Genetic approaches towards overcoming water deficit in plants - special emphasis on LEAs

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ABSTRACT

Water deficit arises as a result of low temperature, salinity and dehydration, thereby affecting plant growth adversely and making it imperative for plants to surmount such situations by acclimatizing/adapting at various levels. Water deficit stress results in significant changes in gene expression, mediated by interconnected signal transduction pathways that may be triggered by calcium, and regulated *via* ABA dependent and/or independent pathways. Hence, adaptation of plants to such stresses involves maintaining cellular homeostasis, detoxification of harmful elements and also growth alterations. Stress in general cause excess production of reactive oxygen species (ROS) and the plants overcome the same by either preventing the accumulation of ROS or by eliminating the ROS formed. Ion homeostasis includes processes such as cellular uptake, sequestration and export in conjunction with long distance transport. Requisite amounts of osmolytes are hence synthesized under stress to maintain turgor along with maintaining the macromolecular structures and also for scavenging ROS. Another noteworthy response is the accumulation of novel proteins, including enzymes involved in the biosynthesis of osmoprotectants, heat-shock proteins (HSPs), late embryogenesis abundant (LEA) proteins, antifreeze proteins, chaperones, detoxification enzymes, transcription factors, kinases and phosphatases. The LEAs belong to a redundant protein family and are highly hydrophilic, boiling-soluble, non-globular and therefore have been defined and classified accordingly. The precise function of LEAs is still unknown, but substantial evidence indicates their involvement in desiccation tolerance as the expression of LEAs confers increased resistance to stress in heterologous yeast system and also significantly improves water deficit tolerance in transgenic plants. Genetic manipulation of plants towards conferring abiotic stress tolerance is a daunting task, as the abiotic stress tolerance mechanism is highly complex and various strategies have been exploited to address and evaluate the stress tolerance mechanism, and the molecular responses to water deficit via complex signaling networks. Genomic technologies have recently been useful in integrating the multigenicity of the plant stress responses through, transcriptomics, proteomics and metabolite profiling and their interactions. This review deals with the recent developments on genetic approaches for water stress tolerance in plants, with special emphasis on LEAs. [Physiol. Mol. Biol. Plants 2008; 14(4) : 277-298] E-mail : param@genomeindia.org; paramjitkhurana@hotmail.com

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Abbreviations : DHN – Dehydrins, LEA – Late embryogenesis abundant; LTP – Lipid transfer proteins, HSP – Heat shock proteins, ROS – Reactive oxygen species

Stress is delineated as an external factor that exerts negative influence on plants (Levitt, 1980). Water deficit conditions, low temperature (chilling and freezing), heat stress, salinity and oxygen deficiency are some of the

major stress factors that affect plant growth and therefore to surmount such situations, plants acclimatize and adapt by undergoing various biochemical and physiological changes. Understandably, approximately half to two-thirds of the yield potential of major crops is typically lost due to adverse environmental conditions (Bray *et al.*, 2000). Among the various abiotic stresses, water deficit is the major factor that limits crop productivity worldwide (Vallivodan and Nguyen, 2006). Moreover it is also predicted that by the year 2025, one-third of the world's population would be living in regions experiencing severe water scarcity (www.iwmi.cgiar.org).

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Exposure of plants to a water-limiting environment during various developmental stages appears to accumulate various physiological and developmental stages. Understanding of the basic physiological, biochemical and molecular mechanisms for drought stress perception, transduction and tolerance is still a major challenge in biology. Plant modification for enhanced drought tolerance is mostly based on the manipulation of either transcription and/or signaling factors or genes that directly protect plant cells against water deficit. Hence efforts need to be targeted apropos to overcome such adverse stress conditions. This review focuses on mechanisms of gene regulation and genetic engineering for enhanced drought tolerance in various crops.

Biological water deficit

Water deficit is one of the major limiting factors for plant growth and productivity around the world. Water deficit may be mild or severe, occurring due to either a distinct or a concerted effect of environmental stresses such as, salinity, drought and low temperature (freezing and chilling). Drought stress occurs when water uptake from the soil cannot balance water loss through transpiration. Plants cope with water deficit by reducing water loss and/or by increasing water uptake (Bray *et al.*, 2000). They have evolved two major mechanisms to deal with water deficit: stress **avoidance** and **tolerance**. **Drought avoidance** refers to adaptations that help the plant in escaping the stressful situations; for instance, short life cycles that cease before drought occurs (e.g. desert ephemerals). **Drought tolerance** refers to adaptations that allow the plant to withstand stress, with or without a decline in performance. **Desiccation avoidance** involves approaches so as to minimize evaporational water loss along with harnessing as much water as possible through - deep root systems, maintaining high internal resistance to water transport, small and thick leaves, leaf angle, spines and waxy coats. **Desiccation tolerance** encompasses osmotic adjustments and possession of stiff cell walls. Many plants use a combination of both mechanisms (Gaspar *et al.*, 2002). The resurrection plants such as *Craterostigma plantagineum* is desiccation tolerant (poikilohydric) and can survive losses of up to 95 % water content by entering a quiescent stage and rehydrating rapidly. Resurrection plants have been widely used as model system for dehydration studies and their comparative evaluation vis-à-vis the non-tolerant plants suggests the genetic basis of tolerance mechanism to be a quantitative characteristic (Vinocur and Altman, 2005). Plant response to stress may thus involve a range of temporal scales, from a matter of seconds to evolutionary

time scales resulting in acclimation and/or adaptation. Seed maturation is a natural phase of plant growth that results in the removal of approximately 90 % water and therefore, water deficit is customary during this phase and occurs ubiquitously in higher plants. Seeds withstand this desiccation only for few days during the late maturation period by accumulating proteins such as the late embryogenesis abundant (LEA) proteins and also the sHSPs (small heat shock proteins) (Wehmeyer and Vierling, 2000).

High salt concentration impinges both ionic as well as osmotic stress. High Na^+ and Cl^- concentration leads to an influx of ions which consequently alters the ion homeostasis of plant cells. Osmotic stress on the other hand is instigated due to lowering of the soil water potential leading to reduced water uptake which in due course leads to cellular dehydration. Salt tolerance mechanism thus entail, minimizing the uptake of salts along with minimizing the salt concentration in the cytoplasm of the plant. Salt tolerant plants (halophytes) implement both types of mechanisms such as exclusion of Na^+ uptake, as well as compartmentalization of Na^+ in the vacuole along with active salt excretion through special salt glands (Thomas and Bohnert, 1993). Glycophytes on the other hand may exclude the salt, but are incapable of effectively compartmentalizing the residual salt as do halophytes (Munns, 2002).

Adaptation to saline stress is often accompanied by induction of genes encoding for protective proteins such as osmotin, LEA-proteins (Espelund *et al.*, 1992), ion transporters, while others encode for enzymes that partake in metabolic processes primarily triggered by salinity stress. Based on the analysis of few stress sensors it is too early to assess whether cross-talk occurs at the level of sensors (Chinnusamy *et al.*, 2004). Ion transporter genes induced under salt (NaCl) stress towards maintaining ionic homeostasis include plasma membrane Na^+/H^+ antiporters for Na^+ extrusion, vacuolar Na^+/H^+ antiporters for Na^+ compartmentation in the vacuole (Apse *et al.*, 1999) and also high-affinity K^+ transporters for K^+ acquisition. Overexpression of a plasma membrane Na^+/H^+ antiporter gene improves salt tolerance in *Arabidopsis thaliana* (Shi *et al.*, 2003). Also, Overexpression of K^+ , Na^+ transporters and of H^+ pumps in transgenics bestowed considerable stress tolerance (see Table 1) e.g. sustained growth and tolerance to salt stress was observed by overexpressing *AtNHX1* (*A. thaliana*, Na^+/H^+ antiporter) in *A. thaliana* (Apse *et al.*, 1999), tomato (Zhang and Blumwald, 2001) as well as in *Brassica napus* (Zhang *et al.*, 2001) and overexpression of *SOS1* in transgenic *Arabidopsis*

conferred sufficient stress tolerance (Shi *et al.*, 2003). Verma *et al.* (2007) reported the functional validation of *PgNHX1* in rice. Overexpression of *PgNHX1* resulted in extensive root growth and improved the ability of transgenic rice plants to withstand salt without adversely affecting plant growth and development.

