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Review

WRKY transcription factor superfamily: Structure, origin and functions

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WRKY transcription factors are transcriptional regulatory factors in which N-terminal ends contain the WRKYGQR amino acid sequence and a zinc-finger motif. WRKY transcription factors can regulate the expression of target genes that contain the W-box elements (C/T)TGAC(C/T) in the promoter regions by specifically binding to the (C/T)TGAC(C/T) sequence. WRKY transcription factors regulate the expression of pathogen-induced, senescence-induced, abscisic acid (ABA)-induced, gibberellic acid (GA)-induced and salcylic acid (SA)-induced genes and play an important role in the regulation of plant growth and development as well as in their response to many kinds of biotic and abiotic stress. The progress of research on the basic structure, origin and biological function of plant WRKY transcription factor in recent years was reviewed in this paper.

Key words: WRKY, transcription factors, structure, origin, abiotic stress, function.

INTRODUCTION

Origin of the WRKY family

The WRKY family is among the ten largest families of transcription factors in higher plants and is found throughout the green lineage (green algae and land plants) (Ulker and Somssich, 2004). WRKY factors are generally regarded as being plant specific, but the fact that they are found in the protist *Giardia lamblia* and the slime mold *Dictyostelium discoideum* imply an earlier origin (Ulker and Somssich, 2004; Pan et al., 2009). The WRKY proteins contain 109 and 74 representatives in rice and *Arabidopsis*, respectively (Eulgem and Somssich, 2000). To date, all of the higher plants analyzed contain numerous members of the three major WRKY groups, which differ in the number of *WRKY* domains and in the pattern of the zinc-finger motif. In rice, WRKY members contain nineteen variants, in which WRKYGEK and WRKYGKK are two common variants; others are WRICGQK, WRMCGQK, WKKYGQK, WIKYGQK, WKRYGQK, WSKYEQK and WRKYSEK (Xie et al., 2005). However, these variants mainly belong to group III. In *Arabidopsis*, the majority of the group III members are found to respond both to pathogen infection and to salicylic acid (Kalde et al., 2003). Interestingly, the WRKY genes found in the unicellular eukaryote *Chlamydomonas*, the non-photosynthetic eukaryotic slime mold *D. discoideum,* the unicellular protist *G. lamblia,* lower plant *Physcomitrella paterts* and *Ceratopteris richardii* belong to group I. The single WRKY present in the green alga *Chlamydomonas reinhardtii* also belongs to group I. These findings reveal that group I may be the ancestral type of WRKY gene (Ulker and Somssich, 2004), and group III is evolved from other groups, perhaps as a consequence of increasing

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environmental pressures.

The WRKY genes are believed to have originated some 1.5 to 2 billion years ago in eukaryotes; that is, before the divergence of the plant phyla (Zhang et al., 2005). During their long evolutionary history, the WRKY gene family greatly expanded, as demonstrated by the increased numbers of WRKY genes in higher plants (Guo et al., 2008), and this expansion may be primarily due to segmental duplications of genomic fragments as a result of independent polyploidy events (Bowers et al., 2003; Cannon et al., 2004; Thomas et al., 2006), it is likely to be associated with the ongoing development of highly sophisticated defence mechanisms co-evolving in land plants together with their adapted pathogens. Although not completely restricted to the plant kingdom, this family has expanded enormously in higher plants, whereas they appear to have been lost in yeast, prokaryotes and animal lineages (Bhattarai et al., 2010).

