



ISSN (E): 2277-7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.23  
TPI 2023; SP-12(10): 2102-2112  
© 2023 TPI  
[www.thepharmajournal.com](http://www.thepharmajournal.com)  
Received: 07-07-2023  
Accepted: 11-08-2023

**RT Shende**  
Sardar Vallabhbhai Patel  
University of Agriculture,  
Meerut, Uttar Pradesh, India

**RM Shinde**  
Vasantrao Naik College of  
Agricultural Biotechnology,  
Dr. PDKV, Akola, Maharashtra,  
India

**DN Damse**  
Mahatma Phule Krishi  
Vidyapeeth, MPKV, Rahuri,  
Maharashtra, India

**PP Dande**  
Department of Microbiology,  
RA Arts, Shri Mithulalji  
Kacholiya Commerce  
& Shri Satyanarayanji  
Ramkrishnaji Rath  
Science College, Washim  
Maharashtra, India

**Corresponding Author:**  
**RM Shinde**  
Vasantrao Naik College of  
Agricultural Biotechnology,  
Dr. PDKV, Akola, Maharashtra,  
India

## Exploring biotic and abiotic responses in plants: A systems biology perspective on the role of WRKY transcription factors

**RT Shende, RM Shinde, DN Damse and PP Dande**

### Abstract

The WRKY transcription factor (TF) family is central to regulating plant development and responses to biotic and Abiotic stressors. Escalating stress frequencies, including temperature shifts, water scarcity, and soil salinity, emphasizes the need to enhance stress resilience in crucial crops. WRKY TFs mechanistically govern plant processes, like stomatal adjustment, photosynthesis fine-tuning, osmolyte accumulation, and stress-responsive gene activation. The conserved WRKY domain, marked by the "WRKYGQK" pattern, is pivotal for TF function. WRKY TFs exhibit structural flexibility due to sub classification based on WRKY domains and zinc-finger motifs. For biotic stress, WRKY TFs play a vital role in fortifying plant defenses against pathogens and herbivores. They activate defense genes via signal molecules like salicylic acid and jasmonic acid, mediate immune responses, and coordinate cross-talk between defense pathways, offering potential for pathogen-resistant crops. Regarding abiotic stress, WRKY TFs translate external stress signals into internal adjustments by interacting with specific DNA sequences (W-boxes) in gene promoters. They also engage in signal cross-talk, hormonal interactions, and post-translational modifications, expanding their influence. WRKY TFs contribute to trichome formation, seed germination, and senescence. In short this review discuss about, WRKY TFs are pivotal for plant stress resilience and regulate diverse physiological and developmental aspects. Integrating genomics, transcriptomics, proteomics, and metabolomics has deepened our understanding of WRKY TFs, offering biotechnological avenues to enhance crop resilience and global food security.

**Keywords:** WRKY family, transcription factors (TFs), plant growth, abiotic stresses, genetic engineering

### Introduction

The WRKY family of transcription factor (TF) is extensively distributed throughout plants that play crucial roles in the plants growth development and response to biotic and abiotic stresses. A global threat to essential crops is posed as plants experience increasing interactions with various stressors such severe temperatures, drought, and salinity. This has a significant impact on the growth and overall output of the crops. The WRKY TFs stand out among the variety of TF genes as possible candidates for selective crop breeding with the aim of enhancing stress resilience.

WRKY TFs work as molecular switches that regulate plant developmental pathways and speed up responses to various stresses. Plants undergo a variety of molecular, cellular, and physiological changes in response to stress, including the closing of stomata, a reduction in photosynthesis, an increase in the accumulation of osmolality, and the activation of numerous genes involved in stress responses. As a strategy to amplify stress resilience in crops, genetic manipulation has risen as a viable option, with numerous genes and TFs, including WRKY, AP2/ERF, and NAC, being pinpointed for their participation in responses to non-living stress factors.

The structural features and homology of the WRKY TFs are characterized by a conserved WRKY domain and DNA-binding motif. The WRKY domain has a Heptapeptide sequence WRKYGQK, enabling the TFs to bind to specific CIS-elements called W-boxes in the promoter regions of target genes, thereby regulating their expression in response to stresses. The WRKY TFs are classified into subgroups based on the number of WRKY domains and the presence of other conserved motifs like the zinc-finger-like motifs C2H2 and C2HC, which determine their functional specificity. This high diversity and evolutionary divergence within the WRKY family lead to distinct roles of different WRKY members in stress responses and developmental processes.

The WRKY family of TFs plays critical roles in plant stress responses, making them potential targets for improving stress tolerance in crops through genetic engineering. The conserved WRKY domain and DNA-binding motif, as well as their classification into subgroups based on structural features, contribute to the functional diversity and specificity of the WRKY TFs in regulating stress-responsive genes (Table 1). Understanding the structural features and homology of the WRKY TFs is vital for comprehending their complex roles in plant stress adaptation and crop improvement.

**Table 1:** Number of WRKY TFs genes n Plant

Sr. No.	Name of Plants	Number of WRKY TF gene
1.	<i>A. thaliana</i>	81
2.	<i>B. distachyon</i>	81
3.	<i>G. max</i>	179
4.	<i>M. esculenta</i>	117
5.	<i>M. domestica</i>	123
6.	<i>Z. mays</i>	180
7.	<i>O. sativa Indica</i>	116

### Structure of WRKY transcription factors

The structural attributes of WRKY transcription factors cover a compact domain of roughly 60 amino acids, recognized as the WRKY domain, which is a hallmark shared among all members of the WRKY protein family (Eulgem *et al.*, 2000) [18]. This distinctive domain features a conserved heptapeptide motif, WRKYGQK, situated at the N-terminus, and is accompanied by a unique zinc finger-like structure at the C-terminus. While WRKYGQK represents the predominant signature, it is noteworthy that there are over 35 variations of this motif, found across diverse species including both plants and non-plants (Eulgem *et al.*, 2000; Xie *et al.*, 2005b) [18, 120]. WRKY genes encode transcription factors that primarily reside in the cell nucleus, facilitated by a fundamental nuclear localization signal. These WRKY proteins display a marked preference for binding to a consensus sequence known as the W-box, represented by the motif TTGACC/T. This W-box motif is prevalent in the promoter regions of WRKY target genes, particularly those implicated in stress responses. Notably, the integrity of both the WRKY domain and the zinc finger motif is crucial for ensuring efficient DNA binding and regulatory functions (Maeo *et al.*, 2001) [60].

The categorization of WRKY proteins into three groups based on the presence of one or two WRKY domains remains a foundational classification scheme (Eulgem *et al.*, 2000) [18]. Furthermore, recent studies have highlighted the dynamic nature of WRKY proteins by identifying chimeric WRKY proteins. For example, AtWRKY52/RRS1 is a chimeric protein encompassing a group III WRKY domain and a TIR-NBS-LRR domain, thereby bridging transcriptional regulation with immune response pathways (Deslandes *et al.*, 2003) [9].

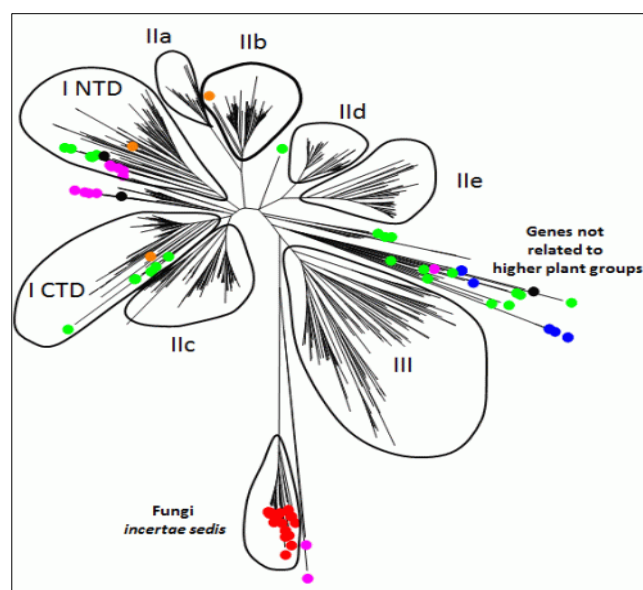
Recent advancements in structural biology, employing techniques such as cryo-electron microscopy and X-ray crystallography, have provided high-resolution insights into the structural attributes of WRKY transcription factors (Dai *et al.*, 2021; Meng *et al.*, 2022) [7, 65]. These studies have illuminated the intricate details of WRKY domain-DNA interactions and conformational dynamics, deepening our comprehension of their diverse functional roles in various biological processes.

