/MYBRS, ABRE and NACRS (Agarwal and Jha 2010). The analysis of ABA mutants has thrown light into the ABA-dependent and ABA-independent pathways for osmotic and cold stresses interactions (Kobayashi *et al.* 2008). Further, SNAC2/OsNAC6 transgenic rice plants had improved drought tolerance because of the enhanced expression of a large number of genes encoding proteins with predicted stress tolerance functions such as detoxification, redox homeostasis and proteolytic degradation (Nakashima *et al*. 2007, Hu *et al*. 2008). When an *AtDREB* homologous gene *GmDREB2* from soybean was overexpressed constitutively, it increased the survival rate of transgenic plants without growth retardation under drought and salinity stress (Chen *et al*. 2007). The overexpression of a GmERF TF family member, the *GmERF089* gene, and a chilling inducible *GmCHI* gene promoted enhanced tolerance to drought stress in tobacco and *Arabidopsis*, respectively (Zhang *et al*. 2008, Cheng *et al*. 2009). The plant Q-type C_2H_2 zinc finger proteins subfamily of TFs in *Triticum aestivum* was found to be involved in modulating the drought adaptation in roots (Kam *et al.* 2008). The expression of TFs regulates the expression of downstream target genes that are involved in the drought stress response and tolerance. The recent progress in TF study has led to the engineering of drought tolerance using both transcriptional activators and repressors.

Transcriptional activators that upregulate stress-responsive genes have been widely used to impart drought tolerance to transgenic plants (Muoma *et al.* 2010). The overexpression of the DREB1/CBF3 TF in *Arabidopsis* was found to control many stress inducible target genes (Seki *et al.* 2001, Fowler and Thomashow 2002, Maruyama *et al.* 2004) apart from increasing tolerance to freezing and salinity (Kasuga *et al.* 1999). Transgenic plants expressing a drought-responsive AP2-type TF, SHINE transcription factor1-3(SHN1-3) or WAX inducible protein 1(WXP1), induced wax-related genes leading to enhanced cuticular wax accumulation and increased drought tolerance (Aharoni *et al.* 2004, Zhang *et al.* 2005). Transgenic rice plants overexpressing rice NAC transcription factor 45 (OsNAC045) were reported to have enhanced tolerance to drought and salt treatments and thus can be a potential tool for rice genetic engineering for better stress response (Zheng *et al.* 2009). Recently, drought induced ABA-responsive element (ABRE)-binding basic region/leucine zipper (bZIP) TF rice ABA responsive element binding factor (OsABF2) as a positive regulator of abiotic stress and ABA signaling genes expression in transgenic rice was reported (Hossain *et al.* 2010). Consequently, the increasing use of point mutations in TFs is also holding a platform for evolving an important strategy to produce active forms of transcriptional activators for tailoring drought tolerance. A point mutation at the phosphorylation site of a rice bZIP transcription factor (TRAB1), from serine to aspartic acid has resulted in significant upregulation of transcriptional activation even in the absence of the inducer ABA in the protoplast transient assay. (Kagaya *et al.* 2002). Recently, transgenic plants expressing a phosphorylated active form of AREB1(ABA responsive element binding protein) with multisite mutations also resulted in the induction of many ABA-responsive genes without exogenous ABA application suggesting that altered TFs activation by point mutations may have the potential to enhance drought tolerance ability (Furihata *et al.* 2006).

Transcriptional repressors that down regulate gene expression under stress have also been used to engineer drought tolerance (Zhang 2003). *AtMYB60*, an *R2R3-MYB* transcriptional repressor in *Arabidopsis*, functioned in the regulation of stomatal movements. This gene was specifically expressed in guard cells and its expression was negatively regulated during drought stress (Cominelli *et al.* 2005). A null, T-DNA insertion mutation in *AtMYB60* resulted in the constitutive reduction of stomatal opening and minimized wilting under water stress. Interestingly, the *Atmyb60-1* mutation resulted in guard-cell-specific defects with no apparent deleterious effects on other developmental and physiological processes (Cominelli *et al.* 2005). This was very important when considering the yield of agricultural crops, because most transgenic plants that constitutively express TFs showed growth retardation and alterations in basic metabolism (Vinocur and Altman 2005). Although such undesirable traits can be improved to some extent through the use of stress inducible promoters that regulate the expression of TFs (Kasuga *et al.* 1999, 2004), the engineering of stomatal response as a means to reduce water loss is an attractive approach to confer drought tolerance in crops (Schroeder *et al.* 2001). Thus, the knowledge of drought inducible TFs will open up avenues for development of the drought tolerant crop plants which will survive well in acute field condition.

Drought induced signal transduction pathways

With the bulk of information generated by recent studies, it has been demonstrated that the signal transduction for drought and salt stress can be divided into three major signaling pathways: *1*) oxidative stress signaling – MAPK modulates based generation of ROS scavengers and antioxidant compounds, *2*) Ca^{2+}-dependent signaling – activation of a *LEA*-type genes involved in the expression of stress-responsive proteins and *3*) Ca^{2+}-dependent salt overlay sensitive (SOS) signaling that regulates ion homeostasis. Recently, there has been a report of $CaCl_2$ mediated increase in dry mass, proline and phenolic contents of leaf with a decrease in H_2O_2 and lipid peroxidation and increased activities of SOD, CAT, POX and GR during post drought recovery of *Camellia sinensis* (Upadhyay *et al.* 2011). This suggests the involvement of Ca^{2+} mediated stress response signaling pathways. The advanced serial analysis of gene expression (SuperSAGE) analysis of drought responsive transcriptome of chickpea roots has revealed a network of genes involved in Ca^{2+}-dependent signaling, general signal transduction, transcription regulation, protein-protein interactions, stress induced ABA-dependent signaling, damage and repair responses, and chromatin organization (Molina *et al.* 2008). Furthermore, the transcriptome analysis of wheat revealed multilevel regulation, including transcriptional regulation, RNA binding, kinase activity and Ca and ABA signaling implicated in stomatal closure signifies an interrelated signaling network during drought stress (Krugman *et al.* 2010).