The wrky domain and w-box

Since a novel DNA-binding protein, designated SWEET POTATO FACTOR1 (SPF1) (Ishiguro and Nakamura, 1994) was identified from sweet potato fifteen years ago, similar proteins were subsequently cloned from various plant species (Rushton et al., 1995, 1996). The WRKY families have 109 and 74 representatives in rice and *Arabidopsis*, respectively (Eulgem et al., 2007; Ross et al., 2007). The WRKY proteins contain a DNA-binding region of approximately 60 amino acids (the WRKY domain) at its amino-terminal end and a novel zinc-finger motif at the carboxyl terminus, these features give this transcription factor family the name 'WRKY'. The WRKY amino acid sequences are not conserved in all WRKY proteins and have many variants (WRRY, WSKY, WKRY, WVKY or WKKY) in a few WRKY proteins (Xie et al., 2005). Previous studies have reported that the divalent metal chelators 1, 10-o-phenanthroline and ethylenediaminetetraacetic acid (EDTA) abolish DNA-binding, showing the existence of a zinc-finger structure within the WRKY domain (Hara et al., 2000). However, it has not yet been proven that zinc is actually complexed in the WRKY domain. Yamasaki et al. (2005) reported the first NMR solution structure of a WRKY domain, which consists of a four-stranded β-sheet with a zinc-binding pocket formed by the conserved Cys/His residues. Therefore, the binding domain of WRKY transcription factors contains spatial structures features directly combined with DNA and Zn^{2+}.

WRKY proteins can be categorized into three distinct groups on the basis of both the number of WRKY domains and the features of their zinc-finger-like motif. WRKY proteins with two WRKY domains belong to group I (*PcWRKY1*, *AtZAP1, NtWRKY* and *NtWRKY2*), whereas most proteins with one WRKY domain belong to group II (*PcWRKY3*, *AfABF2*, *PcWRKY4* and *NtWIZZ*) and group III (*PcWRKY5*, *NtWRKY4* and *NtWRKY5*). However, the WRKY domains of the group I and group II members have the same type of finger motif ($C–X_{4-5}–C–X_{22–23}–H–X_1–H$). The WRKY domains of group III contain a $C_2–HC$ motif ($C–X_7–C–X_{23}–H–X_1–C$). In *Arabidopsis*, group II can be categorized into five subgroups (IIa – IIe) based on additional short conserved structural motifs (Park et al., 2005). In rice, group II can be categorized into ten subgroup (IIa - IIj), and group III can be categorized into two subgroups (IIIa and IIIb) (Qiu et al., 2004). A few WRKY proteins do not fit neatly into any (sub) group. For example, *At*WRKY10 has only one WRKY domain, but is more related to group I. Moreover, the two WRKY domains of the group I members have distinct function. The C-terminal domain plays a major role in binding to the W-box, while the N-terminal WRKY domain might be involved in binding process and affects binding affinity and specificity (de Pater et al., 1996; Eulgem et al., 1999). In addition, some zinc-finger-like domains in N-terminal WRKY domain might provide an interface for protein–protein interactions (Mackay et al., 1999). The single WRKY domains of the group II and III family members are more similar in sequence to the C terminal than to the N-terminal WRKY domain of group I. In other words, the C-terminal and single WRKY domains are functionally equivalent and constitute the major DNA-binding domain.

An additional common feature of the *WRKY* genes is the existence of an intron within the region encoding the C terminal WRKY domain of the group I members or the single WRKY domain of the group II and III members. This intron position is highly conserved, but its function is unclear and may regulate post-transcriptional processing, like the different intron splicing mechanism in TLR (toll-like receptor) signaling molecules which plays an important role in signal transduction (Jordan et al., 2002). A typical plant transcription factor usually contains a DNA-binding domain, a transcription regulation domain, an oligomerization site and a nuclear localization domain. Transcription factors interact with *cis*-elements and regulate the expression of target genes through these domains. The WRKY domain contains one or two sets of approximately 60 amino acids with a WRKYGQK core sequence plus a novel zinc-finger motif. The WRKY proteins show high affinity binding to a DNA sequence, termed the W-box sequence (C/T)TGAC(C/T), which is found in the promoter region of many genes. Gel-shift experiments, random binding site selection, DNA-ligand binding screens, yeast one-hybrid studies and co-transfection assays performed with different plant WRKY proteins have indicated that with an altered amino acid in TGAC, WRKY-W-box interactions are reduce or abolished (Ciolkowski et al., 2008; Dong et al., 2003). The W-box is the minimal consensus sequence required for specific DNA binding. The W-box consensus alone is necessary, but is not sufficient for the binding of WRKY proteins, and additional neighboring nucleotides of W-box elements also contribute to determining high-affinity

binding *in vitro*. *At*WRKY6 and *At*WRKY11 bind to W-boxes that have a G residue directly 5' adjacent to the element in preference to any others, whereas *At*WRKY-26, 38 and 43 bind to the same motif if this residue is a T, C or A (Ciolkowski et al., 2008).

