

# Applications of Genetic Engineering in Addressing Adverse Environmental Conditions for Agricultural Productions

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## Summary

Abiotic stresses (i.e., drought, salinity, frost, heat, etc.) are the major causes of crop loss worldwide. Plant adaptation to these adverse conditions is dependent upon the activation of a number of genetic/molecular events involved in stress perception, signal transduction, and the expression of specific stress-related genes and metabolites. Identification of stress-related genes that are either up- or down-regulated, either in the originator or suspect candidate recipient organism, might provide a specific focus for the relative necessity for plant transformation. Transgenic technology can help search for the cellular mechanisms that underlie tolerance, but the complexity of the trait often indicates that engineering abiotic stress tolerance into non-tolerant species is difficult, but not impossible. In general, genetic transformation with abiotic stress-related genes might represent the sole avenue to overcome the adverse effects on economically important crops like wheat, rice, maize, tomato, etc..

**Keywords:** Genetic engineering; Environmental conditions; Abiotic stress; Gene expression.

## Background

Abiotic stresses, such as drought, salinity, high temperatures, frost, chemical toxicity and oxidative stresses are serious threats to agriculture and result in the deterioration of the environment. They are the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Boyer, 1982 and Bray *et al.*, 2000). Half of the land surface on the earth is a perennial desert or drylands (UNDP, <http://www.undp.org/seed/unso/pub-htm/dryland-population.pdf>). A strong link with soil salinization is becoming widespread in many regions in the world (Ghassemi *et al.*, 1995). It was speculated that drought and salinity may cause serious salinization of more than 50% of all arable lands by the year 2050. Besides, world population is growing at an annual rate of about 1.5%, and expected to be over 10 billion by 2050. Given the amount by which food production ought to be increased, it can be assumed that changing abiotic stress tolerance of crops will be an important aspect of plant breeding programs in the near future. Therefore, much attention is being directed towards biotechnological approaches for increasing the productivity of food crops under such adverse conditions.

These approaches have been started by producing stress tolerant plants using classical genetic as well as improved plant breeding methods. However, the strategy of gene transfer to crop plants from their wild relatives using classical genetic methods has been of limited success (Flowers and Yeo, 1995). The genetically complex responses to abiotic stresses are multigenic, required modification of a large number of parameters and thus more difficult to control. On the other hand, genetic engineering provides flexibility in manipulation of crops by altering

the expression levels of indigenous genes (gene regulation) or by incorporating alien genes (gene addition) for a given desired trait, in a relatively short time frame (Sahi *et al.*, 2006).

Most of the recent molecular studies on plant stress tolerance have used *Arabidopsis* (in addition to a limited number of crop plants), a typical glycophyte that is not adapted to salt or drought stress. A substantial number of halophytes (adapted to salt), as well as xerophytes (adapted to drought) or desiccation-tolerant plants, can use mechanisms of stress tolerance that are similar to those found in glycophytes. Regular drought-tolerant plants can withstand moderate dehydration conditions of about 30% water loss. By contrast, desiccation-tolerant plants (generally referred to as resurrection plants) are tolerant to further cell dehydration, around 90% water loss, and also have the ability to rehydrate successfully. Resurrection plants have been widely used as model plants for dehydration studies (Bartels and Sunkar, 2005). Several studies suggest that desiccation tolerance in the vegetative tissues of *C. plantagineum* is unlikely to result from the presence of genes that are unique to resurrection plants, as the relevant genes are also present in the genome of non-tolerant plants (e.g., LEA proteins, Hilbricht *et al.*, 2002). Thus, the difference between desiccation-tolerant and non-tolerant plants is likely to reside in the expression patterns and rates of the genes and is, in part, a quantitative characteristic (Deng *et al.*, 2002).

Further complications arise from the large variability in stress sensitivity at different periods during the life cycle of a given plant. Of the various general types of plant response to salinity or drought stress, avoidance mechanisms mainly result from morphological and physiological changes at the whole-plant level. These are less amenable to practical manipulations. By contrast, tolerance mechanisms are caused by cellular and molecular biochemical modifications that lend themselves to biotechnological manipulation.

