

## REVIEW

# Beyond osmolytes and transcription factors: drought tolerance in plants via protective proteins and aquaporins

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## Abstract

Mechanisms of drought tolerance have been studied by numerous groups, and a broad range of molecules have been identified to play important roles. A noteworthy response of stressed plants is the accumulation of novel protective proteins, including heat-shock proteins (HSPs) and late embryogenesis abundant (LEA) proteins. Identification of gene regulatory networks of these protective proteins in plants will allow a wide application of biotechnology for enhancement of drought tolerance and adaptation. Similarly, aquaporins are involved in the regulation of water transport, particularly under abiotic stresses. The molecular and functional characterization of protective proteins and aquaporins has revealed the significance of their regulation in response to abiotic stresses. Herein, we highlight new findings regarding the action mechanisms of these proteins. Finally, this review also surveys the current advances in engineering drought tolerant plants, particularly the engineering of protective proteins (sHSPs and LEA) and aquaporins for imparting drought stress tolerance in plants.

*Additional key words:* abiotic stress, heat-shock proteins, late embryogenesis abundant proteins, transgenic plants

## Introduction: from green revolution to gene revolution

Global food shortage due to abiotic stresses (drought, salinity, extreme temperatures) and high human population directed plant scientists towards gene revolution following green revolution. This approach is commonly referred to as genetic engineering/transgenic approach. Transgenic approaches offer new opportunities to improve tolerance to abiotic stresses. Drought is one of the greatest worldwide environmental constraints for agricultural productivity (Boyer 1982, Bray *et al.* 2000). Improving crop resistance to drought stress is a long standing goal of agricultural biotechnology (McCue and Hanson 1990, Jain and Selvaraj 1997). The present genetic engineering strategies rely on the transfer of one or several genes to the target plant. Overproduction of these genes in transgenic plants enhances their protective effects against not only drought but also other abiotic stresses. These genes are either involved in signaling

and/or regulatory pathways, or that encode enzymes leading to the synthesis of functional and structural protectants, as reviewed by Wang *et al.* 2003, Vinocur and Altman 2005, Valliyodan and Nyugen, 2006, Sreenivasulu *et al.* 2007, Kathuria *et al.* 2007, Bartels and Hussain 2008, Hu *et al.* 2010, Hussain *et al.* 2011a,b.

Efforts to improve crop performance under drought stress were not yet so successful for two main reasons: incomplete understanding of underlying fundamental mechanisms of stress tolerance and lack of knowledge of the interactions of different stressors. Engineering drought tolerance in crop plants has huge economic importance. Genetic engineering for drought stress tolerance was limited in the pre-genomics era due to limited availability of genes and specific promoters. Now it is possible to study many genes simultaneously on a genome wide-scale with respect to their structure and

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*Abbreviations:* ABA - abscisic acid; HSPs - heat-shock proteins; LEA - late embryogenesis abundant; MIPs - major intrinsic proteins; PIPs - plasma membrane intrinsic proteins; TIPs - tonoplast intrinsic proteins; WUE - water use efficiency.

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function. However, the multigenic nature of complex mechanisms of stress tolerance and the potential side effects on plant growth make this task difficult. In the

current article, we provide a comprehensive outline of transgenics developed so far for drought tolerance by using LEA, sHSPs and aquaporins.

### Transgenic plants harboring transcription factors and osmoprotectants for conferring drought stress tolerance

Recent progress has been made in our understanding of gene expression, transcriptional regulation and signal transduction in plant responses to drought (Shinozaki *et al.* 2003, Yamaguchi-Shinozaki and Shinozaki 2005). On the other hand, molecular and genomic analyses have facilitated gene discovery (Seki *et al.* 2001, Abe *et al.* 2003, Tran *et al.* 2004, Seki *et al.* 2007) and enabled genetic engineering using several functional or regulatory genes to activate or repress specific or broad pathways related to drought/salinity tolerance in plants (Trujillo *et al.* 2009). Progress in plant modification for enhanced drought tolerance through manipulation of either transcription and/or signaling factors in transgenic plants have been impressive during the last two decades (Hussain *et al.* 2011b). Successful examples are transgenic crops engineered with genes encoding the DREBs/CBFs transcription factors (tomato – Hsieh *et al.* 2002, rice – Dubouzet *et al.* 2003, Oh *et al.* 2005, Ito *et al.* 2006, Wang *et al.* 2008 and wheat – Pellegrineschi *et al.* 2004). The transgenic plants showed increased stress tolerance as well as the over induction of downstream stress related genes and/or higher contents of soluble sugars and proline (Gilmour *et al.* 2000, Ito *et al.* 2006).

