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REVIEW

Unraveling the involvement of WRKY TFs in regulating plant disease defense signaling

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Abstract

Main conclusion **This review article explores the intricate role, regulation, and signaling mechanisms of WRKY TFs in response to biotic stress, particularly emphasizing their pivotal role in the trophism of plant-pathogen interactions.**

Abstract Transcription factors (TFs) play a vital role in governing both plant defense and development by controlling the expression of various downstream target genes. Early studies have shown the differential expression of certain WRKY transcription factors by microbial infections. Several transcriptome-wide studies later demonstrated that diverse sets of WRKYs are significantly activated in the early stages of viral, bacterial, and fungal infections. Furthermore, functional investigations indicated that overexpression or silencing of certain WRKY genes in plants can drastically alter disease symptoms as well as pathogen multiplication rates. Hence the new aspects of pathogen-triggered WRKY TFs mediated regulation of plant defense can be explored. The already recognized roles of WRKYs include transcriptional regulation of defense-related genes, modulation of hormonal signaling, and participation in signal transduction pathways. Some WRKYs have been shown to directly bind to pathogen effectors, acting as decoys or resistance proteins. Notably, the signaling molecules like salicylic acid, jasmonic acid, and ethylene which are associated with plant defense significantly increase the expression of several WRKYs. Moreover, induction of WRKY genes or heightened WRKY activities is also observed during ISR triggered by the beneficial microbes which protect the plants from subsequent pathogen infection. To understand the contribution of WRKY TFs towards disease resistance and their exact metabolic functions in infected plants, further studies are required. This review article explores the intrinsic transcriptional regulation, signaling mechanisms, and hormonal crosstalk governed by WRKY TFs in plant disease defense response, particularly emphasizing their specific role against different biotrophic, hemibiotrophic, and necrotrophic pathogen infections.

Keywords Transcription factors (TFs) · WRKY TFs · Transcription regulation · Biotic stress · Defense signaling

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Introduction

Plants are essential for our existence as they provide us with oxygen, food, and numerous other resources. However, plants are not invincible and susceptible to various stresses that can significantly impact their growth and production. Stress imparted on plants can be broadly categorized as biotic stress and abiotic stress. Abiotic stress is conveyed by non-living factors such as drought, heat, cold, salt, heavy metals, etc. On the other hand, living organisms like fungi, viruses, bacteria, insects, and numerous nematodes can cause biotic stress leading to severe damage to crop health and productivity loss of up to 40% (Khan et al. 2021). Fungi, bacteria, and viruses can cause various diseases such as rust, blight, canker, and mosaic (Kumar et al. 2014; Wang et al. 2020, 2015; Freeborough et al. 2021; Yoda et al. 2002). Nematodes and insects can cause damage by feeding on plant tissues, leading to stunted growth and reduced yield (Nicol et al. 2011). These stresses not only impact the quantity but also the quality of the produce, reducing their economic value (Nakashima et al. 2014). To defend against these biotic stresses, plants have evolved various immunity-inducing pathways. One such pathway is pattern-triggered immunity (PTI), which is triggered upon the recognition of conserved motifs known as pathogen-associated molecular patterns (PAMPs) and microbial-associated molecular patterns (MAMPs) present on the pathogen's surface (Jones and Dangl 2006). Upon recognition, a cascade of downstream signaling is triggered, which ultimately builds up activated defense mechanisms including the formation of certain compounds like reactive oxygen species (ROS), phytoalexins, phytohormones, as well as pathogenesis-related (PR) proteins (Zipfel 2014). Another pathway is effector-triggered immunity (ETI), which is prompted when the plant recognizes specific effector molecules of pathogen delivered into the plant cell. This recognition leads to a robust and heightened defense response known as Hypersensitive Response (HR) that includes localized cell death which paves the way for the stimulation of systemic acquired resistance (SAR) (Cui et al. 2015).

Phytohormones like salicylic acid (SA), ethylene (ET) and jasmonic acid (JA) play important roles in modulating the defense response of plants. Biosynthesis of SA leads to enhanced protection against biotrophic and hemibiotrophic pathogens. Similarly, it is reported that ET and JA are essentially involved in defense response against necrotrophic pathogens. These hormones control the defense response of plants against specific pathogens largely by transcriptional reprogramming of the pathogen-responsive genes through the activity of specialized proteins called transcription factors.

Transcription factors have a significant influence on the differential expression of stress-responsive genes upon pathogen attacks (Javed et al. 2020). The binding of these TFs to specific cis-acting elements present in the promoters either activates or represses the transcription of their downstream target genes (Wani et al. 2021; Qiu et al. 2007; Qiu et al. 2008a; Gao et al. 2020; Tolosa and Zhang 2020). WRKY TFs, known for their involvement in abiotic stress tolerance mechanisms, have also demonstrated their regulatory role in plant defense mechanisms towards various biotic stresses. Successful execution of the plant defense is largely dependent on finetuning of different hormone signaling pathways upon their exposure to different types of pathogens. WRKY transcription factors act as regulators for this interplay and are essential for the coordination of defense responses (Xu et al. 2006). Moreover, the differential expression pattern in different tissues, developmental stages, and under different stress conditions, makes these WRKY TFs very exclusive in regulating defense response.

The involvement of WRKY TFs in response to different abiotic and biotic stress has been investigated in various plant species including pearl millet, foxtail millet, cotton, grapevine, wheat, and others, in multiple studies (Chanwala et al. 2020; Ning et al. 2017; Li et al. 2017; Javed et al. 2022; Wei et al. 2016; Goel et al. 2016; Muthamilarasan et al. 2015; Wang et al. 2014; Dou et al. 2014; Huang et al. 2012). Understanding the molecular mechanisms involved in plant defense, particularly their response during pathogen attacks based on trophism and signal transduction mediated by WRKY transcription factors is crucial. With the aid of this knowledge, crops that are more resilient to biotic stresses can be developed, reducing the financial impact they have on agricultural production.

Structure and classification of WRKY

The term "WRKY" is derived from the evolutionarily conserved WRKY domains which are comprised of nearly sixty amino acids that are found in the members of this TF family. These WRKY domains are characterized by the presence of a conserved seven amino acid sequence WRKYGQK at their N-terminal end. Furthermore, the C-terminal end of these proteins also contains an additional characteristic zinc-finger-like DNA binding motif. Both of these motifs contribute significantly to the specific binding of these TFs to the highly conserved cis-acting element called W-box present in the promoters of downstream target genes. The number of these DNA-binding domains (DBDs) that WRKY TFs contain decides how well each of them binds to its target, even though WRKY TFs share a highly conserved W-box. These structural characteristics provide the basis for the division of WRKY proteins into three groups (Eulgem et al. 2000): Group I has 2 WRKY DBDs, whereas Group II contains only

one DBD with different C_2-H_2 (C–$X_{4–5}$–C–$X_{22–23}$–H–X–H) zinc finger element. Group III is comprised of single DBD and C_2-HC (C–X_7–C–X_{23}–H–X–C) zinc finger (Fig. 1). Another uncharacterized group of WRKY proteins i.e. Group IV; is made of incomplete WRKY domain and they also don't have any zinc finger motif (Xie et al. 2005). Further, divisions in group II (IIa, IIb, IIc, IId, and, IIe) are an exception from the other three groups which are monophyletic in nature. This division was made based on primary amino acid sequences and phylogenetic analysis (Rushton et al. 2010; Eulgem et al. 2000). The primary WRKYGQK motif present in the DBD displays certain anomalies such as WKKY, WRMC, WSKY, and WVKY (Villacastin et al. 2021). WKKY and WRMC are only identified in Group IIc proteins whereas WSKY and WVKY are found in Group IIb and III. In addition, they possess leucine zippers, a region rich in serine and threonine, a basic nuclear localization domain, a glutamine-rich region, a kinase domain, a proline-rich region, and a TIR-NBS-LRR domain (Chen et al. 2012; Phukan et al. 2016). Studies on structures of WRKY TFs show that the pre-WRKY structures (Pro-WRKY) likely originated from a single domain. This domain was likely duplicated, leading to the development of group I WRKY TFs. The loss of the N-terminal WRKY domain resulted in the emergence of group IIc members. Group IIc may have subsequently diverged prophylactically to produce additional subgroups within group II, while group III is the most recent and least varied of the three groups (Wu et al. 2017; Song and Gao 2014). The sequence similarity that group II and III WRKY domain share with the C-terminal domain of group I WRKY TFs suggests the emergence of group II and III as the evolutionary result of group I (Chen et al. 2019).

Substitutions in the amino acid sequences of the WRKYGQK domain has also been reported in many plants. For example, maize, banana, populus, mulberry and soybean contain the WRKYGKK; populus and banana contain WRKYGRK; populus contains FWRKYGQK; and rice and banana contain WRKYGEK (Eulgem et al. 2000). Nineteen variants of these WRKY domains have been identified in rice, where WRKYGEK and WRKYGKK are more prevalent than variants like WRICGQK, WSKYEQK, WRMCGQK, WKKYGQK, WIKYGQK, WRKYGQK and WRKYSEK (Zhang and Wang 2005). In some cases, the WRKY motif is also substituted by motifs like WRMC, WIKY, WRIC, WKKY, WVKY, WSKY, etc. (Jiang et al. 2017). WRKY TFs have been studied in several monocot crops, revealing that their distribution across the genome is not uniform and can vary even within individual chromosomes (Xu et al. 2016; Chanwala et al. 2020).

Role of WRKY TFs in response to biotic stress

Biotic stress in plants refers to the negative impact of living organisms such as pathogens, insects, mammals, and weeds on plant growth and productivity. These biotic stressors cause various types of damage to plants, including tissue damage, reduced photosynthesis, and altered nutrient uptake, ultimately leading to decreased plant yield and quality. Plant pathogens, such as bacteria, fungi, and viruses, can cause a range of diseases in plants resulting in visible symptoms such as wilting, necrotic lesions, chlorosis, etc. (Jones and Dangl 2006). Insects, such as aphids, mites, and beetles, can cause damage to plants by feeding on leaves, stems, and fruits (Agrios 2008). Mammals, such as deer, rodents, and rabbits, cause damage to plants by browsing on leaves, stems, and fruits, and trampling plants (Stout et al. 2006). Weeds compete with plants for resources such as water, light, and nutrients, ultimately leading to reduced growth of plants and yield (Booth et al. 2004). Phytopathogens can be classified based on the type of interaction between the plant

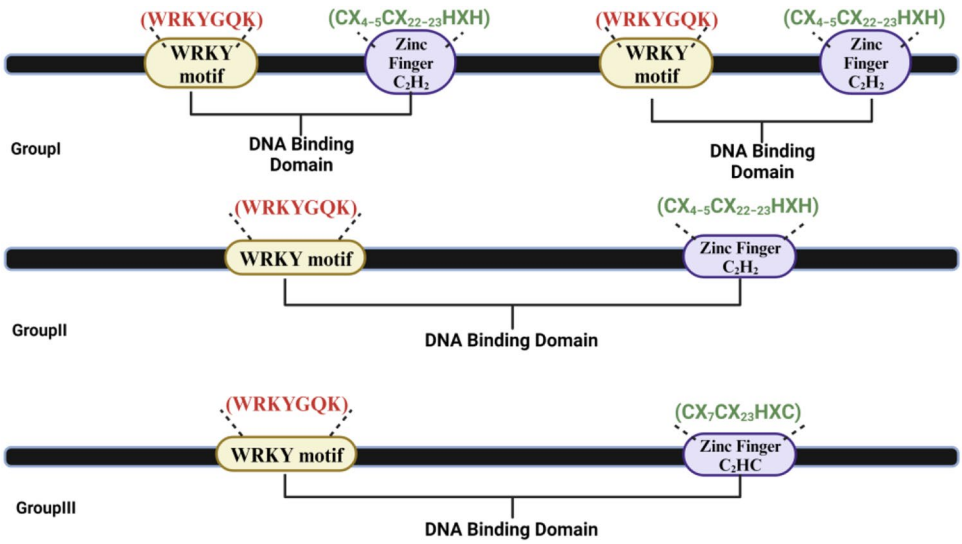

Fig. 1 Schematic diagram of three different groups of WRKY DNA binding domain. The WRKY proteins are divided into three different groups depending upon the number of DNA binding domain and the type of zinc-finger-elements they possess

and the biotic stressor. The three types of interactions are necrotrophic, biotrophic, and hemibiotrophic. In response to pathogen-specific infection, plants have developed various defense mechanisms to encounter the challenges (Sun et al. 2015; Jha et al. 2020).

