Insights into the Role of WRKY Superfamily of Protein Transcription Factor in Defense Response

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Preeti Singh, Shashi Shekhar, Anjana Rustagi,
Vinay Sharma, and Deepak Kumar

Abstract

Plants are constantly challenged by a variety of biotic and abiotic stresses. To combat these challenges, plants have developed intricate mechanisms to perceive external signals and respond with the proper physiological and morphological changes. Generally, plants regulate the expression of many stress-related genes by activating or repressing their transcription upon signal perception and transduction of the external stimuli. The WRKY transcription factors comprise a large family of plant-specific zinc-finger-type regulatory proteins and regulate many plant defense responses to diverse biotic and abiotic stresses. WRKY proteins possess either one or two WRKY domains, a 60-amino-acid region that contains the amino acid sequence WRKYGQK, and a zinc-finger-like motif. In spite of the strong conservation of their DNA-binding domain, the overall structures of WRKY proteins are highly divergent and can be categorized into distinct groups, which might reflect their different functions. Based on the number of conserved WRKY domains and the features of the zinc-finger motif, the WRKY superfamily can be divided into three distinct groups: I, II, and III. Previous studies have demonstrated that WRKY transcription factors participate in regulating defense gene expression at various levels, partly by directly modulating immediate downstream target genes, by activating or repressing other TF genes, and by

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P. Singh · S. Shekhar
School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

A. Rustagi
Department of Botany, Gargi College, New Delhi, India

V. Sharma
Department of Bioscience and Biotechnology, Banasthali University,
Banasthali, Rajasthan, India

D. Kumar (✉)
Department of Botany, Central University of Jammu, Jammu, J&K, India
e-mail: deepakkumar@cujammu.ac.in

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regulating WRKY genes. WRKY proteins also seem to be involved in other plant-specific processes, such as trichome development and the biosynthesis of secondary metabolites. In this chapter, we will focus our attention to the role of WRKY TFs in plant defense response.

Keywords
Biotic stress · Plant defense · Transcription factor · WRKY · Zinc-finger protein

8.1 Introduction

The global climate change leads to the dynamic interaction between climatic and biological factors. It is not only confined up to modification of physiology and resistance of plants, but rather it also modifies the rates and stages of pathogen development, which will ultimately lead to the shifting of host-pathogen physiological interactions. Pathogen would be following the hosts and may infect vegetation of natural plant communities which were previously not exposed to the more aggressive strains. Therefore, new combinations of species are evolving. So the emphasis is shifted to develop new strategies to cope up with these biotic constraints in present scenario. However, the nature orchestrated by plants with an inherent defense system can be generally divided into two levels: the first is PAMP-triggered immunity (PTI), which confers resistance to most pathogens, and the second begins in the cytoplasm and mainly relies on the recognition of microbial effectors which is called effector-triggered immunity (ETI). Bostock (2005) reported that both PTI and ETI activate local as well as systemic defense responses, modulated by jasmonic acid (JA) and salicylic acid (SA). Activation of these two pathways extensively shares downstream signaling pathway, which in turn induces expression of defense gene and their corresponding defense responses (Tsuda et al. 2013). The response leads to adaptive plasticity of plants, which is mainly achieved by enforcement of a network of various transcription factors (TFs). TFs are multimers of polypeptide mediating different cellular responses through recognizing the specific cis-regulatory DNA sequences at the promoters of their targets genes (Franco-Zorrilla et al. 2014) and the rearrangement of the multimeric subunits leading to different functions through their differential expression patterns (Berk and Schmidt 1990). The binding of TFs with *cis*-elements of stress-related genes results in either overexpression or suppression of these genes, which may improve the plant's tolerance potential against different biotic stresses. Approximately 6% of the plant genome encodes for TFs, and among all, WRKY TFs are one of the largest families of transcriptional regulators in plants (Eulgem and Somssich 2007; Bakshi and Oelmüller 2014), involved in regulation of various physiological processes. WRKY TFs are emerging players in plant signaling, which regulate diverse cellular programs by relaying extracellular signals to intracellular responses and involved in multiple defense responses, development, metabolism, etc. The reprogramming of

WRKY network under biotic stress efficiently deteriorates the pathogens, and at the same time, it restricts defense responses, which can be detrimental for plant growth, development, and reproductive fitness. However, in the contemporary time of scientific advancement, enormous role of WRKY TFs in abiotic stresses has also been revealed.