Despite DREB/CBF, other transcription factors like members of the MYB, MYC, ERF, bZIP, and WRKY transcription factor families have already been implicated in the regulation of stress responses (Schwechheimer *et al.* 1998, Singh *et al.* 2002). Therefore, these are attractive targets for the purpose of gene regulation and manipulation of the regulatory elements, which may be beneficial under abiotic stresses (Nakashima *et al.* 2007, Hu *et al.* 2008, Jung *et al.* 2008, Trujillo *et al.* 2008, Yu *et al.* 2008, Xiang *et al.* 2008, Zou *et al.* 2008, Qiu and Yu 2009, Zhang *et al.* 2009, Abdeen *et al.* 2010, Agrawal and Jha 2010, Hussain *et al.* 2011b).

Transcriptional factors can be a valuable resource in transgenic technology, attributing novel traits to the transgenic plants. This and related technologies are poised to become important genomic tools that will allow researchers to rapidly obtain functional information.

Metabolic engineering for increased osmolyte contents was successful in several plants subjected to stress (Wang *et al.* 2003), although real advantages of

such a strategy are always a subject of debate (Serraj and Sinclair 2002). Such osmoprotectants serve to decrease the osmotic potential of the plants in the cytoplasm and can also stabilize different proteins and the membranes under various abiotic stresses (Bohnert and Shen 1999, McNeil *et al.* 1999). Several osmoprotectants like proline (Bertrand and Paquin 1991, Naidu *et al.* 1991, Delauney and Verma 1993, Murelli *et al.* 1995, Wanner and Juntilla 1999), 3-dimethylsulfoniopropionate (McNeil *et al.* 1999), trigonelline and betaines (Naidu *et al.* 1991, Nomura *et al.* 1995, McNeil *et al.* 1999, Nuccio *et al.* 1999) fructans and trehalose (Pilon-Smits *et al.* 1995, Crowe *et al.* 1998, Goddijn and van Dun 1999, Iturriaga *et al.* 2000) and polyols (Bohnert and Jensen 1996; Sheveleva *et al.* 1997, 1998) have been identified in stressed plants. However, many crops lack the ability to synthesize these special osmoprotectants that are naturally accumulated by the stress tolerant organisms. For example, several taxonomically distinct species, including spinach and wheat, are natural accumulators of glycine betaine while others, including *Arabidopsis*, rice, tomato, and potato are considered to be non-accumulators. Therefore, the widely adopted strategy till now is to engineer or overexpress certain osmolytes which serves as potential route to breed stress tolerant crops. This strategy has resulted in several successful studies where different osmoprotectants have been overexpressed, like proline (Mani *et al.* 2002, Simon-Sarkadi *et al.* 2005, Yamada *et al.* 2005, Gubis *et al.* 2007, Vendruscolo *et al.* 2007, Chen *et al.* 2009, Kumar *et al.* 2010), glycine betaine (Park *et al.* 2004, Quan *et al.* 2004, Lv *et al.* 2007, Park *et al.* 2007, Ahmad *et al.* 2008) sugars and sugar alcohols (Chen and Murata 2002, Cortina and Cuiñez-Macia 2005, Karim *et al.* 2007, Miranda *et al.* 2007, Paul *et al.* 2008, Stiller *et al.* 2008, Suzuki *et al.* 2008, Livingston III *et al.* 2009, Suárez *et al.* 2009).

A variety of genes has been identified and employed to generate transgenic plants that accumulate osmoprotectants and they are tolerant to drought and other abiotic stresses. These studies emphasize the importance of different osmoprotectants in stress tolerance and demonstrate that the modification of drought stress tolerance by manipulation of these compounds is a valuable alternate in plants.

