

# Molecular characterization and roles of AP2 transcription factors on drought tolerance in plants

Jincai LI<sup>1</sup>, Yongsheng ZHANG<sup>2</sup>, Juntao GU<sup>3</sup>, Chengjin GUO<sup>2</sup>, Shumin WEN<sup>3</sup>, Guiru LIU (✉)<sup>2</sup>, Kai XIAO (✉)<sup>2</sup>

<sup>1</sup> Science and Technology Management Office, Agricultural University of Hebei, Baoding 071001, China

<sup>2</sup> College of Agronomy, Agricultural University of Hebei, Baoding 071001, China

<sup>3</sup> College of Life Science, Agricultural University of Hebei, Baoding 071001, China

© Higher Education Press and Springer-Verlag Berlin Heidelberg 2011

**Abstract** The APETALA2 (AP2) domain defines a large family of DNA binding proteins. It has been demonstrated that the AP2 proteins have important functions in the transcriptional regulation of a variety of biologic processes related to growth and development in various responses to drought and other abiotic stresses. In this essay, recent researches on the AP2 transcription factors, such as the molecular characterization, expression patterns in responses to drought and other abiotic stresses, the roles of ABA on drought responding which were mediated by AP2 transcription factors, transcription regulation mechanisms, and the roles of overexpression of AP2 transcription factor on plant drought tolerance, etc. have been overviewed. Deepening the understanding of signaling and the corresponding transduction pathways that are initiated via drought stress stimuli will play crucial roles for providing the theoretical basis for variety breeding with promising drought tolerance in the future.

**Keywords** transcription factor, AP2 domain, molecular characterization, transcriptional activation, drought tolerance

## Introduction

Drought, as well as other abiotic stresses, such as high salinity, low temperature, and inorganic deficiencies, affect plant growth and decrease crop productivity extremely, by which it is important to improve stress tolerance of the crop plants to implement the crop yield increase under the stress conditions. For a long time of evolution, plants have established the mechanisms of responding and adaptation to the environmental stresses with physiologic and developmental changes, via delicate signal transduction pathways.

Upon exposure to drought conditions, many stress-related genes are induced in plants. The modifications of the related genes on transcription and further changes of the translation products are thought to lead to the plants to modify their normal metabolic responses and alter their physiologic and developmental programs (Seki et al., 2002), which finally function as cellular protectants of drought stress-induced damage (Thomashow, 1999; Shinozaki et al., 2003; Khan-

delwal, et al., 2008).

Through interaction with specific DNA sequences and thereby controlling the transfer (or transcription) of genetic information from DNA to mRNA (Karin, 1990; Latchman, 1997), transcription factors play critical roles in regulation of the expression patterns of downstream specific genes (Roeder, 1996; Nikolov et al., 1997; Lee et al., 2000). In the genomes of *Arabidopsis* (*Arabidopsis thaliana*) and rice (*Oryza sativa*), more than 1300 genes covering about 6% of the estimated total number of genes are functional grouped in transcriptional regulators. Of these, nearly 45% were reported to be plant-specific and were involved in regulation of multitude gene expressions initiated by the internal and external stimuli (Riechmann et al., 2000; Kikuchi et al., 2003).

Based on the features of DNA binding domain and the interaction mechanisms with the regulatory acting cis-elements in the promoters of the target genes, transcription factors have been classified into various families (Kikuchi et al., 2003). Of these, AP2 factors appear to be widespread in plants, with the genomes of rice and *Arabidopsis* predicted to contain 139 and 122 AP2 genes, respectively (Nakano et al., 2006). Studies have found that members of the AP2 family

Received April 28, 2011; accepted May 9, 2011

Correspondence: <sup>a</sup>Guiru LIU; <sup>b</sup>Kai XIAO

E-mail: <sup>a</sup>nxlgr@hebau.edu.cn; <sup>b</sup>xiaokai@hebau.edu.cn

have been implicated in diverse functions in cellular processes such as drought tolerance (Ingram and Bartels 1996, Thomashow 1999; Shinozaki et al., 1997; 2003), in addition to the flower development, spikelet meristem determinacy, plant growth, and stress tolerance (Chuck et al., 1998; Liu et al., 1998; Haake et al., 2002; Dubouzet et al., 2003; Gutterson et al., 2004; Oh et al., 2009). In this essay, the molecular characterization, transcriptional regulation, and the roles of AP2 transcription factors, especially overexpression of distinct AP2 transcription factors on drought tolerance in plants have been overviewed, aiming at deepening the understanding of signaling and corresponding transduction pathways that are initiated via the drought stress, which provides the theoretical basis for crop variety breeding with improvement of the drought tolerance in the future.

## Molecular characterizations of AP2 transcription factors in plants

The AP2 domain was first identified as a repeated motif within the *Arabidopsis* AP2 protein, which was involved in regulation of various functions in the cellular process (Jofuku et al., 1994). The presence of AP2 DNA binding domain characterized the AP2/ERF family of transcription regulators by consisting of 57–66 amino acids in size (Okamura et al., 1997; Riechmann et al., 1998; Sakuma et al., 2002).

As one of the largest plant transcription factor families that covered in total 139 members in *Arabidopsis* (Nakano et al., 2006), the proteins of AP2/ERF transcription factors are divided into four subfamilies referred to AP2, CBF/DREB, ERF, and RAV, respectively, based on their sequence similarities and numbers of AP2/ERF domains (Sakuma et al., 2002). Among the four subfamilies, AP2 subfamily proteins contain two AP2/ERF domains, and genes in this subfamily chiefly participate in the regulation of developmental processes, especially playing crucial roles in responding to the abiotic stresses, such as drought (Elliott et al., 1996; Chuck et al., 1998; Boutilier et al., 2002). The RAV subfamily proteins contain one AP2/ERF domain and a B3 domain, which differ in biologic functions and are involved in distinct types of the transcription. It is found that the RAV subfamily members are associated with the ethylene response (Alonso et al., 2003), brassinosteroid response (Hu et al., 2004), and biotic and abiotic stress responses (Sohn et al., 2006) in plants. In contrast to the AP2 and RAV subfamily members, the CBF/DREB and ERF subfamily proteins contain single AP2/ERF domains. The genes in the CBF/DREB subfamily play crucial roles in the resistance of plants to abiotic stresses by recognizing the dehydration responsive or cold-repeat element (DRE/CRT) with a core motif of A/GCCGAC (Yamaguchi-Shinozaki et al., 1994; Thomashow, 1999). The fourth subfamily of AP2 transcription factors, ERF, is mainly involved in the response to biotic stresses like pathogenesis by recognizing the *cis*-acting element AGCCGCC, known as

the GCC box (Hao et al., 1998). Some of the ERF subfamily members also bind DRE/CRT elements to endow their roles on the transcription regulation of downstream genes with different metabolic pathways (Lee et al., 2004; Xu et al., 2007).