The recent research has yielded valuable insights into the structural features of WRKY transcription factors, uncovering the nuanced interplay between their domains and their

involvement in governing gene expression. These new perspectives hold promise for enhancing our understanding of how WRKY proteins contribute to stress responses, developmental processes, and immune modulation in plants.

### The Evolution of WRKY Transcription Factors

The increasing accessibility to sequenced genomes has prompted a fresh examination of the evolutionary path of the WRKY TF protein family having crucial function in plant response to stresses and development. Plants, including modern ones, descended from charophyte green algae that colonized land around 430 to 470 million years ago. The genome sequence of *Klebsormidium flaccidum*, a charophyte, revealed interesting insights. While unicellular green algae usually possess 1-3 WRKY genes and mosses have around 30-40, *K. flaccidum* has only two. Surprisingly, one of them belongs to Group IIb, previously thought to appear in mosses. This suggests an earlier origin of this group. Interestingly, WRKY genes have also been found in non-plant organisms, like diplomonads, social amoebae, fungi, and amoebzoa. This sporadic distribution implies lateral gene transfer. These events seem to have occurred before the establishment of WRKY groups in flowering plants. Notably, flowering plants harbor proteins containing domains of both WRKY transcription factors and resistance (R) proteins. Such chimeric proteins, termed R protein-WRKY genes, have emerged multiple times in flowering plants, each restricted to specific lineages. These proteins possess unique combinations of domains, influencing signaling pathways and potentially diversifying or expediting signaling processes. To explain the evolution of WRKY transcription factors, two hypotheses are proposed. The "Group I Hypothesis" suggests that all WRKY genes evolved from Group I C-terminal WRKY domains. This hypothesis seeks to link the evolution of different WRKY groups. Alternatively, the "Group-Specific Hypothesis" suggests that each group evolved independently from different WRKY domain types. This hypothesis emphasizes the diversity among WRKY groups.



**Fig 1:** Evolution of WRKY TF image from Rinerson *et al.* (2015) [93]

The evolutionary relationships within the WRKY gene family are depicted in a phylogenetic tree constructed using a Neighbor Joining method and a Maximum Likelihood approach. This tree includes WRKY domains from diverse

species such as *Arabidopsis thaliana*, *Glycine max*, *Brachypodium distachyon*, and others. Each species is represented by distinct colors, such as red for fungal genes, green for unicellular green algae, blue for diplomonads, black for amoebozoa, purple for social amoebae, and orange for *Klebsormidium flaccidum*. The higher plant WRKY groups I-III are labeled, and Group I proteins are further distinguished by N-terminal (NTD) and C-terminal (CTD) domains. The construction of the tree involved advanced computational methods and the utilization of MEGA 6 software. Another Maximum Likelihood tree was also created, reinforcing the evolutionary insights drawn from this comprehensive analysis. This phylogenetic exploration provides a visual representation of the intricate relationships and diversification of WRKY genes across various organisms which were reported by Rinerson *et al.* in 2015<sup>[93]</sup>.

### Biological functions of WRKY transcription factors

Several investigations have underscored the importance of WRKY's in a myriad of plant phenomenon, encompassing developmental events, hormonal signaling cascades, and reactions to both biotic and abiotic stresses (Rushton *et al.*, 2010)<sup>[97]</sup>. It has been observed that a solitary WRKY transcription factor can orchestrate the rewiring of transcriptional patterns linked to various signaling pathways. Conversely, within a singular physiological process, several WRKY proteins have the capacity to function cooperatively or exhibit antagonistic behavior (Pandey and Somssich, 2009)<sup>[77]</sup>. The intricate web of signaling, with its multitude of inputs and outputs, constitutes the organized framework of WRKY factors (Rushton *et al.*, 2010)<sup>[97]</sup>.

### Role of WRKY TF in Biotic Stress

Biotic stressors, such as pathogenic infections and insect herbivory, pose significant threats to agricultural produce and food security. In response to these challenges, plants have developed complex defense strategies, which encompass the participation of WRKY factors. The WRKY family's diverse members regulate gene expression and signaling cascade that are pivotal for effective defense against various biotic stressors.

### WRKY Transcription Factors and Pathogen Response

WRKY TF has been comprehensively studied for their involvement in plant-pathogen interactions. They act as central players in orchestrating defense responses by regulating defense-related genes. WRKYs often function downstream of key regulators, such as salicylic acid (SA) and jasmonic acid (JA), in response to different classes of pathogens. Birkenbihl *et al.*, (2012)<sup>[2]</sup> reported that in *Arabidopsis*, WRKY33 is crucial for regulating defense through JA-dependent signaling against necrotrophic pathogens.

### WRKYs in Insect Herbivory and Insect-Plant Interactions

Beyond combating pathogenic infections, WRKY transcription factors are also involved in shielding plants from insect herbivores. They oversee both direct and indirect defense strategies, influencing the synthesis of secondary metabolites, anti-insect proteins, and volatile organic compounds. WRKYs further contribute to controlling the activation of genes associated with systemic acquired resistance (SAR) and induced systemic resistance (ISR) triggered by herbivore attack (Pandey *et al.*, 2017)<sup>[75]</sup>.

### Cross-Talk with Other Regulatory Pathways

The intricate nature of plant responses to biotic stress involves intricate interplay between different signaling pathways. WRKY transcription factors play a key role in connecting these pathways, especially those controlled by hormones like salicylic acid, jasmonic acid, and ethylene. This helps plants effectively adjust their defense responses to various challenges. They act as integrators of various signals, enabling plants to prioritize different defense strategies based on the nature of the biotic stress (Liu *et al.*, 2018)<sup>[55]</sup>.

### WRKYs in Effector-Triggered Immunity (ETI) and Pattern-Triggered Immunity (PTI)

WRKY TF are also involved in both effector-triggered immunity (ETI) and pattern-triggered immunity (PTI) responses. They regulate the expression of pathogen recognition receptors (PRRs) and downstream defense genes upon recognition of pathogen-associated molecular patterns (PAMPs) or effectors, contributing to the reinforcement of plant protection against pathogens (Lai *et al.*, 2013)<sup>[44]</sup>.

### Engineering Biotic Stress Tolerance

Insights from understanding WRKY-mediated defense responses paved the way for new strategies to increase crop resistance to biotic stressors. Genetic modification of WRKY genes by expression or gene therapy offers a promising method for breeding plants with good resistance to various diseases and pests (Ramamoorthy *et al.*, 2018)<sup>[87]</sup>. Additionally, in 2006 Jones and Dangl widely reported that plants develop a two-stage defense against invading pathogens. Immune interactions, termed MAMP-induced immunity (MTI) and effector-triggered immunity (ETI), result from the recognition of microbe-associated molecular patterns (MAMPs) or specific pathogens. Plant hormones such as jasmonic acid (JA) and salicylic acid (SA) modulate local responses and plant defenses elicited by ETI or MTI (Durrant and Dong, 2004; Pandey and Somssich, 2009)<sup>[15, 77]</sup>. This response includes the importance of transcriptional regeneration supported by transcriptional mechanisms, including the WRKY protein mentioned by Eulgem (2005)<sup>[19]</sup>, Naoumkina (2008)<sup>[72]</sup>, Ryu (2006)<sup>[100]</sup>, and Wang (2006)<sup>[113]</sup>. This reprogramming enables coordinated activation and suppression of different targets in plant resistance, highlighting the important role of WRKY factors as respected regulators at the center of the plant immune system, as noted by Eulgem and Somssich (2007)<sup>[21]</sup>.