Low temperature stress encompasses - **chilling stress** (at $\sim 10^\circ\text{C}$), as well as, **freezing stress**. Chilling stress impairs cytoplasmic streaming, reduces respiration, photosynthesis and also protein synthesis while freeze-induced cellular dehydration results in membrane injury by virtue of low temperature and also due to mechanical stress caused by ice formation in the apoplast. Resistance or sensitivity to low temperature stress depends on the species, genotype, and the developmental age of the plant (Bray *et al.*, 2000). Plants avoid freezing injury by various **avoidance** or **tolerance** mechanisms (Levitt, 1980). **Avoidance** of freezing is usually achieved by deep supercooling wherein the liquid in the intercellular space never undergoes transition to a solid phase, while **tolerance** to freezing involves cold acclimation/hardening (Levitt, 1980). Plants overcome adverse temperature conditions by acclimatizing and adapting by synthesizing various cold-induced genes such as the fatty acid desaturases, lipid transfer proteins, proteins involved in osmoprotectant biosynthesis, antifreeze proteins, chaperones, signal transduction components, such as transcription factors, kinases and phosphatases and also other genes with unidentified functions such as the low temperature induced/cold regulated genes (*LTI/COR*).

Responses upon exposure to osmotic stress at the molecular, cellular and at the whole plant level — include, morphological (development of specialized structures in the leaf to curtail the rate of transpiration, leaf area diminution, sunken stomata, etc.) modifications, developmental changes (inhibition of shoot growth and enhanced root growth), adjustments in ion transport (ion uptake, export exclusion/extrusion, sequestration of ions) and also metabolic alterations (carbon metabolism, the synthesis of compatible solutes) (Levitt, 1980; Xiong and Zhu, 2002). As a corollary to the stress conditions primary osmotic stress signals and secondary stress signals including phytohormones (e.g. ABA, ethylene), reactive oxygen species (ROS) and also the intracellular second messengers (e.g. phospholipids) ensue the co-ordination of whole-plant stress signal responses (Xiong and Zhu, 2002). It is apparent that the linear pathways that we have been discussing are actually only part of a more complex signaling network and that there is much overlap between its branches,

with many genes inducible by more than one particular stimulus. Cross-talk also occurs at the interaction between different genes. Many drought- and cold-inducible genes contain both DRE/CRT and ABRE motifs in their promoters. These *cis*-acting elements are thought to function independently. However, precise analysis of these *cis*-acting elements in RD-29A gene expression revealed that DRE/CRT functions cooperatively with ABRE as a coupling element in ABA-responsive gene expression in response to drought stress (Narusaka *et al.*, 2003). Genes of the *CBF/DREB1* family are mainly induced by cold stress but the drought-inducible gene *CBF4* functions to provide crosstalk between *DREB2* and *CBF/DREB1* regulatory systems (Shinozaki *et al.*, 2007). Not only this, members of MAPK cascades are activated by more than one type of stress, which suggests that MAPK cascades act as points of convergence in stress signaling (Fig. 1).

Stress signaling network

Calcium and Its Role

Calcium, a critical component in the signal transduction pathway initiates gene expression in response to a number of abiotic stresses comprising low temperature, osmotic stress, heat, oxidative stress, anoxia and mechanical perturbation. Environmental stresses lead to rapid transient elevations in cytosolic free calcium concentration ($[\text{Ca}^{2+}]_{\text{cyt}}$) in plants that further lead to increased expression of stress-responsive genes (Knight, 2000). The $[\text{Ca}^{2+}]_{\text{cyt}}$ signaling network has thus been equated to a basic cellular ‘memory’ (Trewavas, 1999). Stress or ABA induced $[\text{Ca}^{2+}]_{\text{cyt}}$ increases can be attributed to release of Ca^{2+} from intracellular stores (ER, vacuoles, mitochondria, chloroplasts and nucleus) and also through influx from plasma membrane channels (Reddy, 2001). Inositol 1,4,5-trisphosphate (IP3), cyclic ADP ribose (cADPR) and Ca^{2+} channels play an important role in elevating $[\text{Ca}^{2+}]_{\text{cyt}}$. IP3-gated Ca^{2+} channels have been implicated in dehydration and salt stress-induced cytosolic Ca^{2+} elevations (De Wald *et al.*, 2001; Takahashi *et al.*, 2001). The Ca^{2+} signaling circuit has been postulated to consist of three major “nodes” — generation of a Ca^{2+} signature in response to a signal, recognition of the signature by Ca^{2+} sensors, and the further transduction of the signature message to downstream targets that participate in producing signal-specific responses (Reddy and Reddy, 2004). Ca^{2+} transmits the stress signal downstream by interacting with sensors such as calmodulin (Cam), Cam like and other EF-hand containing Ca^{2+} -binding proteins, Ca^{2+} -regulated protein kinases (e.g. CDPKs) and Ca^{2+} -binding

Fig. 1. Networks of osmotic stress responses elucidates the speculative targets based on literature reports as discussed in the text. However, many details have been omitted for simplicity. Abbreviations: AtHK1 (*Arabidopsis thaliana* two-component histidine kinase), NtC7 (*Nicotiana tabacum* C7), R? (unknown receptor), G? (undeciphered activity of G proteins), PLD (Phospholipase D), PLC (Phospholipase C), PIP₂ (phosphatidylinositol 3,5-bisphosphate), PLA₁ (Phospholipase A₂), PLA₂ (Phospholipase A₂), Ca²⁺ (calcium ions), SOS (salt overly sensitive), IP₃ (inositol 1,4,5-trisphosphate), DAG (diacylglycerol), PtdOH (phosphatidic acid), ROS (reactive oxygen species), MAPK (mitogen-activated protein kinase), CDPK (calcium dependent protein kinase), ABRE (abscisic acid responsive element), CRT/DRE (C-repeat/dehydration-responsive element), bZIP (basic leucine zipper), Pr (red light absorbing biologically inactive form), Pfr (far-red light absorbing biologically active form), PhyB (Phytochrome B), DREB/CBF (DRE-binding protein/C-repeat-binding factor), COR/RD/KIN/LTI (cold-regulated/responsive to dehydration/low-temperature-induced).

proteins without EF-hand motifs (Reddy, 2001; Sanders *et al.*, 2002; Ray *et al.*, 2007). Gene expression in both plants and animals is regulated *via* Ca^{2+} /calmodulin interaction with transcription factors or *via* specific protein kinases. Several abiotic challenges result in an immediate, transient increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ that is restored to basal levels within minutes (Knight, 2000; White and Broadley, 2003). However, the mechanisms that bring about the changes in cytosolic Ca^{2+} levels are far from being identified. The $[\text{Ca}^{2+}]_{\text{cyt}}$ signatures elicited by one environmental challenge can be modified by prior exposure to a contrasting one (Knight, 2000). Furthermore, transformants obtained *via* manipulation of one or more components of Ca^{2+} signaling pathway were observed to be more tolerant to adverse stress conditions, especially water deficit (Saijo *et al.*, 2000, Pandey *et al.*, 2002, Cheong *et al.*, 2003, Wang *et al.*, 2005, Liu *et al.*, 2006, Ma *et al.*, 2007).