There are many strategies to identify abiotic stress-related genes and proteins suitable for manipulation (Vij and Tyagi, 2007). These strategies include:

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- Map-based cloning loci and Quantitative Trait Loci (QTLs).
- Transgenics for ectopic expression of abiotic stress response genes.
- Gene expression patterns by microarray analysis, and
- Proteomics to identify targets beyond the gene to be manipulated

Using the information available from different strategies, it will be possible to understand the abiotic stress matrix and manipulation.

The primary objective of this review is to report recent advances in the stress-response mechanisms and their biotechnological applications in agriculture. Emphasis is given to transgenic plants that were engineered for stress tolerance, based on different straight-forward mechanisms of stress response.

### Abiotic Stress Cross-Talk

All types of abiotic stress evoke cascades of physiological and molecular events, some of which can result in similar responses (Fig. 1). For example, drought, salinity and freezing can all be manifested at the cellular level as physiological dehydration. In other words, exposure of a plant to different abiotic stress conditions will result in the co-activation of certain stress-response pathways. This involves a series of physiological, biochemical and molecular events that might have a synergistic or antagonistic effect on one another (Mittler, 2006). Drought or salt stress leads to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm into the extracellular space resulting in a reduction of the cytosolic and vacuolar volumes. Early responses to water stress are largely identical except for the ionic component. Cross-talk between co-

activated pathways is likely to be mediated at different levels; different stress hormones, transcription factors and Mitogen-Activated Protein Kinase (MAPK) cascades (Xiong and Yang, 2003), Reactive Oxygen Species (ROS) signaling (Bowler & Fluhr, 2000 and Mittler *et al.*, 2004), different receptors and signaling complexes (Casal, 2002). This cross-talk leads to the induction of certain pathway (ex., Hsps/chaperons network) as regulatory molecules, participate in stress sensing, signal transduction and transcription activation of stress genes (Wang *et al.*, 2004). We are still far beyond the full understanding as to how this cross-talk network participates in stress response. But the availability of genome sequences and the new molecular-omic tools, will open up new avenues in understanding stress biology.

### Plant Genetic Engineering Strategies

Plant genetic engineering strategies for abiotic stress tolerance rely on the expression of genes that are involved in one of the following approaches:

- Signaling and regulatory pathways (Seki *et al.*, 2003 and Shinozaki *et al.*, 2003).
- Genes that encode proteins conferring stress tolerance (Wang *et al.*, 2004)
- Enzymes present in pathways leading to the synthesis of functional (Park *et al.*, 2004) and structural metabolites (Rontein *et al.*, 2002).

Current efforts to improve plant stress tolerance by genetic transformation have resulted in several important achievements. However, the genetically complex mechanisms of abiotic stress tolerance make the task extremely difficult. For this reason and resolve this task, biotechnology should be fully integrated with classical physiology and breeding (Wang *et al.*, 2003 and Flowers, 2004). Some of the developed transgenic plants with abiotic stress-related genes that were produced, so far, are summarized in Table (1).

The following are major mechanisms and aspects taken into consideration to produce transgenics with abiotic stress tolerance.

#### a. Regulatory control or transcription factors:

To activate or repress transcription of a given gene, transcription factor(s) located in the nucleus, bind DNA, and interact with the basal transcription apparatus. Therefore, environmental signals that regulate transcription factor activity may affect one or a combination of processes. Regulation of a transcription factor is achieved by either reversible phosphorylation or by *de novo* synthesis of transcription factors (Bartels and Sunkar, 2005). Transcription factors could regulate the production of special proteins such as chaperons (heat shock proteins) under high temperature and CBFs (C-repeat binding proteins) under dehydration stress (Cherian *et al.*, 2006). The latter include leucine zipper (bZIP) proteins, Zn-finger pro-