Among various plant-specific TFs, WRKY TFs represent one of the largest groups that regulate gene expression in response to biotic stress (Fig. 2 and also see Table S1). Upon a specific pathogen attack, the activation of several genes associated with defense mechanisms depends on the type of defense mechanism that is being triggered. (De Vos et al. 2005; Reymond and Farmer 1998). The WRKY TFs play a crucial role in regulating these defense mechanisms by regulating their expression upon pathogen attack. They either activate or repress the downstream target genes by binding specifically to the cis-acting elements present in their promoter (Cai et al. 2008; Rushton et al. 2010). Response of WRKY TFs to biotic stress depends upon the variety of pathogen types. For instance, WRKY33 from *Arabidopsis* is increased in response to fungal necrotroph *Alternaria brassicicola* and *Botrytis cinerea*, which activates defense-related genes such as PR proteins and genes implicated in JA signaling (Zheng et al. 2006). *AtWRKY18* and *AtWRKY40* genes were shown to negatively regulate the defense against the biotrophic pathogen, *Golovinomyces orontii* which causes powdery mildew infection in Arabidopsis (Pandey et al. 2010). Involvement of WRKY genes against hemibiotrophic pathogens has also been noted, for example, *AtWRKY48* negatively affects the basal resistance against *Pseudomonas syringae* (Xing et al. 2008). Understanding the type of interaction between the plant and the biotic stressor is important for developing effective strategies to manage biotic stress in plants. However, the mechanisms by which WRKY TFs regulate the defense response against these pathogens are more complex, as these pathogens have evolved strategies to evade and manipulate plant defense. In some cases, WRKY TFs might play a positive role in the regulation of defense responses, while in other cases, they

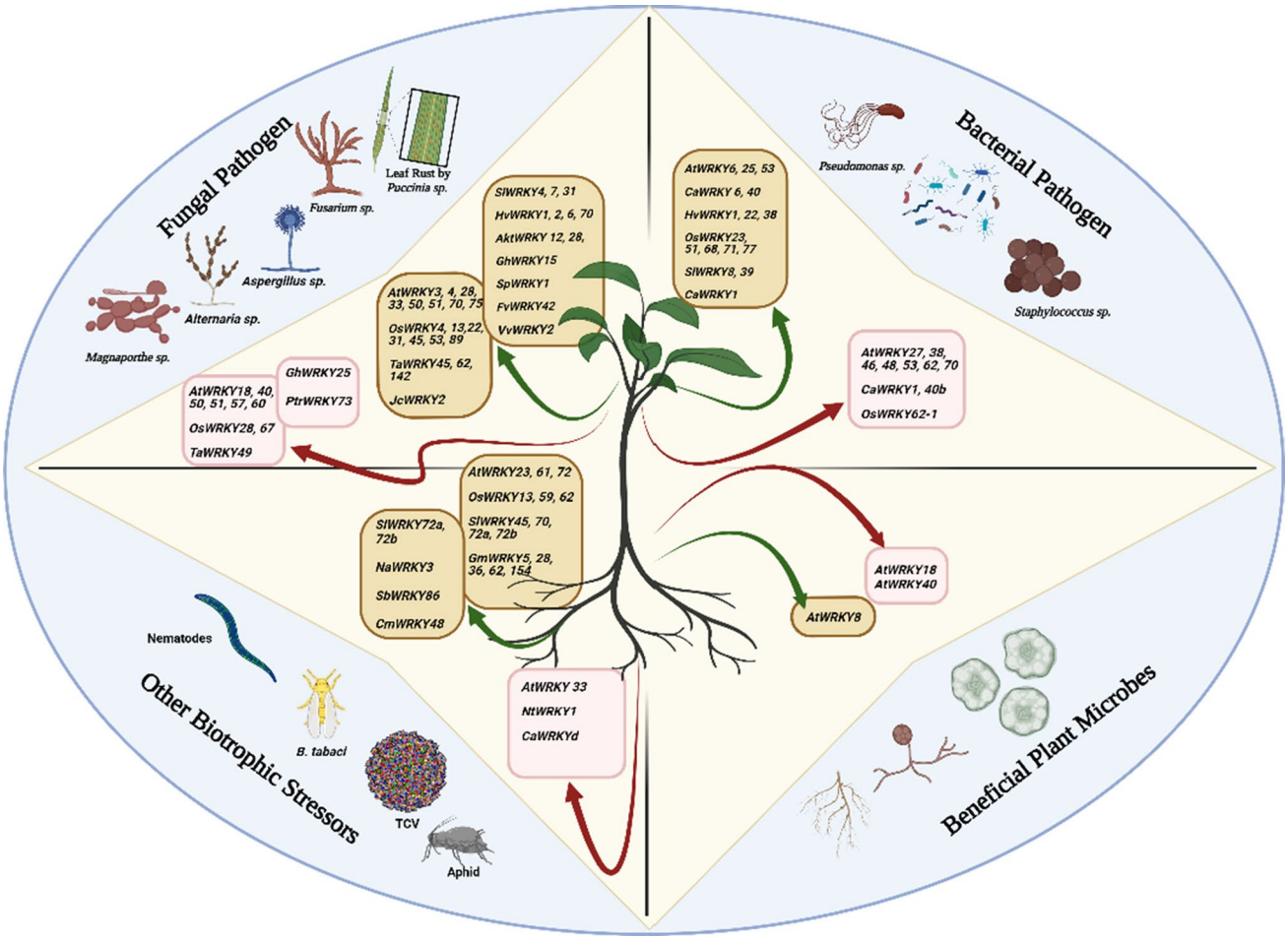

Fig. 2 Involvement of WRKY TFs under different biotic stress conditions. To regulate the defense mechanism against various external stimuli, plants employ different WRKY proteins. This regulation can either be beneficial/positive (indicated by green arrows) or harmful/negative (indicated by red arrows)

might act negatively to avoid excessive defense responses that could harm the plant (Vo et al. 2017; Ifnan Khan et al. 2018). Overall, understanding the role of WRKY TFs in plant-pathogen interactions is critical for developing effective strategies for plant disease management. Considering various plant and pathogen interaction models, we have categorized the function of WRKYs in various species in this section, with a particular focus on Arabidopsis, Rice, Tomato, and Tobacco as well as several other economically significant plants.

WRKY TFs in plant–fungus interaction

Plants are infected mainly by three types of pathogenic fungi based on their mode of infection: biotrophic, necrotrophic, and hemibiotrophic. Necrotrophic fungi acquire nutrition by consuming infected plant tissues, whereas biotrophic fungi depend on living plant cells and tissues to invade a host. Another unique group of pathogenic fungi that infect plants is Hemibiotrophs, which exhibit both biotrophic and necrotrophic phases during pathogenesis. They start with a biotrophic phase before transitioning into a necrotrophic phase (Barna et al. 2012; Spanu and Panstruga 2017). This section compiles data from various sources to discuss the regulation of WRKY-mediated defense against necrotrophic, biotrophic, and hemibiotrophic fungi across several species and the contribution of different WRKYs toward defense signaling against various fungal pathogens.

Necrotrophic fungi

Necrotrophic fungi, obtain nutrients from dead or dying plant tissues and cause extensive damage by killing plant cells. WRKY TFs play a pivotal role in regulating the defense responses against necrotrophic fungi through the transcriptional manipulation of defense-associated genes or through interaction with certain defense-related proteins. The resistance mechanism of JA is shared with necrotrophic fungi and insect pests. Studies have shown that constitutive expression of certain WRKY genes can enhance plant resistance to necrotrophic fungi by activating the expression of defense-related genes participating in the JA and ET signaling pathways (Niu et al. 2011; Martinez-Medina et al. 2013; Kravchuk et al. 2011). While *AtWRKY4* enhances resistance to the necrotrophic pathogen as well as the biotrophic pathogen, *AtWRKY3* enhances resistance to the necrotrophic fungal pathogen *Botrytis cinerea*. Pathogen-induced PR1 is inhibited by *AtWRKY3* and *AtWRKY4* overexpression (Lai et al. 2008). Similarly, *AtWRKY33* functions as a positive regulator for protection against infection with *Alternaria brassicicola* and *B. cinerea* (Zheng et al. 2006). Microarray screening reveals that *AtWRKY28* and *AtWRKY75* are increased after *Sclerotinia sclerotiorum* infection and oxalic acid treatment. The involvement of *AtWRKY28* and *AtWRKY75* in SA and JA/ET-dependent defense signaling pathways leads to increased resistance towards oxalic acid and fungal infection in *Arabidopsis* (Chen et al. 2013b). In *Arabidopsis, AtWRKY57* was found to be associated with suppression of immune response against necrotroph *B. cinerea*. To bind to the promoters of SIB1, SIB2, JAZ1, and JAZ5, *AtWRKY57* and *AtWRKY33* must compete with one another, which alters the JA-mediated defensive signal pathway (Jiang and Yu 2016). The susceptibility against *B. cinerea* has also been found to be increased in overexpressing lines of *AtWRKY18* in combination with *AtWRKY40* or *AtWRKY60* forming homo and heterocomplex. It suggests that they interact together to control plant defense either physically or functionally by regulating the JA and SA pathways (Xu et al. 2006). Studies conducted by Li et al. (2006) have shown that *AtWRKY70* regulates the equilibrium between SA and JA-dependent pathways necessary for R-gene-mediated resistance against necrotroph *Alternaria brassicicola*, where suppression of JA-signaling is accomplished by NPR1 in *Arabidopsis* (Li et al. 2006). Studies have also proven that some WRKYs be involved both as positive and negative regulators in defense, for example, *AtWRKY50 and AtWRKY51* regulate SA- and low oleic acid-induced suppression of JA signaling, which results in improved and reduced performance of the plant against *A. brassicicola* and *B. cinerea*, respectively (Gao et al. 2011). *PtrWRKY73* isolated from Poplar (*Populus trichocarpa*) is the closest homolog of *AtWRKY33. PtrWRKY73* overexpression in *Arabidopsis* in contrast (Zheng et al. 2006) decreases resistance towards fungal necrotroph *B. cinerea* (Duan et al. 2015), which indicates their involvement in plant defense mechanism mediated by SA-dependent pathway. Identification of 42 WRKY genes has been done very recently in *Akebia trifoliata*. Among several WRKYs induced after infection with *Colletotrichum acutatum; AktWRKY03, 12, 28,* and *33* showed evident expression changes in all tested varieties suggesting their involvement in defense against this phytopathogen (Wen et al. 2022). *VvWRKY2* from Grapes (*Vitis vinifera)* when overexpressed in tobacco, reduced susceptibility against the pathogen found in transgenic tobacco (Mzid et al. 2007). Remarkable changes in expression were observed for *GhWRKY15* transcripts upon infection with the *Phytophthora parasitica* spores and conidial suspensions of *Colletotrichum gossypii* in cotton plants (*Gossypium hirsutum*). Constitutive expression of *GhWRKY15* in tobacco exhibits improved resistance for both *P. parasitica* and *C. gossypii* infection (Yu et al. 2012), whereas overexpression of *GhWRKY25* in transgenic tobacco resulted in enhanced susceptibility against *B. cineria* (Liu et al. 2016). Similarly, overexpression of *JcWRKY2* from Jatropha (*Jatropha cucas*) reduces the susceptibility against Collar rot disease due to the pathogen *Macrophomina phaseolina* (Dabi et al. 2020).