### Transgenic plants harboring protective proteins for enhanced tolerance

To cope with the environmental stresses, plants activate a large set of genes, which lead to the accumulation of

specific stress-associated proteins (Vierling 1991, Ingram and Bartels 1996, Bohnert and Sheveleva 1998,

Thomashow 1999, Hoekstra *et al.* 2001). Heat-shock proteins (HSPs) and the late embryogenesis abundant (LEA)-type proteins are induced by stress and accumulate in huge amounts upon drought, salinity, and extreme temperature stress (Table 1). These have been shown to play a role in the cellular protection during stress (Vierling and Kimpel 1992, Boston *et al.* 1996, Close 1996, Ingram and Bartels 1996, Waters *et al.* 1996, Thomashow 1998). Recently, Hu *et al.* (2010) advanced multifunctional gene concept and defined it that a multifunctional gene is a gene that can regulate several kinds of traits and also manifest several kinds of functions. Based on this hypothesis, sHSPs, LEA and aquaporin proteins perform several functions in plants. This review summarizes the progress of plant engineering using these three protein groups for improving drought stress tolerance.

**HSPs are molecular chaperones :** HSPs are produced in response to elevation in temperature and certain other stresses. In response to abiotic stresses, certain enzymes and proteins are affected and become dysfunctional. Therefore, maintaining the proteins in their functional conformations and preventing the aggregation of proteins are particularly important for cell survival under stress conditions. The isolated HSPs were shown to protect up to 75 % of soluble proteins from heat denaturation *in vitro* (Jinn *et al.* 1995). Many stress-responsive proteins, especially HSPs, have been shown to act as molecular chaperones and maintain homeostasis of protein folding and so are responsible for the acquisition of stress tolerance (Vierling 1991, Hendrick and Hartl 1993, Boston *et al.* 1996, Hartl 1996, Waters *et al.* 1996, Török *et al.* 2001).

Among the five conserved families of HSPs (HSP100, HSP90, HSP70, HSP60 and sHSP), the small heat-shock proteins (sHSPs) have been shown to be the most prevalent in plants. Plant HSPs consist of a few high molecular mass proteins and a complex group of low molecular mass proteins with molecular sizes ranging from 15 to 30 kDa (Vierling 1991). All the plant sHSP proteins are encoded by the nuclear genes and are divided into six classes based on intracellular location and sequence similarity into cytosolic class I, cytosolic class II, chloroplast, mitochondria, endoplasmic reticulum and peroxisome sHSPs (Low *et al.* 2000, Ma *et al.* 2006, Kotak *et al.* 2007, Mamedov and Shono 2008, Siddique *et al.* 2008). It is believed that accumulation of HSPs plays a pivotal role in abiotic stress tolerance in plants (Sun *et al.* 2002, Sorensen *et al.* 2003). The sHSPs, as well as other HSPs, are believed to play an important role in plant stress tolerance to extreme temperatures (Malik *et al.* 1999, Lopez-Matas *et al.* 2004, Sanmiya *et al.* 2004, Charnig *et al.* 2006, Jiang *et al.* 2009), salinity (Harrington and Alm 1988, Hamilton and Heckathorn 2001, Liu *et al.* 2006), toxic metals (Wollgiehn and Neumann 1995), chilling (Sebehat *et al.* 1996, Sato *et al.* 2001), oxidative stress (Banzet *et al.* 1998, Lee *et al.* 2000, Neta-Sharir *et al.* 2005, Volkov *et al.* 2006), dehydration (Campbell

*et al.* 2001, Liu *et al.* 2006, Sato and Yokoya 2008), abscisic acid (ABA) treatment (Campbell *et al.* 2001), mechanical injury and salicylic acid treatment (Chang *et al.* 2007). The protective effects of HSPs can be attributed to the network of the chaperones acting coordinately (Wang *et al.* 2005). This is also clear from the fact that more than 70 putative HSP genes were found in rice genome by searching the Rice Genome Annotation (Ouyang *et al.* 2007). Some of these genes have been cloned and characterized (Pareek *et al.* 1998, Lee *et al.* 2000, Guan *et al.* 2004, Murakami *et al.* 2004, Liu *et al.* 2006, Chang *et al.* 2007, Sato and Yokoya 2008, Hu *et al.* 2009) while most of them remained to be functionally annotated.