In the present chapter, we emphasize on WRKY TFs and their action on downstream regulation of different molecular switches under biotic stress. This will provide important insights in understanding of regulatory networks and its associated functions to develop strategies for crop improvement and value addition in plants, which could be useful to the humankind.

8.2 WRKY Domains and Classification

WRKY proteins constitute a novel family of plant-specific TFs and are characterized by the presence of WRKY domain which consists of ~60 amino acid residues at the N-terminus and a zinc-finger-like motif C-C-H-H/C at the C-terminus (Rushton et al. 1996). The WRKY domains contain the conserved heptapeptide "WRKYGQK" also referred to as the "signature sequence" at the N-terminus of DNA-binding domain. WRKY proteins bind to W-box elements containing the consensus motif TGACC/T, which occur either as single hexamers, TTGACC/T; as palindromic sequence, TGACC/T-A/GTCA; or as tandem repeats, TGACC/C--TGACC/T in the promoter of target genes (Eulgem et al. 1999; Yang et al. 1999). WRKY proteins have been categorized into three groups based on the number of WRKY domains and the type of their zinc-finger-like motif (Kumar et al. 2016). Generally, group I member contains two WRKY domains both at N- and C-terminal and C2H2-type zinc-finger motif (C–X4–5–C–X22–23–H–X1–H), and group II has one WRKY domain with C2H2-type zinc-finger motif. Group II members have been further divided into subgroups a–e based upon additional amino acid motifs present outside the WRKY domain. Group III also has one WRKY domain but with C2HC-type zinc-finger motif (C–X7–C–X23–H–X–C) at C-terminal (Eulgem et al. 2000). It has been reported for group I WRKY proteins from *Arabidopsis thaliana*, parsley (*Petroselinum crispum*), and sweet potato (*Ipomoea batatas*) that sequence-specific DNA binding occurs at the C-terminal of WRKY domain, but not the N-terminal domain (Ishiguro and Nakamura 1994; Agarwal et al. 2011) (Table 8.1).

The first WRKY TF has been identified as DNA-binding protein (SPF1) from *Ipomoea batatas* and shown to regulate gene expression in sucrose inducibility (Ishiguro and Nakamura 1994). WRKY proteins have been identified in a wide range of plants due to successive duplication events, resulting in large gene families including up to 74 members in *Arabidopsis* (Ülker and Somssich 2004), >109 in rice (Shimono et al. 2012), 197 in *Glycine max* (Schmutz et al. 2010), 66 in papaya, 104 in *Populus*, 68 in sorghum (Pandey and Somssich 2009), and 45 in barley (Mangelsen et al. 2008). WRKY TFs play a broad-spectrum regulatory role as a positive and negative regulator to control gene expression (Eulgem and Somssich 2007).

Table 8.1 WRKY TFs involved in plant defense and its associated regulatory pathways

S. No.	Gene/pathway	Action/role	Plant species	Tolerance/resistance	References
1.	*AtWRKY18*	Modulation of defense-related genes	*Arabidopsis thaliana*	*Pseudomonas syringae*	Chen and Chen. (2002)
2.	*AtWRKY3* and *AtWRKY4*	Regulation of SA and JA-/ET-mediated signaling pathway	*Arabidopsis thaliana*	*Pseudomonas syringae* and *Botrytis cinerea* *A*	Lai et al. (2008)
3.	*AtWRKY33*	Reduced expression of the salicylate and jasmonate defense genes	*Arabidopsis thaliana*	*Botrytis cinerea*, *Pseudomonas syringae*	Zheng et al. (2006)
4.	*AtWRKY6*	Regulation of genes associated with leaf senescence	*Arabidopsis thaliana*	*Pseudomonas syringae*	Robatzek and Somssich. (2001)
5.	*AtWRKY7*	Reduced expression of defense-related genes	*Arabidopsis thaliana*	*Pseudomonas syringae*	Kim et al. (2006)
6.	*AtWRKY70*	Involvement of R-gene	*Arabidopsis thaliana*	*Hyaloperonospora parasitica*	Knoth et al. (2007)
7.	*BnWRKY33*	Induction of salicylic acid, jasmonic acid, ethylene, and glucosinolate synthesis pathway	Oilseed rape	*Sclerotinia sclerotiorum*	Wang et al. (2014)
8.	*BrWRKY12*	By upregulation of defense-related proteins and jasmonic acid signaling pathway	*Brassica rapa*	*Pectobacterium carotovorum*	Kim et al. (2013)
9.	*FaWRKY1* and *WRKY75*	Uncoupled to PATHOGENESIS-RELATED (PR) gene expression but strongly associated with oxidative burst and glutathione S-transferase (GST) induction	Strawberry	*Colletotrichum acutatum*	Encinas-Villarejo et al. (2009)
10.	*GhWRKY15*	Through increased RNA expression of pathogen-related genes, NONEXPRESSOR OF PR1, ET biosynthesis, and antioxidant enzymes POD and APX	*Gossypium hirsutum*	*C. gossypii*, *Fusarium oxysporum*, and *Rhizoctonia solani*	Yu et al. (2012)
11.	*GhWRKY25*	Through co-expression of large number of resistance- and defense-related genes	*Gossypium hirsutum*	*Magnaporthe grisea*, *Blumeria graminis, and M. oryzae*	Liu et al. (2015)

