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Review

DRE-binding Transcription factor (DREB1A) as a master regulator induced a broad range of abiotic stress tolerance in plant

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Environmental stresses, such as drought, high salinity, and low and high temperature, affect plant growth. Abiotic stresses cause losses worth hundreds of million dollars of agricultural industry each year and extremely decrease crop productivity. Many genes are induced in response to environmental stresses. The *DREB1A* gene is a stress-inducible DRE-binding transcription factor that strongly up-regulates many downstream genes, resulting in adaptation of plants to stress conditions and exercise specific tolerance mechanisms, thereby increase efficiency of plants production. In this review, we presented evidences which show that the *DREB1A* gene can increase tolerance to drought, salinity, heat and low temperature stresses. Given that the molecular control mechanisms of abiotic stress tolerance are similar and *DREB1A* may have effects on majority of them, it is also possible that over-expression of *DREB1A* leads to production of plants resistant against heavy metal toxicity and harmful rays or mechanical injuries.

Key words: Abiotic stress, *DREB1A* gene, tolerance plant.

INTRODUCTION

The world population is increasing with an alarming rate and is expected to reach about six billion at the end of the first-half of the 21st century. On the other hand, food productivity is decreasing due to the effect of various abiotic stresses; therefore, minimizing these losses is a major area of concern for all nations to cope with increasing food requirements (Mahajan and Tuteja, 2005). Abiotic stresses, including drought, high salinity, heat and freezing, disturb the water balance of the cell, leading to a series of morphological, physiological, biochemical and molecular changes that have adverse effect on plant growth and productivity, thereby causing increasingly great damages to crop productivity (Yamaguchi-Shinozaki et al., 2002). Drought and salinity also are becoming particularly widespread in many regions, and may cause serious salinization of more than 50% of all arable lands by the year 2050 (Wang et al., 2001a). As a consequence, appreciable effort has been made to determine the adaptive mechanisms plants evolved to survive this adverse environmental condition (Thomashow, 1999; Bartels, 2001; Rizhsky et al., 2004; Nakashima and Yamaguchi-Shinozaki, 2006; Agarwal et al., 2006), and indicated that the cellular and molecular responses of plants to stress are very complicated, including perceiving of environmental signals and transmitting the signals to cellular machinery. Moreover, it has been shown that expression of a variety of genes is induced by these stresses (Maruyama et al., 2004; Vogel et al., 2005).

Although tolerance or susceptibility to abiotic stresses is a very complex phenomenon, in part, because stress may occur at multiple stages of plant development and often more than one stress simultaneously affects the plant (Chinnusamy et al., 2004), a comprehensive and virtual method for enhancing tolerance consisting of the expression of a set of stress tolerance genes, could be achieved by introducing genes encoding stress-inducible transcription factors. Toward this end, many stress-inducible transcription factors responding to drought, low temperature and high salinity, such as *AREB*, *DREB*, *MYB* and *WRKY*, have been isolated (Mare et al., 2004; Xiong and Fei, 2006; Mukherjee et al., 2006; Agarwal et

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al., 2006). Among them, *DREB* is a well characterized transcription factor known to play an important role in regulating gene expression in response to abiotic stresses via abscisic acid (ABA)-independent manner (Sreenivasulu et al., 2007).

