Transcriptional Regulatory Networks in Response to Abiotic Stresses in Arabidopsis and Grasses[1]

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Various abiotic stresses such as drought, high salinity, high temperature, and low temperature negatively impact plant growth and productivity of crops. Plants have adapted to respond to these stresses at the molecular, cellular, physiological, and biochemical level, enabling them to survive. Various adverse environmental stresses induce the expression of a variety of genes in many plant species (Xiong et al., 2002; Shinozaki et al., 2003; Bartels and Sunkar, 2005). Numerous stress-induced genes have been identified using microarray experiments (Kreps et al., 2002; Seki et al., 2002). The products of these genes are thought to promote stress tolerance and to regulate gene expression through signal transduction pathways (Xiong et al., 2002; Shinozaki et al., 2003).

Abscisic acid (ABA) is produced under water deficit conditions and plays an important role in the stress response and tolerance of plants to drought and high salinity. Exogenous application of ABA induces a number of genes that respond to dehydration and cold stress (Zhu, 2002; Shinozaki et al., 2003). Several reports have characterized genes that are induced by dehydration and cold stress, but do not respond to exogenous application of ABA in Arabidopsis (*Arabidopsis thaliana*; Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2006). This suggests the existence of ABA-independent and ABA-dependent signal transduction pathways that convert the initial stress signal into cellular responses. To better understand the molecular mechanisms regulating gene expression in response to abiotic stresses, including dehydration and cold stress, studies have initially focused on the analysis of Arabidopsis cis- and trans-acting elements and their role in mediating stress responses (Yamaguchi-Shinozaki and Shinozaki, 2006). Recently, abiotic stress-inducible genes and their cis- and trans-acting elements were also studied in rice (*Oryza sativa*), a preferred crop plant to study stress responses because of its commercial value, relatively small genome size (approximately 430 Mb), diploid origin (2x = 24), and close relationship to other important cereal crops.

Transcription factors (TFs) are master regulators that control gene clusters. A single TF can control the expression of many target genes through specific binding of the TF to the cis-acting element in the promoters of respective target genes. This type of transcriptional regulatory system is called regulon. Several major regulons that are active in response to abiotic stress have been identified in Arabidopsis. Dehydration-responsive element binding protein 1 (DREB1)/C-repeat binding factor (CBF) and DREB2 regulons function in ABA-independent gene expression, whereas the ABA-responsive element (ABRE) binding protein (AREB)/ABRE binding factor (ABF) regulon functions in ABA-dependent gene expression (Fig. 1). In addition to these major pathways, other regulons, including the NAC and MYB/MYC regulons, are involved in abiotic stress-responsive gene expression. Recent studies demonstrated that DREB1/CBF, DREB2, AREB/ABF, and NAC regulons have important roles in response to abiotic stresses in rice (Fig. 1). In this article, we focus on the regulation of gene expression in response to dehydration, high salinity, cold, and heat stresses, with particular emphasis on the role of DREB1/CBF, DREB2, AREB/ABF, and NAC regulons in grasses, including important crops such as rice, wheat (*Triticum aestivum*), maize (*Zea mays*), and barley (*Hordeum vulgare*), in comparison to Arabidopsis. For a more comprehensive overview on the very complex signal transduction pathways controlling abiotic stress responses, we refer the reader to the many excellent review articles that have recently been published (Chinnusamy et al., 2004; Bartels and Sunkar, 2005; Sunkar et al., 2007).

DREB1/CBF AND DREB2 REGULONS

Identification and Expression of DREB1/CBF and DREB2 in Arabidopsis

The dehydration-responsive element (DRE) containing the core sequence A/GCCGAC was identified as an ABA-independent cis-acting element important for