Transcription factors and cross talk

Plant stress responses are thus regulated by multiple signaling pathways that activate gene transcription and its downstream machinery. Plant genomes therefore contain a large number of transcription factors (TFs), few of which have been engineered in plants to confer abiotic stress tolerance. Constitutive expression of *ABF3* or *ABF4* demonstrated enhanced drought tolerance in *Arabidopsis* plants with an altered expression of ABA/stress-responsive genes (Kang *et al.*, 2002). A generally noticed negative effect of transcription factor-modified plants is the growth retardation in transgenic plants that constitutively express the same. This may perhaps be accounted due to stress responsive transcription factors triggering overexpression of downstream stress-associated multiple genes, along with additional non-stress genes that adversely influence the normal phenotype of the plant (*see* Table 1). NAC genes belonging to one of the largest family of plant-specific transcription factors are involved in various aspects of plant functioning including abiotic stress responses (Olsen *et al.*, 2005). Isolation and functional analysis of stress-inducible NAC transcription factors binding to drought-responsive *cis*-element in early response to dehydration stress promoter has been done in *Arabidopsis* (Trans *et al.*, 2004). Genome-wide identification and expression analysis of the NF-Y family of transcription factors was done in *Triticum aestivum*. The expression of nine NF-Y and two Dr1 genes in wheat leaves appeared to be responsive to drought stress. Three of these genes were up-regulated under drought conditions, indicating that these members of

the NY-1 and Dr-1 families are involved in plant drought adaptation (Stephenson *et al.*, 2007).

ABA Signaling Mechanism

ABA is a 15-carbon (a sesquiterpene) plant growth regulator synthesized *via* the carotenoid intermediates. ABA regulates many aspects of plant growth and development embracing embryo maturation, seed dormancy, germination, cell division and elongation, and also functions in response to environmental stresses such as drought, salinity, cold, pathogen attack and UV radiation (Shinozaki and Yamaguchi-Shinozaki, 2000). ABA accumulates in shoot and root tissues subjected to water deficit to initiate processes involved in adaptation to drought and other environmental stresses (Bray, 1993). ABA biosynthesis is known to up-regulate in response to osmotic stress but however, the signalling pathway by which ABA biosynthetic genes are up-regulated is still obscure. Nevertheless, both ABA-dependent and ABA-independent signal transduction cascades exist subsequent to the initial perception of the stress signal (Shinozaki and Yamaguchi-Shinozaki, 2007). ABA-independent stress-responsive gene expression has been thought to be regulated through DRE *cis*-elements, while ABA-dependent pathways activate gene expression through ABRE *cis*-elements. When the DRE/CRT-binding protein DREB1/CBF was overexpressed in transgenic *Arabidopsis* plants, changes in the expression of more than 40 stress inducible genes were identified and these changes led to increased freezing, salt and drought tolerance (Seki *et al.*, 2001; Maruyama *et al.*, 2004). *ABF3* or *ABF4* overexpression conferred several ABA-associated phenotypes such as ABA hyper-sensitivity, sugar hypersensitivity and enhanced drought tolerance, along with altered expression of ABA- or stress-responsive genes such as *rd29B*, *ABA-insensitive (ABI 1)* and *ABI 2*. The identification of these transcriptional activators of ABA signaling has promise for genetic engineering for enhanced drought tolerance (Vallivodan and Nguyen, 2006).

Stress injury and cellular responses

Plant water deficit induced dehydrative stress results in concentration of solutes within cells, changes in cell volume and membrane shape, disruption of water potential gradients, inhibition of photosynthesis, loss of turgor, disruption of membrane integrity, denaturation of proteins and oxidative stress (Bray *et al.*, 2000). Hence, to overcome subsequent injuries, plants respond by maintaining cellular homeostasis, detoxification of

Table 1. Transgenic efforts to overcome water deficit stress

Gene	Functional Protein	Species	Functional role	Trans-host	Reference
Regulation of Calcium					
<i>OsCDPK7</i>	Calcium-dependent protein kinase	<i>O. sativa</i>	protein kinase	<i>O. sativa</i>	Saijo <i>et al.</i> , 2000
<i>AtGluR2</i>	Homolog of the mammalian ionotropic glutamate receptor	<i>Arabidopsis</i>	Ca ²⁺ permeable channel	<i>A. thaliana</i>	Kim <i>et al.</i> , 2001a
<i>EhCaBP</i>	Calcium binding protein	<i>Entamoeba histolytica</i>	calcium binding protein involved in Ca ²⁺ signaling	<i>N. tabacum c_ Xanthi</i>	Pandey <i>et al.</i> , 2002
<i>CBL1</i>	Ca sensing protein	<i>Arabidopsis thaliana</i>	calcium sensor molecule	<i>Arabidopsis</i>	Cheong <i>et al.</i> , 2003
Regulation of ABA biosynthesis					
<i>LeNCED1</i>	9-cis-epoxy carotenoid dioxygenase	Tomato	ABA biosynthesis	Tobacco and Tomato	Thompson <i>et al.</i> , 2000
<i>AtNCED3</i>	<i>Arabidopsis thaliana</i> 9-cis- epoxy carotenoid dioxygenase	<i>A. thaliana</i>	ABA biosynthesis	<i>A. thaliana</i>	Iuchi <i>et al.</i> , 2001
<i>PvNCED1</i>	9-cis-epoxy carotenoid dioxygenase	<i>Phaseolus vulgaris</i>	ABA biosynthesis	<i>N. plumbagini-folia</i>	Qin and Zeevaart, 2002
Regulatory genes					
<i>CBF1</i>	CRT/DRE binding factor	<i>A. thaliana</i>	transcription factor	<i>A. thaliana</i>	Jaglo-Ottosen <i>et al.</i> , 1998
<i>DREB1 and DREB2</i>	DRE-binding protein	<i>A. thaliana</i>	transcription factor	<i>A. thaliana</i>	Liu <i>et al.</i> , 1998
<i>DREB1A</i>	DRE-binding protein	<i>A. thaliana</i>	transcription factor	<i>A. thaliana</i>	Kasuga <i>et al.</i> , 1999
<i>CBF3</i>	CRT/DRE binding factor	<i>A. thaliana</i>	transcription factor	<i>A. thaliana</i>	Gilmour <i>et al.</i> , 2000
<i>SCOF-1</i>	<i>Soybean cold-inducible factor-1</i>	<i>Glycine max</i>	transcription factor	<i>A. thaliana</i> and <i>N. tabacum</i>	Kim <i>et al.</i> , 2001b
<i>TS11</i>	Tobacco stress- induced gene1	<i>N. tabacum</i>	transcription factor	<i>N. tabacum</i>	Park <i>et al.</i> , 2001
<i>ABI3</i>	Absciscic acid induced protein	<i>A. thaliana</i>	transcription factor	<i>A. thaliana</i>	Tamminen <i>et al.</i> , 2001
<i>CBF1</i>	CRT/DRE binding factor	<i>A. thaliana</i>	transcription factor	Tomato	Hsieh <i>et al.</i> , 2002