DRE*B*-type transcription factor genes have been found in various plants, such as *AtDREB* (*1A*, *1B*, *1C*, *2A*, *2B*) from Arabidopsis (Liu et al., 1998; Kohan et al, 2009), *OsDREB* (*1A*, *1B*, *1C*, *1D*, *2A*) from rice (Dubouzet et al., 2003), *AhDREB1* from *Atriplex hortensis* (Shen et al., 2003a) and *TaDREB1* from wheat (Shen et al., 2003b), *HvDREB1* from barley (Choi et al., 2002), *HsDREB1A* from wild barley (James et al., 2008), *ScDREB1* from rye, *BnDREB1* from *Brassica napus* (Jaglo et al., 2001), *AsDREB1* from oat (Brautigam et al., 2005), *ZmDREB1* from maize (Qin et al., 2004), *LpDREB1* from perennial ryegrass (Xiong and Fei, 2006), *GmDREB* (*a*, *b*, *c*, *2A*, *3*) from soybean (Li et al., 2005; Chen et al., 2007, 2009) and also from other plant species such as sweet cherry (Kitashiba et al., 2004), *Populus* spp. (Benedict et al., 2006), eucalyptus (El Kayal et al., 2006), grape (Xiao et al., 2006), citrus (Champ et al., 2007) and birch (Welling and Palva, 2008). It is apparent that the *DREB1/CBF* regulon is conserved and plays a key role in regulating stress responses of higher plants, and those *CBF*-like family genes may be useful for improving the stress tolerance of crops (Yamaguchi-Shinozaki and Shinozaki, 2006).

There are three *DREB1/CBF* proteins that are encoded by genes that lie in tandem on chromosome 4 in the order *DREB1B/CBF1*, *DREB1A/ CBF3* and *DREB1C/ CBF2*, and there are two *DREB2* proteins (*DREB2A* and *DREB2B)* in *Arabidopsis thaliana* (Liu et al., 1998; Gilmour et al., 1998). The *CBFs/DREB1s* belong to the AP2/EREBP family of transcription factors and bind to the cold- and dehydration-responsive DNA regulatory element designated C-repeat (CRT)/dehydration response element (DRE) that contain the conserved CCGAC core sequence (Yamaguchi-Shinozaki and Shinozaki, 1994).

Fowler and Thomashow (2002) were able to understand how abiotic stress regulates gene expression and reported 41 target genes of *CBFs/DREBs* using Affymetrix GeneChip arrays, and showed that most of these target genes contained the DRE or DRE-related core motifs in their promoter regions. Some products of these genes are among carbohydrate metabolism-related proteins (*SPS*, *SuSy*), prolin biosynthetic genes (*P5CS*), *COR* protein synthesis (Gilmour et al., 2000; Zhao et al., 2007); and transcription factors, phospholipase C, RNA binding protein, sugar transport protein, desaturase, late embryo abundant (*LEA*) proteins, *KIN* (cold inducible) proteins, molecular chaperones, osmoprotectant biosynthesis protein, protease inhibitors, and so on (Seki et al., 2001; Maruyama et al., 2004; Ito et al., 2006). Any one of these genes or related genes is individually transformed to plants and confers tolerance to one or more of the various abiotic stresses (Konstantinova et al., 2002; Ndong et al., 2002), but a simple reasoning says to us that transformation of a transcription factor, which regulates expression of all of these genes, can activate those pathways and increase the amount of their products. Consequently, tolerance plants with more productivity would be obtained under stress conditions.

Subsequently, we will discuss about a transcription factor (*DREB1A*), which was introduced by abiotic stresses, and over-expression of it induced strong expression of stress-responsive genes in transgenic plants, resulting in increased tolerance to drought, salinity, heat and low temperature and even oxidative stress; therefore, it particularly increased plant productivity. Furthermore, since the molecular control mechanisms of abiotic stress tolerance, at least, are similar in part, it is expected that the *DREB1A* gene is involved in the tolerance to heavy metal toxicity and harmful rays or mechanical injury stresses.

DREB1A AND DROUGHT TOLERANCE

Water deficit stress known as drought stress has many physiological effects on plants, to include reduction in vegetative growth, leaf expansion and transpiration. As a secondary effect, abscisic acid (ABA) concentration increased and the stomata almost prevented the transpirational water loss that results in limited photosynthesis due to decline in Rubisco activity (Bota et al., 2004). On the other hand, decline in intracellular CO_2 levels results in the generation of ROS components, such that they lead to photo-oxidation and finally cause extensive peroxidation and de-esterification of membrane lipids, as well as lead to protein denaturation and mutation of nucleic acids (Bowler et al., 1992).