W-box-dependent binding activity requires the invariable WRKY amino-acid signature. Tobacco *Nt*WRKY12 has a WRKYGKK amino acid sequence rather than the more common WRKYGQK, and binds specifically to the WK box (TTTTCCAC) in the PR-1a promoter, which is significantly different from the consensus sequence of a W-box (van Verk et al., 2008). A few studies indicated that WRKY proteins can bind to non-W box sequences. Previously, the barley WRKY protein SUSIBA2 was reported to specifically bind to the sugar responsive *cis*-element (SURE) (TAAAGATTACT-AATAGGAA) in addition to a W-box (Sun et al., 2003). Furthermore, another WRKY protein binding site PRE4 (TACTGCGCTTAGT), which was identified in the promoter of *Os*WRKY13, participates in the self-regulation of *Os*WRKY13 (Qiu et al., 2009).

BIOLOGICAL FUNCTIONS OF THE WRKY TRANSCRIPTION FACTORS

Expression modes of WKRY transcription factors

Expression of the WRKY factors is not constitutive, but is strongly and rapidly induced in response to biotic or abiotic stresses in numerous plant species. Induced *WRKY* mRNA accumulation is often extremely rapid and transient, and does not seem to require protein synthesis. In addition, more than 500 WRKY ESTs are identified from various tissue sources, including roots, leaves, inflorescences, abscission zones, seeds and vascular tissue, as well as from drought- or salt-stressed, or pathogen-infected tissue. WRKY factors appear to be selective expressed in numerous cell types and under different physiological conditions and could therefore participate in the control of a wide variety of biological processes (Journot-Catalino et al., 2006; Kim et al., 2006; Xu et al., 2006; Shen et al., 2006).

Dual activities of transcription factors can be dependent on the cell environment and the type or level of signal input (Hoecker et al., 1995). Concentration-dependence is one mechanism of dual functionality by which transcription factors can act as activators or repressors (Rushlow et al., 2001). Dual functionality, whereby the gene functions either as a positive or negative regulators in defense signaling is observed for AtWRKY41 (Higashi et al., 2008). *AtWRKY41*-overexpressing plants showed enhanced resistance to virulent *Pseudomonas* but susceptibility to *Erwinia carotovora*. However, Atwrky41 mutants did not display a differential phenotype. Functional redundancy is a common occurrence with structurally related transcription factors. For example, in *Arabidopsis*, the structurally related *At*WRKY18, *At*WRKY40 and *At*WRKY60 proteins have been shown to have partly functional redundancy. *Atwrky18 Atwrky40* and *Atwrky18 Atwrky60* double mutants and *Atwrky18 Atwrky40 Atwrky60* triple mutant are more resistant to *Pseudomonas syringae*, but more susceptible to *Botrytis cinerea* infection. However, single mutants behaved similarly to wild-type plants in response to the two distinct types of pathogens (Xu et al., 2006).

