HANS SHODH SUDHA , Vol. 1, Issue. 1, (2020) pp. 34-50

WRKY transcription factor super family: Role in plant disease management

Jyotsna Bharti[1#], Sahil Mehta[1#] and Satyakam Guha[2*]

Abstract

Plants face numerous challenges in their natural habitat by various biotic stresses, which in turn trigger their stress-response related machinery. This is achieved by activation or repression of a complex system consisting of key genes coding for miRNAs, siRNAs, and most importantly transcriptional factors (TFs). Among transcriptional factors (TFs), a superfamily of WRKY TFs is considered most important as they regulate maximum number of downstream genes as well as proteins, by both direct (auto and cross-regulation) and indirect mechanisms (physical interactions within themselves or other TFs, proteins and small RNAs) reported hitherto. All the WRKY TF members possess a conserved WRKY domain consisting of nearly 60 amino acids with a specific heptapeptide sequence (WRKYGQK) with a Zn^{2+}-finger motif that binds to a specific cis-regulatory element of a defense gene called as W-box (TTGAC[C/T]). This W-box has been reported to be present in the promoter region of genes related to plants' innate immunity including PAMP triggered immunity (PTI), effectors triggered immunity (ETI), basal defense and systemic acquired resistance (SAR). As a result of this specific molecular orchestration primarily in plant immunity, this superfamily has established as a good target in plant disease management. Therefore, in the present review, the focus will be on highlighting the application of the WRKY TFs and plant disease management specifically.

Keywords: W-box, *Pseudomonas*, Classification, *Botrytiscinerea,* Virus.

1. Introduction

Due to the sedentary life and changing climatic conditions, plants have to face various environmental stresses on a regular basis. Among all the stresses, biotic stresses incited by plant pathogens especially affect the growth of various food crops such as rice, barley, sugarcane, lentil, fava bean, chickpea and many more (APS 2020). Upon being challenged by phytopathogens, the plants must change their growth as well as Défense pattern which lead to modification of host-pathogen interaction (Madhusudan *et al.* 2019). Due to continuous changes in climatic conditions, new virulent races or pathovars develop, that can further infect the crops which were resistant to pathogens previously (APS 2020). Therefore, in order to combat these new entities, there is a pressure to enhance the current, as well as to develop new management strategies for phytopathogens.

To improve the strategies, special focus is also given in understanding changes occurring at the genic, transcriptional, protein, metabolic as well as the cellular levels (Chen *et al.* 2019). The converging point of all these changes can be traced back to the transcriptional regulation controlled by transcription factors (TFs). The various TFs related to stress-responsive genes are WRKY "worky", AP2/ ERF, bZIP, MYC, MYB, MADS, NAM, ATAF, CUC and NAC (Chen *et al.* 2019). Each TF consist of a specific polypeptide binding domain which binds to a sequence/stretch of DNA bases together known as *cis*-regulatory

---

1. International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi, Delhi-110067, India.
2. Department of Botany, Hansraj College, University of Delhi, Delhi-110007, India.

*Corresponding author: **Satyakam Guha.** Department of Botany, Hansraj College, University of Delhi, Delhi-110007, India. email: satyakamg173@gmail.com

#Equal Contribution

elements (CREs) in response to specific stress (Mittal *et al.* 2018).

Among all of the TFs reported till date, WRKY TF superfamily is of great importance as they are reported to be involved in a diverse range of plant development, defence (**Figure 1**), metabolism, senescence, wounding, stress response and much more (Chen *et al.* 2019). The WRKY TF superfamily members interact among themselves or with other key TFs, proteins, and small RNAs to regulate the target genes (Chen *et al.* 2019). Our objective in this article is mainly to discuss the WRKYTFs modulation in complex defence gene network, which is a key step for signal transduction pathways related to plant immunity. We have summarised in present article, the structure and classification of WRKY as well as downstream regulation or interaction with same or other TFs, proteins and small RNAs to regulate the defence gene for various plant pathogens such as fungi, bacteria, and viruses.

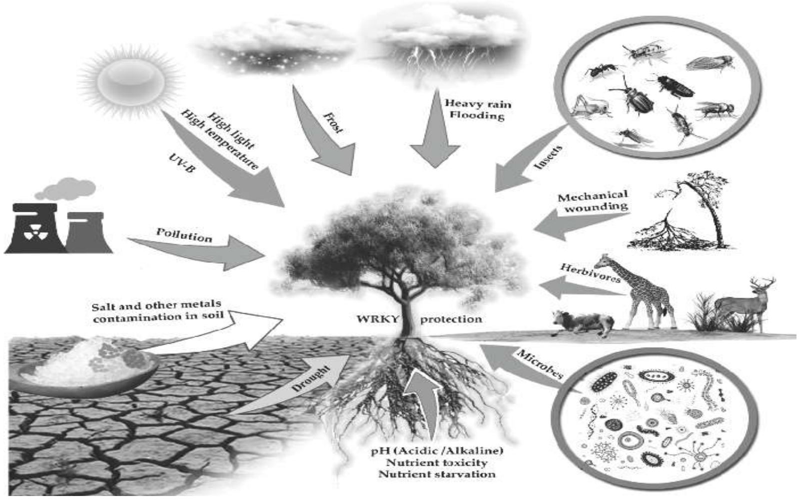


Figure 1. Schematic representation of the WRKY TFs imparted protection in plants against all biological and non-biological stresses.

Sources: Google Scholar (https://scholar.google.co.in/), PubMed (https://www.ncbi.nlm.nih.gov/pubmed) and Web of Science (https://clarivate.com/webofsciencegroup/solutions/web-of-science/) till 10th July, 2020.

2. WRKY TFs: structure

A specific and conserved DNA-binding domain is the distinguishing character of WRKY TFs which is partly protruding region of almost 60 amino acids in plants (Chen *et al.* 2019). In this region, there is an almost invariable sequence at N terminal of protein, WRKYGQK (W; Tryptophan, R; Arginine, K; Lysine, Y; Tyrosine, G; Glycine Q; Glutamine, K; Lysine).WRKY TFs generally binds to a considerably conserved region of DNA identified as the W-box elements having the conserved motif TGACC/T singly or in tandem repeats situated in the promoter region of defense genes (Chen *et al.* 2019). Recently, the WRKY domain structure with W-box as a binding site was identified, and structurally four-stranded β-sheet enters the major grove of DNA in an atypical mode termed the β-wedge, where the sheet is nearly perpendicular to the helical axis of the DNA (Yamasaki *et al.* 2012)._ Whereas, C-terminal of the protein consist of zinc-finger-like motif $Cx_{4-5}Cx_{22-23}HxH$ or $Cx_7Cx_{23}HXC$ formation (Li *et al.*, 2015).Tryptophan (Y) makes the core of the conserved (WRKYGQK) sequence, whereas rest of the amino acids binds to DNA. Glycine (G) helps in the formation of protrusion to make the grove for binding to W box by hydrophobic interaction with the methyl groups of thymine nitrogenous bases of the DNA. Mutation in the thymine base or Zn^{2+}-binding site drastically reduced the DNA-binding activity due to the destruction in the active structure of DNA binding domain protein (Yamasaki *et al.* 2013). Only in very few crops like *Arabidopsis* (WRIY), soybean (WHQY), potato (WHKC and WRKC), black cottonwood (FRKY), *tomato (*WRKR, WIKY, WSKY, and WQKY*) and* French bean (ARKM, WWKN, and WRMY), WRKY proteins have been reported with changes in the conserved sequence of WRKY (Mohanta *et al.* 2016).

3. Classification of WRKY TFs

In the initial days of researchInitially, WRKY TF was identified for the regulation of sporamin and β-amylase production from sweet potato as SPF1

(Ishiguro and Nakamura 1994). However, later, Rushton and group identified three different WRKY TFs (WRKY1, WRKY2 and WRKY3) from parsley in a stress response against elicitor Pep25 of *Phytophthora parasitica* and given the name of "worky" in 1996 (Rushton *et al.* 1996). This opened a way for identification of stress responsive WRKY TFs which has resulted now in a superfamily. As a result, based on the total number of WRKY domains and presence/absence of Zn^{2+}-finger-like motif, WRKY proteins are classified into three groups*i.e.*, Group I, Group II and Group III (Chen*et al.* 2019). The group I members consist of two WRKY domains, whereas Group II members have one WRKY domain along with Cys2-His2-type of Zn^{2+}-finger motif. Group II members are further divided into five subgroups IIa to IIe based on the presence of additional amino acid motifs in the WRKY domain. Group III members also contain one WRKY domain with Cys2-His/Cysor Cys2-His2 type of zinc-finger motif (Eulgem *et al.* 2000). The criterion of this classification method is exclusively based on protein structure; however, this classification does not includeevolution, origin, and duplications of a gene for WRKY TFs. So, in the year 2005, Zhang and Wang (2005) again reclassified WRKY TFs into five groups *i.e.*, group 1, group 2a + 2b, groups 2c, group 2d + 2e and group 3, based on phylogenetic analysis, domains conservation and intron position in WRKY domain. WRKY TFs were again classified into two groups where Group 1 includes R-type of the intron in the WRKY domain whereas Group 2 members include V-type of an intron (Zhang and Wang 2005).

4. Role of host plant WRKY against viral diseases

As described earlier, the WRKY TFs are one of highly studied regulatory protein family which play both positive and negative roles in plant immunity (Chen *et al.* 2019). One of the major challenges to plant immunity is the onset of viral diseases. There are already many reports in the literature by various research groups for the bolstering support of the role of WRKY TFs against plant viruses. Out of all published articles, the first preliminary report was by Yoda *et al*. (2002). In their pioneer work, they screened a set of defense reaction genes up-regulated during the hypersensitive response (HR) in wild tobacco (Cv. Xanthinc) upon *Tobacco mosaic virus* (TMV) infection using fluorescent differential display (Yoda *et al.* 2002). The full-length deduced TIZZ protein contained a single WRKY domain which showed high similarity to one of WRKY family member namely WIZZ. Their results indicated the presence of a novel type of WRKY protein(s) that might play a critical role in HR signal activation.

Dardick *et al.* (2007)observed significant changes in gene expression concomitant with*Plum pox poty virus* (PPV), *Tomato ringspot virus* (ToRSV) and *Prunus necrotic ring spot ilar virus* (PNRSV) symptoms in *Nicotiana benthamiana* leaves. The number of pathogens associated are? identified including WRKY transcription factors, which were consistent with the severity of the observed symptoms in all the three viruses (Dardick *et al.*2007). In a similar study, McGregor, and colleagues(McGregor *et al.* 2009), focused on one of the most devastating *Ipomoea batatas* disease namely sweet potato virus disease. They used a similar global gene expression approach in two different sweet potato cultivars NASPOT1 (resistant) and Beauregard (susceptible), and found that cell expansion genes, as well as chloroplastic genes, were suppressed while stress-related and various transcription family genes (WRKY, homeodomain proteins, and NAC-like proteins) were induced highly. After virus infection, the protein synthesis-related genes induction was in co-relation with virus accumulation in susceptible plants. This switch in the expression of all these specific host-encoded genes was established as a reason to cause developmental defects in susceptible plants (McGregor *et al.* 2009).

