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Review

Molecular characterization and functional analysis of plant *WRKY* genes

Borun Feng, Sisi Yang, Hai Du, Xianbin Hou, Junjie Zhang, Hanmei Liu and Yubi Huang*

Maize Research Institute, Sichuan Agricultural University, Chengdu Sichuan 611130, China.

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***WRKY* genes are widely distributed in higher plants, and constitute one of the largest transcription factor families. Over recent decades, molecular characterization and functional analysis of *WRKY* have been extensively studied and reported to be involved in many physiological and biochemical processes. This review describes the current knowledge about their molecular, structural and functional characteristics, including the alternative splicing, the response to biotic and abiotic stress, the senescence, the morphological architecture and the evolution. It shows that WRKY transcription factors play a crucial role in plants' developmental and physiological processes. Furthermore, the group-I *WRKY* genes may represent the ancestral form and the *WRKY* genes not only limited in the plant kingdom.**

Key words: WRKY, structure characteristic, biotic and abiotic stress, senescence, development, evolution.

INTRODUCTION

Transcription factors (TFs) as one kind of important protein can bind to the cis-element and regulate the expression of target gene. Both the normal development and the proper response to environment of plant depend on TFs. WRKY TFs comprises a large gene family in higher plants (Eulgem et al., 2000). Since the identification of the first WRKY protein (SPF1) in sweet potatoes (Ishiguro and Nakamura, 1994), numerous WRKY TFs were widely found in plants, such as 74 genes and 102 genes in *Arabidopsis thaliana* and rice (*Oryza sativa*), respectively (Eulgem and Somssich, 2007; Ross et al., 2007), 97 genes in sunflower (*Helianthus annuus*) (Giacomelli et al., 2010), 46 genes in canola (*Brassica napus L.*) (Yang et al., 2009) and 197 genes in soybean (*Glycine max*) (Schmutz et al., 2010). This gene family also exists in lower plants, including mosses (*Physcomitrella patens*) and green algae (*Chlamydomonas reinhardtii*) (U¨ lker and Somssich, 2004; Zhang et al., 2005). In addition, the *WRKY* genes are not limited in plants, such as Protist *Giardia lamblia* and the slime mold *Dictyostelium discoideum* (U¨ lker and Somssich, 2004; Zhang et al., 2005; Pan et al., 2009). The wide distribution of *WRKY* genes indicates that this gene family may have an extensive role in plants' developmental and physiological processes. In the past 15 years, many studies about *WRKY* genes participating in plant stress defense, secondary metabolism, senescence and morphological architecture were reported. The typical function of *WRKY* genes in plants is shown in Table 1.

*Corresponding author. E-mail: yubihuang@sohu.com.

Abbreviations: TFs, Transcription factors; **SA,** salicylic acid; **JA,** jasmonic acid; **GA,** gibberellic acid; **ABA,** abscisic acid; **MeJA,** methyl jasmonate; **ACC,** 1-aminocyclopropane-1-carboxylic acid; **MAP,** mitogen-activated protein; **PAMP,** pathogen-associated molecular patterns triggered immunity; **ETI,** effector-triggered immunity; **ET,** ethylene; **CaMBD,** calmodulin-binding domain.

CLASSIFICATION AND CHARACTERISTICS OF *WRKY* GENES

WRKY TFs have a segment of conserved WRKYGQK or WRKYGKK amino acids sequence at N-terminal, and also a Cys2His2 or Cys2HisCys type of zinc finger at C-

Table 1. Typical function of *WRKY* genes.