Many plant transcription factors form hetero- or homo-dimers, affecting the DNA-binding specificity and affinity for *cis*-elements. Xu et al. reported that three different kinds of *Arabidopsis* WRKY proteins (*At*WRKY18, *At*WRKY40, and *At*WRKY60) can interact with each other and form hetero-complexes, and the interactions between these WRKY factors influence their DNA binding activities. In addition, rice (*Os*WRKY71) and barley (*Hv*WRKY1, *Hv*WRKY2) WRKY proteins were found to engage in homomeric associations *in vitro* (Xie et al., 2006). In addition, majority of WRKY genes contain numerous W-box in their promoters, and could therefore is auto-regulated by oneself and cross-regulated by other WRKY factor suggesting that several *WRKY* genes are under direct positive or negative control by WKRY factor; that is, a self-feedback or mutual manipulation channel might exist among *WRKY* genes. For example, *AtWRKY6* (Robatzek and Somssich, 2002) suppresses its own and *AtWRKY42* expression, and *PcWRKY1* represses its own expression (Turck et al., 2004). In addition, upon herbivore attack, *NaWRKY6* transcript accumulation was shown to be dependent on *NaWRKY3* expression (Skibbe et al., 2008).

Functions of WRKY transcription factors in defense signaling

Plants are subjected to attack by a variety of microbial pathogens and herbivores, and through a long history of co-evolution they have developed a set of defense mechanisms that are activated by multiple defense signaling pathways. The plants innate immunity system consists of two layers mechanisms: Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) (Abramovitch et al., 2006; Chisholm et al., 2006; Jones et al., 2006). Both PTI and ETI are controlled by the signaling hormones including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) (Kunkel and Brooks, 2002; Thatcher et al., 2005). The expression of NPR1 (non-expresser of PR-1), one important regulator of SA-mediated defense pathways, which is induced by SA or pathogen infection (Cao et al., 1997), and SA-dependent signaling can be broadly divided into NPR1-dependent and NPR1-independent responses (Durrant and Dong, 2004). NPR1 is partly controlled by WRKY factors interacting with two W-box elements in its promoter (Yu et al., 2001). Studies

indicate that in Arabidopsis expression of at least nine *WRKY* genes, *AtWRKY18, -38, -53, -54, -58, -59, -62, -66, and -70*, is dependent on NPR1, suggesting that they may be under TGA factor control (Wang et al., 2006; Fan and Dong, 2002; Mao et al., 2007). A majority of studies reported on *WRKY* genes predominantly point to their involvement in SA- mediated defense (Asai et al., 2002; Zheng et al., 2007; Dellagi et al., 2000; Lai et al., 2008). SA plays a crucial role in mediating responses of plant to biotic stress. In *Arabidopsis*, 49 out of 72 tested *WRKY* genes respond to SA treatment.

In the complex network of regulatory interactions during plant resistance responses, an antagonistic relationship between SA and JA signaling pathways is evident. SA-dependent defenses are often triggered by biotrophic pathogens, whereas JA and ET are required for resistance to necrotrophic pathogens (Glazebrook, 2005). *AtWRKY70* (Li et al., 2004, 2006) appears to control the balance between SA- and JA-dependent responses pathways. It exerts its function on resisting pathogens through activating SA-biosynthesis and SA-response genes while suppressing JA signaling. In addition, *OsWRKY13* (Qiu et al., 2007) is an important regulator of rice- *Xanthomonas oryzae* pv *oryzae* (*Xoo*) and rice-*Magnaportha grisea* and, similar to *AtWRKY70* in pathogen-induced defense responses, mediates disease resistance to bacterial blight (*Xoo*) and fungal blast through the activation of SA-dependent pathway and suppression of JA-dependent pathway. *AtWRKY33*, which plays an important role in resistance to necrotrophic pathogens, acts not only as a positive regulator of JA- and ET-mediated defense signaling but as a negative regulator of SA-mediated responses (Zheng et al., 2006). Similarly, *AtWRKY25* acts as a negative regulator of the SA-mediated signaling pathway to *P. syringae*. *AtWRKY33* and *AtWRKY25* can be phosphorylated by the MAP kinase4 (MPK4)-mediated JA/ET-activating and SA-repressing signaling pathways (Andreasson et al., 2005). Moreover, *AtWRKY22* and *AtWRKY29* are shown to be important downstream components of a PAMP-induced MAPK (mitogen-activated protein kinase) signaling pathway that mediates resistance responses to both bacterial and fungal pathogens. Overexpression of *AtWRKY29* leads to enhanced tolerance to the otherwise virulent pathogens *P. syringae* and *B. cinerea*.

