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Recent advances in molecular breeding of drought tolerance in rice (*Oryza sativa* L.)

Khela Ram Soren, Kishwar Ali, Vandana Tyagi[1] and Aruna Tyagi*

Division of Biochemistry, Indian Agricultural Research Institute, New Delhi 110 012, India
[1]Germplasm Exchange Unit, National Bureau of Plant Genetic Resources, New Delhi 110 012, India

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Rice is an ideal plant species for genomic studies for its relative small genome size (~430 Mb), diploid origin (2x=24) and close relationship with other important crops. Rice has been grown under diverse ecological conditions and gets exposed to different environmental stresses like drought, salinity, cold, etc. Drought is generally avoided in irrigated rice production system but it is more prone to 63.5 mha of rainfed rice grown annually in different parts of world. Severe osmotic stress causes detrimental changes in cellular components. Yet in response to various environmental stresses, plants have developed different physiological and biochemical strategies to adapt stress conditions, such as, stress associated changes in metabolites and amino acids (proline), amines (glycin-betaine and polyamines), and variety of sugar and sugar alcohols (manitol and trehalose). There is also activation of cascade of molecular networks involved in stress perception, signal transduction and the expression of specific stress related genes. To understand these genetically complex mechanisms of abiotic stress tolerance, an integrated approach of molecular breeding, classical physiology and conventional breeding is necessary, and the present review is an effort to deal these issues.

Keywords: Drought stress, osmoprotectent, rice, transcription factor

Introduction

Crop performance is sensitive to a number of biotic and abiotic factors, wherein drought stress constitutes an important yield-limiting determinant. Drought not only causes the delay of flowering[1] but also affects the process of starch deposition in pollen grains and, thus, leading to enhanced anther dehiscence. Drought stress refers to a situation when the amount of water in the plant is not sufficient to meet the transpiration requirements, which leads to altered visible symptoms, such as, leaf curling. Drought should also be quantifiable through important physiological parameters, such as, relative water content (RWC), water use effeciency (WUE), harvest index (HI), total dry matter (TDM), crop duration (C), transpiration efficiency (TE), etc. Plant response to water-deficit are dependent on the amount of water lost, the rate of water loss, the duration of drought stress, the plant variety/species under consideration, developmental stages of the plant and other environmental variables, such as, temperature, relative humidity, etc.

Stress affects many metabolic pathways and structures, which may be the result of some up or down-regulated genes. These gene products can be classified into two groups. First group includes many of the water-deficit induced gene encoded products, predicted to protect cellular functions. One commonly observed response of the plant is the accumulation of metabolically compatible solutes, such as, proline, glycine-betaine, pinitol, carnitine, mannitol, sorbitol, polyols, trehalose, sucrose, oligosachharides and fructans in large quantities. These are chemically dissimilar and are excluded from the surface of the proteins; thus, keeping the proteins preferentially hydrated. Accumulation of these compounds results in decreased water potential, facilitating water movement in the cell that helps in maintaining the turgor, a mechanism proposed to safeguard against water deficit. Second group of the gene products, such as, transcription factors, consists of proteins that are involved in regulation of gene expression and signal transduction[2]. These genes may be useful for improving the stress tolerance of plants as they can regulate many stress inducible genes involved in stress tolerance.

Various approaches of genetic engineering have been attempted to improve the stress tolerance of plants by gene transfer[2]. The genes selected for transformation were those involved in encoding enzymes required for biosynthesis of various

*Author for correspondence:
Mobile: 91-9868288687
E-mail: at_bio@iari.res.in

osmoprotectants, late embryogenesis abundant (LEA) proteins and detoxification enzymes.

Engineering for Osmoprotectant Accumulation

Osmolytes are the non-toxic compounds that accumulate during drought and salinity stress (Table 1). These compounds stabilize proteins and cell membranes against the denaturing effect of stress conditions on cellular functions. Their accumulation result in the decrease of cell osmolytic potential and, thus, in the maintenance of water absorption and cell turgor pressure, which might contribute to sustaining physiological processes, such as, stomatal opening, photosynthesis and expansion of growth[3-6]. Many major crops lack the ability to synthesize the special osmoprotectants that are naturally accumulated by stress-tolerant organisms. It has, therefore, been hypothesized that engineering the introduction of osmoprotectant synthesis pathways is a potential strategy for improving the stress tolerance of crop plants.

The genetic engineering of metabolic pathways for the production of osmolytes, such as, mannitol, fructans, trehalose, ononitol, proline or glycine-betaine among others, might increase the resistance to drought (Table 2). However, the mechanism by which these osmolytes provide protection is not yet completely understood[7]. Genetic modulation for osmolyte accumulation, whereas does not always lead to osmotic adjustment in plants in response to stress. Other responses like production of scavenging reactive oxygen species (ROS) and induction of chaperone-like activities that protect protein structure and metabolic detoxification are also being reported during drought stress. Water deficit threatens crop survival and offers little yield to grower[8]. The stimulated osmotic adjustment in cell is the ability to readily induce over-expression in the production of non-toxic compounds, resulting in a number of putative benefits for crop production in drought stress environment. A number of genes and cDNAs encoding enzymes involved in osmolyte biosynthesis have been isolated from different organisms and genetically transformed into transgenic plants[9].

Table 1—Osmolyte that accumulates in plant during drought

Carbohydrate	Nitrogenous compound	Organic acid
Sucrose	Protein	Oxalate
Sirvutik sorbitol	Glycine-betain	Malate
Mannitol	Glutamate	
Glycerol	Aspertate	
Arabinitol	Glycine	
Pinitol	Choline	
Other polyols	Putrescine	

Glycine-betaine

Choline plays a vital role as the precursor for phosphatidylcholine, a dominant constituent of membrane phospholipids in eukaryotes. A large proportion of free choline is diverted to glycine-betaine in plants that naturally accumulates in response to stress. Glycine-betaine is synthesized by a two-step oxidation of choline *via* betaine aldehyde. In bacterium *Escherichia coli* (Migula) Castellani & Chalmers, choline is oxidized by a membrane-bound choline dehydrogenase (CDH) to betaine aldehyde and then oxidized to glycine-betaine by a soluble betaine aldehyde dehydrogenase (BADH)[10]. In contrast, in soil inhabiting *Arthrobacter* spp., choline is oxidized by choline oxidase (COX), a soluble enzyme that generates hydrogen peroxide during the reaction[11]. In plants, choline oxidation to betaine aldehyde is carried out by choline monooxygenase (CMO) enzyme, an iron sulphur enzyme[12]. Then, betainealdehyde oxidation to glycine-betaine is catalysed by BADH, a non-specific aldehyde dehydrogenase[13,14]. Both these enzymes are stress-inducible stromal enzymes[15,16].