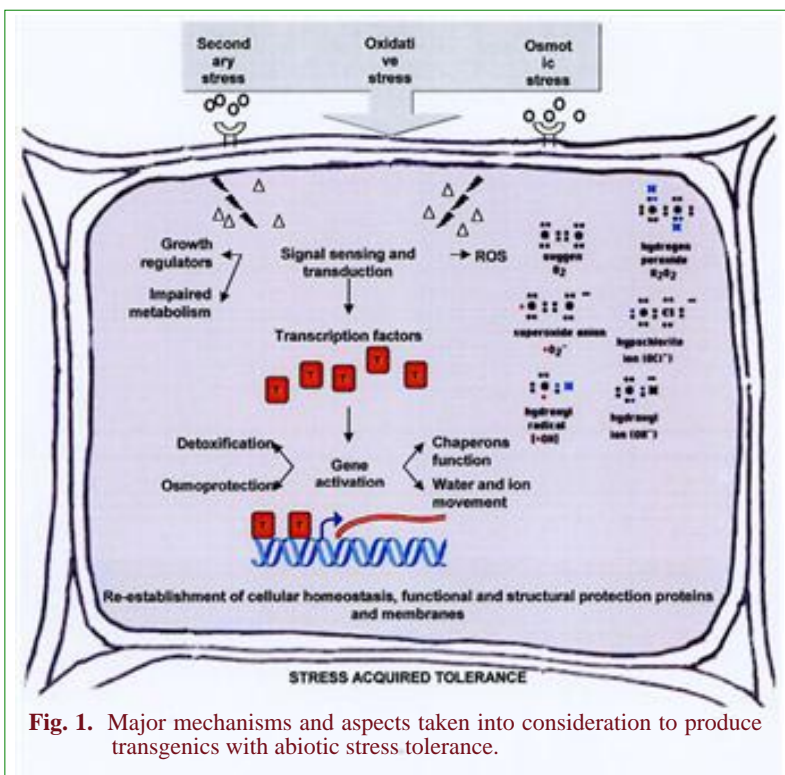


Fig. 1. Major mechanisms and aspects taken into consideration to produce transgenics with abiotic stress tolerance.

**Table 1.** List of some recently produced transgenic plants with abiotic stress-related genes

Mechanism of tolerance	Transgenic	References
<b>Regulatory control</b>		
<i>DREB1A</i>	<i>Arabidopsis</i> Tobacco Wheat	Kasuga <i>et al.</i> , 1999; Liu <i>et al.</i> , 1998 Kasuga <i>et al.</i> , 2004 Pellegrineschi <i>et al.</i> , 2004
<i>AtMYC2</i>	<i>Arabidopsis</i>	Abe <i>et al.</i> , 2003
<i>OsDREB1A</i>	<i>Arabidopsis</i>	Dobouzet <i>et al.</i> , 2003
<i>Alfin1</i>	Alfalfa	Winicov, 2000
<i>Tsi1</i>	Tobacco	Park <i>et al.</i> , 2001
<i>CBF1</i>	Tomato	Hsieh <i>et al.</i> , 2002
<i>CBF4</i>	<i>Arabidopsis</i>	Haake <i>et al.</i> , 2002
<i>ABF3</i>	<i>Arabidopsis</i>	Kang <i>et al.</i> , 2002
<i>CpMYB10</i>	<i>Arabidopsis</i>	Villalobos <i>et al.</i> , 2004
<b>Ion transporter</b>		
<i>AtNHX1</i>	<i>Arabidopsis</i> Tomato <i>Brassica</i> Wheat	Apse <i>et al.</i> , 1999 Zhang and Blumwald, 2001 Zhang <i>et al.</i> , 2001 Xue <i>et al.</i> , 2004
<i>SOS1</i>	<i>Arabidopsis</i>	Shi <i>et al.</i> , 2003
<i>AVP1</i>	<i>Arabidopsis</i>	Gaxiola <i>et al.</i> , 2001
<i>Avp1/SsNHX1</i>	Rice	Zhao <i>et al.</i> , 2006
<i>HAL1</i>	Tomato Melon <i>Arabidopsis</i>	Rus <i>et al.</i> , 2001 Bordas <i>et al.</i> , 1997 Yang <i>et al.</i> , 2001
<i>HAL2</i>	Citru	Cervera <i>et al.</i> , 2000
<b>Protective proteins</b>		
<i>AtHSP17.7</i>	<i>Arabidopsis</i>	Sun <i>et al.</i> , 2001
<i>HVA1</i>	Rice Wheat	Xu <i>et al.</i> , 1996 Sivamani <i>et al.</i> , 2000; Bahieldin <i>et al.</i> , 2005
<b>Osmoprotectants</b>		
<i>betaA</i>	Tobacco	Lilius <i>et al.</i> , 1996
<i>BADH</i>	Tobacco	Liang <i>et al.</i> , 1997; Holmstrom <i>et al.</i> , 2000
<i>CodA</i>	<i>Arabidopsis</i> Rice <i>Brassica juncea</i> Carrot	Hayashi <i>et al.</i> , 1997 Sakamoto <i>et al.</i> , 1998 Prasad <i>et al.</i> , 2000 Kumar <i>et al.</i> , 2004
<i>COX</i>	<i>Arabidopsis</i>	Huang <i>et al.</i> , 2000
<i>TUR1</i>	<i>Arabidopsis</i>	Smart and Flores, 1997
<i>IMT1</i>	Tobacco	Sheveleva <i>et al.</i> , 1997
<i>P5CS</i>	Tobacco Rice Wheat	Kishor <i>et al.</i> , 1995 Zhu <i>et al.</i> , 1998 Sawahel and Hassan, 2002
<i>ProDH</i>	<i>Arabidopsis</i>	Nanjo <i>et al.</i> , 1999
<i>GS</i>	Rice	Hoshida <i>et al.</i> , 2000
<i>adc</i> and <i>samdc</i>	Rice	Capell <i>et al.</i> , 2004
<b>Sugars and sugar alcohols</b>		
<i>sacB</i>	Sugar beet Wheat	Pilon-Smits <i>et al.</i> , 1999 Bahieldin <i>et al.</i> , 2005
<i>mtlD</i>	<i>Arabidopsis</i> Tobacco chloroplasts Wheat	Tarczynski <i>et al.</i> , 1993 Shen <i>et al.</i> , 1997 Abebe <i>et al.</i> , 2003
<i>TPS1</i>	Tobacco Potato	Romero <i>et al.</i> , 1997 Yeo <i>et al.</i> , 2000
<b>Reactive oxygen species scavengers</b>		
<i>Fe SOD</i>	Tobacco chloroplasts	Van Camp <i>et al.</i> , 1996
<i>Mn SOD</i>	Rice	Tanaka <i>et al.</i> , 1999
<i>gtX</i>	Tobacco	Yoshimura <i>et al.</i> , 2004
<i>AtALDH3</i>	<i>Arabidopsis</i>	Sunkar <i>et al.</i> , 2003