Using *Bean pod mottle virus*-based VIGS technology, the role of WRKY6, as well as WRKY30 in Rsv1-mediated resistance was elucidated in soybean (Zhang *et al.* 2012). Similarly, the over expression of cotton *Gh*WRKY 15 and *Gh* WRKY11 in transgenic tobacco plants activated the expression of several PR, POD, and APX genes, therefore, triggering systemic acquired resistance (SAR) to protect the plant against viral pathogens such as *TMV* and *cucumber mosaic virus* (CMV) as compared with the wild type (Yu *et al.* 2012). Inoculation of *Rice tungro spherical virus* (RTSV) on susceptible rice (Cv. TN1) changed the transcripts levels of multiple stress-related genes including multiple members of the WRKY gene family (*Os*WRKY1, *Os*WRKY5, *Os*WRKY9, *Os*WRKY28, *Os*WRKY29 and *Os*WRKY45) (Satoh *et al.* 2013).

The role of six tomato WRKYs (WRKY41, WRKY42, WRKY53, WRKY54, WRKY 80 and

WRKY81) in *tomato yellow leaf curl virus* (TYLCV) infection were elucidated using subcellular localization analysis, interaction network analysis and TRV-VIGS by Huang *et al.*(2016). Time-course analysis of the effect of *Ugandan cassava brown streak virus* grafting on resistant and susceptible cassava varieties transcriptome revealed the up-regulation of differentially expressed-defence genes response genes including LRR-containing, NBARC-containing, PR, LEA, WRKY, GATA, NAC and HSPs (Amuge *et al.* 2017).

Madronero *et al.* (2018) adopted the global gene expression analysis approach on *Papaya meleira virus* complex induced changes in infected papaya at pre-and post-flowering stages. They reported that at the pre-flowering stage, a total of 633 DEGs was observed including multiple SA-, ethylene (ET)-pathway genes, PR genes, ROS genes, and WRKY TF encoding genes (Madronero *et al.* 2018). Recently, the combinatorial effect of low light intensity/shading and *Soybean mosaic virus* on the transcriptome level of soybean plants was assessed (Zhang *et al.* 2019). Among all the 24 DEGs related to plant-pathogen interaction, a total of two WRKY genes (WRKY33 and WRKY62) were differentially expressed under both light conditions. More recently, Kumar and Dasgupta (2020) studied the effect of infection by both rice tungro viruses (*Rice tungro bacilliform virus* and RTSV) at the rice transcriptomic landscape was deduced by using global gene expression changes using Illumina Hiseq 2500 platform followed by qRT-PCR. About 959 DEGs were related to stress-responsive pathways and hormonal homeostasis. Among all DEGs, the reported WRKY transcription factors were LOC_Os05g25770, LOC_Os08g38990, LOC_Os09g25060, and LOC_Os11g02520 (Kumar and Dasgupta 2020). By summarising all these studies by various authors, we can conclude that the WRKY TFs regulate host defence against viruses at various levels directly or indirectly.

5. Role of host plant WRKY against bacterial diseases

Unlike viruses, the bacteria grow in the spaces between plant cells and cause multiple symptoms including cankers, wilts, soft rots, blights, scabs, galls and leaf spots (APS 2020). Dellagi *et al.* (2000) were the first to report the elicitor-induced nature of WRKYs in response to bacteria where they have isolated an upregulated potato *St* WRKY1 protein using the SSH technique upon inoculation of *Erwiniacarotovora* subsp. *atroseptica* culture filtrate (Dellagi *et al.* 2000). Another evidence of the involvement of WRKY against bacterial diseases in plants was forwarded by Robatzek and Somssich (2002), studied the targets of senescence- and defence-associated *AtWRKY6* factors. Their study revealed the WRKY6 negative regulation on its promoter activity as well as promoters of *At*PR1, *AtSIRK* and other closely related WRKY family members (Robatzek and Somssich 2002). A WRKY gene '*Ca*WRKY' was isolated by using a domain-specific differential display procedure, during the infection of*Xanthomonas campestris*pv. *vesicatoria* (Park *et al.* 2006). Dang *et al.* (2013) clarified the role of pepper *Ca*WRKY40 in imparting resistance against *R. solanacearum* infection and reported that the overexpression of *Ca*WRKY27 enhanced the resistance of tobacco transgenic plants to*Ralstonia solanacearum* (Dang *et al.* 2014).

A positive role of *Os*WRKY51 and *Os* WRKY67 in defence against *X. oryzae* pv. *Oryzae* was established using overexpression study (Hwang *et al.* 2016; Liu *et al.* 2018). By performing temporal transcript profiling, Nemchinov *et al.* (2017) selected and inoculated bacterial stem blight-resistant and susceptible alfalfa (*Medicago sativa* L.) plants. Their analysis revealed that there were plenty of differentially expressed genes (DEGs) in two contrasting genotypes at the molecular level. The reason for resistance appeared to be mediated primarily by 20 WRKY family transcription factors and other function-related genes (Nemchinov*et al.* 2017).

Recently, the constitutive overexpression of wild grapevine *VdWRKY53* in *Arabidopsis* resulted in multi-fold enhancement in resistance to multiple pathogens including *P. syringae* pv. *tomato* (DC3000) (Zhang *et al.* 2019). Gao *et al.* (2020), characterized the role of *SlWRKY8* in the resistance to *P. syringae* pv. *tomato* DC3000 (Pst DC3000) along with other abiotic stresses. The constitutive over expression in the tomato plants (Cv. Ailsa Craig) resulted in increased resistance to Pst DC3000 by enhancing expression levels of PR genes namely *Sl*PR1a as well as *Sl*PR7. Overall, their report suggested the role of *Sl*WRKY8 in plant immunity against bacterial pathogen and other prominent abiotic stresses (Gao *et al.* 2020).Thus, from the above reports by various authors clearly indicates the role of *WRKY* transcription factor superfamily against bacterial diseases in plants. Though enough evidence is available about the role of WRKY defense against bacterial diseases in plants, but the exact mechanism of signal transduction is not yet clear.

6. Role of host plant WRKY against fungal diseases

Another major challenge to plant immunity apart from bacteria and viruses is the fungal diseases (APS 2020). There are many reports on the role of WRKY against fungal diseases in plants, out of which, the first preliminary report by Rushton *et al.* (1996) needs a special mention, as they used both gain and loss of function experiments in parsley (*Petroselinum crispum*) and identified the presence of WRKY1, WRKY2 and WRKY3 TFs binding W-box in the promoters of PR1-1 and PR1-2 genes (Rushton *et al.* 1996). Furthermore, they confirmed the Pep25 elicitor treatment in parsley cells induced a rapid increase in the mRNA levels of only WRKY1 and WRKY3. Their work suggested that WRKY TFs might play a role in the signal transduction pathway (Rushton*et al.* 1996).

Using suppression subtractive hybridization (SSH), a putative StWRKY1 protein-encoding gene was identified in potato after inoculation of *P. infestans* as well as *E. carotovora* subsp. *atroseptica* filtrate (Dellagi *et al.* 2000). Ryu *et al.* (2006) confirmed the changes in the expression level of a total of 15 host WRKY genes upon inoculation of *M. grisea* (Philippines isolate PO6-6). Their extensive profiling analysis work revealed that the transcript levels of *Os* WRKY7, *Os* WRKY10*, Os* WRKY11*, Os* WRKY30*, Os*WRKY45*, Os* WRKY62*, Os* WRKY76*, Os* WRKY82, and *Os* WRKY85 were significantly increased by 6-48 hours.

The role of the so-called SA activator namely benzothiadiazole is well depicted in the literature. Using the group of techniques like microarray screening, RNAi and transient over-expression system, the role of BTH-inducible WRKY45 was identified in providing resistance against rice blast disease (Shimono *et al.* 2007). The constitutive overexpression of rice blast-induced *Os*WRKY31 in Japanese rice cultivar namely Zhonghua 17 leads to enhanced shoot length, root length and resistance against two blast fungus strain P131 and MS220 of *M. oryzae* (Zhang *et al.* 2008). Yang *et al.* (2009) studied the expression of a total of 46 WRKY TFs encoding genes in the canola infected with two devastating fungal pathogens namely *Sclerotinia sclerotiorum* and *Alternaria brassicae* using quantitative real time-PCR (qRT-PCR). Their study revealed that about 13 BnWRKYs transcript abundance changed significantly following the fungal challenge (Yang *et al*. 2009). Overexpression of nuclear-localized *Os*WRKY30 gene in rice plants depicted the enhanced *Rhizoctonia solani* and *M. grisea* resistance. This occurred due to the activated expression of JA- and PR- synthesis-related genes (Peng *et al.* 2012). The similar role of *Os*WRKY76 (Yokotani *et al.* 2013), *Bo*WRKY6 (Jiang *et al.* 2016), *Ta*WRKY49 (Wang *et al.* 2017), *Vl*WRKY48 (Zhao *et al.* 2018),*Gm*WRKY31 (Xiong *et al.* 2019) and *Ta*WRKY142 (Kuki *et al.* 2020) using gain-of-function and loss-of-function studies were identified. From the above survey of literature we can gain an idea about the identification of various genes associated with WRKY mediated defence in plants against fungal growth and the flowchart is depicted in the **Figure 2**.