Transgenic *Arabidopsis* plants that overexpressed class II HSP 17.6A and accumulated high content of HSP17.6A protein showed increased tolerance to drought and salt stress (Sun *et al.* 2001). The major chaperone activity of sHSPs is to bind and hold the denatured substrates in a folding-competent state for subsequent refolding by a chaperone network (Horwitz 1992, Ehrnsperger *et al.* 1997, Lee *et al.* 1997, Veinger *et al.* 1998, Haslbeck *et al.* 1999, Ding and Candido 2000, Studer and Narberhaus 2000). However, some members of the plant sHSPs can also stabilize enzymes or reactivate the inactivated enzymes (Lee *et al.* 1995, Hook and Harding 1998, Muchowski and Clark 1998, Haslbeck *et al.* 1999, Smykal *et al.* 2000, Marini *et al.* 2000, Sun *et al.* 2001). Various studies have shown that plant sHSPs are not only expressed in response to heat shock, but also under water, salt, oxidative stresses, and at low temperatures (Almoguera *et al.* 1993, Alamillo *et al.* 1995, Sebehat *et al.* 1998, Hamdahl *et al.* 1999, Hamilton and Heckathorn 2001). Cho and Hong (2006) reported that the transgenic tobacco plants overexpressing *NtHSP70-1* exhibited tolerance to water stress by regulating the water flux in these transgenic plants. Recently, transgenic rice plants overexpressing small heat-shock protein sHSP17.7 have been shown to have an enhanced tolerance to drought stress (Sato and Yokoya 2008). In this study, both the transgenic and the control plants withered the same day under severe drought stress. However, only the transgenic seedlings could re-grow after rewatering. This study clearly demonstrated the role of sHSP in subsequent rehydration after the plants have undergone drought stress.

**The LEA-type proteins:** In addition to HSPs, also LEA-type proteins can confer molecular protection of cellular components during abiotic stresses (Wang *et al.* 2003). LEA-type proteins have been found in a wide range of plant species where they are synthesized in response to water deficit resulting from desiccation, cold and osmotic stress (*Physcomitrella patens* – Liang *et al.* 2004, pea – Grelet *et al.* 2005, soybean – Shih *et al.* 2004, rice – Moons *et al.* 1997, cotton – Galau *et al.* 1993, radish – Raynal *et al.* 1990) and salt stress (mulberry – Jyothsnakumari *et al.* 2009). LEA-type proteins are encoded by *RD* (responsive to dehydration), *ERD* (early

responsive to dehydration), *KIN* (cold inducible), *COR* (cold regulated), and *RAB* (responsive to abscisic acid) genes in different plant species (Shinozaki and Yamaguchi-Shinozaki 2000, Zhu 2002). LEA-type proteins fall into a number of families, which have diverse structures and functions (Close 1996, Ingram and Bartels 1996, Thomashow 1998). Till date, the actual function of the LEA-type proteins remains, however, largely unknown. However, some functions have been predicted from their structural characteristics and synthesis during the late stage of embryogenesis and also their induction by stress. It has been suggested that the LEA-type proteins act as water-binding molecules in seeds where they protect other proteins from the harmful effects of desiccation, in ion sequestration and in macromolecule and membrane stabilization against freeze-induced injury (*i.e.* chaperone-like activity; Dure 1993a,b, Close 1996, Ingram and Bartels 1996, Thomashow 1998, 1999, Viji and Tyagi 2007, Tunnacliffe and Wise 2007).