Table 1. Continued

Gene	Functional Protein	Species	Functional role	Trans-host	Reference
<i>CBF4</i>	CRT/DRE binding factor	<i>A. thaliana</i>	transcription factor	<i>A. thaliana</i>	Haake <i>et al.</i> , 2002
<i>OsDREB1</i>	DRE-binding protein	<i>Oryza sativa</i>	transcription factor	<i>A. thaliana</i>	Dubouzet <i>et al.</i> , 2003
<i>HvCBF4</i>	<i>Dehydration responsive element binding factor</i>	<i>Hordeum vulgare</i>	transcription factor	<i>O. sativa</i>	Oh <i>et al.</i> , 2007
<i>MBF1a</i>	<i>Multiprotein bridging factor</i>	<i>A. thaliana</i>	Transcription factor	<i>A. thaliana</i>	Kim <i>et.al.</i> ,2007
<i>JERF1</i>	<i>Ethylene responsive factor</i>	<i>Tomato</i>	Transcription factor	<i>Nicotiana tabacum</i>	Wu <i>et. al.</i> , 2007
LEA and LEA like genes					
<i>HVA1</i>	Lea protein	<i>H. vulgare</i>	unknown	<i>T. aestivum</i>	Sivamani <i>et al.</i> , 2000
<i>HVA1</i>	Lea protein	<i>H. vulgare</i>	unknown	Rice	Rohila <i>et al.</i> , 2002
<i>HVA1</i>	Lea protein	<i>H. vulgare</i>	unknown	Oat	Maqbool <i>et al.</i> , 2002
<i>CuCOR19</i>	Lea protein dehydrin	Citrus unshiu Marcov	unknown	<i>Nicotiana tabacum</i>	Hara <i>et al.</i> , 2003
LEA3-L2 <i>WCS19</i>	chloroplast LEA-like protein (WCS19)	<i>A. thaliana</i>	protein of unknown function	<i>T. aestivum</i>	Ndong <i>et al.</i> , 2002
<i>Rab 16a</i>	Lea protein	<i>O. sativa</i>	unknown	<i>N. tabacum</i>	Roychoudhury <i>et. al.</i> , 2007
<i>HVA1</i>	Lea protein	<i>H. vulgare</i>	unmnown	<i>Agrostis stolonifera</i>	Fu <i>et.al.</i> , 2007
<i>Dehydrin</i>	Lea protein	<i>T. aestivum</i>	unknown	<i>A. thaliana</i>	Brini <i>et.al.</i> ,2007
Transporter protein genes					
<i>PIP1b</i> ; <i>PIP2b</i>	Anti sense construct targeted to the <i>PIP1b</i> gene	<i>Arabidopsis thaliana</i>	plant plasma membrane intrinsic protein	<i>Arabidopsis thaliana</i>	Kaldenhoff <i>et al.</i> , 1998
<i>AtNHX1</i>	Na ⁺ /H ⁺ antiporter	<i>A. thaliana</i>	vacuolar Na ⁺ /H ⁺ antiporter	<i>A. thaliana</i>	Apse <i>et al.</i> , 1999
<i>HAL1</i>	Protein involved in regulation of K ⁺ transport	<i>Saccharomyces cerevisiae</i>	regulation of K ⁺ transport	<i>Lycopersicon esculentum</i>	Gisbert <i>et al.</i> , 2000

Table 1. Continued

Gene	Functional Protein	Species	Functional role	Trans-host	Reference
<i>ALAI</i>	Aminophospho-lipid ATPase1	<i>A. thaliana</i>	P-type ATPase	<i>A. thaliana</i>	Gomes <i>et al.</i> , 2000
<i>AtNHX1</i>	Na ⁺ /H ⁺ antiporter	<i>A. thaliana</i>	vacuolar Na ⁺ /H ⁺ antiporter	<i>Lycopersicon esculentum</i>	Zhang and Blumwald, 2001
<i>AVP1</i>	vacuolar H ⁺ - pyrophosphatase	<i>A. thaliana</i>	vacuolar proton pump established proton gradients	<i>A. thaliana</i>	Gaxiola <i>et al.</i> , 2001
<i>AtNHX1</i>	Na ⁺ /H ⁺ antiporter	<i>A. thaliana</i>	vacuolar Na ⁺ /H ⁺ antiporter	<i>Brassica napus</i>	Zhang <i>et al.</i> , 2001
<i>HKT1</i>	Potassium transporter	Wheat	regulation of K ⁺ transport	Florida Wheat	Laurie <i>et al.</i> , 2002
<i>NtAQP1</i>	Antisense tobacco <i>PIP1</i> (plasma membrane aquaporin)	Tobacco	protein-mediated membrane water transport	Tobacco	Siefritz <i>et al.</i> , 2002
<i>PIP1b</i>	Plasma membrane aquaporin overexpression	<i>Arabidopsis thaliana</i>	symplastic water transport	<i>N. tabacum</i>	Aharon <i>et al.</i> , 2003
<i>PIP2;2</i>	Plasma membrane aquaporin knockout	<i>Arabidopsis</i>	plasma membrane intrinsic protein 2;2 (PIP2;2)	<i>Arabidopsis</i>	Javot <i>et al.</i> , 2003
<i>SOS1</i>	Plasma membrane Na ⁺ /H ⁺ antiporter	<i>Arabidopsis</i>	plasma membrane localized Na ⁺ /H ⁺ antiporter	<i>Arabidopsis</i>	Shi <i>et al.</i> , 2003
<i>TNHX1</i> and <i>TVP1</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>T. aestivum</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>A. thaliana</i>	Brini <i>et al.</i> , 2007
<i>AtNHX1</i>	Tonoplast Na ⁺ /H ⁺ antiporter	<i>Arabidopsis thaliana</i>	Tonoplast Na ⁺ /H ⁺ antiporter	<i>Fagopyrum esculentum</i>	Cheng <i>et al.</i> , 2007
<i>PgTIP1</i>	Tonoplast aquaporin	<i>Panax ginsang</i>	Tonoplast intrinsic protein	<i>A. thaliana</i>	Peng <i>et al.</i> , 2007
<i>PgNHX1</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>Pennisetum glaucum</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>O. sativa</i>	Verma <i>et al.</i> , 2007
<i>AtNHX1</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>Arabidopsis thaliana</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>Festuca arundinacea</i>	Zhao <i>et al.</i> , 2007

harmful elements and by altering growth (Zhu, 2002, 2003; Xiong and Zhu, 2002).

Membranes

Plasma membrane and associated proteins undergo biochemical changes under water deficit conditions. Water deficit results in membrane damage as the lipid bilayers undergo a phase transition (T_m) from the liquid crystalline to the gel phase (Levitt, 1980) and thereby reduce the permeability of water and aqueous solutes. The phase transition temperature (T_m) depends on the fatty acid length, the number and position of double bonds, the lipid head group and the surrounding medium. Freeze induced dehydration and drought leads to more severe membrane damage as efflux of water from the cells results in shrinkage of cell walls and plasma membranes and an eventual collapse of cells.

Protein complexes involved in Water Deficit Responses

Aquaporins are water channel proteins belonging to the major intrinsic protein (MIP) superfamily of membrane proteins, involved in regulating the hydraulic conductivity of membranes by achieving 10 to 20-fold increase in water permeability (Maurel and Chrispeels, 2001). Aquaporins present in the plasma membrane and also in the tonoplast are regulated by environmental factors such as drought, salinity, hormones, and blue light (Guerrero *et al.*, 1990; Yamaguchi-Shinozaki *et al.*, 1992; Yamada *et al.*, 1995). Antisense PIP2 transformed lines resulted in reduced hydraulic conductance (Javot *et al.*, 2003) and alterations in plant morphology (Kaldenhoff *et al.*, 1998) while, overexpression of a plasma membrane aquaporin PIP1b, in transgenic tobacco improved plant vigor under favorable growth conditions but not under drought or salt stress (Aharon *et al.*, 2003). Therefore, approaches involving alterations in plant plasma membrane aquaporins should, however, be viewed with prudence. Using a proteomic approach, Hajheidari *et al.* (2005), found an osmotin-like protein up-regulated in drought conditions.

Antioxidants and Mechanisms of Detoxification

Both abiotic and biotic stresses cause excess production of ROS that result mainly due to decline in CO_2 fixation leading to higher leakage of electrons to O_2 . Reduction of O_2 advances through numerous steps and finally results in formation of ROS such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and also the hydroxyl radical ($\text{OH}^\cdot$) (Apel and Hirt, 2004). Plants overcome the ROS

mediated stress response either by preventing the accumulation of ROS or by effectively eliminating the ROS thus formed e.g. The gamma-amino butyrate (GABA) shunt prevents the accumulation of ROS and has been implicated in plant defense against environmental stress (Bouche *et al.*, 2003). Elimination of ROS is achieved by antioxidant compounds such as ascorbic acid, glutathione, thioredoxin and carotenoids, and by ROS scavenging enzymes (e.g. superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase). Over-expression of the above mentioned enzymes leads to increased tolerance to abiotic stresses such as low temperature and water stress. Over-expression of ascorbate peroxidase in tobacco chloroplasts enhanced tolerance to salt stress and water deficit (Badawi *et al.*, 2004). Transgenic alfalfa expressing Mn-superoxide dismutase cDNA reduced injury during water-deficit stress (McKersie *et al.*, 1996, 2000).