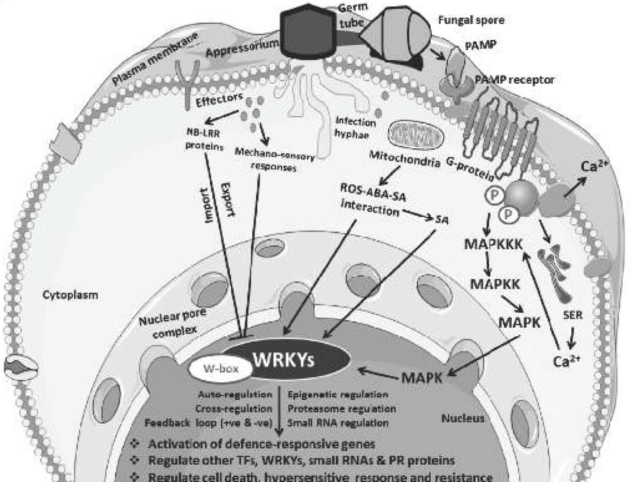


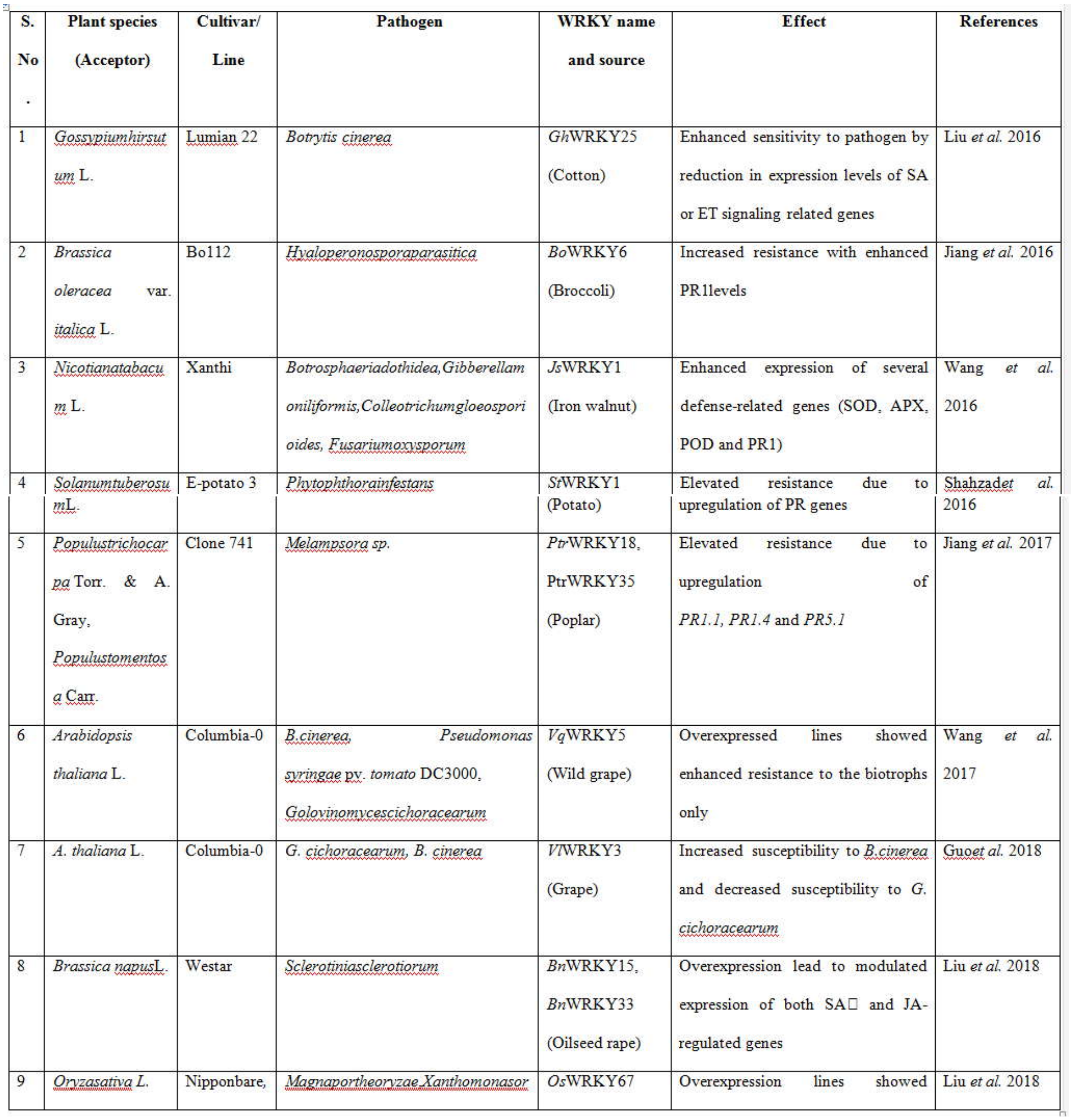
Figure 2.A generalized model depicting the role of WRKYgenes in cellular defense signalling against fungal pathogens.

Under fungal stress, WRKY TFs are regulated by various upstream receptors (RLKs, G-protein) and regulators and impart resistance by controlling expression of defence-related genes as well as PR proteins.

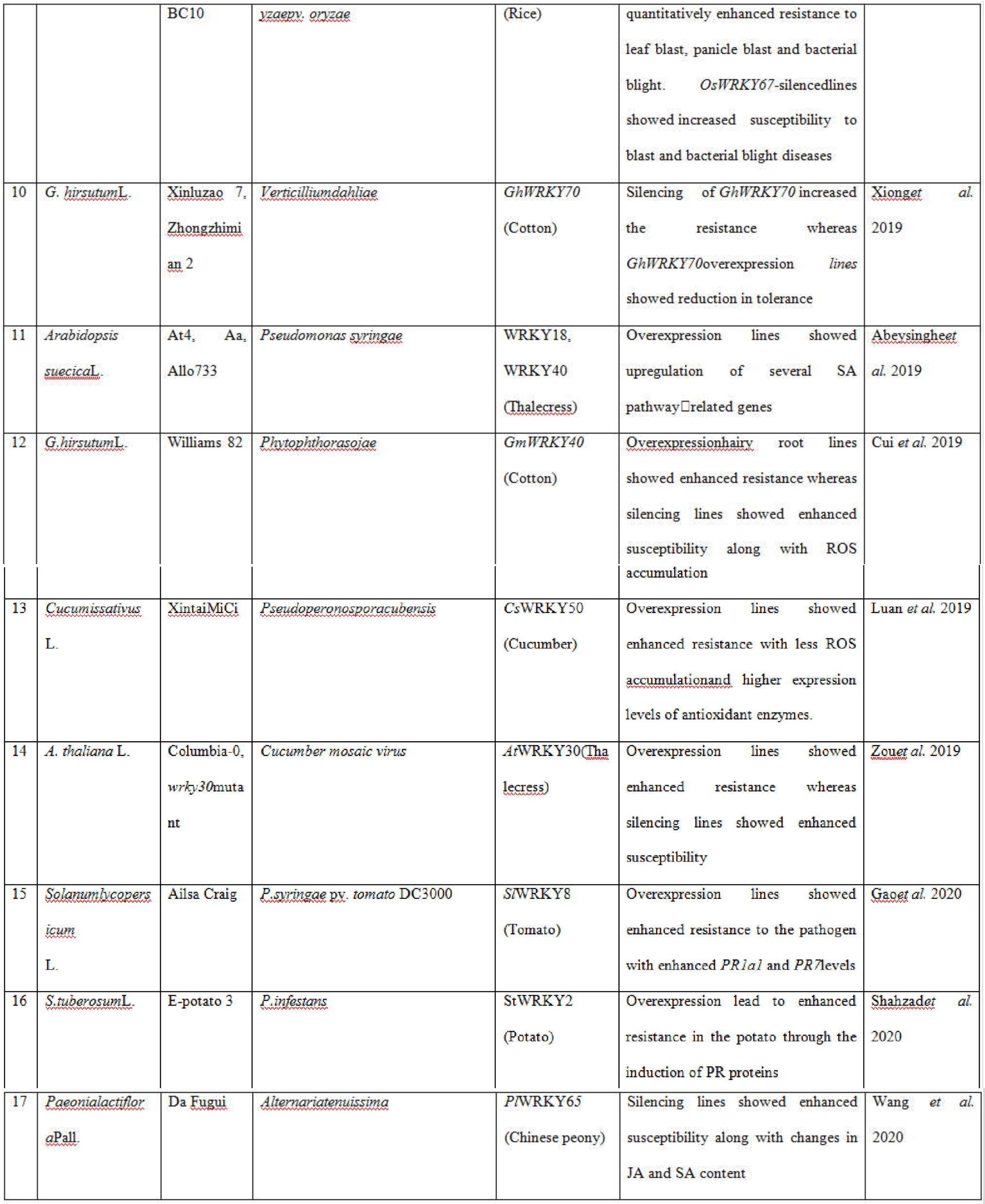
7. Conclusion

Under natural conditions, the plant faces multiple biological and non-biological stresses which creates a complex agronomical environment and corroborates the plant's ability to develop, grow, and reproduce. Within this, biotic stresses are considered as the foremost limiter. The most important aspect is that the major effect is reflected on agriculturally important crop plants *i.e.* the major pillar of food security. As per the data, a 16% share of total yield loss is caused by the microbes annually. Within this, a hefty loss of more than 70% arises due to unavoidable fungal infections. The establishment of proper loss assessment as well as research in this field is important for the process of crop improvement. In this regard, many researchers through their efforts have identified key genes, factors, small RNAs, and epigenetic modifications that control the resistance mechanism. One such key regulator is a superfamily

Table 1. Case studies showing use of transgenic approach (over-expression or downregulation) to modify or manipulate the expression of WRKY TFs for plant defense against microbial diseases.



S. No.	Plant species (Acceptor)	Cultivar/ Line	Pathogen	WRKY name and source	Effect	References
1	*Gossypiumhirsutum* L.	Lumian 22	*Botrytis cinerea*	*Gh*WRKY25 (Cotton)	Enhanced sensitivity to pathogen by reduction in expression levels of SA or ET signaling related genes	Liu *et al.* 2016
2	*Brassica oleracea* var. *italica* L.	Bo112	*Hyaloperonosporaparasitica*	*Bo*WRKY6 (Broccoli)	Increased resistance with enhanced PR1levels	Jiang *et al.* 2016
3	*Nicotianatabacum* L.	Xanthi	*Botrosphaeriadothidea,Gibberellamoniliformis,Colleotrichumgloeosporioides, Fusariumoxysporum*	*Js*WRKY1 (Iron walnut)	Enhanced expression of several defense-related genes (SOD, APX, POD and PR1)	Wang *et al.* 2016
4	*Solanumtuberosum*L.	E-potato 3	*Phytophthorainfestans*	*St*WRKY1 (Potato)	Elevated resistance due to upregulation of PR genes	Shahzad*et al.* 2016
5	*Populustrichocarpa* Torr. & A. Gray, *Populustomentosa* Carr.	Clone 741	*Melampsora sp.*	*Ptr*WRKY18, PtrWRKY35 (Poplar)	Elevated resistance due to upregulation of *PR1.1, PR1.4* and *PR5.1*	Jiang *et al.* 2017
6	*Arabidopsis thaliana* L.	Columbia-0	*B.cinerea, Pseudomonas syringae* pv. *tomato* DC3000, *Golovinomycescichoracearum*	*Vq*WRKY5 (Wild grape)	Overexpressed lines showed enhanced resistance to the biotrophs only	Wang *et al.* 2017
7	*A. thaliana* L.	Columbia-0	*G. cichoracearum, B. cinerea*	*Vl*WRKY3 (Grape)	Increased susceptibility to *B.cinerea* and decreased susceptibility to *G. cichoracearum*	Guo*et al.* 2018
8	*Brassica napus*L.	Westar	*Sclerotiniasclerotiorum*	*Bn*WRKY15, *Bn*WRKY33 (Oilseed rape)	Overexpression lead to modulated expression of both SA□ and JA-regulated genes	Liu *et al.* 2018
9	*Oryzasativa L.*	Nipponbare,	*Magnaportheoryzae,Xanthomonasor*	*Os*WRKY67	Overexpression lines showed	Liu *et al.* 2018