The AP2 domain in the AP2 transcription factors are approximately 60-amino acid in length, playing a crucial role in interacting with the regulatory *cis*-acting elements that generally are GC-rich (GCC box/C-repeat) in the promoter of their target genes (Riechmann et al., 1998; Krizek, 2003). The proteins in AP2 subgroup contain two copies of the DNA binding domain (BD) which are separated by a spacer region (Meyerowitz, 1994; Okamura et al., 1997).

With the rapidly progress of modern molecular biology, the mechanisms how CBF/DREBs proteins are involved in mediation of signaling transductions of the abiotic stresses, such as the drought stress, have been gradually elucidated. Except for the conserved AP2 domain, the signature motifs of CBF/DREB (PKK/RPAGRxKxFxETRHP and DSAWR) are identified in the proteins that are directly flanking the AP2 domain (Jaglo et al., 2001). In *Arabidopsis*, there are identified quantities of DREB/ERF family proteins that are classified into 12 groups (Nakano et al., 2006; Khandelwal, et al., 2008). Over previously one decade, *CBF/DREB* orthologs have also been identified in diverse plant species, such as canola, tomato, wheat, rye, barley, and rice (Jaglo et al., 2001; Choi et al., 2002; Xue, 2003; Skinner et al., 2005; Oh et al., 2007). The *AP2* gene family from other plant species, including *DBF1* and *DBF2* (Kizis et al., 2002) from maize, *AhDREB1* (Shen et al., 2003) from *Atriplex hortensis*, *CaPF1* (Yi et al., 2004) from pepper, *HvRAF* (Jung et al., 2007) from barley, and *SodERF3* (Trujillo et al., 2008) from sugarcane, have been identified and found to be involved in responses to drought and various other abiotic stress conditions.

## Expression and transcriptional regulation

### Expression patterns in response to drought and other abiotic stresses

In plants, cells in different tissues and organs can communicate with each other by the signaling cascades initiated via the internal or the external signals based on which to fulfill the plants responses to the developmental cues and the biotic/abiotic stresses. As one of the indispensable components of signaling cascade, transcription factors act as first the downstream (Pawson, 1993) and on the other hand, as the upstream of signaling cascades related to internal and external stimuli, leading the signaling to the downstream. Therefore, the transcription factor genes that respond to the abiotic stress, such as drought, can be modulated at the expression level when plants are subjected to drought and other abiotic stresses.

Spatial and temporal expression patterns of the AP2 transcription factors have been widely studied over the past decade. It has been found that AP2 transcription factor genes respond to diverse signals, in addition to drought and other abiotic stresses. In *Arabidopsis*, *PLETHORA1* and *PLETHORA2* (*PLT1* and *PLT2*), two genes encoding repeated AP2 domain proteins, are transcribed in response to auxin in the basal embryo region, embryonic root primordium, and later in root meristem stem cells, as well as are essential in maintaining the position of the stem cell niche in the root (Aida et al., 2004). However, in tobacco, *ESR1* gene (enhancer of shoot regeneration) encodes a protein and referred to as the ethylene-responsive element binding protein, containing a single copy of the AP2 domain (Ohme-Takagi et al., 1995). Ectopic expression of *ESR1* in *Arabidopsis* causes a promotion of cytokinin-independent shoot regeneration from the *Arabidopsis* root explants (Banno et al., 2001). These results suggest that the AP2 transcription factors, such as *PLT1*, *PLT2* and *ESR1*, can not only sense the auxin signal, but also play a vital role in the mediation of the auxin signal to the downstream.

In the past several years, more and more studies have reported that the AP2 transcription factors are widely involved in the drought stimuli responses, in accordance with an expression pattern to be generally upregulated. In rice, two AP2 transcription factor genes, *AP37* and *AP59*, representing subgroups I and II, respectively, were found to be induced by over 2 h of exposure to drought stress, but differ in their expression profile upon exposure to low temperature and abscisic acid (Oh et al., 2009). In chickpea, the transcript level of *CAP2*, one AP2 transcription factor gene, was increased by dehydration, as well as by other abiotic stress treatments, such as sodium chloride, abscisic acid, and auxin, but not by low temperature, salicylic acid, and jasmonic acid (Shukla et al., 2006). Further studies based on yeast one-hybrid assay suggested that the recombinant *CAP2* protein was bound specifically to C-repeat/dehydration-responsive element in gel-shift assay and transactivated reporter genes in yeast (Shukla et al., 2006).

As one subfamily of AP2 transcription factors, the CBF/DREB type plays vital roles in responses to drought and other abiotic stresses. It is demonstrated that the members of subgroup IIIc belonging to CBF/DREB subfamily have been shown to play crucial roles in the regulations of drought, salt, and low-temperature stress-responsive gene expression (Gilmour et al., 1998; Liu et al., 1998; Haake et al., 2002; Dubouzet et al., 2003; Magome et al., 2004).

### **The roles of ABA on drought responding and ABA responses mediated by AP2 transcription factors**

As one of important phytohormones, the abscisic acid (ABA) is an important regulator for plant growth and development and plant responses to environmental stress such as drought, cold, and salt (Finkelstein et al., 2002). Generally, drought

and salt stress can result in the accumulation of ABA, which initiates many adaptive responses (Leung et al., 1998). Under water-deficit conditions, for example, ABA-induced stomatal closure reduces transpirational water loss from plants (Assmann et al., 2001; Schroeder et al., 2001).

Presently, although ABA receptors have not been found, many components involved in ABA signaling have been identified. By dissection of the dehydration-induced genes, it is found that a number of genes that respond to dehydration and cold stress are associated with the elevated ABA levels in plants. The genes are also induced by exogenous application of ABA (Zhu, 2002; Shinozaki et al., 2003). Whereas there are also a part of genes induced by dehydration stress but not responded to exogenous application of ABA (Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2006). This suggests that there are two signal transduction pathways, known as ABA-independent and ABA-dependent, respectively. The latter plays roles in converting the initial stress signal into cellular responses (Yamaguchi-Shinozaki et al., 2006).

It was observed that the type 2C protein phosphatases ABI1 and ABI2 are central regulators of ABA responses (Merlot et al., 2001). The dominant *abi1-1* and *abi2-1* mutations render *Arabidopsis* plants insensitive to ABA in seed germination, root growth, stomatal closure, and gene regulation (Koornneef et al., 1984; Leung et al., 1997; Allen et al., 1999; Hoth et al., 2002). The Ser/Thr protein kinase OST1, an *Arabidopsis* ortholog of *Vicia faba* AAPK, is activated by ABA as a positive role in guard cell ABA responses (Mustilli et al., 2002; Assmann, 2003). The only heterotrimeric G protein present in *Arabidopsis* is also a positive regulator of ABA responses in guard cells (Wang et al., 2001). In another case, it is observed that PKS3, one Ser/Thr protein kinase, can be physically interacted with ABI2, acting as a global negative regulator of ABA responses (Guo et al., 2002). Other negative regulators of ABA responses including the farnesyl transferase ERA1 (Cutler et al., 1996), inositol phosphatase FRY1 (Xiong et al., 2001a), and several proteins involved in RNA metabolism have also been reported (Lu and Fedoroff, 2000; Hugouvieux et al., 2001; Xiong et al., 2001b).