Table 2—Engineering of compatible solutes

Classification	Gene name	Transgene	Origin	Expression	Experiment	Parameter	Year
Proline	P5CS	Rice	Mothbean	AIPC-ABA	Water holding	Shoot growth	1998
Trehalose	TPSP-OtsA-OtsB	Rice	*Escherichia coli*	ABA-inducible	Water holding	Photosynthesis, shoot growth	2002
Trehalose	TPSP-OtsA-OtsB	Rice	*E. coli*	ABA-inducible	Water holding	Photosynthesis, shoot growth	2003
Polyamines	ADC	Rice	*Datura stramonium*	Maize-Ubi-IP	20% PEG	Shoot growth	2004
DREB/CBF	DREB/CBF3	Rice	*Arabidopsis*	Maiz Ubi-IP	Water holding	Photosynthesis, surviviality	2005

Overexpression of the BADH gene in the tobacco showed increased tolerance to the drought stress[17]. Recent works demonstrated that the activity and regulation of phosphoethanolamine methylation is the limiting factor in choline synthesis in tobacco[18,19]. Genetic analysis of *Arabidopsis* t365 mutant having an impaired S-adenosyl-L-methionine phosphoethanolamine-N-methhyl transferase (PEMT) gene, involved in glycine-betaine biosynthesis, exhibited hypersensitivity to salt stress. Thus, glycine-betain accumulation is considered critical for salt tolerance.

Transgenic rice plants expressing BADH converted high levels of exogenously applied betaine aldehyde to glycine-betaine than did wild type plants. The elevated levels of glycine-betaine in transgenic plants conferred significant tolerance to salt, cold and heat stress[20]. Glycine-betaine stabilizes the quaternary structure of proteins and the highly ordered state of membranes and also reduces lipid peroxidation during salinity stress. Furthermore, compartmentation of these osmoprtectants may also be required for enhanced tolerance, e.g., transgenic rice plants that overexpressed choline oxidase targeted to chloroplasts showed better tolerance to photoinhibition under salt and low temperature stress than did plants overexpressing choline oxidase targeted to cytoplasm. It is also possible that choline transport into the chloroplast differs among species[20,21].

Mannitol

Mannitol is a major photosynthetic product in many algae and higher plants, and enhances tolerance to water-deficit stress primarily through osmotic adjustment. The introduction of a mannitol dehydrogenase (mtlD) gene into wheat produced a considerable increase in water stress tolerance. There was, however, no significant difference in osmotic adjustment between the mtlD transgenic wheat and control plants, at either the callus or whole-plant level, suggesting that the beneficial effect of mannitol resulted from protective mechanisms other than osmotic adjustment[22]. These mechanisms are likely to involve scavenging of hydroxyl radicals (OH^-) and the stabilization of macromolecules. Su e*t al*[23] obtained three rice lines with bacterial mltD and demonstrated that biosynthesis and accumulation of mannitol in plants were correlated with the salt stress tolerance of plants. *Arabidopsis* plants transformed with bacterial mltD had a high mannitol content and were able to withstand NaCl salinity up to 400 mol m^{-3}, whereas wild type seeds did not germinate at 100 mol m^{-3} NaCl [9]. In tobacco, mannitol has been shown to protect thioredoxin, glutathione and thiol regulated enzyme phosphoribulokinase from the effect of OH^- [24]. Another example of the protection of sensitive enzymes and membranes from ROS is provided by D-ononitol and myo-inositol in cytoplasm. Transgenic tobacco plants that overexpressed the inositol methyl transferase gene (*Imt*1) from the ice plant (*Mesembryanthemum crystallinum* L.) showed increased drought and salt stress tolerance by the accumulation of D-ononitol, a methylated form of inositol[25].

Raffinose and Galactionol

Water-deficit alters the synthesis and partitioning of metabolically important carbohydrates in plants. Some of these effects on carbohydrate metabolism might be required for the photosynthetic assimilation of carbon and its conversion to metabolically usable forms. Other stress-induced changes in carbon metabolism might reflect adaptations for stress tolerance. For example, raffinose-family oligosaccharides, such as, raffinose, stachyose and galactinol, play important roles in the desiccation tolerance of plants. Seven galactinol synthase (GolS) related genes have been identified in the *Arabidopsis* genome, but little is known about their roles in the accumulation of galactinol and raffinose in plants under water-deficit conditions. *Arabidopsis* plant that overexpressed the *AtGolS1* and *AtGolS2* genes showed enhanced tolerance to drought stress owing to their accumulation of galactinol and raffinose[26]. The endogenous production of these sugar compounds in transgenic plants provided membrane protection and a reduced rate of transpiration from the leaves, which resulted in drought tolerance. Hence, galactinol and raffinose act as osmoprotectants that provides an adaptation to water stress conditions rather than by conferring an osmotic adjustment.

Fructan

Fructans are polyfructose molecules that are soluble carbohydrates and are located in the vacuoles of many plants. Fructan metabolism plays a significant role in drought and cold-stress tolerance in plants. These compounds are soluble and might play a role in the osmotic adjustment for multiple stress tolerance. The value of any gene or pathway for drought tolerance in crop plants can only be judged by

evidence of solid field adjustment of natural fructan accumulators to different abiotic stresses by varying the degree of polymerization of the fructan pool. Tobacco and sugar beet plants that were engineered with the bacterial fructan gene (*Sac*B) showed enhanced tolerance to drought stress conditions[27].

Trehalose

Trehalose (α-D-glucopyranosyl-1, 1-α-D-gluco-pyranoside) is a non-reducing disaccharide that is present in many different organisms and functions as reserve carbohydrate and stress protectant, stabilizing protein membranes and protecting them from denaturation. In yeast, trehalose is synthesized from UDP-glucose and glucose-6-phosphate in two reactions, which are catalyzed by trehalose phosphate synthase (TPS) and trehalose-6-phoshate-phosphatase (TPP). A family of 11 TPS genes including TPPs and a subfamily of TPPs has been identified in *Arabidopsis*. Metabolic engineering of yeast TPS gene in tobacco plants resulted in an elevated level of drought-stress tolerance in tobacco[17]. The TPP enzyme plays a significant role in sugar and abscisic acid (ABA) signaling during vegetative development and the overexpression of *AtTPS1* produces increased drought tolerance through these signaling processes. The level of trehalose accumulation in transgenic plants that overexpressed *AtTPS1* was slightly changed, whereas the trehalose-6-phosphate level was increased without affecting plant morphology[28]. Often engineered alterations in osmoprotectant accumulation resulted in infertility depending upon the concentration of osmoprotectants. Use of a stress-inducible promoter to overexpress osmoprotectants biosynthesis may help overcome the growth defects, while protecting the plants during osmotic stress[29]. Controlled trehalose overproduction in rice owing to the stress-inducible or tissue-specific expression of bifunctional TPS fusion gene resulted in drought tolerance without any detrimental effect on plant growth or grain yield. In addition, the expression of this gene resulted in elevated soluble carbohydrate levels, including subtle changes in the levels of glucose, fructose, and sucrose. These results confirm the role of trehalose in sugar sensing and carbon metabolism.