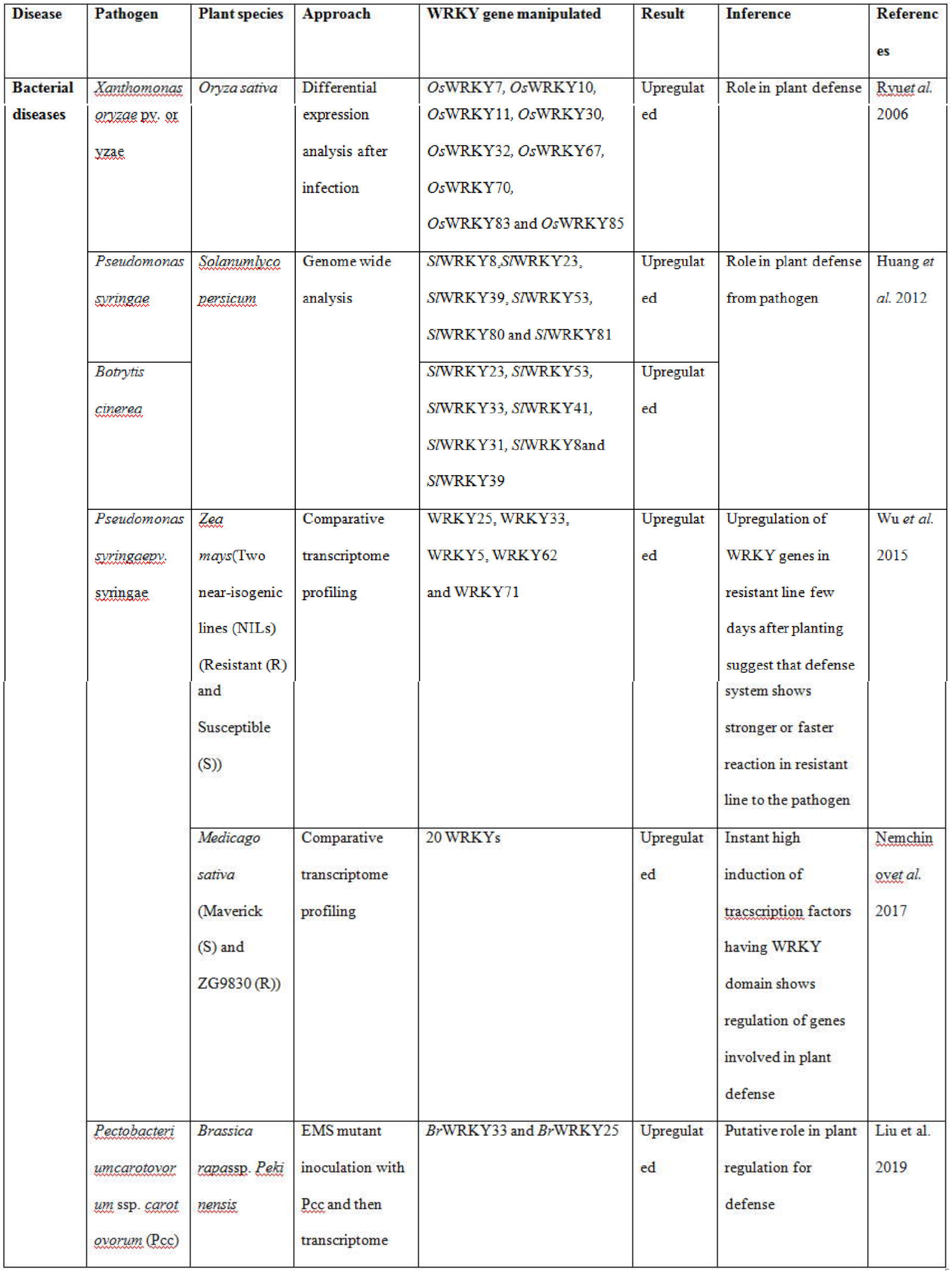
		BC10	*yzaepv. oryzae*	(Rice)	quantitatively enhanced resistance to leaf blast, panicle blast and bacterial blight. *OsWRKY67*-silencedlines showed increased susceptibility to blast and bacterial blight diseases	
10	*G. hirsutum*L.	Xinluzao 7, Zhongzhimian 2	*Verticilliumdahliae*	*GhWRKY70* (Cotton)	Silencing of *GhWRKY70* increased the resistance whereas *GhWRKY70*overexpression *lines* showed reduction in tolerance	Xionget *al.* 2019
11	*Arabidopsis suecica*L.	At4, Aa, Allo733	*Pseudomonas syringae*	WRKY18, WRKY40 (Thalecress)	Overexpression lines showed upregulation of several SA pathway􀀀related genes	Abeysingheet *al.* 2019
12	*G.hirsutum*L.	Williams 82	*Phytophthorasojae*	*GmWRKY40* (Cotton)	Overexpressionhairy root lines showed enhanced resistance whereas silencing lines showed enhanced susceptibility along with ROS accumulation	Cui *et al.* 2019
13	*Cucumissativus* L.	XintaiMiCi	*Pseudoperonosporacubensis*	*Cs*WRKY50 (Cucumber)	Overexpression lines showed enhanced resistance with less ROS accumulationand higher expression levels of antioxidant enzymes.	Luan *et al.* 2019
14	*A. thaliana* L.	Columbia-0, *wrky30*mutant	*Cucumber mosaic virus*	*At*WRKY30(Thalecress)	Overexpression lines showed enhanced resistance whereas silencing lines showed enhanced susceptibility	Zou*et al.* 2019
15	*Solanumlycopersicum* L.	Ailsa Craig	*P.syringae* pv. *tomato* DC3000	*Sl*WRKY8 (Tomato)	Overexpression lines showed enhanced resistance to the pathogen with enhanced *PR1a1* and *PR7*levels	Gao*et al.* 2020
16	*S.tuberosum*L.	E-potato 3	*P.infestans*	StWRKY2 (Potato)	Overexpression lead to enhanced resistance in the potato through the induction of PR proteins	Shahzad*et al.* 2020
17	*Paeonialactiflora*Pall.	Da Fugui	*Alternariatenuissima*	*Pl*WRKY65 (Chinese peony)	Silencing lines showed enhanced susceptibility along with changes in JA and SA content	Wang *et al.* 2020

of WRKY transcription factors that have been reported to control key processes in plants including resistance to biotic stresses (**Figure 1, 2**). Due to the dedicated scientific research more than two decades, the connection of WRKY TFs with plant defense and immunity has been established. Interestingly, it has already been highlighted that WRKY TFs are responsive in many stresses and their modification or manipulation increased the plant tolerance towards specific stresses.

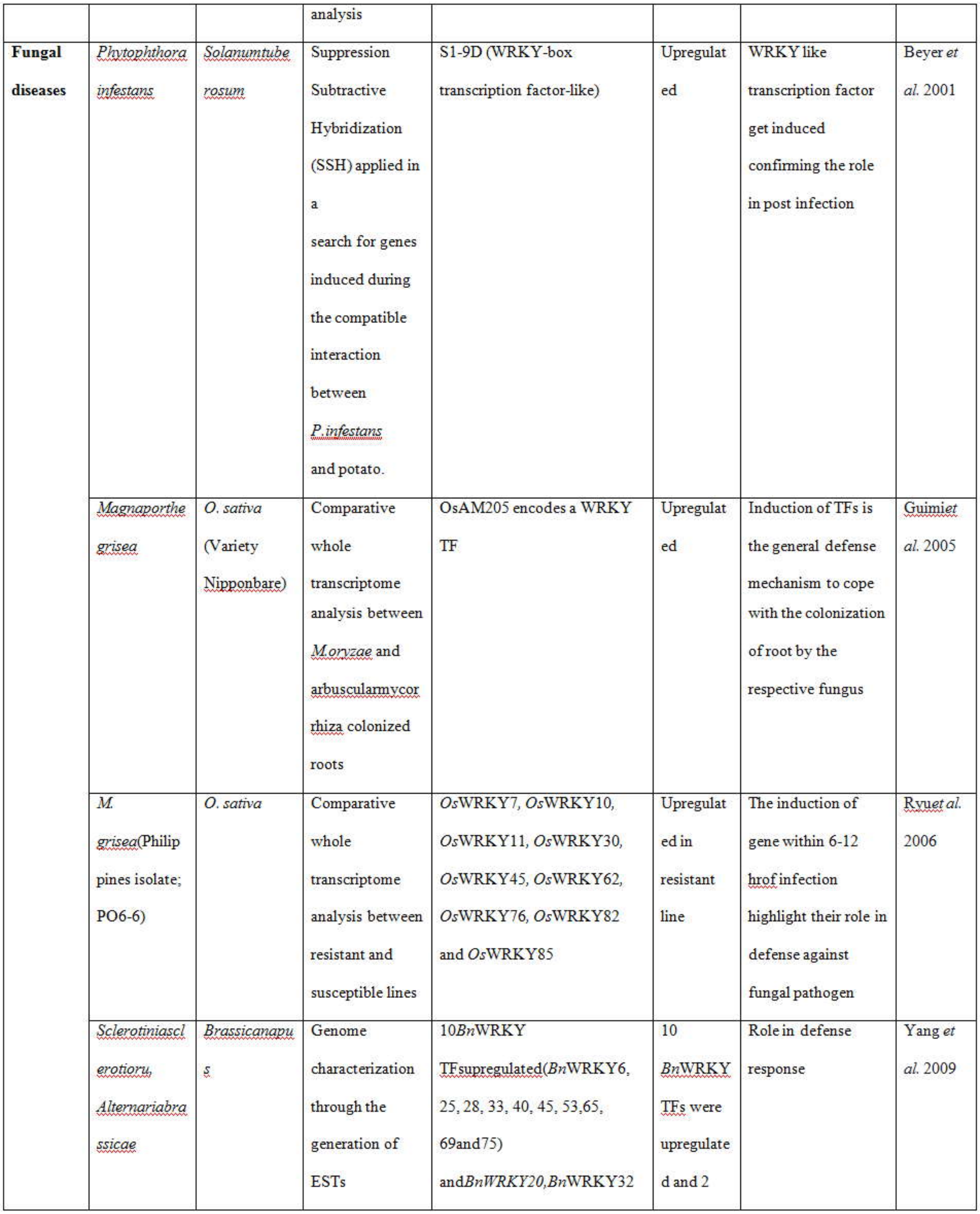
The data in the table also highlights the importance of characterization of the gene in one crop (source) as the same gene can be expressed in acceptor crop for enhancing resistance. For more details, the readers are encouraged to go through the references.

