

From Laboratory to Field. Using Information from Arabidopsis to Engineer Salt, Cold, and Drought Tolerance in Crops¹

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ARABIDOPSIS: A MODEL PLANT FOR STUDYING PLANT BIOLOGY

After almost a century since its first appearance in the scientific literature, Arabidopsis has now been widely adopted as a model plant of choice for biological research (Somerville and Koornneef, 2002). Its many advantages include a small genome, short life cycle, small stature, prolific seed production, and ease of transformation. In addition, a wealth of genomics resources exists, such as a completely sequenced genome, a near saturation insertion mutant collection, a genome array that contains the entire transcriptome, and more than 50,000 molecular markers. Add in a vibrant collaborative community, and it is easy to see why Arabidopsis is often the system of choice for plant research. While the culminating achievement of Arabidopsis research—the release of its complete genome sequence—is still fresh in memory, a new 10-year project of comparable importance has already begun. The ambitious goal of this program is to know the function of all Arabidopsis genes by 2010 (Somerville and Dangl, 2000). This knowledge will facilitate the development of a virtual plant—a computer model that will use information about each gene product to simulate the growth and development of a plant under many environmental conditions. However, it has not escaped many plant biologists that another goal is to use knowledge gained from research on Arabidopsis to facilitate the understanding of biological phenomena in other plant species. One specific challenge is how to utilize information gained from Arabidopsis research to produce new commercial plant varieties. In this perspective, we reflect on the critical role Arabidopsis is playing in unraveling abiotic stress signal transduction (focusing primarily on salt, cold, and drought stress), and how these new insights on the mechanisms of tolerance to these stresses are suggest-

ing novel approaches to engineer the next generation of biotech crops.

ARABIDOPSIS AND BEYOND: FROM CONCEPTS TO ENGINEERING STRESS TOLERANCE IN CROP PLANTS

Salt Stress in Arabidopsis

The potential of manipulating ion transporters to improve ion homeostasis is well recognized. For example, several classes of transporters are required in regulating sodium homeostasis under salt stress (Fig. 1). The influx of Na⁺ is controlled by AtHKT1, a low affinity Na⁺ transporter (Schachtman and Schroeder, 1994; Rus et al., 2001), and other nonselective cation channels, while the efflux is controlled by Salt Overly Sensitive1 (SOS1), a plasma membrane Na⁺/H⁺ antiporter (Shi et al., 2000). Vacuolar membrane ion transporters, such as the tonoplast Na⁺/H⁺ antiporter AtNHX1 (Apse et al., 1999; Gaxiola et al., 1999), also play a vital role in regulating cytoplasm Na⁺ homeostasis by sequestering Na⁺ ions in the vacuole. The sensing of Na⁺ in plant cells is still unknown but is speculated to involve SOS1 (Fig. 1). It has been suggested that the long C-terminal tail predicted to reside in the cytoplasm might be a sensor of cytoplasm Na⁺ (Shi et al., 2000; Zhu, 2003). Similarly, evidence suggests that the C terminus of AtNHX1, present in the vacuolar lumen, may also have a regulatory role (Yamaguchi et al., 2003). It is speculated that Na⁺ sensors regulate the cytoplasmic Ca²⁺ level, which triggers the SOS signal transduction chain: SOS3, a myristoylated calcium binding protein, interacts with and activates the SOS2 kinase, which phosphorylates and activates SOS1, the plasma membrane Na⁺/H⁺ antiporter (Zhu, 2002). There is also evidence that the SOS2 kinase positively regulates the activities of AtNHX1 and CAX1 (a vacuolar Ca²⁺/H⁺ exchanger) and may negatively regulate AtHKT1 (Cheng et al., 2004; Qiu et al., 2004). Therefore, coordination exists between the transporters in the tonoplast and plasma membranes in part through the SOS2 kinase (Cheng et al., 2004; Qiu et al., 2004).