On the cellular level, removal of water from the membrane disrupts the normal bilayer structure and results in membrane damage, displacement of membrane proteins and reduced activity ion transporters, or may even complete denaturation of enzymes and cytosolic and organelle proteins, so that they can cause disruption of cellular metabolism. Plants tend to protection of membranes as well as macromolecules by synthesis of a large number of osmolytes compounds, such as proline, glutamate, glycine-betaine, carnitine, mannitol, sorbitol, fructans, polyols, trehalose, sucrose, oligosaccharides and inorganic ions like K^+ (Ramanjulu and Bartels, 2002). The primary function of compatible solutes is to maintain cell turgor and thus the driving gradient for water uptake. Some studies indicate that compatible solutes can also act as free-radical scavengers or chemical chaperones by directly stabilizing membranes and/or proteins (Diamant et al., 2001, Garg et al., 2002).

Analyses of the expression of dehydration-inducible genes have shown that at least four independent

signaling pathways function in the induction of stress-inducible genes in response to dehydration: Two are ABA dependent (Uno et al., 2001; Velasco et al., 1998) and two are ABA independent (Savoure et al., 1997). Dehydration responsive element (DRE) or C-repeat (CRT), a *cis*-acting element, plays an important role in regulating gene expression in response to stress in an ABA independent manner (Yamaguchi-Shinozaki et al., 2002). DRE/CRT exists in many dehydration responsive genes and *DREB1A*, as a DRE/CRT-binding transcription factor, can up-regulate them. Consequently, over-expression of *DREB1A* gene will significantly introduce expression of dehydration responsive genes and can result in high tolerance to drought stress in transgenic plants.

Kasuga et al. (1999) test whether over-expression of the *DREB1A* gene enhanced tolerance to dehydration, allowed the wild-type and transgenic plants to grow in pots that were not watered for 2 weeks. Nearly all the wild-type plants died within this 2-week period, whereas 69.2% or even more of the *35S:DREB1A* transgenic plants survived this level of drought stress and continued to grow when watering started again [similar results were obtained for *AtDREB1A*-transgenic tobacco by Kasuga et al. (2004); rice by Oh et al. (2005); tall fescue by Zhao et al. (2007); and *HsDREB1A*-transgenic bahiagrass by James et al. (2008)]. Pellegrineschi et al. (2004) transferred *AtDREB1A* into wheat and found that transgenic plants show water stress symptoms 5 days later than wild type plants. This drought tolerance is attributable to the transgenic plants' reduced evapo-transpiration or simply to an increase in the osmolarity of the cell. Interestingly, the *DREB1A* plants consistently had a higher total number of heads and better head development. These plants also developed a more branched root phenotype, in that the root system could enable the *DREB1A* plants to use the moisture in the pots more efficiently, allowing them to survive longer. Vadez et al. (2007) analyzed *DREB1A*-transgenic groundnut and reported that the total evapotranspiration under water deficit was higher by 14 to 31% in all transgenic plants when compared to the wild type (WT). Moreover, *DREB1A* clearly induced a root response under water deficit conditions. This response enhanced root growth under water deficit, in particular in the deep soil layers. Consequently, water uptake under water deficit was enhanced, up to 20 to 30% in some transgenics as compared to the WT. Finally, it appeared that the putative effect of *DREB1A* on root under water stress conditions was due to an effect of the root/shoot ratio, which was dramatically increased under water stress in all transgenic lines.

