

WRKY Transcription Factor: Role in Abiotic and Biotic Stress

Alka Shankar • Amita Pandey • Girdhar K. Pandey*

Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, Dhaula Kuan, New Delhi-110021, India

Corresponding author: * gkpandey@south.du.ac.in

ABSTRACT

One of the fundamental behaviors of plants, being sessile allows them to develop an intricate molecular machinery to adapt themselves to biotic and abiotic challenges experienced in the environment. In response to adapt against a particular stress, a massive transcriptional activity of several genes triggered the defense responses in the plant cell. Extensive research on stress related studies have introduced the role of transcription factors for regulation of the plants responses. In many cases, transcription factors acts as a “master or key regulator” of gene expression under one or multiple stress conditions. In plants, WRKY has emerged as a major and largest transcription factor family. *Arabidopsis* and rice has 74 and 109 WRKY members respectively, which play a major role during stress and development. WRKY gene family has been studied to be induced in response to several phytohormones such as SA, JA, ABA and pathogen attack, thus have been found to be a key player in plant defense mechanism. This gene family also forms a highly interacting regulatory network with stress response, by acting as either transcription activator or repressor thereby modulating the gene expression. Beside stress and developmental conditions, WRKY is also induced in nutrient deficient conditions such as phosphate deficiency and starvation. The main emphasis of this review is to summarize the progress in WRKY transcription factor research under stress and developmental conditions. At the same time an attempt has also been made to comment upon interaction with a wide range of signaling networks such as MAP kinase proteins, 14-3-3 proteins, calmodulin and regulator of chromatin such as histone deacetylases, and plant-pathogen defense proteins. Moreover, a crosstalk or overlap is also discussed among these different components or pathways involving WRKY transcription factor.

Keywords: abscisic acid signaling, abiotic stress, biotic stress, nutrient deficiency, plant defense, signal transduction, thermal tolerance, transcription

Abbreviations: ABA, abscisic acid; ABF, ABA responsive elements-binding factor; ABI, ABA insensitive; APX1, ascorbate peroxidase1; BTH, benzothiadiazole; EMSA, electrophoresis mobility shift assay; ETI, effector-triggered immunity; FHB, fusarium head blight; FLS2, flagellin receptor; JA, jasmonic acid; LRR, leucine rich-repeat; MAPK, mitogen-activated protein kinase; MBF1, multiprotein bridging factor1; MLA, mildew A; NB, nucleotide binding; NPR1, non-expresser of pathogenesis-related gene1; PTI, PAMP triggered immunity; ROS, reactive oxygen species; SA, salicylic acid; SAR, systemic acquired resistance

CONTENTS

INTRODUCTION.....	26
Structural features and classification	27
ROLE IN ABIOTIC STRESSES	27
Role in nutrient deficiency.....	27
Role in drought, abscisic acid signaling and stomatal opening.....	29
Role in oxidative and thermal tolerance	30
ROLE IN BIOTIC STRESSES	30
WRKY transcription factors in plant defense: Case study in <i>Arabidopsis</i>	30
WRKY transcription factors in plant defense: case study in other plants	31
CONCLUSIONS AND FUTURE PROSPECTS	32
ACKNOWLEDGEMENTS	32
REFERENCES.....	32

INTRODUCTION

The WRKY family is among the largest family of transcription factors in higher plants and almost specific to green lineage like green algae and land plants. WRKY class of transcription factors binds to W-box, which begin with the sequence TTGACC or TTGACT. A large gene family of the WRKY superfamily of transcription factors have been identified in several plants, 74 members in *Arabidopsis* (Dong *et al.* 2003), 109 members in the rice, *Oryza sativa* (Ross *et al.* 2007), 197 in soybean, *Glycine max* (Schmutz *et al.* 2010), 66 in papaya, *Carica papaya*, 68 in sorghum, *Sorghum bicolor*, 104 in poplar, *Populus alba*, 38 in moss, *Physcomitrella patens* (Pandey and Somssich 2009), 80 in

pine, *Pinus monticola* (Liu *et al.* 2009), 45 in barley, *Hordeum vulgare* (Mangelsen *et al.* 2008), 55 in cucumber, *Cucumis sativus* (Ling *et al.* 2011), 47 in castor bean, *Ricinus communis* (Li *et al.* 2012), 81 in tomato, *Solanum lycopersicum* (Huang *et al.* 2012), and 119 in maize, *Zea mays* (Wei *et al.* 2012). Their presence in such large numbers in several plants species points towards their important role in evolution. The phylogenetic analysis of WRKY genes based on the conserved amino acid sequence showed these are present in a few non-plants species such as *Giardia lamblia* and *Dictyostelium discoideum* (Glockner *et al.* 2002; Pan *et al.* 2009). The phylogenetic analysis also suggests that WRKY genes might have originated approx. 1.5-2 billion years ago in eukaryotes but absent in animals

(Ulker and Somssich 2004).

The first reported WRKY genes were *SPF1* from sweet potato (*Ipomoea batatas*), *ABF1* and *ABF2* from wild oat (*Avena fatua*), which play a major role in the regulation of gene expression in sucrose metabolism and during germination (Ishiguro and Nakamura 1994; Rushton *et al.* 1995). *PcWRKY1* and *PcWRKY3* from parsley (*Petroselinum crispum*) provided the first evidence that these transcription factors play important role in plant defense and termed the name WRKY after the conserved WRKY amino acid sequence in their DNA binding domains (Rushton *et al.* 1996). Besides regulating responses to biotic stress, the WRKY proteins have been implicated in the regulation of many other biological processes such as wounding (Hara *et al.* 2000), senescence (Hinderhofer *et al.* 2001; Miao *et al.* 2004), development (Johnson *et al.* 2002), dormancy, drought tolerance (Pnueli *et al.* 2002) and hormone signaling pathways (Zhang *et al.* 2004; Xie *et al.* 2005). Being a large gene family of transcription factors involved in many physiological and developmental processes in plants, WRKY group of genes enticed us to discuss their structure and function in greater detail. Moreover, this report will emphasize the functional role of WRKY in different signaling networks, which require alteration in gene expression to bring about a final response.

Structural features and classification

DNA-binding domain is the defining feature of WRKY proteins and binds to W-box (C/TTGACT/C) in the *cis*-regulatory element of the target gene promoter (Rushton *et al.* 1996). Each WRKY protein has at least one WRKY domain of approximately 60 amino acids containing the conserved amino acid sequence WRKYGQK at its N-terminal end, coupled with a novel metal chelating zinc-finger-like motif at the C-terminal, CX₄₋₇CX₂₂₋₂₃HXH/C. For proper binding of the WRKY proteins both the WRKY and Zn finger motif are required (Maeo *et al.* 2001). Based on the comparison of WRKY domain sequences and their numbers from *AtWRKY* proteins, Euglem *et al.* (2000) classified the *AtWRKY* into three groups, group I, group II and group III. Group I proteins contain two WRKY domains and a C2H2 type zinc-finger motif, group II proteins have a single WRKY domain and a zinc-finger motif same as that of group I. Group III proteins are similar to group II possessing a single WRKY domain but differ from both the groups in containing a unique C2HC type zinc-finger motif. On the basis of the primary amino acid sequence, the group II proteins were further divided into five subgroups IIa, IIb, IIc, IId, and IIe (Euglem *et al.* 2000). Based on phylogenetic analysis, conserved domain structures and intron positions of the WRKY domains in a range of eukaryotes (slime mold, green alga and higher plants such as *Pinus*, rice, *Arabidopsis*), a new classification system was proposed in which the genes were reorganized into five independent groups i.e., group 1, group 2a+2b, group 2c, group 2d+2e, and group 3. The new system of classification retained group I and group III as such from classification done by Euglem *et al.* (2000) but the subgroup IIc was promoted to group 2c, while subgroup IIa and IIb, IIc and IId were merged to form group 2a+2b and group 2d+2e respectively (Zhang *et al.* 2005).

Based on the structure and numbers of WRKY domain and zinc finger motifs, the WRKY proteins of rice were grouped into four groups. Group I proteins possess two WRKY domains and two zinc finger motifs of either C2H2 or C2HC type. Group II proteins have a single WRKY domain and C2H2 type zinc finger motif, while group III proteins have a single WRKY domain like that of group II but differ in the sequence of their zinc finger motif (C2HC). The unique group IV proteins possess a single WRKY domain and an incomplete zinc finger motif designated as C2XX motif (Ross *et al.* 2007).

The NMR solution structure of a WRKY domain revealed that this domain consists of a four-stranded β -sheet

and a zinc-binding pocket formed by the conserved Cys/His residues (Yamasaki *et al.* 2005). The crystal structure of *AtWRKY1-C* (C-terminal WRKY domain of *AtWRKY1*) showed that it is composed of five β -strands forming an antiparallel β -sheet with DNA-binding site located at β 2 and β 3 strands (Duan *et al.* 2007). Beside the WRKY domain, WRKY proteins contain characteristic features of transcription factor such as nuclear localization signals, activation or repression domains, and domains associated with protein-protein interactions such as leucine zippers and TIR-NBS-LRRs (Chen *et al.* 2011). The presence of the characteristic features of transcription factors and DNA binding domain enables WRKY proteins to play a major role in regulation of gene expression.

ROLE IN ABIOTIC STRESSES

WRKY transcription factors are now known to be involved in regulating responses involved in abiotic stresses such as drought, cold, high salinity, heat shock, ultraviolet B-radiation, and in developmental and physiological processes like senescence (Miao *et al.* 2004), dormancy (Pnueli *et al.* 2002) and hormonal responses (Xie *et al.* 2005). However, very little is known about their mechanistic role in abiotic stresses as compared to biotic stress. WRKY genes are central regulators in signal transduction pathways either acting as activators or repressors of gene expression (Liu *et al.* 2006). WRKY family of transcription factors has many members in different plants and considering the fact that they play diverse roles, many of which are still unknown under different environmental conditions, it becomes a challenging task to determine their mode of action. Here, we describe a few of the possible abiotic processes where WRKY gene family members are known to be involved.