Recently, there has been a report that the pair of allelic *WRKY* genes，named *OsWRKY45-1* and *OsWRKY45-2* (Tao et al., 2009), play opposite roles in defense responses. *OsWRKY45-1* and *OsWRKY45-2* were respectively identified from *japonica* rice var. Nipponbare and *indica* rice var. Minghui 63. *OsWRKY45-1* and *OsWRKY45-2* act as a negative regulator and a positive regulator in rice resistance against *Xanthomonas oryzae* pv *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv *oryzicola* (*Xoc*), respectively. *OsWRKY45-1*-mediated resistance is associated with increased accumulation of SA and JA and suppressed expression of *OsWRKY13*. However, *OsWRKY45-2*-mediated resistance is only associated with increased accumulation of JA and increased expression of *OsWRKY13*. In addition, they are positive regulators in rice resistance against the fungal pathogen *M. grisea* through different defense signaling pathway, in which *OsWRKY45-1* is independent of SA of JA and *OsWRKY45-2* is dependent of JA.

AtWRKY3 and *AtWRKY4* play a positive role in plant resistance toward necrotrophic pathogens, as *Atwrky4*, *Atwrky3*, and *Atwrky3 Atwrky4* double mutants showed increasing susceptibility toward the fungus *B. cinerea*, whereas *AtWRKY4* enhanced susceptibility toward the biotrophic bacterial pathogen and suppressed pathogen-induced PR1 gene expression. *AtWRKY53* (Murray et al., 2007) increase in response to *P. syringae* and acts as a positive regulator. *AtWRKY48* (Xing et al., 2008) negatively influences basal resistance toward virulent *P. syringae*.

Functions of *WRKY* transcription factors in development

Recently, there has been more and more reports published on how WRKY proteins play a variety of developmental roles in plants. Some WRKY transcription factors regulate biosynthesis of starch, and sesquiterpene (Xu et al., 2004) and embryogenesis (Lagace and Matton, 2004), seed size (Luo et al., 2005), senescence (Ishida et al., 2007; Johnson et al., 2002), seed coat and trichome development (Miao et al., 2004; Jing et al., 2009). *MINI3*, which encodes the WRKY10 protein, is expressed in pollen and in the developing endosperm from the two nuclei stage to the cellularization stage. The endosperm growth plays a key role in controlling seed size and is under the regulation of the KIU pathway (Garcia et al., 2003; Wang et al., 2010). The homozygous mutants of *MINI3* and *IKU1/2* (a leucine-rich repeat kinase) can produce smaller seeds than wild plants. *IKU1* and *MINI3* form a complex and regulate the downstream gene *IKU2*, and *MINI3* associates with the putative W-box motif identified in the *IKU2* promoter. IKU–MINI pathway plays an important role in early endosperm growth and seed size. SHORT HYPOCOTYLUNDER BLUE 1 (SHB1) binds to the promoters of *IKU2* and *MINI3* controls the seeds size (Zhou et al., 2009).

The production of a-amylases in aleurone layers is believed to be necessary for seed germination and post-germination in cereal grains, which is antagonistically regulated by gibberellic acid (GA) and abscisic acid (ABA) (Bethke et al., 1997; Sun and Gubler, 2004). ABA can inhibit seed dormancy, seed maturation and the embryo development (Lopez-Molina et al., 2001), while GA promotes seed germination, seed development stem and root elongation, floral initiation, and flower and fruit development (Hedden and Phillips, 2000). Rice *OsWRKY71* and *OsWRKY51* were found to act as regulators of ABA-inducible pathway and GA-repressible

