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THE ROLE OF DREB TRANSCRIPTION FACTORS IN ABIOTIC STRESS TOLERANCE OF PLANTS

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ABSTRACT
DREBs belong to the ethylene response factor (ERF) family of transcription factors that bind to the DRE/CRT element in the promoter regions of a large number of genes involved in biotic and abiotic stress signaling in plants. In the recent past, there has been substantial progress towards understanding the regulatory role of DREBs in abiotic stress responses of crop plants. Following isolation from various plant species, DREB genes have been introduced into a number of transgenic plants, which showed tolerance to multiple abiotic stresses. Despite the initial success of achieving abiotic stress tolerance with DREBs, there are several issues that need further research to ensure increased plant growth and yield under abiotic stress conditions. This review focuses on the current status of research on DREB transcription factors, their role in abiotic stress tolerance in transgenic plants, and challenges confronting deployment of these plants.

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Introduction
Abiotic stresses are constantly threatening plant growth, biomass production and crop productivity (9). Due to the polygenic nature of abiotic stress responses, development of abiotic stress tolerance in crop plants through conventional approaches has been a challenge for plant breeders (35). Genetic engineering of plants with individual genes somehow paved the way for achieving abiotic stress tolerance with less effort and time. Over the last two decades, considerable progress has been made to engineer crop plants with individual genes from various sources that conferred tolerance to biotic and abiotic stresses. Unlike the successful manipulation of individual genes conferring biotic stress tolerance, genetic transformation of crop plants for abiotic stress tolerance has met with limited success (73).

Plant responses to abiotic stresses are highly complex and involve expression of a large number of genes encoding stress-related proteins and enzymes working in biosynthetic pathways of osmoprotectants and other stress-related metabolites (65). The molecular determinants governing abiotic stress signaling responses in plants are complex and difficult to dissect and due to this the success rate of achieving abiotic stress tolerance with transfer of individual genes is very low (58). Also, the constitutive expression of these single genes with strong promoters has deleterious effects on plant health and overall energy balance.

In the quest to find genetic factors working in concert in abiotic signaling pathways, various transcription factors have been discovered (2). Some of these important transcription factors responding to drought, low temperature and high salinity stress include the ethylene responsive element binding factors (ERF), MYC, MYB, basic-domain leucine-zipper (bZIP), WRKY binding (WRKY), NAC and DELLA transcription factors (**Fig. 1**) (2, 41, 71). Among these, the DREB transcription factors have gained much attention due to their involvement in the regulation of many stress-related genes that play an important role in cascading a response to environmental stimuli (2, 40). During the last one and a half decade, transcription factors including DREBs, have been focused in relation to their expression under abiotic stress condition and regulation of stress-related genes working in different plant stress defense pathways.

Genetic engineering of plants for abiotic stress tolerance could be achieved through expression of DREB transcription factors that, in turn, regulate the expression of abiotic stress-related downstream genes (2). Research on genetic manipulation of plants with DREB transcription factors is still in its infancy. Despite a large number of transgenic plants harboring DREBs and the initial success in achieving abiotic stress tolerance under control experimental conditions, many issues still need to be resolved to fully exploit the potential of DREB-transgenic plants under natural stress environments. This review focuses on the current status of research on DREB transcription factors, their role in abiotic stress tolerance in transgenic plants, and challenges confronting deployment of DREB-transgenic plants.

DREB transcription factors
One prominent class of transcription factors is the DREB/CBF that binds to the drought responsive *cis*-acting elements. DREB/CBF belongs to the ERF (ethylene responsive element binding factors) family of transcription factors. ERF proteins are a subfamily of the APETLA2 (AP2)/ethylene responsive