Oxidative stress signaling

Apparently all abiotic stresses including drought lead to formation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radical that hamper photosynthesis and cause oxidative damage to proteins, DNA and lipids (Apel and Hirt 2004). Thus, oxidative stress is one of the major causes of cellular damage in plants during stresses (Miller *et al.* 2010). Oxidative stress signaling includes a MAPK cascade (Tuteja and Mahajan 2007) leading to enhanced production of osmolytes by activating the protein tyrosine kinases, G-protein receptors, two-component histidine kinases, *etc.* (Hasegawa *et al.* 2000). The highly conserved MAPK pathways in eukaryotes are intracellular signals that mediate signal transduction from the cell surface to the nucleus. The MAP kinase kinase kinase (MAPKKK), upon activation, phosphorylates a MAP kinase kinase (MAPKK) on serine and threonine residues. This MAPKK in turn phosphorylates a MAP kinase (MAPK) on conserved tyrosine and threonine residues (Nadarajah and Sidek 2010). The activated MAPK can then either migrate to the nucleus to activate the transcription factor directly, or phosphorylates the activators and sensors which carry on the downstream signals (Xiong *et al*. 2002). The MAPK can also remain in the cytoplasm to mediate cytoplasmic events such as phosphorylation of protein kinases, phospholipases and cytoskeleton associated proteins (Reiser *et al.* 1999). This information provides a clue that modulating MAPK will lead to altered expression of specific regulatory TFs and protein kinases towards enhanced drought tolerance in plants. Compatible solutes of three major types: amino acids (*e.g*., proline), quaternary amines (*e.g.* glycine-betaine and dimethyl-sulfoniopropionate) and sugars (*e.g*., mannitol and trehalose) were also reported to act as free radical scavengers (Wang *et al*. 2003).

Ca^{2+}-dependent signaling that activate *LEA*-type genes

Various intercellular signaling processes in both plants and animals are Ca^{2+} dependent. Drought and salinity have been shown to induce transient Ca^{2+} influx into the cell cytoplasm. Calcium dependent protein kinases (CDPKs) are implicated as important sensors of Ca^{2+} influx in plants in response to these stresses (Tuteja and Mahajan 2007). CDPKs are serine or threonine protein kinases with a C-terminal calmodulin-like domain with up to 4EF-hand motifs that can directly bind Ca^{2+} (Harmon *et al.* 2001). The CDPK pathway seems more connected to increasing the expression of LEA proteins (Serrano *et al*. 2003). The pathways leading to the activation of *LEA*-type genes including the DRE/C-repeat (CRT) class of stress-responsive genes may be different from the pathways regulating osmolyte production. The activation of *LEA*-type genes may actually mean for development of stress tolerance through damage repair pathways (Xiong *et al*. 2002). In higher plants, LEA proteins have been reported to accumulate under extreme desiccation condition (Garay-Arroyo *et al*. 2000). LEA proteins were reported to act as chaperones in protection of macromolecules like lipids, enzymes and mRNA from degradation (Campos-Alvarez *et al*. 2002). Hence the modification of the expression levels of CDPKs can be a mode to achieve drought tolerance in plants.

Ca^{2+}-dependent SOS signaling that regulate ion homeostasis

In plants, it is well known that Ca^{2+} dependent salt overly sensitive (SOS) signaling governs ion transporters that control ion homeostasis under salt stress and drought. The SOS pathway is likely to be associated with excess extracellular or intracellular Na^+, which somehow triggers a cytoplasmic Ca^{2+} signal (Zhu 2002). The ionic aspect of salt stress is signaled *via* the SOS pathway where a calcium responsive SOS3-SOS2 protein kinase complex controls the expression and activity of ion transporters such as SOS1 (Boudsocq and Lauriere 2005). The genetic analysis indicated that SOS1, SOS2 and SOS3 function in a common pathway in controlling salt tolerance. An important group of Ca^{2+}sensors in plants is the SOS3 family of Ca^{2+} binding proteins. The amino acid sequence of SOS3 is most closely related to the regulatory subunit of yeast calcineurin (CNB) and animal neural calcium sensors. A loss-of-function mutation in the *Arabidopsis* SOS3 gene renders the mutant hypersensitive to NaCl. In addition, the SOS2-SOS3 kinase directly phosphorylates and activates the SOS1 transporter (Xiong *et al*. 2002, Wise and Tunnacliffe 2004). Furthermore, the elevated intracellular Ca^{2+} content due to drought stress may activates several protein kinases and phospholipid systems generating a diverse array of messenger molecules which may function upstream of the osmotic stress-activated protein kinases to mediate osmotic homeostasis and/or detoxification responses (Shao *et al.* 2008). Thus, in-depth understanding of the SOS signaling pathway and alterations of key kinases will pave ways for the development of drought tolerant plants.