Role in nutrient deficiency

Mineral nutrient deficiency is a major limitation for plant growth and development, having a direct bearing on the agricultural yield around the world (Luan *et al.* 2009). Plant mineral nutrient deficiency also imposes abiotic stress conditions in plant. Plants absorb minerals from the soil through their roots, which is then transported and finally distributed and assimilated to rest of the plant. In response to low-nutrient conditions, plants adapt by augmenting their competence of nutrient uptake from the soil. Plants need to “monitor” the external nutrient concentration and “respond” to the condition and like many biological processes, this “nutrient sensing-responding” process could involve a signaling pathway. Unfortunately, very little is known about the components involved in this signaling network. Recently, it has been shown that several WRKY transcription factors like *AtWRKY6*, *AtWRKY45* and *AtWRKY75* also participate in the nutrient deficiency response signaling pathways (Chen *et al.* 2009). WRKY75 is the first member of the WRKY transcription factor family reported to be involved in regulating phosphate starvation response and root development. In the absence of phosphate (Pi), the expression of WRKY75 was strongly induced and the expression is rapidly suppressed by resupply of Pi. RNAi mutants of WRKY75 in *Arabidopsis* further revealed the expression pattern of some Pi-starvation associated genes, such as phosphatases, Mt4/TPS1-like genes (Metallothionein4/Trehalose-6-Phosphate Synthase1), and high-affinity Pi transporters, which are reduced upon suppression of WRKY75 (Devaiah *et al.* 2007). This resulted in reduced Pi uptake in this RNAi transgenic plant. Phenotypically these mutants also showed significant increase in the number of root hair and increase in the number and length of the lateral roots. This confirmed the role of *AtWRKY75* in regulation of Pi starvation response and also in the regulation of root architecture (Wang *et al.* 2002; Devaiah *et al.* 2007). This gene WRKY75, which is activated in response to Pi deficient condition, is also induced in nitrogen, potassium and iron starvation conditions, but not by other abiotic stresses such

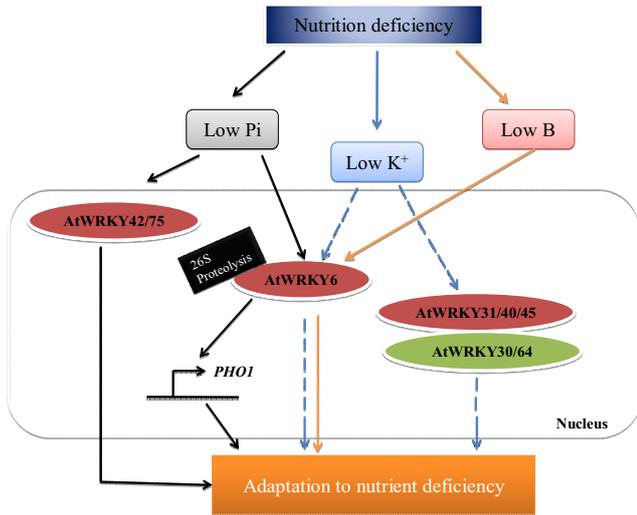


Fig. 1 Model depicting the role of WRKY in regulating responses to nutrient deprivation conditions. In low Pi condition some of the WRKY genes like *AtWRKY6/42/75* are playing a very important role to adapt accordingly and *AtWRKY6* is regulated by degradation via 26S proteasome-mediated proteolysis and thought to relieve the expression of *PHO1* promoter. Under low potassium conditions, *AtWRKY31/40/45* are tentatively up regulated and *AtWRKY30/64* are down regulated as determined by microarray analysis under potassium starvation conditions. Also in low boron conditions, *AtWRKY6* is highly up regulated as found in the expression analysis study.

as cold, drought and salt stresses (Devaiah *et al.* 2007). Another member of Arabidopsis WRKY family, *AtWRKY6*, also plays an important role in phosphate deficiency response (Chen *et al.* 2009). WRKY transcription factors have a DNA binding domain, which binds to W-box motifs in the promoters of their target genes and regulate their expression. One of the targets of WRKY6 transcription factor is PHOSPHATE1 (*PHO1*), which is known to be involved in regulating Pi loading of the xylem possibly through a signal transduction cascade or by affecting the activity of transporter proteins (Fig. 1). The *PHO1* promoter is known to contain six W-box motifs (four named as W_0 , W_X , W_Y and W_Z) close to the coding region. ChIP-qPCR (Chromatin Immunoprecipitation Quantitative PCR) analysis confirmed that WRKY6 strongly interacted with W_Y and W_Z motifs, thereby, suggesting that WRKY6 regulates the transcription of *PHO1*. WRKY6 negatively regulates *PHO1* expression was confirmed by the observation that the transcription of

PHO1 in roots of *Arabidopsis* was greatly reduced in WRKY6-overexpression lines (Chen *et al.* 2009). Another transcription factor *AtWRKY42* regulates the *PHO1* transcription in phosphate deficient conditions through binding to the W-box, similar to that *AtWRKY6*. Previously, *AtWRKY6* was identified to be involved in the process of leaf senescence and defense related processes through the expression of its target gene *SIRK*, a receptor like protein kinase (Robatzek and Somssich 2002). *AtWRKY6* act as a positive regulator under boron deficiency condition as shown genetically in the T-DNA insertion of *AtWRKY6* rendering reduced root length in the mutant plant (Fig. 1) (Kasajima *et al.* 2010).

Potassium is one of the important macronutrient for plant growth and development, which perform vital functions in metabolism, growth, and stress adaptation (Leigh and Jones 1984). Deficiency of this macronutrient leads to inhibition of growth, protein synthesis and susceptibility to pathogen due to increased level of nitrogen and sugar compounds (Armengaud *et al.* 2004). Previously, Armengaud *et al.* (2004) used full genome microarray to monitor the transcriptome of *Arabidopsis* seedlings exposed to long-term K^+ starvation and short-term K^+ resupply after starvation. Their gene expression data showed that some of the WRKY transcription factors were also regulated under potassium starvation condition. By using a potassium expression database (http://www.brc.dcs.gla.ac.uk/rb106x/arabidopsis_results.htm) from Armengaud *et al.* (2004), we have analyzed the whole WRKY gene family, and found that some of the *Arabidopsis* WRKY transcription factors were substantially up regulated during K^+ starvation and down regulated upon external short term K^+ replenishment. Expression of *AtWRKY6*, *AtWRKY31*, *AtWRKY40*, and *AtWRKY45* genes in the roots and shoots were significantly up-regulated upon K^+ starvation and the transcript levels were quickly reduced after K^+ was replenished, except *AtWRKY45*, which was highly up-regulated upon potassium replenishment specifically in roots (Fig. 2). In contrast, *AtWRKY49* and *AtWRKY72* were up-regulated upon external potassium resupply but slightly down regulated in potassium starvation condition. Expression levels of *AtWRKY30* and *AtWRKY64* went down during starvation condition but no significant change was observed in resupply (Fig. 2). Transcripts for *AtWRKY33* showed significant down regulation in K^+ resupply in both root and shoot. Also from whole *AtWRKY* family some of the WRKY genes like *AtWRKY3*, *AtWRKY10* and *AtWRKY29* did not show any significant changes in transcript level in prolonged starvation as well as upon resupply i.e. all of them showed lower transcript level in root and shoot. Similarly,