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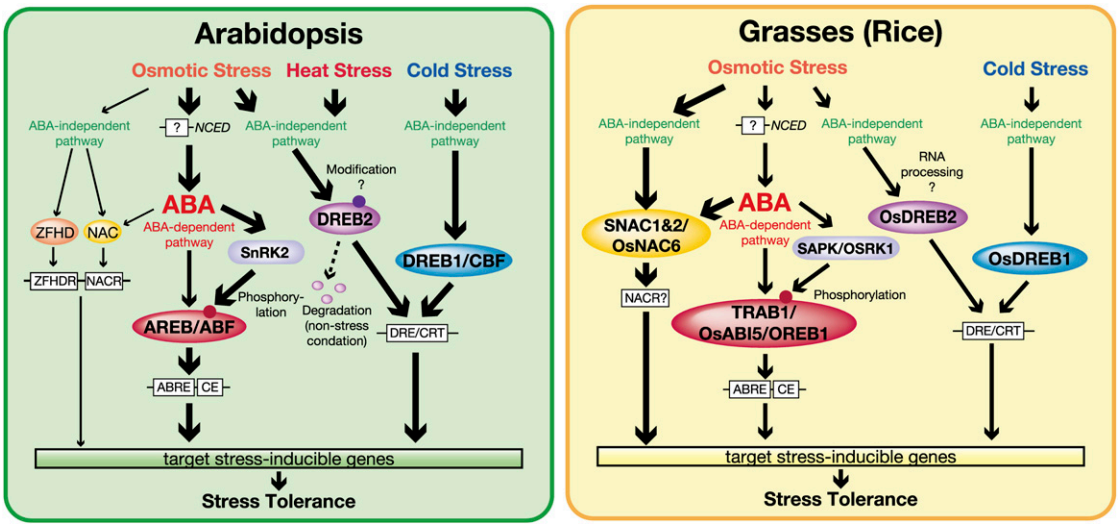

Figure 1. Major transcriptional regulatory networks of cis-acting elements and TFs involved in abiotic stress-responsive gene expression in Arabidopsis and grasses such as rice. TFs controlling stress-inducible gene expression are shown in ellipses. cis-Acting elements involved in stress-responsive transcription are shown in white boxes. Protein kinases involved in phosphorylation of TFs are shown in light purple boxes. Small filled circles illustrate modifications of TFs, for example, through phosphorylation, in response to stress signals.

the regulation of gene expression in response to drought, high salinity, and cold stresses in Arabidopsis (Yamaguchi-Shinozaki and Shinozaki, 1994). Similar cis-acting elements, named C-repeat (CRT) and low-temperature-responsive element, both containing the DRE core motif, are present in cold-inducible genes (Baker et al., 1994; Jiang et al., 1996). DRE/CRTs were identified in many promoters of stress-inducible genes from various plants, including Arabidopsis and grasses. The Arabidopsis cDNAs encoding DREB1/CBF and DREB2 were isolated using yeast one-hybrid screening (Stockinger et al., 1997; Liu et al., 1998). These proteins contain APETALA2 (AP2)/ethylene-responsive element binding factor (ERF) motifs and specifically bind to the DRE/CRT sequence, thereby activating transcription of genes driven by the DRE/CRT sequence in Arabidopsis. The AP2/ERF motif is specific to plants and functions as a DNA-binding domain. The Arabidopsis genome contains six *DREB1/CBF* genes and eight *DREB2* genes (Sakuma et al., 2002), including *DREB1A*, *DREB1B*, and *DREB1C*, which are rapidly and transiently induced by cold, but not by dehydration and high-salinity stress (Liu et al., 1998; Fig. 1). By contrast, *DREB2A* and *DREB2B* genes are induced upon dehydration and high salinity, but not by cold stress (Liu et al., 1998; Nakashima et al., 2000; Fig. 1). Transcript levels of three other *DREB1*- and six other *DREB2*-related genes were low under these stress conditions (Sakuma et al., 2002). DREB1A, DREB1B, and DREB1C proteins are major TFs required for cold-inducible gene expression, whereas the Arabidopsis DREB2A and DREB2B are major TFs required for high salinity- and drought-inducible gene expression, respectively. However, *CBF4/DREB1D*, *DREB1E/DDF2*, and *DREB1F/DDF1* are weakly induced by osmotic stress, suggesting the existence of cross talk between the DREB1/CBF and the DREB2 pathways under osmotic stresses (Haake et al., 2002; Sakuma et al., 2002; Magome et al., 2004).