Furthermore, multiple transcriptome studies of treated or challenged samples have provided a list of putative candidate genes in many crop species that will be considered for ensuring food security.

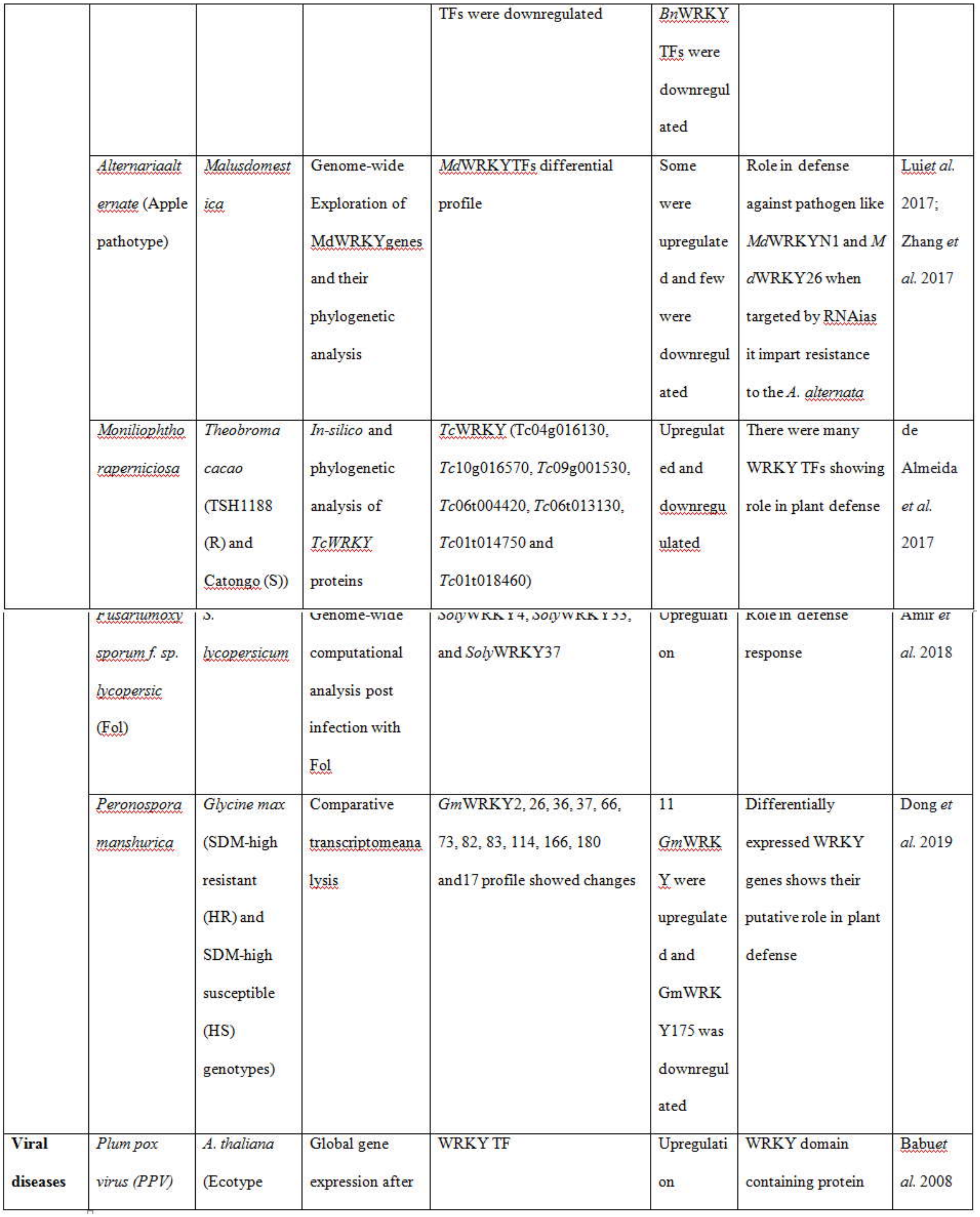
Table 2. Descriptive case studies showing importance of the member of WRKY TF family in plant defense against microbial diseases.



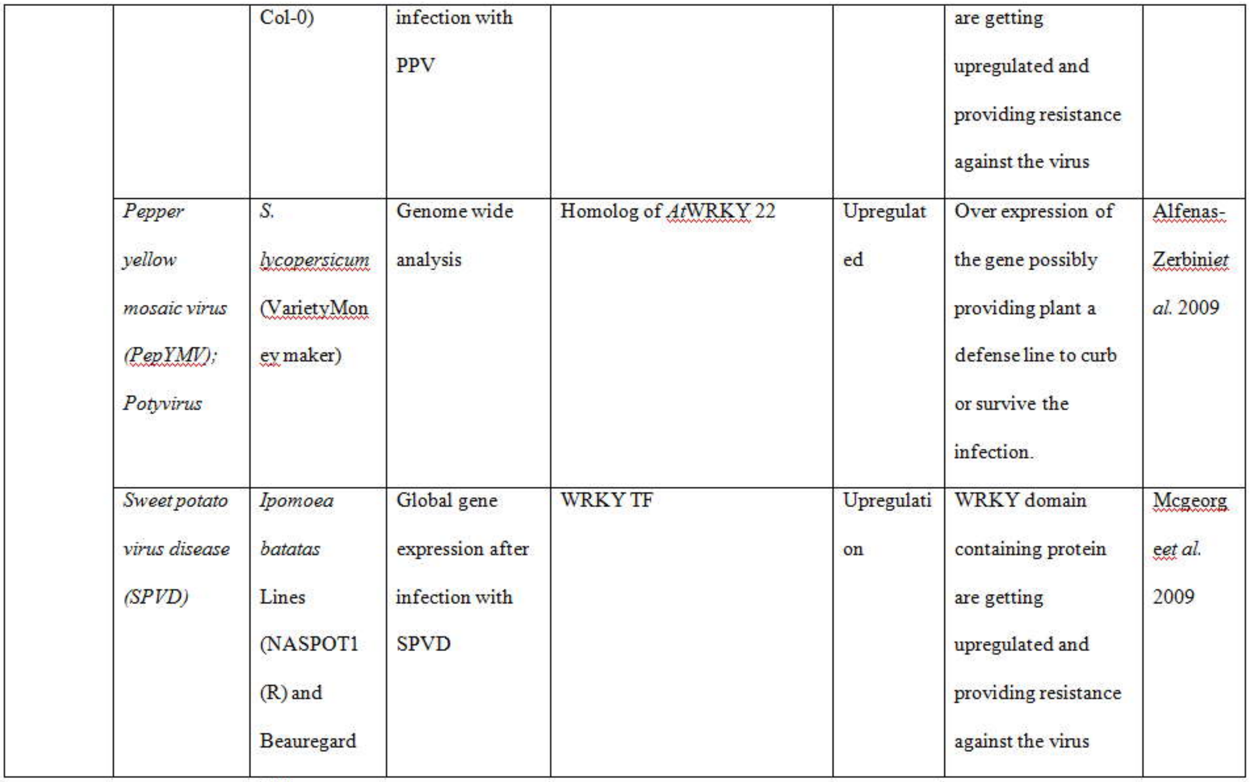
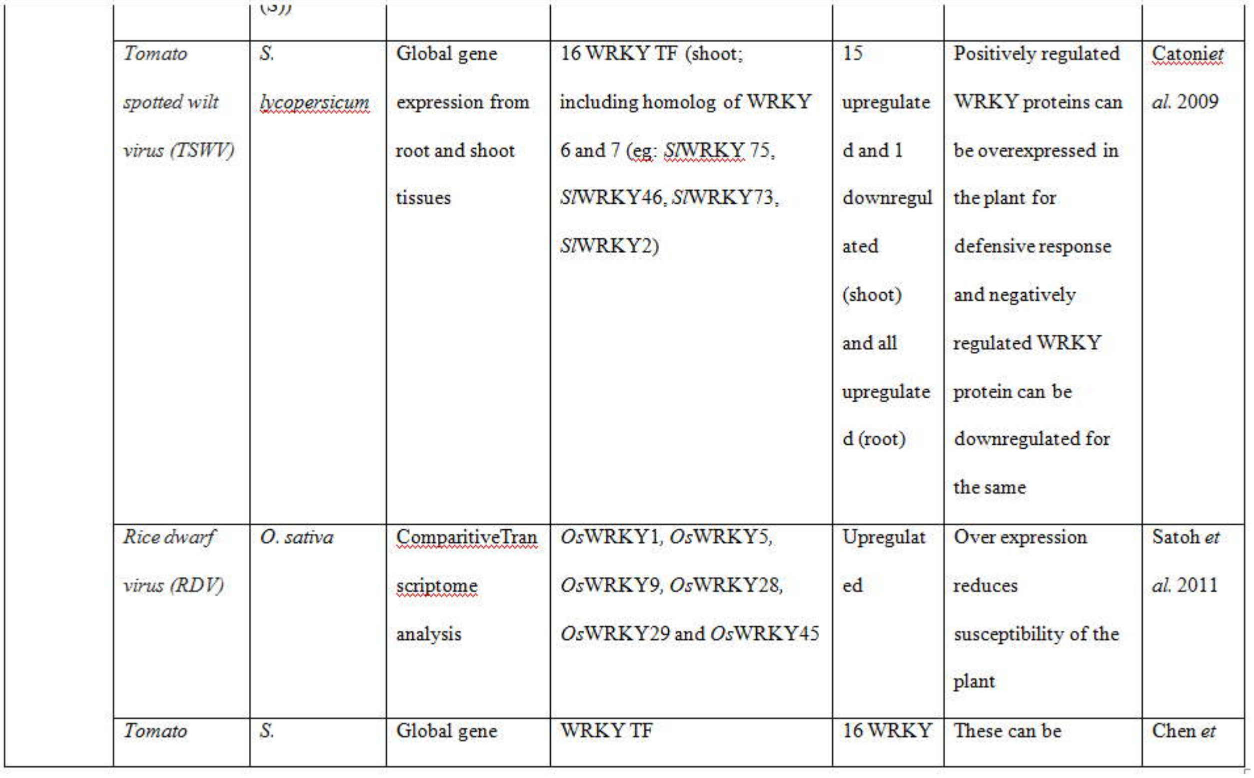
Disease	Pathogen	Plant species	Approach	WRKY gene manipulated	Result	Inference	References
Bacterial diseases	*Xanthomonas oryzae* pv. *oryzae*	*Oryza sativa*	Differential expression analysis after infection	*Os*WRKY7, *Os*WRKY10, *Os*WRKY11, *Os*WRKY30, *Os*WRKY32, *Os*WRKY67, *Os*WRKY70, *Os*WRKY83 and *Os*WRKY85	Upregulated	Role in plant defense	Ryu*et al.* 2006
	Pseudomonas syringae	*Solanumlycopersicum*	Genome wide analysis	*Sl*WRKY8,*Sl*WRKY23, *Sl*WRKY39, *Sl*WRKY53, *Sl*WRKY80 and *Sl*WRKY81	Upregulated	Role in plant defense from pathogen	Huang *et al.* 2012
	Botrytis cinerea			*Sl*WRKY23, *Sl*WRKY53, *Sl*WRKY33, *Sl*WRKY41, *Sl*WRKY31, *Sl*WRKY8and *Sl*WRKY39	Upregulated		
	*Pseudomonas syringae*pv. *syringae*	*Zea mays*(Two near-isogenic lines (NILs) (Resistant (R) and Susceptible (S))	Comparative transcriptome profiling	WRKY25, WRKY33, WRKY5, WRKY62 and WRKY71	Upregulated	Upregulation of WRKY genes in resistant line few days after planting suggest that defense system shows stronger or faster reaction in resistant line to the pathogen	Wu *et al.* 2015
		Medicago sativa (Maverick (S) and ZG9830 (R))	Comparative transcriptome profiling	20 WRKYs	Upregulated	Instant high induction of tracscription factors having WRKY domain shows regulation of genes involved in plant defense	Nemchinov*et al.* 2017
	Pectobacterium carotovorum ssp. *carotovorum* (Pcc)	*Brassica rapa*ssp. *Pekinensis*	EMS mutant inoculation with Pcc and then transcriptome	*Br*WRKY33 and *Br*WRKY25	Upregulated	Putative role in plant regulation for defense	Liu et al. 2019