pathway in aleurone cell (Zhang et al., 2004). *OsWRKY71* can bind to TGAC motifs in the *Amy32b* promoter *in vitro*, whereas *OsWRKY51* itself does not. *OsWRKY51* and *OsWRKY71* form a complex that enhances the binding affinity of *OsWRKY71* to *Amy32b* promoter, which synergistically suppresses GA-induced α-amylase expression by functionally interfering with GAMYB, a transcriptional activator of GA signaling, in barley aleurone cells (Lu et al., 2002; Washio, 2003). In addition, *OsWRKY24* (Zhang et al., 2009) also suppresses the expression of the *Amy32b* by interfering with GAMYB in the GA signal transduction pathway. In contrast to *Os*WRKY71, OsWRKY24 fusion proteins are stable under exogenous GA treatment. Furthermore, *OsWRKY24* represses ABA induction of the *HVA22* promoter in barley aleurone cells, whereas *OsWRKY51* and *OsWRKY71* have almost no effect. A barley *WRKY* gene, *Hv*WRKY38, orthologue of *OsWRKY71*, acts as a transcriptional repressor of GA-induction of a-amylases gene expression in barley aleurone cells (Xie et al., 2007; Zou et al., 2008). *HvWRKY38* represses the GA-induced transactivating activity of *Amy32b* by competing with the binding of *Hv*GAMYB to the *cis*-acting elements in the a-amylase promoter.

Recent studies indicate that the transcription factors ABI5 and ABI3 are important regulators of ABA-dependent post germination developmental arrest (Zhang et al., 2005; Lopez-Molina et al., 2002). *Atwrky2* mutants can increase mRNA levels of *ABI5, ABI3* and *ABI5*-induced *Em1* and *Em6*, which are more sensitive to ABA responses than the wild type during seed germination and post germination early seedling establishment. In addition, *AtWRKY2* mediated ABA responses are independent of miR159 and its target genes MYB33 and MYB101 (Jiang and Yu, 2009). Auxin regulates the formation of lateral branches in roots and shoots, and regulates other aspects of plant growth and development (Leyser, 2006). Overexpression of the *OsWRKY31* gene induces constitutive expression of early auxin-response genes, such as the *OsIAA4* and *OsCrl1* genes, and reduces lateral root formation and elongation, which suggests that *OsWRKY31* might be a key regulator in the signal transduction pathways of auxin response and transport in rice (Zhang et al., 2008). In addition, *GmWRKY13*-transgenic plants also showed an increase in lateral roots (Zhou et al., 2008).

AtWRKY70 (Ulker et al., 2007) and *AtWRKY53* are not only involved in the regulation of plant pathogen defense, but also function as regulators during leaf senescence. WRKY70 and WRKY53 are induced by SA and repressed by JA/ET signaling in a similar manner. But WRKY53 and WRKY70 appear to have opposite functions in plant senescence. *AtWRKY53* knock-out plants delayed and overexpression plants accelerated leaf senescence, while *AtWRKY70* knock-out plants accelerated it. Therefore, *AtWRKY70* and *AtWRKY53* act as negative and positive regulators during senescence, respectively. *MEKK1* not only directly interacts with the *AtWRKY53* promoter, but also with the AtWRKY53 protein to switch from an expression dependent on leaf age to plant age (Miao et al., 2007). *AtWRKY6* is also shown to be expressed during pathogen defense, wounding and senescence. AtWRKY6 is strongly unregulated and acts upstream of SENESCENCE-INDUCED RECEPTOR KINASE (SIRK) in process of leaf senescence. Developmental expression of SIRK is induced specifically during leaf senescence. Amorpha-4, 11-diene synthase (ADS) of *Artemisia annua* is the first committed step in the biosynthesis of the anti-malarial drug artemisinin. *AaWRKY1* (Ma et al., 2009) interacts with the W-box *cis*-acting element (TTGACC) in the promoter region to activate ADS expression, which regulates artemisinin biosynthesis. It also activates other genes related to artemisinin biosynthesis, such as *CYP71AV1, DBR2* and *HMGR.*