Proline

Proline accumulation plays a highly protective role in plants that are exposed to abiotic stresses, conferring osmotic adjustment together with an increase in the levels of other osmolytes. Other suggested functions of proline are the detoxification of ROS generated by these stresses and interaction with the hydrophobic residues of proteins[30]. Proline acts as an antioxidant and stablizes subcellular structures (membrane and proteins) and buffers cellular redox potential under stress[18]. The proline biosynthetic pathway has been well characterized[31]. Although proline can be synthesized from either glutamate or ornithine, glutamate is primary precursor in osmotically stressed cells. The biosynthetic pathway consists of two important enzymes, viz., pyrroline carboxylate synthetase and pyrroline-carboxylate reductase. Transcripts correlating to both cDNAs accumulate in response to NaCl treatments. Both these regulatory steps are key to developing strategies for overproducing proline in selected plant species. A pyrroline-5 carboxylate synthetase (P5CS) cDNA from mothbean was introduced into rice. The expression of this P5CS transgene under the control of a stress inducible promoter led to stress induced overproduction of the P5CS enzyme and proline accumulation in transgenic rice plants. Second generation transgenic rice plants showed an increase in biomass under salt and water stress conditions[19,32]. The drought tolerant cultivar of rice (N-22) showed high RWC, proline content and gene expression compared to susceptible one (Panidhan). The proline levels were positively correlated with P5CS activities and expressions of P5CS[33]. The involvement of proline in response to water deficit has been demonstrated in transgenic tobacco that overexpressed proline biosynthesis enzymes[34]. The suppression of proline synthesis in transgenic plants that contain the P5CS gene in the antisense direction resulted in increased sensitivity to water deficit. Recently, it was reported that transgenic petunia plants that overexpressed the *AtP5CS* gene from *Arabidopsis* and the *OsP5CS* gene from rice can withstand drought conditions for longer durations than wild type plants[35]. The expression of P5CS also resulted in drought tolerance in soybean. The sense transformants, which demonstrated the earliest proline accumulation, experienced the least water loss when compared to the antisense transformants, which possessed the slowest proline accumulation[36]. In *Lathyrus sativus* L., a grain legume that can withstand drought, high proline accumulation was observed in leaves and roots under water stress[37].

Late Embryogenesis Abundant (LEA) Proteins

LEA proteins were first characterized in cotton and wheat[38]. They are produced in abundance during seed development comprising upto 4% of cellular proteins. In both plants and animals, LEA proteins are associated with tolerance to water stress resulting from desiccation and cold shock. Genes encoding LEA proteins (9 to 200 kDa) have been identified in many plant species, and at least six different groups of LEA proteins have been identified on the basis of expression pattern and sequence. Group 1 (*Gossypium hirsutum* L. D19, *Triticum aestivum* L. EM & *Hordeum vulgare* L. B19) is characterized by an internal 20-amino acid signature motif repeated upto four times depending on the species and a high proportion of Gly, Glu, and Gln residues. Group 2, also referred to as dehydrin, is characterized by a highly conserved 15-amino acid lysine-rich sequence, or K-segment, with a consensus EKKGIMDKIKEKLPG. Dehydrins are induced by dehydration-related stresses, such as, low temperature, drought and high salinity[39], and in response to wounding[40,41]. Group 3 (*H. vulgare* HVA1 and *Daucus carota* L. Dc8) has an 11-amino acid fragment whose consensus sequence has been broadly defined as ΦΦE/QXΦKE/QKΦXE/D/Q (where Φ represents a hydrophobic residue)[42]. This group has been increased by the discovery of homologues in organisms other than plants, including the nematodes—*Caenorhabditis elegans* Maupas, *Steinernema feltiae* Filipjev and *Aphelenchus avenae* Bastian—and the prokaryotes—*Deinococcus radiodurans* Brooks & Murray, *Bacillus subtilis* (Ehrenberg) Cohn and *Haemophilus* influenzae (Lehmann & Newmann) Winslow[44]. Group 3 LEA proteins, from a hydrobiotic nematode *A. avenae*[44] and a putative example from bullrush[45], are natively unfolded in solution but seem to become more structured on drying. A mutational study of genes in *D. radiodurans*[46] supported the role for Group 3 LEA proteins in the sequestration of ions that were concentrated during cellular dehydration but again their precise function is unknown. Group 4 LEA proteins contain conserved N-terminal domains that form a helix and less conserved C-terminus, rich in glycine and amino acids that contain hydroxyl groups, and form an unstructural random coil[46]. They have been suggested to bind water molecules and may also act as reserve chaperones, stabilizing the surface of membranes and possibly protect proteins by binding water and functioning as a salvation film. Genes encoding group 4 LEA are expressed in vegetative tissue in response to salinity, drought, ABA and low temperature[48,49]. The Group 4 consists of LEA14 and cotton D113. Group 5 (D34, D29 and DcECP31) also has an 11-mer repeat, in which each amino acid has the chemical properties similar to Group 3. Group 5 of LEA proteins are predicted to sequester ions during water loss but well defined function is not yet known. Group 6 (D95) has not been well defined.

In addition to determine the structure of the LEA proteins, it is also important to investigate the molecular mechanisms that regulate LEA genes in response to ABA, drought and salt stress[50,51]. After completion of genome sequencing of both *indica* and *japonica* rice the draft genome sequence available in the public database were used to investigate the rice LEA (*OsLEA*) gene with the help of bioinformatics approach throughout the rice genome and performed an evolution analysis of the *OsLEA* gene family. A total of 34 rice LEA (*OsLEA*) genes were identified[52], of which 25 LEA genes were new. All the LEA genes were distributed on all rice chromosomes, except 10 and 12. In salt-tolerant rice varieties, LEA proteins were induced to higher levels by salt or ABA in comparison to those of salt-sensitive rice varieties[53]. Genetically engineered rice plants constitutively over-expressing a barley LEA gene (HVA1) driven by rice actin 1 promoter showed better salt and water stress tolerance and faster recovery once the stress was relieved. Wilting, dying of old leaves and necrosis of young leaves were delayed in transgenic rice, when compared to control plants under both salt and water stress[54]. On the other hand, LEA proteins alone showed *in vitro* anti-aggregation activity upon desiccation and freezing of the enzymes, suggesting the involvement of LEA proteins in preventing the formation of aggregates of denatured proteins during water stress[55].