Signaling factors as prospects for engineering drought tolerance

Various signal transduction systems function in plants underlying abiotic stress responses, involving protein phosphorylation and dephosphorylation, phospholipid metabolism, calcium sensing and protein degradation

(Vinocur and Altman 2005, Goyal *et al.* 2005, Boudsocq and Lauriere 2005). Although these complex signaling processes are not yet fully understood, several genes encoding signaling factors that function in the drought response have been identified (Shinozaki *et al.* 2003, Chinnusamy *et al.* 2004). An added advantage of signaling factors engineering is that they can control the signal output involved in stress resistance. In many cases, signaling factors may get activated or inactivated in response to specific stress stimuli. An Suc nonfermenting 1 (SNF1)-related protein kinase 2 (SnRK2) was shown to be activated by ABA or osmotic stress, suggesting that SnRK2 might be important for signal transduction in the stress response (Wang *et al.* 2005, Mikolajczyk *et al.* 2000). There are reports of ten members of the SnRK2 family in *Arabidopsis* (SRK2A-J/SnRK2.1-10) and rice genome (SAPK1-10). Almost all SnRK2s were found to be specifically activated by ABA or hyper-osmolarity (Kobayashi *et al.* 2004, Boudsocq *et al.* 2004). The most crucial finding in SnRK2 function was established in guard cells: ABA-activated protein kinase (AAPK) in fava bean was essential for ABA-dependent stomatal closure (Li *et al.* 2000). In *Arabidopsis*, the physiological function of SRK2E/OST1 (*SnRK2.6*), an ortholog of AAPK, has been established: *srk2e* or *ost1* mutants showed hypersensitivity to drought because of their lack of control of transpiration (Mustilli *et al.* 2002, Yoshida *et al.* 2006). These data suggest that *SnRK2* serves as a central hub to mediate ABA signaling in guard cells (Fujii *et al.* 2007). In addition, one of the *Arabidopsis SnRK2s, SRK2C (SnRK2.8*), is an osmotic stress-activated protein kinase and its overexpression significantly improves drought tolerance in *Arabidopsis* (Umezawa *et al.* 2004). The stress-dependent activation or deactivation of signal components might function as a molecular switch for the biotechnological manipulation of the stress response in plants. Calcineurin B-like protein/SOS3-like calcium-binding protein (CBL/SCaBP) and its interacting partner calcineurin B-like protein (CBL)-interacting protein kinases/salt overly sensitive 2 (SOS2)-like protein kinases (CIPK/PKS), which is identical to SnRK3, form a complex that is known to be an important regulator of signal transduction in the presence of ABA and under drought, salt or cold stress (Cheong *et al.* 2003, Hrabak *et al.* 2003, Kolukisaoglu *et al.* 2004). Plants overexpressing CBL1 exhibited elevated drought and salt tolerance and upregulated expression of stress responsive genes (Cheong *et al.* 2003). Although *SnRK3* seems to be important for engineering drought tolerance, it is not a simple case. The manipulation of *SnRK3* is complicated because its kinase activity is regulated by a Ca^{2+}-dependent interaction of CBL/SCaBP proteins which sense internal or external stimuli. The PKS kinases were reported to have FISL motif in regulatory domain responsible for binding to CBL/SCaBPs and has a consensus sequence XL (or M, I, F, Y) N (or T)**A**F**D** (or E, Q) I (or L, F, M)**IS** (or A, T, G, L, I) L (or M, F, Y, S, T, G)**S**XG (or F, D, E, S) F (or L, S) D (or N, S, G)**LS** (or A, E, G) G (or N, S, T, P) L (or F)**FE** (or D, G, A) (where X represents amino acid residue and residues in bold are absolutely conserved. (Guo *et al.* 2001). As demonstrated in the case of SOS2 or PKS18 kinases, mutations in the activation loop of the catalytic domain or deletion of the FISL motif enables the production of a constitutively active form of SnRK3 (Park *et al.* 2003). These findings also highlight the complexity of the signal transduction network with cross-talk, feedback and physical interactions delivering appropriate signals to suitable targets at the correct time. As with the yeast 'scaffold' hypothesis, signaling protein may have several signal outputs which may probably explain the different functions of the MAPKKK, Ste11p (Zhang 2003, Cominelli *et al.* 2005). For example, AtMPK6 functions in at least two different MAPK cascades MKK2-MPK6 and MKK4/MKK5-MPK6, and each combination transduces different signals from cold or salt stress, and bacterial pathogens, respectively (Nakagami *et al.* 2005). Further functional or biochemical analyses will be required to gain a precise understanding of the functions of various signaling factors in plants, which can then be used to promote the engineering of drought tolerance using signaling factors.

Conclusions and future prospects

The rapid development in transcriptome, proteome and metabolome studies has been the guiding force to identify and characterize crucial genes that are induced by drought stress, and the coupled signaling and regulatory networks in plants. Recent efforts to dissect the key abiotic stress signaling pathways have also provided potential target genes for stress tolerance. However, the majority of these studies have been conducted using model plants, and engineering for drought tolerance in crop plants is still in its infancy. Although transgenic approaches have been shown to be powerful tools to manipulate the responses of plants to stress and develop drought tolerant plants, this can be achieved only when supported by precise physiological and biochemical validation under field conditions. A thorough analysis needs to be made while combining genes and priority may be given to pyramid a set of genes contributing to the whole regulatory pathway. For this, the foremost challenge is to identify molecular events for better understanding of how to improve drought tolerance. This demands a thorough understanding of the target genes at transcriptional and translational level and signal transduction cascades under drought stress. Consequently, the value of any genes or pathways for drought tolerance in crop plants can only be judged by their precise field performance. Apart from tedious phyto-engineering efforts, researchers have to be familiar with the actual field conditions and the present species and cultivars favored by farmers. Hence, an endeavor has to be made to establish linkages and collaboration along basic and applied research to release drought tolerant crop plants at the field level.

In the development of tolerant plants, the co-incidence of several abiotic stresses rather than a single stress in the field condition will always exist. Since the response of the plants to concurrent occurrence of more than one stress is different compared to one particular abiotic stress, therefore phytoengineering for drought stress, possibly need to consider about other associated abiotic stresses and attempt to alter the plant response. Attaining combined tolerance in plants for synchronously occurring abiotic stresses might lead to a significant access for yield enhancement.