Macromolecular Homeostasis

Osmolytes are organic molecules synthesized evidently in most organisms under hyperosmotic stress to maintain turgor. They are highly soluble and non-toxic to cells and comprise of polyhydroxylated sugar alcohols, amino acids and their derivatives, tertiary sulphonium compounds and quaternary ammonium compounds (Bohnert and Jensen, 1996). Extreme desiccation promotes crystallization of proteins and solutes and one way to avoid this is by vitrification (glass formation). Osmolytes (sugars) promote vitrification as water is removed from cells while the glass phase prevents collapse of cells by maintaining the macromolecular structures. Osmolytes are also acknowledged to be involved in scavenging of ROS. Moreover the intracellular concentration of proline in yeast cells as a corollary increased ploidy level, vacuolation and altered accumulation of transcripts associated with cell division and gene expression control (Maggio *et al.*, 2002). High levels of glycine betaine was attained by expressing *BETA* in tobacco (Liljus *et al.*, 1996; Holmstrom *et al.*, 2000), or *CODA* in rice (Mohanty *et al.*, 2003) enhancing the stress tolerance of the transgenics. Substantial interest has focused on the study of the protective effect of osmolytes and enzymes that synthesize different osmolytes in transformants in order to improve tolerance. For instance, the transgenic maize plants accumulated higher levels of glycine betaine and were more tolerant to drought stress than non-transgenic plants at germination and young seedling stage (Quan *et al.*, 2004).

Stress Genes and Proteins - Hsps, LEAs

Although the exact molecular and biochemical mechanism of adaptation of plants to water deficit is still enigmatic, nevertheless, the most noteworthy response is the amassing of novel proteins alongwith the induction of many stress-induced genes encoding for enzymes involved in the biosynthesis of osmoprotectants, HSPs, LEA proteins, antifreeze proteins, chaperones, detoxification enzymes, transcription factors, kinases and enzymes involved in phosphoinositide metabolism (Ingram and Bartels, 1996; Bray *et al.*, 2000; Vallivodan and Nguyen, 2006).

HSP's as a molecular chaperone

HSPs play an important role in cellular protection under stress in addition to participation in developmental processes, such as embryogenesis, embryo development, seed germination, pollen development, and fruit maturation (Ingram and Bartels, 1996; Waters *et al.*, 1996; Wang *et al.*, 2004). HSPs as molecular chaperones engage in protein synthesis, targeting, maturation and degradation under normal and stress conditions and have been classified accordingly (Waters *et al.*, 1996; Wang *et al.*, 2004). In plants, the small heat-shock proteins (SHSPs) are most prevalent among the five conserved families of HSPs (HSP100, HSP90, HSP70, HSP60 and SHSP). Plant SHSPs are not only expressed in response to heat shock but also under water stress, salt stress, oxidative stress and also under low temperature stress. SHSPs display a protective role throughout the seed especially in imparting desiccation tolerance (Wehmeyer and Vierling, 2000). Certain members of plant SHSPs are concerned with stabilization or reactivation of inactivated enzymes and over-expression studies have conferred stress tolerance in the transgenics. Expression of *HSP101* (Queitsch *et al.*, 2000; Katiyar-Agarwal *et al.*, 2003), *HSP17.6A* (Sun *et al.*, 2001) and antisense expression of *HSP70* (Lee and Schoffl, 1996) in plants has been accomplished resulting in enhanced thermotolerance. Overexpression of *DNAK1*, an HSP70 member from the halotolerant *Cyanobacterium aphanothece*, in the cytosol of transgenic tobacco plants bestowed salt tolerance (Sugino *et al.*, 1999).

LEA proteins

Water deficit also results in accumulation of LEA proteins whose precise function is still recondite even after twenty years of discovery. Nonetheless, substantial evidence indicates their involvement in dessication tolerance (Bray, 1993; Ingram and Bartels, 1996; Tunaclyff

and Wise, 2007). They were first identified, in cotton and wheat (Dure *et al.*, 1981; Grzelczak *et al.*, 1982). LEA proteins are principally found in plant seeds and seedlings. They have also been discovered in the prokaryotes (Stacy and Aalen, 1998) as well as in the eukaryotes (Garay-Arroyo *et al.*, 2000) such as the nematodes (*Aphelenchus avenae*) with functional evidences (Battista *et al.*, 2001). They are usually considered to be single domain proteins, although perhaps with a single domain appearing multiple times. It is therefore worth noting that a likely LEA protein has been found with LEA domains appearing in a true multi-domain architecture, though it is not yet clear how LEA protein domains might function in multi-domain proteins (Tunaclyff and Wise, 2007). LEA proteins have been defined and classified on the basis of their expression pattern and sequence (Bray, 1993; Dure, 1993; Soulages *et al.*, 2002). The computational method called POPP (Protein or Oligonucleotide Probability Profile) analysis (Wise and Tunnacliffe, 2004) undertook protein comparison based on similarities in the peptide composition and questioned the logic involved in using parameters such as hydrophilicity and high glycine content in the "hydrophilin" classification as undertaken by Garay-Arroyo *et al.* (2000). Bies-Etheve *et al.* (2003) classified 61 LEA genes in *A. thaliana* according to their sequence homologies into 13 groups. LEAs are typically highly hydrophilic, boiling-soluble, non-globular with a preponderance of hydrophilic amino acids resulting in their characterisation as "hydrophilin" proteins (Garay-Arroyo *et al.*, 2000). The LEA proteins confer increased resistance to osmotic or freeze stress when introduced into yeast and also improved water deficit tolerance in the transformants (Wang *et al.*, 2003, also see Table 1). Structural information in both hydrated and dry states is known in a group 3 LEA-like protein (AavLEA1) from *Aphelenchus avenae* provides new insights in to the molecular mechanism especially under stress conditions (Goyal *et al.*, 2003).

The hydrophilic, random coil structure of the proteins (e.g. LEA1) possibly serve as water-binding domains, act as hydration buffer to regulate water status, or may also interact with the surface of macromolecules as a water matrix to oppose protein denaturation in the dehydrating tissues thereby solvating the cytosolic structures (McCubbin *et al.*, 1985; Baker *et al.*, 1988; Dure, 1993; Cuming, 1999). The charged amino acid motifs in LEA3 may aid in sequestering ions that accumulate under water deficit conditions (Dure, 1993). LEA proteins exhibit increase in folding when water activity is decreased using trifluoroethanol, high salt,

SDS or even under dehydration conditions (Lisse *et al.*, 1996; Ismail *et al.*, 1999; Soulages *et al.*, 2002). These LEA proteins are natively unfolded or intrinsically disordered but nevertheless this does not mean lack of function as increased folding is known to occur on binding to biological targets. The LEAs (rGmDHN1, LEA2) are known to exist in equilibrium between two extended conformational states: unordered and left-handed extended helical or poly (L-proline)-type II structures, a decrease in extended helix gradually to 15% as the temperature is increased. Another assumption is that the LEAs might perform their function in the unstructured state, as a hydration buffer, ion sink or as water replacement molecule. Structural shifts such as superhelical filamentous, coiled coil like structures in group 3a (SF2) and other categories of LEA proteins depend on the availability of water suggesting that LEAs may form part of the cytoskeleton (Wise and Tunnacliffe, 2004). However, recent studies indicate that LEA proteins do not behave as classical molecular chaperones but nevertheless do exhibit protective synergistic effect in the presence of trehalose suggesting the likelihood of its functioning as a molecular shield towards preventing the formation of damaged protein aggregates (Goyal *et al.*, 2005). Blackman *et al.* (1995) observed that in ABA-treated immature embryo axes, despite having high levels of LEA-like proteins, the electrolyte leakage was noticeably higher than that of the mature desiccation tolerant embryo axes and therefore surmised that the protection conferred by the LEAs might be during the recovery phase.