The DREB1/CBF Regulon in Response to Cold Stress in Arabidopsis

Transgenic Arabidopsis plants overexpressing *DREB1B/CBF1* or *DREB1A/CBF3* under control of the cauliflower mosaic virus (CaMV) *35S* promoter showed strong tolerance to drought, high salinity, and freezing (Jaglo-Ottosen et al., 1998; Liu et al., 1998; Kasuga et al., 1999), suggesting that DREBs/CBFs target multiple genes. In fact, more than 40 target genes of DREB1/CBF have been identified using both cDNA and GeneChip microarrays (Seki et al., 2001; Fowler and Thomashow, 2002; Maruyama et al., 2004). Most of those target genes contained the DRE/CRT or related core motifs in their promoters (Maruyama et al., 2004). Examples of the target genes include TFs, phospholipase C, an RNA-binding protein, a sugar transport protein, a desaturase, carbohydrate metabolism-related proteins, LEA (late embryogenesis abundant) proteins, KIN (cold-inducible) proteins, osmoprotectant biosynthesis proteins, and protease inhibitors. Most of the target proteins are known to function in response to stress and are thought to be responsible for the observed stress tolerance of the transgenic overexpressor lines. In fact, the transgenic plants overexpressing *DREB1A/CBF3* accumulated osmoprotectants, such as Pro and various sugars, under control (i.e. non-stress condition; Gilmour et al., 2000).

However, overexpression of DREB1/CBF also caused severe growth retardation under optimal growth conditions. To overcome the negative impact on growth and development, the stress-inducible *RD29A* promoter instead of the strong constitutive CaMV *35S* promoter was used for overexpression of *DREB1A* (Kasuga et al., 1999).

DREB1/CBF Regulon in Response to Cold Stress in Grasses

DREB1/CBF-homologous genes were identified in various grasses such as rice, wheat, diploid wheat (*Triticum monococcum*), barley, wild barley (*Hordeum spontaneum*), maize, sorghum (*Sorghum bicolor*), rye (*Secale cereale*), oat (*Avena sativa*), and perennial ryegrass (*Lolium tremulentum*; Dubouzet et al., 2003; Xue, 2003; Qin et al., 2004; Bräutigam et al., 2005, Skinner et al., 2005; Vágújfalvi et al., 2005; Xiong and Fei, 2006; James et al., 2008; Zhao and Bughrara, 2008). In rice, a low-temperature-sensitive cereal, four *DREB1/CBF* homologous genes, *OsDREB1A*, *OsDREB1B*, *OsDREB1C*, and *OsDREB1D*, have been isolated (Dubouzet et al., 2003). The DREB1/CBF-type TF ZmDREB1A was also identified in maize (Qin et al., 2004). Skinner et al. (2005) reported that barley, a diploid Triticeae model and a low-temperature-tolerant cereal, contains a large DREB1/CBF family consisting of at least 20 genes (*HvCBF*s). The increase in size and complexity of the barley DREB1/CBF family might be derived from the genome size (barley, 5 Gb; rice, 430 Mb; Arabidopsis, 145 Mb). Most of these *DREB1* genes showed a rapid response to cold stress (Fig. 1). Overexpression of *DREB1/CBF* homologous genes from rice, maize, barley, perennial ryegrass, and wheat in transgenic Arabidopsis or tobacco (*Nicotiana tabacum*) plants resulted in growth retardation and in overexpression of stress-inducible genes downstream of Arabidopsis DREB1A under nonstress conditions. Furthermore, these overexpressor lines exhibited tolerance to abiotic stresses such as drought and freezing (Dubouzet et al., 2003; Qin et al., 2004; Skinner et al., 2005; Xiong and Fei, 2006; Zhao and Bughrara, 2008). These results suggest that the *DREB1/CBF* genes from various grasses can function as TFs similar to the Arabidopsis DREB1A TF.