Readers are referred to a recent publication for an extensive review of the regulation of ion homeostasis

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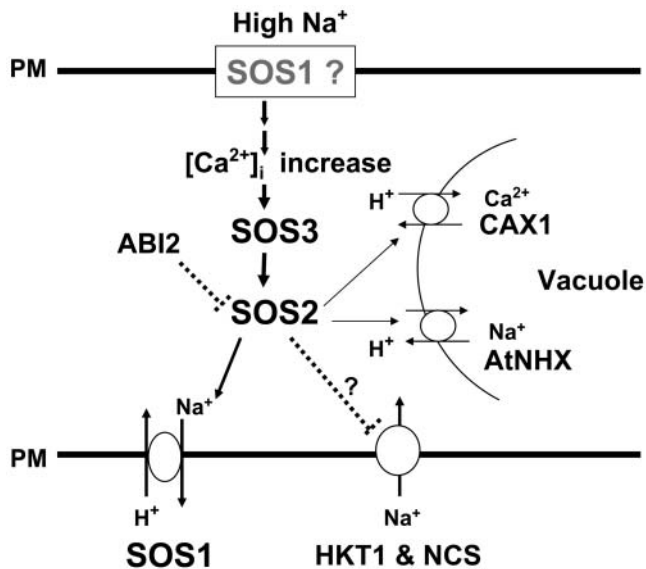


Figure 1. The SOS signaling pathway for the regulation of Na⁺ homeostasis and salt tolerance in Arabidopsis. High Na⁺ stress triggers a calcium signal that activates the SOS3-SOS2 protein kinase complex, which then stimulates the Na⁺/H⁺ exchange activity of SOS1 at the plasma membrane. SOS2 also activates Na⁺/H⁺ (AtNHX) and Ca²⁺/H⁺ (CAX1) exchangers on the vacuolar membrane. The protein phosphatase ABI2 has been shown to physically interact with SOS2 and is proposed to inactivate SOS2. The SOS pathway may down-regulate the activity of Na⁺ influx transporters (AtHKT1 and NCS). SOS1 in gray indicates that this transporter may also have a sensory role. Dotted lines indicate possible regulation.

under salt stress (Zhu, 2003). It is noteworthy that Arabidopsis has played a vital role in many investigations of the basic processes that occur during salt stress. For example, SOS1/2/3 were identified by Arabidopsis mutants and position-based cloning (Liu and Zhu, 1998; Liu et al., 2000; Shi et al., 2000); AtNHX1 was discovered from Arabidopsis genome sequences (Apse et al., 1999; Gaxiola et al., 1999); and HKT1, initially identified in wheat (*Triticum aestivum*; Schachtman and Schroeder, 1994), has been shown through the study of Arabidopsis knockout mutants to be an important Na⁺ influx system in plant roots (Rus et al., 2001) and to play a critical role in controlling the long distance transport of Na⁺ (Maser et al., 2002; Berthomieu et al., 2003). These and other examples illustrated how mutant isolation, position-based cloning, and gene expression analysis in Arabidopsis can be combined with biochemical and physiological studies to unravel the inner workings of salt stress signal transduction (Zhu, 2002).

Translation of Arabidopsis Models to Engineer Salt Tolerance in Crop Plants

It is generally accepted that maintaining a low cytosolic Na⁺ concentration is essential to achieve salt tolerance and can be achieved by restricting inflow, increasing outflow, or increasing vacuole sequestra-