As further studies were carried out in plants and more recently in other types of organisms, including invertebrates and microorganisms, the concept of what constitutes LEA protein has become increasingly blurred. Classifying proteins solely on the basis of expression profile is fraught with danger, as it is negated each time an exception is discovered. In plants, many exceptions are known where LEA expression is not restricted to embryonic tissues or where expression is associated with other stresses besides desiccation, chiefly cold stress, a few LEA protein genes are constitutively expressed (Wise, 2003). While the nomenclature is unsatisfactory in plants, the nature and categorization of LEA proteins became even more problematic when they were discovered in organisms outside the plant kingdom. Clearly, the idea of defining a nematode protein with reference to expression of plant seed proteins has no sense (Tunnacliffe and Wise, 2007).

Although most LEA proteins fall into three main categories, a few other minor groups have also been proposed: group 4 (D-113 family), group 5 (D-29 family), (Bray, 1993).

Group 1 LEA

Group 1 LEA proteins are characterised by a conserved 20-amino-acid motif (GGQTRKEQLGEEGYREMGGHK) arranged in tandem up to four times with high glycine content (~20 %) and is envisaged to be involved in water binding under water deficit conditions. Such signature repeats manifest even in *Bacillus subtilis* GsiB stress protein (Stacy and Aalen, 1998), induced by oxygen limitation, heat, oxidation and salinity stress. NMR spectroscopy of a recombinant carrot (*Daucus carota*) group 1 protein, EMB-1, expressed in *Escherichia coli* revealed no defined secondary or tertiary structure of the protein in solution (Eom *et al.*, 1996). UV absorption and CD spectroscopy studies disclosed the unstructured protein (rGmD-19), with 6 % to 14 % of the protein occupying a left-handed extended helical or poly (L-Pro)- type (PII) conformation instead of a α -helical or β -sheet structure (Soulages *et al.*, 2002). Circular dichroism (CD) spectroscopy and hydrodynamic modeling of the wheat group 1 LEA protein, Em divulged 70 % random coil or nonregular and a flexible secondary structure (McCubbin *et al.*, 1985). Heterologous expression of *Em* in yeast expounds its feature as an ion-scavenger (Swire-Clark and Marcotte, 1999). POPPs, together with other machine-learning techniques, have been employed to scrutinize the relatedness of LEA protein sequences and their grouping into families and superfamilies (SF) based on which subgroups of group 1 proteins have also been recognized (Wise and Tunnacliffe, 2004).

Group 2 LEA protein/Dehydrins (DHNs)

Group 2 proteins include 15-amino acid conservative structure (EKKGIMD-KIKEKLPG) at the C-terminus and play an important role in metabolism as molecular chaperones and defending protein structure with a close connection to higher plants resistance to drought. Four names have been designated for this protein family-RAB, LEA D-11, LEA (II) and DHNs (dehydrins) (Baker *et al.*, 1988; Dure *et al.*, 1989). Dehydrins (dehydration induced) are water deficit-induced genes customarily expressed in the embryos during the late stages of embryogenesis and are also induced in vegetative tissues during normal growth conditions and in response to stress leading to cellular dehydration (e.g. drought, low temperature and salinity). Dehydrins are localized in

different cell compartments, such as the cytosol, nucleus mitochondria, vacuole and the vicinity of plasma membrane, however, they are primarily localized to the cytoplasm and nucleus. (Close, 1997; Campbell and Close, 1997; Rorat, 2006). They are distributed in a wide range of organisms including algae, yeast, cyanobacteria and higher plants. The DHNs are a redundant protein family and efforts are underway to clone and characterize them. Eleven *Dhn* genes have been identified in barley and since hexaploid wheat contains three homeologous Triticeae genomes it may predictably have 33–39 *Dhn* genes (Choi *et al.*, 1999). The DHNs are characterized by the conserved sequences Y, S and K segments and have been further classified based on sequence homology (see *Structure and functional studies of DHNs*). Localization of specific dehydrins at the plasma membrane (Danyluk *et al.* 1998) and also on the endomembranes (Dure, 1993) is consistent with their role in stabilizing cellular structures during dehydration stress (Kazuoka and Oeda, 1994; Close, 1997; Nylander *et al.*, 2001). Dehydrin-like proteins (DLPS) are known to accumulate in organelles such as mitochondria of *Triticum aestivum*, *Secale cereale* and in also in *Zea mays* in response to drought, freezing, cold, and exogenous ABA treatment (Borovskii *et al.*, 2002). These group 2 LEA proteins have been further categorized under POPPs classification, into two subgroups: group 2a, that are not specifically induced under cold stress along with expression during late embryogenesis and group 2b LEA proteins, that show cold tolerance and are explicitly not produced during late embryogenesis (Wise and Tunnacliffe, 2004).

Structure and functional studies of DHNA

Structurally DHNs are characterized by three conserved sequences; the Y, S and K segments, (i) a consensus Y-segment in the N-terminal region (T/VDEYGNP), found in one to three copies followed by (ii) the S segment, a phosphorylatable serine cluster [(LHRSGS4–10(E/D)3] in tandem with three acidic amino acids downstream of which is (iii) a 15-amino-acid lysine-rich sequence, or K-segment (EKKGIMDKIKEKLPG) that forms a putative amphipathic α -helical structure, possibly involved in hydrophobic interactions acting as solubilizing agents and preventing coagulation during environmental stress (Dure, 1993; Close, 1997; Campbell and Close, 1997).

Functional analysis of DHNs has been done in transgenic plants and also in yeast and studies highlight their potential use in conferring stress adaptability. Transformants expressing spinach CAP85 in tobacco (Kaye *et al.*, 1998) and *C. plantagineum* DSP16 in

tobacco (Iturriaga and Bartels, 1992) had no profound influence on stress tolerance. However, constitutive expression of barley DHN (*ABA2*) in *Arabidopsis* enhanced germination rate under salt and osmotic stress but no significant difference in germination at low temperature or protection from freeze induced damage of adult plants was detected (Calestani *et al.*, 1998). The expression of tomato group 2 LEA protein (Le4) in yeast partially ameliorated the detrimental effects of ionic and freezing stress by conferring tolerance to high concentration of KCl but not to NaCl or sorbitol (Zhang *et al.*, 2000). However, overexpression studies undertaken using DHNs such as CAP85 (Kaye *et al.*, 1998) as well as *C. plantagineum* DHN (Iturriaga and Bartels, 1992) and RAB18 (Lang *et al.*, 1994) had no effect on freezing or drought tolerance. Overproduction of citrus dehydrin (CoCOR19) in tobacco resulted in slight decrease in ion leakage under chilling and freezing stress. Moreover, recent strategies such as overexpression of multiple DHNs [Chimeric double constructs *RAB18* and *COR47*(pTP9) or *LTI29* and *LTI30*(pTP10)] genes in *Arabidopsis* resulted in improved survival especially when exposed to freezing stress (Puhakainen *et al.*, 2004). The intracellular translocation of the acidic dehydrin LTI29 from the cytosol to vicinity of the membranes during cold acclimation in transgenic plants suggests its plausible membrane protection mechanism.