teins, AP2/ERF-Type and Myb-like proteins.

The results of genetic transformation by ABF3 and ABF4 overexpression in *Arabidopsis* indicated an enhanced drought tolerance accompanied by decreased transpiration, where both ABF3 and ABF4 are in-

involved in stomatal closure mediated by ABA accumulation (Kang *et al.*, 2002). Overexpression of the *Alfin1* gene in transgenic alfalfa resulted in enhanced NaCl tolerance, where the *Alfin1* gene product regulates *MsPRP2* (NaCl-responsive gene) expression *in vivo* (Winicov and Bastola, 1999). Overexpression of *OsDREB1A* in transgenic *Arabidopsis* improved stress tolerance (Dubouzet *et al.*, 2003). The *Tsi1* (tobacco stress-inducible gene 1), belonging to the ERF subfamily (Sakuma *et al.*, 2002) and induced by NaCl, salicylic acid, and ethylene, showed an enhanced resistance to osmotic stress and pathogen attack (Tsugane *et al.*, 1999). Ectopic expression of *CpMYB10* whose expression is upregulated by dehydration and ABA in transgenic *Arabidopsis* plants resulted in drought and salinity tolerance (Villalobos *et al.*, 2004).

Interestingly, genetic engineering *via* transcription factors was more efficient when using stress-inducible promoters rather than the constitutive promoters commonly used in genetic transformation (Pellegrineschi *et al.*, 2004).

**b. Ion transporters:** Regulation of ion transport is one of the important factors for salt tolerance of plants. Many physiological studies have demonstrated that Na<sup>+</sup>-toxicity is not only due to toxic effects of Na<sup>+</sup> in the cytosol, but also because K<sup>+</sup>-homeostasis is disrupted possibly due to the ability of Na<sup>+</sup>-competing for K<sup>+</sup>-binding sites. Enhancing plant tolerance to abiotic stress conditions by overexpression of ion transporters resulted in a novel way of sequestering excess Na<sup>+</sup> levels to cause increased salt-tolerant phenotype.