Experimental studies using gain-of-function and loss-of-function methods have identified the important functions of WRKY proteins in complex immune systems. For example, as described by Deslandes *et al.*, the R genotype protein AtWRKY52 confers strong resistance to the pathogen *Ralstonia solanacearum* through nuclear interaction with the bacterial effector PopP2. (2002, 2003). In addition, AtWRKY52 confers dual resistance against fungal and bacterial infections through a synergistic effect with the R protein RPS4. AtWRKY70 is a co-factor in SA- and JA-dependent defense pathways and is essential for facilitating crosstalk between these pathways and promoting R gene-mediated resistance and receptor-like protein (RLP)-mediated immunity. (Li *et al.* 2004, 2006; Wang *et al.* 2006)<sup>[47, 49, 113]</sup> (Table 2). Lai *et al.* (2008)<sup>[43]</sup> pointed out that other positive regulators such as AtWRKY3, -4 and -33 play an important role in defense against necrotrophic bacteria such as *Botrytis cinerea* and *Alternaria brassicicola*. Recently, according to

Chen *et al.*, AtWRKY8 appeared to be a positive regulator of basal resistance against *Botrytis cinerea* and a negative regulator against *Pseudomonas syringae*. (2010a).

Although many insights into WRKY function come from the model dicot *Arabidopsis thaliana*, their importance in disease resistance extends to monocots such as rice and barley. For example, Ramamoorthy (2008) [87] and Ryu (2006) [100] showed that rice has many OsWRKY genes that respond to competitive pathogens, abiotic stress, and plant hormones. Published studies indicate that several WRKYs (OsWRKY3, -13, -31, -45, -53, -71 and -89) are involved in rice resistance to *Magnaporthe grisea* and/or *Xanthomonas oryzae*. as noted by oryzae, Chujo *et al.* (2007) [5], Liu *et al.* (2005, 2007) [56, 53], Qiu *et al.* (2007, 2008a) [82-83], Shimono *et al.* (2007) [106], Tau *et al.* (2009) [111], Wang *et al.* (2007) [115] and Zhang *et al.* (2008) [132]. For example, overexpression of OsWRKY13 increases resistance to Xoo and *Magnaporthe grisea* blight. Qi *et al.* showed that *Aspergillus oryzae* is controlled by modulating SA and JA pathways. (2007, 2008a). Similarly, OsWRKY71 is inducible by SA and its overexpression enhanced rice resistance to Xoo through indirect activation of OsPR1b and OsNPR1, as demonstrated by Liu *et al.* (2007) [53]. OsWRKY45, Shimono *et al.* (2007, 2011) [105-106]. Tai *et al.* showed that overexpression of japonica-derived WRKY45-1 increased susceptibility to Xoo, while indicator-derived WRKY45-2 promoted resistance. (2009). Chujo *et al.* Overexpression of OsWRKY53 protects rice plants against *M. grisea* and induces expression of PR proteins and peroxidase. (2007).

The effects of WRKY proteins on immunity may be different for certain diseases, as demonstrated for AtWRKY8. However, some WRKY transcription factors often act as negative regulators in plant defense responses. In *Arabidopsis*, AtWRKY18, -40 and -60 function in a partially redundant manner, negatively regulating resistance to the pathogen *Pseudomonas syringae*. For example, the wrky18wrky40 double mutant exhibits a strong resistance to the biotrophic fungus *Golovinomyces orontii*, while an increased susceptibility to the necrotrophic fungus *Botrytis cinerea*. This mutant exhibits a wide variety of immune-related diseases. Pandey *et al.* suggesting that activation of WRKY40 in the wrky18 wrky40 double mutant restores susceptibility, suggesting that the feedback inhibition system controls basal resistance. (2010). Similarly, barley orthologs HvWRKY1 and HvWRKY2 negatively regulate MTI and interact with the resistance protein MLA, resulting in

suppression of MTI in response to the fungal effector AVR10, as described by Eckey *et al.* (2004) [16] and Shen *et al.* (2007) [104].

As reported by Grunewald *et al.*, many other negative regulators in *Arabidopsis* include AtWRKY7, -11, -17, -23, -25, -27, -38, -48, -53, -58, and -62. Includes. (2008), Journot-Catalino *et al.* (2006) [36], Kim EJ *et al.* (2006, 2008) [39, 40], Mao *et al.* (2007) [62], Muhtar *et al.* (2008) [69], Wang *et al.* (2006) [113] and Xing *et al.* (2008) [124]. AtWRKY38 and -62 negatively regulate basal resistance in P. Kim *et al.* showed that the expression of *Syringa* is regulated by PKS5, an SNF1-related kinase. (2008) and Xie *et al.* (2010) [118]. Moreover, according to Journot-Catalino *et al.*, AtWRKY7, -11 and -17 play a negative role in the defensive response against *Pseudomonas syringae*. (2006) and Kim *et al.* (2006) [40]. In rice, several WRKY genes, including OsWRKY62 and OsWRKY76, act as negative regulators of the immune system, as suggested by Peng *et al.* (2008, 2010) [80, 79] and Seo *et al.* (2011) [103]. Overexpression of OsWRKY62 or OsWRKY76 impairs basal resistance and XA21-mediated resistance to Xoo, a blight-causing pathogen, as reported by Peng *et al.* (2008) [79] and Seo *et al.* (2011) [103]. Giacomelli *et al.* Note that recent findings from other plant species such as fruit, pepper, sunflower and coffee further highlight the importance of WRKY proteins in regulating biotic responses. (2010), Guo *et al.* (2011) [24], Li *et al.* (2010a) [46], Marchive *et al.* (2007) [63], Molan and El-Komy (2010) [68], Mzid *et al.* (2007) [71], Ramiro *et al.* (2010) [89], Ren *et al.* (2010b) [92], Skibbe *et al.* (2008) [107] Thiab van Eyck *et al.* (2010). For example, Li *et al.* showed that overexpression of VvWRKY1 and VvWRKY2 in grapevines reduced the susceptibility of tobacco to various fungi. (2010a), Marchive *et al.* (2007) [63] and Mzid *et al.* (2007) [71]. In pepper, CaWRKY1 appears to play a negative role in resistance, Oh *et al.* (2008) [73]. In addition, WRKY4 and WRKY8 have been recently described by Ishihama *et al.* (2011) [30] and Ren *et al.* (2010b) [92]. Skibbe *et al.* showed that NaWRKY3 and NaWRKY6 regulate the herbivore response in the natural tobacco *Nicotiana attenuata*. (2008). Van Eck *et al.* showed that silencing of TaWRKY53 in wheat by virus-induced gene silencing (VIGS) resulted in increased susceptibility to aphid infestation. (2010). The abundance of WRKY proteins is revealed by recent genome sequencing of many plants such as poplar, sorghum, papaya and kelp. However, their specific functions in plant protection need to be fully characterized, as discussed by Pandey and Somssich (2009) [77].

**Table 2:** List of WRKYs playing important role in biotic stresses

Sr. No.	Tolerance to Stress	Gene	Species	Mode of Expression	References
1	Bacterial Infection	AtWRKY33	<i>Arabidopsis</i>	Induced	Li, J., Brader, G., & Palva, E. T. (2018) [48]
2	Fungal Pathogens	PtWRKY45	Poplar	Induced	Chen, J., Xia, X., Yin, W., Wu, S., Li, P., & Dong, J. (2020) [3]
3	Soybean Cyst Nematode	GmWRKY27	Soybean	Induced	Zhang, Z., Xu, Y., Xie, Z., Li, X., He, Z., & Peng, X. (2018) [135]
4	Verticillium Wilt	GhWRKY40	Cotton	Induced	Ma, Q., <i>et al.</i> (2019) [59]
5	Rice Blast	OsWRKY45	Rice	Induced	Peng, X., <i>et al.</i> (2012) [78]
6	Powdery Mildew	TaWRKY33	Wheat	Induced	Li, C., <i>et al.</i> (2019) [45]

### WRKY in Abiotic Stresses

Abiotic stresses, such as drought, salinity, extreme temperatures, and heavy metal toxicity, significantly impact global agriculture and threaten crop productivity. Researchers have extensively investigated the intricate molecular mechanisms underlying plant responses to these challenges. Among the key regulators, the WRKY transcription factors

have gained attention for their vital task in modulating plant Abiotic stress.

The WRKY transcription factor family is characterized by the conserved WRKY domain and is central to plant stress responses. Recent research provides insights into the multifaceted functions of WRKY transcription factors in shaping gene expression and signaling cascades during abiotic

stress conditions.