The tissue distribution of DHNs revealed their localization being affected by post translational modifications. DHNs localize primarily to the nucleus or cytoplasm (Houde *et al.*, 1995; Bracale *et al.*, 1997), but studies also corroborate their presence either in the plasma membrane (Danyluk *et al.*, 1998), endoplasmic reticulum (Neven *et al.*, 1993), chloroplast (Wisniewski *et al.*, 1999), mitochondria (Borovskii *et al.*, 2002) or even in the vacuole (Heyen *et al.*, 2002). DHNs may thus be expressed either constitutively [e.g. *Arabidopsis* DHNX] or confined to the root tip (ERD14 and LTI29), vascular tissues (ERD14, LTI29 and RAB18) and stomatal guard cells (RAB18). DHNs accumulate within vascular tissues (Godoy *et al.*, 1994; Houde *et al.*, 1995; Danyluk *et al.*, 1998; Nylander *et al.*, 2001), pollen sacs, guard cells and root meristems (Nylander *et al.*, 2001), endosperm and zygotic embryos of mature seeds, developing root primordia (Godoy *et al.*, 1994; Bracale *et al.*, 1997), wheat crown tissues (Houde *et al.*, 1995) and peach shoots (Wisniewski *et al.*, 1999). DHNs found only in the cytosol include those of *C. plantagineum* DSP16 (Schneider *et al.*, 1993) and rice RAB21 (Mundy and Chua, 1988). DHNs in birch improve enzyme activity

under conditions of low water activity and were found localized in storage protein bodies and starch rich amyloplasts (Rinne *et al.*, 1999).

Group 3 LEA

Group 3 LEA proteins are characterised by a repeat of 11-mer amino acid motif with the consensus amino acid sequence TAQAAKEKAGE sometimes repeated as much as 13 times in *Brassica napus* Lea76 (Harada *et al.*, 1989) and *Hordeum vulgare* HVA1. The consensus sequence could be postulated to form an alpha helical dimer suitable for the sequestration of positively and negatively charged ions that accumulate under water deficit conditions (Dure, 1993). Group 3 LEA proteins are abundant, cytosolic proteins, ubiquitous and conserved in plants. Functional analyses of LEA proteins have been carried out under both *in vitro* and *in vivo* conditions. Studies with HVA1, a barley group 3 LEA protein, conferred tolerance to water deficit and other stress conditions (Xu *et al.*, 1996; Sivamani *et al.*, 2000; Maqbool *et al.*, 2002; Rohila *et al.*, 2002). Expression of HVA1 in *japonica* rice (Xu *et al.*, 1996) and Basmati rice (Rohila *et al.*, 2002) conferred water deficit and salt stress tolerance. In transgenic rice, ion leakage due to stresses is reduced by 90 % compared to the wild type plant. The transgenic plants wherein the HVA1 gene was regulated by a stress inducible promoter, displayed less ionic leakage in comparison to plants expressing the same gene under a constitutive promoter (Rohila *et al.*, 2002). Transgenic plants maintained higher leaf relative water content (RWC) showed lesser reduction in plant growth under drought stress, delayed wilting and better cell membrane protection (Babu *et al.*, 2004). These results indicate that the production of HVA1 might have helped in better performance of transgenic rice plants by protecting cell membrane from injury under drought stress (Babu *et al.*, 2004). In transgenic wheat, water use efficiency (WUE) improved by about 20 % leading to higher biomass accumulation as compared to the wild type plants (Sivamani *et al.*, 2000). Oat transgenics showed delayed wilting under drought stress, 37 % more tolerance to salt stress and 60 % more tolerance to mannitol stress (Maqbool *et al.*, 2002). Heterologous expression of HVA1 in yeast (*Saccharomyces cerevisiae*) improved the growth rate under both ionic (NaCl and KCl) as well as low temperature stresses (Zhang *et al.*, 2000). Overexpression of HVA1 gene from barley generates tolerance to salinity and water stress in transgenic mulberry, *Morus indica* (Lal *et al.*, 2008) and in *Agrostis stolonifera* (Fu *et al.*, 2007).

The grass species, including wheat, are known to have at least three groups of LEA3-like proteins (two small proteins - LEA3-L1 and LEA3-L2 and a large protein - LEA3-L3). WCS19 (encoding for a wheat LEA3-L2 protein) constitutively expressed in *Arabidopsis* resulted in significant freezing tolerance and resistance to photoinhibition (Ndong *et al.*, 2002). Under *in vitro* conditions, an algal (*Chlorella vulgaris*) LEA 3 protein diminished the freeze-induced damage of the enzyme lactate dehydrogenase (Honjoh *et al.*, 2000). POPPs (Protein or Oligonucleotide Probability Profile) analysis has categorized group 3 LEA proteins into two subgroups: corresponding to SF2 (group 3a) and SF5 (group 3b) (Wise and Tunnacliffe, 2004) based on similarities in their peptide compositions and reflecting their natural distribution.

Group 4, 5 and 6 LEA

Group 4 LEA proteins lack repeat motifs, but contain a well conserved N-terminal domain that forms amphiphilic α -helices thereby interacting with ions or membranes (Bray, 1993; Imai *et al.*, 1995). An alternative function of water-binding protein was proposed for the cotton group 4 LEA protein, D113, based on the predicted random coil domain at its C-terminal (Dure, 1993). However, since this domain is not as long in tomato and sunflower proteins, it may not be a common function of group 4 LEA proteins. Heterologous expression of tomato LE25, in *Saccharomyces cerevisiae* resulted in the yeast cells displaying shorter lag times with respect to the control cells when challenged with high NaCl concentration thereby suggesting that the protein might be functioning as an ion-scavenger (Imai *et al.*, 1995). Groups 4 and 5 LEAs disappear altogether under the POPP computational method, with former members of these groups consistently reallocating into LEA groups 2 and 3. Group 5 protein sequences have a similar repeat (11 mer) motif as found in group 3 members (Wise and Tunnacliffe, 2004). *Arabidopsis thaliana* AtECP31, carrot DcECP31 and cotton D34, D29 are involved in group 5 proteins, in which there is little higher amino acid-residue specificity. On the basis of this structure it is predicted that group 5 proteins play roles in seed maturation, dehydration and combining concentrated ions (Yu *et al.*, 1999). The group 6 LEA has a balanced hydrophobicity plot unlike the other LEA protein members (Baker *et al.*, 1988; Pla *et al.*, 1991) and is predicted to have 4-6 α -helices and a globular structure. CaLEA6 (LEA Group 6) protein displayed potentially protective role when water deficit is induced by dehydration and high salinity but non-inducible under low temperature (Kim *et al.*, 2005).

Prospects for genetic engineering

Abiotic stress such as drought, salinity, extreme temperature and oxidative stress are usually interrelated and often activate similar cell signaling pathways and cellular responses. Therefore, genetic manipulation of plants towards conferring abiotic stress tolerance is a daunting task, as the abiotic stress tolerance mechanism is interconnected and is not monogenic in nature. Many mechanisms involving engineering of plants towards conferring abiotic stress have been undertaken successfully by various researchers, but the pertinent question that arises is the relative importance of the different strategies. A fundamental question to be addressed is whether detoxification (by use of ROS scavenging enzymes) or homeostasis (by use of osmolytes, LEAs, HSPs) is more imperative for engineering tolerance. The need of the hour is to engineer stress tolerance in major crop plant species.

Expression of transcription factors such as *DREB1A*, *CBF1*, *CBF3*, *CBF4*, *ABI3*, *ALFIN1*, *ABF3*, *ABF4*, *AtMYC2*, *AtMYB2*, *HSF1* and *HSF3* (also see Table 1) shows landmark achievements in overcoming the stress response. However, constitutive expression of several stress-related genes, including *CBF1*, *DREB1A*, *ATHB7* and yeast trehalose synthase, has resulted in slow growth of transgenic plants (Liu *et al.*, 1998; Holmstrom *et al.*, 2000) and therefore the need for comprehensive evaluation of the transformants arises. Hence, various transgenic plants should be comparatively assessed under identical test conditions to identify the most useful genes. For example, the evaluation of the relative utility of transgenes was undertaken by comparing *CBF1* and *DREB1A* transgenic plants (Steponkus *et al.*, 2000) and it was observed that *CBF1* improved freezing tolerance by 1 °C whereas *DREB1A* improved the same trait by ~10 °C.