Protons are used as coupling ions for ion transport systems, and the proton gradient, generated by proton pumps in the membrane systems, is the driving force for Na<sup>+</sup> transport across membranes. An antiporter cloned from *Arabidopsis*, i.e., *AtNHX1* (coding for a vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter), when overexpressed in *Arabidopsis*, tomato and *Brassica*, increased levels of salt tolerance (Apse *et al.*, 1999; Zhang & Blumwald, 2001 and Zhang *et al.*, 2001). When *AtNHX1* was overexpressed in wheat, the grain yield of a transgenic line was reduced by about 50% under salt stress, compared with 65% in the non-transformed control plants (Xue *et al.*, 2004). Overexpression of

the plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter *SOS1* was found to increase stress tolerance in *Arabidopsis* (Shi *et al.*, 2003). Zhang and Blumwald (2001) indicated that transgenic tomato plants overexpressing *AtNHX1* produced similar yield when grown at 200 mM NaCl

to the control non-transgenic plants grown under normal condition.

The activity of Na<sup>+</sup>/H<sup>+</sup> antiporters could be limited by their number or by the H<sup>+</sup> differences across the membranes. In such a case, increasing the capacity of a proton pump would increase the salt tolerance of the plant. The plasma membrane H<sup>+</sup>-ATPase (P-H<sup>+</sup>-ATPases) and vacuolar ATPases (V-ATPases) coupled with vacuolar H<sup>+</sup>-PPase (*pyrophosphatase*) (V-H<sup>+</sup>-PPases) act at the plasma membrane and tonoplast, respectively. Overexpression of *Avp1* (vacuolar H<sup>+</sup>-PPase, pyrophosphatase), which is important to energize the vacuole membrane, leads to enhanced salt and drought tolerance in transgenic plants (Gaxiola *et al.*, 2001). The *HAL1* gene from yeast controls K<sup>+</sup>/Na<sup>+</sup> selectivity and salt tolerance of yeast cells. Expression in tomato increased fruit yield and enhanced K/Na<sup>+</sup> selectivity in leaves (Rus *et al.*, 2001). This suggests that pumping of H<sup>+</sup> across the vacuolar membrane is an additional driving force for vacuolar sodium accumulation. Therefore, salt adaptation is correlated with V-ATPase in many plants.

**c. Protective proteins:** Heat-shock proteins (Hsps) and molecular chaperones, as well as Late Embryogenesis Abundant (LEA) protein families, are involved in plant abiotic stress tolerance (Wang *et al.*, 2003 and Wang *et al.*, 2004). Hsps and LEA proteins help in protecting against stress by controlling the proper folding and conformation of both structural (i.e., cell membrane) and functional (e.g., enzymes) proteins. Positive correlations between the levels of several Hsps and stress tolerance have been described (Sun *et al.*, 2002; Sung & Guy, 2003 and Wang *et al.*, 2004). The protective effects of Hsps/chaperones can be attributed to the network of the chaperone machinery, in which many of them act in concert.

During stress, many enzymes and structural proteins undergo deleterious structural and functional changes. Therefore, maintaining proteins in their functional conformations, preventing aggregation of non-native proteins, refolding of denatured proteins to regain their functional conformation and removal of non-functional but potentially harmful polypeptides (arising from misfolding, denaturation or aggregation) are particularly important for cell survival under stress (Wang *et al.*, 2004). Thus, the different classes of Hsps/chaperones cooperate in cellular protection and play complementary and sometimes overlapping roles in the protection of proteins from stress.

Overexpression of LEA proteins was correlated in several cases of genetic transformation with desiccation tolerance. Although the precise mechanism of these proteins is still unknown (Villalobos *et al.*, 2004), it may function as chaperone-like protective molecules to combat cellular damage (Umezawa *et al.*, 2006). The *HVA1* gene encoding a LEA protein is naturally accumulated during seed desiccation (Hong

*et al.*, 1988) and found to be induced by ABA and several stresses including salt (Hong *et al.*, 1992). Transgenic rice plants expressing the barley *HVA1*, driven by the constitutive promoter from the rice *actin1* gene, showed significant increased tolerance to salt stress (Xu *et al.*, 1996). The transgenic plants showed greater shoot height and root fresh weight than wild-type plants. The *HVA1* gene was also introduced into wheat, and several transgenic lines showed improved productivity and Water Use Efficiency (WUE) in the glasshouse (Sivamani *et al.*, 2000). The homozygous T4 plants of these transgenics were tested in the field under drought as well as under rainfed conditions over six consecutive seasons (Bahieldin *et al.*, 2005) and plants showed improved drought tolerance in most environments, indicating that this approach has the potential to improve wheat crop performance under drought conditions.