References

Agarwal, P.K., Jha, B.: Transcription factors in plants and ABA dependent and independent abiotic stress signaling. - Biol. Plant. **54**: 201-212, 2010.

Aharoni, A., Dixit, S., Jetter, R., Thoenes, E., Van Arkel, G., Pereira, A.: The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when over expressed in *Arabidopsis.*- Plant Cell **16**: 2463-2480, 2004.

Apel, K., Hirt, H.: Reactive oxygen species: metabolism, oxidative stress, and signal transduction. - Annu. Rev. Plant Biol. **55**: 373-399, 2004.

Ashraf, M.: Inducing drought tolerance in plants: recent advances. - Biotechnol. Adv. **28**: 169-183, 2010.

Assmann, S.M., Simoncini, L., Schroeder, J.L.: Blue light activates electrogenic ion pumping in guard cell protoplasts in *Vicia faba* L. - Nature **318**: 285-287, 1985.

Bartels, D., Sunkar, R.: Drought and salt tolerance in plants. - Crit. Rev. Plant Sci. **24**: 23-58, 2005.

Batistic, O., Kudla, J.: Integration and channeling of calcium signaling through the CBL calcium sensor/CIPK protein kinase network. - Planta **219**: 915-924, 2004.

Boudsocq, M., Barbier-Brygoo, H., Lauriere, C.: Identification of nineSNF1-related protein kinases 2 activated by hyperosmotic and saline stresses in *Arabidopsis thaliana*. - J. biol. Chem. **279**: 41758-41766, 2004.

Boudsocq, M., Lauriere, C.: Osmotic signaling in plants: multiple pathways mediated by emerging kinase families. - Plant Physiol. **138**: 1185-1194, 2005.

Boyer, J.S.: Plant productivity and environment. - Science **218**: 443-448, 1982.

Boyer, J.S, Wong, S.C., Farquhar, G.D.: CO_2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials. - Plant Physiol. **114**: 185-191, 1997.

Cai, Y.F., Zhang, S.B., Hu, H., Li, S.Y.: Photosynthetic performance and acclimation of *Incarvillea delavayi* to water stress. - Biol. Plant. **54**: 89-96, 2010.

Campos-Alvarez, F., Cruz-Garcia, F., Torres-Espinosa, A., Sanchez-Jimenez, M., Colmenero-Flores, J.M., Smith-Espinoza, C.: Expression of late embryogenesis abundant (LEA) proteins codifying genes during osmopriming of maize and bean seeds. - Agrociencia **36**: 461-470, 2002.

Chaitanya, K.V., Masilamani, S., Jutur, P.P., Ramachandra Reddy, A.: Variation in photosynthetic rates and biomass productivity among four mulberry cultivars. - Photosynthetica **40**: 305-308, 2002.

Chaitanya, K.V., Sundar, D., Jutur, P.P., Ramachandra Reddy, A.: Water stress effects on photosynthesis in different mulberry cultivars. - Plant Growth Regul. **40**: 75-80, 2003.

Chaves, M.M., Maroco, J.P., Periera A.S.: Understanding plant responses to drought from genes to the whole plant. - Funct. Plant Biol. **30**: 239-264, 2003.

Chen, M., Wang, Q.Y., Cheng, X.G., Xu, Z.S., Li, L.C., Ye, X.G., Xia, L.Q., Ma, Y.Z.: GmDREB2, a soybean DRE-binding transcription factor, conferred drought and high-salt tolerance in transgenic plants. - Biochem. biophys. Res. Commun. **353**: 299-305, 2007.

Cheng, L., Huan, S., Sheng, Y., Hua, X., Shu, Q., Song, S.J.X.: GMCHI, cloned from soybean (*Glycine max* (L.) Merr.) enhances survival in transgenic *Arabidopsis* under abiotic stress. - Plant Cell Rep. **28**: 145-153, 2009.

Cheong, Y.H., Kim, K.N., Pandey, G.K., Gupta, R., Grant, J.J., Luan, S.: CBL1, a calcium sensor that differentially regulates salt, drought, and cold responses in *Arabidopsis*. - Plant Cell **15**: 1833-1845, 2003.

Chinnusamy, V., Schumaker, K., Zhu, J.K.: Molecular genetic perspectives on cross-talk and specificity in abiotic stress signaling in plants. - J. exp. Bot. **55**: 225-236, 2004.

Clement, M., Lambert, A., Herouart, D., Boncompagni, E.: Identification of new up-regulated genes under drought stress in soybean nodules. - Gene **426**: 15-22, 2008.

Cominelli, E., Galbiati, M., Vavasseur, A., Conti, L., Sala, T., Vuylsteke, M., Leonhardt, N., Dellaporta, S.L, Tonelli, C.: A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. - Curr. Biol. **15**: 1196-1200, 2005.

Cornic, G.: Drought stress inhibits photosynthesis by decreasing stomatal aperture not by affecting ATP synthesis. - Trends Plant Sci. **5**: 187-188, 2000.

Davies, J.P., Yildiz, F.H., Grossman, A.R.: Sac3, an Snf1-like serine/threonine kinase that positively and negatively regulates the responses of *Chlamydomonas* to sulfur limitation. - Plant Cell **11**: 1179-1190, 1999.

Ebel, J., Cosio, E.G.: Elicitors of plant defense responses. - Int. Rev. Cytol. **148**: 1-36, 1994.

Fowler, S., Thomashow, M.F.: *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. - Plant Cell **14**: 1675-1690, 2002.

Fujii, H., Verslues, P.E., Zhu, J.: Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in *Arabidopsis*. - Plant Cell **19**: 485-494, 2007.

Furihata, T., Maruyama, K., Fujita, Y., Umezawa, T., Yoshida, R., Shinozaki, K., Yamaguchi-Shinozaki, K.: Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AtDREB1. - Proc. nat. Acad. Sci. USA **103**: 1988-1993, 2006.