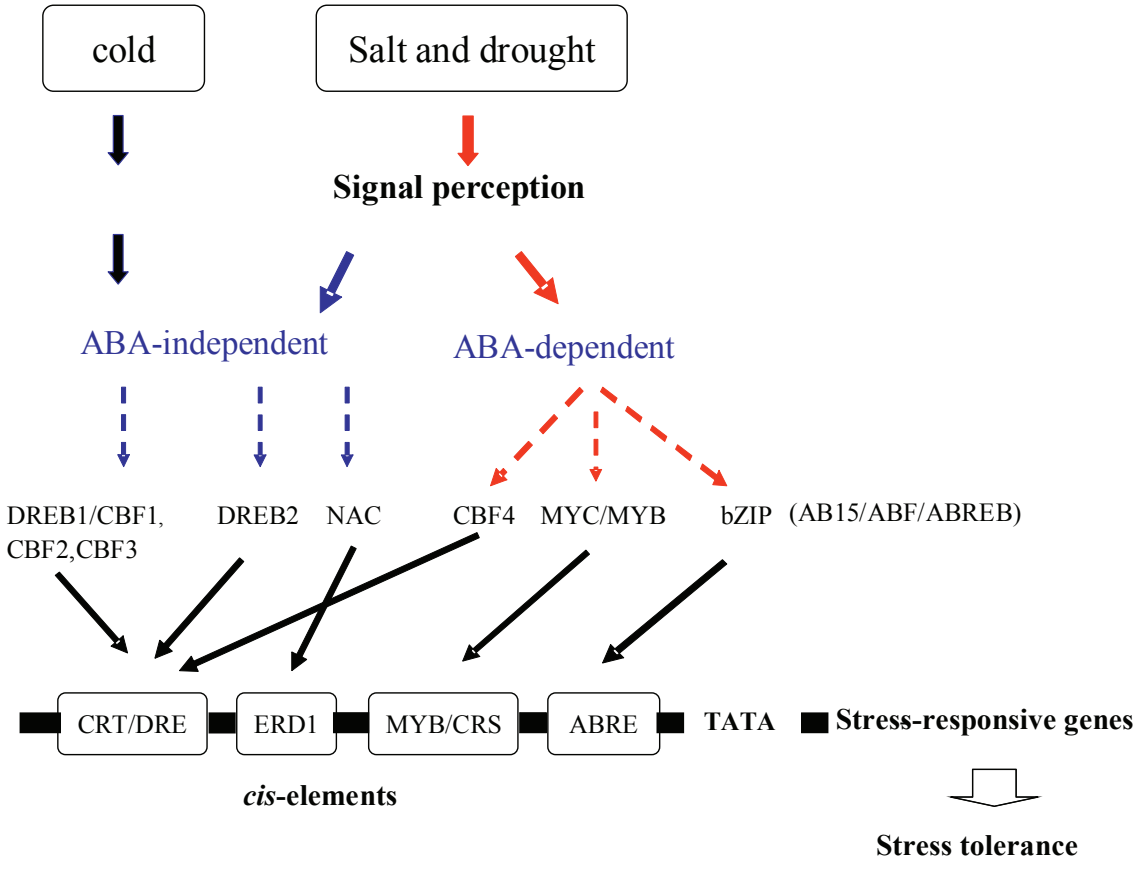

Fig. 1. A schematic representation of cellular signal transduction pathways leading from signal perception to gene expression. DREB1/CBF genes are induced by cold stress, whereas DREB2 are induced by dehydration and slat stress. Both work in ABA-independent pathways. CBF4, MYC/MYB and bZIP are induced by dehydration/salt stress and work in ABA-dependent pathway. DREB1/CBF and DREB2 bind to DRE/CRT, MYC/MYB to MYB/CRS and bZIP to ABRE cis-acting elements. DRE: drought responsive element, ABRE: abscisic acid responsive binding element, MYBRS: MYB recognition site, MYCRS: MYC recognition site, bZIP: basic-domain leucine-zipper (modified from Agarwal et al. 2006 (2)).

element binding proteins (EREBP), which are characteristic of plants. The ERF family comprises two subfamilies, the EREBP (single AP2 domain) and AP2/family (two copies of AP2). The EREBP subfamily is further divided into two classes, i.e. ERFs and DREBs/CBFs. ERFs bind to the GCC box found in the promoters of many pathogenesis-related (PR) genes conferring ethylene responsiveness (20). DREBs/CBFs bind to the dehydration responsive element (DRE/CRT) in the promoters of cold and dehydration responsive LEA genes including *rd29A, rd17, cor6.6, cor15a, erd10* and *kin1* (33, 74). From the promoter of a stress inducible *rd29A* gene, a nine base pair conserved sequence (TACCGACAT) was identified that is essential for *rd29A* induction under dehydration and cold stress in transgenic Arabidopsis (74). The DREBs/CBFs are further divided into two subclasses, i.e. DREB1/CBF and DREB2, induced by cold and dehydration stress respectively.

Isolation of DREBs and their role in abiotic stress responses

Transcription factors belonging to the ERF family play a vitally important role in biotic and abiotic stress responses. DREB transcription factors are prominent members of this family. DREB1 and DREB2, the two classes of DREBs, are induced by cold and dehydration stress respectively and work in an ABA-independent pathway except CBF4, which requires CRT/DRE elements in an ABA-dependent pathway (**Fig. 1**). DREB transcription factors were first isolated from Arabidopsis, the CBF1 (*DREB1A* and *DREB2A*) (39). Since then, several homologs of DREB1 and DREB2 have been reported from several other plants (**Table 1**). From Arabidopsis, two DREB1A homologs (*DREB1B*, *DREB1C*) and one DREB2A homolog (*DREB2B*) were isolated (39). In the same way, several CBFs (CBF1, CBF2, CBF3 and CBF4) were isolated from Arabidopsis (19, 21, 43). Several CBF homologs have also been reported from a wide range of plants including barley (12, 59), canola (30), Bell pepper (24), soybean (37), tobacco (49), tomato (30) and wheat (66). Typically the DREB1 and DREB2 genes are induced by cold and dehydration stress, respectively. However, the expression of these genes has shown mixed responses to abiotic stresses in their respective plants. In Arabidopsis, expression of *DREB1A* was induced by cold, while *DREB2A* was induced by dehydration and salt stress (39). Expression