In rice, overexpression of *OsWRKY4* results in the enhanced defense response against *Rhizoctonia solani* which causes sheath-blight disease. *R. solani* infection upregulated several pathogenesis-related genes like *PR1a, PR1b, PR5*, and *PR10* in overexpressed plants. (Wang et al. 2015). In response to the necrotrophic pathogen *Sclerotinia sclerotiorum,* the accumulation of 13 WRKY transcripts in Canola (*Brassica napus*) plants was significantly modulated, 10 among them being increased, 2 (*BnWRKY20* and *BnWRKY32*) being decreased and left one initially decreased (12 h after infection) followed by an increase after 72 h. *BnWRKY33* and *BnWRKY75* transcripts accumulation was significantly increased after 48-h post-pathogen challenge with *Alternaria brassicae* (Yang et al. 2009).

Biotrophic fungi

Biotrophic fungi are a group of plant pathogens that rely on living plant tissue to complete their life cycle. These fungi establish intimate relationships with their hosts and are often considered obligate biotrophs because they require a living host to grow and reproduce. Many WRKY genes are often shown to be differentially expressed in plants in response to infection caused by these biotrophic fungi. This indicates that the WRKY TFs are essential for the plant in terms of defense mechanisms against these pathogens. Double and triple mutant lines of *Arabidopsis* like *AtWRKY18/40* and *AtWRKY18/40/60* showed enhanced resistance against the biotroph *Golovinomyces orontii*, which causes powdery mildew disease, although the double mutants do not show constitutive expression of several defense-associated genes (Schon et al. 2013). In contrast, the mutation in the *AtWRKY33* gene in *Arabidopsis* resulted in increased susceptibility in the transgenic lines compared to wild types when infected with *Hyaloperonospora parasitica*. Further experiments showed that *AtWRKY33* binds directly to the promoter of the camalexin biosynthesis gene *CYP71A12* and activates its expression. The researchers also found that overexpression of *AtWRKY33* increases camalexin levels and enhances resistance to *H. parasitica* infection (Lippok et al. 2007). Overall, the study demonstrates that *AtWRKY33* plays a critical role in plant defense against *H. parasitica* by regulating the production of the antimicrobial phytohormone camalexin. Similarly, in rice upregulation of *OsWRKY22* is observed during powdery mildew disease which is caused by *Blumeria graminis*. Overexpression of *OsWRKY22* in rice plants enhances their resistance to *B. graminis* infection, while knockdown of *OsWRKY22* expression leads to increased susceptibility against this fungal pathogen (Abbruscato et al. 2012). Kuki et al. (2020) have shown that during the compatible interaction of wheat leaf with wheat blast fungus (Br-48), the transcript level of four WRKY genes namely *TaWRKY49, 92, 112,* and *142* been significantly increased, with a maximal peak at 3 days after infection. These four WRKYs were found to be upregulated in various abiotic stress treatments including cold, drought, dark, and salinity, etc. To understand the biological function of these genes, transgenic overexpression lines of *Arabidopsis* were created and challenged with the fungal pathogen *Colletotrichum higginsianum.* While wild-type plant leaves showed severe disease symptoms, no symptoms were observed in *TaWRKY142* overexpressing lines. This result suggests that the expression of *TaWRKY142* conferred resistance against *C. higginsianum* (Kuki et al. 2020). In a recent study on wheat, RNA-Seq analysis revealed that the expression of two WRKY genes (*TaWRKY49* and *TaWRKY62*) differed in response to high-temperature seedling-plant resilience to stripe rust (*Puccinia striiformis* f. sp. *tritici*). To validate the RNA-Seq results, the researchers conducted gene silencing experiments and found out that *TaWRKY62*-silencing reduces and *TaWRKY49*-silencing improves resistance against *P striiformis*. The study projected that *TaWRKY49* and *TaWRKY62* played negative and positive regulatory roles, respectively, by differentially regulating SA, JA, ethylene, and ROS pathways in high-temperature seedling-plant resistance to Pst (HTSP) (Wang et al. 2017). During powdery mildew infection, the expression of *FvWRKY42* from woodland strawberries (*Fragaria vesca*) was observed to increase in the transgenic overexpression lines of *Arabidopsis* using the 35S:FvWRKY42-YFP construct. This overexpression in *Arabidopsis* improves resistance against powdery mildew with higher PR1 expression compared to wild-type plants (Wei et al. 2018). Cloning and overexpression of *SpWRKY1* from wild tomatoes (*Solanum pimpinellifolium*) and in cultivated tomatoes (*Solanum lycopersicum*), resulted in enhanced resistance to *Phytophthora infestans*. This effect was achieved by regulating the expression of abscisic acid (ABA) biosynthesis genes (Li et al. 2015a). In a distinct experiment, the transformation of tobacco plants with *SpWRKY1* resulted in decreased malondialdehyde accumulation and relative electrolyte leakage, additionally increased activity of antioxidant enzymes for example peroxidase (POD) and superoxide dismutase (SOD), and phenylalanine ammonia-lyase (PAL). These findings suggest an increased resistance of the overexpression lines against *Phytophthora nicotianae* (Li et al. 2015b). *Hordeum vulgare*, commonly known as barley, exhibits isolate-specific resistance to the powdery mildew caused by *Blumeria graminis* by utilizing intracellular mildew resistance protein A (MLA). Researchers have demonstrated a physical interaction between MLA and HvWRKY1 and -2, which are two repressors of PAMP-triggered basal defense in the nucleus. This interaction interferes with the functions of WRKY repressors, resulting in resistance against the powdery mildew fungus (Shen et al. 2007). Two WRKY transcription factors from Barley, *HvWRKY6,* and *70* overexpressed in

transgenic wheat resulted in an increased level of resistance against the pathotype CYR32 of *Puccinia striiformis* f. sp. *tritici* and pathotype E20 of *B. graminis* f. sp. *tritici*. (Li et al. 2020a).

Hemibiotrophic fungi

Hemibiotrophic fungi are a group of plant pathogens that initially behave as biotrophs, living in close association with living plant cells, before switching to a necrotrophic phase and killing plant tissues. Several studies have established the involvement of WRKY TFs in defense responses against various fungal pathogens that come in this group. Overexpression of *OsWRKY13* has shown enhanced resistance against the fungus *Magnaporthe grisea* causing blast disease in rice by turning on the genes for SA-biosynthesis and SA responses while deactivating JA signaling (Qiu et al. 2007, 2008a). *OsWRKY13* is in turn also regulated by two alleles of another WRKY TF from rice, i.e., *OsWRKY41-1* and *45-2* found in *Japonica* and *Indica* varieties, respectively (Tao et al. 2009; Cheng et al. 2015). A similar result was found by Chujo et al. (2007), where overexpression lines of *OsWRKY53* showed increased resistance to *M. grisea*. When compared to transgenic rice plants overexpressing native *OsWRKY53*, those overexpressing a phosphomimic mutant of *OsWRKY53* (*OsWRKY53SD*) exhibited an even greater resistance to the blast fungus (Chujo et al. 2007). Furthermore, the *OsWRKY53SD*-overexpressing plants displayed significantly higher upregulation of the genes involved in defense, together with *PR* genes, compared to the *OsWRKY53*-overexpressing plants proving that the function of the WRKY gene is caused by its modified state (Chujo et al. 2014). *OsWRKY45* overexpressing lines showed increased resistance to *M. grisea* in the experiment, however in that same experiment, plants overexpressing *OsWRKY19, -62*, and *-76* did not. In this instance, *OsWRKY45* knockdown lines reduced the host's ability to withstand the fungal invasion. *OsWRKY45* seems to function independently of *NH1* (Rice homolog of *Arabidopsis* NPR1) in SA signaling (Shimono et al. 2007). The orthologous protein of *OsWRKY45* found in wheat, *TaWRKY45* was found to be involved in the defense against *Fusarium* head blight disease caused by *Fusarium graminearum.* Overexpression of *TaWRKY45* showed enhanced defense against the disease pathogen in transgenic wheat plants (Bahrini et al. 2011). The overexpression of *OsWRKY31* led to an elevated resistance against fungal blast pathogens. In addition, it caused changes in lateral root formation and induced the expression of two early auxin-responsive genes (Zhang et al. 2008). *OsWRKY67* activation by T-DNA tagging has greatly enhanced the resistance against the fungal pathogen *Magnaporthe oryzae* (Vo et al. 2017). Positive regulation of *OsWRKY89* has been reported for resistance against fungal blast by Wang et al. (2007), whereas negative regulation of *OsWRKY28* was reported against the fungal blast pathogen by overexpressing the gene (Wang et al. 2007; Chujo et al. 2013). An *in-silico* microarray study conducted on tomato plants against *Fusarium oxysporum* f. sp. *Lycopersici* infection revealed differential gene expression of *SlWRKY4*, *SlWRKY33*, and *SlWRKY37* (Aamir et al. 2018). Significant upregulation of three WRKY transcripts i.e., *SlWRKY4, 31*, and 7 have been found after 96 h of post-inoculation with the fungal pathogen *Fusarium solani*. Overexpression of the WRKY was found to be beneficial for the plants against *F. solani* (Abd-Ellatif et al. 2022).

WRKY TFs in plant–bacterium interaction

Plant-bacterium interactions involve complex mechanisms of recognition and response, with both the plant and the bacterium employing various strategies to gain an advantage over one another. The members of the WRKY TFs family play a crucial role in fine-tuning the defense response of plants against bacterial pathogens. This WRKY protein regulates the efficiency of gene transcription of downstream genes associated with the biosynthesis of signaling molecules and other defense-related processes, thus serving as a significant mechanism utilized by plants to protect themselves against bacterial pathogens. A negative impact on plant immunity of *AtWRKY27* has been identified by Mukhtar et al. (2008), in response to the necrotrophic bacteria *Ralstonia solanacearum, AtWRKY27* knockout transgenic lines showed delayed symptom development with a reduced expression of *PR* genes (Mukhtar et al. 2008). *CaWRKY40b* in pepper (*Capsicum annuum*) is another example of how WRKY genes negatively influence plant immunity. It controls a group of defense-related genes under *Ralstonia solanacearum* infection. Silencing of *CaWRKY40b* with the help of the Virus Inducing Gene Silencing (VIGS) method has resulted in reduced susceptibility of the plants against *R. solanacearum.* Transient overexpression of the chimeric repressor version of *CaWRKY40b* (*CaWRKY40b-SRDX*) increased susceptibility to the pathogen, while overexpression of *CaWRKY40b* had the opposite effect of decreasing susceptibility (Ifnan Khan et al. 2018). Additionally, it has been noted that increased *CaWRKY40* transcript levels during *R. solanacearum* infection activate the JA, SA, and ethylene-mediated pathways. Overexpression of *CaWRKY40* controls genes related to pathogenesis and the hypersensitive response (HR), and provides resistance to *R. solanacearum* (Dang et al. 2013). This event demonstrates the negative regulation of *CaWRKY40b* by modifying the defense-associated gene *CaWRKY40.* Furthermore, *CaWRKY6* triggers *CaWRKY40* to positively modulate the resistance of *R. solanacearum* (Cai et al. 2015). In peanut plants (*Arachis hypogea*) out of 174 identified WRKY