Garay-Arroyo, A., Colmenero-Flores, J.M., Garciarrubio, A., Covarrubias, A.: Highly hydrophilic proteins in prokaryotes and eukaryotes are common during condition of water deficit. - J. biol. Chem. **275**: 5668-5674, 2000.

Gong, D., Guo, Y., Schumaker, K.S., Zhu, J.K.: The SOS3 family of calcium sensors and SOS2 family of protein kinases in *Arabidopsis*. - Plant Physiol. **134**: 919-926, 2004.

Gong, D., Zhang, C., Chen, X., Gong, Z., Zhu, J.K.: Constitutive activation and transgenic evaluation of the function of an *Arabidopsis* PKS protein kinase. - J. biol. Chem **277**: 42088-42096, 2002.

Gong, P., Zhang, J., Li, H., Yang, C., Zhang, C., Zhang, X., Khurram, Z., Zhang, Y., Wang, T., Fei, Z., Ye, Z.: Transcriptional profiles of drought-responsive genes in modulating transcription signal transduction, and biochemical pathways in tomato. - J. exp. Bot. **61**: 3563-3575, 2010.

Goyal, K., Walton, L.J., Tunnacliffe, A.: LEA proteins prevent protein aggregation due to water stress - Biochemistry **388**: 151-157, 2005.

Guo, P., Baum, M., Grando, S., Ceccarelli, S., Bai, G., Li, R., Korff, M.V., Varshney, R.K., Graner, A., Valkoun, J.: Differentially expressed genes between drought-tolerant and drought-sensitive barley genotypes in response to drought stress during the reproductive stage. - J. exp. Bot. **60**: 3531-3544, 2009.

Guo, Y., Halfter, U., Ishitani, M., Zhu, J.-K.: Molecular characterization of functional domains in the protein kinase SOS2 that is required for plant salt tolerance. - Plant Cell **13**: 1383-1400, 2001.

Hajheidari, M., Abdollahian-Noghabi, M., Askari, H., Heidai, M., Sadeghian, S.Y., Ober, E.S., Salekdeh, G.H.: Proteome analysis of sugar beet leaves under drought stress. - Proteomics **5**: 950-960, 2005.

Harmon, A.C., Gribskov, M., Gubrium, E., Harper, J.F.: The CDPK superfamily of protein kinase. - New Phytol. **151**: 175-183, 2001.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J.: Plant cellular and molecular responses to high salinity. - Annu. Rev. Plant Physiol. Plant mol.Biol. **51**: 463-499, 2000.

Haupt-Herting, S., Fock. H.P.: Oxygen exchange in relation to carbon assimilation in water-stressed leaves during photosynthesis. -Ann. Bot. **89**: 851-859, 2002.

Hossain, M.D., Chob, J., Hanb, M., Ahna, C.H., Jeon, J.S., An, G., Park, P.B.: The ABRE-binding bZIP transcription factor OsABF2 is a positive regulator of abiotic stress and ABA signaling in rice. - J. Plant Physiol. **167**: 1512-1520, 2010.

Hrabak, E.M., Chan, C.W., Gribskov, M., Harper, J.F., Choi, J.H., Halford, N., Kudla, J., Luan, S., Nimmo, H.G., Sussman, M.R.: The *Arabidopsis* CDPK-SnRK super family of protein kinases. - Plant Physiol. **132**: 666-680, 2003.

Hu, H., You, J., Fang, Y., Zhu, X., Qi, Z., Xiong, L.: Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice. - Plant mol. Biol. **67**: 169-181, 2008.

Hu, W.H., Xiao, Y.A., Zeng, J.J., Hu, X.Y.: Photosynthesis, respiration and antioxidant enzymes in pepper leaves under drought and heat stresses. - Biol. Plant. **54**: 761-765, 2010a.

Hu, X.J., Zhang, Z.B., Xu, P., Fu, Z.Y., Hu, S.B., Song, W.Y.: Multifunctional genes: the cross-talk among the regulation networks of abiotic stress responses. - Biol. Plant. **54**: 213-223, 2010b.

Ingram, J., Bartels, D.: The molecular basis of dehydration tolerance in plants. - Annu. Rev. Plant Physiol. **47**: 377-403, 1996.

Johnson, R.C., Mornhinweg, D.W., Ferris, D.M., Heithol, J.J.: Leaf photosynthesis and conductance of selected *Triticum* species at different water potentials. - Plant Physiol. **83**: 1014-1017, 1987.

Kagaya, Y., Hobo, T., Murata, M., Ban, A., Hattori, T.: Abscisic acid induce transcription is mediated by phosphorylation of an abscisic acid response element binding factor, TRAB1. - Plant Cell. **14**: 3177-3189, 2002.

Kam, J., Gresshoff, P.M., Shorter, R., Xue, G.P.: The Q-type C_2H_2 zinc finger subfamily of transcription factors in *Triticum aestivum* is predominantly expressed in roots and enriched with members containing an EAR repressor motif and responsive to drought stress. - Plant mol. Biol. **67**: 305-322, 2008.

Kasuga, M., Liu, Q., Miura, S., Yamaguchi, S., Shinozaki, K.: Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. - Nat. Biotechnol. **17**: 287-291, 1999.

Kasuga, M., Miura, S., Shinozaki, K., Yamaguchi-Shinozaki, K.: A combination of the *Arabidopsis DREB1A* gene and stress inducible rd29A promoter improved drought and low temperature stress tolerance in tobacco by gene transfer. - Plant Cell Physiol. **45**: 346-350, 2004.

Kiani, S.P., Tália, P., Maury, P., Grieu, P., Heinz, R., Perrault, A., Nishinakamasu, V., Hopp, E., Gentzbittel, L., Paniego, N., Sarrafi, A.: Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments. - Plant Sci. **172**: 773-787, 2007.