**d. Osmoprotectants:** A wide range of metabolites that can prevent the detrimental changes due to abiotic stress have been identified, including amino acids (e.g., proline), quaternary and other amines (e.g., glycinebetaine and polyamines) and a variety of sugars and sugar alcohols (e.g., fructan, mannitol and trehalose). Two general strategies for the metabolic engineering of abiotic stress tolerance have been proposed: increased production of specific desired compounds and reduction in the levels of unwanted toxic compounds (Capell and Christou, 2004).

#### 1. Amines:

**Proline:** proline is the most common osmoprotectant that accumulates in plants in response to water stress (Hanson & Hitz, 1982 and Yoshida *et al.*, 1995). It is synthesized either from glutamate (Glu) or from ornithine (Delauney *et al.*, 1993). Genetic engineering of proline biosynthesis pathway, by either increasing its synthesis (P5CS) or avoiding its degradation (proline dehydrogenase, PDH), led to increased osmotolerance in transgenic plants. Tobacco plants overexpressing proline resulted in an enhanced biomass production under salt stress (Kishor *et al.*, 1995). Overproduction of proline in rice increased its biomass under water stress (Zhu *et al.*, 1998). Transgenic wheat plants with high content of proline increased tolerance to salt stress were reported (Sawahel and Hassan, 2002). Antisense suppression of proline degradation also improved salt tolerance (Nanjo *et al.*, 1999).

**Glycinebetaine:** Glycinebetaine is a quaternary ammonium compound that occurs naturally in a wide variety of plants, animals and microorganisms (Jagendorf and Takabe, 2001). It accumulates in cells of a number of halophytes and bacteria as an adaptive response to high salt (Borsani *et al.*, 2003). At high concentrations, Glycinebetaine does not interfere with cytoplasmic functions and stabilizes the structure and function of many macromolecules. A major role of Glycinebetaine might be to protect membranes and macromolecules from the damaging effects of stress. Murata *et al.* (1992) determined that Glycinebetaine protects photosystem

II by stabilizing the association of PS II core complex extrinsic proteins during salt stress. Transgenic tobacco plants harboring the *E. coli betA* gene, encoding a coline dehydrogenase, were more tolerant to salt conditions than wild type (Lilius *et al.*, 1996). Transformation of *A. thaliana* with *codA* (encoding choline oxidase) enabled the plant to accumulate glycinebetaine and enhanced its tolerance to salt stress (Hayashi *et al.*, 1997). Seeds of the transgenic plants were able to germinate in 300 mM NaCl, whereas seeds of wild type could not germinate at all. Transgenic rice plants carrying the *codA* gene, with the encoded protein directed to the chloroplast, were more tolerant than the transgenic plants with the protein localized in the cytosol (Sakamoto *et al.*, 1998). This result suggested that the protective function of glycinebetaine is more efficient when present in a photosynthetic organelle (Sakamoto *et al.*, 1998). The successful integration of *codA* in *Brassica juncea* enhanced tolerance to salt stress (Prasad *et al.*, 2000). Another step in the glycinebetaine synthesis is catalyzed by the enzyme betaine dehydrogenase (BADH). Transgenic tobacco plants expressing the *BADH* gene accumulated a higher amount of glycinebetaine in cytosol and chloroplasts and exhibited increased tolerance to salt stress (Holmstrom *et al.*, 2000). A recent study showed that transgenic carrot plants expressing BADH can grow in the presence of high concentrations of NaCl (up to 400 mM); the highest level of salt tolerance reported, so far, among genetically modified crop plants (Kumar *et al.*, 2004).