Several DREB1/CBFs were overexpressed in various grasses such as rice, wheat, tall fescue (*Festuca grundinacea*), and bahiagrass (*Paspalum notatum*; Lee et al., 2004; Pellegrineschi et al., 2004; Oh et al., 2005; Ito et al., 2006; Zhao et al., 2007; James et al., 2008). These transgenic plants showed tolerance to drought, high salinity, or cold stress. In transgenic rice plants, some downstream genes were induced under control conditions by overexpression of Arabidopsis *DREB1B/CBF1*, *DREB1A/CBF3*, or rice *OsDREB1A* (Lee et al., 2004; Oh et al., 2005; Ito et al., 2006). Transgenic rice constitutively overexpressing Arabidopsis *DREB1A* accumulated osmoprotectants, including Pro and various sugars, under control conditions (Ito et al., 2006). Transgenic tall fescue, which contained *DREB1A* driven by the stress-inducible *RD29A* promoter, accumulated a significantly higher level of Pro under drought conditions than control plants (Zhao et al., 2007). These data suggest that the DRE/DREB1 regulon exists in a variety of grasses and can be used to improve tolerance to drought, high salinity, and cold stresses by gene transfer in economically important cereals. Interestingly, an *Fr-A^{m}2* or *Fr-H2* locus was identified as a quantitative trait locus that functions in tolerance to cold stress. Some *DREB1/CBF* genes map to these loci in diploid wheat and barley, respectively (Vágújfalvi et al., 2005; Francia et al., 2007). In addition, mRNA levels of the *DREB1/CBF* genes within the *Fr-A^{m}2* or *Fr-H2* locus correlated with frost resistance (Vágújfalvi et al., 2005; Stockinger et al., 2007).

Taken together, the DREB1/CBF regulon responds to cold stress and functions in stress tolerance without posttranscriptional modification in most grasses. Therefore, induction of the *DREB1/CBF* genes in response to cold appears to be important for regulation of this regulon (Fig. 1).

DREB2 Regulon in Response to Osmotic and Heat Stress in Arabidopsis

The *DREB2A* gene was isolated as a gene encoding a DRE/CRT-binding protein and was shown to be induced by osmotic stresses (Liu et al., 1998). However, investigations studying the function of *DREB2* genes did not proceed as rapidly as those analyzing the role of *DREB1* genes because overexpression of *DREB2A* did not result in any phenotypic changes in transgenic plants (Liu et al., 1998). Domain analysis of DREB2A using Arabidopsis protoplasts revealed that the central region of DREB2A contains a negative regulatory domain and that deletion of this region makes DREB2A constitutively active (DREB2A-CA). Overexpression of *DREB2A-CA* in transgenic Arabidopsis induced expected phenotypic changes, including dwarfism, up-regulation of many stress-inducible downstream genes, and drought tolerance. When DREB2A-CA protein was fused to GFP, stable expression (i.e. green fluorescence) was observed in the nucleus. However, full-length DREB2A proteins fused to GFP protein were not stable in the nuclei of transgenic Arabidopsis plants, suggesting that the central region of DREB2A is required for regulation of the stability of DREB2A proteins. DREB2A protein degradation is thought to be mediated by DRIPs (DREB2A-interacting protein, C3HC4 RING domain-containing proteins), which bind to DREB2A. DRIP proteins function as E3 ubiquitin ligases and are capable of mediating DREB2A ubiquitination, as was revealed in an in vitro ubiquitination assay (Qin et al., 2008).

Although DREB2A was isolated together with DREB1A (Liu et al., 1998), it was found that some DREB2A downstream target genes were different from those of DREB1A. The reason for this probably lies in the fact that the DNA-binding specificity of DREB2A slightly differs from that of DREB1A. Specifically, DREB1A has a high affinity to A/GCCGACNT sequences, whereas

DREB2A preferentially binds ACCGAC motifs (Sakuma et al., 2006a). Overexpression of *DREB2A-CA* also induced expression of heat shock (HS)-related genes and improved thermotolerance in transgenic plants (Sakuma et al., 2006b). Furthermore, overexpression of *DREB2C* was found to induce the expression of many HS stress-inducible genes, resulting in thermotolerance in transgenic Arabidopsis (Lim et al., 2007). Collectively, these data indicate that DREB2 regulons function in both osmotic and HS stress responses (Fig. 1).