Bhatnagar-Mathur et al. (2007) observed that all the selected *DREB1A*-transgenic peanuts were able to maintain a transpiration rate equivalent to the well-watered control in soils dry enough to reduce transpiration rate in wild type JL.24. However, all of them except one achieved higher transpiration efficiency (TE) under well-watered conditions and this appeared to be explained by a lower stomatal conductance. Under water limiting conditions, one of the selected transgenic plants showed 40% higher TE than the untransformed control. The results obtained by Zhao et al. (2007) demonstrate that the drought and cold inducible over-expression of the *AtDREB1A* can increase the drought tolerance of tall fescue plants, without stunting growth. In a hydroponic growth system, James et al. (2008) showed that over-expression of *HsDREB1A* in bahiagrass enhanced survival and biomass production under severe dehydration as compared to wild-type plants, under controlled environmental conditions. Following repeated cycles of dehydration, transgenic plants recovered more quickly than wild-type plants due to more rapid growth of new roots. Moreover, surprising results were obtained by Al-Abed et al. (2007), in that 80.2 and 62.4% of *AtDREB1A*-transgenic maize plants survived the 14 and 21 days dehydration periods, whereas 22.6 and 0.3% of the wild-type plants survived this drought stress. Also, 64.5 and 87.7% of the ions leaked from the wild type plants after 7 and 14 days of dehydration, while only 23.2 and 26.1% of the ions leaked from transgenic plants after these water-stress periods. Hence, the plants expressing the *CBF3* gene were significantly more resistant to water stress than wild-type plants ($p < 0.0001$).

All evidences indicate that *DREB1A* up-regulated many drought-responsive genes. These genes have critical roles in processes where plants are adaptive to drought, such as stomatal configuration, photosynthesis activity, transpiration efficiency, shoot and root growth and water uptake, osmolytes accumulation, free-radical scavengers, and stabilizing membranes and macromolecules. Hence, over-expression of *DREB1A* gene helps plants, by strongly inducing the protection processes, to recover themselves and show high tolerance to drought stress.

DREB1A AND SALINITY TOLERANCE

According to the U.S. Department of Agriculture Salinity Laboratory, saline soil can be defined as soil having an electrical conductivity of the saturated paste extract (EC_e) of 4 dS m^{-1} (4 dS m^{-1} is about 40 mM NaCl) or more. Over 800 million hectares of land throughout the world are salt-affected, either by salinity (397 million ha) or the associated condition of sodicity (434 million ha) (FAO, 2005). This is over 6% of the world's total land area. On the other hand, high salinity stress is the most severe environmental stress, which impairs crop production on at least 20% of the irrigated land worldwide (Tuteja, 2007). Salinization is manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell (Zhu, 2001a). Also, there are numerous documented changes, such as cell wall alterations, declines in photosynthesis, protein synthesis,

injured cells in transpiring leaves, which leads to growth inhibition (Locy et al. 1996, Tuteja 2007) and decrease in potassium and sodium, proline, late embryogenesis abundant (LEA) proteins, glycine-betaine, and polyols contents (Wang et al., 2003; Rorat, 2006; Vijayan et al., 2008a), in cellular activities found inside higher plants in response to salt stress. Prevention, compartmentation or exclusion of sodium ions under salinity stress is extremely important for plant survival and growth, which result in maintenance and re-establishment of cellular ion homeostasis during stress. Plants possess specific mechanisms to overcome the hyper-saline environment and thrive in such conditions by adjusting their internal osmotic status (Mahajan and Tuteja, 2005). To activate these mechanisms, mRNAs of many stress-inducible genes are accumulated in response to high salinity stress (Liu and Zhu, 1997). For example, Shi et al. (2003) suggested that the *A. thaliana* plasma membrane Na^+/H^+ antiporter, encoded by the SOS1 gene and catalyzed by the exchange of Na^+ for H^+ across membranes which have a variety of functions, such as regulating cyto-plasmic pH, sodium levels and cell turgor (Zhu 2003), may be essential for salt tolerance; however, salinity is a quantitative trait and the activity of multiple genes is required for salt resistance. Therefore, it is possible that over-expression of a transcription factor, which up-regulated many salt stress-inducible genes, can directly or indirectly enhance plant tolerance to salinity. For example, the LEA family genes can protect plants against salt stress by lowering intracellular water potential, stabilizing membrane structure, binding metal ions and scavenging active species (Wang et al., 2003), regulated by *DREB1A* as a transcription factor.