AGI	Function	Name	Roots 2h		Shoots 2h		Shoots 6h		Roots 14d		Shoots															
			Na	-K	Na	-K	Na	-K	starved	starved																
AT4G22070	Regulate defense response	<i>AtWRKY31</i>	-0.6	-0.7	-1.1	-0.6	-0.6	0	0	0.1	0	0.2	0.1	-0.1	-0.1	-0.1	-0.2	0.2	1.4	0.1	0	0.6				
AT1G62300	Regulate <i>PHO1</i> in low Pi and defense response	<i>AtWRKY6</i>	0	0	-0.1	-0.5	-0.5	-0.3	0	0.1	-0.1	0	-0.1	-0.2	0.3	0	0	-0.4	-0.6	-0.5	0.5	0.6	0.3	0.3	0.8	0.7
AT1G80840	Regulate ABA signaling and defense response	<i>AtWRKY40</i>	-0.1	0.1	-0.2	-0.4	0	-0.1	-0.3	-0.3	-0.3	-0.3	-0.5	-0.2	-0.4	-0.5	-0.8	-0.9	-1	-0.8	-0.3	-0.2	-0.1	0.6	0.5	0.2
AT3G01970	Regulate carbon starvation	<i>AtWRKY45</i>	0.1	-0.1	-0.3	0.5	0.7	0.4	0.1	0	0	-0.3	0	0	0.1	0	0	-0.1	-0.1	-0.1	0.9	0.2	0.9	0	0.9	0.3
AT5G43290	Regulate cold stress	<i>AtWRKY49</i>	0	0.1	0	0.1	0.9	0.7	0.1	0	0.3	0	0.5	0.1	0.4	0.5	-0.1	0.4	0.2	0.1	0.1	0	0	-0.2	-0.1	0
AT5G15130	Regulate ABA signaling, sugar starvation, defense response	<i>AtWRKY72</i>	0.4	0.4	0.4	0.6	0.5	-0.1	0.1	0.1	-0.4	0	-0.1	0.1	0.4	0.3	0	0	0.1	0.4	0.2	0.1	0.5	-0.5	0.3	0.1
AT5G24110	Regulate oxidative stress	<i>AtWRKY30</i>	0	-0.3	-0.2	0	-0.1	-0.1	0.1	0.2	-0.1	-0.1	0	0.1	0.1	0	0.4	0.1	0	0.1	-0.4	-0.4	0.3	0	-0.2	-0.2
AT1G66560	Regulate defense response	<i>AtWRKY64</i>	0.1	-0.2	-0.1	0.1	-0.2	-0.1	-0.2	0.1	-0.1	0.1	0	0	0	0.4	0	0.2	0	0.2	0	-0.1	-0.1	-0.1	-0.1	-0.1
AT2G38470	Regulate salt and oxidative stress	<i>AtWRKY33</i>	-0.6	-0.2	0	0.8	-1	-0.6	0	0	-0.1	0	-0.1	-0.1	0.1	-0.1	-0.1	-0.6	-0.7	-0.6	0.1	0	0.6	-0.1	-0.1	0.2
AT1G69810	Regulate osmotic stress	<i>AtWRKY36</i>	0.2	0.1	0.2	0	0.2	0.1	0.1	0.2	0.2	-0.2	0.1	0.1	0.3	0.3	0	0.1	0.1	0.1	0.3	0.2	0.5	0.1	0	0.2
AT4G31800	Regulate ABA signaling and defense response	<i>AtWRKY18</i>	0.2	0.3	0.3	-0.1	0.5	-0.1	0.4	-0.1	0.6	-0.2	0.2	0.4	0.3	0	0.3	0.1	0.4	0.4	0.4	0	0.2	0.1	0.2	0.2
AT4G23550	Regulate defense response	<i>AtWRKY29</i>	-0.2	-0.2	-0.2	-0.7	-0.4	-0.3	0	0	-0.4	-0.1	-0.2	-0.2	-0.3	-0.1	-0.2	-0.2	-0.3	-0.1	0.1	0.3	0.6	-0.1	-0.2	0
AT2G03340	Regulate defense response	<i>AtWRKY3</i>	-0.2	-0.3	-0.2	-0.2	-0.6	-0.5	-0.2	0.1	-0.4	0.1	-0.4	-0.2	-0.1	-0.3	-0.2	0	-0.2	-0.4	-0.2	-0.1	-0.2	-0.1	-0.2	-0.1
AT1G55600	Regulate defense response and seed development	<i>AtWRKY10</i>	0	-0.1	0	-0.1	-0.1	-0.1	-0.1	0	-0.2	-0.1	-0.1	0.1	-0.1	-0.1	0.4	0.1	-0.1	-0.1	-0.1	0	-0.1	-0.1	-0.1	0

Fig. 2 Expression profile of K^+ responsive WRKY genes. On the left side, AGI number (*Arabidopsis* gene index number), gene name and functions of each gene are given based on the already published reports and TAIR *Arabidopsis* database. On the right side, expression profile of some of the selected genes is shown on the basis of differential expression. Green color indicates the down regulation and pink color is for up regulation of the transcript level of individual WRKY genes. Tissue types, conditions and treatment controls are mentioned on the top of the heat map. Also for each treatment three replicate experiments are used and their respective expression is shown as relative expression value.

the higher levels of transcripts were observed for *AtWRKY18* and *AtWRKY36* during starvation as well as re-supply conditions (Fig. 2).

Like WRKY transcription factor, SUSIBA2 (Sugar Signaling in Barley 2) (WRKY homolog in *Hordeum vulgare*), also binds strongly to the W-box and SURE (a SUGAR Responsive cis Element) present in the sugar metabolism genes *ISO1* (Iso amylase1) and *SBE1b* (Starch-Branching Enzymes) promoter (Sun *et al.* 2003). Another WRKY member, *AtWRKY4* and *AtWRKY34* regulates *AtNDPK3* (Nucleoside Diphosphate Kinase), which is known to be induced during sucrose and glucose deficiency. Sequence analysis of NDPK3 reveals the presence of two WBOXHWISO1 boxes in the promoter region that is shown to interact with WRKY protein 4 and 34 (Hammargren *et al.* 2008). In carbon deficient condition, *AtWRKY45* and *AtWRKY65* regulate the expression of the downstream genes. In carbon starvation condition the whole genome transcriptome profiling revealed that the expression level of *AtWRKY45* and *AtWRKY65* were up regulated (Contento *et al.* 2004).

Role in drought, abscisic acid signaling and stomatal opening

Drought is one of the major abiotic stresses, which restrict plant growth and crop production. Drought stress induces the accumulation of ABA, which leads to stomatal closure, thereby, reducing water loss caused by transpiration (Schroeder *et al.* 2001). The accumulation of cytosolic ABA is sensed by the ABA receptors PYR/PYLs of the START domain superfamily, which then interacts with a phosphatase PP2C, and represses the PP2C activity. When ABA is not available, PP2C acts as a constitutive negative regulator of a family of kinases (SnRK2) whose autophosphorylation is required for kinase activity. Activation of the kinase activity of SnRK2 leads to the activation of downstream targets like *ABF2* (ABA responsive elements-binding factor 2) and *ABI5* (ABA insensitive5) and finally the regulation of expression of ABA responsive genes (Fujii and Zhu 2009; Ma *et al.* 2009; Nishimura *et al.* 2009; Park *et al.* 2009). Some of the WRKY genes are known to be involved in drought and salinity stress response. For example *OsWRKY11*, expressed under heat shock inducible HSP101 promoter led to enhanced heat and drought tolerance and this is the first report of enhancement of tolerance to abiotic stress by over expression of WRKY genes (Wu *et al.* 2009). Recently, it was shown that over-expression lines of two allele of rice *OsWRKY45*; *OsWRKY45-1* and *OsWRKY45-2* showed reduced and increased ABA sensitivity, respectively. This suggests that *OsWRKY45-1* is a negative and *OsWRKY45-2* is a positive regulator of ABA signaling. In addition *OsWRKY45-2* also negatively regulates responses to salt stress in rice. The differential regulations of ABA signaling and salt stress signaling by the two alleles of *OsWRKY45* suggested that they regulate the transcription through different signaling pathways (Tao *et al.* 2011). Rice *OsWRKY72*, and *Arabidopsis AtWRKY75*, belonging to group II of the WRKY superfamily are induced in salinity and heat stresses as well as by phytohormone ABA and naphthalene acetic acid (NAA) (Kato *et al.* 2007; Song *et al.* 2010). Besides the abiotic stress, WRKY transcription factors also form an important part of stomatal closure mechanism, which is induced by pathogens to prevent infection of the plants (Schulze-Lefert and Robatzek 2006). Abscisic acid is a plant hormone that mediates different abiotic stresses in plants and hence is known as 'stress hormone'. Abiotic stress in plants is mediated through two pathways, ABA-independent and ABA-dependent pathways (Shinozaki and Yamaguchi-Shinozaki 2000). Studies reveal that in cold stress, there is no ABA accumulation, which means that response to cold stress is mediated through an ABA-independent pathway (Thomashow *et al.* 1999; Shinozaki and Yamaguchi-Shinozaki 2000). Salt and drought stress signal transduction activate both of these pathways involving *ABFs* (ABRE binding factor), *MYC/*

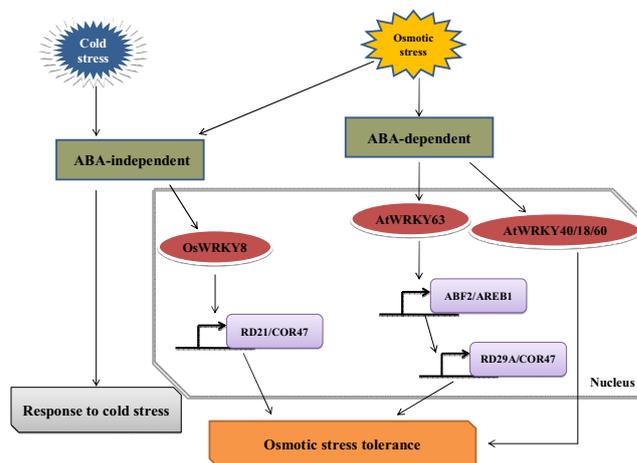


Fig. 3 Model showing the role of WRKY genes in ABA-dependent and -independent responses. In the case of osmotic stress where no ABA accumulates i.e. in ABA independent pathway, *OsWRKY8* confers osmotic stress tolerances by positively regulating stress inducible *RD21* and *COR47*. In the second case where ABA accumulates i.e. in ABA-dependent pathway, *AtWRKY40/18/60* directly induced osmotic stress tolerance while *AtWRKY63* acts by up regulating *ABF2/AREB1*, which in turn increases the expression of *RD29A* and *COR47*.

MYB, *DREB2* (Drought Responsive Element Binding) and *NAC* domain containing transcription factors. In case of rice, the expressional analysis of the WRKY genes showed that most of them are involved in abiotic stress signaling pathway in an ABA-dependent manner (Ramamoorthy *et al.* 2008). Since *AtCOR47* and *AtRD21*, two ABA-independent abiotic stress responsive genes, were up regulated in *OsWRKY08* overexpression line, which suggested that *OsWRKY08* enhances the osmotic stress tolerance of transgenic *Arabidopsis* through an ABA-independent signaling pathway (Fig. 3) (Song *et al.* 2009).