12.	*GhWRKY27a*	Through increased RNA expression of pathogen-related genes, NONEXPRESSOR OF PR1, ET biosynthesis, and antioxidant enzymes POD and APX	*Gossypium hirsutum*	*Rhizoctonia solani*	Yan et al. (2015)
13.	*MaWRKY1* and *MaWRKY2*	Upregulation of defense-associated genes	*Poplar tomentosa* Carr	*Dothiorella gregaria* Sacc	Ye et al. (2014)
14.	*MrWRKY30*	Uncoupled to PATHOGENESIS-RELATED (PR) gene expression but strongly associated with oxidative burst and glutathione S-transferase (GST) induction	*Muscadinia rotundifolia*	*Peronospora parasitica*	Jiang et al. (2015)
15.	*OsWRKY22*	Through co-expression of large number of resistance- and defense-related genes	Rice	*Magnaporthe grisea*, *Blumeria graminis*, *and M. oryzae*	Abbruscato et al. (2012)
16.	*OsWRKY45*	By upregulation of defense-related proteins and jasmonic acid signaling pathway	*Oryza sativa*	Brown planthopper (BPH, *Nilaparvata lugens*)	Huangfu et al. (2016)
17.	*OsWRKY53*	Upregulation of defense-related genes	*Oryza sativa*	*Magnaporthe grisea*	Chujo et al. (2007)
18.	*OsWRKY77*	Through enhanced expression of defense-related PR1, PR2, and PR5 genes	*Oryza sativa* L.	*Pseudomonas syringae*	Lan et al. (2013)
19.	*PtoWRKY60*	Upregulation of defense-associated genes	*Poplar tomentosa* Carr	*Dothiorella gregaria* Sacc	Ye et al. (2014)
20.	*PtrWRKY89*	Through enhanced expression of defense-related PR1, PR2, and PR5 genes and downregulating the marker gene of SA and JA pathways	*Populus trichocarpa*	*Pseudomonas syringae*	Jiang et al. (2016)
21.	*SpWRKY1*	Enhanced expression of SA- and JA-associated genes (*NtPR1*, *NtPR2*, *NtPR4*, *NtPR5*, and *NtPDF1.2*), as well as various defense-related genes (*NtPOD*, *NtSOD*, and *NtPAL*)	*Solanum pimpinellifolium* L3708	*Phytophthora nicotianae*	Li et al. (2015)
22.	*VvWRKY1*	Involvement of SA- and JA-/ET-mediated signaling pathway	*Vitis vinifera*	*Downy mildew*, *Plasmopara viticola*	Marchive et al. (2013)