TABLE 1

DREB and DREB-like homologs isolation from various plant species

Plant species	Transgene	Stress induction	Reference
Aloe vera	*DREB1*	cold	Wang and He 2007 (69)
Arabidopsis thaliana	*DREB1A*, *DREB2A*	cold, dehydration	Liu et al. 1998 (39)
Arabidopsis thaliana	CBF1, CBF2, CBF3	cold	Gilmour et al. 1998 (19)
Atriplex hortensis	*AhDREB1*	salt	Shen et al. 2003 (57)
Brassica juncea	*BjDREB1B*	drought, salt, low temperature	Cong et al. 2008 (13)
Brassica napus	BNCBFs 5, 7 and 16	cold	Gao et al. 2002 (17)
Brassica napus	Group I and II DREBs	cold	Zhao et al. 2006 (78)
Chrysanthemum	*DmDREBa*, *DmDREBb*	cold	Yang et al. 2007 (75)
Festuca arundinacea	*FaDREB1*	cold	Tang et al. 2005 (61)
Glycine max	*GmDREBa, GmDREBb, GmDREBc*	salt, drought, cold	Li et al. 2005 (37)
Glycine max	*GmDREB2*	drought, salt, low temperature	Chen et al. 2007 (11)
Gossypium hirsutum	*GhDBP2*	drought, salt, low temperature	Huang et al. 2008 (27)
Hordeum Vulgare	*CBF3*	cold	Choi et al. 2002 (12)
Hordeum Vulgare	*DREB2*-type *HvDRF1*	drought, salt	Xue and Loveridge 2004 (72)
Lolium perenne	*LpCBF3*	cold	Xiong and Fei 2006 (71)
Oryza sativa	*OsDREB1A*, *OsDREB1B*	cold	Dubouzet et al. 2003 (16)
Oryza sativa	*OsDREB2A*	salt, dehydration	Dubouzet et al. 2003 (16)
Oryza sativa	*OsBZ8*	salt	Kakali et al. 2006 (32)
Oryza sativa	*OsDREB1B*	cold, heat	Qin et al. 2007 (52)
Oryza sativa	*OsDREB1F*	salt, drought, cold	Wang et al. 2008 (68)
Pennisetum glaucum	*PgDREB2A*	salt, dehydration	Agarwal et al. 2007 (3)
Physcomitrella patens	*PpDBF1*	salt, drought, cold	Liu et al. 2007 (38)
Triticum aestivum	*TaDREB1*	low temperature, salinity, drought	Shen et al. 2003 (56)
Zea Mays	*ZmDREB2A*	cold, dehydration, salt, heat	Qin et al. 2007 (52)

of CBF1, CBF2 and CBF3 was induced only by cold stress in Arabidopsis (19). In *Brassica napus*, the BnCBFs 5, 7 and 16 were induced by cold stress (17), while more recently, the isolation of *BjDREB1B* was reported to be induced by drought, salt and low temperature (13). Similarly, in rice, cold stress induced expression of *OsDREB1A* and *OsDREB1B*, while exposure to salt and dehydration stress induced expression of *OsDREB2A* (16). Recently, Wang et al. (68) reported a new member of the DREB family, called *OsDREB1F* from rice. *OsDREB1F* was induced under salt, drought and cold stress. Various DREB homologs, e.g. *GmDREBa*, *GmDREBb* and *GmDREBc* from *Glycine max*, were induced by salt, drought and cold stress (37). Chen et al. (11) reported the induction of *GmDREB2* under drought, salt and low temperature. In canola, wheat, rye and tomato, expression of CBF like transcription factors was induced by cold stress only (30). In barley, a *DREB1A* homolog CBF3 was induced by cold stress (12). A homolog of *DREB2*-type gene found in wheat was induced by cold, while dehydration and salt stress had small effects on its expression (57). The expression of several DREB homologs i.e. *FaDREB1* (*Festuca arundinacea), DREB1 (Aloe vera)* and *DmDREBa* and *DmDREBb* (Chrysanthemum) were induced by cold stress (61, 69, 75). Isolation of a number of DREBs and their homologs from various plant species clearly indicates the consistent presence and important role of *DREB* transcription factors in abiotic stress responses.

DREB expression confers abiotic stress tolerance in transgenic plants

Keeping in view the important role of DREBs in abiotic stress tolerance, various plants have been transformed with DREB transcription factors that conferred multiple abiotic stress tolerance on plants (**Table 2**). Overexpression of *AtDREB1A* under a constitutive *CaMV35S* promoter conferred enhanced freezing and dehydration tolerance in transgenic Arabidopsis (39) and tobacco (34) plants respectively. However, transgenic Arabidopsis and tobacco plants showed growth retardation under non-stressed conditions. Liu et al. (39) reported up-regulation of 12 stress-related genes in *CaMV35S::AtDREB1A* plants that showed two-fold higher expression than in control plants. Six of these genes were known as stress-related, while the other six were found to have sequence similarities with cold acclimatization proteins. In *35S::OsDREB1A*