Arabidopsis mutant, over sensitive to ABA, *abo3* (ABA overly sensitive 3) had a T-DNA insertion in the gene encoding WRKY transcription factor, *AtWRKY63*. The expression of ABA-responsive transcription factor *ABF2/AREB1* and stress inducible genes *RD29A* and *COR47* was lower in the *abo3* mutant than in the wild type upon ABA treatment (Fig. 3). The *abo3* mutant also showed reduced sensitivity to ABA mediated stomatal closure. Electrophoresis mobility shift assay (EMSA) showed that ABO3 (*AtWRKY63*) protein can recognize and binds to the TGAC core sequence of W-box in the promoter of *ABF2* and regulates the expression of *ABF2/AREB1*, a key transcriptional activator for ABA-responsive genes (Fig. 3) (Ren *et al.* 2010). During seed germination and post germination growth of plants, three WRKY genes regulate ABA response in a negative manner. The key players involved are *AtWRKY18*, *AtWRKY60* and *AtWRKY40*; and the Mg-chelatase H subunit/putative ABA receptor (ABAR). ABAR spans chloroplast envelope and the cytosolic C-terminus binds ABA. When ABA level is high in the cell, ABAR binds to ABA and recruits *AtWRKY40*, *AtWRKY18* and *AtWRKY60* repressor proteins from the nucleus to cytosol. As a result, the repression of expression of ABA-responsive genes like *ABF4*, *ABI4*, *ABI5*, *DREB1A*, *MYB2* and *RAB18* was relieved and ABA mediated transcription takes place (Rushton *et al.* 2011). In another work, it was shown that *AtWRKY40*, *AtWRKY18* and *AtWRKY60* were involved in plant responses to ABA and abiotic stresses such as salt and osmotic stress (Chen *et al.* 2010). By examining their single, double and triple mutants and over-expression lines, it was revealed that *AtWRKY40* negatively regulate ABA responses during seed germination and post-germination growth, however, *AtWRKY18* and *AtWRKY60* were found to regulate ABA responses positively. In the mutants of *AtWRKY40* and *AtWRKY18*, the expression of *AtWRKY60* was significantly reduced, which conferred that the *AtWRKY60* might

be a direct target of *AtWRKY40* and *AtWRKY18*. Also, it was suggested that the expression of the *AtWRKY60* was controlled by *AtWRKY18/AtWRKY40* hetero-complex (Chen *et al.* 2010). All these studies indicated that WRKY proteins play a major role in ABA signaling as well as in abiotic stresses.

Role in oxidative and thermal tolerance

In the cell, reactive oxygen species (ROS) play an important role as a signaling molecule and on the other hand is a toxic by-product of aerobic metabolism. Due to its dual role, a complex network of genes termed as 'ROS gene network' controls the regulation of the cellular ROS homeostasis. Antioxidants such as ascorbate, glutathione, tocopherol along with different enzymes and proteins regulate the ROS homeostasis and production. Under oxidative stress, the induction of *AtWRKY25* depends on *Zat12*, a member of the zinc-finger protein (Rizhsky *et al.* 2004a). Similarly, the expression of *AtWRKY70* also increases in transgenic line of *Zat7* and the expression is repressed in EAR-mutated *Zat7* since *Zat* family contains the EAR domain (ERF-associated amphiphilic repression), that could act as a repressor (Ciftci-Yilmaz *et al.* 2007). Other WRKY members like *AtWRKY18*, *AtWRKY33*, *AtWRKY40* and *AtWRKY70* were constitutively expressed in *Atapx1* (Ascorbate Peroxidase1) knockout mutant, which scavenges ROS during high light stress (Davletova *et al.* 2005a). Therefore, WRKY transcription factor play a major role in response to ROS stress and may act down or upstream to *Zat* proteins. These findings suggested complex interactions between different transcription factors and pathways that might be occurring in a cell upon oxidative stress.

Temperature beyond an organism's optimal tolerance range is considered as a major abiotic stress. Changes in the optimal temperature affect cellular homeostasis, which leads to impaired plant growth and development. WRKY proteins are now known to be involved in regulating plant responses to heat and dehydration stress as well (Wang *et al.* 2011). In *Arabidopsis* transgenic plants, where a highly conserved transcriptional coactivator, MBF1c (Multiprotein Bridging Factor1) was overexpressed led to increased expression of WRKY proteins *AtWRKY18*, *AtWRKY33*, *AtWRKY40*, and *AtWRKY46*. The overexpression lines showed enhanced thermo-tolerance compared to the wild-type plants. These results indicated that *AtWRKY18*, *AtWRKY33*, *AtWRKY40*, and *AtWRKY46* have a role in conferring thermo-tolerance (Suzuki *et al.* 2005). *OsWRKY11* in rice has also been reported to interact with HSP101 promoter and hence play a role in heat and drought stress tolerance (Wu *et al.* 2009). Also the *AtWRKY25* and *AtWRKY39* are positive regulators of thermo-tolerance (Li *et al.* 2009, 2010). *AtWRKY25*, *AtWRKY26* and *AtWRKY33* were involved in the regulation of heat stress. Expression of *AtWRKY25* and *AtWRKY26* was induced upon treatment with high temperature, whereas *AtWRKY33* expression was repressed. *AtWRKY33* along with *AtWRKY25* and *AtWRKY26* positively regulate heat stress tolerance (Li *et al.* 2011). Mutant plants of these three genes showed sensitive phenotype rather than over expression plants, which showed enhanced resistance to heat stress (Li *et al.* 2011). *Arabidopsis* transgenic plants over-expressing soybean gene *GmWRKY21* was tolerant to cold stress, whereas *GmWRKY54*-transgenic plants function in stress tolerance through the regulation of *DREB2A*, *RD29B* and *STZ* (Zhou *et al.* 2008). *HvWRKY38*, a barley gene coding for a WRKY protein, has been reported to be involved in cold and drought responses (Mare *et al.* 2004). In the case of *Brassica campestris*, *BcWRKY46* plays an important role in responding to ABA and abiotic stress, as the constitutive expression of *BcWRKY46* under the control of the CaMV35S promoter reduced the susceptibility of transgenic tobacco to freezing, ABA and salt.

ROLE IN BIOTIC STRESSES

In the environment, plants are vulnerable to attack by a variety of pathogens like bacteria, virus, fungi, insects and to counteract this attack, they have developed active defense mechanism to protect themselves. One of the mechanisms, they adopt is transcriptional activation of a large number of genes to induce defense responses (Yang *et al.* 1997; Rushton and Somssich 1998).

Plants mainly have two layers of innate immunity system against pathogen attacks. The first layer is activated by pathogen associated molecular patterns (PAMPs), which includes flagellin, elongation factor Tu and chitin, and is known as PAMP triggered immunity (PTI). These PAMPs molecules are not present in host cells and evolutionarily conserved in microorganisms. Plant resistance (R) protein specifically recognized some effector proteins and activates effector-triggered immunity (ETI), the second layer of innate immunity. Most of the known R proteins belong to the NBS-LRR protein, named after their characteristic nucleotide binding (NB) and leucine rich-repeat (LRR) domains. R gene-activated ETI involves a complex defense program including production of reactive oxygen species (ROS) and salicylic acid (SA), rapid programmed cell death (hypersensitive responses, HR) and induction of a large number of host genes including pathogenesis-related (PR) genes (Jones and Dangl 2006). The plant hormones, salicylic acid (SA), jasmonic acid (JA) and ethylene play a very important role in defense responses (Chen *et al.* 2000; Dellagi *et al.* 2000; Eulgem *et al.* 2000; Kim *et al.* 2000; Asai *et al.* 2002; Ulker *et al.* 2004). Systemic acquired resistance (SAR) is induced defense mechanism in plants that brings about enduring and broad range protection against microorganism by the accumulation of PR proteins via SA signaling. SA activates the positive regulator protein NPR1 (non-expresser of pathogenesis-related gene1, also known as NIM1 and SAI1) to mediate the signaling cascade that confers resistance against the microorganism (Durrant and Dong 2004).

WRKY transcription factors in plant defense: Case study in *Arabidopsis*

WRKY proteins play important roles in plant defense responses augmenting the W-box sequences (C/TTGACT/C), which binds to WRKY domain and induces the expression of many defense-related genes (Rushton *et al.* 1996; Eulgem *et al.* 1999; Yang *et al.* 1999; Du and Chen 2000). Like the WRKY gene family, W-boxes are present in promoters of many defense-regulated genes like PR genes, which regulate their own expression (Rushton *et al.* 1996; Maleck *et al.* 2000). Also, it was shown that some of the WRKY genes were induced in pathogen infection but not detectable in SA treated plants and *vice versa*. This suggested that WRKY gene families were regulated by two different pathways i.e. NPR1-dependent and NPR1-independent pathway. NPR1-dependent pathway is mediated by SA. When SAR inducers are absent, NPR1 is shown to be as an oligomer and present in the cytosol whereas, after SAR induction the oligomer is transformed into monomer and is localized in the nucleus (Mou *et al.* 2003). Plant signaling molecules like SA and JA/ET act antagonistically, as mutation of COI1 and JAR1-JA signaling molecule causes increased SA accumulation and this accumulation further promotes JA signaling (Kloek *et al.* 2001; Kunkel and Brooks 2002; Spoel *et al.* 2003). WRKY TFs are known to negatively regulate defense responses. Loss-of-function mutant of *AtWRK62* showed enhanced expression of JA-responsive genes like *LOX2* and *VSP2* and on the other hand the over-expression of *AtWRKY62* led to the repression of JA-responsive genes (Mao *et al.* 2007). Also in another study, it was shown that the expression of SA responsive gene PR1 is enhanced in *AtWRK62* loss-of-function mutants and the expression is repressed in *AtWRK62* overexpression lines (Kim *et al.* 2008). Other WRKY pro-