To examine the tolerance of the *DREB1A*-transgenic *Arabidopsis t.* plants to salt stress, Kasuga et al. (1999) removed plants grown on agar plates from the plates, soaked in 600 mM NaCl solution for 2 h, and then grow them in pots for 3 weeks. Only 17.9% of the wild-type plants survived this treatment when compared with 29.4% of the *35S:DREB1A* plants. In contrast, the *rd29A:DREB1A* plants were highly tolerant to the salt stress (78.6% survival). In the other research, Oh et al. (2005) used the *Ubi1:CBF3* construct and found that levels of the fluorescence ratio (Fv/Fm) (reductions in the maximum photochemical efficiency of PSII in the dark-adapted state) were approximately 30% higher in *Ubi1:CBF3* transgenic rice than in control plants under drought and high salinity stress. Hong et al. (2006c) also reported increase in tolerance for *DREB1A*-transgenic chrysanthemum plants to drought and salinity stress. Behnam et al. (2006) transferred *DREB1A* into tetrasomic tetraploid potato and found that *DREB1A* act in enhancing the tolerance of salinity (1M NaCl solution for 2 h) in the transgenic plants. Interestingly, they showed a correlation between the number of inserted gene and salinity tolerance, which suggested that increasing the *DREB1A* inserted copy number, increased the salinity tolerance in the transgenic potato lines, with some exceptions [similar results were shown by Celebi-Toprak et al. (2005), with the same transgenic plants that were watered with 20 ml of a 2 M NaCl solution overnight].

Ito et al. (2006) examined the tolerance of the trans-genic rice to high-salt stress by keeping the transgenic plants removed from the pots in 250 mM NaCl solution for 3 days, and then growing them in pots for 19 days. Only 5% of the wild-type plants survived this treatment, while 13 to 83% of the transgenic plants over-expressing *OsDREB1* or *DREB1* survived, showing that both *OsDREB1* and *DREB1* genes improved the tolerance to high-salt stress in transgenic rice plants. Similarly, *HsDREB1A*-transgnic and control bahiagrass plants were supplemented with 100 mM NaCl for one week, followed by 200 mM NaCl for two weeks. Plants were then allowed to recover for 10 days without NaCl; moreover, none of the wild-type plants recovered, whereas one to three plants from each transgenic line showed new growth of leaves and roots (James et al., 2008). These results are in complete agreement with those obtained by Al-Abed et al. (2007), who reported that when plants were given two weeks of recovery by removing any salt residues with regular watering, a significant difference in the survival rate was observed between *AtDREB1A*-transgenic maize and wild-type plants subjected to high salt stress ($p < 0.0001$). Only 2.1% of the wild-type plants survived the 200 mM L^{-1} treatments and none survived the 400 mM L^{-1} treatment; as such, this compares with 82.3 and 75.4% of transgenic plants, respectively.

Levels of osmolytes compounds change in salinity stress, and it is well known that accumulation of these compounds decreases the adverse effects of stress on the cells. Also, the modification of different pathways by over-expressing the concerned genes has greater effect on salinity tolerance in plants (Vijayan, 2009). Based on the fact that expression of *DREB1A* gene regulated ion homeostasis and turgor pressure by increase in levels of osmolytes and reduced ions leakage by increase in membrane stabilization, it appears that over-expression of *DREB1A* gene can result in high tolerance to salt stress.

DREB1A AND FREEZING TOLERANCE

Cold and freezing conditions are two relevant factors limiting the distribution of plant species and they limit the geographic locations where crops can be grown. Cold stress often affects plant growth and crop productivity, which causes significant crop losses (Xin and Browse, 2000). Results also showed that a slight temperature variation can induce dramatic effect on flowering time and variation among accessions (Lefebvre et al., 2009). Many genes respond to low temperature and encode a diverse number of proteins, including enzymes involved in respiration and metabolism of carbohydrates, lipids,

phenylpropanoids and antioxidants, molecular chaperones, antifreeze proteins, among others, with a believed function in freezing tolerance (Thomashow, 1999).