genes, *AhWRKY76* and 77 were found to be targeted by ahymiR3512, which might be involved in peanut disease defense response towards the pathogen *R. solanacearum* (Yan et al. 2022). In rice, *OsWRKY51* enhances the resistance against the biotrophic pathogen *Xanthomonas oryzae* by activating the defense-related gene *OsPR10a,* through binding to its promoter cis-element W-box and WLE1 (Hwang et al. 2016). In rice, the *Xa21* gene provides resistance against *Xoo*, by recognizing their Juxtra Membrane (JM) region *OsWRKY62* interacts with *Xa21*. Furthermore, the overexpression of the splice variant *OsWRKY62-1* was found to reduce basal resistance against *Xoo*, along with the suppression of stimulation of genes involved in defense (Peng et al. 2008). *OsWRKY68*, another WRKY gene in rice, controls *Xa21*-mediated plant disease resistance against *Xoo* by interacting with the W-boxes present in the *PR1b* cis-element region, leading to the activation of the gene (Yang et al. 2016). Similarly, *OsWRKY71* has also enhanced the resistance against the bacterial pathogen *Xoo* (Liu et al. 2007). Upon hemibiotrophic pathogen *Pseudomonas syringae* infection*,* numerous WRKY genes have shown remarkable changes in their expression in *Arabidopsis*. The overexpression of *AtWRKY48* resulted in the downregulation of *PR1* expression, which indicates that negative regulation of PR genes mediated by *AtWRKY48* can suppress plant immunity during infection (Xing et al. 2008). *AtWRKY38* and *AtWRKY62* adversely affect the basal resistance against the bacterial pathogen (Kim et al. 2008a). In overexpression lines, *AtWRKY62* interacts with *HDA19* (*Histone Deacetylase 19*) protein leading to their disruption, which results in compromised resistance during pathogen attack (Kim et al. 2008a). The *atwrky46* single mutant line shows enhanced PR1 gene expression, which is greater in *atwrky46-atwrky53* and *atwrky46-atwrky70* double mutants. Conversely, *AtWRKY46*, *AtWRKY53*, and *AtWRKY70* exhibit functional redundancy and work together to enhance the immune response. The transcript level of *AtWRKY46* is triggered by SA and *P. syringae*. Double-knockout mutants of the combination of *atwrky46-atwrky53* or *atwrky46-atwrky70,* as well as the knockout of three genes, were studied mutant *atwrky46-atwrky53-atwrky70*, exhibit increased sensitivity to *P. syringae* and reduced expression of the PR1 gene (Hu et al. 2012). Interestingly *AtWRKY53* was found to have dual roles in defense signaling. The *atwrky53* mutants exhibited delayed symptom development when infected with *R. solanacearum*. However, these same mutants showed heightened susceptibility to *P. syringae* (Murray et al. 2007). Likewise, *AtWRKY25* overexpression Showed increased disease symptoms in *P. syringae* infections while the *atwrky25* mutant showed normal growth of the pathogen (Zheng et al. 2007). The knockdown mutant of *AtWRKY6* demonstrated a larger infection area on its leaves compared to the wild type, indicating the involvement of *AtWRKY6* in regulating a particular cell layer around the infected area against the virulent strain of *P. syringae pv.* tomato *DC3000* (Robatzek and Somssich 2002). In rice, a series of PR genes were found to be activated by the overexpression of *OsWRKY23* against *Pseudomonas syringae* (Jing et al. 2009). *OsWRKY77,* overexpressed in *Arabidopsis* resulted in better resistance against *P. syringae* associated with heightened expression of the genes involved in defense, namely *PR-1*, *PR-2*, and *PR-5* (Lan et al. 2013). In tomatoes, overexpression of *SlWRKY8* and *39* provided enhanced resistance toward the biotrophic pathogen *P. syringae (*Gao et al. 2020; Sun et al. 2015*).* In barley (*Hordeum vulgare*) bacteria-induced systemic immunity was found to be linked with the local and/or systemic induction of *HvWRKY22*, and *HvWRKY38/1* gene transcript (Dey et al. 2014) against both *Pseudomonas syringae* and *Xanthomonas translucens*. *CaWRKY1* from *Capsicum annuum* was induced strongly when challenged with *P. syringae, Xanthomonas axonopodis pv vesicatoria,* and signaling molecule SA. The silencing of *CaWRKY1* using VIGS resulted in the reduced growth rate of *Xanthomonas axonopodis* (Oh et al. 2008).

WRKY TFs in beneficial plant–microbes interaction

Recent studies have also suggested that WRKY proteins can play a role in beneficial plant/microbe interactions (Table 1), such as those that occur between plants and mycorrhizal

Table 1 List of WRKY TFs involved in plant interaction with different beneficial microbes

Group	WRKYs	Phytozome Identifier/ TAIR gene symbol	Regulation	Pathogen	References
I	*AtWRKY33*	AT2G38470.1	✓	*T. atroviridae*	Sáenz-Mata et al. (2014)
IIa	*AtWRKY18*	AT4G31800.1	–	*T. asperelloides*	Brotman et al. (2013)
	AtWRKY40	AT1G80840.1	–	*T. asperelloides*	Brotman et al. (2013)
IIc	*AtWRKY8*	AT5G46350.1	+	*T. atroviridae*	Sáenz-Mata et al. (2014)
	AtWRKY57	AT1G69310.1	✓	*T. atroviridae*	Sáenz-Mata et al. (2014)

+: Indicates positive regulation in defense signaling

✓: Indicates involvement in defense signaling, regulation unknown

−: Indicates negative regulation in defense signaling

fungi. Mycorrhizal fungi and plant roots form a mutualistic relationship where fungi obtain carbon sources from plants in exchange for phosphorus and nitrogen. After the successful establishment of the arbuscular mycorrhizal association, significant changes occur in the root morphology and transcriptome at different stages of root colonization, including pre-, early-, and late-stage colonization. Recent research has shown the involvement of WRKY proteins in the transcriptional regulation of genes involved at the initial stage of mycorrhizal colonization. It was observed that 9 WRKY genes were upregulated in the pre-colonization phase whereas one WRKY gene was upregulated during the later stage of colonization of potato roots with *Glomus interadices* (Gallou et al. 2012). *Trichoderma spp*., which are plant symbionts, colonize the apoplast of plant roots. Root transcriptome microarray analysis has shown enhanced expression of *AtWRKY18 and 40*, which stimulate the JA-signaling by suppressing JAZ repressor and regulates the expression of defense genes *FMO1* (flavin mono-oxygenase 1)*, PAD3,* (phytoalexin deficient 3) and *CYP71A13* (cytochrome P450 family 71 polypeptides) during *Trichoderma asperelloides* T203 colonization (Brotman et al. 2013). The relationship between *Arabidopsis* and *Trichoderma* has evolved into a paradigm for studying advantageous plant–microbe interactions (Contreras-Cornejo et al. 2009; Lorito et al. 2010; Shoresh et al. 2010). According to the microarray results, eight WRKY TFs namely *AtWRKY8, 33, 38, 42, 54, 57, 60,* and *70* have shown significant differential expression patterns upon interaction of *Arabidopsis* with *Trichoderma atroviride.* The transcript level of *AtWRKY8* is upregulated by 9.4-fold during the early stages of interaction. In contrast, the *AtWRKY33* and *AtWRKY57* gene's expression was considerably suppressed in the early stages of the interaction, however, a modest rise was seen after 96 hpi (Sáenz-Mata et al. 2014). Microarray analysis of the plants treated with *T. harzianum* T34 revealed extensive alterations in *AtWRKY54* gene expression in the aerial region with a reduction of around 2.02 fold (Moran-Diez et al. 2012).

WRKY TFs interaction with nematodes, viruses and insects

WRKY proteins are also very well known for playing a substantial role in plant defense against pests and diseases, including nematodes, aphids, viruses, and insects (Table 2). Nematodes are one of the most damaging plant pathogens, causing severe yield losses worldwide. WRKY TFs are known to be intricate in regulating the plant's defense response against nematodes. For instance, knockdown of the *WRKY23* gene has been shown to confer reduced resistance to parasitic nematodes *Heterodera schachti* in *Arabidopsis thaliana*. During the early stage of feeding site establishment, *WRKY23* expression was induced (Grunewald et al. 2008). Similarly, in *Solanum lycopersicum* knockdown of *SlWRKY72a* and *SlWRKY72b* gene showed lower *Mi-1* (*R*-gene from tomato) mediated resistance and basal defense against root-knot nematodes (RKN) *Meloidogyne incognita*, which also has been confirmed with *Arabidopsis* with the T-DNA insertion mutants, in *Arabidopsis* ortholog, *AtWRKY72*, upon same pathogen infestation (Bhattarai et al. 2010). Roots of transgenic plants overexpressing SlWRKY45 result in an increase in the development of giant cells upon infection by the nematode *Meloidogyne javanica* (Chinnapandi et al. 2017). High resistance to the soybean cyst nematode has been observed in overexpression lines of around 30 WRKY genes in soybeans (*Glycine max*). Five among them (*GmWRKY154, 62, 36, 28, and 5*) showed remarkably increased resistance with more than a 70% reduction in cyst numbers (Yang et al. 2017). In rice infection with RKN *M. graminicola* resulted in significant upregulation of three WRKY transcript encoding *OsWRKY62, 59,* and *13* (Nguyễn et al. 2014; Kyndt et al. 2012).

Aphids are sap-sucking insects that cause significant damage to crops by reducing plant growth, transmitting viruses, and inducing plant deformities. Numerous WRKY TFs have been described to be engaged in the plant's defense response against aphids. For instance, significant upregulation of *CmWRKY48* (around seven-fold after three hours of infestation) was observed against *Macrosiphoniella sanborni* aphid infestation in *Chrysanthemum morifolium* transgenic plants. Overexpression of *CmWRKY48* inhibited the reproductive capacity (Li et al. 2015c). In tomatoes, *SlWRKY70* was found to regulate the defense response against aphid infestation. During an infestation, *SlWRKY70 was* found to be significantly upregulated while silencing made the plants more susceptible to aphid infestation, which occurred along with decreased expression of defense-associated genes (Atamian et al. 2012). Sorghum plants, overexpressing the *SbWRKY86* gene exhibited increased resistance to *Melanaphis sacchari*, as evidenced by reduced aphid survival and fecundity on the plants. Additional analysis revealed that *SbWRKY86* plays a crucial role in regulating the expression of genes involved in the plant's defense response to aphids, including the biosynthetic genes of defense compounds including flavonoids (Poosapati et al. 2022).