Channels Protein

Apart from the osmolytes assisting in maintaining the hydration status, drought or osmotically stressed plants have several genes which produce water channel proteins/water transport proteins, such as, membrane proteins of family aquaporins that can alter the cellular water potential and, thus, protect against water deficit[56-58]. Two proteins, osmotin and a nonspecific lipid transfer protein are stress induced and thought to play a role in controlling pathogens[59]. Salt-overly-sensitive (SOS) regulatory pathway is induced due to the excessive intracellular or extracellular Na^+, which triggers a cytoplamic Ca^{2+}

signal[59]. Initially the salt stress signaling is perceived by the SOS3 protein and it interacts with and activates SOS2, a serine/threonine protein kinase[61,62]. SOS3 and SOS2 regulate the expression level of SOS1, a salt tolerance effector gene encoding a plasma membrane Na^+/H^+ antipoter[63]. *OsNHA*1 full-length cDNA (3612 bp), cloned from rice[64] (*Oryza sativa* L.), encodes a putative plasma membrane Na^+/H^+ antiporter. Its deduced protein has 11 transmembrane domains and a significant similarity to a plasma membrane Na^+/H^+ antiporter *AtNHA1* from *A. thaliana* (L.) Heynh. This was confirmed through semi-quantitative RT-PCR assay, which revealed that the expression of *OsNHA*1 was up-regulated in both shoots and roots of rice seedlings under salt stress.

Antioxidant and ROS Scavenger

Stress-induced production of ROS, singlet oxygen ($^1O_2$), superoxide radical (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) is another aspect of environmental stress in plants[65], which can damage chlorophyll, protein, DNA, lipids and other important macromolecules[66-68]; thus, affecting plant metabolism and ultimately growth and yield. However, the plant possesses both enzymatic and non-enzymatic mechanisms for scavenging of ROS by overproducing enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione-synthesizing enzymes. These enzymes are overproduced during the stress and detoxify the ROS. Plants overexpressing *Chlamydomonas* glutathione peroxidase in the cytosol and in the chloroplast displayed increased tolerance to oxidative stress, which was imposed by using methylviologen, chilling and salt stress[69]. Overexpression of the aldehyde dehydrogenase (*AtALDH3*) gene in *Arabidopsis* conferred tolerance to drought and salt stress[70]. Aldehyde dehydrogenase catalyzes the oxidation of toxic aldehydes, which accumulate as a result of side reactions of ROS with lipids and proteins[71]. ROSs play a key signaling role in plants and are controlled in cells by a complex network of ROS metabolizing enzymes found in several different cellular compartments. To study different ROSs signal, a double mutant lacking in thylakoid ascorbate peroxidase (*tylapx*) and cytosolic ascorbate peroxidase 1 (*apx1*) genes was generated. It was suggested that two different signals were generated in plants lacking cytosolic APX1 or tylAPX. The absence of a chloroplastic H_2O_2 removing enzyme triggered a specific signal in cells that resulted in enhanced tolerance to heat stress, whereas the absence of a cytosolic H_2O_2 removing enzyme triggered a different signal that resulted in stunted growth and enhanced sensitivity to oxidative stress. When the two signals were co-activated in cells (*tylapx/apx1*), a new response was detected that manifested in late flowering, low protein oxidation during light stress and enhanced accumulation of anthocyanins[72]. Transgenic rice overexpressing yeast manganese superoxide dismutase (Mn-SOD) exhibited increased level of APX and chloroplastic SOD in transformed rice compared to wild-type[73]. The transformed rice plants also showed more salinity tolerance than the wild type.

Heat-shock Protein and Molecular Chaperons

Heat-shock proteins (HSPs), molecular chaperones as well as LEA protein families are involved in plant abiotic stress tolerance[74,75]. High temperature, salinity and drought stress can cause denaturation and dysfunction of many proteins. The *in vivo* function of small heat shock proteins (sHSPs) in thermoprotection was successfully demonstrated by the constitutive expression of anti-sHSP single-chain fragment variable (scFv) antibodies in tobacco[76]. When scFv-transgenic plants were subjected to prolonged high temperature conditions, they became yellow and then died, suffering mainly from total destruction of cellular membranes. The expression of the specific scFv prevented the formation of functional heat stress granules (HSGs) and resulted consequently in the collapse of different cell compartments at sublethal temperatures. Expression of HSPs is primarily regulated by heat dependent activation of heat shock transcription factors (HSFs). Constitutive expression of heat shock transcription factor, HSF3 has been shown to cause increased expression of small HSPs (HSP17.1, HSP17.6, HSP18.2, HSP18.4) and, thus, providing direct evidence for the significance of transcription factors in the expression of stress genes[77]. The constitutive expression of heat shock transcription factor HSF3 resulted in HSP synthesis at 25°C and increased the level of thermotolerance in transgenic *Arabidopsis* without prior exposure to elevated temperatutres[78]. Transgenic *Arabidopsis* plants expressing less than normal amounts of HSP101, a result of either antisense inhibition or co-suppression, grew at normal rates but had a diminished capacity to acquire heat

tolerance[79]. Conversely, plants constitutively expressing HSP101 tolerated sudden shifts to extreme temperatures better than controls. Association of small HSPs products has been demonstrated with plant desiccation tolerance[80]. HSPs that are induced by water deficit may be involved in refolding of proteins in order to regain their function or the prevention of protein aggregation during drought[81-83]. Small HSPs might act as molecular chaperones during seed dehydration and first few days of rehydration. OsHSP110 accumulated in shoots of rice seedlings in response to salinity, drought and low temperature apart from heat shock. It has been shown that two of the HSPs, HSP70 in maize and HSP27 in soybean, could also be induced by water stress[84]. Transgenic *Arabidopsis* plant overexpressing AtHSP17.7 accumulated high level of AtHSP17.7 protein and showed enhanced tolerance to drought and salinity[85].

Identification of Drought and High-salinity Stress Responsive Genes Using Rice Whole Genome Oligomer Microarray