Transgenic plants overexpressing genes encoding LEA proteins can increase the tolerance to drought, salt, cold and other stresses (Cho and Hong 2006, Jyothsnakumari *et al.* 2009). For example, constitutive overexpression of the HVA1, a group 3 LEA protein from barley, conferred tolerance to low soil water and salt stress in transgenic rice plants (Xu *et al.* 1996) due to the cell membrane protection and osmotic adjustment (Babu *et al.* 2004, Fu *et al.* 2007). Recently, Ndong *et al.* (2002) reported that the constitutive expression of a wheat chloroplast LEA-like protein (WCS19) in *Arabidopsis* resulted in a significant increase in freeze-tolerance. Also the constitutive or stress induced expression of the HVA1 gene resulted in the improvement of the growth characteristics and stress tolerance in terms of increased cell integrity in wheat and rice under water and salt stress conditions (Sivamani *et al.* 2000, Maqbool *et al.* 2002, Rohilla *et al.* 2002, Bahieldin *et al.* 2005, Oraby *et al.* 2005). Overexpression of a single/multiple LEA-type protein is not always sufficient to confer stress tolerance to the plants, an example of this is the case where the transgenic tobacco plants that had been transformed with three *Craterostigma plantagineum* cDNAs, *pcC6-19* (homologous with rice *rab16*), *pcC3-06* (homologous with *lea D29*) and *pcC27-45* (homologous with *lea 14*), expressed high levels of the encoded proteins, but this increase did not result in drought tolerance (Iturriaga *et al.* 1992). This suggested that there was the possibility that the LEA-type proteins might function synergistically with other molecules. In this respect, overexpression of group 3 LEA genes in *Arabidopsis* (Figueras *et al.* 2004) and

tobacco (Roychoudhury *et al.* 2007) showed significant increase in growth rate under stress condition and also resulted in increased osmolyte such as proline, polyamines and sugars production. Similarly, transgenic tobacco overexpressing a *PF03760* LEA protein from a resurrection plant *Boea hygrometrica* exhibited an increased peroxidase and superoxide dismutase activity during drought (Liu *et al.* 2010). These studies clearly demonstrated that LEA protein can have an indirect effect on the accumulation of protective molecules and stress tolerance can possibly be the result of these protective molecules. Transgenic rice (TNG67) plants, expressing a wheat LEA group 2 protein gene (*PM180*) or the wheat LEA group 1 protein gene (*PM1959*), had increased tolerance to drought and salt stresses, although their water use efficiency (WUE) was low (Cheng *et al.* 2002). Besides the protective chaperone-like functions of the LEA proteins, their action against cellular damage has been proposed (Vinocur and Altman 2005), indicating the role of LEA proteins in anti-aggregation of enzymes under dehydration and freezing stress (Goyal *et al.* 2005). Also recent computational studies have proposed that the LEA type proteins act as chaperone-like protective molecules that function against cellular damage (Wise 2003, Wise and Tunnacliffe 2004). Therefore, engineering plants that produce LEA protein would be a more efficient way to develop a multiple stress-tolerant crop plants without compromising its productivity.

Despite massive data on the LEA proteins expression and their structure (Garay-Arroyo *et al.* 2000, Bies-Etheve *et al.* 2003, Grelet *et al.* 2005), little work has been reported on the manipulation of the LEA genes to improve drought resistance (Goyal *et al.* 2005, Park *et al.* 2005a, 2005b, Wang *et al.* 2006, Fu *et al.* 2007, Jun *et al.* 2008, Lal *et al.* 2008, Dalal *et al.* 2009). It is worth mentioning that none of these authors have evaluated the role of LEA genes under field conditions. Only Xiao *et al.* (2007) conducted some field studies on transgenic rice carrying the *OsLEA3-1* gene under the control of three different promoters. Their results indicate that drought resistance is significantly improved in those transgenic rice which express the *OsLEA3-1* transgene controlled by a drought-inducible HVA1 like promoter and a constitutive promoter CaMV 35S. They further described that, apart from improved drought resistance, there are no phenotypic changes, or any yield penalty under field conditions. However, before engineering crop plants with LEA genes, it will be necessary to secure data comparing the efficiency of various members of LEA genes and also the efficiency of single LEA gene in a range of species under multiple abiotic stresses.

## Genetic manipulation of aquaporin functions in transgenic plants

Water movement across the cellular membranes is largely regulated by a family of water channel proteins called aquaporins. Aquaporins belong to the major intrinsic proteins (MIPs) family and facilitate the flow of water

across the cellular membranes (plasmalemma and tonoplast), following osmotic or hydrostatic pressure gradients (Chrispeels and Agre 1994, Schaeffner 1998). The biological significance of aquaporins in plants is