Numerous studies reveal the direct interaction between WRKY transcription factors and the W-box motif (TTGACC/T) within stress-responsive gene promoters. This interaction precisely modulates genes involved in stress tolerance, osmotic adjustment, and antioxidant defense mechanisms (Pandey *et al.*, 2020; Liu *et al.*, 2021) [74, 52].

Emerging evidence highlights intricate crosstalk between WRKY transcription factors and various stress-related signaling pathways. Interaction between WRKY factors with other families such as MYB, AP2/ERF, and bZIP forms complex networks that intricately modulate plant reactions to abiotic stresses (Liu *et al.*, 2020) [54].

Recent advances emphasize the significance of post-translational modifications in regulating WRKY transcription factor activity under abiotic stress. Phosphorylation, acetylation, and sumoylation mechanisms govern WRKY factors' stability and DNA-binding capacity, modulating their regulatory roles (Chen *et al.*, 2022) [4].

Epigenetic alterations, encompassing DNA methylation and histone modifications, hold a pivotal significance in governing the regulation of WRKY genes during abiotic stress conditions. These changes contribute to the flexibility

and adaptability of WRKY-mediated stress responses, adding complexity to their functions (Zhang *et al.*, 2022) [131] (Table 3).

Research reveals intricate interplay between WRKY transcription factors and hormone signaling pathways, such as abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA). WRKY factors integrate hormonal signals with abiotic stress cues, enabling precise plant responses for optimal survival (Gupta *et al.*, 2021) [26].

The growing understanding of WRKY transcription factors has led to innovative strategies for enhancing stress tolerance in crops. Genetic engineering approaches, like WRKY gene over expression and regulatory network manipulation, hold promise for developing stress-resistant plants with improved agricultural productivity (Cui *et al.*, 2022) [6].

In brief, WRKY transcription factors play a pivotal role in orchestrating plant abiotic stress responses. Recent research unveils their intricate functions in gene regulation, signaling integration, post-translational modifications, epigenetic regulation, and hormone crosstalk. This knowledge offers potential avenues for engineering stress-tolerant crops and ensuring global food security.

**Table 3:** List of WRKYs in Plants Abiotic stress

Sr. No.	Tolerance to Stress	Gene	Species	Mode of expression	References
1.	Drought	AtWRKY57	<i>Arabidopsis thaliana</i>	Gain of Function Mutant	Jiang <i>et al.</i> (2012) <sup>[32]</sup>
		AtWRKY63/ABO3	<i>Arabidopsis thaliana</i>	Knockout	Ren <i>et al.</i> (2010) <sup>[91]</sup>
		HvWRKY38	Barley ( <i>Hordeum</i> )	Overexpression	Xiong <i>et al.</i> (2010) <sup>[125]</sup>
		OsWRKY05 OsWRKY43	<i>Oryza sativa</i>	Overexpression	Ramamoorthy <i>et al.</i> (2008) <sup>[87]</sup>
2.	Cold	SdSTHP64	Bitter sweet	Overexpression	Huang and Duman (2002) <sup>[28]</sup>
3.	Salinity	AtWRKY25 AtWRKY33	<i>Arabidopsis thaliana</i>	Knockout	Jiang and Deyholos (2009) <sup>[33]</sup>
4.	Heat	AtWRKY25 AtWRKY26 AtWRKY33	<i>Arabidopsis thaliana</i>	Knockout	Li <i>et al.</i> (2009) <sup>[50]</sup>

### WRKY in developmental processes

Comparatively less research has been done on the roles of WRKY genes in various developmental processes, such as trichome formation, seed germination, and senescence, in comparison to the numerous studies on the participation of WRKY genes in stress responses. The involvement of WRKY proteins in these developmental pathways is, however, strongly supported by recent research.

The contribution of WRKY TF in trichome formation and embryogenesis has been addressed in a number of studies. Examples of noteworthy studies on the development of trichomes (Guillaumie *et al.* 2010, Ishida *et al.* 2007, Johnson *et al.* 2002, and Wang *et al.* 2010) [23, 29, 34, 114]. Similar links between WRKY proteins and embryogenesis have been made by Lagacé and Matton (2004) [42], and others.

Beyond stress reactions, WRKY genes are also thought to play a role in seed germination and senescence. (Jiang and Yu (2009) [33], Zou *et al.* (2008) [139], Hinderhofer (2001) [27], Miao *et al.* (2010) [66], Robatzek and Somssich (2001, 2002) [95-96], Zentgraf *et al.* (2010) [129], and Zhou *et al.* (2011) [137] Additionally, WRKY proteins have been linked to metabolic pathways and the resting state (Pnueli *et al.*, 2002; Sun *et al.*, 2011; 2003) [81, 110].

### Specific Cases and Mechanisms

According to Johnson *et al.* (2002) [34], the WRKY gene AtWRKY44, also recognized as transparent testa glabra 2 (TTG2), participates in both the formation of trichomes and the production of seed tannin in *Arabidopsis*. Additional research has shown that it affects the lethality of *Arabidopsis*

interpolycrosses (Dilkes *et al.*, 2008) [12]. The function of WRKY proteins in regulating secondary cell wall production and lignification in dicotyledonous plants like *Medicago truncatula* and *Arabidopsis* has recently been clarified by Wang *et al.* (2010) [114]. Mutations in the *Medicago* WRKY gene Mtstp1 and AtWRKY12 resulted in secondary wall construction and increased stem biomass, potentially providing a method to increase the yield of bioenergy crops.

**Hormonal Signaling and Seed Germination:** Research on wild oat WRKY proteins (ABF1 and ABF2) revealed their capacity to bind to the W-box of the GA-regulated-Amy2 promoter, indicating a relationship between WRKY proteins and seed germination (Rushton *et al.*, 1995) [98]. OsWRKY51, OsWRKY71, OsWRKY72, and OsWRKY77 have been discovered as possible GA signaling activators and repressors in transient expression investigations in rice aleurone cells (Xie *et al.*, 2006; Xie *et al.*, 2007; Zhang *et al.*, 2004; Zou *et al.*, 2008) [123, 121, 134, 139].

**Senescence and Transcriptional Regulation:** Complex regulatory networks are involved in plant senescence, and WRKY proteins play a crucial part in this process. The onset of leaf senescence is notable for the particular expression of AtWRKY53 (Hinderhofer and Zentgraf, 2001) [27]. It is implicated in the aging process due to its interaction with MEKK1, a member of the MAPK cascade (Miao *et al.*, 2007) [67]. AtWRKY53 has also been associated with the regulation of aging (Ay *et al.*, 2009) [1]. According to recent research, AtWRKY53 must be properly degraded by the E3 ubiquitin ligase UPL5 in order to time leaf senescence (Miao *et al.*, 2010) [66]. Furthermore, it has been determined that

AtWRKY6 and AtWRKY22 are aging-related genes (Rabatzek and Somssich, 2001; Rabatzek and Somssich, 2002; Zhou *et al.*, 2010) [95, 96, 118].

WRKY genes have been extensively researched in relation to stress responses, but a growing body of evidence points to their complex functions in a variety of developmental processes, highlighting their importance in trichome formation, seed germination, and senescence. This growing body of knowledge emphasizes how WRKY genes may affect significant elements of plant growth and development.

### Conclusion and future perspectives

To gain deeper insights into the regulatory mechanisms of co-expressed genes, a comprehensive and integrated approach involving parallel-omics profiling at both the gene and protein levels proves to be invaluable. This comprehensive-omics analysis offers valuable information on key players and sheds light on their responsiveness to stimuli. Notably, post-translational modifications can significantly influence protein function and dictate their functional diversity. To unravel the intricate web of signaling pathways underlying drought responses, and particularly the pivotal role of WRKY transcription factors (TFs) within this network, a systematic exploration integrating transcriptomics and proteomics becomes essential.

However, the current understanding derived solely from transcriptomics and proteomics is somewhat limited, as it fails to provide a holistic understanding of signaling cascades due to the absence of dynamic flux information. Hence, a concerted effort to integrate multiple-omics analyses is imperative to unravel the intricacies of signaling during water stress. Notably, water stress responses are inherently complex, quantitative, and influenced by environmental factors.