			analysis				
Fungal diseases	*Phytophthora infestans*	*Solanumtuberosum*	Suppression Subtractive Hybridization (SSH) applied in a search for genes induced during the compatible interaction between *P.infestans* and potato.	S1-9D (WRKY-box transcription factor-like)	Upregulated	WRKY like transcription factor get induced confirming the role in post infection	Beyer *et al.* 2001
	Magnaporthe grisea	*O. sativa* (Variety Nipponbare)	Comparative whole transcriptome analysis between *M.oryzae* and arbuscularmycorrhiza colonized roots	OsAM205 encodes a WRKY TF	Upregulated	Induction of TFs is the general defense mechanism to cope with the colonization of root by the respective fungus	Guimi*et al.* 2005
	M. grisea(Philippines isolate; PO6-6)	*O. sativa*	Comparative whole transcriptome analysis between resistant and susceptible lines	*Os*WRKY7, *Os*WRKY10, *Os*WRKY11, *Os*WRKY30, *Os*WRKY45, *Os*WRKY62, *Os*WRKY76, *Os*WRKY82 and *Os*WRKY85	Upregulated in resistant line	The induction of gene within 6-12 hrof infection highlight their role in defense against fungal pathogen	Ryu*et al.* 2006
	Sclerotiniasclerotioru, Alternariabrassicae	*Brassicanapus*	Genome characterization through the generation of ESTs	10*Bn*WRKY TFsupregulated(*Bn*WRKY6, 25, 28, 33, 40, 45, 53,65, 69and75) and*BnWRKY20*,*Bn*WRKY32	10 *Bn*WRKY TFs were upregulated and 2	Role in defense response	Yang *et al.* 2009



				TFs were downregulated	*Bn*WRKY TFs were downregulated		
	Alternariaalternate (Apple pathotype)	*Malusdomestica*	Genome-wide Exploration of MdWRKYgenes and their phylogenetic analysis	*Md*WRKYTFs differential profile	Some were upregulated and few were downregulated	Role in defense against pathogen like *Md*WRKYN1 and *Md*WRKY26 when targeted by RNAias it impart resistance to the *A. alternata*	Lui*et al.* 2017; Zhang *et al.* 2017
	Moniliophthoraperniciosa	*Theobroma cacao* (TSH1188 (R) and Catongo (S))	*In-silico* and phylogenetic analysis of *TcWRKY* proteins	*Tc*WRKY (Tc04g016130, *Tc*10g016570, *Tc*09g001530, *Tc*06t004420, *Tc*06t013130, *Tc*01t014750 and *Tc*01t018460)	Upregulated and downregulated	There were many WRKY TFs showing role in plant defense	de Almeida *et al.* 2017
	Fusariumoxysporum f. sp. *lycopersic* (Fol)	*S. lycopersicum*	Genome-wide computational analysis post infection with Fol	*Soly*WRKY4, *Soly*WRKY33, and *Soly*WRKY37	Upregulation	Role in defense response	Amir *et al.* 2018
	Peronospora manshurica	*Glycine max* (SDM-high resistant (HR) and SDM-high susceptible (HS) genotypes)	Comparative transcriptomeanalysis	*Gm*WRKY2, 26, 36, 37, 66, 73, 82, 83, 114, 166, 180 and17 profile showed changes	11 *Gm*WRKY were upregulated and GmWRKY175 was downregulated	Differentially expressed WRKY genes shows their putative role in plant defense	Dong *et al.* 2019
Viral diseases	*Plum pox virus (PPV)*	*A. thaliana* (Ecotype	Global gene expression after	WRKY TF	Upregulation	WRKY domain containing protein	Babu*et al.* 2008

		Col-0)	infection with PPV			are getting upregulated and providing resistance against the virus	
	Pepper yellow mosaic virus (PepYMV); Potyvirus	*S. lycopersicum* (VarietyMoney maker)	Genome wide analysis	Homolog of *At*WRKY 22	Upregulated	Over expression of the gene possibly providing plant a defense line to curb or survive the infection.	Alfenas-Zerbini*et al.* 2009
	Sweet potato virus disease (SPVD)	*Ipomoea batatas* Lines (NASPOT1 (R) and Beauregard (S))	Global gene expression after infection with SPVD	WRKY TF	Upregulation	WRKY domain containing protein are getting upregulated and providing resistance against the virus	Mcgeorgeet *al.* 2009
	Tomato spotted wilt virus (TSWV)	*S. lycopersicum*	Global gene expression from root and shoot tissues	16 WRKY TF (shoot; including homolog of WRKY 6 and 7 (eg: *Sl*WRKY 75, *Sl*WRKY46, *Sl*WRKY73, *Sl*WRKY2)	15 upregulated and 1 downregulated (shoot) and all upregulated (root)	Positively regulated WRKY proteins can be overexpressed in the plant for defensive response and negatively regulated WRKY protein can be downregulated for the same	Catoni*et al.* 2009
	Rice dwarf virus (RDV)	*O. sativa*	ComparitiveTranscriptome analysis	*Os*WRKY1, *Os*WRKY5, *Os*WRKY9, *Os*WRKY28, *Os*WRKY29 and *Os*WRKY45	Upregulated	Over expression reduces susceptibility of the plant	Satoh *et al.* 2011
	Tomato	*S.*	Global gene	WRKY TF	16 WRKY	These can be	Chen *et*

	yellow leaf curl virus (TYLCV)	*lycopersicum* (Lines CLN2777A (R) & TMXA48-4-0 (S))	expression after infection with TYLCV		genes downregulated (S) and 7 upregulated (R)	possible candidate for identification and characterization of new gene	*al.* 2013
	Tomato yellow leaf curl virus (TYLCV)	*S. lycopersicum*	Subcellular localization analysis	WRKY41, WRKY42, WRKY53, WRKY54, WRKY80, and WRKY81	Upregulated	Role in plant defense	Huang *et al.* 2016
	Papaya ringspot virus (PRSV)	*Carica papaya*	qRT-PCR of stressed samples	TF12.199, TF807.3, TF21.156 and TF18.51	Upregulated	Over expression reduces susceptibility of the plant	Pan *et al.* 2014
	Ugandan cassava brown streak virus (UCBSV)	*Manihotesculentus* (Lines Namikonga(R) and Albert (S))	Time-course analysis of transcriptome analysis	WRKY	Upregulated	Role in plant defense	Amugeet *al.* 2017
	Cucumber green mottle mosaic virus (CGMMV)	*Citrulluslanatus*	RNASeq	WRKY13, WRKY31, WRKY46, WRKY48, WRKY53, and WRKY70	Upregulated	Plant defense	Li *et al.* 2017
	Soybean mosaic virus	*G. max*	Transcriptome analysis	WRKY genes (WRKY33 and WRKY62)	Upregulated	Plant defense	Zhang *et al.* 2019
	Rice tungro bacilliform virus	*O. sativa*	ComparitiveTranscriptome analysis	*LOC_Os05g25770,LOC_Os08g38990, LOC_Os09g25060, and LOC_Os11g02520*	Upregulated	Over expression reduces susceptibility of the plant	Kumar and Dasgupta 2020

The members of the WRKY TF family expressed under specific stress condition are putative candidates that can be tested for gaining resistance against plant microbial pathogens

In this regard, the science of molecular breeding has the potential to accelerate process of resistant line development for sustainable agricultural practices. To accelerate the research and to screen the putative candidates, CRISPR genome editing can be put to use, as there are many negative regulators of the WRKY family that can be downregulated for achieving resistance in the plant.

Acknowledgements

All the authors wish to thank DrBaljinder Singh (NIPGR, New Delhi, India) for critical reading of the manuscript. S.G. wants to acknowledge the administration of Hans Raj College and Principal Dr Rama specially. Furthermore, all the authors together acknowledge the peer reviewers in advance.

Declarations

J.B. and S.M. acknowledges the University Grants Commission (UGC) and Council of Scientific & Industrial Research (CSIR) for awarding a Senior Research Fellowship respectively.

The authors declare no conflict of interests.