tion of Na⁺. Intuitively, increasing plasma membrane Na⁺ exporters and tonoplast Na⁺ importers and/or restricting the amount of Na⁺ influx by lowering the amount of plasma membrane Na⁺ importers should suffice. Indeed, a number of successes have ensued when these strategies were used (see Table I). For example, increased expression of the Arabidopsis tonoplast membrane Na⁺/H⁺ antiporter, AtNHX1, under a strong constitutive promoter was reported to result in salt-tolerant Arabidopsis (Apse et al., 1999), *Brassica napus* (Zhang et al., 2001), and tomato (*Lycopersicon esculentum*; Zhang and Blumwald, 2001). AgNHX1, a AtNHX1 ortholog from the halophytic plant *Atriplex gmelini* (Hamada et al., 2001), when overexpressed in rice (*Oryza sativa*) plants, improved salt tolerance of the transgenic rice (Ohta et al., 2002). AtNHX1 orthologs from many plant species have been isolated, mostly based on their sequence homology to the Arabidopsis gene. Examples include NHX1 genes from rice (Fukuda et al., 1999), wheat (Chen et al., 2002), Japanese morning glory (Yamaguchi et al., 2001), *A. gmelini* (Hamada et al., 2001), ice plant (Chauhan et al., 2000), and sugar beet (Xia et al., 2002). Thus, the NHX1 system seems to be highly conserved between many different plant species, and manipulation of this system in crop species will likely result in improved salt tolerance. In addition to NHX1, other transporters have also been used successfully. Overexpression of the plasma membrane Na⁺/H⁺ antiporter SOS1 in Arabidopsis (Shi et al., 2003), increased expression of vacuolar H⁺-ATPase AVP1 in Arabidopsis (Gaxiola et al., 2001), and antisense of the wheat high affinity K⁺ transporter HKT1 in wheat (Laurie et al., 2002) also have resulted in improved salt tolerance (Table I).

Cold and Drought Stress in Arabidopsis

The signal transduction events that occur during cold and drought stress have recently been reviewed (Shinozaki et al., 2003). In contrast to ion homeostasis, a plant's adaptation to cold and drought is to a greater extent under transcriptional control—some processes are regulated by abscisic acid (ABA), while others are ABA independent (Shinozaki et al., 2003). In this perspective, we will look at the transcription factors (TFs) involved in the adaptation to cold and drought stress (Fig. 2).

The regulation of gene expression by ABA has been reviewed in great detail elsewhere (Finkelstein et al., 2002; Himmelbach et al., 2003; Kuhn and Schroeder, 2003). Mediators of ABA-triggered gene expression include TFs belonging to many different classes, including the bZIP, MYC/MYB, homeodomain Leu zipper (or HD-Zip), Zn finger, and ABI3/VP1 families. The bZIP TFs bind as dimers to ABA response elements (ABREs), with optimal ABA responsiveness usually requiring a second cis-element or coupling element (CE; Shen and Ho, 1995). The CE element is sometimes similar to a dehydration-responsive element

Table 1. Engineered abiotic stress tolerance: from ion transporters to transcription factors

Gene Name	Gene Source	Transgenic Species	Intervention Method	Traits	Reference
Ion Transporters					
AtNHX1 (vacuolar Na ⁺ /H ⁺ antiporter)	Arabidopsis	Arabidopsis	Overexpression	Salt tolerance	Apse et al. (1999)
AtNHX1 (vacuolar Na ⁺ /H ⁺ antiporter)	Arabidopsis	<i>B. napus</i>	Overexpression	Salt tolerance	Zhang et al. (2001)
AtNHX1 (vacuolar Na ⁺ /H ⁺ antiporter)	Arabidopsis	Tomato	Overexpression	Salt tolerance	Zhang and Blumwald (2001)
AtNHX1 (vacuolar Na ⁺ /H ⁺ antiporter)	<i>A. gmelini</i>	Rice	Overexpression	Salt tolerance	Ohta et al. (2002)
SOS1 (plasma membrane Na ⁺ /H ⁺ antiporter)	Arabidopsis	Arabidopsis	Overexpression	Salt tolerance	Shi et al. (2003)
AVP1 (vacuolar H ⁺ -ATPase)	Arabidopsis	Arabidopsis	Overexpression	Salt tolerance	Gaxiola et al. (2001)
HKT1 (high affinity K ⁺ transporter)	Wheat	Wheat	Antisense	Salt tolerance	Laurie et al. (2002)
Transcription Factors					
CBF1, DREB1a (CBF3)	Arabidopsis	Arabidopsis	Overexpression	Freezing, salt, and drought tolerance	Jaglo-Ottosen et al. (1998); Liu et al. (1998); Haake et al. (2002)
CBF1, CBF2, CBF3	Arabidopsis	<i>B. napus</i>	Overexpression	Freezing and drought tolerance	Jaglo et al. (2001); Mendel Biotechnology (unpublished data)
CBF1	Arabidopsis	Tomato	Overexpression	Drought, chilling, and oxidative stress tolerance	Hsieh et al. (2002a, 2002b); Lee et al. (2003)
CBF1	Arabidopsis	Strawberry	Overexpression	Freezing tolerance	Owens et al. (2002)
ZmCBF	Maize	Maize	Overexpression	Cold tolerance	Chaiappetta (2002)
DREB1a (CBF3)	Arabidopsis	Wheat	Overexpression	Drought tolerance	Pellegrineschi et al. (2002)
OsDREB1A	Rice	Arabidopsis	Overexpression	Drought, salt, and freezing tolerance	Dubouzet et al. (2003)
CBF3	Arabidopsis	Rice	Overexpression	Stress tolerance	J. K. Kim (personal communication)
ICE1	Arabidopsis	Arabidopsis	Overexpression	Freezing tolerance	Chinnusamy et al. (2003)
ABF3/4	Arabidopsis	Arabidopsis	Overexpression	Drought tolerance	Kang et al. (2002)
Tsi1	Tobacco	Tobacco	Overexpression	Salt tolerance	Park et al. (2001)
SCOF-1	Soybean	Arabidopsis, Tobacco	Overexpression	Low temperature stress tolerance	Kim et al. (2001)
AtMYC2/AtMYB2	Arabidopsis	Arabidopsis	Overexpression	Drought tolerance	Abe et al. (2003)