Acquired freezing tolerance involves extensive reprogramming of gene expression and metabolism. Determining the nature of these genes and mechanisms and the sensing and regulatory mechanisms that activate the cold-acclimation response provide the potential for new strategies to improve the freezing tolerance of agronomic plants (Jan et al., 2009). The *CBF/DREB1* cold response pathway is the best characterized genetic control of the cold acclimation process that is executed in Arabidopsis and crop plants (Chinnusamy, 2006; Yamaguchi-Shinozaki and Shinozaki, 2006), and it has also been described in trees, whose cold acclimation capacity exceeds that of any other plant species, including sweet cherry (Kitashiba et al., 2004), Populus spp. (Benedict et al., 2006), Eucalyptus (El Kayal et al., 2006), Grape (Xiao et al., 2006), Citrus (Champ et al., 2007) and Birch (Welling and Palva, 2008).

In this pathway, *SIZ1*-dependent sumoylation of *ICE1* at K393 (Sumoylation of *ICE1* is a key regulatory process in cold signaling and tolerance), regulates the expression of *CBF3/DREB1A* and cold tolerance. SUMO conjugation to *ICE1* induces *CBF3/DREB1A* expression (Miura et al., 2007), and then *CBF3/DREB1A* induces the cold-responsive genes, such as *cor15a*, *rd29A*, *erd10*, *kin1*, *cor6.6* and *cor47*/ *rd17* in both Arabidopsis and rice (Gilmour et al., 2000; Maruyama et al., 2004; Ito et al., 2006), and *Lip5, Dip1, Jacalin1-2* and *LOX* in rice (Oh et al., 2005). The *DREB1A*-target genes have important roles in freezing tolerance; for example, the major harmful effect of freezing is membrane damage, in that plants that contain higher proportion of saturated fatty acids in their membranes are more sensitive. Formation of hexagonal II phase lipids is a major cause of membrane damage in non-acclimated plants. *COR15a* expression decreases the propensity of the membranes to form hexagonal II phase lipids in response to freezing (Uemura, 1997), due to the fact that *COR15a*-encoded the protein stabilizing membranes against freezing injury (Steponkus et al. 1998).

It was reported that over-expression of *CBF3* in Arabidopsis increased freezing tolerance and, more interestingly, it resulted in multiple biochemical changes associated with cold acclimation: Elevated levels of proline and total soluble sugars, including sucrose, raffinose, glucose and fructose (Gilmour et al., 2000; Maruyama et al., 2004). Recently, Maruyama et al. (2009) compared cold-exposed and *35S:DREB1A* plants, and found that the levels of 155 and 37 metabolites increased significantly in cold-exposed and *35S:DREB1A* plants, respectively. Most metabolites (89%) were increased in both sample plants and seventeen of them were unique. These metabolites contained sugars, such as Suc, galactinol, myoinositol, raffinose and unknown metabolites. Previously, the evidences presented also indicated the role of *DREB1A* gene in cold tolerance.

Kasuga et al. (1999) reported that when plants grown in pots were exposed to temperatures of -6°C for 2 days, they were returned to 22°C and grown for 5 days. It was observed that less than 10% of the wild-type plants survived, whereas 77.9 and 96.2% of the *35S:DREB1Ab* and *rd29A:DREB1Aa* plants survived, respectively. To quantify the increase in freezing tolerance, Gilmour et al. (2000) carried out electrolyte leakage tests. The results indicated that non-acclimated control plants had an EL_{50} (temperature that caused a 50% leakage of electrolytes) of approximately - 4.5°C, whereas the three *CBF3*-expressing lines had EL_{50} values of approximately - 8°C. They also reported that *P5CS* transcript levels and the amount of total soluble sugars were approximately 4-fold and 3-fold higher in non acclimated *CBF3*-expressing plants than they were in non acclimated control plants, respectively. Oh et al. (2005) found that the levels of Fv/Fm were approximately 10% higher in *Ubi1:CBF3* plants than in non-transgenic control plants under low-temperature. Ito et al. (2006) examined the tolerance of the transgenic rice to cold stress and they found that all wild type plants died after treatment, whereas 25 to 60% of *CBF3*-overexpression rice survived. In addition, they identified 12 stress-regulated genes in rice that are up-regulated by *CBF3*. Hong et al. (2006b) reported similar results in transgenic chrysanthemum that over-expressed *DREB1A* gene. The ryegrass *CBF3* (*LpCBF3*) gene, when introduced into Arabidopsis, functions in cold-responsive signaling pathway. The *LpCBF3* gene was constitutively expressed, but the level of expression was stronger in the transgenic Arabidopsis plants exposed to low temperature than normal growth condition (Xiong and Fei, 2006).