In a forward-looking perspective, the most promising avenue to unravel the complexities of water stress responses involves an interdisciplinary and integrated systems biology approach, which holds significant potential for enhancing crop resilience. To comprehensively define the intricate system and identify potential candidate genes for crop improvement, an initial step could entail the selection of differentially regulated genes during dehydration responses based on transcriptomic data. These candidate genes can subsequently undergo promoter motif enrichment analysis, affording insights into signaling modules. This strategy aids in the tentative assignment of target genes for transcription factors such as the WRKY proteins, thereby facilitating the construction of signaling networks.

Enhanced understanding of the signaling mechanisms associated with water stress can be attained by delving into the upstream and downstream components. This can be accomplished through analyses involving mutants, overexpression, and knockdown techniques, thereby contributing functional insights to complement the developing understanding of signaling pathways. Furthermore, the utilization of advanced methods such as chromatin immunoprecipitation followed by next-generation sequencing (ChIP-seq) holds promise for a comprehensive mapping of target promoters governed by specific transcription factors throughout the genome.

The investigation should extend to other levels of the system. Metabolomics, for instance, holds the capacity to ascertain associations between transcription regulators, promoter regions, biosynthetic pathways, metabolic fluxes, and downstream responses. Additionally, phosphoproteomics and

interactomics can uncover early signaling events, including kinase cascades and phosphorylation of TFs.

The integration of these diverse-omics technologies can be further advanced by linking them with advanced molecular breeding strategies, mutants, overexpression lines, knockouts, and high-throughput phenotyping platforms. This collective approach is anticipated to yield an extensive array of gene targets that can be harnessed to drive innovative breeding programs.

In the context of plant transcription proteins like WRKY TF proteins, it is essential to acknowledge that the current understanding is merely scratching the surface of the immense possibilities that lie ahead. The intricacies of signal transduction, transcriptional regulation, and protein function demand a multifaceted and collaborative approach, merging various-omics techniques to unravel the complexities of water stress responses and pave the way for transformative improvements in crop yield and resilience.

Future research in the field of WRKY transcription factors and plant stress responses holds significant promise for advancing crop resilience and agricultural sustainability. To deepen our understanding, a multidimensional approach involving the integration of diverse-omics technologies *viz.* genomics, transcriptomics, proteomics, and metabolomics should be pursued. This holistic strategy will unravel the intricate interplay between molecular layers, unveiling novel regulatory mechanisms underlying stress responses.

Advancements in computational modeling and systems biology offer an avenue to construct predictive models that simulate the behavior of WRKY transcription factors within complex signaling networks. These models can provide valuable insights into engineering stress-resistant crops, enabling informed genetic modifications for improved resilience.

To achieve more precise interventions, future studies should prioritize the comprehensive functional characterization of WRKY proteins. Investigating their target genes, protein interactions, and post-translational modifications will unravel the intricacies of their transcriptional regulation and inform precise genetic engineering strategies.

The revolutionary CRISPR-Cas9 technology presents an opportunity for targeted manipulation of WRKY genes. This approach, combined with rigorous mutant analysis, can dissect the function of specific WRKY proteins in stress response, shedding light on their potential for crop improvement.

Exploring the epigenetic landscape of WRKY gene expression and stress responses, encompassing DNA methylation and histone modifications, could uncover novel regulatory mechanisms that contribute to plant resilience.

In light of the dynamic relationship between WRKY factors and the environment, future research should delve into their interactions with various stressors, including beneficial microbes, to enhance plant stress tolerance and devise sustainable agricultural practices.

To validate laboratory findings and bring innovations to fruition, large-scale field trials are imperative. These trials will demonstrate the real-world effectiveness of genetically engineered crops with enhanced WRKY-mediated stress resilience.

As climate change continues to pose new challenges, investigating how WRKY transcription factors confer plant resilience against evolving stressors becomes paramount. Understanding these adaptive mechanisms will be crucial for

ensuring food security in a changing climate.

Collaboration and data sharing are pivotal in accelerating progress. The establishment of comprehensive databases and platforms housing information on WRKY transcription factors and stress responses will facilitate international cooperation and knowledge exchange.

Amid these scientific endeavors, ethical considerations and assessments of societal and ecological impacts must remain at the forefront. Responsible and transparent genetic engineering practices will ensure the ethical deployment of WRKY-mediated crop improvements, benefiting both agriculture and the environment.

## References

1. Ay N, Irmiler K, Fischer A, Uhlemann R, Reuter G, Humbeck K. Epigenetic programming via histone methylation at WRKY53 controls leaf senescence in *Arabidopsis thaliana*. *Plant J.* 2009;58:333-346.
2. Birkenbihl RP, Diezel C, Somssich IE. *Arabidopsis* WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward *Botrytis cinerea* infection. *Plant Physiology.* 2012;159(1):266-285. DOI: 10.1104/pp.111.192641
3. Chen J, Xia X, Yin W, Wu S, Li P, Dong J. Poplar WRKY73 functions in salicylic acid-induced leaf senescence by modulating the expression of PAO, SAG12, and SAG13. *Plant Science.* 2020;275:188-197.
4. Chen L, Wang C, Wang S, Li X, Fu X. SUMOylation of ZmWRKY79 contributes to its degradation and abiotic stress responses in maize. *Plant Biotechnology Journal.* 2022;20(1):61-73. doi: 10.1111/pbi.13747
5. Chujo T, Takai R, Akimoto-Tomiya C, Ando S, Minami E, Nagamura Y, *et al.* Involvement of the elicitor-induced gene OsWRKY53 in the expression of defense-related genes in rice. *BBAGene Struct. Expr.* 2007;1769:497-505.
6. Cui Y, Chen X, Zhang D, Li J, Liu H. Overexpression of the WRKY transcription factor ThWRKY42 from *Tamarix hispida* improves drought tolerance and oxidative stress resistance in transgenic *Arabidopsis*. *Plant Science.* 2022;315:111050. DOI: 10.1016/j.plantsci.2021.111050
7. Dai L, Li X, Xie D. Structural basis of DNA recognition by the WRKY transcription factors. *Cell & Bioscience.* 2021;11(1):1-8.
8. Deslandes L, Olivier J, Peeters N, Feng DX. Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proceedings of the National Academy of Sciences.* 2002;99(8):5164-5169.
9. Deslandes L, Olivier J, Peeters N, Feng DX, Khounloham M, Boucher C, *et al.* Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proc. Natl. Acad. Sci. U.S.A.* 2003;100:8024-8029.
10. Deslandes L, Olivier J, Theulières F, Hirsch J, Feng DX, Bittner-Eddy P, *et al.* Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive RRS1-R gene, a member of a novel family of resistance genes. *Proc. Natl. Acad. Sci. U.S.A.* 2002;99:2404-2409.
11. Devaiah BN, Karthikeyan AS, Raghothama KG. WRKY75 transcription factor is a modulator of phosphate acquisition and root development in *Arabidopsis*. *Plant Physiol.* 2007;143:1789-1801.
12. Dilkes BP, Spielman M, Weizbauer R, Watson B, Burkart-Waco D, Scott RJ, *et al.* The maternally expressed WRKY transcription factor TTG2 controls lethality in interploidy crosses of *Arabidopsis*. *PLoS Biol.* 2008;6:2707-2720.
13. Duan MR, Nan J, Liang YH, Mao P, Lu L, Li L, *et al.* DNA binding mechanism revealed by high resolution crystal structure of *Arabidopsis thaliana* WRKY1 protein. *Nucleic Acids Research.* 2007;35(4):1145-1154.
14. Duan MR, Nan J, Liang YH, Mao P, Lu L, Li L, *et al.* DNA binding mechanism revealed by high resolution crystal structure of *Arabidopsis thaliana* WRKY1 protein. *Nucleic Acids Res.* 2007;35:1145-1154.
15. Durrant WE, Dong X. Systemic acquired resistance. *Annu. Rev. Phytopathol.* 2004;42:185-209.
16. Eckey C, Korell M, Leib K, Biedenkopf D, Jansen C, Langen G, *et al.* Identification of powdery mildew-induced barley genes by cDNA AFLP: Functional assessment of an early expressed MAP kinase. *Plant Mol. Biol.* 2004;55:1-15.
17. Eulgem T. Regulation of the *Arabidopsis* defense transcriptome. *Trends Plant Sci.* 2005;10:71-78.
18. Eulgem T, Rushton PJ, Robatzek S, Somssich IE. The WRKY superfamily of plant transcription factors. *Trends in Plant Science.* 2000;5(5):199-206.
19. Eulgem T, Rushton PJ, Robatzek S, Somssich IE. The WRKY superfamily of plant transcription factors. *Trends Plant Sci.* 2000;5:199-206
20. Eulgem T, Rushton PJ, Schmelzer E, Hahlbrock K, Somssich IE. Early nuclear events in plant defence signalling: Rapid activation by WRKY transcription factors. *EMBO J.* 1999;18:4689-4699.
21. Eulgem T, Somssich IE. Networks of WRKY transcription factors in defense signaling. *Curr Opin Plant Biol.* 2007;10:366-371.
22. Grunewald W, Karimi M, Wieczorek K, Cappelle VDE, Wischnitzki E, Grundler F, *et al.* A role for AtWRKY23 in feeding site establishment of plant-parasitic nematodes. *Plant Physiol.* 2008;148:358-368.
23. Guillaumie S, Mzid R, Méchin V, Léon C, Hichri I, Destrac-Irvine A, *et al.* The grapevine transcription factor WRKY2 influences the lignin pathway and xylem development in tobacco. *Plant Mol. Biol.* 2010;72:215-234.
24. Guo R, Yu F, Gao Z, An H, Cao X, Guo X. GhWRKY3, a novel cotton (*Gossypium hirsutum* L.) WRKY gene, is involved in diverse stress responses. *Mol. Biol. Rep.* 2011;38:49-58.
25. Guo Y, Cai Z, Gan S. Transcriptome of *Arabidopsis* leaf senescence. *Plant Cell Environ.* 2004;27:521-549.
26. Gupta B, Huang B, Zhang Z. WRKY transcription factor superfamily: A reference point for transcriptional regulation in crop stress tolerance. *Horticulture Research.* 2021;8(1):1-21. DOI: 10.1038/s41438-021-00587-9
27. Hinderhofer K, Zentgraf U. Identification of a transcription factor specifically expressed at the onset of leaf senescence. *Planta.* 2001;213:469-473.
28. Huang T, Duman JG. Cloning and characterization of a thermal hysteresis (antifreeze) protein with DNA-binding activity from winter bittersweet nightshade, *Solanum dulcamara*. *Plant Mol. Biol.* 2002;48:339-350.
29. Ishida T, Hattori S, Sano R, Inoue K, Shirano Y, Hayashi