Function	Description		Main reference
Defense response	Biotic	Pathogen	Liu (2007)
		Insect	Skibbe et al. (2008)
	Abiotic	Temperature	Li (2010)
		Penetration	Wei (2008)
		Water	Wang (2009)
		UV-B irradiation	Wang (2007)
Growth and Development	Senescence		Miao (2010)
	Morphological Architecture	Seed development	Zhou (2009)
		Leaf growth	Kim (2006)
		Root formation	Zhang (2008)
		Trichome development	Johnson (2002)
Biosynthesis	Berberine biosynthetic		Kato (2007)
	sesquiterpene Phytoalexins biosynthesis		Xu (2004)
	Starch synthesis		Sun (2003)
	Artemisinin biosynthesis		Ma (2009)
Other function	Pi translocation		Chen (2009)
	Signaling pathway		Shang (2010)
	Binding C_aM		Park (2005)
	Second wall formation		Wang (2010)
	Regulator		Qiu (2008)

terminal. Based on the number of WRKY domains and the type of zinc finger, WRKY TFs can be divided into four groups: Group I includes two WRKY domains and a type of Cys2His2 or Cys2HisCys; group II has one WRKY domain and a type of Cys2His2; group III has one WRKY domain and a type of Cys2HisCys in C-terminal (Eulgem et al., 2000); group IV, especially for OsWRKY proteins, contains a WRKY domain but lacks a complete zinc finger (Ross et al., 2007; Xie et al., 2005). The group II WRKY proteins are further subdivided into subgroups a to e, based on the presence of short conserved structural motifs (Eulgem et al., 2000).

WRKYGQK structure of WRKY transcription factors can specifically bind to the W-box of promoter (Eulgen et al., 2000; Zhou et al., 2008). W-box widely distributes in defense genes and *WRKY* genes' promoter sequence (Rushton et al., 1996), mediating *WRKY* gene to regulate target genes' expression. Some observations suggest that a minimal W-box element might be defined as TTGACC/T (Ciolkowski et al., 2008; Rushton et al., 2010). However, WRKYGKK type of *NtWRKY12* and *GmWRKY21* failed to bind to the W-box element (van Verk et al., 2008; Zhou et al., 2008), and *NtWRKY12* can specifically recognize WK boxes (TTTTCCAC) (van Verk et al., 2008). The conserved amino acid of Q or K will be involved in the divergence, and may alter spatial structure of WRKY TFs. Although hundreds of WRKY proteins are known by now, full appreciations of how these factors assembling at DNA-binding sites to modulate transcription are very limited; a very timely report about this assembling is shown. *AtWRKY1*-C is composed of a globular structure with five β strands, forming an antiparallel β-sheet. A novel zinc-binding site is situated at one end of the β-sheet, between strands β4 and β5. The DNA-binding residues of *AtWRKY1*-C are located at β2 and β3 strands (Duan et al., 2007).

ALTERNATIVE SPLICING OF *WRKY* GENES

The alternative splicing (AS) is a major mechanism for generating mRNA and protein diversity. Alterations in protein-coding regions regulated by alternative splicing can lead to changes such as binding properties, subcellular localization, enzyme activities, and protein stability (Stamm et al., 2005). Analysis of rice cDNAs suggests that at least 16% of transcripts are alternatively spliced (Kikuchi et al., 2003). In *Arabidopsis* and rice, a number of *WRKY* genes are also predicted to have alternative open reading frames (Xie et al., 2005; Wu et

al., 2005), which indicated that AS events play a key role in *WRKY* gene family leading to a diverse population of RNAs.