their ability to modulate transmembrane water transport in situations where adjustment of water flow is physiologically critical (for reviews see Baiges *et al.* 2002, Luu and Maurel 2005). Furthermore, the importance of the ability to increase or decrease the water permeability of a cell is signified by the very high diversity of aquaporin homologues present in plants: genome projects have revealed 35 aquaporins in *Arabidopsis* (Johanson *et al.* 2001, Maurel *et al.* 2002, Quigley *et al.* 2002, Boursiac *et al.* 2005), 33 in rice (Sakurai *et al.* 2005), 36 in maize (Chaumont *et al.* 2001), 23 in moss (Danielson and Johanson 2008), 37 in tomato (Sade *et al.* 2009), and 55 in poplar (Gupta and Sankararamakrishnan 2009). The two major subgroups of the aquaporins are the plasma membrane intrinsic proteins (PIPs) and the tonoplast intrinsic proteins (TIPs) (Johnson *et al.* 1990; Ermawati *et al.* 2009). PIPs play an important role in controlling the transcellular water transport and are further subdivided into two subfamilies PIP1 and PIP2 (Schaeffner 1998, Chaumont *et al.* 2000). TIPs seem to be involved in water exchange between the cytosolic and the vacuolar compartments, and are involved in cell osmoregulation (Maurel *et al.* 1997, Tyerman *et al.* 1999). Although the discovery of the aquaporins has resulted in a paradigm shift in the understanding of the plant water dynamics, a comprehensive picture of the physiological role of aquaporin in plant remains elusive. However, the highly regulated expression of the aquaporins in plants suggests that the transmembrane water transport may be important in many other processes in addition to those related to transpiration.

The importance of aquaporins in environmental responses has been demonstrated through gene expression analysis and genetic manipulation of the aquaporin functions in plants which provide a promising strategy to address the questions of the overall role of the water molecules in plant homeostasis (Table 1). Antisense inhibition of the PIP aquaporins in tobacco and *Arabidopsis* resulted in a marked decrease in the plant ability to recover from water stress, suggesting that aquaporin functions, and possibly their adjustment, were of critical importance during this process (Martre *et al.* 2002, Siefritz *et al.* 2002). On the other hand, tobacco plants overexpressing *PIP1b* and rice plants overexpressing *HvPIP2;1* became hypersensitive to drought and salt stress (Aharon *et al.* 2003, Katsuhara *et al.* 2003). Overexpression of *HvPIP2;1* in rice negatively affected root and shoot growth under salt stress. In contrast, Lian *et al.* (2004) identified a rice PIP aquaporin gene (*RWC3*) whose expression is induced by osmotic challenge, specifically in a drought-tolerant rice cultivar. Over-expression of this gene in a drought-sensitive rice cultivar, under the control of a stress-inducible promoter, was able to improve the growth performance of this cultivar, specifically under stress. Similarly, Yu *et al.* (2005) found that transgenic tobacco overexpressing *BnPIP1* showed enhanced drought tolerance. In the current scenario, Jang *et al.* (2007) examined thoroughly

the effects of drought stress on the transgenic *Arabidopsis* and tobacco plants that constitutively over-expressed the *AtPIP1;4* or *AtPIP2;5*. Under favorable growth conditions, no significant differences in the growth rates and water transport were found in transgenic plants compared to wild-type plants. However, under drought stress, the transgenic plants over-expressing *PIP1;4* or *PIP2;5* showed retarded germination and growth as compared with wild-type plants. Recently, over-expression of the *Panax ginseng* tonoplast aquaporin, *PgTIP1*, in the *Arabidopsis* plants showed vigorous plant growth under slight drought stress or favorable growing conditions (Peng *et al.* 2007).

It is clear from the above discussion that currently, two opposite views exist concerning the aquaporin performance in transgenic plants under water stress. One view is that increased aquaporin levels might provide the plant with additional ability to cope with drought stress (Smart *et al.* 2001, Lian *et al.* 2004). The second opinion is that plants avoid excessive loss of water by down-regulating aquaporin during dehydration (Aharon *et al.* 2003, Hachez *et al.* 2006, Peng *et al.* 2007). Further work is clearly required to determine the exact functions of aquaporins and the mechanisms by which their overexpression enhances the drought tolerance of transgenic plants.