8.3 Structure of DNA-Binding Domain of WRKY Proteins

The first structure of the C-terminal WRKY domains of AtWRKY1 and AtWRKY4 protein of *Arabidopsis thaliana* revealed that the WRKY domain consists of a four-stranded antiparallel β-sheet and zinc-binding pocket formed by the coordination of zinc atom with the conserved two cysteine and histidine residues (Yamasaki et al. 2005). The crystal structure of the C-terminal of WRKY domain of AtWRKY1 consists of five β-strands, with DNA-binding residues located at *β2* and *β3* strands (Duan et al. 2007). The N-terminal region of the β-strand consisting of WRKY signature sequence partly protrudes from one surface of the protein, thereby enabling access to the major DNA groove, and binds to its cognate W-box. Recently, solution structure of WRKY domain with W-box-binding site has been determined, and it revealed that four-stranded β-sheet enters the major groove of the DNA in an atypical mode where the plane of the sheet is nearly perpendicular to the helical axis of DNA (Yamasaki et al. 2012). In the WRKYGQK signature sequence, the tryptophan residue forms the core of the structure, while all the other amino acids (RKYGQK) are directly involved in DNA binding. The glycine residue helps in bending of the strand and thus enables deep penetration into the DNA groove. Recognition of the W-box sequence occurs mainly through the hydrophobic interaction with the methyl groups of thymine (T) bases of the DNA strand. Mutations in the residues involved either in DNA binding or in Zn binding significantly impaired the DNA-binding activity due to the disruption of the tertiary structure, which is important in DNA binding (Duan et al. 2007; Yamasaki et al. 2013).

8.4 Regulation of WRKY TFs

8.4.1 Autoregulation and Cross-Regulation

WRKY proteins are involved in diverse pathways and regulate the expression of downstream target genes either as a positive or negative regulator. The regulation of WRKY-dependent signaling pathways is very extensive and complex. In response to the internal or external stimuli, WRKY TFs trigger the expression of the target genes by binding to their W-box elements in the promoter regions. W-box elements are also present in the promoters of the majority of the WRKY genes, and this suggests that they are regulated via specific feedback mechanisms (autoregulation by themselves or cross-regulation by other WRKY TFs) (Eulgem and Somssich 2007; Rushton et al. 2010). For example, chromatin immunoprecipitation (ChIP) analysis of PcWRKY1 of parsley (*Petroselinum crispum*) revealed that it binds not only to the W-box of its own promoter but also has affinity toward binding the promoters of PcWRKY3 and marker gene PcPR1 (Eulgem et al. 1999; Turck et al. 2004). Likewise, WRKY33 expression is activated by the MAPK3/6, and it autoregulates its expression via a positive feedback loop by binding to its own promoter (Mao et al. 2011). WRKY18, WRKY40, and WRKY60 act as a negative regulator of ABA signaling and could directly bind to the W-box in the promoter region of their respective genes and thus repress the expression of all three WRKY genes (Chen et al. 2010; Yan et al. 2013). The above finding suggests the importance of autoregulation and cross-regulation of WRKY TFs in maintaining the homeostasis of WRKY protein expression in the cell during abiotic and biotic stress conditions.

8.4.2 Regulation of WRKY TFs by MAP Kinases

Some WRKY TFs are also regulated via MAPK (mitogen-activated protein kinase) pathway (Adachi et al. 2015). WRKY TFs act downstream of various MAPKs to regulate defense-related plant genes (Phukan et al. 2016). The AtMPK3, AtMPK6, and AtMPK4 get activated during both biotic and abiotic stresses (Banerjee and Roychoudhury 2015). Group I WRKY TFs are the first protein, which gets phosphorylated by MAP kinases in response to PAMP-triggered MAPK signaling. Two WRKY proteins AtWRKY22 and AtWRKY29 act downstream of the bacterium flagellin receptor FLS2, are upregulated by a PAMP-induced MAPK cascade, and contain multiple W-boxes within their respective promoters. AtWRKY33, involved in the production of phytoalexin during pathogen attack, forms a complex with MPK4-MKS1 (MPK4 substrate) in the nucleus. Upon infection MPK, MKK (MAP kinase kinase), and MEKK (MAP kinase kinase kinase) are activated. The activated MPK4 phosphorylate MKS1, which lead to the dissociation of the MPK4-MKS1-WRKY33 complex, and AtWRKY33 was released. Then AtWRKY33 binds to the promoter of the target gene PAD3 (phytoalexin deficient 3) that is required for the synthesis of antimicrobial compound camalexin (Qiu et al. 2008). WRKY33 could be phosphorylated by two other MPKs, MPK3 and MPK6, which led to binding to its own and the PAD3 promoters in response to *B. cinerea*. Mao et al. (2011) had shown that in *wrky33* mutant, camalexin production was abolished and mutation in the phosphorylation sites of WRKY33 also had the same effect. Taken together, these results suggested that AtWRKY33 works downstream of the MPK3/MPK6 and phosphorylation of WRKY is important for the production of camalexin upon bacterial infection. In rice, OsWRKY33 is phosphorylated by BWMK1 (blast- and wounding-activated MAP kinase 1) and binds to the promoter of PR genes during salicylic acid-dependent defense responses (Koo et al. 2009). OsWRKY53 suppresses herbivore-induced defense in rice by negative feedback modulation of MPK3/MPK6 activity (Hu et al. 2015). Therefore, phosphorylation and activation of WRKY proteins by MPKs is an important regulatory mechanism which increases the capacity of WRKYs to bind to the promoters of target gene which are involved in the plant defense responses.