DNA microarray provides a high throughput means of analyzing genome expression, which has been used to study patterns of gene expression in response to drought or high-salinity stresses in several plant species[86]. A microarray containing ~1,300 full-length cDNA clones from *Arabidopsis* was used to study gene expression under drought and cold stress. This study resulted in the identification of 44 and 19 cDNA clones as drought and cold inducible genes, respectively[87]. Other study employed an improved microarray containing around 7,000 *Arabidopsis* full-length cDNA clones to profile gene expression in response to ABA treatment[88] as well as cold, drought, and high-salinity stresses[89]. In another study, an Affymetrix Gene Chip covering approximately 8,100 genes from *Arabidopsis* was employed to monitor changes in gene expression under salt, osmotic and cold stresses. The study revealed that resulting expression changes varied significantly between root and leaf with only minor overlap[90]. Similar studies have also been performed in barley to assess the drought and high-salinity gene expression responses using a microarray containing 1,463 DNA elements[91]. Rice is a model plant for cereal crops and has one of the richest set of resources available for plant genomic studies[92-96]. In rice, the high-salinity stress response has been analyzed with a microarray containing 1,728 cDNA clones from a root cDNA library of salt-tolerant rice[96]. In another microarray study containing 8,987 DNA elements, 509 possible ABA or gibberellin responsive genes were detected[98]. Later, the same group used a 22,000 rice cDNA based oligonucleotide array to identify ABA and GA-responsive genes and made comparison of ABA-responsive genes and their putative responsible promoter elements between rice and *Arabidopsis*[99]. This analysis gave an initial global view of the ABA and GA-responsive genes in rice. Using a microarray containing 1,700 full length cDNA clones, a total of 73 genes were identified, which were induced by cold, drought, high-salinity and ABA treatment (Table 3)[100]. Genes associated with water-stress response in rice were identified using expressed sequence tags (ESTs) generated from a normalized cDNA library, constructed from drought-stressed leaf tissue of an *indica* cultivar, Nagina-22[101]. Analysis of 7794 cDNA sequences led to the identification of 5815 rice ESTs. Of these, 334 exhibited no significant sequence homology with any rice ESTs or full-length cDNAs in public databases, indicating that these transcripts are enriched during drought stress. Analysis of these 5815 ESTs led to the identification of 1677 unique sequences. To characterize this drought transcriptome further and to identify candidate genes associated with the drought-stress response, the rice data were compared with those for abiotic stress-induced sequences obtained from expression profiling studies in *Arabidopsis*, barley, maize, and other rice. This comparative analysis identified 589 putative stress-responsive genes (SRGs) that are shared by these diverse plant species. Further, the identified leaf SRGs were compared to expression profiles for a drought-stressed rice panicle library to identify common sequences. Significantly, 125 genes were found to be expressed under drought-stress in both tissues. The functional classification of these 125 genes showed that a majority of them are associated with cellular metabolism, signal transduction, and transcriptional regulation. A total of 582, 1,257 and 614 drought up-regulated genes and 795, 646 and 1,305 drought down-regulated genes were identified in rice flag leaf, shoot and panicle, respectively. Most of the up and down-regulated genes during dehydration have been isolated but very few rehydration-specific proteins are known[102].

Table 3—No. of clones involved in different functional group up-regulated by cold, drought and high salinity or ABA application

Functional Category	No.	Description
Transcriptional factor	6	bZip DNA-binding protien, C2H2 type zinc finger, DNA-binding protein, C3HC2 type RING finger protein, Myb type DNA-binding protein and NAC type DNA-binding protein
Receptor-like protein kinase	1	Receptor-like protein kinase
Protien phosphatase	1	Protien phasphatase 2C
Compatible solutes	6	LEA protein, dehydrin and lectin
Detoxification	3	Catalase, O-methyltransferase and aldehyde dehydrogenase
Photosynthesis	1	Chlorophyll a/b-binding protein
Membrane protein	1	Chloroplast membrane protein
Carbohydrate metabolism phosphoglycerate	7	Glycoside hydrolases, glycosyl transferase, kinase, pyruvate dehydrogenase kinase 1, trehlose-6 phosphate phosphatase, UPD-Glc 4-epimerase and carboxy phosphonoenol pyruvate mutase
Electron transport system	1	Thioredoxin
Amino acid metabolism	2	4-Hydroxyphenylpyruvate dioxygenase and S-adenosylmethionine decarboxylase
Fatty acid metabolism	3	Choline kinase, lipase and lipoxygenase
Nucleotide synthesis	1	Adenylate kinase
Harmone biosynthesis	1	Zeaxanthin epoxidase
F-box protein	1	F-box protien
Protease inhibitor	1	Protease inhibitor
Protease	1	Papain Cys protease
Dehydrogenase	3	3-Hydroxyacyl-Co A dehydrogenase, dyhydroorotate dehydrogenase and glutamate dehydrogenase
Iron homeostasis	2	Ferritin and metellothionein-like type 2
Cytoskeleton	2	Actin
Transporter	1	Sugar transporter
Unknown protein	28	Unknown protein

Drought Perception, Signal Transduction and Response

Water-deficit stress seems to be sensed by a membrane-bound two-component histidine kinase[103], which is activated by high osmolarity. Increase of a cell's osmolarity upon water loss during drought triggers the drought response signal chain. The active signal receptor activates phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate to yield the second messengers, inositol 1,4,5-trisphosphate (IP3) and diacylglycerol[104,105]. IP3 releases calcium from internal stores and the Ca^{2+} sensor (calcineurin B-like protein, CBL) activates down stream protein kinases and phosphatases. Drought inducible genes display characteristic promotor *cis*-acting elements, the dehydration-responsive elements (DREs), which at least partially resemble those of the cold-induced genes[50]. In contrast to the cold response, in which ABA appears to play a minor role, ABA triggers a major signaling pathway in drought stress response. Activation of the ABA responsive elements (ABREs) by several transcription factors, such as, the DRE-binding factors, bZIP-proteins, MYB, MYC and CBF (CBF1, 2, 3 & 4), leads to the expression of drought-stress tolerance effectors, such as, dehydrins or enzymes catalyzing synthesis of low mol wt osmolytes. The signal transduction pathway of ABA involves cADP ribose, NADP and Ca^{2+}as second messenger[106].

Regulatory Genes

Molecular mechanisms of water stress response have been investigated primarily in *A. thaliana*[107,108]. All the genes associated directly or indirectly with stress survival and stress tolerance[108] are mediated through ABA-depended and independent signal pathways. Some of these induced genes are responsible for accumulation of osmoprotectents and some are for transcriptional regulators (Table. 4)[109-113]. There are four independent signal pathways in response to dehydration, two are ABA-dependent[114] and two are ABA-independent[115].

Transcription factors that bind to the C-repeat element (CRT)/dehydration responsive element (DRE) are termed CRT binding factor (CBF)/DRE-

Table 4—Transcription factor genes induced in each organ by drought or high salinity