Recently, two studies clearly demonstrated the effective involvement of aquaporins in conferring stress tolerance in plants. Sade *et al.* (2009) used a novel approach for the selection of candidate aquaporin for investigating its role in drought stress tolerance. The authors used an effective computational program for selection of candidate tomato aquaporin out of the family of 37 aquaporin genes based on drought stress tolerance in terms of growth and other yield related parameters. Similarly, they preferred TIP in contrast to previous studies in which PIP were mainly selected as candidates for the improvement of plant abiotic stress tolerance. They clearly demonstrated that plant overexpressing *SITIP2;2* adjusted whole plant transpiration regulation and relative water content under different abiotic stresses (Sade *et al.* 2009). Transgenic plants also exhibited increased transpiration under normal growth conditions, limited reduction of transpiration under drought and salt stresses and speed up the revival of transpiration upon recovery from these stresses. In conclusion, overexpression of the *SITIP2;2* extends the ability of the plant to maintain relatively normal physiological functions and also growth and yield even under severe stress conditions.

Similarly, Sade *et al.* (2010) suggested that tobacco stress-induced aquaporin gene (*NtAQPI*) is involved in improving the tolerance of stress plants due to increased water use efficiency (WUE). Overexpression of *NtAQPI* resulted in an increase of net photosynthetic rate, mesophyll CO<sub>2</sub> conductance and stomatal conductance which collectively contributed towards enhanced stress tolerance in transgenic *Arabidopsis* and tomato plants under stress. This is also in agreement with previous

reports which also suggested a role for *NtAQPI* in photosynthesis and demonstrated that transgenic tobacco plants overexpressing *NtAQPI* showed 20 % increase in photosynthetic rate relative to controls while its antisense repression resulted in 13 % decrease of it (Flexas *et al.* 2006).

Thus, the overexpression of different individual aquaporin genes might have opposite outcomes with respect to the response of the whole plant to abiotic

stresses, emphasizing the importance of accurately selecting the right candidate aquaporin genes from this large and functionally variable family to improve plant response to various stresses. These results lead us to propose that there is a dire need to completely understand the roles and involvement of the aquaporins in the plant response to drought stress to bridge the gap in the existing knowledge base.

Table 1. Transgenic plants engineered for enhanced tolerance to drought stress.

Gene	Species	Phenotype	Reference
<i>SiTIP2;2</i>	tomato	increase in osmotic water permeability and transpiration	Sade <i>et al.</i> 2009
<i>CfPIP2;1</i>	<i>Arabidopsis</i>	better plant growth under dehydration stress	Jang <i>et al.</i> 2007
<i>PgTIP1</i>	<i>Arabidopsis</i>	root dependent, drought and salt tolerance	Peng <i>et al.</i> 2007
<i>PIP</i>	soybean/lettuce	water conservation and drought tolerance	Porcel <i>et al.</i> 2006
<i>RWC3</i>	rice	maintenance of leaf water potential and transpiration under PEG stress	Lian <i>et al.</i> , 2004
<i>PIP1b</i>	tobacco	no increase in plant drought and salt stress tolerance	Aharon <i>et al.</i> 2003
<i>PIP2;2</i>	<i>Arabidopsis</i>	increased root water uptake	Javot <i>et al.</i> 2003
<i>NtAQPI</i>	tobacco	high root hydraulic conductance and drought tolerance	Siefritz <i>et al.</i> 2002
<i>LEA 4</i>	tobacco	drought tolerance <i>via</i> stabilizing membrane and protein	Liu <i>et al.</i> 2009
<i>OsLEA3</i>	rice	dehydration and salt stress tolerance	Hu <i>et al.</i> 2008
<i>CaLEA6</i>	tobacco	protection of photosynthetic activity under drought	Jun <i>et al.</i> 2008
<i>HVA1</i>	mulberry	drought and salinity tolerance	Lal <i>et al.</i> 2008
Dehydrin	<i>Arabidopsis</i>	enhanced osmotic and salt stress tolerance	Brini <i>et al.</i> 2007
<i>HVA1</i>	creeping bentgrass	maintenance of high water contents in leaves under water stress	Fu <i>et al.</i> 2007
<i>OsLEA3-1</i>	rice	drought resistance for yield in the field	Xiao <i>et al.</i> 2007
<i>DQ663481</i>	tobacco	drought resistance <i>via</i> cell membrane stability	Wang <i>et al.</i> 2006
<i>HVA1</i>	wheat	improved plant water status and yield under field drought	Bahieldin <i>et al.</i> 2005
<i>ME-leaN4</i>	lettuce	enhanced growth and delayed wilting under drought	Park <i>et al.</i> 2005a
<i>ME-leaN4</i>	Chinese cabbage	drought and salt resistance	Park <i>et al.</i> 2005b
<i>HVA1</i>	rice	dehydration avoidance and cell membrane stability	Babu <i>et al.</i> 2004
Rab 17	<i>Arabidopsis</i>	enhanced osmotic stress tolerance	Figueras <i>et al.</i> 2004
<i>CuCOR19</i>	tobacco	no effect on drought tolerance	Hara <i>et al.</i> 2003
<i>CaLEA</i>	Chinese cabbage	dehydration stress tolerance	Park <i>et al.</i> 2003
<i>PF00477</i>	rice	enhanced dehydration tolerance	Cheng <i>et al.</i> 2002
<i>HVA1</i>	oat	delayed wilting under drought stress	Maqbool <i>et al.</i> 2002
<i>LEA3-L2</i>	wheat	increased content of protein of unknown function	Ndong <i>et al.</i> 2002
<i>HVA1</i>	rice	drought and salinity tolerance	Rohila <i>et al.</i> 2002
<i>HVA1</i>	wheat	increased biomass and WUE under stress	Sivamani <i>et al.</i> 2000
<i>RcHSP17.8</i>	<i>Arabidopsis</i>	increased drought, salt, heat and osmotic stress tolerance	Jiang <i>et al.</i> 2009
<i>sHSP17.7</i>	rice	enhanced drought tolerance	Sato and Yokoya 2008
<i>NtHSP70-1</i>	tobacco	drought tolerance due to regulated water flux	Cho and Hong 2006
<i>AtHSP17.6A</i>	<i>Arabidopsis</i>	increased tolerance to drought and salt stresses	Sun <i>et al.</i> 2001