8.4.3 Regulation of WRKY TFs via Histone Modification

A few WRKY TFs have been shown to be regulated by histone-modifying complex. *AtWRKY70* gets activated by the *Arabidopsis* homolog of trithorax (ATX1) leading to nucleosomal histone H3K4 trimethylation which results in the activation of

SA-responsive gene PR1 and JA-responsive gene *THI2.1* (*THIONIN2.1*). This finding suggests that *PR1* and *THI2.1* genes are the downstream targets of WRKY70 and regulated epigenetically (Alvarez-Venegas et al. 2007). In response to senescence, H3K4 dimethylation and H3K4 trimethylation by histone methyltransferase occur at 5′ end and coding regions of *AtWRKY53*gene (Ay et al. 2009). Another example of histone modification has been shown in two type III WRKY TFS, *AtWRKY38* and *AtWRKY62*. During bacterial infection, HDA19 (histone deacetylase 19) removes acetyl group from histone tails and represses transcription of *AtWRKY38* and *AtWRKY62* and thus negatively regulates basal defense (Kim et al. 2008). Similarly, methylation at the promoter of *AtWRKY40* inhibits expression of *ABI5* and negatively regulates ABA signaling in seed germination and post-germination growth (Shang et al. 2010). Wang et al. (2012) showed that the protein encoded by chromatin remodeling linker histone H1gene (*MaHIS1*) and MaWRKY1 could interact and regulate physiological processes like fruit ripening and stress responses in banana. MaHIS1 has also been shown to be induced by other factors like JA, ABA, and hydrogen peroxide and under cold stress.

8.4.4 Interaction of WRKY TFs with Other Factors

8.4.4.1 VQ Proteins

It has been reported in the literature that many interacting partners like coactivators regulate the expression of many WRKY TFs. One of the interacting partners is VQ protein, which is a group of cofactors containing a short conserved VQ-related motif (FxxxVQxLTG). The conserved valine and glutamine residues in the conserved motif are important and required for the interaction with the C-terminal domain of WRKY TFs. In *Arabidopsis* and rice, 34 and 40 VQ members were identified, respectively, and shown to be involved in disease resistance and in the plant response to environmental stresses (Cheng et al. 2012; Kim et al. 2013). The first VQ proteins were identified as a MPK4 substrate (MKS) in *Arabidopsis* by using a yeast two-hybrid assay. The VQ protein MKS has been shown to form complex with AtWRKY25 and AtWRKY33, which are involved in the regulation of plant defense responses (Andreasson et al. 2005). Binding of VQ proteins with WRKY TFs changes the binding affinity of the latter for the nucleotides flanking the conserved W-box. It has been shown that C-terminal domain of AtWRKY33 interacts with two VQ proteins, SIGMA FACTORBINDINGPROTEIN 1 (SIB1) and SIB2, to regulate plant defense response against necrotrophic pathogens Lai et al. (2011). These results demonstrate that VQ proteins are crucial cofactors in regulating WRKY-mediated gene expression (Cheng et al. 2012; Chi et al. 2013).

8.4.4.2 Calmodulin (CaM) Proteins

CaM binds to the conserved Ca^{+2}-dependent calmodulin-binding domain (CaBD) (DxxVxKFKxVISLLxxxR) present in WRKY group II members like *AtWRKY7* (Park et al. 2005). Increasing concentration of calcium triggers the interaction of CaM and WRKY members over WRKY-WRKY interaction (Chi et al. 2013).