Cellular processes that contribute to cold tolerance include protection and stabilization of cellular membranes, enhancement of antioxidative mechanisms, and synthesis and accumulation of cryoprotectant solutes and unique cryoprotective proteins (Mahajan and Tuteja, 2005), in which *DREB1A* directly or indirectly affected majority of them and strongly introduced and regulated these pathways (Maruyama et al., 2009). All together, they indicated that over-expression of *DREB1A* gene can confer high tolerance to cold stress in transgenic plants.

DREB1A AND HEAT TOLERANCE

Temperatures above the normal optimum are sensed as heat stress by all plants. Heat stress disturbs cellular homeostasis and can lead to severe retardation in growth and development, and even death. The heat stress response is characterized by inhibition of normal transcription and translation, higher expression of heat shock proteins (HSPs) and induction of thermo-tolerance (Krishna, 2003). HSPs are believed to play an important role in plant stress tolerance and not only expressed in

response to heat shock, but also under water, salt, and oxidative stress, and at low temperature. Moreover, HSPs are involved in many developmental processes, such as embryo development, seed germination, somatic embryogenesis, pollen development, and fruit maturation (Waters et al., 1996).

Response to and survival of heat stress is a complex phenomenon in plants (Kotak et al., 2007). The induction and accumulation of HSPs (chaperones) through the HSFs (heat stress transcription factors) network is clearly important, but other transcription factors and multiple signaling pathways also orchestrate the heat stress response, regulating a range of effectors components, all of which contribute to survival under high temperature stress (Kotak et al., 2007). Among transcription factors of *DREB* family, the *DREB2A* has been reported to play an important role in heat stress response through regulating its downstream genes in Arabidopsis and maize (Sakuma et al., 2006; Qin et al., 2007). Although, until now, little information is available concerning the effect of *DREB1* on heat stress, Hong et al. (2009) have recently reported that over-expression of *AtDREB1A* in chrysanthemum enhances tolerance to heat stress.

Hong et al. (2009) exposed WT and 35S plants with 7 to 8 leaves to the heat treatment at 45^{o}C for 0 to 36 h, and then recovered under normal growth conditions at 22^{o}C for 3 weeks. However, higher complete recovery (50%), significantly lower electrolyte leakage and higher activities of Rubisco and sucrose-phosphate synthase ($p < 0.05$), and higher transcript level of *DREB1A* were observed in 35S plants as compared to WT plants under heat stress. On the other hand, among 74 *DREB1A* regulon members in chrysanthemum (Ma and Gao, in press), 55 genes were identified as significantly up-regulated by heat stress including the genes encoded for phosphatase type 2C, rab-type small GTP-binding protein, serine/threonine kinase, RNA binding protein, constans B-box zinc finger family protein, HSP18.6, HSP70, PIP1A, peptidase and cellular biogenesis/ chromatin binding protein, SPS, Rubisco SSU, NADH dehydrogenase and dehydrin (a cell defense protein), in which there were some members in the 35S plants whose enhanced expression ratios were over tenfold greater than the WT plants.

Based on these observations, Hong et al. (2009) indicated that the improved heat tolerance is related to the enhanced expression of HSPs and photosynthesis by increase of the SPS and Rubisco activity in *AtDREB1A* transgenic plants. Although the effects of *DREB1A* on photosynthesis activity, osmolytes and chaperons accumulation, free-radical scavengers, cellular homeostasis and stabilizing membranes, which were all affected by heat stress, were first reported on the directed role of *DREB1A* in heat tolerance, it appears that over-expression of *DREB1A* gene can confer high tolerance to heat stress in other transgenic plants species, similarly to chrysanthemum.