References

1. Abeysinghe, J.K., Lam, K.M., & Ng, D.W.K. (2019). Differential regulation and interaction of homoeologous WRKY 18 and WRKY 40 in Arabidopsis allotetraploids and biotic stress responses. *The Plant Journal*, 97(2), 352-367.
2. Alfenas-Zerbini, P., Maia, I.G., Fávaro, R.D., Cascardo, J.C., Brommonschenkel, S.H., & Zerbini, F.M. (2009). Genome-wide analysis of differentially expressed genes during the early stages of tomato infection by a potyvirus. *Molecular plant-microbe interactions*, 22(3), 352-361.
3. Amuge, T., Berger, D.K., Katari, M.S., Myburg, A.A., Goldman, S.L., & Ferguson, M.E. (2017). A time series transcriptome analysis of cassava (Manihotesculenta Crantz) varieties challenged with Ugandan cassava brown streak virus. *Scientific reports*, 7(1), 1-21.
4. Babu, M., Griffiths, J.S., Huang, T.S., & Wang, A. (2008). Altered gene expression changes in Arabidopsis leaf tissues and protoplasts in response to Plum pox virus infection. *BMC genomics*, 9(1), 325.
5. Beyer, K., Binder, A., Boller, T.,&Collinge, M. (2001). Identification of potato genes induced during colonization by Phytophthora infestans. *Molecular Plant Pathology*, 2(3), 125-134.
6. Catoni, M., Miozzi, L., Fiorilli, V., Lanfranco, L., & Accotto, G.P. (2009). Comparative analysis of expression profiles in shoots and roots of tomato systemically infected by Tomato spotted wilt virus reveals organ-specific transcriptional responses. *Molecular plant-microbe interactions*, 22(12), 1504-1513.
7. Chen, L., Zhang, L., & Yu, D. (2010). Wounding-induced WRKY8 is involved in basal defense in Arabidopsis. *Molecular Plant-Microbe Interactions*, 23(5), 558-565.
8. Chen, L., Zhang, L., Li, D., Wang, F., & Yu, D. (2013). WRKY8 transcription factor functions in the TMV-cg defense response by mediating both abscisic acid and ethylene signaling in Arabidopsis. *Proceedings of the National Academy of Sciences*, 110(21), E1963-E1971.
9. Chen, X., Li, C., Wang, H., & Guo, Z. (2019). WRKY transcription factors: Evolution, binding, and action. *Phytopathology Research*, 1(1), 13.
10. Cui, X., Yan, Q., Gan, S., Xue, D., Wang, H., Xing, H., Zhao, J., & Guo, N. (2019). Gm WRKY40, a member of the WRKY transcription factor genes identified from Glycine max L., enhanced the resistance to Phytophthorasojae. *BMC Plant Biology*, 19(1), 1-15.
11. Dang, F., Wang, Y., She, J., Lei, Y., Liu, Z., Eulgem, T., Lai, Y., Lin, J., Yu, L., Lei, D., & Guan, D. (2014). Overexpression of CaWRKY27, a subgroup IIe WRKY transcription factor of Capsicum annuum, positively regulates tobacco resistance to Ralstoniasolanacearum infection. *Physiologiaplantarum*, 150(3), 397-411.
12. Dang, F.F., Wang, Y.N., Yu, L.U., Eulgem, T., Lai, Y.A.N., Liu, Z.Q., Wang, X.U., Qiu, A.L., Zhang, T.X., Lin, J.,& Chen, Y.S. (2013). CaWRKY40, a WRKY protein of pepper, plays an important role in the regulation of tolerance to heat stress and resistance to Ralstonia-solanacearum infection. *Plant, Cell & Environment*, 36(4), 757-774.
13. Dardick, C. (2007). Comparative expression profiling of Nicotianabenthamiana leaves systemically infected with three fruit tree viruses. *Molecular plant-microbe interactions*, 20(8), 1004-1017.
14. de Almeida, D.S.M., Do Amaral, D.O.J., Del-Bem, L.E., dos Santos, E.B., Silva, R.J.S., Gramacho, K.P., Vincentz, M., & Micheli, F. (2017). Genome-wide identification and characterization of cacao WRKY transcription factors and analysis of their expression in response to witches' broom disease.*PloS one*, 12(10).
15. Dellagi, A., Heilbronn, J., Avrova, A.O., Montesano, M., Palva, E.T., Stewart, H.E., Toth, I.K., Cooke, D.E., Lyon, G.D., & Birch, P.R.

(2000). A potato gene encoding a WRKY-like transcription factor is induced in interactions with Erwiniacarotovora subsp. atroseptica and Phytophthorainfestans and is coregulated with class I endochitinase expression. *Molecular plant-microbe interactions*, 13(10), 1092-1101.

16. Dong, H., Tan, J., Li, M., Yu, Y., Jia, S., Zhang, C., Wu, Y., & Liu, Y. (2019). Transcriptome analysis of soybean WRKY TFs in response to Peronosporamanshurica infection. *Genomics*, 111(6), 1412-1422.
17. Eulgem, T., Rushton, P.J., Robatzek, S., & Somssich, I.E. (2000). The WRKY superfamily of plant transcription factors. *Trends in plant science*, 5(5), 199-206.
18. Gao, Y.F., Liu, J.K., Yang, F.M., Zhang, G.Y., Wang, D., Zhang, L., Ou, Y.B., & Yao, Y.A. (2020). The WRKY transcription factor WRKY8 promotes resistance to pathogen infection and mediates drought and salt stress tolerance in Solanumlycopersicum. *Physiologiaplantarum*, 168(1), 98-117.
19. Güimil, S., Chang, H.S., Zhu, T., Sesma, A., Osbourn, A., Roux, C., Ioannidis, V., Oakeley, E.J., Docquier, M., Descombes, P., & Briggs, S.P. (2005). Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. *Proceedings of the National Academy of Sciences*, 102(22), 8066-8070.
20. Guo, R., Qiao, H., Zhao, J., Wang, X., Tu, M., Guo, C., Wan, R., Li, Z., & Wang, X. (2018). The grape VlWRKY3 gene promotes abiotic and biotic stress tolerance in transgenic Arabidopsis thaliana. *Frontiers in Plant Science*, 9, 545.
21. Guo, R., Yu, F., Gao, Z., An, H., Cao, X., &Guo, X. (2011). GhWRKY3, a novel cotton (Gossypiumhirsutum L.) WRKY gene, is involved in diverse stress responses. *Molecular biology reports*, 38(1), 49-58.
22. Huang, Y., Li, M.Y., Wu, P., Xu, Z.S., Que, F., Wang, F., & Xiong, A.S. (2016). Members of WRKY Group III transcription factors are important in TYLCV defense signaling pathway in tomato (Solanumlycopersicum). *BMC genomics*, 17(1), 788.
23. Huang, S., Gao, Y., Liu, J., Peng, X., Niu, X., Fei, Z., Ca, S., & Liu, Y. (2012). Genome-wide analysis of WRKY transcription factors in *Solanumlycopersicum.* Molecular Genetics and Genomics, 287, 495–513.
24. Hwang, S.H., Kwon, S.I., Jang, J.Y., Fang, I.L., Lee, H., Choi, C., Park, S., Ahn, I., Bae, S.C., & Hwang, D.J. (2016). OsWRKY51, a rice transcription factor, functions as a positive regulator in defense response against Xanthomonasoryzaepv.oryzae. *Plant cell reports*, 35(9), 1975-1985.
25. Ishiguro, S., & Nakamura, K. (1994). Characterization of a cDNA encoding a novel DNA-binding protein, SPF1, that recognizes SP8 sequences in the 52 upstream regions of genes coding for sporamin and β-amylase from sweet potato. *Molecular and General Genetics MGG*, 244(6), 563-571.
26. Jiang, M., Liu, Q.E., Liu, Z.N., Li, J.Z.,& He, C.M. (2016). Over-expression of a WRKY transcription factor gene BoWRKY6 enhances resistance to downy mildew in transgenic broccoli plants. *Australasian Plant Pathology*, 45(3), 327-334.
27. Jiang, Y., Guo, L., Ma, X., Zhao, X., Jiao, B., Li, C., &Luo, K. (2017). The WRKY transcription factors PtrWRKY18 and PtrWRKY35 promote Melampsora resistance in Populus. *Tree physiology,* 37(5), 665-675.
28. Kalde, M., Barth, M., Somssich, I.E.,&Lippok, B. (2003). Members of the Arabidopsis WRKY group III transcription factors are part of different plant defense signaling pathways. *Molecular Plant-Microbe Interactions*, 16(4), 295-305.
29. Kim, C.Y., Lee, S.H., Park, H.C., Bae, C.G., Cheong, Y.H., Choi, Y.J., Han, C.D., Lee, S.Y., Lim, C.O & Cho, M.J. (2000). Identification of rice blast fungal elicitor-responsive genes by differential display analysis. *Molecular plant-microbe interactions*, 13(4), 470-474.
30. Kuki, Y., Ohno, R., Yoshida, K., & Takumi, S. (2020). Heterologous expression of wheat WRKY transcription factor genes transcriptionally activated in hybrid necrosis strains alters abiotic and biotic stress tolerance in transgenic Arabidopsis. *Plant Physiology and Biochemistry*, https://doi.org/10.1016/j.plaphy.2020.02.029.
31. Kumar, G., &Dasgupta, I. (2020). Comprehensive molecular insights into the stress response dynamics of rice (Oryza sativa L.) during rice tungro disease by RNA-seq-based comparative whole transcriptome analysis. *Journal of*

Biosciences, 45(1), 27. Li, C., Li, D., Shao F., Lu, S. (2015). Molecular cloning and expression analysis of WRKY transcription factor genes in *Salvia militiorrhiza*. BMC Genomics 34, 533-537.