8.4.4.3 14-3-3 Proteins

14-3-3 proteins specifically bind to phosphoserine and phosphothreonine and regulate many processes like plant development, plant defense, and stress responses (Roberts 2003; Denison et al. 2011). They function as homo- or heterodimers and each dimer binds two substrates. In *Arabidopsis*, seven WRKY members including WRKY6, WRKY16, WRKY18, WRKY19, WRKY27, WRKY32, and WRKY40 have been identified as putative interacting partners for 14-3-3 proteins by tandem affinity purification tag assay (Chang et al. 2009). 14-3-3 proteins interact and phosphorylate AtWRKY18 and AtWRKY40 to regulate ABA signaling (Shang et al. 2010; Shen et al. 2003). These results suggest that 14-3-3 proteins might have potential roles in regulating biotic and abiotic stress responses via WRKY TFs (Chang et al. 2009; Rushton et al. 2010; Chi et al. 2013).

8.5 WRKY TFs in Defense Response

The plant innate immunity is mainly responsive to two interconnected pathways termed PTI or ETI (Jones and Dangl 2006). PTI is initiated by the recognition of molecular patterns of pathogens and activates MAP kinase cascade pathway and defense-related genes, while ETI is associated with plant disease resistance (R) proteins that activate defense reactions upon specific recognition of pathogen effectors (Chisholm et al. 2006). PTI and ETI activate local as well as long-distance defense reactions like systemic acquired resistance (SAR) (Durrant and Dong 2004; Bostock 2005).

8.5.1 Interaction of WRKY TFs with SA and JA Signaling Pathway

SA and JA are two important signaling molecules in defense response. JA-dependent plant defense pathways are activated by necrotrophic pathogens, whereas SA-dependent defenses are triggered by biotrophic pathogens. JA and SA signaling pathway act antagonistically in regulating defense response (Koornneef and Pieterse 2008). During the past few years, much attention has been focused on TFs involved in the regulation of gene expression upon pathogen challenge. Expression profiling studies have revealed that a large set of the *WRKY TF* gene family members are responsive to pathogen challenge and regulate plant defense responses either as a positive or negative regulator (Eulgem and Somssich 2007). Expression of WRKY genes has been shown to get induced by pathogen infection and pathogen elicitors or by SA treatment in a number of plants (Agarwal et al. 2011). In *Arabidopsis* and rice, more than 75 and 109 WRKY genes have been reported (Shimono et al. 2012). Upon infection, pathogens induce SAR leading to accumulation of SA. Many WRKYs are positively regulated by SA through the receptors NPR1 and its paralogues NPR3 and NPR4 (Wang et al. 2006; Fu et al. 2012; Wu et al. 2012). A few WRKY genes including WRKY18, WRKY38, WRKY53, WRKY54, WRKY58, WRKY59, WRKY66, and WRKY70 bind to the W-box sequences in the promoter region of NPR1 genes in *Arabidopsis*; this suggests that WRKY genes act upstream of NPR1 genes and involved in the positive regulation of WRKY TFs during pathogen-induced signaling (Wang et al. 2006; Ishihama and Yoshioka 2012). Many WRKY TFs are common component in the SA-/JA-mediated plant defense pathway (Koornneef and Pieterse 2008; Thaler et al. 2012). For example, WRKY70 works at a convergence point for maintaining balance between SA- and JA-mediated signaling pathways as well as also plays a crucial role for R-gene-mediated resistance. Overexpression of AtWRKY70 induces the expression of SA-induced PR genes and acts as a positive transcriptional regulator of SA signaling while for JA-responsive pathways acts as a negative regulator. Overexpression of AtWRKY70 improved resistance to biotrophic pathogen *Erysiphe cichoracearum* and necrotrophic bacteria *Erwinia carotovora* (Ecc) but reduced resistance to fungal necrotroph *Alternaria brassicicola*. Similar dual roles have also been observed for WRKY53. It positively regulates plant defense response during *P. syringae* infection, while its mutant displayed delayed symptom development toward *Ralstonia solanacearum* (Murray et al. 2007; Hu et al. 2008). Moreover, during *P. syringae* infection, WRKY11 and WRKY17 have shown to positively regulate the JA biosynthesis pathway genes, *LOX2* and *AOS*, while negatively regulate the expression of WRKY70 (Li et al. 2004, 2006; Journot-Catalino et al. 2006). AtWRKY53 was reported to positively regulate the basal defense response during *P. syringae* infection while negatively regulate during JA and ethylene signaling pathway (Murray et al. 2007).