It is clear now that ABA modulates the expression of many genes important for plant to be adapted to drought and other abiotic stresses. Among them, transcriptional regulation mediated by the distinct transcription factors plays crucial roles for above biologic processes. Song et al. (2005) found that an AP2/EREBP-type transcription factor, AtERF7, played an important role in ABA responses via its interaction with the protein kinase PKS3, a global regulator of ABA responses. After interaction occurs between the AP2 domain of AtERF7 with the GCC box and *cis*-regulatory DNA elements, the downstream gene transcription was repressed. Overexpression of *AtERF7* in *Arabidopsis* showed a reduced sensitivity of guard cells to ABA and concomitantly led to an increased transpirational water loss. These results suggest that AtERF7 plays an important role in ABA responses and may

be a part of transcriptional repressor in the ABA signaling pathway (Song et al., 2005).

In addition to the case that AP2 transcription factor acts as the transcriptional repressors, the members of this transcription factor family can also act as the transcriptional activators, being involved in ABA responses (Himmelbach et al., 2003). *ABI4*, a transcription factor in the AP2 domain family, is found to be involved in the plant responses to ABA, based on the binding to the ABA-responsive element G box (Finkelstein et al., 1998; Finkelstein et al., 2000). Analysis of the mutants of *ABI4* suggests that the ABA insensitivity can be caused specifically in seeds (Koorneef et al., 1984; Finkelstein et al., 2000; Finkelstein et al., 2002). Taken together, ABA responses in plants mediated by AP2 transcription factors that are markedly associated with drought tolerance may involve two transcriptional forms, activators and repressors. The two pathways of drought responses and tolerances in plants, namely ABA-dependent and ABA-independent, respectively, especially the former, needs to be elucidated further. On the other hand, as the indispensable components in the ABA-dependent/independent pathways, it is also necessary to identify the upstream and downstream members, as well as the transcriptional regulation mechanisms for the AP2 transcription factors.

### Transcription regulation mechanisms of AP2 transcription factors in plants

Promoter analysis has been widely used to explore the *cis*-regulatory DNA elements that the transcription factor interacted (Ingram et al., 1996). In *Arabidopsis*, it is demonstrated that a 9-bp conserved sequence (TACCGAC), referred to as dehydration-responsive element (DRE)/C-repeat, which is independent of ABA, and is found essential and sufficient for the high expression of an *Arabidopsis* gene, *rd29A*, under drought, low temperature, and high salinity conditions (Yamaguchi-Shinozaki et al., 1994). Further studies have verified that the dehydration response element (DRE, TACCGACAT) functioned as the *cis*-acting DNA element recognized by proteins of the DREB subfamily (Yamaguchi-Shinozaki et al., 1994; Stockinger et al., 1997) and confirmed that the sequence CCGAC inside the DRE element is the minimal sequence motif for binding, and C4, G5, and C7 are essential for specific interaction (Hao et al., 2002; Sakuma et al., 2002). DREB factors are known to also bind the C-repeat and the low-temperature-responsive element, which share the CCGAC motif with the DRE element (Baker et al., 1994; Jiang et al., 1996; Thomashow, 1999; Shinozaki et al., 2003).

In addition to the DRE-related sequences, other *cis*-regulatory DNA elements that interact with AP2 transcription factors have also been identified. ANT, (gCAC(A/G)N(A/T)TcCC(a/g)ANG(c/t)), a motif recognized and bound by AP2 transcription factor, contains an important stretch of three Cs (Nole-Wilson and Krizek, 2000; Krizek, 2003). Some AP2/

ERF proteins show cross-affinity *in vitro* for these binding motifs, such as the ERF Tsi1 and DREB1B and DREB2A, which can bind both the GCC box and DRE element. Analysis of ANT indicates that it can also bind the DRE minimal motif (CCGAC) in the *COR78* and *COR15* promoters (Nole-Wilson et al., 2000; Park et al., 2001; Hao et al., 2002; Sakuma et al., 2002). Therefore, AP2 domains are suggested to have evolved into different DNA binding specificities, maintaining a strong affinity for G/C rich motifs.

More and more DNA binding specificities have also been shown for members of the ERF and RAV subfamilies. It is reported that several ERF proteins can bind the GCC box (AGCCGCC) where G2, G5, and C7 are essential for binding (Ohme-Takagi et al., 1995; Buttner et al., 1997; Zhou et al., 1997; Hao et al., 1998; Fujimoto et al., 2000; Hao et al., 2002). The *Arabidopsis* RAV1 transcription factor can bind a bipartite recognition sequence with the B3 and AP2 domain recognizing the sequences CACCTG and CAACA, respectively (Kagaya et al., 1999).

With the research progressing, the secondary structures of AP2 transcription factors have been elucidated. It is observed that the secondary structure of the AtERF1 AP2 domain shares structural similarities with other DNA binding proteins. The Structural Classification of Proteins database (Murzin et al., 1995) has classified the DNA binding domain of the Tn916 integrase (Connolly et al., 1998), the  $\lambda$ -integrase N-terminal domain (Wojciak et al., 2002), the human methyl-CpG binding domain MBD (Ohki et al., 1999), and the AP2 domain (Allen et al., 1998) into the same superfamily in that all of them share the common three-stranded  $\beta$ -sheet and an  $\alpha$ -helix structure. Despite the similar secondary structure and topology, no apparent sequence similarity has been found between the AtERF1 AP2 domain and the other domains.

The three-dimensional structure of the AP2 domain in *Arabidopsis* AtERF1 has also been established via heteronuclear multidimensional NMR (Allen et al., 1998), which deepens the reorganization of the interaction mechanism between the conserved AP2 domain and the *cis*-acting DNA element. The AP2 domain consists of a three-stranded  $\beta$ -sheet and one  $\alpha$ -helix almost parallel to the  $\beta$ -sheet. Arg and Trp residues are located in the  $\beta$ -sheet consolidated the interaction. Based on the fully understanding of the secondary and three-dimensional structure of AP2 transcription factors, the molecular mechanisms that interact with the *cis*-regulatory DNA elements in the promoter of the downstream genes, transcription activation, and signal transduction mediated by this kind of transcription factors will be explored further.

### Downregulated genes of the AP2 transcription factors

Various adverse environmental stresses result in the induced expression of a variety of genes in many plant species (Xiong et al., 2002; Shinozaki et al., 2003). The products of these genes are thought to promote stress tolerance and regulate gene expression through signal transduction pathways (Xiong

et al., 2002; Shinozaki et al., 2003). Among the induced genes, much more of them are regulated by specific transcription factors. Members of the AP2 transcription factors have been shown to have regulatory roles in drought stress responses (Oh et al., 2009).

Using RNA gel blot analysis and a 1300-bp full-length *Arabidopsis* cDNA microarray approach, 12 genes have been identified as the target stress-inducible genes of *DREB1A* (Seki et al., 2002). These *DREB1A* target genes all contained the DRE or DRE-related core motifs in their promoter regions. Similarly, taken together, these results suggest that overexpression of DREBs/CBFs can result in the induction of multiple stress tolerance genes, leading to the plant physiologic and developmental responses under drought conditions.