Kobayashi, F., Maeta, E., Terashima, A., Kawaura, K., Ogihara, Y., Takumi, S.: Development of abiotic stress tolerance *via* bZIP-type transcription factor LIP19 in common wheat. - J. exp. Bot. **59**: 891-905, 2008.

Kobayashi, Y., Yamamoto, S., Minami, H., Kagaya, Y., Hattori, T.: Differential activation of the rice sucrose nonfermenting1-related protein kinase 2 family by hyperosmotic stress and abscisic acid. - Plant Cell **16**: 1163-1177, 2004.

Kolukisaoglu, U., Weinl, S., Blazevic, D., Batistic, O., Kudla, J.: Calcium sensors and their interacting protein kinases: genomics of the *Arabidopsis* and rice CBL-CIPK signaling networks. - Plant Physiol. **134**: 43-58, 2004.

Kramer, P.J.: Plant Water Relations. - Academic Press, New York 1983.

Krugman, T., Chagué, V., Peleg, Z., Balzergue, S., Just, J., Korol, A.B., Nevo, E., Saranga, Y., Chalhoub, B., Fahima, T.: Multilevel regulation and signaling processes associated with adaptation to terminal drought in wild emmer wheat. - Funct. Integr. Genomics **10**: 167-186, 2010.

Kumar, S.G., Mattareddy, A., Sudhakar, C.: NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. - Plant Sci. **165**: 1245-1251, 2003.

Larsson, K.E., Nyström, B., Liljenberg, C.: A phosphatidylserine decarboxylase activity in root cells of oat (*Avena sativa*) is involved in altering membrane phospholipid composition during drought stress acclimation. - Plant Physiol. Biochem. **44**: 211-219, 2006.

Lata, C., Sahu, P.P., Prasad, M.: Comparative transcriptome analysis of differentially expressed genes in foxtail millet (*Setaria italica* L.) during dehydration stress. - Biochem. biophys. Res. Commun. **393**: 720-727, 2010.

Lawlor, D.W., Cornic, G.: Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. - Plant Cell Environ. **25**: 275-294, 2002.

Lawson, T., Oxborough, K., Morison, J.I.L., Baker, N,R.: The responses of guard and mesophyll cell photosynthesis to CO_2, O_2, light, and water stress in a range of species are similar. - J. exp. Bot. **54**: 1743-1752, 2003.

Li, J., Wang, X.Q., Watson, M.B., Assmann, S.M.: Regulation of abscisic acid-induced stomatal closure and anion channels by guard cell AAPK kinase. - Science **287**: 300-303, 2000.

Ludlow, M.M., Muchow, R.C.: A critical evaluation of traits for improving crop yields in water-limited environments. - Adv. Agron. **43**: 107-153, 1990.

Maheswari, M., Varalaxmi, Y., Vijayalakshmi, A., Yadav, S.K., Sharmila, P., Venkateswaswarul, B., Vanaja, M., Paradha Saradhi, P.: Metabolic engineering using *mtlD* gene enhances tolerance to water deficit and salinity in sorghum. - Biol. Plant. **54**: 647-652, 2010.

Manavalan, L.P., Guttikonda, S.K., Tran, L.S.P., Nguyen, H.T.: Physiological and molecular approaches to improve drought resistance in soybean. - Plant Cell Physiol. **50**: 1260-1276, 2009.

Markandeya, G., Babu, P.R., Lachagari, V.B.R., Reddy, A.M.M., Ramakrishna, W., Jeffrey, L.B., Reddy, A.R.: Identification of stress-responsive genes in an indica rice (*Oryza sativa* L.) using ESTs generated from drought-stressed seedlings. - J. exp. Bot. **58**: 253-265, 2007.

Maruyama, K., Sakuma, Y., Kasuga, M., Ito, Y., Seki, M., Goda, H., Shimada, Y., Yoshida, S., Shinozaki, K., Yamaguchi-Shinozaki, K.: Identification of cold-inducible downstream genes of the *Arabidopsis* DREB1A/CBF3 transcriptional factor using two microarray systems. - Plant J. **38**: 982-993, 2004.

Meyer, S., Genty, B.: Heterogenous inhibition of photosynthesis over the leaf surface of *Rosa rubinosa* L. during water stress and abscisic acid treatment induction of a metabolic component by limitation of CO_2 diffusion. - Planta **210**: 126-131, 1999.

Mikolajczyk, M., Awotunde, O.S., Muszynska, G., Klessig, D.F., Dobrowolska, G.: Osmotic stress induces rapid activation of a salicylic acid-induced protein kinase and a homolog of protein kinase ASK1 in tobacco cells. - Plant Cell **12**: 165-178, 2000.

Miller, G., Suzuki, N., Ciftci-Yilmazi, S., Mittler, R.: Reactive oxygen species homeostasis and signaling during drought and salinity stresses. - Plant Cell Environ. **33**: 453-467, 2010.

Molina, C., Rotter, B., Horres, R., Udupa, S.M., Besser, B., Bellarmino, L., Baum, M.M., Matsumura, H., Terauchi, R., Kahl, G., Winter, P.: SuperSAGE: the drought stress-responsive transcriptome of chickpea roots. - BMC Genomics **9**: 553-579, 2008.

Morgan, J.M.: Osmoregulation and water stress in higher plants. - Annu. Rev. Plant Physiol. **35**: 299-319, 1984.

Muoma, J., Ombori, O., Jesse, M.: Signal transduction one of the current molecular approaches in the management of drought stress in the sub-saharan region. - Biotechnology **9**: 469-476, 2010.

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

Nadarajah, K., Sidek, H.M.: The green MAPKS. - Asian J. Plant Sci. **9**:1-10, 2010.