DREB2 Regulons in Response to Dehydration and High Salinity in Grasses

DREB2 homologous genes were isolated in grasses such as rice, wheat, barley, maize, and pearl millet (*Pennisetum glaucum*; Dubouzet et al., 2003; Shen et al., 2003; Xue and Loveridge, 2004; Egawa et al., 2006; Qin et al., 2007; Agarwal et al., 2007). Most of them respond to high salinity and drought (Fig. 1). The maize *ZmDREB2A* was responsive to high temperature, which was also shown for the Arabidopsis *DREB2A* gene (Qin et al., 2007). Wheat *TaDREB1*, wheat *WDREB2*, maize *ZmDREB2A*, and pearl millet *PgDREB2* are also responsive to cold stress, whereas Arabidopsis *DREB2A* and rice *OsDREB2A* are not (Dubouzet et al., 2003; Shen et al., 2003; Egawa et al., 2006; Agarwal et al., 2007; Qin et al., 2007). *DREB2* transcripts were shown to be regulated by alternative splicing in barley, wheat, and maize. Although nonfunctional forms of transcripts accumulated, functional forms of transcripts were induced by stress treatments (Xue and Loveridge, 2004; Egawa et al., 2006; Qin et al., 2007). Most of these DREB2 proteins were reported to have transactivation abilities in yeast or plant cells. Although pearl millet PgDREB2 preferably binds to the ACCGAC sequence as does DREB2A, the most preferred binding sequence of barley HvDRF1 is TT/ACCGCCTT (Xue and Loveridge, 2004; Agarwal et al., 2007). PgDREB2 was shown to be phosphorylated by total cell extracts and that the phosphorylated protein could not bind to DRE/CRT (Agarwal et al., 2007).

Although overexpression of rice *OsDREB2A* did not result in any phenotypic changes in transgenic Arabidopsis, overexpression of wheat *TaDREB1* and *WDREB2* and maize *ZmDREB2A* caused phenotypic changes in transgenic Arabidopsis and tobacco (Dubouzet et al., 2003; Shen et al., 2003; Kobayashi et al., 2007; Qin et al., 2007). For example, Arabidopsis overexpressing maize *ZmDREB2A* were dwarf, but exhibited improved tolerance to drought and heat stress as did transgenic Arabidopsis overexpressing *DREB2A-CA*. Microarray analysis revealed that 28 of 44 up-regulated genes in transgenic Arabidopsis overexpressing *ZmDREB2A* were also up-regulated in Arabidopsis overexpressing *DREB2A-CA*. Unfortunately, stress-tolerant grasses overexpressing *DREB2* have not yet been generated.

In conclusion, expression of *DREB2* genes is induced by dehydration and high salinity in grasses, while some *DREB2* genes respond to cold or heat stress (Fig. 1). Stability of DREB2 proteins is required for function in Arabidopsis, whereas RNA processing of *DREB2* genes is important in grasses (Fig. 1).

AREB/ABF REGULON

ABRE and Coupling Elements for ABA-Mediated Responses

Many drought and high salinity-inducible genes respond to ABA in Arabidopsis and rice (Seki et al., 2002; Rabbani et al., 2003). Most ABA-inducible genes contain a conserved, ABA-responsive, cis-acting element, designated as ABRE (PyACGTGG/TC), in their promoter regions. ABRE is a major cis-acting element in ABA-responsive genes (Fig. 1). For ABA-dependent transcription, a single copy of ABRE is not sufficient. Rather, ABRE and coupling elements, including coupling element 1 (CE1) and coupling element 3 (CE3), constitute an ABA-responsive complex in the regulation of wheat *HVA1* and *HVA22* genes (Shen et al., 1996). Most known coupling elements are similar to ABREs. For example, rice contains an A/GCGT motif (Hobo et al., 1999a). In Arabidopsis, the DRE/CRT sequence may serve as a coupling element of ABRE in response to ABA (Narusaka et al., 2003), suggesting that the existence of the interaction between the DREB regulons and the ABRE-related regulons.