Three WRKY TFs of subgroup IIa, WRKY18, WRKY40, and WRKY60, function in a partly redundant way in regulating plant disease resistance. Xu et al. (2006) showed that double mutants *wrky18wrky40* and *wrky18wrky60* and the triple mutant *wrky18wrky40wrky60* were found to be more resistant to *P. syringae* infection but susceptible to *B. cinerea.* In other studies, *Atwrky18/Atwrky40* double mutants showed resistance toward avirulent powdery mildew fungus *Golovinomyces orontii*, and complementation of WRKY40 in this mutant partially restored susceptibility (Pandey et al. 2010). The HvWRKY1 and HvWRKY2 homologs of AtWRKY18 and AtWRKY40 in barley act as a suppressor of PAMP-induced basal defense, leading to resistance against virulent pathogen *B. graminis.* During infection, fungal effector AVR10 is recognized by the resistance protein MLA (mildew resistance locus A) in the cytoplasm followed by interaction of HvWRKY 1 and 2 with activated MLA10 in the nucleus (Shen et al. 2007). In addition, AtWRKY33 is another example and was known to act as a positive regulator of resistance to the necrotrophic pathogens *Botrytis cinerea* and *Alternaria brassicicola*, while overexpression leads to susceptibility to *Pseudomonas syringae* DC3000. However, loss of function mutant of AtWRKY33 showed increased resistance toward *R. solanacearum* (Zheng et al. 2006; Birkenbihl et al. 2012). Similarly, WRKY3 and WRKY4, which are structurally similar proteins, confer resistance to necrotrophic pathogens (Lai et al. 2008).

Few of the WRKY TF members act as negative regulator of defense signaling including AtWRKY7, AtWRKY38, AtWRKY62, and AtWRKY52. AtWRKY11, AtWRKY17, AtWRKY38, and AtWRKY62 negatively regulate basal defense response toward bacterial pathogen *P. syringae.* Interaction of AtWRKY38 and AtWRKY62 with HDA19, a positive regulator of plant basal disease resistance, leads to inactivation of defense repressing WRKY38 and WRKY62 TFs (Journot-Catalino et al. 2006; Kim et al. 2008). Expression of AtWRKY62 is induced by SA and JA in a NPR1-dependent manner. Loss of function mutant of AtWRKY62 resulted in enhanced expression of JA-response genes, while overexpression of AtWRKY62 inhibited JA-response gene expression (Mao et al. 2007). In other study, overexpression of WRKY62 leads to elevated transcript levels of PR1 gene, whereas in Atwrky62 mutant, PR1 gene is downregulated (Kim et al. 2008). In addition, AtWRKY48 and AtWRKY8 also negatively regulate basal resistance to *P. syringae* (Xing et al. 2008; Chen et al. 2010). Additionally, WRKY8 was also involved in negative regulation of crucifer-infecting tobacco mosaic virus (TMV-cg) (Chen et al. 2013). AtWRKY48 mutants showed increased expression of PR1 genes found to be associated with reduced bacterial growth, whereas *AtWRKY48* overexpressor lines showed the opposite phenotypes. *AtWRKY58* acts downstream ofNPR1, negatively regulating SAR (Wang et al. 2006). Some WRKY proteins exist as chimeric proteins like AtWRKY52 which possesses TIR-NBS-LRR (Toll/interleukin-1 receptor-nucleotide-binding site-leucine-rich repeat) domain in combination with group III-type WRKY domain and mediates R-gene-based resistance toward bacterial wilt *Ralstonia solanacearum*. The physical interaction of *AtWRKY52*/RRS1 with its cognate bacterial effector PopP2 within the plant cell nucleus has been suggested to inactivate the WRKY domain of RRS1 to activate defense mechanisms by derepression (Deslandes et al. 2003). *AtWRKY16* and *AtWRKY19* also contain NBS-LRR domain reported in *Arabidopsis*.