(CRT/DRE), thus ABRE-binding bZIPs and CRT/DRE-binding AP2s possibly interact to control ABA-regulated gene expression (Narusaka et al., 2003). ABI5 is active mainly during seed maturation and early seedling development (Finkelstein and Lynch, 2000), whereas the AREB/ABF appears to function later in development (Uno et al., 2000; Kang et al., 2002). For some bZIPs, other TFs may be necessary for optimum transcriptional activation. For example, ABI5 is able to form a complex with ABI3 (Nakamura et al., 2001) and thereby recruit ABI3 to ABRE-containing promoters, even though ABI3 does not bind ABREs directly (Lopez-Molina et al., 2002). In another example, the binding efficiency of the bZIP TF SGBF-1 to ABREs is enhanced by another protein, a C2H2 Zn finger protein, SCOF-1, even though SCOF-1 does not bind directly to ABREs (Kim et al., 2001). The activity of some bZIPs may also be regulated by phosphorylation. For example, the phosphorylation of ABI5 stabilizes this TF (Lopez-Molina et al., 2001), perhaps by blocking the

ABI5 BINDING PROTEIN-mediated degradation by the 26S proteasome (Lopez-Molina et al., 2003). The homeodomain TF AtHB6 (Himmelbach et al., 2002), which interacts with ABI1, can also heterodimerize with AtHB5 and possibly with other HD-Zip TFs (Johannesson et al., 2003), while AtMYC2 and AtMYB2 act cooperatively to activate the expression of ABA-inducible genes such as RD22 (Abe et al., 2003).

One class of AP2 TFs that plays a central role in both the ABA-dependent and ABA-independent pathways is the CRT binding factors (CBFs; also called DREB1s). Expression of all CBF genes in Arabidopsis is low under normal growth condition but increases within several minutes after cold (CBF1-3; Gilmour et al., 1998) or drought stress (CBF4; Haake et al., 2002). In addition, the DNA-binding activity of some CBFs can also be modulated by temperature—it was reported that the binding affinity of a barley (*Hordeum vulgare*) CBF to the CRT/DRE element at 0°C was more than 10 times higher than that at 25°C (Xue, 2003). The