**Polyamines:** polyamines are a group of nitrogenous cellular compounds that accumulate under a variety of abiotic stress conditions including salt and drought (Krishnamurthy and Bhagwat, 1989). Genetic manipulation of polyamine metabolism has become a valuable tool for studying their physiological roles in plants. Plant polyamine content has been modulated by the overexpression/down-regulation of arginine decarboxylase (*adc*), ornithine decarboxylase (*odc*), and S-adenosylmethionine decarboxylase (*samdc*) (Capell *et al.*, 1998; Roy & Wu, 2001; Kakkar & Sawhney, 2002; Thu-Hang *et al.*, 2002 and Capell *et al.*, 2004). Overexpression of spermidine synthase in *Arabidopsis* enhanced tolerance to multiple stresses. When compared with wild type plants, the T2 and T3 transgenic *Arabidopsis* plants exhibited a significant increase in spermidine synthase activity and spermidine content in leaves together with enhanced tolerance to various stresses such as chilling, freezing, salinity, hyperosmosis, drought, and paraquat toxicity (Kasukabe *et al.*, 2004). Besides, the overexpression of *adc* and *samdc* genes increased polyamine content in transgenic rice and conferred tolerance to drought stress (Capell *et al.*, 2004).

## 2. Sugars and sugar alcohols:

Sugar and sugar alcohols such as mannitol, fructans and sorbitol occur in as many as 50 families of higher plants. Although their physiological functions are not clear, (Tarczynski *et al.*, 1992), accumulation of these compounds often occurs as a result of water stress. There are many reports on using sugar alcohols for stress tolerance *via* genetic transformation by modifying the responsible enzymes in their bi-

osynthesis pathway.

**Fructans:** Fructans are polyfructose molecules (polyfructosylsucroses) that are less frequently available in plant cells than bacteria as the main storage carbohydrate (Hendry, 1993). Fructan-producing cells respond to changing environmental conditions by shifting the size of their fructan pool (Pontis and del Campillo, 1985). In contrast to starch, fructans are soluble, thus, play a role in osmotic adjustment to changing environmental conditions *via* variation in the Degree of Polymerization (DP) of the fructan pool (Pilon-Smits *et al.*, 1999). The effects of fructans on the abiotic stress tolerance was assessed by transforming different plant species with a construct containing the *sacB* gene for levansucrase from *Bacillus subtilis* fused to the vacuole-sorting signal of carboxypeptidase Y from yeast and placed downstream of the double constitutive 35S promoter (Pilon-Smits *et al.*, 1995). When sugar beet plants were transformed using this construct, transgenic plants accumulated fructans up to 0.5% of root and shoot dry mass and showed significantly better growth under drought stress than wild type plants (Pilon-Smits *et al.*, 1999). In wheat, the root dry weight of the transgenic plants was higher than that of the non-transgenic plants under either normal or drought stress condition (Bahieldin *et al.*, 2003). This indicates that fructan accumulation might stimulate root growth and, hence, promote water uptake under normal as well as drought condition. Fructans might also promote the process of root branching, and thus increasing root surface and water uptake (Datta, 2002).

**Mannitol:** Mannitol is a sugar alcohol that accumulates upon salt and water stress and can thus alleviate abiotic stress. It enhances tolerance to water deficit through osmotic adjustment (Loescher *et al.*, 1992) and through scavenging of hydroxyl radicals (OH<sup>-</sup>) and stabilization of macromolecular structures (Karakas *et al.*, 1997 and Abebe *et al.*, 2003). Tobacco plants transformed with the *mtlD* gene from *E. coli*, which encodes a mannitol-1-phosphate dehydrogenase, accumulated mannitol in their cells and showed increased plant growth under salt stress (Tarczynski *et al.*, 1993). Shen *et al.* (1997) reported an increased resistance to oxidative stress in transgenic tobacco plants by targeting mannitol biosynthesis to chloroplasts. The ectopic expression of the *mtlD* gene for the biosynthesis of mannitol in transgenic wheat improves tolerance to water stress both at the callus and whole plant levels (Abebe *et al.*, 2003). The transgenic wheat plants showed an increased biomass, plant height and number of tillers.

**Trehalose:** Trehalose is a rare, non-reducing sugar, present in many bacteria and fungi and in some desiccation-tolerant higher plants (Vinocur and Altman, 2005). Increasing trehalose accumulation in crop plants could improve drought and salinity tolerance (Penna, 2003). Trehalose is thought to protect biomolecules from environmental stress, as suggested by its reversible water-absorption capacity to protect biological molecules from desiccation-induced damage. Tolerance to multiple abiotic stresses through engineering trehalose overexpression was reported in rice (Garg *et*

*al.*, 2002 and Jang *et al.*, 2003). The modest increase in trehalose levels in the transgenic plants resulted in a higher photosynthetic rate and in a decreased photo-oxidative damage during stress. Transgenic plants maintained optimal K<sup>+</sup>/Na<sup>+</sup> ratios necessary for cellular functions.