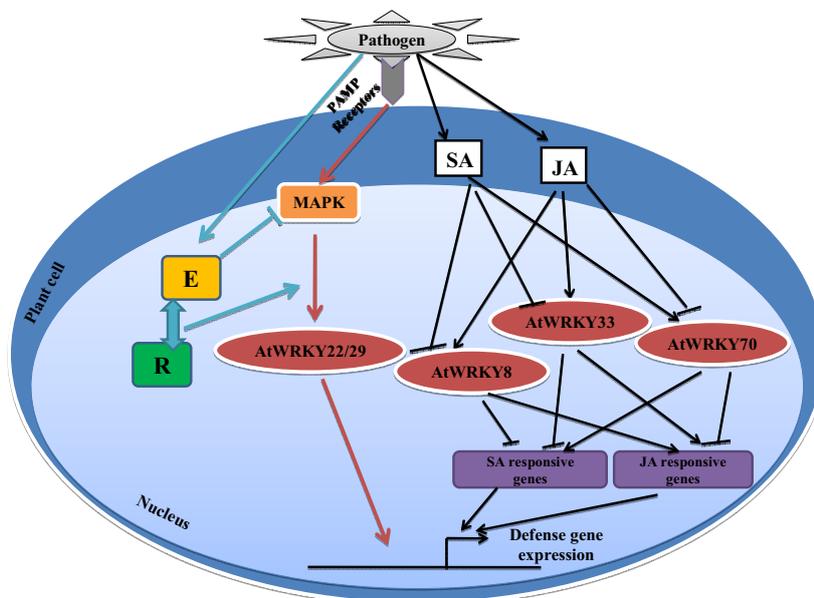


Fig. 4 Simplified model of the WRKY genes involved in the plant defense responses. In plant, defense signaling can be activated via PAMP receptors (PTI-PAMP Triggered Immunity) or through pathogen elicitor, which activated SA or JA pathways. In PTI activated PAMP receptors upon binding to the pathogen, activates MAPK cascade, which in turn up regulate *AtWRKY22* and *AtWRKY29*. *AtWRKY22* and *AtWRKY29* positively regulate defense response genes. This pathway is suppressed by effector protein (E) and is thought to be activated upon interaction with resistant protein (R) and termed as ETI (Effector Triggered Immunity). In the second pathway, which involves SA and JA, *AtWRKY70* positively regulate SA mediated defense response and negatively regulate JA mediated defense response. In contrast to *AtWRKY70*; *AtWRKY8* and *AtWRKY33* negatively regulate SA mediated defense response and positively regulate JA mediated defense response.

teins like *AtWRKY38* have very similar protein structure and expression pattern as *AtWRKY62* protein (Kalde *et al.* 2003).

The JA-dependent plant defense is triggered by biotrophic pathogens but SA-dependent pathway is linked to necrotrophic pathogen. *AtWRKY8* is a negative regulator of plant defense responses to biotrophic bacterial pathogen *Pseudomonas syringae* and positive regulator of defense responses against necrotrophic fungal pathogen, *Botrytis cinerea*. This is proved by the study of mutant line of *AtWRKY8*, which showed increase resistance to *Pseudomonas syringae* and decreased resistance to *Botrytis cinerea*, whereas over-expression lines of *AtWRKY8* showed the opposite results. This also suggested that *AtWRKY8* protein might act downstream to both SA and JA to regulate defense responses (Fig. 4) (Chen *et al.* 2010). *AtWRKY33* over-expressing or mutant plants showed similar kind of regulation as *AtWRKY8* in defense response signaling (Fig. 4) (Andreasson *et al.* 2005; Zheng *et al.* 2006). However, *AtWRKY70* acts in the opposite manner as *AtWRKY8* i.e. it played a positive role in SA signaling and functions as a negative regulator of JA-inducible genes. Mutation in *AtWRKY70* increased the susceptibility to biotrophic and necrotrophic pathogens, *Erwinia carotovora* and the fungal pathogens *Erysiphe cichoracearum* and *Botrytis cinerea* (Fig. 4) (Li *et al.* 2004, 2006; AbuQamar *et al.* 2006). In another instance, the expression of WRKY46 of *Arabidopsis* increases in response to SA and *Pseudomonas syringae* infection, which is structurally linked to WRKY52 and WRKY70 (Hu *et al.* 2012). In *Arabidopsis*, WRKY22 and WRKY29 are downstream components of the flagellin receptor (FLS2), a leucine-rich repeat (LRR) receptor kinase and a mitogen-activated protein kinase (MAPK) cascade, which confers resistance to both bacterial and fungal pathogens. Overexpression of the *AtWRKY29* induces the plant defense response against *P. syringae* and *B. cinerea* (Asai *et al.* 2002). *AtWRKY53*, *AtWRKY54*, and *AtWRKY70* were identified as direct targets of NPR1 since they were shown as structurally similar (Wang *et al.* 2006).

Some of the WRKY proteins act as negative regulator of basal defense responses in plants. The mutant line of *AtWRKY7*, *AtWRKY11*, *AtWRKY17*, *AtWRKY25*,

AtWRKY38, *AtWRKY48* and *AtWRKY62* increase basal plant resistance triggered by a virulent *P. syringae* strain (Journot-Catalino *et al.* 2006; Kim *et al.* 2006; Zheng *et al.* 2007; Xing *et al.* 2008). *Arabidopsis AtWRKY18* transcription factor interacts physically and functionally with *AtWRKY40* and *AtWRKY60* and functions as negative regulators of plant resistance against the *P. syringae* and the fungal pathogen *E. cichoracearum* (Xu *et al.* 2006; Shen *et al.* 2007).

WRKY transcription factors in plant defense: case study in other plants

Some of the other WRKY genes were found to be up-regulated in response to pathogen in a number of plants other than *Arabidopsis* like in rice, *Oryza sativa* (Kim *et al.* 2000; Wen *et al.* 2003; Liu *et al.* 2005), potato, *Solanum tuberosum* (Dellagi *et al.* 2000), sugarcane, *Saccharum officinarum* (Lambais *et al.* 2001) and chamomile, *Matricaria chamomilla* (Ashida *et al.* 2002). Approximately 109 WRKY genes have been identified in rice, which are involved in mainly abiotic and biotic stress responses. Over-expression line of *OsWRKY71* exhibited the constitutive expression of both *OsNPR1* and *OsPR1b* and confers disease resistance to bacterial blight. This indicates that *OsWRKY71* may act as a transcriptional regulator upstream of *OsNPR1* and *OsPR1b* in rice defense response signaling pathways (Liu *et al.* 2007). The overexpression of *OsWRKY13* enhances resistance to the bacterial blight *Xanthomonas oryzae* and the fungal blast *Magnaportha grisea* by activating SA-biosynthesis and SA-response genes while suppressing JA signaling (Qiu *et al.* 2007, 2008a). The over expression of *OsWRKY89* in rice showed reduced growth but an increased resistance to *M. grisea* by regulating the wax deposition on the leaf surface (Wang *et al.* 2007). *OsWRKY31* was strongly induced by the rice blast fungus *M. grisea* and auxin, which suggested that *OsWRKY31* might be a common component in the signal transduction pathways of auxin and defense responses in rice (Zhang *et al.* 2008). *OsWRKY45* is a component of the salicylic acid (SA) signaling pathway in rice and its knockdown lines showed decreased resistance to fungal blast whereas its

over expression in transgenic rice dramatically enhanced resistance to leaf blight (Shimono *et al.* 2007; Tao *et al.* 2009).

Recently, it was shown that the expression level of *GhWRKY3* (*Gossypium hirsutum*) was regulated in response to wounding and pathogen infection via SA, JA/ET signaling pathways (Guo *et al.* 2010). By homology search analysis, 22 WRKY genes were identified in *Coffea arabica*. Among the 22 WRKY genes, five of them, namely, *CaWRKY6*, *CaWRKY11*, *CaWRKY12*, *CaWRKY13* and *CaWRKY14* were shown to be involved in regulating response to pathogens (Ramiro *et al.* 2010). Wheat *TaWRKY45* protein an orthologue of rice OsWRKY45 has been shown to be up regulated in response to a plant immune system strengthener benzothiadiazole (BTH) upon Fusarium head blight (FHB) infection caused by fungus *Fusarium graminearum*. In overexpression of the *TaWRKY45* transgenic lines, an enhanced resistance against *F. graminearum* was observed (Bahrini *et al.* 2011). The over expression of grapevine (*Vitis vinifera*) *VvWRKY1* in tobacco (*Nicotiana tabacum*) act as a negative regulator of plant defense responses to different fungal infections whereas its ectopic expression enhanced resistance to the necrotrophic fungi (Marchive *et al.* 2007). In barley (*Hordeum vulgare*), *HvWRKY1* and *HvWRKY2* showed enhanced resistance against powdery mildew fungus *Blumeria graminis* via repression of PAMP-triggered basal defense through interaction of intracellular mildew A (MLA) (Shen *et al.* 2007). Two insect-responsive WRKY genes native to tobacco *Nicotiana attenuata*, WRKY3 and WRKY6 have been identified, where they coordinate JA-mediated defense responses. Silencing of either WRKY3 and WRKY6 or both, makes plant highly vulnerable to *Manduca sexta* attack (Skibbe *et al.* 2008). The involvement of WRKY in several other plant species in biotic stress responses suggests this class of transcription factors in regulation of gene expression upon pathogen challenge. However, the mechanistic functional role of several of the WRKY transcription factors required further investigation.