Functions of WRKY transcription factors in abiotic stress response

Abiotic stresses, such as drought, cold, higher salt and heat are most severe environmental culprits that greatly restrict plant distribution and crop production. In order to adapt to abiotic stresses, plants have evolved a complicated and comprehensive regulatory network, in which the WRKY factors family plays an important regulated role. Initiating signals of abiotic stresses interaction with gene expression are involved in at least two types of signal transduction: ABA-dependent and ABA-independent. Salt, drought, and to a lesser extent, cold stresses, also increase the biosynthesis and accumulation of abscisic acid (ABA). For example, in *Boea hygrometrica* leaves, *BhWRKY1* is likely to function in an ABA-dependent signal pathway to regulate *BhGolS1* expression, which leads to the accumulation of RFOs and enhanced drought tolerance (Wang et al., 2009). In addition, *AtWRKY33* is partially ABA-dependent in response to salt stress. However, *AtWRKY25* transcript accumulation does not require ABA (Jiang and Deyholo, 2009). Overexpression of *AtWRKY25* and *AtWRKY33* enhance tolerance of salt stress, but causes more sensitivity to oxidative and ABA treatments. Nevertheless, *wrky33 null* mutants and *wrky25wrky33* double mutants are more sensitive to NaCl treatment. The stress sensitivity of *wrky25* null mutants is similar to wild-type plants under any assay conditions. In addition, overexpression of *AtWRKY25* enhances heart tolerance through increasing the expression of heat-inducible genes (Li et al., 2009). *AtWRKY39* acts as a positive regulator of SA-dependent heat stress defense pathways (Li et al., 2010).

Accumulation of ABA leads to stomatal closure in plant cells in water-stressed conditions and enhances tolerance of plants to drought. ABO3 (Ren et al., 2010), encoding a WRKY transcription factor AtWRKY63, is able

to regulate expression of ABF2 by binding the W-box in the promoter of ABF2, and directly or indirectly regulate the expression of *RD29A* and *COR47.* The *abo3* mutants are more sensitive to drought stress than the wild- type plants through impaired stomatal closure and lower expression of some downstream ABA-responsive genes. *ABO3* overexpression lines do not induce any ABA- or drought-related phenotypes. ABO3 is a component of ABA-mediated drought stress response pathway. Recently, *AtWRKY6* has been identified in response to reduced Pi conditions. PHOSPHATE1 (PHO1) plays a key role in Pi translocation from root to shoot (Hamburger et al., 2002). In Pi-deficient conditions *AtWRKY6* can bind to the PHO1 promoter, and negatively regulates *PHO1* expression. Hence *AtWRKY6* overexpression lines are more sensitive to low Pi stress and have lower Pi contents in shoots compared with wild-type seedlings and the *Atwrky6-1* mutant. In addition, *AtWRKY42* has a function similar to *AtWRKY6*, which interacts with the PHO1 promoter and negatively regulates *PHO1* transcription. However, *AtWRKY6* and *AtWRKY42* regulate *PHO1* expression via different pathways (Chen et al., 2009). In contrast to *AtWRKY6*, *AtWRKY75* acts as a positive regulator of Pi acquisition under Pi-deficient conditions (Devaiah et al., 2007).

In barley, the expression of a *WRKY* gene, *Hv-WRKY38*, is involved in the cold and drought stress response (Mare et al., 2004). The *VvWRKY11* gene is involved in the response to dehydration stress (Liu et al., 2010). *GmWRKY21*-transgenic *Arabidopsis* plants were tolerant to cold stress, whereas overexpression of *GmWRKY54* enhances tolerance to salt and drought stress, possibly through the regulation n of *DREB2A, RD29B* and *STZ*. Transgenic plants over-expressing *GmWRKY13* showed increased sensitivity to salt and mannitol stress, but decreased sensitivity to abscisic acid compared with wild-type plants. However, the identity of natural target genes of WRKY factors that function in abiotic tolerance is currently unknown.