TABLE 2

Transcription factors confer abiotic stress tolerance on plants

Target	Transgene	Tolerance	Promoter	Physiological and biochemical effects on plant growth	Growth conditions	Reference
Arabidopsis thaliana	*AtCBF1*	freezing	*CaMV35S*	-	Growth chamber	Jaglo-Ottosen et al. 1998 (29)
Arabidopsis thaliana	*AtDREB1A/ AtDREB2A*	freezing, dehydration	*CaMV35S*	Growth retardation under non-stress condition	Laboratory	Liu et al. 1998 (39)
Arabidopsis thaliana	*AtCBF3*	freezing	*CaMV35S*	High accumulation of proline and soluble sugars	Laboratory	Gilmour et al. 2000 (18)
Arabidopsis thaliana	*AtCBF4*	freezing, dehydration	*CaMV35S*	Growth retardation	Laboratory and growth room	Haake et al. 2002 (21)
Arabidopsis thaliana	*OsDREB1A*	freezing, salt	*CaMV35S*	High plant survival under freezing and salt stress. Mild growth retardation	Laboratory and growth chamber	Dobouzet et al. 2003 (16)
Arabidopsis thaliana	*AtDREB2A*	dehydration	*CaMV35S*	High plant survival under 45°C temperature	Laboratory	Sakuma et al. 2006 (54)
Arabidopsis thaliana	*GmDREB2*	drought, salt	*CaMV35S/ rd29A*	High survival at 200 mM NaCl. Higher free proline content under drought. No growth retardation	Growth room	Chen et al. 2007 (11)
Arabidopsis thaliana	*OsDREB1B*	cold, high temperature	*CaMV35S*	High plant survival under low and high temperature	Growth room	Qin et al. 2007 (53)
Arachis hypogaea	*DREB1A*	dehydration	*rd29A*	High root/shoot ratio, 20-30% higher water uptake	Green house	Vadez et al. 2007 (64)
Brassica napus	*AtCBF1*	freezing	*CaMV35S*	-	-	Jaglo-Ottosen et al. 2001 (30)
Brassica napus	*BNCBF5/ BNCBF17*	freezing	-	Higher photosynthetic activity	-	Savitch et al. 2005 (55)
Chrysanthemum	*AtDREB1A*	drought, salt	*CaMV35S/ rd29A*	Higher proline and SOD activity. Growth retardation in *35S::AtDREB1A* plants	Green house	Hong et al. 2006 (23)
Chrysanthemum	*AtDREB1A*	heat stress	*CaMV35S*	High Rubisco and sucrose phosphate synthase activity. Higher photosynthetic activity	Growth chamber	Hong et al. 2009 (22)
Medicago sativa	*GmDREB1*	salt	*rd29A*	Better plant growth at 200-300 mM NaCl. High proline and soluble sugars at 200 mM NaCl. No growth retardation	Green house	Jin et al. 2010 (31)

transgenic Arabidopsis, six genes were found with two-fold expression compared to that in control plants (16, 33, 39). They concluded that up-regulation of stress-related genes in transgenic Arabidopsis plants under normal growth conditions was responsible for growth retardation. The negative effects on plant growth in transgenic Arabidopsis and tobacco were diminished, when *AtDREB1A* was expressed under a stress inducible *rd29A* promoter (34, 39). Similarly the constitutive expression of *OsDREB1A* in Arabidopsis conferred freezing, dehydration and salt tolerance (16).

There are limited reports of transgenic plants for *DREB2* genes. Liu et al. (39) first reported *AtDREB2A* expression in Arabidopsis; however they did not detect any stress tolerance in transgenic Arabidopsis. Recently, it was found that a post-translational modification was necessary for the function of *AtDREB2A*. A post-translational modification of deleting a portion between the 135th and 165th amino acid enabled *AtDREB2A* to up-regulate the expression of downstream genes in transgenic Arabidopsis (54). With up-regulation of stress-related genes (LEA proteins, dehydrins, COR15a, AtHsfA3), transgenic plants showed dehydration stress tolerance. Constitutive expression of *AtCBF1* in Arabidopsis (29) and canola (30) conferred freezing stress tolerance, while in transgenic tomato, the *AtCBF1* expression showed tolerance to water deficit (25). Overexpression of some other DREB homologs such as *AtCBF3* and *AtCBF4* was investigated in transgenic Arabidopsis (18, 21). Transgenic Arabidopsis plants that expressed *AtCBF3* showed freezing tolerance, while overexpression of *AtCBF4* conferred freezing and dehydration tolerance. Similarly, transgenic Arabidopsis that expressed *GmDREB2* under both constitutive and stress inducible promoters showed drought and salt stress tolerance (11). Transgenic Arabidopsis plants showed no symptoms of growth retardation. Dehydration stress tolerance was also achieved in transgenic groundnut with stress inducible expression of *DREB1A* (64). Transgenic plants showed high root/shoot ratio that resulted in 20-30% more water uptake under dehydration condition. Expression of several CBF-type genes in transgenic canola resulted in freezing tolerance and high photosynthetic activity (55). Transgenic chrysanthemum with expression of *AtDREB1A* showed drought and salt tolerance and accumulated higher proline content and SOD activity (23). The same group reported heat stress tolerance in *AtDREB1A* transgenic