CONCLUSIONS AND FUTURE PROSPECTS

WRKY transcription factor super family plays important role in many plant processes and is mainly responsible for regulation of responses to biotic and abiotic stresses. The WRKY regulates the gene expression by directly or indirectly modulating downstream target genes, by activating or repressing other TF genes or by self-regulating their own expression. Recent molecular and mutant studies have shed light on some aspect of their functions and the mode of action. However, this information is just the tip of the iceberg and future studies will have to focus on answering questions pertaining to the downstream targets of WRKY, the individual and redundant roles of WRKY genes in response to different environmental stress and finally the complex crosstalk among different signaling pathways coordinated by WRKY to generate response to a particular stress. One of the major implications of WRKY transcription factors could be in generating the transgenic plants, where some of the “key regulators” or “master-regulator” WRKY leads to enhancement of productivity and yield of crop plants by providing higher tolerance to both abiotic and biotic stresses, which causes the major havoc in crop productivity worldwide.

ACKNOWLEDGEMENTS

We are thankful to Delhi University (DU), University Grant Commission (UGC) and Department of Science and Technology (DST), India for funding research work in GKP's lab. AS acknowledge Department of Biotechnology (DBT), India for the Junior Research Fellowship.

REFERENCES

- AbuQamar S, Chen X, Dhawan R, Bluhm B, Salmeron J, Lam S, Dietrich RA, Mengiste T (2006) Expression profiling and mutant analysis reveals complex regulatory networks involved in *Arabidopsis* response to *Botrytis* infection. *Plant Journal* **48**, 28-44
- Andreasson E, Jenkins T, Brodersen P, Thorgrimsen S, Petersen NH, Zhu S, Qiu JL, Micheelsen P, Rocher A, Petersen M, Newman MA, Nielsen HB, Hirt H, Somssich I, Mattsson O, Mundy J (2005) The MAP kinase substrate MKS1 is a regulator of plant defense responses. *The EMBO Journal* **24**, 2579-2589
- Armengaud P, Breiting R, Amtmann A (2004) The potassium-dependent transcriptome of *Arabidopsis* reveals a prominent role of jasmonic acid in nutrient signaling. *Plant Physiology* **136**, 2556-2576
- Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* **415**, 977-983
- Ashida Y, Nishimoto M, Matsushima A, Watanabe, J Hirata T (2002) Molecular cloning and mRNA expression of geraniol-inducible genes in cultured shoot primordia of *Matricaria chamomilla*. *Bioscience, Biotechnology and Biochemistry* **66**, 2511-2514
- Bahrini I, Sugisawa M, Kikuchi R, Ogawa T, Kawahigashi H, Ban T, Handa H (2011) Characterization of a wheat transcription factor, TaWRKY45, and its effect on Fusarium head blight resistance in transgenic wheat plants. *Breeding Science* **61**, 121-129
- Chen C, Chen Z (2000) Isolation and characterization of two pathogen- and salicylic acid-induced genes encoding WRKY DNA-binding proteins from tobacco. *Plant Molecular Biology* **42**, 387-396
- Chen YF, Li LQ, Xu Q, Kong YH, Wang H, Wu WH (2009) The WRKY6 transcription factor modulates PHOSPHATE1 expression in response to low Pi stress in *Arabidopsis*. *Plant Cell* **21**, 3554-3566
- Chen H, Lai Z, Shi J, Xiao Y, Chen Z, Xu X (2010) Roles of *Arabidopsis* WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. *BMC Plant Biology* **10**, 281
- Chen L, Song Y, Li S, Zhang L, Zou C, Yu D (2012) The role of WRKY transcription factors in plant abiotic stresses. *Biochimica et Biophysica Acta* **1819**, 120-128
- Chen L, Zhang L, Yu D (2010) Wounding-induced WRKY8 is involved in basal defense in *Arabidopsis*. *Molecular Plant Microbe Interaction* **23**, 558-565
- Ciftci-Yilmaz S, Morsy MR, Song L, Coutu A, Krizek BA, Lewis MW, Warren D, Cushman J, Connolly EL, Mittler R (2007) The ear-motif of the C2H2 zinc-finger protein ZAT7 plays a key role in the defense response of *Arabidopsis* to salinity stress. *Journal of Biological Chemistry* **282**, 9260-9268
- Contento AL, Kim SJ, Bassham DC (2004) Transcriptome profiling of the response of *Arabidopsis* suspension culture cells to Suc starvation. *Plant Physiology* **135**, 2330-2347
- Davletova S, Rizhsky L, Liang H, Shengqiang Z, Oliver DJ, Coutu J, Shulaev V, Schlauch K, Mittler R (2005) Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of *Arabidopsis*. *Plant Cell* **17**, 268-281
- Dellagi A, Helibronn J, Avrova AO, Montesano M, Palva ET, Stewart HE, Toth IK, Cooke DE, Lyon GD, Birch PR (2000) A potato gene encoding a WRKY-like transcription factor is induced in interactions with *Erwinia carotovora* subsp. *atroseptica* and *Phytophthora infestans* and is coregulated with class I endochitinase expression. *Molecular Plant-Microbe Interactions* **13**, 1092-1101
- Devaiah BN, Karthikeyan AS, Ragothama KG (2007) WRKY75 transcription factor is a modulator of phosphate acquisition and root development in *Arabidopsis*. *Plant Physiology* **143**, 1789-1801
- Dong J, Chen C, Chen Z (2003) Expression profiles of the *Arabidopsis* WRKY gene superfamily during plant defense response. *Plant Molecular Biology* **51**, 21-37
- Duan MR, Nan J, Liang YH, Mao P, Lu L, Li L, Wei C, Lai L, Li Y, Su XD (2007) DNA binding mechanism revealed by high resolution crystal structure of *Arabidopsis thaliana* WRKY1 protein. *Nucleic Acids Research* **35**, 1145-1154
- Durrant WE, Dong X (2004) Systemic acquired resistance. *Annual Review of Phytopathology* **42**, 185-209
- Eulgem T, Rushton PJ, Robatzek S, Somssich IE (2000) The WRKY superfamily of plant transcription factors. *Trends in Plant Science* **5**, 199-206
- Eulgem T, Rushton PJ, Schmelzer E, Hahlbrock K, Somssich IE (1999) Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors. *The EMBO Journal* **18**, 4689-4699
- Fujii H, Chinnusamy V, Rodrigues A, Rubio S, Antoni R, Park SY, Cutler SR, Sheen J, Rodriguez PL, Zhu JK (2009) *In vitro* reconstitution of an abscisic acid signalling pathway. *Nature* **462**, 660-664
- Glockner G, Eichinger L, Szafranski K, Pachebat JA, Bankier AT, Dear PH, Lehmann R, Baumgart C, Parra G, Abril JF, Guigo R, Kumpf K, Tunggal B, Cox E, Quail MA, Platzer M, Rosenthal A, Noegel AA (2002) Sequence and analysis of chromosome 2 of *Dictyostelium discoideum*. *Nature* **418**, 79-85