## Roles of overexpression of AP2 transcription factor on plant drought tolerance

### Various functions of AP2 transcription factors in plants

Genes in the AP2 family have diverse functions in plants, such as the regulation of developmental processes, e.g. flower development (Elliott et al., 1996), spikelet meristem determinacy (Chuck et al., 1998), leaf epidermal cell identity (Moose et al., 1996), and embryo development (Boutilier et al., 2002). In the past several years, the members of the RAV family involved in the mediation of ethylene response (Alonso et al., 2003) and brassinosteroid response (Hu et al., 2004) have also been reported. Over the previous decade, many proteins in the ERF family, such as tobacco ERFs (Ohme-Takagi and Shinshi, 1995), have been identified and implicated in many diverse functions in cellular processes, including hormonal signal transduction (Ohme-Takagi et al., 1995), responses to biotic (Yamamoto et al., 1999; Gu et al., 2000) and abiotic stresses (Stockinger et al., 1997; Dubouzet et al., 2003), and regulation of metabolism (van der Fits et al., 2000; Aharoni et al., 2004; Zhang et al., 2005), in developmental processes (Banno et al., 2001; Chuck et al., 2002) of various plant species.

In *Arabidopsis*, the homeotic gene *AP2* was found to play a central role in establishment of floral meristem, determination of sepal and petal identity, and expression of other floral homeotic genes (Irish and Sussex, 1990; Bowman et al., 1993). Mutants that loss-of-function of *AP2* caused, relatively increased in the seed mass (Ohto et al., 2005). *AP2* gene was also expressed in the nonfloral organs, suggesting its involvement in the development processes of other organs as well (Jofuku et al., 1994). Another homeotic gene of this subgroup, *AINTEGUMENTA* (*ANT*), was demonstrated to be critical in regulation of ovule and female gametophyte development (Klucher et al., 1996). Thus, AP2 transcription regulators were involved in various plant growth and

development processes, such as flower organogenesis or seed development, in addition to many stress responses, such as drought stress (Riechmann et al., 1998).

### Roles of overexpressing AP2 transcription factors on improvement of drought tolerance

To date, a number of studies have suggested that overexpression of stress-related genes can improve drought tolerance in plants to some extent (Xu et al., 1996; Garg et al., 2002; Jang et al., 2003; Ito et al., 2006; Hu et al., 2006, 2008; Nakashima et al., 2007). In *Arabidopsis*, overexpression of the *DREB1A* (*CBF3*) cDNA under the control of the CaMV 35S promoter resulted in strong expression of target stress-inducible genes and the transgenic plants acquired higher tolerance to drought, high-salinity, and freezing stresses (Liu et al. 1998).

Similarly, ectopically expressing *Arabidopsis* CBF1/*DREB1B* in tomato plants showed enhanced resistance to water deficit, chilling and oxidative stresses (Hsieh et al. 2002). These results indicated that the CBF/DREB gene could be used to improve the multi-stress tolerance of agriculturally important crops by gene transfer. *CBF/DREBs* were also heterologously effective in canola (Jaglo et al., 2001), tomato (Hsieh et al., 2002), tobacco (Kasuga et al., 2004), and rice (Oh et al., 2005), enhancing stress tolerance, such as drought, in the corresponding transgenic plants.

Heretofore, the functional genes regulated by AP2 members on improving the drought tolerance in transgenic plants are also characterized to some extent. Transgenic analysis of a chickpea AP2 transcription factor, *CAP2*, suggests that the ectopic expression of *CAP2* in tobacco can improve the plant tolerance to dehydration and salinity. The elevated drought tolerance in transgenic tobacco plants is possibly owing to expression of native genes like *ERD10B* and *ERD10C* coding for LEA proteins in tobacco (Shukla et al., 2006). In *Arabidopsis* and tobacco, overexpression of *AtDREB1A* causes similar expression of *RD29A* and *NtERD10* genes, respectively, but in contrast to *CAP2*-transgenic plants, results in stunted growth (Liu et al., 1998; Kasuga et al., 2004). These results suggest that the gene downstream of *AtDREB1A* is also regulated by the latter.

Although lots of transgenic studies and mutants analysis suggest that distinct AP2 transcription factors play vital roles in improving drought tolerance in plants, such efforts were performed mostly indoors. Recently, a few studies have also been conducted in the field with promising results to improve crop grain yields under field conditions. As for example, overexpression of *SNAC1* and *OsLEA3* in rice, was shown to improve grain yield under field drought conditions (Hu et al., 2006; Xiao et al., 2007).

Under the control of the constitutive promoter *OsCcl1*, the overexpression of *AP37* and *AP59* in rice increased the tolerance to drought and high salinity at the vegetative stage in the field experiments, in addition to the increased tolerance

to low temperatures in *OsCcl:AP37* rice plants. More importantly, the *OsCcl:AP37* plants showed significantly enhanced drought tolerance around the whole life circle in the field, causing a 16% to 57% grain yield increase under severe drought conditions, yet exhibiting no significant difference under normal growth conditions (Oh et al., 2009). Microarray experiments identified 10 and 38 genes upregulated by AP37 and AP59, respectively, in addition to 37 genes that were commonly induced by both factors. These results suggested that the *AP37* gene has the potential to improve drought tolerance in rice or other crops without causing undesirable growth phenotypes (Oh et al., 2009).

### Value of suitable promoter in the generation of transgenic plants

Under the control of CaMV35S, overexpressing *DREB1A* caused severe growth retardation under normal growth conditions in transgenic *Arabidopsis*. Overexpressing *CBF1* also showed the dwarf phenotype under unstressed normal growth conditions in transgenic tomato (Hsieh et al., 2002). Therefore, suitable promoters were essential for generation of the transgenic crop cultivars with potential goals to drastically improve drought tolerance.

The stress-inducible *rd29A* promoter instead of the constitutive CaMV35S promoter for the overexpression of *DREB1A* in transgenic *Arabidopsis* was used to minimize the negative effects on plant growth (Kasuga et al., 1999). In transgenic tobacco plants, overexpression of the *DREB1A* driven by a modified CaMV35S promoter or the stress-inducible *rd29A* promoter showed varied plant phenotypes (Kasuga et al., 1999). Under normal growing conditions, the growth of the 35S:*DREB1A* plants showed growth retardation and were classified into two groups including two plants for each group (35S:*DREB1Aa* and 35S:*DREB1Ab*). Of the two groups, the 35S:*DREB1Ab* plants showed severer growth retardation than the 35S:*DREB1Aa* plants. By contrast, most of the *rd29A:DREB1A* plants exhibited slight growth retardation (Kasuga et al., 1999). Thus, the stress-inducible *rd29A* promoter could be used in generation of the crop cultivars with strong tolerance in the field drought conditions. On the other hand, it showed a potential value to isolate and identify more and more suitable novel promoters that will contribute largely to the establishment of the transgenic crop research and application in the future.