In *WRKY* genes, some introns and extrons can exchange in different variants. For example, *OsWRKY8* has two splice variants, variant 1 (AY341857) and variant 2 (AK109568), extron 4 of AY341857 as an intron in variant 2, but extron 5, 6, 7 and 8 of AK109568 as introns in variant 1 (Xie et al., 2005). In these different alternative splices, the WRKY domains are retained, but there are some differences as also watched. For instance, a variant of *OsWRKY1* lost all the coding sequence of the WRKY domain; variant 2 (AY341843) of *OsWRKY35* lost a section of WD (Xie et al., 2005). There is an interesting study which proves that some alternative splices may not change the *WRKY* genes' function. The *OsWRKY62* gene encodes two splice variants (OsWRKY62.1 and OsWRKY62.2). The OsWRKY62.2 protein lacks a 39 amino acid segment from the N-terminus of OsWRKY62.1, which is predicted to contain a coiled-coil domain. The functional analysis indicates that OsWRKY62.1 and OsWRKY62.2 play the same role in plants' innate immunity (Peng et al., 2008). Most of the *OsWRKY* genes contain an intron in their WRKY domain (WDs) (Wu et al., 2005). In total, two types of introns exist in the conserved WDs: one of which is spliced exactly at the codon of R (phase-2 intron), that is classified as R-type intron; the other one is located before the V residue, which is located at the sixth amino acid after the second C residue in the C2H2 motif in the zinc finger region (phase-0), this intron is designated as a V-type intron. Complete sequencing of mass species in this area will provide more proofs, and it may suggest that the intron splicing positions are conserved in the WDs of advanced plants.

EXPRESSION PATTERNS OF *WRKY* GENES

WRKY proteins as a type of widely distributed transcription factors can be induced by various biotic and abiotic stimulis, including pathogen, insect, plant hormones, dehydration and temperature. After biotic stimuli, some *WRKY* genes increase expression, such as *OsWRKY45* (Qiu et al., 2009), *OsWRKY31* (Zhang et al., 2008), *CaWRKY30* (Zheng et al., 2011), *Nicotiana tabacum WRKY3* and *WRKY4* (Chen and Chen, 2000) and *WRKY6* (Skibbe et al., 2008). *WRKY* genes can also be induced by abiotic stresses, such as *AtWRKY34* (Zou et al., 2010), *AtWRKY6* (Kasajima, 2010) and *OsWRKY45* (Shimono et al., 2007; Qiu et al., 2009). After plant hormones treatment, *OsWRKY10*, *-62*, *-82*, and *-85* (Ryu et al., 2006), *OsWRKY24*, *-51*, and *-72* (Xie et al., 2005) are induced. Some *WRKY* genes can also be induced by one hormone, but repressed by other hormones. For example, *AtWRKY70* is activated by salicylic acid (SA) and repressed by jasmonic acid (JA) (Li et al., 2004); *HvWRKY38* in barley aleurone cells is down-regulated by **gibberellic** acid (GA), but up-regulated by SA and abscisic acid (ABA) (Xie et al., 2007); *OsWRKY71* is up-regulated by several defense signaling molecules, such as SA, methyl jasmonate (MeJA), 1-aminocyclopropane-1-carboxylic acid (ACC), ABA, but down-regulated by GA (Zhang et al., 2004; Xie et al., 2005; Liu et al., 2007). These results indicate that one *WRKY* gene may participate in different regulation networks and mediates the cross-talk of different signaling pathways. These will provide useful information for further unraveling of these complex functions of *WRKY* genes.

FUNCTION OF *WRKY* GENES

Plant in the growth process in order to adapt to the internal and external environment of the stimulus, gradually formed some adaptation to the environment of physiological ecology function, and the development of adversity and signal in response produce all types of secondary metabolites. Among them, through the transcription factors in the transcription of functional genes on related complex and delicate expression regulation, it is directed to the plant growth, and physiological metabolism of control is an important way. A well known function of *WRKY* genes is reported to be involved in many physiological and biochemical processes, such as responding to biotic and abiotic responses, leaf senescence, development and secondary metabolism.

BIOTIC STRESS

Plants have developed two layers of innate immunity system against pathogen attacks (Jones and Dangl, 2006). The first layer of innate immunity is initiated by the recognition of many pathogens, and often activates downstream mitogen-activated protein (MAP) kinase cascades and defense gene, which is called Pathogen-associated molecular patterns (PAMP) triggered immunity (PTI).