Viruses are also major pathogens that cause significant damage to crops worldwide. In tobacco plants suppression of *NtWRKY1* by tobacco rattle virus (TRV)- induced gene silencing resulted in reduced MMDaV (mulberry mosaic dwarf-associated virus)—RepA-induced cell death (Sun et al. 2022). The involvement of HRR3 (WRKY-like TF) is reported in the early phase of hypersensitive response (HR) upon Tobacco Mosaic Virus (TMV) infection (Yoda et al. 2002). Similarly in *Capsicum annum*, silencing of the *CaWRKYd* gene results in reduced HR lesions caused by the infection of TMV (Huh et al. 2012). It was also observed that

Table 2 List of WRKY TFs involved in plant interaction with nematodes, viruses and insects

Group	WRKYs	Phytozome identifier/ TAIR gene symbol	Regulation	Type of pathogen	Organism	References
I	*AtWRKY33*	AT2G38470.1	−	Insect	White fly	Wang et al. (2019)
	GmWRKY5	Glyma.01G128100	+	Nematode	SCN	Yang et al. (2017)
	GmWRKY62	Glyma.18G056600	+	Nematode	SCN	Yang et al. (2017)
	NtWRKY1	XP_016482656.1	−	Virus	MMDAV, TRV	Sun et al. (2022)
	NaWRKY3	AAS13439.1	+	Herbivore	*M. sexta*	Skibbe et al. (2008)
	SbWRKY86	SOBIC.009G238200.1	+	Aphid	*M. sacchari*	Poosapati et al. (2022)
IIa	*OsWRKY62*	LOC_Os09g25070.2	+	Nematode	*M. graminicola*	Nguyễn et al. (2014) and Kyndt et al. (2012)
IIb	*AtWRKY61*	AT1G18860.1	+	Virus	TCV	Gao et al. (2016)
	SlWRKY72a	Solyc02g067430.2.1	−	Nematode	*M. incognita*	Bhattarai et al. (2010)
	SlWRKY72b	Solyc02g067430.2.1	−	Nematode	*M. incognita*	Bhattarai et al. (2010)
	GmWRKY36	Glyma.13G310100	+	Nematode	SCN	Yang et al. (2017)
IIc	*AtWRKY23*	AT2G47260.1	−	Nematode	*H. schachti*	Grunewald et al. (2008)
	OsWRKY72	LOC_Os11g29870.1	✓	Insect	White backed planthopper	Khan et al. (2022)
	OsWRKY59	LOC_Os01g51690.1	+	Nematode	*M. graminicola*	Nguyễn et al. (2014) and Kyndt et al. (2012)
	SlWRKY45	Solyc02g094270.1.1	+	Nematode	*M. javanica*	Chinnapandi et al. (2017)
	GmWRKY28	Glyma.01G056800	+	Nematode	SCN	Yang et al. (2017)
	NtWRKY10	XP_016458903.1	+	Insect	White fly	Yao et al. (2020)
	CmWRKY48	AJF11724.1	+	Aphid	*M. sanbourni*	Li et al. (2015)
IIe	*OsWRKY13*	LOC_Os01g54600.1	+	Nematode	*M. graminicola*	Nguyễn et al. (2014) and Kyndt et al. (2012)
	GmWRKY154	Glyma.15G135600	+	Nematode	SCN	Yang et al. (2017)
III	*SlWRKY70*	Solyc03g095770.2.1	+	Aphid		Atamian et al. (2012)
	NtWRKY4	XP_016459189.1	+	Insect	White fly	Yao et al. (2020)
	NtWRKY6	XP_016436463.1	+	Insect	White fly	Yao et al. (2020)

+: Indicates positive regulation in defense signaling

✓: Indicates involvement in defense signaling, regulation unknown

−: Indicates negative regulation in defense signaling

many genes involved in defense like *CaBPR1, CaDEF1,* and *CaPR10* were downregulated in *CaWRKYd*-silenced plants. In *Arabidopsis,* overexpression of *AtWRKY61* resulted in reduced symptoms of infection due to Turnip Crinkle Virus (TCV) in comparison to both wild-type and knockout mutants (Gao et al. 2016).

Insects, such as caterpillars and beetles, are major pests that cause significant damage to crops worldwide. Some studies have investigated the WRKY TF's role in regulating plant response to insect attacks. When cotton plants were challenged with the whitefly complex insect, the expression levels of six WRKY genes were significantly altered (*Bemisia tabaci*); further analysis has established that *GhWRKY40* was one of the key regulators in defense response towards whitefly infestation (Li et al. 2016). Negative modulation of plant defense by *AtWRKY33* was reported in *Arabidopsis* against the same insect attack (Wang et al. 2019). Significant upregulation in the expression of three different WRKY genes from tobacco plants; *NtWRKY4, 6,* and *10* was reported after 72 h of whitefly infestation. Survival of the female flies was not affected by either overexpressing or silencing these genes. However, a significant reduction in the number of eggs laid by the females was observed in the overexpressing plants, while there was an increase in the number observed in the silenced plants compared with control plants (Yao et al. 2020). Upregulation of two WRKY genes, namely *NaWRKY3* and *6* in native tobacco (*N. attenuata*) plants, were identified during herbivory attack due to *Manduca sexta*. Although overexpression of these two genes did not provide any defense phenotype, the silencing of the same genes has increased the susceptibility severely (Skibbe et al. 2008b). In response to white-backed planthopper infestation in rice plants, several *WRKY* gene expression changes have been reported. Among them, *OsWRKY50, 62, 104, 75,* and *52* were found to be upregulated significantly whereas *OsWRKY79* and *116* were downregulated initially but after 3 h they also started to upregulate (Khan et al. 2022).

In summary, WRKY transcription factors have a critical function in regulating the plant's defense response against a range of pests and diseases, such as nematodes, viruses, and both phloem-feeding and chewing insects. Their role in plant defense makes them a promising target for developing pest and disease-resistant crops.

Structural feature of WRKY TFs involved in plant defense

Improved knowledge about the structural features and functions of WRKY TFs is crucial for unraveling the complex defense network of plants. The classification of WRKY TFs is based on their conservation of associated peptide motifs and their evolutionary relationship (Eulgem et al. 2000; Xie et al. 2005; Zhang and Wang 2005). The structure of the WRKY domain was first reported in *Arabidopsis WRKY4* using a computational approach (Yamasaki et al. 2005). While no topological information is available for subgroup-specific motifs, some structural hallmarks have been associated with defined molecular or biological functions. Certain group I WRKY TFs have a conserved "D motif" at their N-termini which can play a role in defense signaling after being phosphorylated by MAP-kinases. *AtWRKY25* and *AtWRKY33* are examples of such WRKYs that have been reported to be phosphorylated *in-vitro* by an SA repressive MAP-kinase MPK4. These WRKYs do not directly interact with MPK4, but rather associate using a coupling factor MKS1 that is localized in the nucleus (Andreasson et al. 2005). The conserved pattern of the 'Ser-Pro' dimer is one of the notable features of 'D-motif', which is a preferential site for MAP-kinase phosphorylation (Davis 1993).

The members of group IIa WRKY proteins of *Arabidopsis* either homodimerize or heterodimerize within themselves using the N-terminal leucine zipper motif for efficient transcriptional activity. For instance, a group IIa member *AtWRKY18* in combination with *AtWRKY40* or *AtWRKY60* forms homo and heterocomplex to respond against interaction with *B. cinerea* (Xu et al. 2006). This combinatorial dimer-forming ability can regulate the plant defense both positively (Wang et al. 2006) and negatively (Xu et al. 2006).

WRKY transcription factors (TFs) recognize and bind to a specific DNA sequence called the W-box, which has a consensus sequence of TTGAC-C/T to control the expression of target genes. The nucleotide sequences present on either side of W-box determine the binding affinity of WRKY TFs to it (Maeo et al. 2001; Rushton et al. 1995; Rinerson et al. 2015). *AtWRKY11,* the member of the WRKY superfamily, specifically a group IId member, has been reported to bind to the eleventh and second W-box sequences of the senescence-induced receptor-like kinase (*AtSIRK*) promoter, while another group I member namely *AtWRKY26* binds to the eighth W-box of the same promoter (Ciolkowski et al. 2008). The event indicates that the surrounding region of the W-box sequence strongly influences the DNA-binding preferences of related WRKY transcription factors, impacting their recognition of specific sequences in a profile of sequence recognition.

The conserved "C motif" found among subgroup IId WRKY TFs has been recognized as calmodulin (CaM) -binding domain and hence may function as Ca^{2+} sensors, and react to rapid Ca^{2+} influxes induced by pathogens. For example, in *AtWRKY7,* a group IId WRKY TF contains a CaM binding domain (DxxVxKFKxVISLLxxxR) that may enhance their DNA affinity (Park et al. 2005). The functions of IId WRKY TFs in the modulation of gene expression remain unresolved, but they have been reported to regulate the defense response negatively by either directly inhibiting transcription or indirectly activating an unknown defense suppressor (Kim et al. 2006).

To identify if there is any correlation of a particular group of WRKY proteins involved in a specific plant-pathogen interaction, we have distributed the reported WRKY sequences according to their groups (Table S1). Further, to see the functional relevance with sequence homology amongst the WRKY members of the same group, WRKY sequences were aligned using MUSCLE software with default parameters and imported to MEGA v7.0 to construct an evolutionary relationship tree using Maximum likelihood method with 1000 bootstrap replications. All WRKY proteins were clustered across the major clades and specific groups (I, II, III) of WRKYs were found to be present in the same clades (Fig. 3). This phylogenetic analysis supports the groupwise classification of the WRKY proteins; however, regardless to group wise specific clustering, WRKYs are involved with various type of plant-pathogen interaction (Table S1) irrespective of their evolutionary relationship. However, we have also observed that in the case of plant bacterium interaction group II WRKYs are involved mostly in all three modes of trophism i.e., necrotrophic, biotrophic, and hemibiotrophic.

WRKY in hormone signaling

WRKY TFs play a crucial role in governing the stress and growth processes of plants as discussed in earlier sections. These multifaceted biological functions of WRKY TFs are executed independently or in synergistic coordination with other stress-responsive TFs such as NAC, MYB, WRKY, etc., and their interplay with various phytohormones integrating the environmental and developmental signals (Srivastava and Sahoo 2021, 2022). For the past two decades, extensive genome-wide, functional, and comparative transcriptome studies in various crop species have documented the involvement of WRKYs in both biotic and abiotic stresses that are integrated by hormone signaling