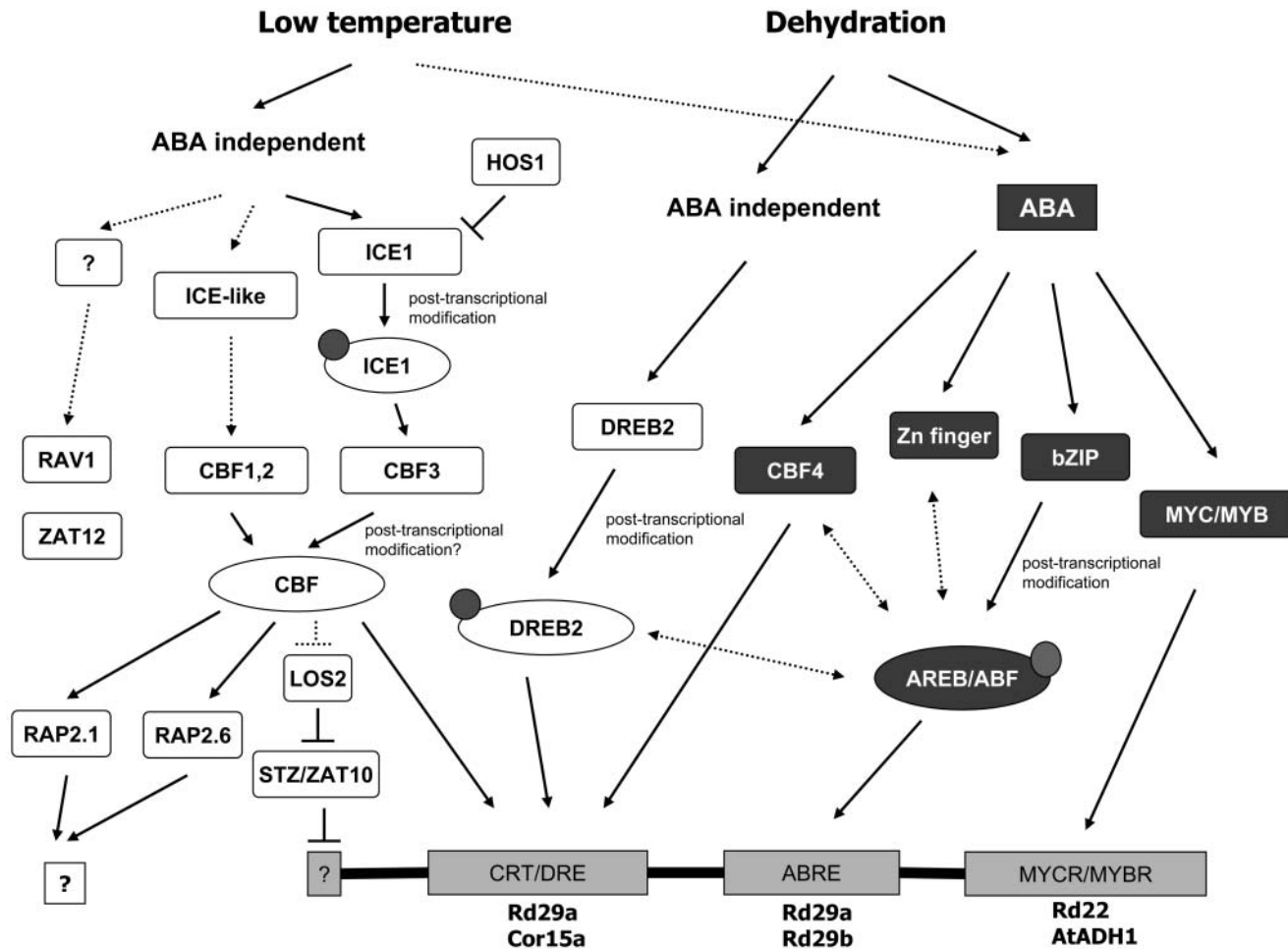


Figure 2. Transcriptional cascades of low temperature and dehydration signal transduction. ABA-dependent transcriptional factors are shaded, while ABA independent factors are not. Small circles indicate posttranscriptional modification, such as phosphorylation. Transcription factor binding sites are represented as rectangles at the bottom of the figure, with the representative promoters listed below. Dotted lines indicate possible regulation. Double arrow lines indicate possible cross talk.

cold induction of the Arabidopsis CBF1-3 genes is ABA independent, while the dehydration induced expression of the CBF4 gene is controlled by ABA (Haake et al., 2002).