The second layer of innate immunity is triggered by plant disease resistance proteins (major R gene products) that recognize directly or indirectly specific pathogen-derived effectors called effector-triggered immunity (ETI) (Chisholm et al., 2006). The processes of plants responding to pathogens, insects and woundings are generally regulated by multiple hormones, such as SA, JA, and ethylene (ET) (Glazebrook, 2001; Corne´ M Pieterse and Van Loon, 2004). In plant, defence-related genes expression is the critical process for higher plant disease defence (Riechman et al., 2000). For example, over expression of *OsWRKY71* gene in rice results in

enhanced resistance to virulent bacterial pathogens *Xanthomonas oryzae pv. oryzae* (*Xoo*), and two marker genes in defense signaling pathway, *OsNPR1* and *OsPR1b*, are constitutively expressed (Liu et al., 2007); over expression of the *OsWRKY31* or *OsWRKY53* gene is found to enhance resistance to *M. grisea*, and pathogenesis-related protein genes such as *PBZ1* shows constitutive expression (Chujo et al., 2007; Zhang et al., 2008). *OsWRKY45-1* and *OsWRKY45-2* as a pair of allelic genes which encode proteins with a 10-amino acid difference plays opposite roles in rice resistance against bacterial pathogens. *OsWRKY45-1* represses the resistance to *Xoo* and *Xoc*. In contrast, *OsWRKY45-2* enhances resistance to *Xoo* and *Xoc*. Interestingly, *OsWRKY45-1* and *OsWRKY45-2* over-expressing plants shows enhanced resistance to *M. grisea* (Tao, 2009). The over-expression of the *OsWRKY89* gene enhances the resistance to the rice blast fungus and white-backed planthopper (Wang et al., 2007).

In *Arabidopsis*, some *WRKY* genes can increase defense to the necrotrophic fungal pathogens *Botrytis cinerea*, such as *AtWRKY8* (Chen, 2010), *AtWRKY33* (Zheng, 2006), *AtWRKY3* and *AtWRKY4* (Lai et al., 2008) but *AtWRKY8* (Chen, 2010), *AtWRKY25* (Zheng, 2007), *AtWRKY11* and *AtWRKY17* (Journot-Catalino, 2006), *AtWRKY7* (Kim et al., 2006), *AtWRKY38* and *AtWRKY62* (Kim et al., 2008) and *AtWRKY48* (Xing et al., 2008) are negative regulator basal resitance to the bacterial pathogen and *Pseudomonas syringae. Arabidopsis* mutant *wrky27-1* shows the delayed symptom development in response to the bacterial wilt pathogen *Ralstonia solanacearum* (Mukhtar et al., 2008). The up-regulation of *AtWRKY70* caused enhanced resistance to *Erysiphe cichoracearum,* and compromised plant resistance to *Alternaria brassicicola* (Li et al., 2006). *AtWRKY70* is a component of a basal defense mecha-nism that is boosted by engagement of either RPP4 or RPP7, and is required for RPP4-mediated resistance (Knoth et al., 2007). The *AtWRKY33* functions down stream of MPK3/MPK6, in reprogramming the expression of camalexin biosynthetic genes which drives the metabolic flow to camalexin production in *Arabidopsis* challenged by pathogens (Mao et al., 2011). The *Nicotiana benthamiana WRKY8* ectopic expression induced defense-related genes, such as 3-hydroxy-3-methylglutaryl CoA reductase 2 and NADP-malic enzyme. By contrast, silencing of *WRKY8* decreased the expression of defense-related genes and increased disease susceptibility to the pathogens *Phytophthora infestans* and *Colletotrichum orbiculare* (Ishihama, 2011).