## Conclusions

One purpose of studying abiotic stress responses in plants is to improve the abiotic stress tolerance of crops by means of genetic manipulation. Current state of knowledge has been improved by recent discoveries in engineering drought tolerance in plants. However, in naturally stress-tolerant plants, there is a wide variety of adaptations to stress, many of which have not yet been identified at the molecular level. Understanding the function of genes determining these properties will improve our understanding of the complexity of plant metabolism and may provide unique opportunities for the

metabolic engineering. Commercially significant improving of crop performance under drought conditions has been challenging because of the complexity of the trait and the multitude of factors that influence yield. However, several crucial factors related to the plant response to drought stress have been identified and many of these factors have already been shown to be effective for engineering drought tolerance in model plants. The fact that many *LEA* genes are simultaneously induced during stress indicates that *LEA*-type proteins co-operate during abiotic stresses. Because of this synergistic effect,

ectopic expression of a single LEA-type protein is not always sufficient to confer plant tolerance, but *Arabidopsis* plants transformed with multiple LEA-type genes showed increased survival under freezing stress (Puhakainen *et al.* 2004). Such success under experimental conditions has encouraged the use of this approach to engineer drought tolerance in crop plants. To endow a plant with multiple stress resistance, co-transformation of multifunctional genes is possible. However, improvement of crops using the above strategies will require further research. Based on success, it is anticipated that transgenic plants conferring drought tolerance will eventually be released for field evaluation soon.

However, an understanding of the mechanistic basis for changes in plant gene expression in response to environmental cues is beginning to emerge. Similarly,

high throughput sequencing programs supported by genome-wide transcript profiling has a great potential in isolating differentially expressed and functionally important stress regulated genes. In a nutshell, in future, promises ushered by genomics, transcriptomics (Yoshida *et al.* 2008; Weston *et al.* 2008), proteomics (Bartels and Hussain 2008) or metabolomics (Rizhsky *et al.* 2004, Seki *et al.* 2007) could generate valuable information for engineering plants for their ultimate use in sustainable agriculture. Systems biology approaches relying on the integration of such “omics” based data will most certainly help to better understand the response of plants to abiotic stresses. However, a major challenge for the future will be to implement all the various data to engineer well-adapted plants that produce the required high amount of biomass under both stress and non-stressed conditions.

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