In order to identify regulators of the CBF genes, Arabidopsis mutants that either impact cold-inducible gene expression under stress or its ability to survive freezing have been isolated and studied. One study identified a bHLH TF, ICE1, which binds specifically to the MYC recognition sequences in the CBF3 promoter and activates CBF3 expression in the cold (Chinnusamy et al., 2003). It is likely that other ICE-like proteins exist because mutational analysis of the CBF2 promoter identified two segments, designated ICer1 and ICer2, that work in concert to impart cold-regulated CBF2 expression (Zarka et al., 2003). The *sfr6* mutant of Arabidopsis was identified on the basis of its inability to gain freezing tolerance after cold acclimation (Warren et al., 1996; Knight et al., 1999). Transcriptome analysis indicates that the *sfr6* mutant is deficient in CRT/DRE-regulated COR gene expression during cold, osmotic stress, or exogenous ABA (Boyce et al.,

2003), indicating that SFR6 could interact with the CBFs or DREB2s. In contrast to the *sfr6* mutation, the *hos1* mutant has higher levels of CBF2 and CBF3 (and downstream COR gene) induction under stress (Lee et al., 2001). HOS1 encodes a RING finger protein that may function in the ubiquitin-mediated degradation of nuclear proteins. It is possible that the elevated CBF transcript levels in *hos1* mutant could be a consequence of decreased turnover of ICE proteins (Lee et al., 2001).

Other TFs that either operate in parallel pathways or downstream of CBF have also been identified from Arabidopsis (Fig. 2). AP2 TF DREB2 plays a role in drought adaptation in an ABA-independent manner (Liu et al., 1998; Nakashima et al., 2000). Two transcription factors, RAV1 (AP2; Kagaya et al., 1999) and ZAT12 (Zn finger; Meissner and Michael, 1997) had patterns of expression that were similar to those of CBF1-3, and because neither RAV1 nor ZAT12 transcript levels were affected in CBF1-3 overexpressing plants, they probably operate in pathways that are parallel to those of the CBFs (Fowler and Thomashow, 2002). By contrast, two AP2 domain proteins, RAP2.6

and RAP2.1 (Okamuro et al., 1997), were induced after the initial wave of CBF1-3, RAV1, and ZAT12 inductions. The RAP2.1 promoter contains two copies of the CCGAC core sequence of the CRT/DRE elements, and RAP2.1 expression was high in transgenic Arabidopsis plants that constitutively express CBF1-3, suggesting that it might be a target of the CBF activators (Fowler and Thomashow, 2002). LOS2, identified from an Arabidopsis mutant screen, may repress expression of a zinc finger transcriptional repressor STZ/ZAT10, which in turn regulates COR/RD gene expression (Lee et al., 2002).

Engineering Cold and Drought Tolerance in Crop Plants Based on Arabidopsis Models

Because many aspects of the cold and drought adaptation process are under transcriptional control, it is not surprising that transcription factors represent one of the best targets for engineering plants to achieve enhanced cold and drought tolerance. Even so, not all TFs involved in the cold and drought signal transduction are suitable targets for biotechnological intervention. For example, even though the DREB2s may play a major role in drought-regulated gene expression, overexpression of the DREB2 cDNAs in transgenic plants only caused weak induction of the downstream genes and did not result in more stress tolerance (Liu et al., 1998). It is speculated that post-translational alterations may be needed for these proteins to be active in transgenic plants (Shinozaki and Yamaguchi-Shinozaki, 2000).