AREB/ABF Regulon in Response to ABA in Arabidopsis

Arabidopsis cDNAs encoding bZIP TFs, referred to as AREB or ABFs, were isolated using yeast one-hybrid screening (Choi et al., 2000; Uno et al., 2000). In the Arabidopsis genome, 75 distinct bZIP TFs exist and 13 members are classified as a subfamily of AREB/ABFs that contain four conserved domains (Bensmihen et al., 2002; Jakoby et al., 2002). Arabidopsis *AREB1/ABF2*, *AREB2/ABF4*, and *ABF3* are mainly expressed in response to dehydration and high salinity in vegetative tissues, but not in seeds (Fujita et al., 2005; Fig. 1). In contrast, Arabidopsis *ABI5*, *AREB3*, *AtDPBF2*, and *EEL* were expressed during seed maturation (Finkelstein and Lynch, 2000; Lopez-Molina and Chua, 2000; Bensmihen et al., 2002). Fujita et al. (2005) reported that transgenic Arabidopsis plants overexpressing the active form of AREB1 showed ABA hypersensitivity and enhanced drought tolerance. In addition, eight genes representing two groups were up-regulated: LEA-class genes and ABA- and dehydration stress-inducible regulatory genes, including linker histone H1 and AAA ATPase.

ABA-responsive 42-kD kinases phosphorylate conserved regions of AREB/ABFs, which suggests that ABA-dependent phosphorylation may be involved in activation of AREB subfamily proteins (Uno et al., 2000). Activation of AREB1 requires ABA-dependent posttranscriptional modification. ABA-activated 42-kD kinase activity phosphorylates Ser/Thr residues of R-X-X-S/T sites in the conserved regions of AREB1

(Furihata et al., 2006). Transgenic plants overexpressing the phosphorylated active form of AREB1 expressed many ABA-inducible genes, including *RD29B*, without ABA treatment (Furihata et al., 2006). Therefore, these results suggest that the ABA-dependent multisite phosphorylation of AREB1 regulates its own activation in plants and that phosphorylation- and dephosphorylation-regulated events are important for ABA signaling.

Several type-2 SNF1-related protein kinases (SnRK2-type), such as OST1/SRK2E in Arabidopsis (Mustilli et al., 2002; Yoshida et al., 2002), were identified as ABA-activated protein kinases. They were also shown to mediate the regulation of stomatal aperture and to function upstream of ABA-responsive gene expression. In Arabidopsis, nine of 10 SnRK2 are activated by hyperosmolarity and five of nine SnRK2 are activated by ABA (Boudsocq et al., 2004). Furihata et al. (2006) demonstrated that ABA-activated SnRK2 protein kinases, including SnRK2.2/SRK2D, SnRK2.3/SRK2I, and SnRK2.6/SRK2E, phosphorylate the AREB1 polypeptide. Recently, Fujii et al. (2007) demonstrated that two protein kinases, SnRK2.2/SRK2D and SnRK2.3/SRK2I, control responses to ABA in seed germination, dormancy, and seedling growth in Arabidopsis. The authors suggested that the mechanism of SnRK2.2 and SnRK2.3 action in ABA signaling involves the activation of ABRE-driven gene expression through phosphorylation of AREB/ABFs (Fig. 1).