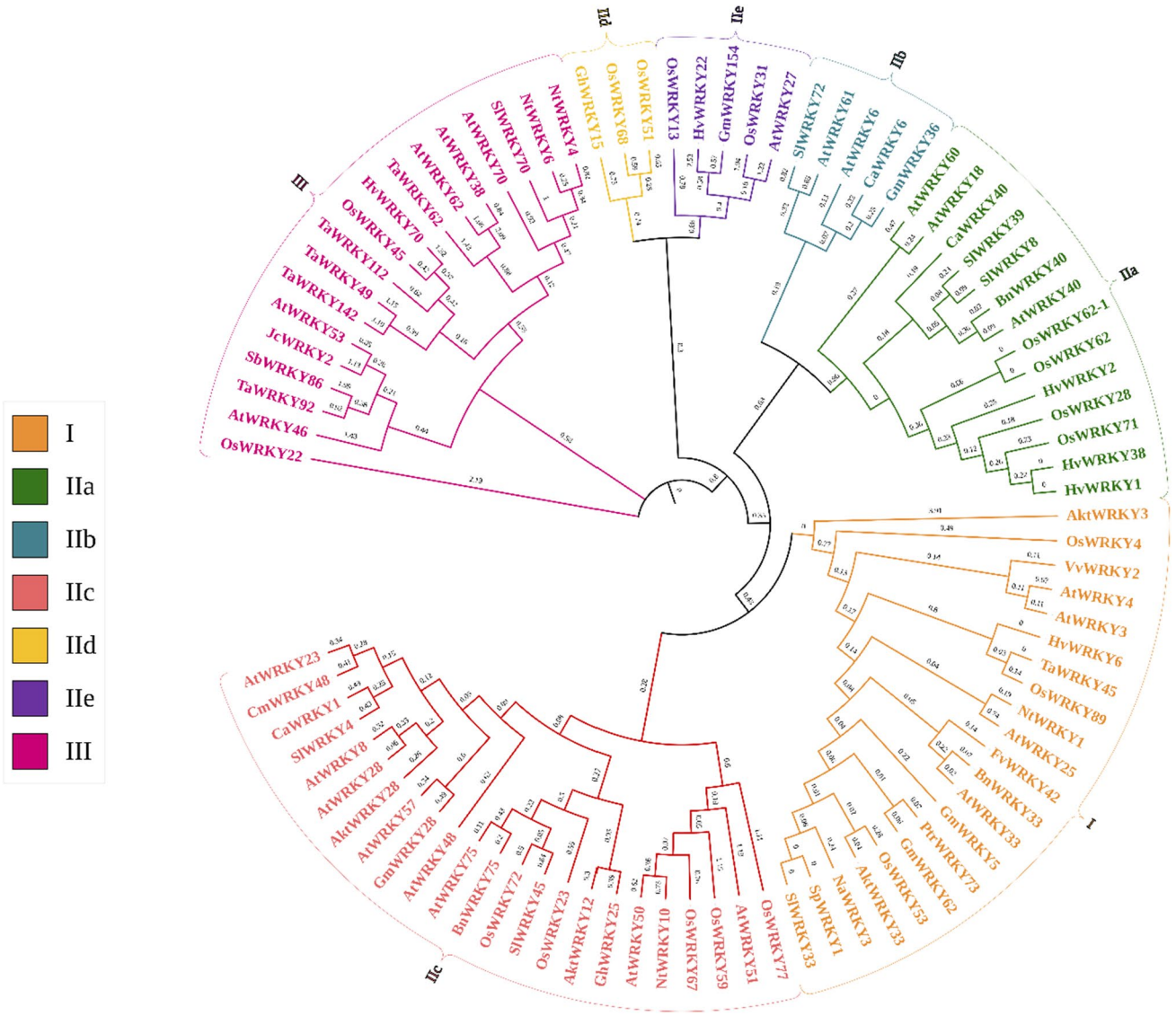

Fig. 3 The circular phylogenetic representation of WRKY proteins from different species. Sequences were aligned using MUSCLE, and a phylogenetic tree was constructed by MEGA v7.0 using the Maximum likelihood method with 1000 bootstrap replications. Each color indicates an individual group (I–III) of ancestral relationship

(SA, JA, ethylene, ABA) and their crosstalk (Yang et al. 2009; Li and Luan 2014; Nuruzzaman et al. 2016; Lui et al. 2017; Srivastava et al. 2018; Fan et al. 2018). Endogenous levels or exogenous applications of hormones change the target WRKY gene's expression in response to stress (Grunewald et al. 2012a; Dang et al. 2013). Indeed, WRKY TFs control downstream hormone signaling and metabolic pathways forming a transcriptional feedback loop of hormonal pathways centered around WRKY proteins (Chinnapandi et al. 2017; Hu et al. 2018; Kang et al. 2020; Singh et al. 2020). The fact that WRKY TFs are directly associated with proteins involved in resistance and stress response highlights the significance of WRKY TFs in plant signaling networks.

Ethylene, SA, and JA signaling

WRKY TFs combine ethylene responses with signals related to growth and development such as lateral root development, leaf senescence, shade avoidance response, etc. (Hu et al. 2018; Yu et al. 2021; Rosado et al. 2022). Furthermore, ethylene level and ethylene signaling pathway are also required for efficient transcriptional reprogramming to potentiate plant immune response towards pathogens. For instance, in *Arabidopsis*, WRKY8 restricts systemic migration of crucifer infecting tobacco mosaic virus (TMVcg) using ABA/ethylene crosstalk. Mutation in *WRKY8* facilitated systemic leaf infection via repression of ABA insensitive 4 (*ABI4*) and induction of 1-aminocyclopropane-1-carboxylic

acid synthase 6 (*ACS6*) and ethylene response factor 104 (*ERF104*) (Chen et al. 2013a). Exogenous application of ABA and *ACS6* inhibited TMVcg accumulation in the infected leaves. Also, systemic immunity activated by the bacterial infection in the monocotyledonous plant, barley (*Hordeum vulgare*), is associated with ethylene-dependent WRKY signaling, unlike the SA-mediated NPR1 gene in *Arabidopsis* (Dey et al. 2014). However, the coordination of ethylene and stress hormones SA and JA is crucial for defense against *R. solanacearum*. Similarly, heat shock tolerance in tobacco is enhanced by the expression of *CaWRKY40*, a WRKY gene identified from pepper (Dang et al. 2013).

Auxin and cytokinin signaling

WRKY TFs are the common component interfacing auxin and cytokinin signal transduction, transport, and plant immunity. Overexpression of the *OsWRKY31* improved resistance towards fungal pathogen *Magnaporthe grisea* which causes Rice blast disease, and the lateral root formation is decreased by modifying auxin transport (Zhang et al. 2008). In tomatoes, auxin/cytokinin-induced *SlWRKY45* supports faster development of root-knot nematode *Meloidogyne javanica* by suppressing SA/JA markers genes by favoring hormonal signals for nematode invasion (Chinnapandi et al. 2017).

Regulation of WRKY TFs

Auto-regulation and cross-regulation

Transducing external stimuli into intracellular signals in response to external stress factors employed in both abiotic and biotic stress, transcription factors work to activate defense-related target genes through particular hormone signaling pathways and gene expression cascade. The idea that WRKY proteins play a crucial role in stress responses necessitates comprehensive regulation of the signaling pathway. In response to both internal and external stimuli, there is a transcriptional upregulation of stress-responsive genes resulting from the binding of the WRKY proteins to the *cis-acting* W-box elements of promoter sequences. WRKY proteins control this expression on their own (auto-regulation) or with the help of other WRKY TFs (cross-regulation) (Rushton et al. 2010). Promoters of WRKY genes comprised of numerous W-boxes (TTTGAC/T) modulate various pathways associated with stress signaling using auto or cross-regulation (Rushton et al. 2010; Dong et al. 2003). WRKY proteins attach to the W-box elements of their own promoter to control their transcription by autoregulation. For instance, in *Arabidopsis*, pathogen invasion triggers the need for WRKY33 to be present for camalexin production (Birkenbihl et al. 2012; Qiu et al. 2008b). Upon pathogen attack, *AtWRKY33* forms a positive feedback regulatory loop by interacting with its own promoter, thereby amplifying the expression of genes responsible for camalexin biosynthesis (Mao et al. 2011). At its promoter region, *AtWRKY18* binds to the W-boxes to establish an equilibrium between growth and defense (Chen and Chen 2002). In *Arabidopsis*, the N-terminal leucine zipper motif enables the interaction between three WRKY proteins, namely AtWRKY18, AtWRKY40, and AtWRKY60, which are classified under Group IIa of the WRKY family (Xu et al. 2006). Similarly in parsley (*Petroselinum crispum*) *PcWRKY1* was reported to bind to the W-box present in the promoters of *PcWRKY3* and some defense marker genes like *PcPR1,* in addition to the binding with its own promoter (Turck et al. 2004). Numerous members of the same TF family often have overlapping and redundant functions in regulating downstream signaling cascades, with mutual transcriptional cross-regulation.

Post-transcriptional regulation

Maintaining precise control over the regulation of WRKY TFs and their downstream activation is essential for maintaining a delicate equilibrium between stress responses and developmental processes in plants. Recent studies reported the involvement of microRNAs (miRNAs) in plant disease defense signaling which changes the expression of certain defense-responsive TFs post-transcriptionally by binding to their 3' untranslated region (UTR). It was discovered that a recently evolved *miR396* targets *HaWRKY6* in sunflowers (*Helianthus annuus*) to control early reactions to temperature stress (Giacomelli et al. 2012). Likewise, *MdWRKYN1* and *MdWRKY26* were shown to be targeted by *Md-miRNA156ab* and *Md-miRNA395* respectively, aiding in the plant's defense against *Alternaria alternate f. sp. Mali,* which causes leaf spot disease in widely grown apple cultivar *(Malus x domestica)* (Zhang et al. 2017). ETI-mediated regulation is necessary for the activation of certain WRKY TFs during biotic stress. For example, a fatty acid amino conjugate (the effector molecule) found in *Manduca sexta* larvae is required to trigger the activation of *NaWRKY6* through *NaWRKY3*. Upon activation through wounding, these genes initiate herbivory responses (Skibbe et al. 2008b). Another instance of herbivory occurs when *Spodoptera littoralis* stimulates the production of JA-isoleucine, which attaches to the receptor COI1 and the repressor JAZ, ultimately triggering the activation of *AtWRKY40* and *AtWRKY18* (Schweizer et al. 2013). Similarly, the overexpression of *AtWRKY23* resulted in an increased response to infection by *Heterodera schachtii* nematode (Grunewald et al. 2008). *OsWRKY33* interacts with the W box motif found within the promoters of PR genes, and this interaction is facilitated by the

phosphorylation activity of *OsBWMK1* (Koo et al. 2009). In *Arabidopsis*, *AtWRKY33* acts as a positive regulator of defense against *B. cinerea* largely controlled by a gene called *Phytoalexin Deficient 4* (*PAD4*) (Qiu et al. 2008b). Auxin Response Factors -7 and 19 control the expression of *AtWRKY23*, which has a pivotal role in regulating the optimal growth and development of roots (Grunewald et al. 2012a). *AtWRKY22* can affect the self-regulation of its gene expression as well as *AtWRKY53* and *AtWRKY70*. It also favorably controls senescence (Zhou et al. 2011). The discovery of snc2-1D (suppressor of npr1-1, constitutive 2) relationship with *AtWRKY70* provides a unique opportunity to study the genetic mechanisms that regulate resistance pathways downstream of RLPs, which are a class of plant receptors that detect pathogens (Zhang et al. 2010).

Regulation by kinases

WRKY transcription factors can be regulated through the activity of kinases, which are enzymes that add phosphate groups to proteins (Fig. 4). Phosphorylation of WRKY transcription factors can affect their DNA-binding activity, stability, and subcellular localization. The MAPK (mitogen-activated protein kinase) cascade is a cardinal signaling system that has been conserved across the evolution of eukaryotes. The tobacco MAPKs, WIPK, and SIPK, along with their orthologs in various plant species, have been demonstrated to be significant immune response controllers (Yoshioka et al. 2003; Katou et al. 2005; Nakagami et al. 2005; Asai et al. 2008; Tanaka et al. 2009; Kishi-Kaboshi et al. 2010). In the *Arabidopsis* plant MPK3, MPK4, and MPK6 were discovered to