The CBF genes have been successfully used to engineer abiotic stress tolerance in a number of different species (Table I). The orthologous genes of CBF have been found in most crop plants examined so far, including canola (*B. napus*), soybean (*Glycine max*), broccoli (*Brassica oleracea*), tomato, alfalfa (*Medicago sativa*), tobacco (*Nicotiana tabacum*), cherry (*Prunus avium*), strawberry (*Fragaria* spp.), wheat, rye (*Secale cereale*), corn (*Zea mays*), rice, and barley (Jaglo et al., 2001; Choi et al., 2002; Gao et al., 2002; Owens et al., 2002; Dubouzet et al., 2003; Francia et al., 2004; Shen et al., 2003a, 2003b; Vagujfalvi et al., 2003; Xue, 2003). Many of the putative orthologs have been functionally tested, indicating conservation of the pathway in those plant species. Constitutive overexpression of the Arabidopsis CBF genes in canola results in increased freezing tolerance (J.Z. Zhang, unpublished data; Fig. 3). Similarly, constitutive overexpression of CBF orthologs from rice (OsDREB1) in transgenic Arabidopsis resulted in salt, cold, and drought tolerance (Dubouzet et al., 2003). Likewise, the overexpression of a ZmCBF gene in maize also resulted in increased cold tolerance (Chaiappetta, 2002). In these and other studies, it was discovered that ectopic overexpression of the CBF genes in plants produced, in addition to increased stress tolerance, dark-green, dwarfed plants with higher levels of soluble sugars and Pro (Liu et al.,

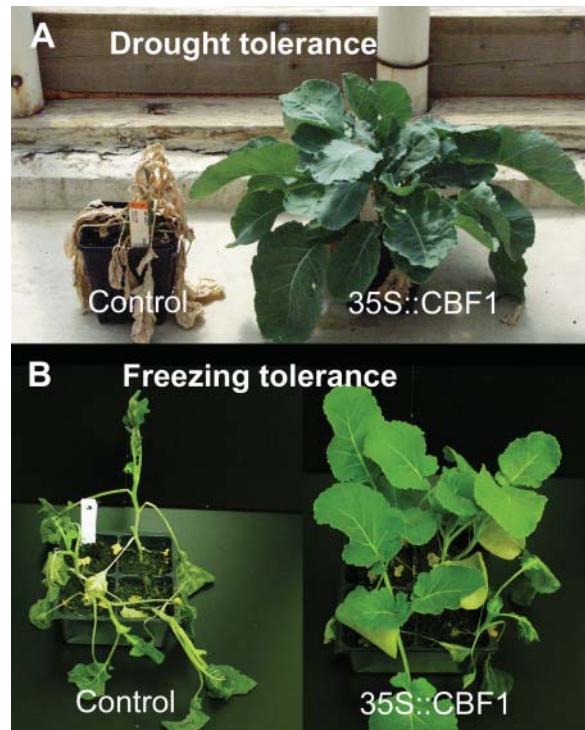


Figure 3. Engineered drought and freezing tolerance in transgenic *B. napus* through constitutive expression of CBF1 (Jaglo et al., 2001). A, Three-week-old plants were frozen at -6°C for 2 d and then let to recover for 2 d at 28°C before pictures were taken. B, Seven-week-old greenhouse grown plants were withheld water for 1 week and then rewatered for 2 weeks before picture was taken.

1998; Gilmour et al., 2000). To overcome these problems, stress-inducible promoters that have low background expression under normal growth condition have been used in conjunction with the CBF genes to achieve increased stress tolerance without the retarded growth (Kasuga et al., 1999; Lee et al., 2003).

CONCLUSIONS AND PERSPECTIVES

As examples from the above discussions have shown, Arabidopsis has been an excellent model plant for the studying of abiotic stress responses and biotechnology applications. In many cases, not only are structural proteins such as the ion transporters conserved between Arabidopsis and other plant species, but also regulatory proteins such as CBF/DREB1 and entire transcriptional regulons can be conserved as well. Only after we thoroughly understand how plants respond to stress—in many cases first in Arabidopsis and then applying the Arabidopsis model to crop plants—will we be able to begin engineering stress tolerance. A case in point: While the overexpression of Na^+/H^+ antiporters is sufficient to achieve measurable improvement in salt tolerance in plants, the engineering of robust cold and drought tolerance requires the coordinated expression of many genes through the altered expression of global regulators

such as CBFs. Therefore, Arabidopsis will continue to play a critical role in the foreseeable future, not only in the understanding of biological mechanisms of abiotic stress tolerance, but also in providing a facile means for testing in biotechnology.

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