AREB/ABF Regulon in Response to ABA in Grasses

The rice TRANSCRIPTION FACTOR RESPONSIBLE FOR ABA REGULATION1 (TRAB1) and barley HvABI5 show high homology to AREB2/ABF4. Expression of *TRAB1* and *HvABI5* genes was detected in ABA-treated and drought-stressed seedlings, respectively (Hobo et al., 1999b; Casaretto and Ho, 2003; Xue and Loveridge, 2004; Fig. 1). HvABI5 binds to cis-elements in the promoter region of *HVA1*, an ABA- and stress-responsive gene (Casaretto and Ho, 2003; Xue and Loveridge, 2004). TRAB1 was shown to be phosphorylated rapidly in response to ABA in rice (Kagaya et al., 2002). The rice bZIP TF OsABI5 was also isolated from rice panicles (Zou et al., 2008). Expression of *OsABI5* was induced by ABA and high salinity, but was down-regulated by drought and cold stress in seedlings (Fig. 1). Overexpression of *OsABI5* in rice conferred high sensitivity to salt stress. In contrast, down-regulation of *OsABI5* promoted stress tolerance, but resulted in decreased fertility of rice. These results suggest that OsABI5 may regulate adaptation to stress and plant fertility. Recently, Nijhawan et al. (2008) surveyed the rice genome for bZIP family proteins and analyzed the expression of 89 *OsbZIP* genes. Their microarray analysis indicated that 26 genes were up-regulated and 11 genes were down-regulated under dehydration, salinity, and/or cold conditions. The transcript level of *TRAB1* (OsbZIP66; Hobo et al., 1999b) was up-regulated under dehydration and salt stress. However, the OsbZIP TFs remain to be analyzed in the context of stress signaling.

Ten SnRK2 protein kinases were identified in rice. All family members are activated by hyperosmotic stress. Three genes of this family are also activated by ABA (Kobayashi et al., 2004). Rice ABA-activated SnRK2 can phosphorylate TRAB1 (Kobayashi et al., 2005). Therefore, ABA-activated SnRK2 protein kinases phosphorylate and activate the AREB/ABF-type proteins in rice as well as Arabidopsis (Fig. 1). Recently, Chae et al. (2007) isolated a dehydration-inducible gene (designated *OSRK1*) that encodes a 41.8-kD protein kinase of the rice SnRK2 family. In vitro kinase assays demonstrated that OSRK1 can phosphorylate both itself and generic substrates. OREB1, a rice ABRE-binding factor, was phosphorylated in vitro by OSRK1 at multiple sites of different functional domains.

In summary, Arabidopsis and rice use similar AREB/ABF regulons in ABA-dependent gene expression during dehydration and high-salinity stresses (Fig. 1). Phosphorylation of AREB/ABF-type TFs by SnRK2 family kinases is important in ABA-dependent stress signaling networks (Fig. 1).

NAC REGULON

NAC Regulon in Response to Dehydration and High Salinity in Arabidopsis

There are additional gene expression pathways that are regulated through NAC TFs under dehydration stress in Arabidopsis (Fig. 1). Two different Arabidopsis cis-acting elements are necessary for induction of gene expression by dehydration in *EARLY RESPONSE TO DEHYDRATION1* (*ERD1*), which encodes a Clp protease regulatory subunit, ClpD (Nakashima et al., 1997; Simpson et al., 2003). Three cDNAs encoding NAC-like proteins, Arabidopsis NAC domain-containing proteins ANAC19 (AT1G52890), ANAC055 (ATNAC3, AT3G15500), and ANAC072 (RD26, AT4G27410), were isolated using yeast one-hybrid screening and were found to bind to one of the cis-elements in the *ERD1* promoter (Tran et al., 2004). Microarray analysis of transgenic plants overexpressing *ANAC019*, *ANAC055*, or *ANAC072/RD26* revealed that several stress-inducible genes were up-regulated, conferring significantly increased drought tolerance in the transgenic lines (Fujita et al., 2004; Tran et al., 2004). However, *ERD1* was not up-regulated in transgenic plants. Through one-hybrid screening, cDNAs encoding zinc-finger homeodomain (ZFHD) TFs that bind to the other cis-element in the *ERD1* promoter were isolated. One of these TFs, ZFHD1, was shown to function as a transcriptional activator in response to dehydration stress (Tran et al., 2007). When both NAC and ZFHD proteins were overproduced, *ERD1* expression increased, suggesting that both cis-acting elements are essential for the expression of *ERD1*. Note that, in Arabidopsis, NAC proteins function alone or act as transcription activators in cooperation with ZFHD proteins (Fig. 1).