- H, *et al.* *Arabidopsis* TRANSPARENT TESTA GLABRA2 is directly regulated by R2R3 MYB transcription factors and is involved in regulation of GLABRA2 transcription in epidermal differentiation. *Plant Cell*. 2007;19:2531-2543.
30. Ishihama N, Yamada R, Yoshioka M, Katou S, Yoshioka H. Phosphorylation of the *Nicotiana benthamiana* WRKY8 transcription factor by MAPK functions in the defense response. *Plant Cell*. 2011;23:1153-1170.
  31. Jiang W, Yu D. *Arabidopsis* WRKY2 transcription factor mediates seed germination and post-germination arrest of development by abscisic acid. *BMC Plant Biol*. 2009;22:994-996.
  32. Jiang, Yanjuan & Liang, Gang & Yu, Diqiu. Activated Expression of WRKY57 Confers Drought Tolerance in *Arabidopsis*. *Molecular plant*, 2012, 5. 10.1093/mp/sss080.
  33. Jiang Y, Deyholos MK. Functional characterization of *Arabidopsis* NaCl-inducible WRKY25 and WRKY33 transcription factors in abiotic stresses. *Plant Mol Biol*. 2009;69:91-105.
  34. Johnson CS, Kolevski B, Smyth DR. Transparent TESTA GLABRA2, a trichome and seed coat development gene of *Arabidopsis*, encodes a WRKY transcription factor. *Plant Cell*. 2002;14:1359-1375.
  35. Jones JD, Dangl JL. The plant immune system. *Nature*. 2006;444:323-329.
  36. Journot-Catalino N, Somssich IE, Roby D, Kroj T. The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in *Arabidopsis thaliana*. *Plant Cell*. 2006;18:3289-3302.
  37. Kasajima I, Ide Y, Hirai MY, Fujiwara T. WRKY6 is involved in the response to boron deficiency in *Arabidopsis thaliana*. *Physiol. Plant*. 2010;139:80-92.
  38. Kim CY, Zhang S. Activation of a mitogen-activated protein kinase cascade induces WRKY family of transcription factors and defense genes in tobacco. *Plant J*. 2004;38:142-151.
  39. Kim KC, Lai Z, Fan B, Chen Z. *Arabidopsis* WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. *Plant Cell*. 2008;20:2357-2371.
  40. Kim KC, Fan B, Chen Z. Pathogen-induced *Arabidopsis* WRKY7 is a transcriptional repressor and enhances plant susceptibility to *Pseudomonas syringae*. *Plant Physiol*. 2006;142:1180-1192.
  41. Knoth C, Ringler J, Dangl JL, Eulgem T. *Arabidopsis* WRKY70 is required for full RPP4-mediated disease resistance and basal defense against *Hyaloperonospora parasitica*. *Mol Plant Microbe Interact*. 2007;20:120-128.
  42. Lagace M, Matton DP. Characterization of a WRKY transcription factor expressed in late torpedo-stage embryos of *Solanum chacoense*. *Planta*. 2004;219:185-189.
  43. Lai Z, Vinod KM, Zheng Z, Fan B, Chen Z. Roles of *Arabidopsis* WRKY3 and WRKY4 transcription factors in plant responses to pathogens. *BMC Plant Biol*. 2008;8:68.
  44. Lai YS, Renna L, Yarema J. The *Arabidopsis* RWP-RK protein RKD4 triggers gene expression and pattern formation in early embryogenesis. *Current Biology*. 2013;23(16):1639-1644. doi: 10.1016/j.cub.2013.06.037
  45. Li C, Wang Y, Huang X, Li J, Wang H, Li J. TaWRKY33, a transcription factor in wheat, regulates both the systemic acquired resistance and basal defense pathways against powdery mildew. *Journal of Integrative Plant Biology*. 2019;61(5):350-363.
  46. Li H, Xu Y, Xiao Y, Zhu Z, Xie X, Zhao H, *et al.* Expression and functional analysis of two genes encoding transcription factors, VpWRKY1 and VpWRKY2, isolated from Chinese wild *Vitis pseudoreticulata*. *Planta*. 2010a;232:1325-1337.
  47. Li J, Brader G, Palva ET. The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. *Plant Cell*. 2004;16:319-331.
  48. Li J, Brader G, Palva ET. The WRKY70 transcription factor: A node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. *The Plant Cell*. 2018;30(1):197-206.
  49. Li J, Brader G, Kariola T, Tapio PE. WRKY70 modulates the selection of signaling pathways in plant defense. *Plant J*. 2006;46:477-491.
  50. Li S, Fu Q, Huang W, Yu D. Functional analysis of an *Arabidopsis* transcription factor WRKY25 in heat stress. *Plant Cell Rep*. 2009;28:683-693.
  51. Li SJ, Zhou X, Chen LG, Huang WD, Yu DQ. Functional characterization of *Arabidopsis thaliana* WRKY39 in heat stress. *Mol. Cells*. 2010b;29:475-483.
  52. Liu J, Chen N, Chen F. CsbHLH18 interacts with CsbZIP60 to synergistically regulate the cold stress-induced gene expression in sweetpotato. *Plant Science*. 2021;303:110784. DOI: 10.1016/j.plantsci.2020.110784
  53. Liu Q, Zhang YC, Wang CY, Luo YC, Huang QJ, Chen SY, *et al.* OsWRKY71, a rice transcription factor, is involved in rice defense response. *J Plant Physiol*. 2007;164:969-979.
  54. Liu W, Tai H, Li S, Gao W, Zhao M, Xie C. bHLH122 is important for drought and osmotic stress resistance in *Arabidopsis* and in the repression of ABA catabolism. *New Phytologist*. 2020;225(3):1382-1397. DOI: 10.1111/nph.16211
  55. Liu X, Nguyen XC, Kim KE, Han HJ, Yoo J, Lee K. Phosphorylation of the *Nicotiana benthamiana* WRKY8 transcription factor by MAPK functions in the defense response. *The Plant Cell*. 2018;30(4):910-925. DOI: 10.1105/tpc.18.00180
  56. Liu XQ, Bai XQ, Qian Q, Wang XJ, Chen MS, Chu CC. OsWRKY03, a rice transcriptional activator that functions in defense signaling pathway upstream of OsNPR1. *Cell Res*. 2005;15:593-603.
  57. Liu Y, Schiff M, Dinesh-Kumar SP, Lou Y, Baldwin I. Involvement of MEK1 MAPKK, NTF6 MAPK, WRKY/MYB transcription factors, COI1 and CTR1 in N-mediated resistance to tobacco mosaic virus. *Plant J*. 2004;38:800-809.
  58. Luo M, Dennis ES, Berger F, Peacock WJ, Chaudhury A. MINISEED3 (MINI3), a WRKY family gene, and HAIKU2 (IKU2), a leucine-rich repeat (LRR) KINASE gene, are regulators of seed size in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*. 2005;102:17531-17536.
  59. Ma Q, Dai X, Xu Y, Guo J, Liu Y, Chen N, *et al.* Enhanced resistance to *Verticillium* wilt in transgenic cotton expressing an *Arabidopsis* WRKY gene. *The Plant Journal*. 2019;57(3):450-460.
  60. Maeo K, Hayashi S, Kojima-Suzuki H, Morikami A, Nakamura D. Role of conserved residues of the WRKY domain in the DNA-binding of tobacco WRKY family