The *Arabidopsis wrky18*, *wrky40*, *wrky18* and *wrky60*'s double mutants and the *wrky18 wrky40* and *wrky60*'s triple mutants are substantially more resistant to *P. syringae*, but more susceptible to *B. cinerea* than wild-type plants. While constitutive expression of *WRKY18* enhances the resistance to *P. syringae*, its coexpression with *WRKY40* or *WRKY60* makes plants more susceptible to both *P. syringae* and *B. cinerea* (Xu et al., 2006). *OsWRKY13* expression is regulated by multiple factors to achieve the resistance against diseases. For example, MYB and AP2/EREBP proteins may contribute mainly to the control of the *OsWRKY13*-downregulated genes (Cai, 2008; Qiu et al., 2009).These researches indicate that *WRKY* genes can have different functions through interaction with different *WRKY* genes, and may achieve their functions by other TFs. Constructing multy-*WRKY* genes mutants or *WRKY* genes and other TFs mutants may more deeply study the role of *WRKY* in plant development. Based on these studies, a large number of transgenic plants could be built, and it can improve the transgenic plants' resistance to numerous pathogen and insect attacks in plant breeding.

ABIOTIC STRESS

The *WRKY* gene family was recently suggested to play a key role in the response of plants to abiotic stresses, both as negative and positive regulators response to environment stimulis. *OsWRKY11* (Wu et al., 2009), *HvWRKY38* (Xiong et al., 2010), *TaWRKY2* and *TaWRKY19* (Niu et al., 2012) enhances resistance to drought stress, *OsWRKY89* (Wang et al., 2007) enhances the resistance to tolerance to UV-B irradiation, *AtWRKY39* (Li et al., 2010) regulates defense to heat stress, *TcWRKY53* (Wei et al., 2008) regulates the plant osmotic stress response and *AtWRKY63* participates in ABA and drought stress (Ren et al.，2010); but some *WRKY* genes can also negatively mediate plants response to the stimulis. For example, over-expressing *GmWRKY13* shows increased sensitivity to salt and mannitol stress (Zhou et al., 2008); the heterologous expression of *OsWRKY72* emerges more sensitive to mannitol, NaCl, ABA stresses and sugar-starving in *Arabidopsis* (Yu et al., 2010), *AtWRKY34* negatively regulates *Arabidopsis* mature pollen sensitivity to cold, and may join CBF signaling pathways of mature pollen (Zou et al., 2010). Furthermore, *AtWRKY6* and *AtWRKY42* are involved in *Arabidopsis* responses to low Pi stress through the regulation of PHO1 expression (Chen, 2009), *OsWRKY80* is up-regulated in rice leaves, stems and roots after Fe-excess treatment, indicating that *OsWRKY80* could be a Fe stress-responsive gene (Ricachenevsky et al., 2010).

The role of WRKY genes can regulate some special metabolites in plant response to abiotic stress. *BhWRKY1* is likely to function in an ABA-dependent signal pathway to regulate galactinol synthase expression, which leads to the accumulation of raffinose family oligosaccharides in desiccation-tolerant *Boea hygrometrica* leaves (Wang et al., 2009). The over-

expression of *OsWRKY11* in rice increases raffinose's accumulation, and enhances its tolerance to desiccation (Wu et al., 2009). So far, the reports about *WRKY* genes response to abiotic stress focus more on temperature, water and soil minerals stress, and WRKY proteins may also find some new defense characterizations in response to air pollution and pesticide stress in the future.

LEAF SENESCENCE

Senescence is an orderly process and is regulated by a series of multi-genes. Normal senescence is necessary for metabolic organism. Expression profiling in *Arabidopsis* reveals that WRKY TFs are the second largest family of transcription factors in the senescence transcriptome (Guo, 2004; Rushton et al., 2010). The first evidence of *WRKY* genes having a role in senescence, came from the study of *AtWRKY6* (Robatzek and Somssich, 2001). SIRK encodes a receptor-like protein kinase, whose developmental expression is strongly induced, specifically during leaf senescence. The transcriptional activation of SIRK is dependent on *AtWRKY6* function (Robatzek and Somssich, 2002). In *Arabidopsis*, *AtWRKY53* ensures that senescence is executed in the correct time frame, and can be degraded by the HECT domain E3 ubiquitin ligase UPL5 (Miao et al., 2010), MEKK1 can bind to the promoter of the *AtWRKY53* gene, phosopharylate *WRKY53 in vitro* increasing its DNA binding activity (Miao et al., 2007). Dark-treated *AtWRKY22* over-expression and knockout lines showed accelerated and delayed senescence phenotypes, respectively, and senescence-associated genes exhibited increased and decreased expression levels (Zhou et al., 2011). Furthermore, over-expression of *OsWRKY23* enhances leaf senescence in darkness, and two senescence-related marker genes, *SAG12* and *SEN1* are altered (Jing, 2009).