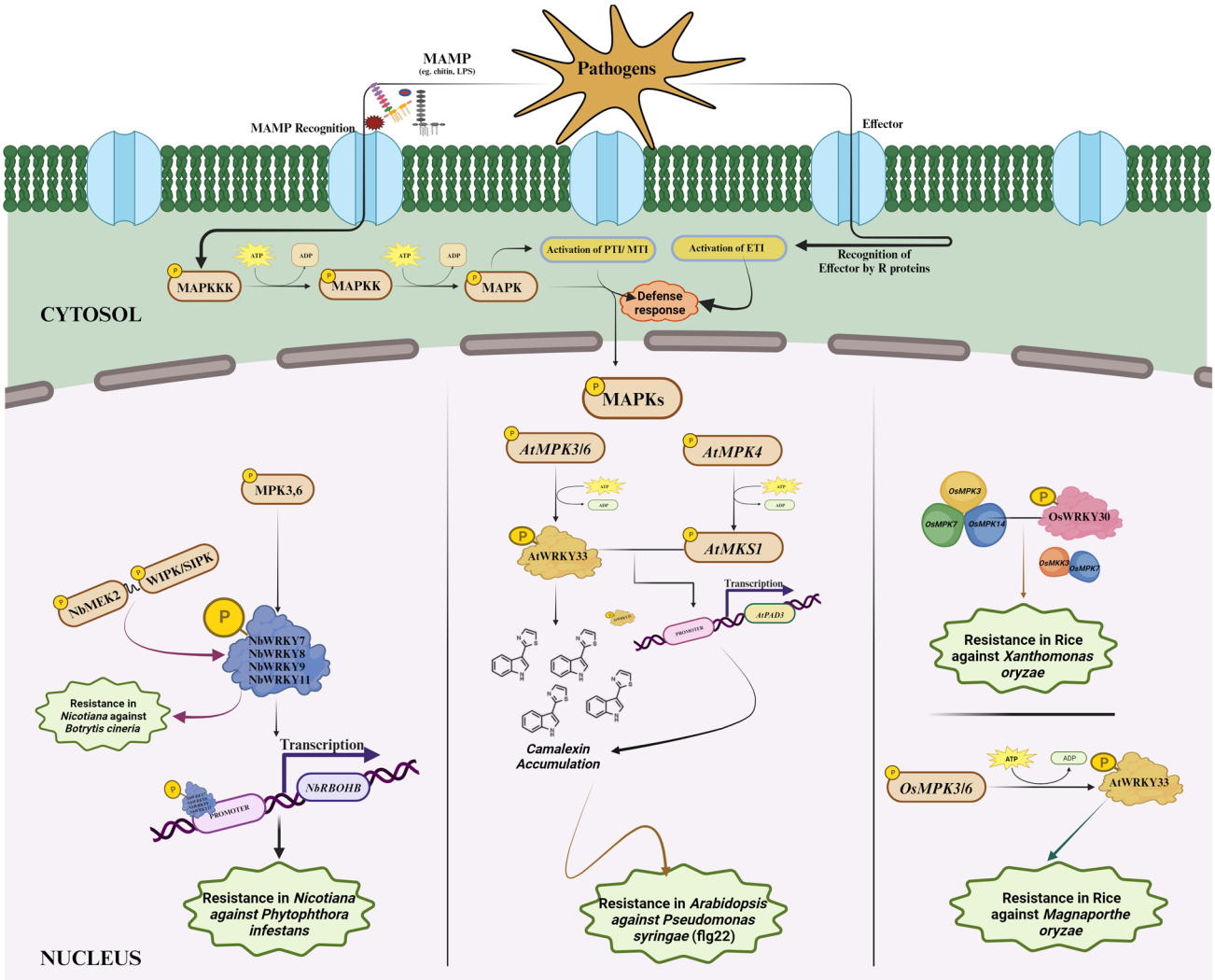

Fig. 4 Regulation of WRKY TFs by Kinases in multiple stress responses. During a pathogen attack different WRKY TFs are controlled by different Mitogen Activated Protein Kinases (MAPKs) upon phosphorylation which finally leads to the activation of defense response. For example, OsWRKY30 is regulated by OsMKK3-OsMPK7 causing enhanced resistance to bacterial pathogen *Xoo*

be pathogen-responsive MAPKs (Cristina et al. 2010). The MPK3 and MPK6 in *Arabidopsis* are orthologous to the WIPK and SIPK in tobacco, respectively (Ichimura et al. 2002). A group I *NbWRKY8* from *Nicotiana benthamiana* has an N-terminal SP (serine/proline residue) cluster which is extremely conserved in some groups of WRKY proteins. In vitro, the *AtMPK3* and *AtMPK6* orthologs WIPK and SIPK can phosphorylate the SP sites in *NbWRKY8*, and *in planta*, the activation of *NbMPKs* by *MEK2* leads to the phosphorylation of only two SP sites in *NbWRKY8* (Ishihama et al. 2011). Later, it was discovered that *NbWRKY8* and its near homologs *NbWRKY9, NbWRKY7*, and *NbWRKY11* interact with the *NbRBOHB* promoter via W-box elements that are $MEK2^{DD}$- and INF1 signal-responsive to favorably control the expression of *NbRBOHB* (Adachi et al. 2015). Moreover, the MPK-WRKY pathway-mediated *NbRBOHB* transactivation required a second burst of ROS during ETI, rather than the PTI-triggered quick ROS burst (Adachi et al. 2015).

Under the influence of the phytopathogen *B. cinerea*, the synthesis of an indolic phytoalexin called camalexin and the phytohormone ethylene occurs in *Arabidopsis*, mediated by *AtMPK3/AtMPK6* (Mao et al. 2011; Li et al. 2012). Upon infection with *B. cinerea*, *AtWRKY33* undergoes phosphorylation through *AtMPK3*/*AtMPK6* both in vitro and in vivo, and mutations in the phosphorylation sites of *AtWRKY33* impairs its capacity to adequately compensate for a deficit in the camalexin build-up. Since *AtWRKY33* targets its own promoter, *AtMPK3*/*AtMPK6* regulation of *AtWRKY33* may result in a regulatory cycle with positive feedback. Furthermore, upon infection with *P. syringae* or treatment with *flg22*, *AtWRKY33* is released from the nuclear ternary complex comprising *AtMPK4* and *AtMSK1* due to the *AtMPK4*-mediated phosphorylation of *AtMKS1* (Qiu et al. 2008b). Following its release, AtWRKY33 specifically associates with the *PAD3* promoters, which are responsible for encoding the P450 enzyme CYP71B15, thereby playing a crucial role in camalexin biosynthesis downstream of *AtMPKs*.

In rice, multiple *OsMPKs* like *OsMPK3*, *OsMPK7*, and *OsMPK14* form associations with *OsWRKY30*, a group I WRKY protein by phosphorylating it (Shen et al. 2012). Furthermore, *OsWRKY30* is under the regulation of the *OsMKK3-OsMPK7* module and enhances resistance to bacterial pathogen *Xoo.* (Jalmi and Sinha 2016). Similarly, *OsMPK3*/*OsMPK6* phosphorylates the conserved SP cluster present in the N-terminus of *OsWRKY53* (Chujo et al. 2014). Although the transactivation activity of phosphorylated *OsWRKY53* is increased, its capacity to engage W-box elements remains unchanged. When compared to the production of these genes, in plants overexpressing native *OsWRKY53*, the upregulation of a phosphomimetic *OsWRKY53* further improved resilience to a virulent *M. oryzae* strain. This example demonstrates the cardinal role of kinase-mediated phosphorylation and activation of regulatory mechanisms for controlling the expression of specific transcription factors.

Epigenetic mode of WRKY regulation

To influence polymerase or TFs attaching to DNA, epigenetic factors directly alter the structure of chromatin through processes like DNA methylation, chromatin remodeling, and histone modifications. The expression of the WRKY gene can be influenced by non-genetic factors, which can have a profound impact on various physiological responses. Under biotic stress conditions, epigenetic modifications like histone methylation and acetylation occurred in promoters of three WRKY TFs, in *Arabidopsis,* namely *AtWRKY29, AtWRKY6*, and *AtWRKY53*, which also facilitated the gene expression in epigenetically primed plants (Jaskiewicz et al. 2011). The activation of *AtWRKY70* by *ATX1* leads to H_3K_4 trimethylations, which trigger defense-responsive genes like *PR1* and *THI2.1*. Similarly, the histone methylation events that occurred at the promoter of *AtWRKY40,* activate the SA-mediated plant defense responses. The SAR-induced priming of *AtWRKY29* and *AtWRKY6* is epigenetically influenced by *FLD* (flowering locus D) at their promoters through histone modifications (Singh et al. 2014). To arbitrate leaf senescence responses, SUVH5 elicits H3K4me2 and H3K4me3 methylation, which epigenetically regulates *AtWRKY53* (Li et al. 2020b). In *Arabidopsis*, the JmjC domain-containing protein 27 (JMJ27), a member of the histone demethylase 2 (JHDM2) family, was responsible for inhibiting the production of the three defense-related transcription factors (TFs) WRKY25, WRKY26, and WRKY33 (Dutta et al. 2017). *Arabidopsis* plants lacking JMJ27, the promoters of two of these TFs, WRKY25 and WRKY33, as well as the PR1 gene, were discovered to be hypermethylated (Dutta et al. 2017; Lippok et al. 2007). Upon exclusion of acetyl groups from the histone tail regions, HDA19 inhibits the production of *AtWRKY62* and *AtWRKY38,* which affects adversely in basal defense (Kim et al. 2008b). To regulate plant immunity, the SA-dependent pathway is found to be activated after the *AtWRKY40* promoter is histone methylated (Alvarez et al. 2010). Additionally, ABI5 expression is inhibited and ABA signaling is adversely regulated by histone methylation of the *AtWRKY40* promoter during the germination of seeds and development post-germination (Shang et al. 2010). To control physiological processes in banana fruit, such as fruit ripening and stress reactions, the linker histone H1 gene *MaHIS1* links with the *MaWRKY1* gene (Wang et al. 2012). These variables affect gene expression and downstream translation, whether it be through covalent changes, structural inheritance, or nucleosome placement. Therefore, it is necessary to handle these epigenetic modes of control before moving to genetic means of alteration.

Regulation by the proteasome system

Proteasome-mediated degradation is one of the mechanisms which regulate the expression of WRKYs under normal conditions. Almost all aspects of plant growth, development, and adaptations to the environment depend on the ubiquitin–proteasome system (UPS). Through the activity of E1, E2, and E3 enzymes in UPS, ubiquitin is covalently linked to target proteins, causing the target genes to be degraded in the 26S proteasome. Transcriptional repressors are frequent targets of UPS in plant signaling and their breakdown results in the derepression of signaling networks (Santner and Estelle 2010). The protein level of *AtWRKY6* in *Arabidopsis* is decreased under minimal P_i stress, which is a repressor of *PHO1* (Chen et al. 2009). A 26S proteasome inhibitor called MG132 prevents low-P_i-induced *AtWRKY6* degradation, which raises the possibility that UPS-mediated *AtWRKY6* repressor degradation is the cause of PHO1 derepression (Chen et al. 2009). A positive regulator of plant senescence called *AtWRKY53* engages with a HECT (homologous to the E6AP carboxyl terminus) domain E3 ubiquitin ligase called UPL5 (Miao and Zentgraf 2010). UPL5 uses *AtWRKY52* as a substrate for polyubiquitination in vitro, and *AtWRKY53* degrades more quickly in vivo when UPL5 is overexpressed (Miao and Zentgraf 2010). Increased senescence is brought on by UPL5 mutation, especially in transgenic plants that overexpress *AtWRKY53* (Miao and Zentgraf 2010). These findings suggest that UPS is negatively regulated to delay early senescence in *AtWRKY53*. In rice, *OsWRKY45* is crucial for defense induced by SA/BTH, which is controlled by nuclear UPS. UPS quickly degrades OsWRKY45 in the nuclei to reduce defense reactions under normal circumstances. But when a pathogen attacks, proteasomes are inhibited, which leads to an accretion of polyubiquitinated *OsWRKY45* (Matsushita et al. 2013). The *OsWRKY45* transactivation domain is near the regions needed for UPS-dependent degradation (Matsushita et al. 2013). In *Vitis pseudoreticulata*, WRKYs are controlled by ubiquitin to enhance defense responses against pathogen attacks. *VpWRKY11* is linked to EIRP1 (E3 ubiquitin ligase Erysiphenecator-induced RING finger protein 1) through its RING domain resulting in its proteolysis through 26S proteasomal degradation (Yu et al. 2013).

Regulatory roles of WRKY transcription factors in defense mechanisms through the production of plant secondary metabolites

Plants exhibit a wide-ranging spectrum of metabolites, categorizable into two primary groups: primary metabolites, essential for fundamental growth and development, and secondary metabolites, also known as plant secondary metabolites (PSMs). Secondary metabolites are assumed to have multifunctional roles in plant defense mechanisms and environmental signaling, particularly in response to stressful conditions (Obata 2019). The intricate mechanisms governing plant defense not only enable survival against stressors but also oversee the accumulation of PSMs (Kajla et al. 2023).