NAC Regulon for Dehydration, High Salinity, and Cold Stress in Grasses

The *OsNAC6* gene is a member of the NAC TF gene family in rice (Ohnishi et al., 2005; Nakashima et al., 2007). Expression of *OsNAC6* is induced by ABA and abiotic stresses, including cold, drought, and high salinity (Fig. 1). *OsNAC6* gene expression is also induced by jasmonic acid, wounding, and blast disease. OsNAC6 was shown to function as a transcriptional activator that localizes to the nucleus. Transgenic rice plants overexpressing *OsNAC6* exhibited growth retardation and low grain yield. However, these transgenic rice plants had improved tolerance to dehydration and high salt stresses and were more tolerant to blast disease. It was found that stress-inducible promoters, such as the *OsNAC6* promoter, are more suitable for overexpression in order to minimize negative effects on plant growth in transgenic rice. Up-regulated genes in rice plants overexpressing *OsNAC6* contained many genes that were induced by abiotic and biotic stresses, as revealed by microarray analyses. This is supported by results of a transient transactivation assay that demonstrated that OsNAC6 activates the expression of two genes, including a peroxidase-encoding gene. Collectively, these results suggest that OsNAC6 functions as a transcriptional activator in response to abiotic and biotic stresses in plants. Recently, Hu et al. (2006, 2008) reported that overexpression of the stress-responsive genes *STRESS-RESPONSIVE NAC1* (*SNAC1*) and *SNAC2* enhance drought and salt tolerance in transgenic rice without growth retardation (Fig. 1). It should be noted that SNAC1 was reported to enhance drought resistance in transgenic rice at the reproductive stage (22%–34% higher seed setting than control) when grown in the field under severe drought stress. These plants, however, were not affected in yield (Hu et al., 2006). Completion of the rice genome project revealed that the rice genome contains six paralogous genes, including *OsNAC6* and *SNAC1* (Ooka et al., 2003). Interestingly, one of the homologous genes, *ONAC010*, encodes a protein showing high similarity with the NAC protein, NAM-B1, regulating senescence, which was shown to improve grain protein, zinc, and iron content in wheat (Uauy et al., 2006).

These results indicate that, in contrast to Arabidopsis, the NAC regulon may have additional functions in grasses (Fig. 1). Therefore, comparative analysis of gene expression patterns, determining the functional role of these genes in growth and tolerance to abiotic and biotic stresses, and identification of target genes of TFs involved in stress responses are important future tasks.

CONCLUSIONS AND PERSPECTIVES

Many plant genes are regulated in response to abiotic stresses, such as dehydration, high salinity, cold, and heat. These genes regulate responses to stress and are thought to be important for stress tolerance. Molecular and genetic studies provided evidence that the dicot Arabidopsis and the monocot rice share common regulatory mechanisms of gene expression. TFs play important roles in the regulation of gene expression in response to abiotic stresses and most TFs are common between grasses and Arabidopsis (Fig. 1). TFs are powerful targets for genetic engineering of stress tolerance because overexpression of a single TF can lead to the up-regulation of a wide array of stress response genes that are controlled by the TF.

The grasses include many agriculturally important plants: major food crops such as rice, wheat, maize, and barley, forage crops such as ryegrass, and biomass/biofuel production species such as sugarcane (*Saccharum* sp.). Many TFs, including DREB1/CBF, DREB2, AREB/ABF, and NAC, can be used to improve stress tolerance to abiotic stresses in various grasses. However, several challenges have to be overcome when using TFs for genetic engineering of abiotic stress tolerance in grasses. First, an effective expression system, including suitable promoters, will be required for each grass because constitutive promoters are not always functional or can have negative effects on plant growth and development. Second, there is an urgent need to establish reliable systems to evaluate abiotic stress tolerance in transgenic grasses, especially under field conditions. Finally, the collective and cooperative efforts of plant molecular biologists, physiologists, and breeders are required to generate stress-tolerant grasses through genetic engineering. It is hoped that, in the future, these collective efforts and results of collaborative studies will positively contribute to sustainable food production in the world and will help to prevent global-scale environmental damage that results from abiotic stress.

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