Nakagami, H., Pitzschke, A., Hirt, H.: Emerging MAP kinase pathways in plant stress signaling. - Trends Plant Sci. **10**: 339-346, 2005.

Nakashima, K., Tran, L.-S.P., Nguyen, V.D., Fujita, M., Maruyama, K., Todaka, D., Ito, Y., Hayashi, N., Shinozaki, K., Yamaguchi-Shinozaki, K.: Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. - Plant J. **51**: 617-630, 2007.

Ober, E.S., Le Bloa, M., Clark, C.J.A, Roya, A., Jaggard, K.W., Pidgeon, J.D.: Evaluation of physiological traits as indirect selection criteria for drought tolerance in sugarbeet. - Field Crop Res. **91**: 231-249, 2005.

Oksman-Caldentey, K.M., Saito, K.: Integrating genomics and metabolomics for engineering plant metabolic pathways. - Curr. Opin. Biotechnol. **16**: 174-179, 2005.

Park, S.H., Zarrinpar, A., Lim, W.A. Rewiring MAP kinase pathways using alternative scaffold assembly mechanisms. - Science **299**: 1061-1064, 2003.

Parry, M.A.J., Andraloj, P.J., Khan, S., Lea, P.J., Keys, A.J.: Rubisco activity: effects of drought stress. - Ann. Bot. **89**: 833-839, 2002.

Posas, F., Saito, H.: Osmotic activation of the HOG MAPK pathway via Ste11p MAPKKK: scaffold role of Pbs2p MAPKK. - Science **276**: 1702-1705, 1997.

Ramachandra Reddy, A.: Fructose-2, 6 bisphosphate modulated photosynthesis in sorghum leaves grown under low water regimes. - Phytochemistry **43**: 319-322, 1996.

Reiser, V., Ruis, H., Ammerer, G.: Kinase activity-dependent nuclear export opposes stress-induced nuclear accumulation and retention of Hog1 mitogen-activated protein kinase in the budding yeast *Saccharomyces cerevisiae*. - Mol. biol. Cell 1**0**: 1147-1161, 1999.

Rodríguez, M., Canales, E., Carlos, J., Borroto, E.C., López, J., Pujol, M., Borrás-Hidalgo, O.: Identification of genes induced upon water-deficit stress in a drought-tolerant rice cultivar. - J. Plant Physiol. **163**: 577-584, 2006.

Sairam, R.K., Veerbhadra, R.K., Srivastava, G.C.: Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. - Plant Sci. **163**: 1037-1046, 2002.

Schafleitner, R., Gutierrez Rosales, R.O., Gaudin, A., Alvarado Aliaga, C.A., Martinez, G.N., Tincopa Marca, L.R., Bolivar, L.A., Delgado, F.M., Simon, R., Bonierbale, M.: Capturing candidate drought tolerance traits in two native Andean potato clones by transcription profiling of field grown plants under water stress. - Plant Physiol. Biochem. **45**: 673-690, 2007.

Schroeder, J.I., Kwak, J.M., Allen, G.J.: Guard cell abscisic acid signaling and engineering drought hardiness in plants. - Nature **410**: 327-330, 2001.

Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozaki, K., Carninci, P., Hayashizaki, Y., Shinozaki, K.: Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. - Plant Cell **13**: 61-72, 2001.

Sengupta, A., Berkowitz, G.A.: Chloroplast osmotic adjustment and water stress effects on photosynthesis. - Plant Physiol. **88**: 200-206, 1988.

Serrano, E.E., Zeiger, E., Hagiwara, S.: Red light stimulates an electrogenic proton pump in *Vicia faba* L. guard cell protoplasts. - Proc. nat. Acad. Sci. USA **85**: 436-440, 1988.

Serrano, R., Gaxiola, R., Rios, G., Forment, J., Vicente, O.R.: Salt stress proteins identified by a functional approach in yeast. - Monatshefts Chemie **134**: 1445-1464, 2003.

Shao, H.B., Song, W.Y., Chu, L.Y.: Advances of calcium signals involved in plant anti-drought. - Crit. Rev. Biol. **331**: 587-596, 2008.

Shinozaki, K., Yamaguchi-Shinozaki, K., Seki, M.: Regulatory network of gene expression in the drought and cold stress responses. - Curr. Opin. Plant Biol. **6**: 410-417, 2003.

Sunkar, R., Bartels, D., Kirch, H.H.: Overexpression of a stress-inducible dehydrogenase gene from *Arabidopsis thaliana* in transgenic plants improves stress tolerance. - Plant J. **35**: 452-464, 2003.

Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., Yamaguchi-Shinozaki, K., Shinozaki, K.: Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. - Plant J. **29**: 417-426, 2002.

Tezara, W., Mitchall, V., Driscoll, **S.P**., Lawlor, D.W.: Effects of water deficit and its interaction with CO_2 supply on the biochemistry and physiology of photosynthesis in sunflower. - J. exp. Bot. **375**: 1781-1791, 2002.

Tezara, W., Mitchel, V., Driscoll, **S.D**., Lawlor, D.W.: Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. - Nature **1401**: 914-917, 1999.

Thippeswamy, M., Chandraobulreddy, P., Sinilal, B., Shivakumar, M., Dudhakar, C.: Proline accumulation and the expression of Δ^1-pyrroline-5-carboxylate synthetase in two safflower cultivars. - Biol. Plant. **54**: 386-390, 2010.

Tuteja, N., Mahajan, S.: Calcium signaling network in plants-an overview. - Plant Signal. Behavior **2**: 79-85, 2007.

Umezawa, T., Fujita, M., Fujita, Y., Yamaguchi-Shinozaki, K., Shinozaki, K.: Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. - Curr. Opin. Biotechnol. **17**: 113-122, 2006.