TABLE 2

continued

Target	Transgene	Tolerance	Promoter	Physiological and biochemical effects on plant growth	Growth condition	Reference
Nicotiana tabacum	*AtDREB1A*	freezing, dehydration	*CaMV35S/ rd29A*	-	-	Kasuga et al. 2004 (34)
Nicotiana tabacum	*GmDREB2*	drought	*CaMV35S*	No growth retardation. Higher proline content than wild type	Growth room	Chen et al. 2007 (11)
Nicotiana tabacum	*BjDREB1B*	drought, salt	*CaMV35S*	Higher proline content under salt stress. Dwarf phenotype	Laboratory	Cong et al. 2008 (14)
Nicotiana tabacum	*PgDREB2A*	salt, osmotic	*CaMV35S*	4-fold higher germination at 200 mM NaCl. 50% higher seed germination under 400 mM mannitol	Laboratory	Agarwal et al. 2010 (4)
Oryza sativa	*AtDREB1A, ABF3*	drought, salt	*UBi1*	No growth inhibition was observed	Green house	Oh et al. 2005 (47)
Oryza sativa	*OsDREB1*	drought, salt, low temperature	*CaMV35S*	High proline and soluble sugar content. Growth retardation under non-stress condition	-	Ito et al. 2006 (28)
Oryza sativa	*NAM, ATAF, NAC*	Drought, salt	-	-	-	Hu et al. 2006 (26)
Solanum lycopersicum	*AtCBF1*	water deficit	*CaMV35S*	Growth retardation, less number of fruit and seeds	Growth room	Hsieh et al. 2002 (25)
Solanum tuberosum	*AtDREB1A*	salt	*Rd29A*	-	Growth room	Celebi-Toprak et al. 2005 (10)
Solanum tuberosum	*AtDREB1A*	salt	*rd29A*	-	Growth room	Behnam et al. 2006 (8)
Solanum tuberosum	*AtDREB1A*	freezing	*rd29A*	-	Growth room	Behnam et al. 2007 (7)
Solanum tuberosum	*AtCBF1-3*	freezing	*CaMV35S/ rd29A*	Growth retardation and delayed flowering. Stress inducible expression eliminated these effects.	Green house	Pino et al. 2007 (51)
Solanum tuberosum	*StEREBP1*	cold, salt	*CaMV35S*	30% increase in tuber yield under cold stress	Growth chamber	Lee et al. 2007 (36)
Solanum tuberosum	*CaPF1*	salt, drought	-	-	-	Youm et al. 2008 (76)
Tall fescue	*AtDREB1A*	drought	*rd29A*	No growth retardation. Higher proline content	Glasshouse	Zhao et al. 2007 (77)
Triticum aestivum	*AtDREB1A*	drought	*rd29A*	Better head development, branched rooting system	Green house	Pellegrineschi et al. 2004 (50)
Triticum aestivum	*DREB*	drought	*rd29A*	Higher proline content	Growth room	Wang et al. 2006 (67)
Triticum aestivum	*TADREB2/ TADREB3*	drought	-	-	Field	OGTR 2008 (45)

(-) Information is not known; NUE, Nitrogen use efficiency.

groundnut (22). Moreover, the stress inducible expression of soybean *GmDREB1* in alfalfa conferred salt tolerance with no negative effects on plant phenotype (31).

Tobacco has been extensively studied for DREB expression under various abiotic stresses. Chen et al. (11) transformed tobacco with *GmDREB2* under *CaMV35S* promoter. Transgenic tobacco plants showed drought tolerance and accumulated 4.5-fold higher proline content. More interestingly, the constitutive expression did not induce phenotypic abnormalities as previously reported in several plants with constitutive expression of DREB genes. Cong et al. (13) reported drought and salt tolerance in transgenic tobacco that expressed *AtDREB1A* under *CaMV35S* and *rd29A* promoters. Unlike the previous experiment, the constitutive expression caused a dwarf phenotype and application of GA3 did not recover the normal phenotype. Recently, Agarwal et al. (4) reported enhanced salt and mannitol stress tolerance in transgenic tobacco transformed with *PgDREB2A* from *Pennisetum glaucum*. Transgenic tobacco plants showed 4-fold higher germination compared to wild type under 200 mM NaCl. Moreover, transgenic plants exhibited better plant growth in terms of leaf area, root number, root length and fresh weight compared to wild type under both stress conditions.

Transgenic rice plants were transformed with Arabidopsis *AtDREB1A* and *ABF3* under *CaMV35S* constitutive promoter (47). The *AtDREB1A* plants showed drought and high salt tolerance and low level cold tolerance. The ABF3 transgenic plants showed only drought stress tolerance. More importantly the constitutive expression of both types of transgenes did not show any growth retardation in transgenic plants. Ito et al. (28) reported drought, salt and low temperature tolerance in transgenic rice plants that expressed DREB1 under *CaMV35S*