32. Li, X., An, M., Xia, Z., Bai, X..& Wu, Y. (2017). Transcriptome analysis of watermelon (Citrulluslanatus) fruits in response to Cucumber green mottle mosaic virus (CGMMV) infection. *Scientific reports*, 7(1), 1-12.
33. Liu, F., Li, X., Wang, M., Wen, J., Yi, B., Shen, J., Ma, C., Fu, T., &Tu, J. (2018). Interactions of WRKY 15 and WRKY 33 transcription factors and their roles in the resistance of oilseed rape to Sclerotinia infection. *Plant biotechnology journal*, 16(4), 911-925.
34. Liu, M., Wu, F., Wang, S., Lu, Y., Chen, X., Wang, Y., Gu, A., Zhao, J., &Shen, S. (2019). Comparative transcriptome analysis reveals defense responses against soft rot in Chinese cabbage. *Horticulture research, 6*(1), 1-18.
35. Liu, Q., Li, X., Yan, S., Yu, T., Yang, J., Dong, J., Zhang, S., Zhao, J., Yang, T., Mao, X., & Zhu, X. (2018). OsWRKY67 positively regulates blast and bacteria blight resistance by direct activation of PR genes in rice. *BMC plant biology*, 18(1), 1-13.
36. Luan, Q., Chen, C., Liu, M., Li, Q., Wang, L., &Ren, Z. (2019). CsWRKY50 mediates defense responses to Pseudoperonosporacubensis infection in Cucumissativus. *Plant Science*, 279, 59-69.
37. Lui, S., Luo, C., Zhu, L., Sha, R., Qu, S., Cai, B., & Wang, S. (2017). Identification and expression analysis of WRKY transcription factor genes in response to fungal pathogen and hormone treatments in apple (Malusdomestica). *Journal of Plant Biology*, 60(2), 215-230.
38. Madhusudhan, P., Sinha, P., Rajput, L.S., Bhattacharya, M., Sharma, T., Bhuvaneshwari, V., Gaikwad, K., Krishnan, S.G., & Singh, A.K. (2019). Effect of temperature on Pi54-mediated leaf blast resistance in rice. *World Journal of Microbiology and Biotechnology*, 35(10), 148.
39. Madroñero, J., Rodrigues, S.P., Antunes, T.F.S., Abreu, P.M.V., Ventura, J.A., Fernandes, A.A.R., & Fernandes, P.M.B. (2018). Transcriptome analysis provides insights into the delayed sticky disease symptoms in Carica papaya. *Plant Cell Rep*. 37(7), 967-980.
40. McGregor, C.E., Miano, D.W., LaBonte, D.R., Hoy, M., Clark, C.A., & Rosa, G.J. (2009). Differential gene expression of resistant and susceptible sweetpotato plants after infection with the causal agents of sweet potato virus disease. *Journal of the American Society for Horticultural Science*, 134(6), 658-666.
41. Mittal, S., Banduni, P., Mallikarjuna, M.G., Rao, A.R., Jain, P.A., Dash, P.K.,&Thirunavukkarasu, N. (2018). Structural, functional, and evolutionary characterization of major drought transcription factors families in maize. *Frontiers in chemistry*, 6, 177.
42. Mohanta, T.K., Park, Y.H., &Bae, H. (2016). Novel genomic and evolutionary insight of WRKY transcription factors in plant lineage. *Scientific reports*, 6(1), 1-22.
43. Nemchinov, L.G., Shao, J., Lee, M.N., Postnikova, O.A.,&Samac, D.A. (2017). Resistant and susceptible responses in alfalfa (Medicago sativa) to bacterial stem blight caused by Pseudomonas syringaepv. syringae. *PloS one*, 12(12).
44. Pan, L.J., & Jiang, L. (2014). Identification and expression of the WRKY transcription factors of Carica papaya in response to abiotic and biotic stresses. *Molecular biology reports*, 41(3), 1215-1225.
45. Pajerowska-Mukhtar, K.M., Emerine, D.K., &Mukhtar, M.S. (2013). Tell me more: roles of NPRs in plant immunity. *Trends in plant science*, 18(7), 402-411.
46. Park, C.J., Shin, Y.C., Lee, B.J., Kim, K.J., Kim, J.K.,&Paek, K.H. (2006). A hot pepper gene encoding WRKY transcription factor is induced during hypersensitive response to Tobacco mosaic virus and Xanthomonascampestris. *Planta*, 223(2), 168-179.
47. Peng, X., Hu, Y., Tang, X., Zhou, P., Deng, X., Wang, H., & Guo, Z. (2012). Constitutive expression of rice WRKY30 gene increases the endogenous jasmonic acid accumulation, PR gene expression and resistance to fungal pathogens in rice. *Planta*, 236(5), 1485-1498.
48. Robatzek, S.,&Somssich, I.E. (2002). Targets of AtWRKY6 regulation during plant senescence and pathogen defense. *Genes & development*, 16(9), 1139-1149.
49. Rushton, P.J., Torres, J.T., Parniske, M., Wernert, P.,Hahlbrock, K., &Somssich, I.E. (1996). Interaction of elicitor induced DNA binding

proteins with elicitor response elements in the promoters of parsley PR1 genes. *The EMBO journal*, 15(20), 5690-5700.

50. Ryu, H.S., Han, M., Lee, S.K., Cho, J.I., Ryoo, N., Heu, S., Lee, Y.H., Bhoo, S.H., Wang, G.L., Hahn, T.R., &Jeon, J.S. (2006). A comprehensive expression analysis of the WRKY gene superfamily in rice plants during defense response. *Plant cell reports*, 25(8), 836-847.
51. Satoh, K., Shimizu, T., Kondoh, H., Hiraguri, A., Sasaya, T., Choi, I.R., Omura, T., & Kikuchi, S. (2011). Relationship between symptoms and gene expression induced by the infection of three strains of Rice dwarf virus. *PLoS One*, 6(3).
52. Satoh, K., Yoneyama, K., Kondoh, H., Shimizu, T., Sasaya, T., Choi, I.L., Yoneyama, K., Omura, T., & Kikuchi, S. (2013). Relationship between gene responses and symptoms induced by Rice grassy stunt virus. *Frontiers in microbiology*, 4, 313.
53. Shahzad, R., Ewas, M., Harlina, P.W., Nishawy, E., Ayaad, M., Manan, A., Maher, M.,&Khames, E. (2020). Multiple Stress Responsive WRKY Transcription Factor, StWRKY2, Enhances Drought and Late Blight Resistance in Transgenic Potato.*International journal of Agriculture & Biology*, 24, 154 164.
54. Shahzad, R., Harlina, P.W., Cong hua, X., Ewas, M., Nishawy, E., Zhenyuan, P.,&Foly, M.M. (2016). Overexpression of potato transcription factor (StWRKY1) conferred resistance to Phytophthora infestans and improved tolerance to water stress. *Plant Omics*, 9(2), 149.
55. Shimono, M., Sugano, S., Nakayama, A., Jiang, C.J., Ono, K., Toki, S.,&Takatsuji, H. (2007). Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. *The Plant Cell*, 19(6), 2064-2076.
56. The American Phytopathological Society(APS) (2020). https://www.apsnet.org/Pages/default.aspx**.** Accessed 10th July, 2020.
57. Wang, G., Chen, C., Li, J., Pu, L., Guan, R., Ge, F., & Liu, D. (2016). Overexpression of a WRKY Transcription Factor Gene from Juglanssigillata Dode Confers Resistance to Colletotrichumgloeosporioides in Transgenic Tobacco Plants. *ActaAgriculturaeBoreali-Sinica*, (3), 26.
58. Wang, J., Tao, F., Tian, W., Guo, Z., Chen, X., Xu, X., Shang, H., & Hu, X. (2017). The wheat WRKY transcription factors Ta WRKY49 and Ta WRKY62 confer differential high-temperature seedling-plant resistance to Pucciniastriiformis f. sp. tritici. *PloS one*, 12(7).
59. Wang, X., Guo, R., Tu, M., Wang, D., Guo, C., Wan, R., Li, Z., & Wang, X. (2017). Ectopic expression of the wild grape WRKY transcription factor VqWRKY52 in Arabidopsis thaliana enhances resistance to the biotrophic pathogen powdery mildew but not to the necrotrophic pathogen Botrytis cinerea. *Frontiers in plant science,* 8, 97.
60. Wang, X., Li, J., Guo, J., Qiao, Q., Guo, X., & Ma, Y. (2020). The WRKY transcription factor PlWRKY65 enhances the resistance of Paeonialactiflora (herbaceous peony) to Alternariatenuissima. *Horticulture Research*, 7(1), 1-12.
61. Xiong, X., Sun, S., Li, Y., Zhang, X., Sun, J.,&Xue, F. (2019). The cotton WRKY transcription factor GhWRKY70 negatively regulates the defense response against Verticilliumdahliae. *The Crop Journal*, 7(3), 393-402.
62. Yamasaki, K., Kigawa, T., Seki, M., Shinozaki, K., & Yokoyama, S. (2013). DNA-binding domains of plant-specific transcription factors: structure, function, and evolution. *Trends in plant science*, 18(5), 267-276.
63. Yamasaki, K., Kigawa, T., Watanabe, S., Inoue, M., Yamasaki, T., Seki, M., Shinozaki, K., & Yokoyama, S. (2012). Structural basis for sequence-specific DNA recognition by an Arabidopsis WRKY transcription factor. *Journal of Biological Chemistry*, 287(10), 7683-7691.
64. Yang, B., Rahman, M.H., Deyholos, M.K., Kav, N.N., & Jiang, Y. (2009). Identification and expression analysis of WRKY transcription factor genes in Brassica napus (canola) in response to fungal pathogens and hormone treatments.*BMC plant biology*, 9(1), 68.
65. Yoda, H., Ogawa, M., Yamaguchi, Y., Koizumi, N., Kusano, T., & Sano, H. (2002). Identification of early-responsive genes associated with the hypersensitive response to tobacco mosaic virus and characterization of a WRKY-type transcription factor in tobacco plants. *Molecular genetics and genomics*, 267(2), 154-161.
66. Yokotani, N., Sato, Y., Tanabe, S., Chujo, T., Shimizu, T., Okada, K., Yamane, H., Shimono, M.,

Sugano, S., Takatsuji, H., &Kaku, H. (2013). WRKY76 is a rice transcriptional repressor playing opposite roles in blast disease resistance and cold stress tolerance. *Journal of experimental botany*, 64(16), 5085-5097.

67. Yu, F., Huaxia, Y., Lu, W., Wu, C., Cao, X., &Guo, X. (2012). GhWRKY15, a member of the WRKY transcription factor family identified from cotton (*Gossypium hirsutum* L.), is involved in disease resistance and plant development. *BMC plant biology*, 12(1), 144.
68. Zhang, Y., & Wang, L. (2005). The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. *BMC evolutionary biology*, 5(1), 1.
69. Zhang, C., Grosic, S., Whitham, S.A., & Hill, J.H. (2012). The requirement of multiple defense genes in soybean Rsv1–mediated extreme resistance to Soybean mosaic virus. *Molecular Plant-Microbe Interactions*, 25(10), 1307-1313.
70. Zhang, J., Peng, Y., &Guo, Z. (2008). Constitutive expression of pathogen-inducible OsWRKY31 enhances disease resistance and affects root growth and auxin response in transgenic rice plants. *Cell Research*, 18(4), 508-521.
71. Zhang, L., Shang, J., Wang, W., Du, J., Li, K., Wu, X., Yu, L., Liu, C., Khaskheli, M.I., & Yang, W. (2019). Comparison of Transcriptome Differences in Soybean Response to Soybean Mosaic Virus under Normal Light and in the Shade. *Viruses*, 11(9), 793.
72. Zhang, Q., Li, Y., Zhang, Y., Wu, C., Wang, S., Hao, L., Wang, S., & Li, T. (2017). Md-miR156ab and Md-miR395 target WRKY transcription factors to influence apple resistance to leaf spot disease. *Frontiers in plant science*, 8, 526.
73. Zhang, Y., Yao, J.L., Feng, H., Jiang, J., Fan, X., Jia, Y.F., Wang, R., & Liu, C. (2019). Identification of the defense-related gene VdWRKY53 from the wild grapevine Vitisdavidii using RNA sequencing and ectopic expression analysis in Arabidopsis. *Hereditas*, 156(1), 14.
74. Zheng, Z., Mosher, S.L., Fan, B., Klessig, D.F.,& Chen, Z. (2007). Functional analysis of Arabidopsis WRKY25 transcription factor in plant defense against Pseudomonas syringae. *BMC plant Biology*, 7(1), 2.
75. Zou, L., Yang, F., Ma, Y., Wu, Q., Yi, K., & Zhang, D. (2019). Transcription factor WRKY30 mediates resistance to Cucumber mosaic virus in Arabidopsis. *Biochemical and biophysical research communications*, *517*(1), 118-124.