Umezawa, T., Yoshida, R., Maruyama, K., Yamaguchi-Shinozaki, K., Shinozaki, K.: SRK2C, a SNF1-related protein kinase 2, improves drought tolerance by controlling stress-responsive gene expression in *Arabidopsis thaliana*. - Proc. nat. Acad. Sci. USA **101**: 17306-17311, 2004.

Upadhyay, H., Panda, S.K.: Responses of *Camellia sinensis* to drought and rehydration. - Biol. Plant. **48**: 597-600, 2004.

Upadhyay, H., Panda, S.K., Dutta, B.K.: Variation of physiological and antioxidative responses in tea cultivars subjected to elevated water stress followed by rehydration recovery. - Acta Physiol. Plant **30**: 457-468, 2008.

Upadhyay, H., Panda, S.K., Dutta, B.K.: $CaCl_2$ improves post-drought recovery potential in *Camellia sinensis* (L) O. Kuntze. - Plant Cell Rep. **30**: 495-503, 2011.

Urano, K., Maruyama, K., Ogata, Y., Morishita, Y., Takeda, M., Sakurai, N., Suzuki, H., Saito, K., Shibata, D., Kobayashi, M., Yamaguchi-Shinozaki, K., Shinozaki, K.: Characterization of the ABA-regulated global responses to dehydration in *Arabidopsis* by metabolomics. - Plant J. **57**: 1065-1078, 2009.

Valliyodan, B., Nguyen, H.T.: Understanding regulatory networks and engineering for enhanced drought tolerance in plants. - Curr. Opin. Plant Biol. **9**: 189-195, 2006.

Vinocur, B., Altman, A.: Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. - Curr. Opin. Biotechnol. **16**: 123-132, 2005.

Wang, W., Vinocur, B., Altman, A.: Plant responses to drought, salinity and extreme temperatures towards genetic engineering for stress tolerance. - Planta **218**: 1-14, 2003.

Wang, W.X., Vinocur, B., Shoseyov, O., Altman, A.: Biotechnology of plant osmotic stress tolerance: physiological and molecular consideration. - Acta Hort. **560**: 285-292, 2001.

Wang, Y., Ying, J., Kuzma, M., Chalifoux, M., Sample, A., McArthur, C., Uchacz, T., Sarvas, C., Wan, J., Dennis, D.T.: Molecular tailoring of farnesylation for plant drought tolerance and yield protection. - Plant J. **43**: 413-424, 2005.

Wise, M.J.: Leaping to conclusions: a computational reanalysis of late embryogenesis abundant proteins and their possible roles. - BMC Bioinformatics **4**: 52, 2003.

Wise, M.J., Tunnacliffe, A.: POPP the question: what do LEA proteins do? - Trends Plant Sci. **9**: 13-17, 2004.

Xiong, L., Schumaker, K.S., Zhu, J.K.: Cell signaling during cold, drought and salt stress. - Plant Cell: S165-S183, 2002.

Yamaguchi-Shinozaki, K., Shinozaki, K.: Organization of *cis*-actin regulatory elements in osmotic- and cold-stress-responsive promoters. - Trends Plant Sci. **10**: 88-94, 2005.

Yancy, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D., Somero, G.N.: Living with water stress: evolution of osmolyte systems. - Science **217**: 1214-1223, 1982.

Yoshida, R., Hobo, T., Ichimura, K., Mizoguchi, T., Takahashi, F., Aronso, J., Ecker, J.R., Shinozaki, K.: ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in *Arabidopsis*. - Plant Cell Physiol. **43**: 1473-1483, 2002.

Yoshida, R., Umezawa, T., Mizoguchi, T., Takahashi, F., Takahashi, S., Shinozaki, K.: The regulatory domain of SRK2E/OST1/SnRK2.6 interacts with ABI1 and integrates ABA and osmotic stress signals controlling stomatal closure in *Arabidopsis*. - J. biol. Chem. **281**: 5310-5318, 2006.

Zeevaart, J.A.D., Creelman, R.A.: Metabolism and physiology of abscisic acid. - Annu. Rev. Plant Physiol. **39**: 439-473, 1988.

Zhang, G., Chen, M., Chen, X., Xu, Z., Guan, S., Li, L.C.: Phylogeny, gene structures, and expression patterns of the ERF gene family in soybean (*Glycine max* L.). - J. exp. Bot. **59**: 4905-4917, 2008.

Zhang, J.Y., Broeckling, C.D., Blancaflor, E.B., Sledge, M.K., Sumner, L.W., Wang, Z.Y.: Over expression of WXP1, a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*) - Plant J. **42**: 689-707, 2005.

Zhang, J.Z.: Overexpression analysis of plant transcription factors. - Curr. Opin. Plant Biol. **6**: 430-440, 2003.

Zhang, R.Y., Zhao, X.U., Li, L.C., Chen, M., You, M.A.: Isolation and expression analysis of a novel abiotic stress-induced gene *W89* from wheat. - Agr. Sci. **6**: 391-398, 2007.

Zhang, X., Wang, T., Li, C.: Different responses of two contrasting wheat genotypes to abscisic acid application. - Biol. Plant. **49**: 613–616, 2005.

Zheng, J., Fu, J., Gou, M., Huai, H., Liu, Y., Jian, M., Huang, Q., Guo, X., Dong, Z., Wang, H., Wang, G.: Genome-wide transcriptome analysis of two maize inbred lines under drought stress. - Plant mol. Biol. **72**: 407-421, 2010.

Zheng, X., Chen, B., Lu, G., Han, B.: Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. - Biochem. biophys. Res. Commun. **379**: 985-989, 2009.

Zhu, J.K.: Salt and drought stress signal transduction in plants. - Annu. Rev. Plant Biol. **53**: 247-273, 2002.