CONCLUSION AND PERSPECTIVE

Due to the various adverse effects of abiotic stresses on plant growth and productivity, it is very important to improve stress tolerance of the crop plants in order to maintain growth and productivity and increase crop yield under stress conditions. Although the tolerance of plants to abiotic stresses is well known to be a multigenic trait, plant improvement using genes that play a role in the abiotic stress response is frequently insufficient to improve stress tolerance significantly. To overcome this, transcription factors that regulate several stress-responsive genes (for example, the *DREB1*s family) have often been used to manipulate plants in order to have a broader response and maintain the function and structure of cellular components (Saibo et al., 2009). The DREB1A is a transcription factor that is induced by abiotic stress, especially low temperature, and strongly up-regulates many downstream genes; thus resulting in adaptation of plants to stress conditions and exercise specific tolerance mechanisms. Many studies indicated that over-expression of *DREB1A* gene in crop plants can result in high tolerance to abiotic stress, thereby increasing the efficiency of plants production. These suggest that we can use the *DREB1A* gene transfer strategy at the trial scale to prevent large and widespread yield reductions under stress conditions and obtain more products in agronomic development. However, the expression of *DREB1A* gene is not only regulated by *SIZ1*-dependent Sumoylation of *ICE1* at K393 in response to low temperature and result in freezing tolerance, but over-expression of it can also induce tolerance to drought, salinity and heat. There are two explanations for this; either the entire genes, inducible by DREB1A, may be involved in various functional processes which result in a broad stress tolerance, or there is a connection between stress tolerance pathways at the various levels, from transcriptional activation of downstream genes to final effectors molecules. On the other means, the *DREB1A* gene play a crucial role in providing tolerance to multiple stresses and display overlapping responses to different stress conditions. In addition, stress treatments analysis has revealed that negative effects induced by abiotic stresses could be overlapped altogether, and on the other hand, Wang et al. (2009) identified ten genes that act as the cross-talkers in all likelihood among the drought, salinity and cold responsive pathways and have DRE motifs within the 300 bp upstream of the start codon.

Most of the environmental stresses, at least in part, have similar adverse effects on plants. These effects include the generation of ROS components, peroxidation and de-esterification of membrane lipids, protein denaturation, mutation of nucleic acids, membrane damage, displacement of membrane proteins, declines in photosynthesis and protein synthesis, reduced activity of ion transporters, disruption of cellular metabolism,

disruption of ion homeostasis in the cell, reduced shoot and root growth and water uptake. At the previous parts, we presented evidences which show that over-expression of *DREB1A* gene confers high tolerance to these harmful damages caused by induced production of carbohydrate metabolism-related proteins (*SPS*, *SuSy*), *COR* and *KIN* proteins, phospholipase C, RNA binding proteins, sugar transport proteins, desaturase, late embryo abundant (*LEA*) proteins, molecular and chemical chaperones, osmoprotectant biosynthesis proteins, protease inhibitors, compatible solutes, free-radical scavengers, and increase in photosynthesis activity, transpiration efficiency, shoot and root growth, water uptake, osmolytes accumulation, and stabilizing membranes and macromolecules.

In this study, we did not find any report about DREB1As role in tolerance to heavy metal toxicity and harmful rays or mechanical injury stresses. These stresses have negative effects on plants, at least in part, and are similar to other environmental stresses effects presented in the foregoing. However, it is expected that plant cellular responses to such stimuli were also similar. In fact some reports have highlighted the connection between disease resistance and drought tolerance (Chini et al., 2004) and have indicated positive effect of tolerance to one stress on increase resistance to other stresses (Chinnusamy et al., 2004). Altogether and given that the common deleterious effects and molecular mechanisms of tolerance about all of these stresses are probably similar and DREB1A effected on majority of them, it is possible that over-expression of DREB1A gene direct or even specially indirect leads to produced tolerance plants again heavy metal toxicity and harmful rays or mechanical injuries. However, the end conclusion is being examined in our studies. It is hoped that these efforts will help to prevent global-scale environmental damage that is resultant from these stress.

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