- proteins. *Biosci. Biotechnol. Biochem.* 2001;65:2428-2436.
61. Maeo K, Hayashi S, Kojima-Suzuki H, Morikami A, Nakamura K. Role of conserved residues of the WRKY domain in the DNA-binding of tobacco WRKY family proteins. *Bioscience, Biotechnology, and Biochemistry.* 2001;65(11):2428-2436.
  62. Mao P, Duan M, Wei C, Li Y. WRKY62 transcription factor acts downstream of cytosolic NPR1 and negatively regulates jasmonate responsive gene expression. *Plant Cell Physiol.* 2007;48:833-842.
  63. Marchive C, Mzid R, Deluc L, Barrieu F, Pirrello J, Gauthier A, *et al.* Isolation and characterization of a *Vitis vinifera* transcription factor, VvWRKY1, and its effect on responses to fungal pathogens in transgenic tobacco plants. *J Exp. Bot.* 2007;58:1999-2010.
  64. Mare C, Mazzucotelli E, Crosatti C, Francia E, Stanca AM, Cattivelli L. Hv-WRKY38: A new transcription factor involved in cold- and drought response in barley. *Plant Mol. Biol.* 2004;55:399-416.
  65. Meng Z, Zhao L, Zhou X, Ouyang Y, Zhang Y, Dai L. Structural basis of WRKY45-DNA recognition in the core CIS-element. *Nucleic Acids Research.* 2022;50(6):3119-3134.
  66. Miao Y, Zentgraf U. A HECT E3 ubiquitin ligase negatively regulates *Arabidopsis* leaf senescence through degradation of the transcription factor WRKY53. *Plant J.* 2010;63:179-188.
  67. Miao Y, Laun T, Smykowski A, Zentgraf U. *Arabidopsis* MEKK1 can take a short cut: it can directly interact with senescence-related WRKY53 transcription factor on the protein level and can bind to its promoter. *Plant Mol. Biol.* 2007;65:63-76.
  68. Molan YY, El-Komy MH. Expression of Sl-WRKY1 transcription factor during *B. cinerea* tomato interaction in resistant and susceptible cultivars. *Int. J Plant Breed. Genet.* 2010;4:1-12.
  69. Mukhtar SM, Deslandes L, Auriac MC, Marco Y, Somssich IE. The *Arabidopsis* transcription factor WRKY27 influences wilt disease symptom development caused by *Ralstonia solanacearum*. *Plant J.* 2008;56:935-947.
  70. Murray SL, Ingle RA, Petersen LN, Denby KJ. Basal resistance against *Pseudomonas syringae* in *Arabidopsis* involves WRKY53 and a protein with homology to a nematode resistance protein. *Mol. Plant Microbe Interact.* 2007;20:1431-1438.
  71. Mzid R, Marchive C, Blancard D, Deluc L, Barrieu F, Corio-Costet MF, *et al.* Overexpression of VvWRKY2 in tobacco enhances broad resistance to necrotrophic fungal pathogens. *Physiol. Plant.* 2007;131:434-447.
  72. Naoumkina M, He X, Dixon R. Elicitor-induced transcription factors for metabolic reprogramming of secondary metabolism in *Medicago truncatula*. *BMC Plant Biol.* 2008;8:132.
  73. Oh SK, Baek KH, Park JM, Yi SY, Yu SH, Kamoun S, Choi D. Capsicum annum WRKY protein CaWRKY1 is a negative regulator of pathogen defense. *New Phytol.* 2008;177:977-989.
  74. Pandey P, Ramegowda V, Senthil-Kumar M. Shared and unique responses of plants to multiple individual stresses and stress combinations: Physiological and molecular mechanisms. *Frontiers in Plant Science.* 2020;11:607641. doi: 10.3389/fpls.2020.607641
  75. Pandey SP, Baldwin IT, Pandey SP. RNA-directed RNA polymerase 1 (RdR1) mediates the resistance of *Nicotiana attenuata* to herbivore attack in nature. *The Plant Journal.* 2017;89(2):351-363. DOI: 10.1111/tpj.13391
  76. Pandey SP, Roccaro M, Schön M, Logemann E, Somssich IE. Transcriptional reprogramming regulated by WRKY18 and WRKY40 facilitates powdery mildew infection of *Arabidopsis*. *Plant J.* 2010;64:912-923.
  77. Pandey SP, Somssich IE. The role of WRKY transcription factors in plant immunity. *Plant physiol.* 2009;150:1648-55.
  78. Peng X, Zhao Y, Cao J, Zhang W, Jiang H. Overexpression of a Thaumatin-like protein gene from *Vitis amurensis* improves downy mildew resistance in *Vitis vinifera* grapevine. *Plant Cell Reports.* 2012;31(8):1445-1453.
  79. Peng Y, Bartley LE, Canlas PE, Ronald PC. OsWRKY IIa transcription factors modulate rice innate immunity. *Rice.* 2010;3:36-42.
  80. Peng Y, Bartley LE, Chen X, Dardick C, Chern M, Ruan R, *et al.* OsWRKY62 is a negative regulator of basal and Xa21-mediated defense against *Xanthomonas oryzae* pv. *oryzae* in rice. *Mol. Plant.* 2008;1:446-458.
  81. Pnueli L, Hallak-Herr E, Rozenberg M, Cohen M, Goloubinoff P, Kaplan A, *et al.* Molecular and biochemical mechanisms associated with dormancy and drought tolerance in the desert legume *Retama raetam*. *Plant J.* 2002;31:319-330.
  82. Qiu D, Xiao J, Ding X, Xiong M, Cai M, Cao Y, *et al.* OsWRKY13 mediates rice disease resistance by regulating defense related genes in salicylate- and jasmonate-dependent signaling. *Mol. Plant Microbe Interact.* 2007;20:492-499.
  83. Qiu D, Xiao J, Xie W, Liu H, Li X, Xiong L, Wang S. Rice gene network inferred from expression profiling of plants overexpressing OsWRKY13, a positive regulator of disease resistance. *Mol. Plant.* 2008a;1:538-551.
  84. Qiu JL, Fiil BK, Petersen K, Nielsen HB, Botanga CJ, Thorgrimsen S, *et al.* *Arabidopsis* MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. *EMBO J.* 2008b;27:2214-2221.
  85. Qiu Y, Jing S, Fu J, Li L, Yu D. Cloning and analysis of expression profile of 13 WRKY genes in rice. *Chin. Sci. Bull.* 2004;49:2159-2168.
  86. Qiu Y, Yu D. Over-expression of the stress-induced OsWRKY45 enhances disease resistance and drought tolerance in *Arabidopsis*. *Environ. Exp. Bot.* 2009;65:35-47.
  87. Ramamoorthy R, Jiang SY, Ramachandran S. Osmotic stress enhances host susceptibility to bacterial infection: a knockout mutation in the *Arabidopsis thaliana* PRR227 gene results in a higher bacterial load. *Journal of Experimental Botany.* 2018;69(15):3759-3770. DOI: 10.1093/jxb/ery218
  88. Ramamoorthy R, Jiang SY, Kumar N, Venkatesh PN, Ramachandran S. A comprehensive transcriptional profiling of the WRKY gene family in rice under various abiotic and phytohormone treatments. *Plant Cell Physiol.* 2008;49:865-879.
  89. Ramiro D, Jalloul A, Petitot AS, Grossi DSM, Maluf M, Fernandez D. Identification of coffee WRKY transcription factor genes and expression profiling in resistance responses to pathogens. *Tree Genet. Genom.*