Under challenging environmental circumstances, the synthesis of PSMs undergoes rigorous regulation at the transcriptome level, involving a number of genes and TFs. The binding of these TFs is sequence-specific and specifically binds to cis-regulatory elements within gene promoter regions. This binding process can either activate or repress gene expression in response to developmental and environmental cues (Patra et al. 2013). WRKY TFs have been documented as regulators of the biosynthesis of several secondary metabolites (Table 3). Their expression underscores their role in governing the biogenesis of defense-related PSMs (Guillaumie et al. 2010; Wang et al. 2010; Grunewald et al. 2012b; Phukan et al. 2016). For instance, NtWRKY3 and NtWRKY6 have been identified for their involvement in terpene biosynthesis in tobacco (Skibbe et al. 2008a), while AaWRKY17 positively regulates artemisinin synthesis, a sesquiterpenoid lactone with significant antimalarial properties (Chen et al. 2021).

Another noteworthy PSM is Hydroxycinnamic acid amide (HCAA), derived from phenylpropanoid metabolism, primarily associated with lignin biosynthesis originating from phenylalanine (Humphreys et al. 1999; Vogt 2010). During infections, StWRKY1 has been observed to enhance resistance against late blight disease in potatoes by binding to the promoters of HCAA biosynthetic genes (Yogendra et al. 2015). Similarly, in barley, HvWRKY23 stimulates the expression of several genes involved in defense, thereby inducing HCAA biosynthesis during *Fusarium*-induced red rot disease (Karre et al. 2019).

Another class of secondary metabolites, phytoalexins, belonging to the stilbene family, significantly regulate plant defense (Jiang et al. 2010; Ahuja et al. 2011). Resveratrol, found in grapes, was the first reported phytoalexin (Lanz et al. 1991). Negative regulation of resveratrol biosynthesis by VvWRKY8 has been reported (Jiang et al. 2019). Additionally, ZmWRKY79 has been associated with increased phytoalexin accumulation in maize, providing resistance against sheath blight disease caused by *Rhizoctonia solani* (Fu et al. 2017). Likewise, GaWRKY has been identified as responsible for enhanced gossypol production in cotton, which exhibits anti-feeding properties (Xu et al. 2004).

In contrast, the presence of WsWRKY1 in *Withania* has been linked to a notable reduction in phytosterol accumulation, resulting in diminished resistance against bacteria, fungi, and insects, as reported by Singh et al. (2017). Furthermore, extensive research has explored the regulatory roles of WRKY TFs in plant secondary metabolite

Table 3 Role of WRKY TFs in defense against biotic stress through the production of PSMs

Plant secondary metabolites (PSM)	WRKYs involved	Plant species	Provide resistance against	References
Hydroxycinnamic acid amide (HCAAs)	*StWRKY1*	*S. tuberosum*	Late blight (*Phytophthora infestans*)	Yogendra et al. (2015)
	TaWRKY70	*T. aestivum*	Head blight (*Fusarium graminearum*)	Kage et al. (2017)
	HvWRKY23	*H. vulgare*	Head blight (*Fusarium graminearum*)	Karre et al. (2019)
Benzylisoquinoline alkaloids	*StWRKY8*	*S. tuberosum*	Late blight (*Phytophthora infestans*)	Yogendra et al. (2017)
Terpenoid phytoalexins	*ZmWRKY79*	*Z. mays*	Sheath blight (*Rhizoctonia solani*)	Fu et al. (2018)
Resveratrol	*VvWRKY24*	*Vitis vinifera*	Bunch rot (*Botrytis cinerea*)	Jiang et al. (2019)
	VvWRKY3			
	VvWRKY8			
Flavonoid	*ZmWRKY83*	*Z. mays*	Stalk rot (*F. graminearum*)	Bai et al. (2021)
Terpenoid	*ZmWRKY83*	*Z. mays*	Stalk rot (*F. graminearum*)	Bai et al. (2021)
Taxol	*TcWRKY1*	*T. chinensis*	Antimicrobial	Li et al. (2013)
Phytosterol	*WsWRKY1*	*W. somnifera*	Bacteria, Fungi and Insect	Singh et al. (2017)
Diterpenoids	*SsWRKY18*	*S. sclarea*	Bacteria and Fungi	Alfieri et al. (2018)
	SsWRKY40			
Artemisinin	*AaWRKY17*	*A. annua*	*Pseudomonas syringae*	Chen et al. (2021)

production, offering valuable insights for engineering biotic stress resistance in transgenic plants.

Conclusion and future perspectives

Although WRKYs are well studied in several model plants such as *Arabidopsis*, still there is a need for more detailed investigations on WRKY genes in crops. Given the economic significance of crops and the various stresses they encounter, there is a pressing need for more comprehensive investigations that specifically focus on WRKY genes. As these genes are found to be the key regulators of plant responses to various stresses, a more thorough understanding of their mechanisms of action could potentially pave the way for the development of more resilient crops that are better equipped to withstand environmental challenges. WRKY TFs have been acknowledged to perform a cardinal role in the regulation of host responses against phytopathogenic organisms, and they may influence defense gene expression at multiple levels. However, to fully comprehend the complex cascade of events that occur in response to a challenge by these organisms, it is essential to investigate the interplay and downstream effects of a single TF. This review emphasizes the need to explore this crosstalk and cascade in greater detail. Techniques such as transgenics, analysis of molecules involved in signaling and interacting partners, and high-throughput transcriptomic, proteomic, and metabolomic platforms should be employed for a comprehensive understanding. By doing so, we can gain a more comprehensive understanding of the intricate mechanisms involved in plant defense against phytopathogens. Multiple WRKY genes/TFs exhibit a variety of behaviors, even to the point wherein homologs respond in several contexts (Cai et al. 2014). Also depending on the external stimuli, a set of WRKY genes can regulate multiple genes with conflicting effects or either induced or suppressed (Fig. 5). For instance, overexpressing transgenic lines of *AtWRKY33* in *Arabidopsis* resulted in heightened resistance towards fungal pathogen *A. brassicicola* (Zheng et al. 2006). *AtWRKY33* also participates in regulating the biosynthesis of terpenes, which are chemical communication signals between plants and whiteflies. In the presence of the MPK6 protein, WRKY33 provides resistance against whiteflies, but the Bsp9 protein from whiteflies can interrupt this interaction. Mutant lines of *AtWRKY33* attract more whiteflies (Wang et al. 2019). On the other hand, overexpression of *AtWRKY70* in Arabidopsis suppressed the expression of a subset of JA and *A. brassicicola*-responsive genes. However, *AtWRKY70* is also involved in SAR activation by the modulation of SA signaling, which results in mounting resistance against *P. syringae* infection. Nevertheless, it also contributes to increased susceptibility to the fungal necrotroph *A. brassicicola* (Li et al. 2006). Overall, these findings underscore the significance of transcription factors in regulating plant defense against different types of pathogens and pests and the complexity of the signaling pathways involved in these responses. Given this complexity, a transgenic approach could be one of the best ways to develop plants with better tolerance towards multiple stress factors. However, the issue with genetically modifying crops is the need for long-term field trials to ensure there are no unintended consequences, such as unwanted traits or transfer of genes to other plants. It is important to carefully monitor and study the modified traits before commercializing genetically modified crops. This will help us understand how plants respond to environmental stresses and improve

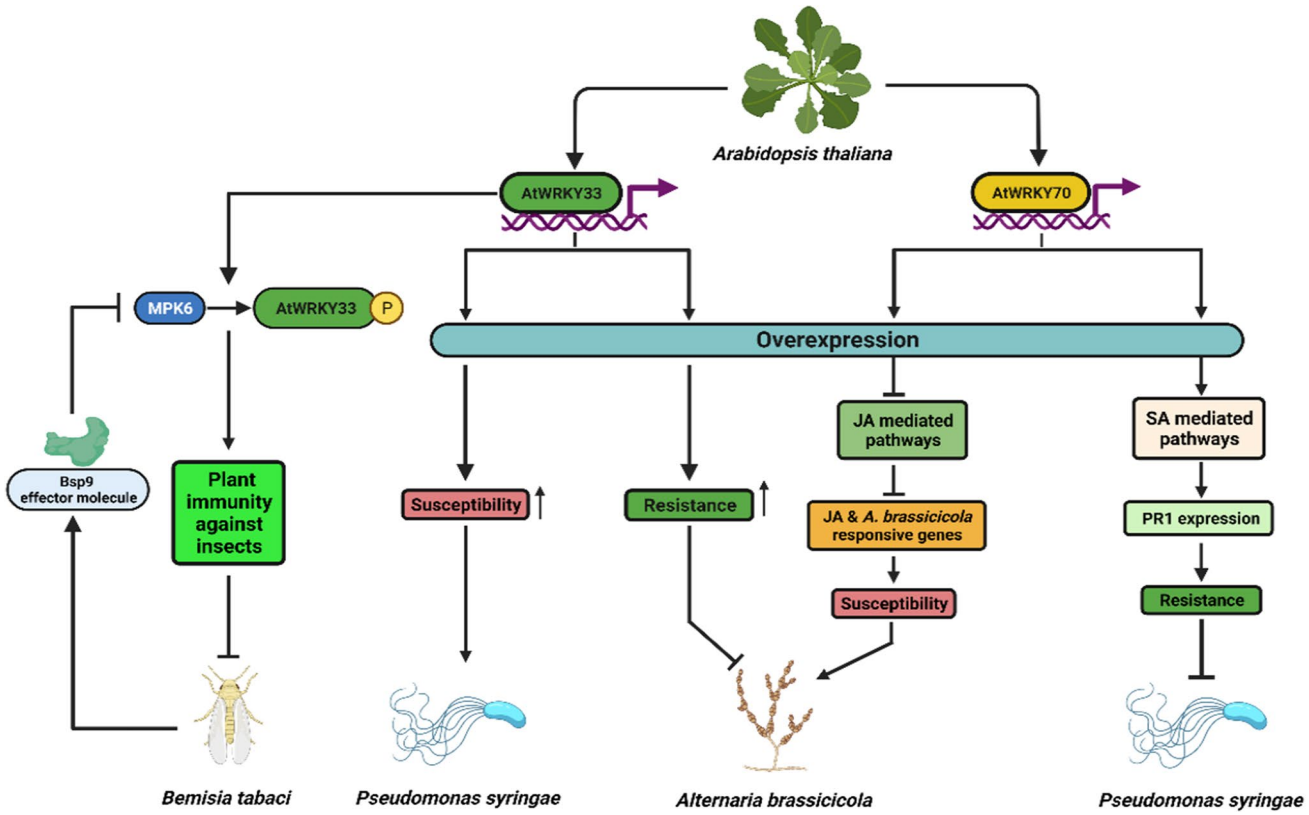

Fig. 5 Overview of the participation of various WRKYs in plant defense in response to different external stimuli. This figure demonstrates that overexpression of *AtWRKY33* enhances resistance to *A. brassicicola* and whiteflies, while *AtWRKY70* suppresses some genes but promotes SAR and resistance to *P. syringae*. SA- Salicylic acid, JA- Jasmonic Acid, PR1- pathogenesis-related protein 1, Bsp9-Whitefly (*Bemisia tabaci*) salivary protein

their survival under changing conditions. The application of CRISPR and CRISPR-associated gene systems holds great potential for examining the functional aspects of WRKYs. Studying how WRKY TFs interact with DNA/chromatin globally will help us understand how they influence metabolic pathways and cellular physiology. This information can also reveal how pathogens interact with the network to counteract host defenses or use it to their advantage.

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