### Remarks and conclusions

Drought stress is among the most serious challenges to crop production worldwide. Under drought stress conditions, many stress-related genes in plants are induced. The expression of stress-related genes is largely regulated by specific transcription factors. As one of large families of the transcription factors, the APETALA2 (AP2)/ethylene-respon-

sive factor (ERF) family of proteins regulates diverse processes of plant development and metabolism, such as vegetative and reproductive development, cell proliferation, secondary metabolism, biotic and abiotic stress responses, and responds to different plant hormones (Shukla et al., 2006). The AP2 domain in the AP2 transcription factors are approximately 60-amino acid in length, playing a crucial role in interacting with the regulatory *cis*-acting elements that generally are GC-rich (GCC box/C-repeat) in the promoter of their target genes.

There are two signal transduction pathways referred to ABA-independent and ABA-dependent, playing roles in converting the initial drought stress signal into cellular responses (Yamaguchi-Shinozaki et al., 2006). So far, it is clearly shown that the ABA responses in plants can be mediated by AP2 transcription factors via activating or repressing way. With the fully understanding of the secondary and three-dimensional structure of AP2 transcription factors, the molecular mechanisms how AP2 proteins interact with the *cis*-regulatory DNA elements in the promoter of the downstream genes and the transcription activation have been gradually understood.

Till now, it is found that some of AP2 transcription factors play crucial roles in improving the drought tolerance in transgenic plants. A few of these members, such as AP37 in rice, have been shown to obtain promising results to improve crop grain yields under field conditions. These results suggest that the *AP37* gene has the potential to be used as the target gene in generation of crop varieties in the future with dramatically improved drought tolerance, yet without causing undesirable growth phenotypes.

Suitable promoters are essential for generation of the transgenic crop cultivars with potential goals to drastically improve drought tolerance. Therefore, it is also necessary to isolate and identify more and more suitable novel promoters that will contribute largely to the establishment of transgenic crop research and application in the future.

### Acknowledgements

This work was supported by the Natural Science Foundation of Hebei, China (No. C2010000752), the National Transgenic Major Program, China (No. 2009ZX08002-012B) and the Key Crop Growth Regulation Laboratory of Hebei Province, China.

### References

- Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira A (2004). The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in *Arabidopsis*. *Plant Cell*, 16(9): 2463–2480
- Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, Nussaume L, Noh Y S, Amasino R, Scheres B (2004). The

- PLETHORA genes mediate patterning of the *Arabidopsis* root stem cell niche. *Cell*, 119(1): 109–120
- Allen G J, Kuchitsu K, Chu S P, Murata Y, Schroeder J I (1999). *Arabidopsis* *abi1-1* and *abi2-1* phosphatase mutations reduce abscisic acid-induced cytoplasmic calcium rises in guard cells. *Plant Cell*, 11(9): 1785–1798
- Allen M D, Yamasaki K, Ohme-Takagi M, Tateno M, Suzuki M (1998). A novel mode of DNA recognition by a beta-sheet revealed by the solution structure of the GCC-box binding domain in complex with DNA. *EMBO J*, 17(18): 5484–5496
- Alonso J M, Stepanova A N, Leisse T J, Kim C J, Chen H, Shinn P, Stevenson D K, Zimmerman J, Barajas P, Cheuk R, Gadrinab C, Heller C, Jeske A, Koesema E, Meyers C C, Parker H, Prednis L, Ansari Y, Choy N, Deen H, Geralt M, Hazari N, Hom E, Karnes M, Mulholland C, Ndubaku R, Schmidt I, Guzman P, Aguilar-Henonin L, Schmid M, Weigel D, Carter D E, Marchand T, Risseuw E, Brogden D, Zeko A, Crosby W L, Berry C C, Ecker J R (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science*, 301(5633): 653–657
- Assmann S M (2003). OPEN STOMATA1 opens the door to ABA signaling in *Arabidopsis* guard cells. *Trends Plant Sci*, 8(4): 151–153
- Assmann S M, Wang X Q (2001). From milliseconds to millions of years: guard cells and environmental responses. *Curr Opin Plant Biol*, 4(5): 421–428
- Baker S S, Wilhelm K S, Thomashow M F (1994). The 5'-region of *Arabidopsis thaliana* *cor15a* has *cis*-acting elements that confer cold-, drought- and ABA-regulated gene expression. *Plant Mol Biol*, 24(5): 701–713
- Banno H, Ikeda Y, Niu Q W, Chua N H (2001). Overexpression of *Arabidopsis* *ESR1* induces initiation of shoot regeneration. *Plant Cell*, 13(12): 2609–2618
- Boutillier K, Offringa R, Sharma V K, Kieft H, Ouellet T, Zhang L, Hattori J, Liu C M, van Lammeren A A, Miki B L, Custers J B, van Lookeren Campagne M M (2002). Ectopic expression of BABY BOOM triggers a conversion from vegetative to embryonic growth. *Plant Cell*, 14(8): 1737–1749
- Bowman J L, Alvarez J, Weigel D, Meyerowitz E M, Smyth D R (1993). Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development*, 119: 721–743
- Choi D W, Rodriguez E M, Close T J (2002). Barley *Cbf3* gene identification, expression pattern, and map location. *Plant Physiol*, 129(4): 1781–1787
- Chuck G, Meeley R B, Hake S (1998). The control of maize spikelet meristem fate by the *APETALA2*-like gene *indeterminate spikelet1*. *Genes Dev*, 12(8): 1145–1154
- Chuck G, Muszynski M, Kellogg E, Hake S, Schmidt R J (2002). The control of spikelet meristem identity by the *branched silkless1* gene in maize. *Science*, 298(5596): 1238–1241
- Connolly K M, Wojciak J M, Clubb R T (1998). Site-specific DNA binding using a variation of the double stranded RNA binding motif. *Nat Struct Biol*, 5(7): 546–550
- Cutler S, Ghassemian M, Bonetta D, Cooney S, McCourt P (1996). A protein farnesyl transferase involved in abscisic acid signal transduction in *Arabidopsis*. *Science*, 273(5279): 1239–1241
- Dubouzet J G, Sakuma Y, Ito Y, Kasuga M, Dubouzet E G, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003). OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *Plant J*, 33(4): 751–763
- Elliott R C, Betzner A S, Huttner E, Oakes M P, Tucker W Q, Gerentes D, Perez P, Smyth D R (1996). *AINTEGUMENTA*, an *APETALA2*-like gene of *Arabidopsis* with pleiotropic roles in ovule development and floral organ growth. *Plant Cell*, 8(2): 155–168
- Finkelstein R R, Gampala S S, Rock C D (2002). Abscisic acid signaling in seeds and seedlings. *Plant Cell*, 14(Suppl): S15–S45
- Finkelstein R R, Lynch T J (2000). The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. *Plant Cell*, 12(4): 599–609
- Finkelstein R R, Wang M L, Lynch T J, Rao S, Goodman H M (1998). The *Arabidopsis* abscisic acid response locus *ABI4* encodes an *APETALA 2* domain protein. *Plant Cell*, 10(6): 1043–1054
- Fujimoto S Y, Ohta M, Usui A, Shinshi H, Ohme-Takagi M (2000). *Arabidopsis* ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. *Plant Cell*, 12(3): 393–404
- Garg A K, Kim J K, Owens T G, Ranwala A P, Choi Y D, Kochian L V, Wu R J (2002). Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc Natl Acad Sci USA*, 99(25): 15898–15903
- Gilmour S J, Zarka D G, Stockinger E J, Salazar M P, Houghton J M, Thomashow M F (1998). Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced *COR* gene expression. *Plant J*, 16(4): 433–442
- Gu Y Q, Yang C, Thara V K, Zhou J, Martin G B (2000). *Pti4* is induced by ethylene and salicylic acid, and its product is phosphorylated by the Pto kinase. *Plant Cell*, 12(5): 771–786
- Guo Y, Xiong L, Song C P, Gong D, Halfter U, Zhu J K (2002). A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signaling in *Arabidopsis*. *Dev Cell*, 3(2): 233–244
- Gutterson N, Reuber T L (2004). Regulation of disease resistance pathways by AP2/ERF transcription factors. *Curr Opin Plant Biol*, 7(4): 465–471
- Haake V, Cook D, Riechmann J L, Pineda O, Thomashow M F, Zhang J Z (2002). Transcription factor *CBF4* is a regulator of drought adaptation in *Arabidopsis*. *Plant Physiol*, 130(2): 639–648
- Hao D, Yamasaki K, Sarai A, Ohme-Takagi M (2002). Determinants in the sequence specific binding of two plant transcription factors, CBF1 and NtERF2, to the DRE and GCC motifs. *Biochemistry*, 41(13): 4202–4208
- Hao D Y, Ohme-Takagi M, Sarai A (1998). Unique mode of GCC box recognition by the DNA-binding domain of ethylene-responsive element-binding factor (ERF domain) in plant. *J Biol Chem*, 273(41): 26857–26861
- Himmelbach A, Yang Y, Grill E (2003). Relay and control of abscisic acid signaling. *Curr Opin Plant Biol*, 6(5): 470–479
- Hoth S, Morgante M, Sanchez J P, Hanafey M K, Tingey S V, Chua N H (2002). Genome-wide gene expression profiling in *Arabidopsis thaliana* reveals new targets of abscisic acid and largely impaired gene regulation in the *abi1-1* mutant. *J Cell Sci*, 115(24): 4891–4900
- Hsieh T H, Lee J T, Charng Y Y, Chan M T (2002). Tomato plants ectopically expressing *Arabidopsis* CBF1 show enhanced resistance to water deficit stress. *Plant Physiol*, 130(2): 618–626
- Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006).