TABLE 3

Up-regulation of abiotic stress-related genes in DREB transgenic plants

Transgene	Target plant	Up-regulated genes	Tolerance	Reference
AtCBF1	*Arabidopsis thaliana*	COR genes	freezing	Jaglo-Ottosen et al. 1998 (29)
AtCBF3	*Arabidopsis thaliana*	COR15a, COR6.6	cold	Gilmour et al. 2000 (18)
AtCBF4	*Arabidopsis thaliana*	COR15a, COR78a	freezing, drought	Haake et al. 2002 (21)
OsDREB1A	*Arabidopsis thaliana*	Cor15, rd29A, rd17, AtGo1S3,FL05-21, F13, FL05-20-N18	drought, salt, freezing	Doubouzet et al. 2003 (16)
AtDREB1A/*ABF3*	*Oryza sativa*	Lip5, Dip1, Jacalin1, 2, LOX, PSLS, Hsp70, Rab21, LEA4, RbcS	drought, salt, low temperature	Oh et al. 2005 (47)
BnCBF5/*BnCNF17*	*Brassica napus*	Photosynthetic and chloroplast development genes induction	freezing	Savatich et al. 2005 (55)
AtDREB2A	*Arabidopsis thaliana*	LEA proteins, dehydrins, COR15a, AtHsfA3	heat	Sakuma et al. 2006 (54)
AtCBF1/*AtCBF3*	*Solanum tuberosum*	DHN10, St-P1	freezing	Pino et al. 2007 (51)
GmDREB2	*Nicotiana tabacum*	Rd29A, Cor15a	drought, salt	Chen et al. 2007 (11)
BjDREB1B	*Nicotiana tabacum*	NtERD10B, NtLEA5	salt	Cong et al. 2008 (13)
PgDREB2A	*Nicotiana tabacum*	NtERD10B, NtERF5, NtHSF2, HSP70-3, HSP18P, PLC	salt, osmotic stress	Agarwal et al. 2010 (4)
AtDREB1A	Chrysanthemum	3 genes of HSP70 group, one HSP 18.6	heat	Hong et al. 2009 (22)

promoter. However, transgenic plants showed growth retardation under normal growth conditions.

The stress inducible expression of *AtDREB1A* conferred on transgenic potato plants salt and freezing stress tolerance (7, 8). Similarly, Pino et al. (51) reported freezing tolerance in transgenic potato that expressed *AtCBF1*, *AtCBF2* and *AtCBF3* under *CaMV35S* and *rd29A* promoters. Transgenic plants with CBF expression under constitutive expression showed growth retardation, delayed flowering and lack of tuber formation. However, the stress inducible expression of *AtCBF* genes eliminated the negative effects on plant growth. Transformation of potato with *StEREBP1* resulted in cold and salt tolerance and transgenic plants showed 30% increase in tuber yield under cold stress (36).

In several experiments, DREB expression conferred drought stress tolerance. Drought stress tolerance was achieved in transgenic tall fescue that expressed *AtDREB1A* under the stress inducible *rd29A* promoter (77). Transgenic plants accumulated higher proline content under drought stress and showed no growth retardation. Pellegrineschi et al. (50) reported drought tolerance in transgenic wheat with *AtDREB1A* expression. Transgenic plants showed branched rooting system that resulted in better and increased number of heads under drought stress condition. In another experiment, similar drought tolerance was reported in transgenic wheat with DREB expression (67). Transgenic wheat plants accumulated higher proline content compared to that of control.

In conclusion, transformation of plants with DREBs is one of the preferred strategies to develop multiple abiotic stress tolerance. From the above examples, it is evident that in response to abiotic stresses, the DREB transcription factors up-regulate the expression of many stress-related genes, the products of which work in various ways to confer protection. In response to drought and salt stress, most of the DREB-transgenic plants accumulated higher proline and soluble sugar content compared to that of wild controls. Proline is an important osmoprotectant that works in osmotic adjustment, protection of macromolecules and scavenging reactive oxygen species (ROS). Soluble sugar accumulation assists in osmotic adjustment. In addition to other protective mechanisms, accumulation of proline and soluble sugars seems to be the main defense strategy against dehydration and salt stress in DREB-transgenic plants.

Induction of abiotic stress-responsive genes in DREB-transgenic plants

DREB transcription factors bind to the DRE/CRT *cis*-acting elements in the promoter regions of many stress-related genes and induce their expression which, in turn, confers abiotic stress tolerance to plants. During early studies, introduction of various DREB1/CBF genes in transgenic Arabidopsis conferred drought, salt and freezing stress tolerance (29, 33, 39, 42). Using cDNA microarrays, more than 40 different target genes of DREB1/CBF were identified in these transgenic Arabidopsis plants. Most of these target genes were found to encode stress-related proteins such as osmoprotectants, LEA proteins, protease inhibitors and transcription factors (44). Since then, identification of stress-related genes has been demonstrated in several transgenic plants transformed with DREBs (**Table 3**). Overexpression of *AtCBF1*, *AtCBF3* and *AtCBF4* in transgenic Arabidopsis up-regulated the expression of many COR genes such as *COR15a*, *COR6.6* and *COR78a* (18, 21, 29). The expression of these genes resulted in tolerance to low temperature and drought stress. Using a cDNA microarray containing 7000 Arabidopsis full-length cDNAs, six genes (*COR15*, *rd29A*, *rd17*, *AtGo1S3*, *FL05-21*, *F13*, *FL05-20-N18*) were identified, whose expression was up-regulated in *OsDREB1A*-transgenic Arabidopsis plants (16). These genes encode stress-related proteins such as cold

inducible protein, LEA proteins and osmotic stress-related proteins such as dehydrin. Oh et al. (47) identified 15-29 genes, whose expression was induced under drought stress in transgenic rice plants transformed with Arabidopsis *CBF3* and *CBF1* genes. Some of these genes *Jacalin1*, *Jacalin2*, *PSLS*, *Wsi18*, *Rab21*, *LEA4*, and *PP2Cb* were confirmed in seedlings exposed to salt, drought and low temperature stress. Similar induction of stress-related genes was reported in some other plants transformed with DREB transcription factors (5, 11, 13, 22, 44).