Gene name	Putative functions		
Panicle induced by high salinity only		S(h)	D(h)
OsIFCCO18668	bHLH transcription factor	1.673	-0.850
OsIFCCO29156	Helix-loop-helix DNA-binding domain	1.720	-0.599
OsJRFA110611	No apical merismatic (NAM) protein	2.234	-1.500
OsJRFA105079	CCAAT-box binding factor HAP5 homolog	3.521	-0.850
OsJRFA070817	RING zinc finger protein	1.708	0.685
OsIFCC039583	Zinc finger C3HC4-type (RING finger)	2.278	0.530
OsJRFA108605	Helix-loop-helix DNA-biding domain	4.173	0.240
OsJRFA108208	AP2 domain	2.429	N/A
OsJRFA101136	C3HC4-type zinc finger	5.559	-0.753
OsIFCA008718	No apical merismatic (NAM) protein	2.542	N/A
OsJRFA066984	Dof domain, zinc finger	1.846	0.164
OsJRFA110661	Zinc finger C-x8-C-x5-C-x3-H-type	1.732	N/A
OsJRFA106969	Myb-like DNA-binding domain	2.012	N/A
OsIFCC016263	Zinc finger,C2H2-type	2.636	N/A
Panicle induce by drought only S		S (h)	D (h)
OsIFCC042866	AP2 domain	N/A	3.050
Panicle induce by both drought and high salinity		S (h)	D (h)
OsJRFA107283	NAM-like protein	7.773	2.080
Shoot induced by drought only		S (h)	D (h)
OsJRFA105599	DRE-binding protein 1A	N/A	1.784
OsIFCC031932	WRKY DNA-binding domain	-2.746	2.186
OsIFCC031182	Myb factor	N/A	2.565
OsIFCC042758	Helix-loop-helix DNA-biding domain	-0.024	2.367
OsJRFA100208	Helix-loop-helix DNA-biding domain	-0.836	3.132
OsJRFA107524	Dof domain, zinc finger	N/A	2.294
OsJRFA106333	Helix-loop-helix DNA-biding domain	0.430	1.762
OsJRFA106282	WRKY DNA-binding domain	N/A	2.026
OsIFCC043271	Helix-loop-helix DNA-biding domain	0.245	2.259
OsIFCC000715	Myb-like DNA-binding domain	0.949	2.617
OsJRFA110587	Similar to DNA-binding domain	0.905	4.057
OsJRFA107146	AP2 domain	0.625	2.746
OsIFCC038336	Zinc finger transcription	0.529	2.407
Shoot induced by both drought and salinity		S (h)	D (h)
OsJRFA072192	Zinc finger protein	2.074	2.059
OsIFCC000984	WRKY DNA	1.744	2.153
Shoot induced by high salinity only		S (h)	D (h)
OsIFCCO17057	B3 DNA-binding domain	2.026	N/A
OsJRFA067496	TRAF-type zinc finger	2.199	0.801
Flag leaf induced by high drought only		S (h)	D (h)
OsIFCC029554	Zinc finger,C2H2-type	-0.553	2.032
Flag leaf induced by high salinity only		S (h)	D (h)
OsIFCC001054	Zinc finger C-×-8-C-×-5-C-×-3-H-type	1.705	0.582

binding proteins (DREBs)[107]. These factors belong to the *Apetala2/*ethylene-responsive element-binding protein (AP2/EREBP) family consisting of two subclasses, i.e., *DREB1/CBF and DREB2* that bind to drought responsive *cis*-acting elements. CRT/DRE is a *cis*-acting element having the conserved A/GCCGAC core sequence that is involved in gene expression responsive to drought and low temprerature stress in higher plants. Genes encoding CBF/DREB1 are rapidly and transiently induced by cold stress and subsequently activate the expression of other target genes. *DREB2* genes are also induced by osmotic stress and may confer induction of target stress responsive genes. It has been possible to engineer stress tolerance in transgenic plants by manipulating the expression of DREBs[116,117].

In *A. thaliana*, *DREB1* and *DREB2* genes encoded transcription factors bind to the *DRE* element present in promoter of genes like *rd29A* and thereby induce expression in response to drought, salt and cold[118-120]. Five cDNA for DREB homologes, OsDREB1A, 1B, 1C, 1D and 2A were isolated and analyzed for their functions in rice[121]. The *DREB1* genes are believed to interact with the *DRE/CRT* and induce expression of stress responsive genes, and function in response to cold, whereas *DREB2* genes are involved in drought-responsive gene expression[87,107]. *DREB1A* has been overexpressed in transgenic *Arabidopsis* plants. The resulting phenotype showed a strong induction of expression of the target genes under unstressed conditions, but also caused dwarf phenotypes in the transgenic plants. These transgenic plants also revealed freezing and dehydration tolerance. In contrast, overexpression of *DREB2A* induced weak expression of the target genes under unstressed conditions and caused growth retardation of the transgenic plants[107,122]. The stress-regulated expression of the *DREB1A* gene by the promoter of *rd29*A produced plants with increased tolerance to freezing, salt and drought stresses without a drastic change in the normal phenotype of the transformed plants[122]. It was reported that *A. thaliana DREB1A* gene when placed under the control of stress inducible promoter of *rd29A* into bread wheat showed substantial resistance to water stress[107]. An AP2/ERF family TF encoding gene has been isolated from rice which was upregulared by water deficit sress and was presumed to be involved in wax biosynthesis [123].

In fact, despite the extensive investigations carried out, understanding of *CBF/DREB1* gene function(s) remains elusive and a clear role of their requirement for stress tolerance has not yet been understood. For example, whether all three *CBF/DREB1* genes are required for freezing tolerance and cold acclimation and how the expression of *CBF/DREB1* genes is regulated in response to low temperatures are essential questions that are still unanswered. To understand the precise role of these genes and to shed some light on these issues, T-DNA mutagenized population of *Arabidopsis* plants was screened for plants containing T-DNA insertions in the *CBF/DREB1*genes. This was the first-time report on isolation and characterization for a mutant plant in which a *CBF/DREB* gene, namely *CBF2/DREB1C*, was disrupted. The results obtained indicated that CBF2/DREB1C played a critical role in the development of *Arabidopsis* tolerance to freezing and other related stresses by controlling the precise expression of *CBF1/DREB1B* and *CBF3/DREB1A* and, hence, that of the downstream genes. Upon over-expression of DREB1A under the control of *rd29A* promoter in *A. thaliana*, a number of stress tolerant genes were expressed and resulted in an improved tolerance under drought and several other stresses[125]. The expression of CBF4 was induced rapidly during drought stress and by exogenous ABA treatment, but not by cold[126]. Similarly, rice OsDREB2A was also induced by dehydration and salt stress. In *Arabidopsis*, when *OsDREB1A* gene of rice was over-expressed, it resulted in the activation of target LEA genes and thereby conferred abiotic stress tolerance including tolerance against salt stress[127]. In other studies also, salt and abiotic stress tolerance of CBF over-expressing transgenic plants was attributed to enhanced expression of LEA genes[128,129], accumulation of compatible osmolytes and enhanced oxidative stress tolerance[130,131]. Genome-wide expression analysis showed that CBF expression also induced transcription factors, such as, AP2 domain proteins and putative zinc finger protein, which might regulate genes involved in osmolyte biosynthesis and antioxidant defense[132]. These results, thus, show that expression of several genes can be manipulated in transgenic plants, engineered with a single CBF transcription factor, and enhanced expression of LEA genes is critical for salt and other abiotic stress tolerance. Recenly, an ICE1 (inducer of CBF expression 1), a MYC-type basic helix-loop-helix transcription factor, was identified in *Arabidopsis* as an upstream regulator of CBFs under cold stress[133].