- Overexpressing a *NAM*, *ATAF*, and *CUC (NAC)* transcription factor enhances drought resistance and salt tolerance in rice. *Proc Natl Acad Sci USA*, 103(35): 12987–12992
- Hu H, You J, Fang Y, Zhu X, Qi Z, Xiong L (2008). Characterization of transcription factor gene *SNAC2* conferring cold and salt tolerance in rice. *Plant Mol Biol*, 67(1–2): 169–181
- Hu Y X, Wang Y X, Liu X F, Li J Y (2004). *Arabidopsis* *RAV1* is down-regulated by brassinosteroid and may act as a negative regulator during plant development. *Cell Res*, 14(1): 8–15
- Hugouvieux V, Kwak J M, Schroeder J I (2001). An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in *Arabidopsis*. *Cell*, 106(4): 477–487
- Ingram J, Bartels D (1996). The molecular basis of dehydration tolerance in plants. *Annu Rev Plant Physiol Plant Mol Biol*, 47(1): 377–403
- Irish V F, Sussex I M (1990). Function of the *apetala-1* gene during *Arabidopsis* floral development. *Plant Cell*, 2(8): 741–753
- Ito Y, Katsura K, Maruyama K, Tajiri T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006). Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant Cell Physiol*, 47(1): 141–153
- Jaglo K R, Kleff S, Amundsen K L, Zhang X, Haake V, Zhang J Z, Deits T, Thomashow M F (2001). Components of the *Arabidopsis* C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. *Plant Physiol*, 127(3): 910–917
- Jang I C, Oh S J, Seo J S, Choi W B, Song S I, Kim C H, Kim Y S, Seo H S, Choi Y D, Nahm B H, Kim J K (2003). Expression of a bifunctional fusion of the *Escherichia coli* genes for trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase in transgenic rice plants increases trehalose accumulation and abiotic stress tolerance without stunting growth. *Plant Physiol*, 131(2): 516–524
- Jiang C, Iu B, Singh J (1996). Requirement of a CCGAC cis-acting element for cold induction of the *BN115* gene from winter *Brassica napus*. *Plant Mol Biol*, 30(3): 679–684
- Jofuku K D, den Boer B G, Van Montagu M, Okamoto J K (1994). Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. *Plant Cell*, 6(9): 1211–1225
- Jung J, Won S Y, Suh S C, Kim H, Wing R, Jeong Y, Hwang I, Kim M (2006). The barley ERF-type transcription factor HvRAF confers enhanced pathogen resistance and salt tolerance in *Arabidopsis*. *Planta*, 225(3): 575–588
- Kagaya Y, Ohmiya K, Hattori T (1999). *RAV1*, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants. *Nucleic Acids Res*, 27(2): 470–478
- Karin M (1990). Too many transcription factors: positive and negative interactions. *New Biol*, 2(2): 126–131
- Kasuga M, Miura S, Shinozaki K, Yamaguchi-Shinozaki K (2004). A combination of the *Arabidopsis* *DREB1A* gene and stress-inducible *rd29A* promoter improved drought- and low-temperature stress tolerance in tobacco by gene transfer. *Plant Cell Physiol*, 45(3): 346–350
- Khandelwal A, Elvitigala T, Ghosh B, Quatrano R S (2008). *Arabidopsis* transcriptome reveals control circuits regulating redox homeostasis and the role of an AP2 transcription factor. *Plant Physiol*, 148(4): 2050–2058
- Rice Full-Length cDNA Consortium; National Institute of Agrobiological Sciences Rice Full-Length cDNA Project Team, Kikuchi S, Satoh K, Nagata T, Kawagashira N, Doi K, Kishimoto N, Yazaki J, Ishikawa M, Yamada H, Ooka H, Hotta I, Kojima K, Namiki T, Ohneda E, Yahagi W, Suzuki K, Li C J, Ohtsuki K, Shishiki T; Foundation of Advancement of International Science Genome Sequencing & Analysis Group, Otomo Y, Murakami K, Iida Y, Sugano S, Fujimura T, Suzuki Y, Tsunoda Y, Kurosaki T, Kodama T, Masuda H, Kobayashi M, Xie Q, Lu M, Narikawa R, Sugiyama A, Mizuno K, Yokomizo S, Niikura J, Ikeda R, Ishibiki J, Kawamata M, Yoshimura A, Miura J, Kusumegi T, Oka M, Ryu R, Ueda M, Matsubara K; RIKEN, Kawai J, Carninci P, Adachi J, Aizawa K, Arakawa T, Fukuda S, Hara A, Hashizume W, Hayatsu N, Imotani K, Ishii Y, Itoh M, Kagawa I, Kondo S, Konno H, Miyazaki A, Osato N, Ota Y, Saito R, Sasaki D, Sato K, Shibata K, Shinagawa A, Shiraki T, Yoshino M, Hayashizaki Y, Yasunishi A (2003). Collection, mapping, and annotation of over 28,000 cDNA clones from *japonica* rice. *Science*, 301(5631): 376–379
- Kizis D, Pagès M (2002). Maize DRE-binding proteins DBF1 and DBF2 are involved in *rab17* regulation through the drought-responsive element in an ABA-dependent pathway. *Plant J*, 30(6): 679–689
- Klucher K M, Chow H, Reiser L, Fischer R L (1996). The *AINTEGUMENTA* gene of *Arabidopsis* required for ovule and female gametophyte development is related to the floral homeotic gene *APETALA2*. *Plant Cell*, 8(2): 137–153
- Koornneef M, Reuling G, Karssen C M (1984). The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. *Physiol Plant*, 61(3): 377–383
- Krizek B A (2003). *AINTEGUMENTA* utilizes a mode of DNA recognition distinct from that used by proteins containing a single AP2 domain. *Nucleic Acids Res*, 31(7): 1859–1868
- Latchman D S (1997). Transcription factors: an overview. *Int J Biochem Cell Biol*, 29(12): 1305–1312
- Lee J H, Hong J P, Oh S K, Lee S, Choi D, Kim W T (2004). The ethylene-responsive factor like protein 1 (CaERFLP1) of hot pepper (*Capsicum annuum* L.) interacts *in vitro* with both GCC and DRE/CRT sequences with different binding affinities: possible biological roles of CaERFLP1 in response to pathogen infection and high salinity conditions in transgenic tobacco plants. *Plant Mol Biol*, 55(1): 61–81
- Lee T I, Young R A (2000). Transcription of eukaryotic protein-coding genes. *Annu Rev Genet*, 34(1): 77–137
- Leung J, Giraudat J (1998). Abscisic acid signal transduction. *Annu Rev Plant Physiol Plant Mol Biol*, 49(1): 199–222
- Leung J, Merlot S, Giraudat J (1997). The *Arabidopsis* *ABSCISIC ACID-INSENSITIVE2 (ABI2)* and *ABI1* genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. *Plant Cell*, 9(5): 759–771
- Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998). Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell*, 10(8): 1391–1406
- Lu C, Fedoroff N (2000). A mutation in the *Arabidopsis* *HYL1* gene encoding a dsRNA binding protein affects responses to abscisic acid, auxin, and cytokinin. *Plant Cell*, 12(12): 2351–2366