8.5.2 Overexpression/Downregulation of WRKY TFs for Biotic Stress Tolerance

Till date WRKY TFs have been reported from many plant species suggesting its importance in regulating plant defense response. Overexpression of many WRKY TFs from rice like *OsWRKY13*, *OsWRKY31*, *OsWRKY45*, *OsWRKY53*, and *OsWRKY47* showed enhanced resistance to fungal pathogen *Magnaporthe grisea*, the causal agent of the devastating rice blast disease (Ryu et al. 2006; Wei et al. 2013). In rice, OsWRKY13, an ortholog of AtWRKY70, is reported to have similar functions. Overexpression of *OsWRKY13* activates the genes related to SA pathways but reduces the expression of genes in JA pathway (Qiu et al. 2007). Overexpression of *OsWRKY3* led to elevated expression of NPR1, PR1b, phenylalanine ammonia-lyase (ZB8), and peroxidase (POX22.3), suggesting that it works as a transcriptional regulator in SA- or JA-dependent defense signaling pathway (Liu et al. 2005). Overexpressor transgenic lines of OsWRKY53 showed resistance against blast disease and induced the expression of PR proteins and peroxidase enzymes (Chujo et al. 2007). Overexpression of *OsWRKY89* showed more tolerance to the rice blast fungus white-backed planthopper (*Sogatella furcifera*), a rice herbivore (Wang et al. 2007). Lan et al. (2013) showed that overexpression of *OsWRKY77* in *Arabidopsis* led to enhanced resistance toward *P. syringae* suggesting its function as a positive regulator of plant defense. The OsWRKY45 showed improved resistance to rice blast fungus and might work independent of NPR1-mediated SA signaling (Shimono et al. 2007). In addition OsWRKY45 is found to negatively modulate the resistance of rice to the brown planthopper *Nilaparvata lugens* (Huangfu et al. 2016). Silencing of *NaWRKY3* and *NaWRKY6* in *Nicotiana attenuata* made plants highly susceptible to lepidopteran herbivore (Skibbe et al. 2008). *CaWRKY1* from pepper (*Capsicum annuum*) negatively regulates plant defense, as silencing of this gene led to decreased growth *of Xanthomonas* (Oh et al. 2008), whereas constitutive overexpression of *CaWRKY40* resulted in enhanced resistance toward *Ralstonia solanacearum* (Dang et al. 2013). Shi et al. (2014) suggested that the overexpression of *GhWRKY39* may positively regulate the plant response against bacterial *R. solanacearum* and bacterial pathogen *R. solani.* Transgenic tobacco plants overexpressing *GhWRKY15* displayed more resistance toward viral and fungal infections and showed induced expression of NPR1 gene (Yu et al. 2012). *GhWRKY25* overexpression resulted in enhanced sensitivity to the fungal pathogen *Botrytis cinerea* by reducing the expression of SA or ET signaling-related genes and inducing the expression of genes involved in the JA signaling pathway (Liu et al. 2015). GhWRKY27a-overexpressing plants conferred reduced resistance to *R. solani* infection as demonstrated by severe disease symptoms in transgenic lines (Yan et al. 2015). Additionally, a number of WRKY TFs which are important players of plant immunity have been found in different plant species, for example, VvWRKY1 and VvWRKY2 from grapevine (*Vitis vinifera*), *PtrWRKY89* from *Populus trichocarpa*, and MaWRKY1 and MaWRKY2 from *Musa* spp. (Marchive et al. 2013; Jiang et al. 2014; Shan et al. 2016).

8.6 Conclusion and Future Perspectives

In this chapter, we have focused on the most recent advances on WRKY TFs. Over the last two decades, significant progress has been made in order to understand the role of WRKY TFs. Current information suggests that the WRKY superfamily of TFs is composed of different types of proteins that have been implicated in plant developmental processes and pathogen-induced defense response. New finding illustrates that they participate in regulating a plethora of genes at various levels, by working as positive or negative regulator, by direct activation of downstream target genes, and by activating or repressing other TF genes. WRKY TFs are itself regulated by a highly intricate mechanism in plants, and they are required to maintain normal cellular homeostasis under normal condition. One WRKY protein is found to regulate several plant processes at a time, and the mechanisms of regulation are not yet clear. Extensive study of these TF families is needed for better understanding of the signaling pathways involved in WRKY-mediated regulation of defense and developmental processes. In the future it would be exciting to explore "how WRKY TF networks exert their functions on DNA/chromatin level" which will certainly allow us to open new vistas of diverse metabolic pathways, their cross-linking, and overall cellular physiology of plants under biotic stress conditions.

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