Upstream transcription factors that regulate the expression of DREB2/CBFs during salt stress have yet to be identified. In rice, gene expression and kinase activity of OsMAPK5 were regulated by ABA, salt, drought, wounding and cold. The transgenic rice overexpressing *OsMAPK5* also showed increased tolerance to several abiotic stresses including salt stress[134]. This evidence demonstrates that diverse abiotic stress signals converge at MAPK3 cascade to regulate stress tolerance.

ABA-responsive *cis*-Eelement in ABA-responsive Gene Expression

The *cis* and *trans*-elements involved in ABA-induced gene expression have been analyzed extensively[41]. The best characterized *cis*-element in context of drought stress is ABA-responsive element (ABRE), which contains the palindromic motif CACGTC. Two ABRE motifs are important in the ABA-responsive expression of the *Arabidopsis* gene *rd29B*. The ABRE-binding proteins (AREBs)/ABRE-binding factors (ABFs) can bind to ABRE and activate ABA-dependent gene expression. The AREB/ABF proteins have had reduced activity in ABA-deficient *aba2* mutants and ABA-insensitive *abi1* mutants, while they showed enhanced activity in the ABA-hypersensitive *era1* mutants. Thus, activation of the AREB/ABF proteins has been shown to require an ABA-mediated signal, which probably involves ABA-dependent phosphorylation. Overexpression of *ABF3* or *AREB2*/*ABF4* caused ABA hypersensitivity, a reduced transpiration rate and enhanced drought tolerance[135-136].

MYBs/MYC Family Transcription Factor Genes

The basic/helix-loop-helix (bHLH) transcription factors and their homologs form a large family in plant and animal genomes. They are known to play important roles in the specification of tissue types in animals. On the other hand, few plant bHLH proteins have been studied functionally. More than 95% of the rice (*japonica* cv. Nipponbare) genome has been sequenced by the International Rice Genome Sequencing Project (http://rgp.dna.affrc.go.jp/cgi-bin/statusdb/irgsp-status.cgi; data from April, 2006). In this study, they have identified 167 *OsbHLH* genes from the rice genomic sequence and carried out phylogenetic analyses to understand the relationships among these rice genes. Cold stress adversely affects plant growth and crop production. Some plants express a series of cold-responsive genes during cold acclimation to reduce the damage of cold stress. Among them, transcription factors play important role in enhancing plant cold tolerance. A bHLH-type gene, *OsbHLH1* was isolated from rice [137]. The predicted OsbHLH1 protein had a putative nuclear-localization signal and a putative DNA binding-domain bHLH. The transcription of the *OsbHLH1* gene was specifically induced in roots of rice seedlings by cold, while NaCl, PEG and ABA treatments did not get any response. These results indicate that the OsbHLH1 may function as a transcription factor in a cold signal-transduction pathway. *CYP72A21*, a rice cytochrome P450 gene, was induced by chloroacetamide herbicides[138]. *OSB2*, a MYC-type transcription factor of rice, induced anthocyanin accumulation in rice leaves. Transgenic *Arabidopsis* plants overexpressing *atMYC2* and *atMYB2* showed enhanced osmotic stress tolerance, although their salt stress tolerance was not determined[139]. In transgenic *Arabidopsis* plants, overexpression of ABA and abiotic stress-inducible *Craterostigma plantagineum* Hochst-MYB10 gene enhanced salinity and desiccation tolerance. MYB subfamily of transcription factor genes was found to be stress-inducible, suggesting that transcription regulation is a part of drought, cold or salt stress signaling[140,141].

bZIP Transcriptional Factor

The ABA-dependent dehydration response involves ABA-responsive element and MYB/MYC promoter elements recognized by basic-region Leu-zipper (bZIP) and MYB/MYC transcription factors, respectively[142-144]. The bZIP family of transcriptional factors, AB15, ABF3 and ABF4 are ABA-inducible and these are positively regulated by SDIR1 (salt and drought induced ring-finger1), a RING FINGER E3 ligase[145]. Salt, drought and ABA upregulated the expressions of *Arabidopsis* bZIP transcription factors, such as, AREB1 and AREB2 genes. Further, constitutive over-expression of ABF3 and AREB2 in *Arabidopsis* enhanced the expression level of target LEA genes. These transgenic plants showed hypersensitivity to ABA, sugar and salt stresses during germination, but drought tolerance was enhanced in seedling stage[146]. ABF3 induced ABA related genes that encoded LEA, *rd29B* and *rab18*, and protein phosphatase 2C[136]. ABF3 alone increased the tolerance to drought stress in transgenic rice[147].

Zinc Finger Network

The zinc finger domain, present in the proteomes of many organisms, enables different proteins to

interact with or bind DNA, RNA or other proteins. Proteins containing zinc finger domain(s) are found to play important roles in eukaryotic cells, regulating different signal transduction pathways and controlling processes, such as, development and programmed cell death. There are many types of zinc finger proteins, classified according to the number and order of the Cys and His residues that bind the zinc ion. Among these, the C2H2-type zinc finger proteins, with 176 members in *A. thaliana*[148], constitute one of the largest families of transcriptional regulators in plants. They are mostly plant-specific and contain a conserved QALGGH sequence within their zinc finger domain. A genome-wide microarray based gene expression analysis involving 14 stages of vegetative and reproductive development alongwith 3 stress conditions has revealed that C2H2 gene family in *indica* rice could be involved during all the stages of reproductive development from panicle initiation till seed maturation. A total of 39 genes were up-regulated more than 2-fold during reproductive development of rice in comparison to its vegetative stages. Of which 18 were specific to panicle development and 12 genes were seed-specific. Twenty-six genes have been found to be up-regulated during three abiotic stresses and of these, 14 genes expressed specifically during the stress conditions analyzed; while 12 are also up-regulated during reproductive development, suggesting that some components of the stress response pathways are also involved in reproduction. The stress responsive zinc-finger protein gene of *Populus euphratica* Oliv (*PSTZ*), which encodes a protein having typical C2H2-zinc finger structure, was introduced into tobacco plants. Transgenic tobacco showed an enhanced salt tolerance, suggesting that *PSTZ* may play a role in plant responsiveness to salt stress. Cytosolic ascorbate peroxidase 1 (Apx1) is a key H_2O_2 removing enzyme in plants. Microarray analysis of Apx1-deficient *Arabidopsis* plants revealed that the expression of two zinc finger proteins (Zat12 and Zat7) and a WRKY transcription factor (WRKY25) was elevated in knock-out Apx1 plants grown under controlled conditions. Because mutants were lacking Apx1-accumulated H_2O_2, the study suggests that Zat12 is an important component of the oxidative stress response signal transduction network of *Arabidopsis*, required for Zat7, WRKY25, and Apx1 expression during oxidative stress[149]. The EAR-domain of C2H2-type zinc finger proteins plays a key role in the defense response of *Arabidopsis* to abiotic stresses[150]. This EAR-motif of Zat7 is directly involved in enhancing the tolerance of transgenic plants to salinity stress. Four different ZPT2-related C2H2-type zinc finger proteins (AZF1, AZF2, AZF3 & STZ) were functionally characterized in *Arabidopsis*[151]. Gel-shift analysis showed that the AZFs and STZ bind to A(G/C)T repeats sequence present in EP2 domain of promoter. This A(G/C)T repeats sequence is known as a target sequence of ZPT2 proteins of petunia (*Petunia hybrida* Hort ex Vilm). These four ZPT2-related proteins were shown to act as transcriptional repressors that down-regulate the transactivation activity of other transcription factors. RNA gel-blot analysis showed that expression of *AZF2* and *STZ* was strongly induced by dehydration, high-salt, cold and abscisic acid treatments. Histochemical analysis of *β*-glucuronidase activities driven by the *AZF2* or *STZ* promoters revealed that both genes were induced in leaves. Transgenic *Arabidopsis* overexpressing *STZ* showed growth retardation and tolerance to drought stress.