- Magome H, Yamaguchi S, Hanada A, Kamiya Y, Oda K (2004). *dwarf and delayed-flowering 1*, a novel *Arabidopsis* mutant deficient in gibberellin biosynthesis because of overexpression of a putative AP2 transcription factor. *Plant J*, 37(5): 720–729
- Merlot S, Gosti F, Guerrier D, Vavasseur A, Giraudat J (2001). The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. *Plant J*, 25(3): 295–303
- Meyerowitz E M (1994). Flower development and evolution: new answers and new questions. *Proc Natl Acad Sci USA*, 91(13): 5735–5737
- Moose S P, Sisco P H (1996). *Glossy15*, an *APETALA2*-like gene from maize that regulates leaf epidermal cell identity. *Genes Dev*, 10(23): 3018–3027
- Murzin A G, Brenner S E, Hubbard T, Chothia C (1995). SCOP: a structural classification of proteins database for the investigation of sequences and structures. *J Mol Biol*, 247(4): 536–540
- Mustilli A C, Merlot S, Vavasseur A, Fenzi F, Giraudat J (2002). *Arabidopsis* OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *Plant Cell*, 14(12): 3089–3099
- Nakano T, Suzuki K, Fujimura T, Shinshi H (2006). Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice. *Plant Physiol*, 140(2): 411–432
- Nakashima K, Tran L S, Van Nguyen D, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, Yamaguchi-Shinozaki K (2007). Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. *Plant J*, 51(4): 617–630
- Nikolov D B, Burley S K (1997). RNA polymerase II transcription initiation: a structural view. *Proc Natl Acad Sci USA*, 94(1): 15–22
- Nole-Wilson S, Krizek B A (2000). DNA binding properties of the *Arabidopsis* floral development protein AINTEGUMENTA. *Nucleic Acids Res*, 28(21): 4076–4082
- Oh S J, Kim Y S, Kwon C W, Park H K, Jeong J S, Kim J K (2009). Overexpression of the transcription factor *AP37* in rice improves grain yield under drought conditions. *Plant Physiol*, 150(3): 1368–1379
- Oh S J, Kwon C W, Choi D W, Song S I, Kim J K (2007). Expression of barley HvCBF4 enhances tolerance to abiotic stress in transgenic rice. *Plant Biotechnol J*, 5(5): 646–656
- Oh S J, Song S I, Kim Y S, Jang H J, Kim S Y, Kim M, Kim Y K, Nahm B H, Kim J K (2005). *Arabidopsis* *CBF3/DREB1A* and *ABF3* in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiol*, 138(1): 341–351
- Ohki I, Shimotake N, Fujita N, Nakao M, Shirakawa M (1999). Solution structure of the methyl-CpG-binding domain of the methylation-dependent transcriptional repressor MBD1. *EMBO J*, 18(23): 6653–6661
- Ohme-Takagi M, Shinshi H (1995). Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. *Plant Cell*, 7(2): 173–182
- Ohta M A, Fischer R L, Goldberg R B, Nakamura K, Harada J J (2005). Control of seed mass by *APETALA2*. *Proc Natl Acad Sci USA*, 102(8): 3123–3128
- Okamoto J K, Caster B, Villarreal R, Van Montagu M, Jofuku K D (1997). The AP2 domain of *APETALA2* defines a large new family of DNA binding proteins in *Arabidopsis*. *Proc Natl Acad Sci USA*, 94(13): 7076–7081
- Park J M, Park C J, Lee S B, Ham B K, Shin R, Paek K H (2001). Overexpression of the tobacco *Tsi1* gene encoding an EREBP/AP2-type transcription factor enhances resistance against pathogen attack and osmotic stress in tobacco. *Plant Cell*, 13(5): 1035–1046
- Pawson T (1993). Signal transduction—a conserved pathway from the membrane to the nucleus. *Dev Genet*, 14(5): 333–338
- Riechmann J L, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe O J, Samaha R R, Creelman R, Pilgrim M, Broun P, Zhang J Z, Ghandehari D, Sherman B K, Yu G (2000). *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science*, 290(5499): 2105–2110
- Riechmann J L, Meyerowitz E M (1998). The AP2/EREBP family of plant transcription factors. *Biol Chem*, 379(6): 633–646
- Roeder R G (1996). The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem Sci*, 21(9): 327–335
- Sakuma Y, Liu Q, Dubouzet J G, Abe H, Shinozaki K, Yamaguchi-Shinozaki K (2002). DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. *Biochem Biophys Res Commun*, 290(3): 998–1009
- Schroeder J I, Kwak J M, Allen G J (2001). Guard cell abscisic acid signalling and engineering drought hardiness in plants. *Nature*, 410(6826): 327–330
- Seki M, Ishida J, Narusaka M, Fujita M, Nanjo T, Umezawa T, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M, Akiyama K, Yamaguchi-Shinozaki K, Carninci P, Kawai J, Hayashizaki Y, Shinozaki K (2002). Monitoring the expression pattern of around 7,000 *Arabidopsis* genes under ABA treatments using a full-length cDNA microarray. *Funct Integr Genomics*, 2(6): 282–291
- Shen Y G, Zhang W K, Yan D Q, Du B X, Zhang J S, Liu Q, Chen S Y (2003). Characterization of a DRE-binding transcription factor from a halophyte *Atriplex hortensis*. *Theor Appl Genet*, 107(1): 155–161
- Shinozaki K, Yamaguchi-Shinozaki K (1997). Gene expression and signal transduction in water-stress response. *Plant Physiol*, 115(2): 327–334
- Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003). Regulatory network of gene expression in the drought and cold stress responses. *Curr Opin Plant Biol*, 6(5): 410–417
- Shukla R K, Raha S, Tripathi V, Chattopadhyay D (2006). Expression of CAP2, an *APETALA2*-family transcription factor from chickpea, enhances growth and tolerance to dehydration and salt stress in transgenic tobacco. *Plant Physiol*, 142(1): 113–123
- Skinner J S, von Zitzewitz J, Szucs P, Marquez-Cedillo L, Filichkin T, Amundsen K, Stockinger E J, Thomashow M F, Chen T H, Hayes P M (2005). Structural, functional, and phylogenetic characterization of a large CBF gene family in barley. *Plant Mol Biol*, 59(4): 533–551
- Sohn K H, Lee S C, Jung H W, Hong J K, Hwang B K (2006). Expression and functional roles of the pepper pathogen-induced transcription factor RAV1 in bacterial disease resistance, and drought and salt stress tolerance. *Plant Mol Biol*, 61(6): 897–915
- Song C P, Agarwal M, Ohta M, Guo Y, Halfter U, Wang P, Zhu J K (2005). Role of an *Arabidopsis* AP2/EREBP-type transcriptional repressor in abscisic acid and drought stress responses. *Plant Cell*, 17(8): 2384–2396