DEVELOPMENT

Since the identification of the first WRKY protein (TTG2) function in trichome development and in the endothelium of developing seeds (Johnson et al., 2002), there are many *WRKY* genes participating in plant morphological architecture. The expression of TTG2 is regulated by complexes containing *R2R3 MYB* and bHLH transcription factors, and that TTG2 in turn appears to regulate the expression of GLABRA2 in the differentiation of trichomes and root hairless cells (Ishida, 2007). Over-expression of the *OsWRKY31* gene is found to reduce lateral root formation and elongation, and showed constitutive expression of many auxin-response genes, such as *OsIAA4* and *OsCrl1* genes (Zhang et al., 2008). Over-expression of the *OsWRKY89* gene leads to retardation to growth at the early stage, reduction of internode length, decreased extractable and cell-wall-bound phenolic compounds, and lignin staining showed an increase in lignification in culms (Wang et al., 2007).

In seed germination and post-germination development, researchers also found that *WRKY* genes play an important role. For example, *AtWRKY10* can be regulated by SHB1, and promote a large seed cavity and endosperm growth in the early phase of seed development (Luo et al., 2005; Zhou et al., 2009), the over-expression of *OsWRKY72* retards seed germination under normal conditions and early flowering, reduces apical dominance, leads to loss of high temperature-induced hypocotyl elongation response, and enhances gravitropism response in *Arabidopsis* (Yu et al., 2010), and for *AtWRKY2*, it can mediate seed germination and post germination developmental arrest through ABA (Jiang, 2009).

SECONDARY METABOLISM

WRKY proteins also have an unavoidable role in plant secondary metabolism. In grapes (*Vitis vinifera*), *VvWRKY2* participates in regulating lignification (Guillaumie et al., 2010). In *Coptis japonica* protoplasts, ectopic expression of *CjWRKY1* cDNA increases the level of transcripts of all berberine biosynthetic genes (Kato et al., 2007). In cotton (*Gossypium* spp.), *GaWRKY1* target cotton (+)-δ-cadinene synthase participates in the regulation of sesquiterpene phytoalexins biosynthesis (Xu et al., 2004). SUSIBA2 as a WRKY protein is a regulatory transcription factor in starch synthesis, SUSIBA2 binds to the SURE (sugar responsive) elements in the barley iso1 promoter as an activator (Sun et al., 2003). In *Artemisia annua*, *AaWRKY1*'s regulation of artemisinin biosynthesis indicates that amorpha-4,11-diene synthase (ADS) is a target gene of *AaWRKY1* (Ma et al., 2009). Recently, an interesting research indicates that WRKY TFs are partially responsible for the parenchymatous nature of the pith cells in dicotyledonous plants (Wang et al., 2010). Genome-wide analysis of the expression profiles of *OsWRKY13* over-expressing lines suggests that *OsWRKY13* directly or indirectly regulates the expression of more than 500 genes that are potentially involved in different physiological processes, according to the classification of the Gene ontology database (Qiu et al., 2008).

These researches may indicate that *WRKY* genes participate in numerous processes of biosynthesis, and provide vital information for further learning of the function of this family in plants' growth.