- Guo R, Yu F, Gao Z, An H, Cao X, Guo X (2010) GhWRKY3, a novel cotton (*Gossypium hirsutum* L.) WRKY gene is involved in diverse stress responses. *Molecular Biology Reports* **38**, 49-58
- Hammargren J, Rosenquist S, Jansson C, Knorr C (2008) A novel connection between nucleotide and carbohydrate metabolism in mitochondria: Sugar regulation of the *Arabidopsis* nucleoside diphosphate kinase 3a gene. *Plant Cell Reports* **27**, 529-534
- Hara K, Yagi M, Kusano T, Sano H (2000) Rapid systemic accumulation of transcripts encoding a tobacco WRKY transcription factor upon wounding. *Molecular and General Genetics* **263**, 30-37
- Hinderhofer K, Zentgraf U (2001) Identification of a transcription factor specifically expressed at the onset of leaf senescence. *Planta* **213**, 469-473
- Hu Y, Dong Q, Yu D (2012) *Arabidopsis* WRKY46 coordinates with WRKY70 and WRKY53 in basal resistance against pathogen *Pseudomonas syringae*. *Plant Science* **185-186**, 288-297
- Huang S, Gao Y, Liu J, Peng X, Niu X, Fei Z, Cao S, Liu Y (2012) Genome-wide analysis of WRKY transcription factors in *Solanum lycopersicum*. *Molecular Genetics and Genomics* **287**, 495-513
- Ishiguro S, Nakamura K (1994) Characterization of a cDNA encoding a novel DNA-binding protein, SPF1, that recognizes SP8 sequences in the 5' upstream regions of genes coding for sporamin and beta-amylase from sweet potato. *Molecular and General Genetics* **244**, 563-571
- Johnson CS, Kolevski B, Smyth DR (2002) TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of *Arabidopsis*, encodes a WRKY transcription factor. *Plant Cell* **14**, 1359-1375
- Jones JD, Dangl JL (2006) The plant immune system. *Nature* **444**, 323-329
- Journot-Catalino N, Somssich IE, Roby D, Kroj T (2006) The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in *Arabidopsis thaliana*. *Plant Cell* **18**, 3289-3302
- Kalde M, Barth M, Somssich IE, Lippok B (2003) Members of the *Arabidopsis* WRKY group III transcription factors are part of different plant defense signaling pathways. *Molecular Plant and Microbe Interaction* **16**, 295-305
- Kato N, Dubouzet E, Kokabu Y, Yoshida S, Taniguchi Y, Dubouzet JG, Yazaki K, Sato F (2007) Identification of a WRKY protein as a transcriptional regulator of benzyloisoquinoline alkaloid biosynthesis in *Coptis japonica*. *Plant Cell Physiology* **48**, 8-18
- Kasajima I, Ide Y, Hirai MY, Fujiwara T (2007) WRKY6 is involved in the response to boron deficiency in *Arabidopsis thaliana*. *Physiologia Plantarum* **139**, 80-92
- Kim CY, Lee SH, Park HC, Bae CG, Cheong YH, Choi YJ, Han C, Lee SY, Lim CO, Cho MJ (2000) Identification of rice blast fungal elicitor-responsive genes by differential display analysis. *Molecular Plant-Microbe Interactions* **13**, 470-474
- Kim KC, Fan B, Chen Z (2006) Pathogen-induced *Arabidopsis* WRKY7 is a transcriptional repressor and enhances plant susceptibility to *Pseudomonas syringae*. *Plant Physiology* **142**, 1180-1192
- Kim KC, Lai Z, Fan B, Chen Z (2008) *Arabidopsis* WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. *Plant Cell* **20**, 2357-2371
- Kloek AP, Verbsky ML, Sharma SB, Schoelz JE, Vogel J, Klessig DF, Kunkel BN (2001) Resistance to *Pseudomonas syringae* conferred by an *Arabidopsis thaliana* coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms. *Plant Journal* **26**, 509-522
- Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. *Current Opinion in Plant Biology* **5**, 325-331
- Lambais MR (2001) *In silico* differential display of defense-related expressed sequence tags from sugarcane tissues infected with diazotrophic endophytes. *Genetics and Molecular Biology* **24**, 103-111
- Leigh RA, Jones RGW (1984) A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. *New Phytologist* **97**, 1-13
- Li J, Brader G, Palva ET (2004) The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. *Plant Cell* **16**, 319-331
- Li J, Brader G, Kariola T, Palva ET (2006) WRKY70 modulates the selection of signaling pathways in plant defense. *Plant Journal* **46**, 477-491
- Li S, Fu Q, Huang W, Yu D (2009) Functional analysis of an *Arabidopsis* transcription factor WRKY25 in heat stress. *Plant Cell Reports* **28**, 683-693
- Li S, Zhou X, Chen L, Huang W, Yu D (2010) Functional characterization of *Arabidopsis thaliana* WRKY39 in heat stress. *Molecular Cells* **29**, 475-483
- Li S, Fu Q, Chen L, Huang W, Yu D (2011) *Arabidopsis thaliana* WRKY25, WRKY26, and WRKY33 coordinate induction of plant thermotolerance. *Planta* **233**, 1237-1252
- Li HL, Zhang LB, Guo D, Li CZ, Peng SQ (2012) Identification and expression profiles of the WRKY transcription factor family in *Ricinus communis*. *Gene* **503**, 248-253
- Ling J, Jiang W, Zhang Y, Yu H, Mao Z, Gu X, Huang S, Xie B (2011) Genome-wide analysis of WRKY gene family in *Cucumis sativus*. *BMC Genomics* **12**, 471
- Liu JJ, Ekramoddoullah AK (2009) Identification and characterization of the WRKY transcription factor family in *Pinus monticola*. *Genome* **52**, 77-88
- Liu X, Bai X, Wang X, Chu C (2007) OsWRKY71, a rice transcription factor, is involved in rice defense response. *Journal of Plant Physiology* **164**, 969-979
- Liu X, Wang X, Pang Y, Liang J, Liu S, Sun X, Tang K (2006) Molecular cloning and characterization of a novel WRKY gene from *Brassica chinensis*. *Molecular Biology (Moskova)* **40**, 816-824
- Liu XQ, Bai XQ, Qian Q, Wang XJ, Chen MS, Chu CC (2005) OsWRKY03, a rice transcriptional activator that functions in defense signaling pathway upstream of OsNPR1. *Cell Research* **15**, 593-603
- López-Bucio J, Cruz-Ramirez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. *Current Opinion in Plant Biology* **6**, 280-287
- Luan S, Lan W, Lee SC (2009) Potassium nutrition, sodium toxicity, and calcium signaling: Connections through the CBL-CIPK network. *Current Opinion Plant Biology* **12**, 339-346
- Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* **324**, 1064-1068
- Maeo K, Hayashi S, Kojima-Suzuki H, Morikami A, Nakamura K (2001) Role of conserved residues of the WRKY domain in the DNA-binding of tobacco WRKY family proteins. *Bioscience, Biotechnology and Biochemistry* **65**, 2428-2436
- Maleck K, Levine A, Eulgem T, Morgan A, Schmid J, Lawton KA, Dangl JL, Dietrich RA (2000) The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. *Nature Genetics* **26**, 403-410
- Mangelsen E, Kilian J, Berendzen KW, Kolukisaoglu UH, Harter K, Jansson C, Wanke D (2008) Phylogenetic and comparative gene expression analysis of barley (*Hordeum vulgare*) WRKY transcription factor family reveals putatively retained functions between monocots and dicots. *BMC Genomics* **9**, 194
- Mao P, Duan M, Wei C, Li Y (2007) WRKY62 transcription factor acts downstream of cytosolic NPR1 and negatively regulates jasmonate-responsive gene expression. *Plant and Cell Physiology* **48**, 833-842
- Marchive C, Mzid R, Deluc L, Barriau F, Pirrello J, Gauthier A, Corio-Costet MF, Regad F, Cailleau B, Hamdi S, Lauvergeat V (2007) Isolation and characterization of a *Vitis vinifera* transcription factor, VvWRKY1, and its effect on responses to fungal pathogens in transgenic tobacco plants. *Journal of Experimental Botany* **58**, 1999-2010
- Mare C, Mazzucotelli E, Crosatti C, Francia E, Stanca AM, Cattivelli L (2004) Hv-WRKY38: A new transcription factor involved in cold- and drought-response in barley. *Plant Molecular Biology* **55**, 399-416
- Melcher K, Ng LM, Zhou XE, Soon FF, Xu Y, Suino-Powell KM, Park SY, Weiner JJ, Fujii H, Chinnusamy V, Kovach A, Li J, Wang Y, Peterson FC, Jensen DR, Yong EL, Volkman BF, Cutler SR, Zhu JK, Xu HE (2009) A gate-latch-lock mechanism for hormone signalling by abscisic acid receptors. *Nature* **462**, 602-608
- Miao Y, Laun T, Zimmermann P, Zentgraf U (2004) Targets of the WRKY53 transcription factor and its role during leaf senescence in *Arabidopsis*. *Plant Molecular Biology* **55**, 853-867
- Mou Z, Fan W, Dong X (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell* **113**, 935-944
- Nishimura N, Hitomi K, Arvai AS, Rambo RP, Hitomi C, Cutler SR, Schroeder JI, Getzoff ED (2009) Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. *Science* **326**, 1373-1379
- Nishimura N, Okamoto M, Narusaka M, Yasuda M, Nakashita H, Shinzaki K, Narusaka Y, Hirayama T (2009) ABA hypersensitive germination2-1 causes the activation of both abscisic acid and salicylic acid responses in *Arabidopsis*. *Plant and Cell Physiology* **50**, 2112-2122
- Pan YJ, Cho CC, Kao YY, Sun CH (2009) A novel WRKY-like protein involved in transcriptional activation of cyst wall protein genes in *Giardia lamblia*. *Journal of Biological Chemistry* **284**, 17975-17988
- Pandey SP, Somssich IE (2009) The role of WRKY transcription factors in plant immunity. *Plant Physiology* **150**, 1648-1655
- Park MY, Chung MS, Koh HS, Lee DJ, Ahn SJ, Kim CS (2009) Isolation and functional characterization of the *Arabidopsis* salt-tolerance 32 (AtSAT32) gene associated with salt tolerance and ABA signaling. *Physiologia Plantarum* **135**, 426-435
- Pnueli L, Hallak-Herr E, Rozenberg M, Cohen M, Goloubinoff P, Kaplan A, Mittler R (2002) Molecular and biochemical mechanisms associated with dormancy and drought tolerance in the desert legume *Retama raetam*. *Plant Journal* **31**, 319-330
- Qiu D, Xiao J, Ding X, Xiong M, Cai M, Cao Y, Li X, Xu C, Wang S (2007) OsWRKY13 mediates rice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. *Molecular Plant-Microbe Interactions* **20**, 492-499
- Qiu D, Xiao J, Xie W, Liu H, Li X, Xiong L, Wang S (2008) Rice gene network inferred from expression profiling of plants overexpressing OsWRKY13, a positive regulator of disease resistance. *Molecular Plant* **1**, 538-551
- Ramamoorthy R, Jiang SY, Kumar N, Venkatesh PN, Ramachandran S (2008) A comprehensive transcriptional profiling of the WRKY gene family in rice under various abiotic and phytohormone treatments. *Plant Cell and Physiology* **49**, 865-879
- Ramiro D, Jalloul A, Petitot AS, Grossi de Sá MF, Maluf M, Fernandez D (2010) Identification of coffee WRKY transcription factor genes and ex-