**e. Reactive Oxygen Species (ROS) scavengers:** A consequence of salt stress, mainly due to its hyperosmotic effect, is a major production of ROS (Ashraf and Harris, 2004). Also, cold stress or drought, combined with high light conditions, result in enhanced production of ROS by the photosynthetic apparatus because these conditions limit the availability of CO<sub>2</sub> for the dark reaction, leaving oxygen as one of the main reductive products of photosynthesis (Mittler, 2002). Plants have evolved both enzymatic and non-enzymatic ROS scavenging system to minimize oxidative damage (Mittler, 2002). Under these conditions, ROS such as superoxide radicals, hydroxyl radical and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) are produced in excess (Asada, 1999; Dionisio-Sese & Tobita, 1998 and Menezes-Benavente *et al.*, 2004). ROS are scavenged by superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT), which exist in different subcellular location (Breusegem *et al.*, 2001 and Foyer *et al.*, 1997).

Alleviation of oxidative damage by the use of different antioxidants and ROS scavengers was found to enhance plant resistance to salt and drought. Overexpression of the genes for Fe SOD and of Mn SOD isoforms in chloroplasts of tobacco and rice plants, respectively, were shown to confer tolerance to oxidative stress and salinity in transgenic plants compared with non-transformed controls (Van Camp *et al.*, 1996 and Tanaka *et al.*, 1999). Transgenic tobacco plants overexpressing *Chlamydomonas* glutathione peroxidase in the cytosol and in the chloroplast displayed increased tolerance to oxidative stress, (Yoshimura *et al.*, 2004). Overexpression of the aldehyde dehydrogenase *AtALDH3* gene in *Arabidopsis* conferred tolerance to drought and salt stress (Sunkar *et al.*, 2003).

### Concluding Remarks

Abiotic stresses, especially salinity, drought, high temperature and oxidative stress, are the primary causes of plant loss worldwide. When studying tolerance to abiotic stress, a number of factors should be taken into consideration. There are sufficient evidences that stress tolerance is a multigenic trait. Mechanisms related to stress tolerance should always be assessed with respect to its cross-talk with other stress-related genes/mechanisms. Current studies use short-term stress treatments, rather than observing the effects of stress over longer periods - conditions that more closely mimic the life span of most crops.

In conclusion, plant biotechnologies aimed at overcoming severe environmental stresses need to be quickly and fully implemented, with intensive molecular-assisted traditional breeding and genetic engineering being at the forefront.

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## تطبيقات الهندسة الوراثية لمواجهة الظروف البيئية غير الملائمة للإنتاج الزراعي

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### الخلاصة

تعتبر العوامل غير الحيوية مثل الجفاف والملوحة والصقيع والحرارة من الأسباب الرئيسية في حدوث فقد في الإنتاج المحصولي على مستوى العالم. ويعتمد تأقلم النباتات مع هذه الظروف على تفعيل عدد من العمليات الوراثية الجزيئية ومنها تعبير عدد من الجينات المرتبطة بالإجهاد البيئي المستولة عن استشعار الإجهاد ونقل الإشارات وكذلك عن التمثيل الحيوي للنبات. إن التعرف على الجينات المرتبطة بالإجهاد البيئي سواء تلك التي يحدث لها زيادة أو نقص في التعبير الجيني يعطي فكرة شاملة عن الاحتياجات الضرورية للنبات قبل حدوث عملية التحول الوراثي بطرق الهندسة الوراثية المختلفة. إن تقانة التحوير الوراثي ممكن أن تساعد في دراسة الأليات الجزيئية المستولة عن التحمل، ولكن تحوير النباتات غير المحتملة للإجهاد البيئي يبدو صعباً لكونها من الصفات المعقدة لكنها غير مستحيلة. وعموماً فإن تقانة التحوير الوراثي قد تمثل أحد الحلول الهامة للتغلب على فقد المحصول الناتج عن الإجهاد البيئي خاصة في المحاصيل الاقتصادية الهامة مثل القمح والذرة الصفراء والبطاطم.

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