In all the above examples, transgenic expression of CBF/DREB genes conferred abiotic stress tolerance through induction of stress-related genes. However, the increased stress tolerance and plant growth achieved in DREB-transgenic plants as discussed in the previous section might not only be due to up-regulation of stress-related gene expression. There may be involvement of other genes working in different plant physiological and developmental processes, whose expression is induced and, in turn, contribute to improved plant growth in DREB transgenic plants. Savitch et al. (55) reported overexpression of two *Brassica* CBF/DREB1-like transcription factors (*BNCBF5* and *BNCBF17*) in transgenic *Brassica napus* cv. Westar. Transgenic Brassica plants accumulated COR gene mRNA and exhibited freezing stress tolerance. They also observed moderate accumulation of transcripts of genes involved in photosynthesis and chloroplast development. The up-regulated transcripts included *GLK1* and *GLK2*-like transcription factors, cyclophilin *ROC4*, ß-amylase and triose-P/Pi translocator. Along changes in these transcript levels, transgenic plants showed improved photosynthetic capacity, and enhanced capacities of enzymes involved in the Calvin cycle and sucrose and starch biosynthesis.

Future prospects and challenges

Although a lot of efforts have been made in the past to develop abiotic stress tolerant cultivars through conventional approaches, these efforts have met with limited success (73). Progress in understanding molecular biology and the technological tools raised hopes of developing transgenic crop plants with enhanced abiotic stress tolerance. However, despite all claims, the level of abiotic stress tolerance in crop plants that was anticipated has not been fully achieved with transgenic technology (5, 62). One of the main reasons of this limited success of developing abiotic stress tolerant transgenic plants is transformation of a single gene that encodes a single protein thus leading to lower level stress tolerance. It is now well established that abiotic stress tolerance is of a multigenic character involving a large number of genes that work in diverse physiological, biochemical and molecular processes (6, 79).

During the recent past, research on the regulatory role of DREBs in transgenic plants under abiotic stress conditions has progressed with enormous proportion. The importance of DREBs in abiotic stress tolerance is evident from the increasing number of transgenic plants transformed with various DREB transcription factors, summarized in several review works (2, 40, 63). The use of DREBs has overcome to some extent the limited success of achieving abiotic stress tolerance associated with manipulation of individual genes that are involved in stress tolerance. DREB transcription factors conferred on transgenic plants enhanced tolerance to multiple abiotic stresses, which is attributed to the diverse function of DREBs through regulating expression of genes involved in abiotic stress responses. In addition to conferring multiple abiotic stress tolerance in crop plants, DREBs may have the potential to increase the water use efficiency of crop plants cultivated under drought-prone environments. In the near future, transgenic plants with DREBs will be ready for deployment to such environments, where these plants will have a profound impact on the surrounding human populations. Despite all these positive developments, research on transgenic plants with DREBs is confronted with several challenges that must be addressed to achieve a real success. Some of these are discussed below.

One such issue is the constitutive expression of DREBs in transgenic plants that not only conferred stress tolerance but also exerted negative effects on plant growth (**Table 2**). An alternative approach is the use of stress inducible promoters that has overcome the phenotypic abnormalities associated with constitutive promoters (33, 34). However, in some cases even constitutive expression of DREBs did not exert any growth abnormalities in transgenic plants. This is still unclear, whether the abnormal growth under constitutive expression has any link with the type of DREB transgene, its origin or the nature of transgenic plant.

Another challenge ahead is evaluation of DREB transgenic plants under natural stressful environments. So far, the abiotic stress tolerance of DREB expressing transgenic plants has been evaluated only under controlled or semi-confined conditions except a few examples where transgenic plants with DREB transgenes were tested under field conditions (**Table 2**). In the near future, these transgenic plants will be tested under field condition, where the actual performance of DREBs will be demonstrated. There is a likelihood that DREB transgenic plants may respond differently to environmental stresses under natural field conditions, where plants are exposed to a number of environmental factors. Under field conditions, combination of different stresses may occur simultaneously and may enhance the severity of stress on plants. For example, different stresses in combination such as drought and heat, drought and light stress, salt and heat, salt and light, and salt and UV may aggravate the damage to plants. It is also important to study plant responses to stresses in combination. In controlled conditions, transgenic plants are subjected to individual stresses and their responses are documented. However, under field conditions, transgenic plants may respond differently to these combinations of stresses. Moreover, in most of the experiments conducted under controlled conditions, stress tolerance was achieved at the early growth stages of plant development. It is still to be investigated in many cases, whether the transgenic plants will maintain the same tolerance during later stages of plant growth and at the whole plant level.