Another important aspect involves the regulated and targeted expression of transgenes, such as by use of stress regulated promoters e.g., *rd29* (Kasuga *et al.*, 1999), *6XARE* (Rahman *et al.*, 2001), *4ABRC-Act1-100P-Hva22* (Garg *et al.*, 2002; Roy and Wu, 2002; Rohila *et al.*, 2002), AIPC (ABA-induced promoter complex, Zhu *et al.*, 1998), *Gmhsp17.6-L* (Lee and Schoffl, 1996). AIPC (ABA induced promoter complex) has also been used in conferring salt and water stress tolerance by engineering *P5CS* in rice (Zhu *et al.*, 1998). Targeted expression has been achieved by using a constitutive promoter along with a transit peptide sequence, consequently targeting the protein specifically to the required organelle (Bowler

et al., 1991; van Camp *et al.*, 1996; Shen *et al.*, 1997; Yu *et al.*, 1999; McKersie *et al.*, 2000). Use of DEX-inducible promoter (Qin and Zeevaart, 2002) or a Tc-Inducible promoter (Thompson *et al.*, 2000) to regulate the expression of *PvNCED1* and *LeNCED1*, respectively, resulted in elevated ABA levels thereby effecting the seed dormancy and water relations in the plant. Furthermore the transformants need to be evaluated experimentally to study their segregation pattern; homozygosity and also the expression levels before conventional breeding into other commercial varieties are undertaken. A thorough examination of the transformants vis-à-vis the untransformed plant at field level would highlight the potential of the same.

Various strategies have thus been exploited to address and evaluate the stress tolerance mechanism in transformants of which the osmolyte engineering has been the most popular one. Examples of osmolyte engineering encompass, targeting the accumulation of proline (by use of *P5CS* and *PRODH*) and also glycine betaine (by use of *CODA*, *BETA* and *BETB*) (also see Macromolecular Homeostasis). However, since many important crops do not accumulate glycine betaine, the main constrain involves maintaining a steady endogenous choline supply to keep up with the glycine betaine production. Sugar alcohols such as mannitol (by use of *MTLD*), *D-ononitol* (by use of *IMT1*) and trehalose (by employing *TPS1*, *OTS A* and *OTSB*) have also been targeted for engineering compatible solutes overproduction. However, incorrect gene expression often results in pleiotropic effects such as necrosis and growth retardation due to disturbance in endogenous metabolic pathways. Therefore, to minimize such impediments the compatible solutes should be engineered such that they are not only stress inducible but also tissue specific. Strategies of detoxification by use of genes encoding for ROS scavenging enzymes such as the *APX3*, *hvaPX1* (encoding for ascorbate peroxidase), *GR*, *GST/GPX* (encoding for glutathione reductase) and also *Mn/Fe-SOD* (encoding for superoxide dismutase) conferred enhanced stress tolerance to most engineered plants (see Table 1). The use of genes involved in the fatty acid metabolism such as *FAD7* and *GPAT* resulted in transformants having more unsaturated fatty acids that further conferred tolerance to temperature stress. The use of heat shock genes such as *HSP17.6A*, *HSP17.7*, *HSP101* and *HSP70* conferred high temperature stress tolerance in most plants. LEAs have also been the target of study by employing *HVA1*, *COR15*, *CuCOR19* and *WCS19* (see Table 1).

Lipid transfer proteins (LTPs) help in transfer of several classes of phospholipids and glycolipids and can be induced by different kinds of abiotic stresses such as cold, drought and osmotic stress (Kader, 1996). Histone H1 variant genes have also been shown to be induced by drought in *Arabidopsis* and *Lycopersicon pennellii* (Wei and O'Connel, 1996). Histone H1-S antisense transgenic plants showed remarkable differences in leaf anatomy and physiological activities (Scippa *et al.*, 2004). The expression of other proteins involved in stress response such as molecular chaperones (Alvim *et al.*, 2001); NAD⁺ dependent glyceraldehyde-3-phosphate dehydrogenase (Jeong *et al.*, 2000) have also conferred tolerance against water stress.

Global expression profiling - Abiotic stress

Efforts towards monitoring the global expression profiles under stress conditions such as ABA, drought, cold, high-salinity and also during the rehydration process after dehydration (Oono *et al.*, 2003) reveal the complexity of the stress mechanism. Genomics has provided a wealth of data and a better understanding of changes in cellular metabolism that are induced by abiotic stresses, but results have been limited with respect to the whole plant level. To some extent, the advent of genomics has shifted away the focus on abiotic stress biology in species that show natural tolerance. Essential requisites for abiotic stress are being assembled on a genome-wide scale in an anthological way rather than by hypothesis-driven experimentation (Bohnert *et al.*, 2006). This information is necessary for the elucidation of abiotic stress interactive pathways. Functional genomics employing genomewide strategies such as transcriptomics, mutant analysis and proteomics, have been widely used to identify stress-responsive genes and to understand the mechanism of stress tolerance. Certain networks involved in the plant stress response have already been proposed, and their extension and refinement in relation to crop plants such as rice will become possible by functional genomics (Vij and Tyagi, 2007). Transcript profiling using gene chips or long-oligonucleotide array slides, provided important insights into the dynamics of the transcriptional changes that accompany abiotic stress treatments. Several contributions have emerged on changes in transcript profiles under cold, drought, and high-salinity conditions, mainly in *A. thaliana* and rice, and other species, focusing on stress responses (Kreps *et al.*, 2002; Rabbani *et al.*, 2003; Buchannan *et al.*, 2005). A new avenue has been opened by the detection of regulatory systems that depend on small non-coding

RNAs in plants. Some siRNAs have been shown to be stress-inducible and affect the translation process. These siRNAs might participate in the alternative splicing of mRNAs and represent important components of an additional level of regulation. A few reports are available in which their function has been correlated with abiotic stress, such as dehydration responsive miRNAs in rice and *Arabidopsis* (Sunkar and Zhu, 2004; Zhao *et al.*, 2007). Transcriptome analysis of drought inducible genes in *Arabidopsis* and rice was done employing microarray technology. More than half of the drought inducible genes were also induced by salinity and/or ABA treatments, implying cross-talk between the drought, high salinity and ABA-response pathways (Shinozaki and Yamaguchi-Shinozaki, 2007).

Advancements in the field of proteomics have also revolutionized the scenario. Given the high throughput and high sensitivity of mass-spectrometry, coupled with advanced softwares to protein databases, proteomics is gaining overwhelming response (Aebersold *et al.*, 2003; Ferguson *et al.*, 2003). As a result we have information on the sequence of a number of proteins in response to environmental stress (Qureshi *et al.*, 2007). The dehydration responsive nuclear proteome of chickpea revealed a coordinated response, which involves both the regulatory as well as functional proteins. Comparative analysis of differentially expressed proteins in chickpea extracellular matrix during dehydration stress demonstrates that over a hundred ECM proteins, impinge on the molecular mechanism of dehydration tolerance in plants (Bhushan *et al.*, 2007). Plomion *et al.* (2006) mapped the proteome of poplar, discovered certain drought stress responsive proteins and compared with transcriptomic data. The understanding of plant stress physiology and of the factors that influence it are beginning to emerge and we can correlate them with the changes occurring in the proteome. Therefore, it can be surmised that molecular inputs are necessary to understand the complex abiotic stress mechanism such that they can be targeted apropos *via* genetic engineering to meet the needs of mankind. In addition, transcriptome analysis using microarray technology offers a powerful platform to find out the expression of genes during osmotic stress at the global level. This perhaps would not have been possible without technical advancements in the realms of gene discovery, high-throughput gene expression, altering gene expression by transformation technologies, functional characterization of genes of interest and genetical/genomic approaches (Sreenivasulu *et al.*, 2007). In a nutshell, promises ushered by genomics, proteomics,

transcriptomics and metabolomics could generate valuable information for engineering plants for their ultimate use in sustainable agriculture.

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