- Stockinger E J, Gilmour S J, Thomashow M F (1997). *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proc Natl Acad Sci USA*, 94(3): 1035–1040
- Thomashow M F (1999). Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Annu Rev Plant Physiol Plant Mol Biol*, 50(1): 571–599
- Trujillo L E, Sotolongo M, Menéndez C, Ochogavía M E, Coll Y, Hernández I, Borrás-Hidalgo O, Thomma B P H J, Vera P, Hernández L (2008). SodERF3, a novel sugarcane ethylene responsive factor (ERF), enhances salt and drought tolerance when overexpressed in tobacco plants. *Plant Cell Physiol*, 49(4): 512–525
- van der Fits L, Memelink J (2000). ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. *Science*, 289(5477): 295–297
- Wang X Q, Ullah H, Jones A M, Assmann S M (2001). G protein regulation of ion channels and abscisic acid signaling in *Arabidopsis* guard cells. *Science*, 292(5524): 2070–2072
- Wojciak J M, Sarkar D, Landy A, Clubb R T (2002). Arm-site binding by lambda-integrase: solution structure and functional characterization of its amino-terminal domain. *Proc Natl Acad Sci USA*, 99(6): 3434–3439
- Xiao B, Huang Y, Tang N, Xiong L (2007). Over-expression of a *LEA* gene in rice improves drought resistance under the field conditions. *Theor Appl Genet*, 115(1): 35–46
- Xiong L, Gong Z, Rock C D, Subramanian S, Guo Y, Xu W, Galbraith D, Zhu J K (2001b). Modulation of abscisic acid signal transduction and biosynthesis by an Sm-like protein in *Arabidopsis*. *Dev Cell*, 1(6): 771–781
- Xiong L, Lee B, Ishitani M, Lee H, Zhang C, Zhu J K (2001a). FIERY1 encoding an inositol polyphosphate 1-phosphatase is a negative regulator of abscisic acid and stress signaling in *Arabidopsis*. *Genes Dev*, 15(15): 1971–1984
- Xiong L, Schumaker K S, Zhu J K (2002). Cell signaling during cold, drought, and salt stress. *Plant Cell*, 14(Suppl): S165–S183
- Xu D, Duan X, Wang B, Hong B, Ho T, Wu R (1996). Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiol*, 110(1): 249–257
- Xu Z S, Xia L Q, Chen M, Cheng X G, Zhang R Y, Li L C, Zhao Y X, Lu Y, Ni Z Y, Liu L, Qiu Z G, Ma Y Z (2007). Isolation and molecular characterization of the *Triticum aestivum* L. ethylene-responsive factor 1 (TaERF1) that increases multiple stress tolerance. *Plant Mol Biol*, 65(6): 719–732
- Xue G P (2003). The DNA-binding activity of an AP2 transcriptional activator HvCBF2 involved in regulation of low-temperature responsive genes in barley is modulated by temperature. *Plant J*, 33(2): 373–383
- Yamaguchi-Shinozaki K, Shinozaki K (1994). A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *Plant Cell*, 6(2): 251–264
- Yamaguchi-Shinozaki K, Shinozaki K (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu Rev Plant Biol*, 57(1): 781–803
- Yamamoto S, Suzuki K, Shinshi H (1999). Elicitor-responsive, ethylene-independent activation of GCC box-mediated transcription that is regulated by both protein phosphorylation and dephosphorylation in cultured tobacco cells. *Plant J*, 20(5): 571–579
- Yi S Y, Kim J H, Joung Y H, Lee S, Kim W T, Yu S H, Choi D (2004). The pepper transcription factor CaPF1 confers pathogen and freezing tolerance in *Arabidopsis*. *Plant Physiol*, 136(1): 2862–2874
- Zhang J Y, Broeckling C D, Blancaflor E B, Sledge M K, Sumner L W, Wang Z Y (2005). Overexpression of *WXPI*, a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*). *Plant J*, 42(5): 689–707
- Zhou J, Tang X, Martin G B (1997). The Pto kinase conferring resistance to tomato bacterial speck disease interacts with proteins that bind a cis-element of pathogenesis-related genes. *EMBO J*, 16(11): 3207–3218
- Zhu J K (2002). Salt and drought stress signal transduction in plants. *Annu Rev Plant Biol*, 53(1): 247–273