A challenging issue confronting deployment of abiotic stress tolerant transgenic plants modified with DREB transcription factors is the formulation of proper environmental risk assessment procedures that could efficiently identify the potential adverse effects of these plants on ecological entities of value (70). The nature of DREB-induced abiotic stress tolerance is different from that of first generation transgenic plants with monogenic traits such as insect and disease resistance and herbicide tolerance. Achieving abiotic stress tolerance is a complex process that involves wide-spread changes at molecular, physiological and biochemical levels and at the whole plant level. Changes in metabolic profiles set a stress response that confers protection to vital cellular proteins, organelles and membranes against osmotic and oxidative stresses. In addition to intended stress tolerance, it is assumed that these changes may also bring unintended or secondary effects with unpredictable non-target and ecological consequences. In one study, the effect of transcription factor ABF3 has been analysed in transgenic plants, however, transcriptome analysis did not reveal any unintended effects (1). DREB transgenes confer stress tolerance through up-regulation of the expression of a large number of stress-related genes working in different pathways. It may be possible that DREBs may have cascading effects on a variety of biosynthetic pathways leading to unintended effects in transgenic plants (48). These unintended effects may lead to changes in interaction of transgenic plants with non-target organisms resulting in adverse effects on biodiversity. The enhanced fitness of transgenic plants with DREB-induced abiotic stress tolerance may also have unpredictable ecological consequences in the form of increased weediness potential of the crop or its weedy relatives upon transgene escape (60). Other issues such as tolerance to more than one stress and the likelihood of changed responses to biotic stress factors due to the cross-talk between biotic and abiotic stress responses are important to consider in the risk assessment. Due to these concerns, the environmental risk assessment of these plants must need critical evaluation of the current risk assessment paradigm. So far, these regulatory frameworks have been used to address environmental constraints of simple monogenic traits of insect resistance and herbicide tolerance. At present some abiotic stress tolerant transgenic plants with stress tolerance genes such as DREBs are under field trials and soon will be ready for commercial cultivation (45, 46). In parallel, a rigorous debate is going on in the international foras regarding potential environmental effects of these transgenic plants (15). The general consensus developed as a result of these discussions is that transgenic plants with abiotic stress tolerance genes will need the same basic risk assessment procedures that have been used for first generation transgenic plants. Despite this, there is a growing need to examine whether these plants will need any additional issues to be considered in the risk assessment and whether additional measures will be required in the risk assessment methodologies. For environmental safety of abiotic stress tolerant transgenic plants with DREB genes, the risk assessment will need to focus on two main issues: 1) the occurrence of unintended effects; 2) and increased weediness and invasiveness potential.

In abiotic stress tolerant transgenic plants, unintended or unanticipated effects may arise due to stress tolerance-associated changes at the molecular, biochemical and physiological levels. These changes later on may result in plants with phenotypic changes, and accumulation of new toxins and anti-nutrients or at least changes in their composition. The combined effects of these modifications may alter plants interaction with target and non-target organisms, such as plant pests, parasitoids, predators and beneficial insects.

Genetic improvement of plants with genes conferring abiotic stress tolerance may increase the persistence and weediness potential of these plants. This concern has long been expressed with first generation insect resistant and herbicide tolerant transgenic plants. However, unlike these plants, abiotic stress tolerant transgenic plants are considered to have high potential of persistence in agricultural environment and invasiveness in natural environment, upon escape of transgenic plants by themselves or transgene escape to weedy relatives. Plant responses to abiotic stresses are mostly overlapping and tolerance to one type of stress often confers tolerance to other stresses such as transgenic plants with DREB genes exhibit tolerance to multiple stresses. Transgenic plants with drought or salt tolerance may also show tolerance to cold stress, resulting in enhanced volunteer potential in the next cropping season. In case the transgene escapes to wild relatives in the natural environment, it may confer to them fitness advantage resulting in increased weediness problem. The fitness advantage over surrounding plant communities may have unpredictable ecological consequences such as displacement of local plant communities, and alteration of plant interaction with non-target organisms. In order to evaluate the increased weediness and invasiveness potential in abiotic stress tolerant transgenic plants engineered with DREBs, emphasis should be placed on the problem formulation during environmental risk assessment.

Conclusions

Although engineering crop plants with DREB transcription factors has resulted in improved stress tolerance unlike the previous reports of limited tolerance with single genes, further efforts are needed to increase stress tolerance and improve plants ability to reduce yield losses under drought and saline conditions. Future research should focus on additional approaches including: 1) better understanding of the complex network of genes and the resultant metabolic and physiological changes during stress tolerance; 2) understanding of molecular determinants that simultaneously regulate responses to stress and plant developmental processes; 3) combining different approaches with multiple gene engineering to target diverse stress response mechanisms and obtain the desired traits and effects; 4) optimizing DREB technologies through reducing growth abnormalities and enhancing the introduced trait to

the level of commercial value. Optimization is achieved by modifying the expression of the DREB transcription factors by developmental, tissue specific and stress-inducible promoters. It can also be achieved through protein modification. Search for the best DREB genes from various plant species, which cause less detrimental effects on growth and yield, and further chimeric DREB transcription factors should be exploited using the DNA binding domain of DREBs and fusing it with optimal transcription activators such as VP16. Moreover, direct evolution of DREBs through DNA shuffling technology has the potential to enhance their DRE binding capacity resulting in enhanced functional activity. These efforts should be integrated to make DREBs more effective, target specific and stress specific.

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