A transcription factor having the zinc finger domain named RAMY was functionally characterized using the yeast one hybrid system from a rice library[152]. The full length RAMY cDNA clone encodes a 218-amino acid protein and is partially homologous to the LEA5. *In vitro* mutagenesis and electrophoretic mobility shift assays confirmed that RAMY can bind with 20-bp O2S sequence of the regulatory region of the Amy2 gene promoter specifically through an unusual zinc finger with a CXCX4CX2H consensus sequence. This gene (RAMY) may act as a trans-acting protein and is probably involved in the GA-induced expression of the rice α-amylase gene. A stress inducible gene named *OsZnI* having a motif like novel zinc finger was characterized from *O. sativa* cv. N22[153].

Molecular Breeding in Rice

The ultimate aim of quantitative trait loci (QTL) analysis is to dissect the complex inheritance of quantitative traits into Mendelian-like factors, amenable to selection through the analysis of flanking molecular markers, and clone the genes underlying the QTLs. In order to identify the QTLs, an experimental population segregating for the traits of interest is created and a linkage map based on molecular markers is developed. Rice genome sequence has made it possible to identify and map

precisely a number of genes through linkage to DNA markers. Markers assisted selection (MAS) can be used for monitoring the presence/absence of the genes of interest in breeding population and can be combined with conventional breeding approaches. Pyramiding different resistance genes using MAS provides opportunities to develop broad spectrum resistance for biotic and abiotic stresses[154]. In rice, rooting traits and osmotic adjustment (OA) are found to be associated with improved drought tolerance[155], but these traits are rarely selected because phenotypic selection for these traits is not possible. Molecular marker technology overcomes these difficulties. QTLs identified on chromosome 1 and 8 by Robin *et al*[156] corresponded to the QTLs identified by Zhang *et al*[157] for OA. In the same region, a QTL for stomatal behaviour was also identified in F_2 mapping population of rice[158]. These findings indicate that this region of genome might have been conserved in plants in response to drought[155]. A total number of 47 QTLs fit for various tolerant water stress indicators, phenology and production traits under control and drought stress conditions in the field were identified. A region on the chromosome no 4 was identified that has major QTLs for plant height, grain yield and number of penicles under drought stress[156]. By using 126 SSR markers in randomly selected lines under stress, a QTL (gt12.1) with large effect on grain yield under stress was detected on chromosome 12[159]. On analysis of genetic basis of drought tolerance and drought avoidance using recombinant inbred lines, 27 QTLs were identified for seven traits of relative performance of fitness yield[160]. A significant proportion of the phenotypic variability of several of these putative drought resistant traits is explained by segregation of relatively few genetic loci; thus, leading to the possibility of indirect selection of these complex traits by means of MAS strategy. Therefore, there is need to analyze whether the QTLs linked to drought resistance traits also affect yield under stress. By comparing the coincidence of QTLs for specific traits and QTLs for plant production under drought, it is possible to test whether a particular constitutive or adaptive response to drought stress is of significance in improving the field level drought resistance[161]. Molecular marker technology is a powerful tool for selecting such traits, and the candidate genes in the area of QTL can be identified directly from the model DNA sequence and their function can be studied in transgenes.

Conclusion

Abiotic stresses, especially, salinity, drought, temperature and oxidative stress are the primary causes of plant loss worldwide. Therefore, plant biotechnologists aim at overcoming severe environmental stresses with the help of intensive molecular-assisted traditional breeding and genetic engineering. In carrying out studies on tolerance to abiotic stress, a number of factors should be taken into consideration. First, it should be kept in mind that a given tolerance-related mechanism should always be assessed with respect to its cross-talk with other stress-related genes/mechanisms. Second, most current studies used short-term stress treatments rather than observing the effects of stress over longer periods that mimic more closely the life span of most crops. As physiological and molecular responses during short and long period of exposures to stress could differ, conclusions regarding actual tolerance, which must involve practical factors, such as, biomass, yield data and survival, can not always be drawn. Third, cycles of stress and recovery from stress, e.g., rehydration, are the prevalent processes occurring under natural conditions during different seasons and under agricultural practices, such as, irrigation and salt leaching. Thus, the degree of recovery from stress, which also has its molecular basis, is as relevant as the response to stress.

Most of the studies on drought stress and engineering for drought tolerance in crop plants using model plants are still in its early stages. The number of transgenic crop plants that have undergone field trials or been tested under natural water-deficit conditions is very small. Private companies and government organizations are evaluating promising genetically modified (GM) new strains of corn, rice, wheat and canola in field, primarily, in Australia, China, Canada and USA[162] for drought tolerance. Drought tolerance is a complex trait, making the transgenic production of tolerant crops a challenging prospect. Nevertheless, advances in our understanding of stress signal perception and transduction, and of the associated molecular regulatory networks, together with high-throughput transformation technology, have improved the possibility of achieving this goal. This progress has made it possible to employ gene pyramiding or co-transformation for resistance to one or more stresses. The identification and characterization of tissue-specific and drought-inducible promoters will enhance these efforts.

Recently, genetic engineering of regulatory elements not only enhanced the plant survival in drought conditions per se but also improved the crop productivity under water deficit conditions. Understanding traits in crop plants that are associated with root architecture and plasticity under water-deficit conditions (osmotic adjustments in roots) and their manipulation might help to advance our knowledge of crop drought tolerance. Another major physiological constraint to sustaining and improving plant production under drought stress is plant reproductive failure under stress. Most of the research on our basic understanding of drought tolerance and its applications is focused on plant developmental stages other than just before flowering and after flowering. In most cases, however, the reproductive parts of crop plants are the harvestable yields and future success in producing drought tolerant crops relies on intensive research efforts to improve reproductive success. A comprehensive screening of metabolites during drought stress will advance our fundamental understanding of major metabolic pathways and provide direction for future metabolic engineering for drought-stress tolerance in crop plants.

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