- pression profiling in resistance responses to pathogens. *Tree Genetics and Genomes* **6**, 767-781
- Ren X, Chen Z, Liu Y, Zhang H, Zhang M, Liu Q, Hong X, Zhu JK, Gong Z** (2010) ABO3, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in *Arabidopsis*. *The Plant Journal* **63**, 417-429
- Ribot C, Zimmerli C, Farmer EE, Reymond P, Poirier Y** (2008) Induction of the *Arabidopsis* PHO1;H10 gene by 12-oxo-phytodienoic acid but not jasmonic acid via a CORONATINE INSENSITIVE1-dependent pathway. *Plant Physiology* **147**, 696-706
- Rizhsky L, Davletova S, Liang H, Mittler R** (2004) The zinc finger protein Zat12 is required for cytosolic ascorbate peroxidase 1 expression during oxidative stress in *Arabidopsis*. *Journal of Biological Chemistry* **279**, 11736-11743
- Robatzek S, Somssich IE** (2002) Targets of AtWRKY6 regulation during plant senescence and pathogen defense. *Genes and Development* **16**, 1139-1149
- Ross CA, Liu Y, Shen QJ** (2007) The WRKY gene family in rice (*Oryza sativa*). *Journal of Integrative Plant Biology* **49**, 827-842
- Rushton PJ, Macdonald H, Huttly AK, Lazarus CM, Hooley R** (1995) Members of a new family of DNA-binding proteins bind to a conserved cis-element in the promoters of alpha-Amy2 genes. *Plant Molecular Biology* **29**, 691-702
- Rushton PJ, Torres JT, Parniske M, Wernert P, Hahlbrock K, Somssich IE** (1996) Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes. *The EMBO Journal* **15**, 5690-5700
- Rushton PJ, Somssich IE** (1998) Transcriptional control of plant genes responsive to pathogens. *Current Opinion Plant Biology* **1**, 311-315
- Rushton DL, Tripathi P, Rabara RC, Lin J, Ringler P, Boken AK, Langum TJ, Smidt L, Boomsma DD, Emme NJ, Chen X, Finer JJ, Shen QJ, Rushton PJ** (2011) WRKY transcription factors: key components in abscisic acid signalling. *Plant Biotechnology Journal* **10**, 2-11
- Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J, Xu D, Hellsten U, May GD, Yu Y, Sakurai T, Umezawa T, Bhattacharyya MK, Sandhu D, Valliyodan B, Lindquist E, Peto M, Grant D, Shu S, Goodstein D, Barry K, Futrell-Griggs M, Abernathy B, Du J, Tian Z, Zhu L, Gill N, Joshi T, Libault M, Sethuraman A, Zhang XC, Shinozaki K, Nguyen HT, Wing RA, Cregan P, Specht J, Grimwood J, Rokhsar D, Stacey G, Shoemaker RC, Jackson SA** (2010) Genome sequence of the palaeopolyploid soybean. *Nature* **463**, 178-183
- Schroeder JI, Kwak JM, Allen GJ** (2001) Guard cell abscisic acid signalling and engineering drought hardness in plants. *Nature* **410**, 327-330
- Schulze-Lefert P, Robatzek S** (2006) Plant pathogens trick guard cells into opening the gates. *Cell* **126**, 831-834
- Shen QH, Saijo Y, Mauch S, Biskup C, Bieri S, Keller B, Seki H, Ulker B, Somssich IE, Schulze-Lefert P** (2007) Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science* **315**, 1098-1103
- Shinozaki K, Yamaguchi-Shinozaki K** (2000) Molecular response to dehydration and low temperature: Differences and crosstalk between two stress signaling pathways. *Current Opinion Plant Biology* **3**, 217-223
- Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, Toki S, Takatsuji H** (2007) Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. *Plant Cell* **19**, 2064-2076
- Skibbe M, Qu N, Galis I, Baldwin IT** (2008) Induced plant defenses in the natural environment: *Nicotiana attenuata* WRKY3 and WRKY6 coordinate responses to herbivory. *Plant Cell* **20**, 1984-2000
- Song Y, Chen L-G, Zhang L-P, Yu D-Q** (2010) Overexpression of OsWRKY72 gene interferes in the abscisic acid signal and auxin transport pathway of *Arabidopsis*. *Journal of Bioscience* **35**, 459-471
- Song Y, Jing SJ, Yu DQ** (2009) Overexpression of the stress induced OsWRKY08 improves the osmotic stress tolerance in *Arabidopsis*. *Chinese Science Bulletin* **54**, 4671-4678
- Spoel SH, Koornneef A, Claessens SM, Korzelius JP, Van Pelt JA, Mueller MJ, Buchala AJ, Metraux JP, Brown R, Kazan K, Van Loon LC, Dong X, Pieterse CM** (2003) NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *Plant Cell* **15**, 760-770
- Stefanovic A, Ribot C, Rouched H, Wang Y, Chong J, Belbahri L, Delesert S, Poirier Y** (2007) Members of the PHO1 gene family show limited functional redundancy in phosphate transfer to the shoot, and are regulated by phosphate deficiency via distinct pathways. *Plant Journal* **50**, 982-994
- Sun C, Palmqvist S, Olsson H, Boren M, Ahlandsberg S, Jansson C** (2003) A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the iso1 promoter. *Plant Cell* **15**, 2076-2092
- Suzuki N, Rizhsky L, Liang H, Shuman J, Shulaev V, Mittler R** (2005) Enhanced tolerance to environmental stress in transgenic plants expressing the transcriptional coactivator multiprotein bridging factor 1c. *Plant Physiology* **139**, 1313-1322
- Tao Z, Kou Y, Liu H, Li X, Xiao J, Wang S** (2011) OsWRKY45 alleles play different roles in abscisic acid signalling and salt stress tolerance but similar roles in drought and cold tolerance in rice. *Journal of Experimental Botany* **62**, 4863-4874
- Tao Z, Liu H, Qiu D, Zhou Y, Li X, Xu C, Wang S** (2009) A pair of allelic WRKY genes play opposite roles in rice-bacteria interactions. *Plant Physiology* **151**, 936-948
- Thomashow MF** (1999) Molecular basis of plant cold acclimation: insights gained from studying the CBF cold response pathway. *Plant Physiology* **154**, 571-577
- Ulker B, Somssich IE** (2004) WRKY transcription factors: from DNA binding towards biological function. *Current Opinion Plant Biology* **7**, 491-498
- Wang D, Amornsiripanitch N, Dong X** (2006) A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. *PLoS Pathogens* **2**, e123
- Wang F, Hou X, Tang J, Wang Z, Wang S, Jiang F, Li Y** (2012) A novel cold-inducible gene from Pak-choi (*Brassica campestris* ssp. *chinensis*), BcWRKY46, enhances the cold, salt and dehydration stress tolerance in transgenic tobacco. *Molecular Biology Reports* **39**, 4553-4564
- Wang H, Hao J, Chen X, Hao Z, Wang X, Lou Y, Peng Y, Guo Z** (2007) Overexpression of rice WRKY89 enhances ultraviolet B tolerance and disease resistance in rice plants. *Plant Molecular Biology* **65**, 799-815
- Wang YH, Garvin DF, Kochian LV** (2002) Rapid induction of regulatory and transporter genes in response to phosphorus, potassium, and iron deficiencies in tomato roots. Evidence for cross talk and root/rhizosphere-mediated signals. *Plant Physiology* **130**, 1361-1370
- Wei KF, Chen J, Chen YF, Wu LJ, Xie DX** (2012) Molecular phylogenetic and expression analysis of the complete WRKY transcription factor family in maize. *DNA Research* **19**, 153-164
- Wen N, Chu Z, Wang S** (2003) Three types of defense-responsive genes are involved in resistance to bacterial blight and fungal blast diseases in rice. *Molecular Genetics and Genomics* **269**, 331-339
- Wu X, Shirotto Y, Kishitani S, Ito Y, Toriyama K** (2009) Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing OsWRKY11 under the control of HSP101 promoter. *Plant Cell Reports* **28**, 21-30
- Xie Z, Zhang ZL, Zou X, Huang J, Ruas P, Thompson D, Shen QJ** (2005) Annotations and functional analyses of the rice WRKY gene superfamily reveal positive and negative regulators of abscisic acid signaling in aleurone cells. *Plant Physiology* **137**, 176-189
- Xing DH, Lai ZB, Zheng ZY, Vinod KM, Fan BF, Chen ZX** (2008) Stress- and pathogen-induced *Arabidopsis* WRKY48 is a transcriptional activator that represses plant basal defense. *Molecular Plant* **1**, 459-470
- Xu X, Chen C, Fan B, Chen Z** (2006) Physical and functional interactions between pathogen-induced *Arabidopsis* WRKY18, WRKY40, and WRKY60 transcription factors. *Plant Cell* **18**, 1310-1326
- Yamasaki K, Kigawa T, Inoue M, Tateno M, Yamasaki T, Yabuki T, Aoki M, Seki E, Matsuda T, Tomo Y, Hayami N, Terada T, Shirouzu M, Tanaka A, Seki M, Shinozaki K, Yokoyama S** (2005) Solution structure of an *Arabidopsis* WRKY DNA binding domain. *Plant Cell* **17**, 944-956
- Yang Y, Shah J, Klessig DF** (1997) Signal perception and transduction in plant defense responses. *Genes and Development* **11**, 1621-1639
- Yang P, Chen C, Wang Z, Fan B, Chen Z** (1999) A pathogen- and salicylic acid-induced WRKY DNA-binding activity recognizes the elicitor response element of tobacco class I chitinase gene promoter. *Plant Journal* **18**, 141-149
- Zhang J, Peng Y, Guo Z** (2008) Constitutive expression of pathogen-inducible OsWRKY31 enhances disease resistance and affects root growth and auxin response in transgenic rice plants. *Cell Research* **18**, 508-521
- Zhang Y, Wang L** (2005) The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. *BMC Evolutionary Biology* **5**, 1
- Zhang ZL, Xie Z, Zou X, Casaretto J, Ho TH, Shen QJ** (2004) A rice WRKY gene encodes a transcriptional repressor of the gibberellin signaling pathway in aleurone cells. *Plant Physiology* **134**, 1500-1513
- Zheng Z, Mosher SL, Fan B, Klessig DF, Chen Z** (2007) Functional analysis of *Arabidopsis* WRKY25 transcription factor in plant defense against *Pseudomonas syringae*. *BMC Plant Biology* **7**, 2
- Zheng Z, Qamar SA, Chen Z, Mengiste T** (2006) *Arabidopsis* WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. *Plant Journal* **48**, 592-605
- Zhou QY, Tian AG, Zou HF, Xie ZM, Lei G, Huang J, Wang CM, Wang HW, Zhang JS, Chen SY** (2008) Soybean WRKY-type transcription factor genes, GmWRKY13, GmWRKY21, and GmWRKY54, confer differential tolerance to abiotic stresses in transgenic *Arabidopsis* plants. *Plant Biotechnology Journal* **6**, 486-503