Functions of *WRKY* transcription factors in Wheat

The gene sequences and function of WRKY family of wheat, which is one of the most important major crops, are both not well understood. Houde et al. (2006) constructed wheat cDNA-mixed genomic libraries and a suppression subtractive library under a variety of abiotic stress conditions. By sequencing a large number of expressed sequence tags (EST) in these libraries, the expression of 28 *WRKY* genes was found to be regulated, including 21 that were up-regulated and 7 that were down-regulated under specific conditions. Through transcriptome patterns from infected wheat spikes by the fungus *Fusarium graminearum* in cDNA microarray hybridization, one *WRKY* gene was found to be significantly up-regulated in the anthers (Golkari et al., 2007). In addition, Gregersen and Holm (2007) reported that two *WRKY* transcription factor ESTs were found in response to senescence in wheat leaf were found. Wu et al. (2008) isolated 15 wheat *WRKY* genes with complete open reading frames (ORFs), including four genes that were strongly up-regulated with the senescence of leaves and eight genes that were involved in low temperature, high temperature, NaCl or PEG treatment.

We obtained 43 *WRKY* unigenes, named *TaWRKY1 to 43*, by using consensus sequences of WRKY and zinc-finger domains for a Basic Local Alignment Search Tool (BLAST) search against the wheat ESTs database. The response patterns of 8 *TaWRKY* genes to abiotic stresses such as drought, salt, freezing, wounding and plant hormone abscisic acid treatment were then investigated. We have screened and cloned 3 *TaWRKYs* in response to abiotic stress and analyzed their chemical characters. *TaWRKY1, 2 and 19* were overexpressed into *Arabidopsis* by a transgenic method respectively and the phenotype of transgenic *Arabidopsis* was analyzed. *TaWRKY1*-overexpressing *Arabidopsis* was more tolerant to freezing than the wild-type *Arabidopsis*. *TaWRKY2*-overexpressing *Arabidopsis* was more tolerant to drought and salt than the wild-type *Arabidopsis*. *TaWRKY19*-overexpressing *Arabidopsis* was more tolerant to drought, salt and freezing than the wild-type *Arabidopsis*.

CONCLUSIONS

WRKY genes act as an important transcription factors super-family and they involved in the response to environmental stimuli, such as high salt, drought, heat, cool and other abiotic stresses. They participate in plant growth and development and material metabolic pathways, and also play an important regulatory role in anti-viral, anti-bacterial and mechanical injury pathways, showing that WRKY transcription factor have a complex and important role in regulation. To date, studies on the WRKY transcription factor family have focused on cloning and expression of genes, and gene functions through gene absence or over-expression. However, their roles in plant defense signaling and abiotic stress response remain obscure. Reverse genetics will be a powerful tool in the functional studies of the WRKY protein, such as through T-DNA or transposon insertion, anti-sense technology or small-molecule RNA interference technology to enable inactivating or silencing of certain *WRKY* gene expression, and then investigate WRKY protein functions through mutant phenotypes. In fact, most of the *Arabidopsis WRKY* genes have a T-DNA insertion mutant. In addition, ectopic overexpression can also provide information that can help define gene function (Zhang et al., 2003).

Currently, our knowledge rests mainly on the strong ectopic expression of *WRKY* genes in transgenic plants, protoplasts or leaves. However, how useful this approach

will be for WRKY factors remains unclear. Specific MAPKs, and possibly calcium-dependent protein kinases (CDPKs) (Ludwig et al., 2004), can be expected to be partners that modify distinct WRKY factors in such pathways. Identifying downstream target genes of WRKY factors will be crucial in understanding their biological functions, which is the most important idea in *WRKY* gene functions to be studied in the future. Integrating such data with similarly obtained information on other transcription factors will allow us to identify combinatorial gene expression programs, and to establish transcriptional regulatory networks in plants like those developed for yeast (Lee et al., 2002). Considering the size of this gene family, there is little doubt that WRKY factors will keep us both fascinated and busy in the coming years.

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