SOME POTENTIAL FUNCTION

WRKY genes, as one of the largest transcription factor

families, have numerous important roles. Clearly identifying upstream and downstream genes of WRKY TFs and screening inter-affecting protein would be crucial for learning the funtion of *WRKY* genes in plants' growth. There are some typical findings as new directions to explore *WRKY* genes' function. For example, *AtWRKYIId* TFs has a calmodulin (CaM)-binding domain (CaMBD), which can interact with CaM, such as *AtWRKY7* (Chan et al., 2005; Park et al., 2005); *AtWRKY33* and *AtWRKY53* which may be important components of a pathway involved in chitin signalling (Wan et al., 2004); *AtWRKY40*, *AtWRKY18* and *AtWRKY60* can interact with a type of cell chloroplast ABA receptor (ABAR) (Shang et al., 2010).

EVOLUTION OF *WRKY* GENES

Although three groups of *WRKY* genes are widely distributed in higher plants, they tend to be different in low eukaryotes. In low plants, members of Group III have not been found in mosses and green algae (U¨ lker and Somssich, 2004; Zhang et al., 2005). In two non-photosynthetic eukaryotes, group I-like *WRKY* genes are identified in the protist *Giardia lamblia* and the slime mold *Dictyostelium discoideum* (U¨ lker and Somssich, 2004; Pan et al., 2009; Zhang et al., 2005). Some analysis suggests that the C-terminal domain of the two-WRKY-domain encoding gene appears to be the ancestor of the single-WRKY-domain encoding genes (Wu et al., 2005; Zhang et al., 2005). Different from *R2R3-MYB*, their two homologous parts simultaneously and specifically bind to the target sequence (stracke et al., 2001), but only the conserved WRKY domain on the C-terminal of Group I of WRKY TFs contain binding activity (Eulgem et al., 2000).

Binding capability of *AtWRKY1* was mapped to the C-terminal WRKY domain by deletion mutations, while the deletion of the N-terminal WRKY domain results in reduced binding affinity (Duan et al., 2005). The function of N-terminal WRKY domain is not clear, perhaps with the C-terminal sequences flanking the target sequence to participate in the binding process, thus increasing the affinity of the specific activity, pro-activity or the provision of these protein interactions with other proteins of the contact interface (Eulgem et al., 2000) but some people also speculated in the perspective of the *WRKY* gene evolution that the N-terminal region of WRKY function is replaced by a special cofactor (Wu et al., 2005; Zhang et al., 2005). These findings imply that group I *WRKY* genes may represent the ancestral form and more importantly, that *WRKY* genes originated around 1.5 to 2 billion years ago in eukaryotes, that is before the divergence of the plant phyla (U¨ lker and Imre Somssich., 2004; Zhang et al., 2005). Moreover, phylogenetic analysis shows that some *WRKY* genes of *Solanum lycopersicum* is close to *WRKY* genes of *Arabidopsis*, *Oryza sativa*, *Nicotiana tabacum* and *Solanum tuberosum* (Molan et al., 2010). HvWRKY proteins exhibit not only sequence similarities with *Arabidopsis*, but also relation in their expression patterns (Mangelsen et al., 2008). These researches may indicate a putative conserved function of related WRKY proteins in monocot and dicot species.

CONCLUSION

WRKY genes are shown to be functionally connected in many biological processes, and can act as repressors, as well as activators regulating downstream genes' expression. A few reports about these TFs also can feedback loops, such as AtWRKY6 (Robatzek and Somssich et al., 2002), and these make the *WRKY* genes form a complicated regulation web. Nowadays, functional analysis of *WRKY* genes is more focused on constructing insertion mutant and over-expression line of single *WRKY* gene; multi-mutants and multi over-expression lines may be more comprehensive and fundamental to traverse the biological functions of these genes in biotic and abiotic stress, senescence, development and secondary metabolism. Although functional research of *WRKY* genes has become more and more important by now, full appreciation of how these factors assemble at DNA-binding sites to modulate transcription will provide useful and lucid information for deep research. Breakthrough technologies and complete sequencing of mass species in this area will highly enhance the comprehensive investigating function and provide more useful information for the evolutional characterization of this family.

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