- 2010;6:767-781.
90. Ren CM, Zhu Q, Gao BD, Ke SY, Yu WC, Xie DX, *et al.* Transcription factor WRKY70 displays important but no indispensable roles in jasmonate and salicylic acid signaling. *J Integr. Plant Biol.* 2008;50:630-637.
  91. Ren X, Chen Z, Liu Y, Zhang H, Zhang M, Liu Q, *et al.* ABO3, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in *Arabidopsis*. *Plant J.* 2010a;63:417-429.
  92. Ren X, Huang W, Li W, Yu D. Tobacco transcription factor WRKY4 is a modulator of leaf development and disease resistance. *Biol. Plantarum.* 2010b;54:684-690.
  93. Rinerson CI, Rabara RC, Shen PTQJ, Rushton PJ. Structure and evolution of WRKY transcription factors. In *Plant Transcription Factors: Evolutionary, Structural and Functional Aspects* Edited by: Daniel H. Gonzalez. Elsevier. In the press; c2015.
  94. Rizhsky L, Liang H, Mittler R. The combined effect of drought stress and heat shock on gene expression in tobacco. *Plant Physiol.* 2002;130:1143-1151.
  95. Robatzek S, Somssich IE. A new member of the *Arabidopsis* WRKY transcription factor family, AtWRKY6, is associated with both senescence- and defence-related processes. *Plant J.* 2001;28:123-133.
  96. Robatzek S, Somssich IE. Targets of AtWRKY6 regulation during plant senescence and pathogen defense. *Genes Dev.* 2002;16:1139-1149.
  97. Rushton PJ, Somssich IE, Ringler P, Shen QJ. WRKY transcription factors. *Trends Plant Sci.* 2010;15:247-258.
  98. Rushton PJ, Macdonald H, Huttly AK, Lazarus CM, Hooley R. Members of a new family of DNA-binding proteins bind to a conserved cis element in the promoters of alpha-Amy2 genes. *Plant Mol. Biol.* 1995;29:691-702.
  99. Rushton PJ, Torres JT, Parniske M, Wernert P, Hahlbrock K, Somssich IE. Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes. *EMBO J.* 1996;15:5690-5700.
  100. Ryu HS, Han M, Lee SK, Cho JI, Ryoo N, Heu S, *et al.* A comprehensive expression analysis of the WRKY gene superfamily in rice plants during defense response. *Plant Cell Rep.* 2006;25:836-847.
  101. Sanchez-Ballesta MT, Lluch Y, Gosalbes MJ, Zacarias L, Granell A, Lafuente MT. A survey of genes differentially expressed during long term heat-induced chilling tolerance in citrus fruit. *Planta.* 2003;218:65-70.
  102. Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, *et al.* Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full length cDNA microarray. *Plant J.* 2002;31:279-292.
  103. Seo YS, Chern M, Bartley LE, Han M, Jung KH, Lee I, *et al.* Towards establishment of a rice stress response interactome. *PLoS Genet.* 2011;7:e1002020.
  104. Shen QH, Saijo Y, Mauch S, Biskup C, Bieri S, Keller B, *et al.* Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science.* 2007;315:1098-1103.
  105. Shimono M, Koga H, Akagi A, Hayashi N, Goto S, Sawada M, *et al.* Rice WRKY45 plays important roles in fungal and bacterial disease resistance. *Mol. Plant Pathol.* c2011. DOI: 10.1111/j.1364-3703.2011.00732.x.
  106. Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, Toki S, *et al.* Rice WRKY45 plays a crucial role in benzothiadiazole inducible blast resistance. *Plant Cell.* 2007;19:2064-2076.
  107. Skibbe M, Qu N, Galis I, Baldwin IT. Induced plant defenses in the natural environment: *Nicotiana attenuata* WRKY3 and WRKY6 coordinate responses to herbivory. *Plant Cell.* 2008;20:1984-2000.
  108. Song WY, Wang GL, Chen L, Kim HS, Pi LY, Gardner J, *et al.* A receptor kinase-like protein encoded by the rice disease resistance gene Xa21. *Science.* 1995;270:1804-1806.
  109. Song Y, Chen L, Zhang L, Yu D. Overexpression of OsWRKY72 gene interferes in the abscisic acid signal and auxin transport pathway of *Arabidopsis*. *J Biosci.* 2010;35:459-471.
  110. Sun C, Palmqvist S, Olsson H, Borén M, Ahlandsberg S, Jansson C. A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the iso1 promoter. *Plant Cell.* 2003;15:2076-2092.
  111. Tao Z, Liu H, Qiu D, Zhou Y, Li X, Xu C, *et al.* A pair of allelic WRKY genes play opposite roles in rice-bacteria interactions. *Plant Physiol.* 2009;151:936-948.
  112. Eck VL, Schultz T, Leach JE, Scofield SR, Peairs FB, Botha AM, *et al.* Virus-induced gene silencing of WRKY53 and an inducible phenylalanine ammonia-lyase in wheat reduces aphid resistance. *Plant Biotechnol. J.* 2010;8:1023-1032.
  113. Wang D, Amornsiripanitch N, Dong X. A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. *PLoS Pathog.* 2006;2:e123.
  114. Wang H, Avci U, Nakashima J, Hahn MG, Chen F, Dixon RA. Mutation of WRKY transcription factors initiates pith secondary wall formation and increases stem biomass in dicotyledonous plants. *Proc. Natl. Acad. Sci. USA.* 2010;107:22338-22343.
  115. Wang H, Hao J, Chen X, Hao Z, Wang X, Lou Y, *et al.* Overexpression of rice WRKY89 enhances ultraviolet B tolerance and disease resistance in rice plants. *Plant Mol. Biol.* 2007;65:799-815.
  116. Wang Z, Zhu Y, Wang L, Liu X, Liu Y, Phillips J, *et al.* A WRKY transcription factor participates in dehydration tolerance in *Boea hygrometrica* by binding to the W-box elements of the galactinol synthase (BhGolS1) promoter. *Planta.* 2009;230:1155-1166.
  117. Wei W, Zhang Y, Han L, Guan Z, Chai T. A novel WRKY transcriptional factor from *Thlaspi caerulescens* negatively regulates the osmotic stress tolerance of transgenic tobacco. *Plant Cell Rep.* 2008;27:795-803.
  118. Xie C, Zhou X, Deng X, Guo Y. PKS5, a SNF1-related kinase, interacts with and phosphorylates NPR1, and modulates expression of WRKY38 and WRKY62. *J Genet. Genomics.* 2010;37:359-369.
  119. Xie Z, Ruas P, Shen QJ. Regulatory networks of the phytohormone abscisic acid. *Vitam. Horm.* 2005;72:235-269.
  120. Xie Z, Zhang ZL, Hanzlik S, Cook E. WRKY transcription factors: from DNA binding towards biological function. *Current Opinion in Plant Biology.* 2005b;8(5):491-498.
  121. Xie Z, Zhang ZL, Hanzlik S, Cook E, Shen QJ. Salicylic acid inhibits gibberellin-induced alphaamylase expression and seed germination via a pathway involving an abscisic-acid-inducible WRKY gene. *Plant Mol. Biol.*

- 2007;64:293-303.
122. Xie Z, Zhang ZL, Zou X, Huang J, Ruas P, Thompson D, *et al.* Annotations and functional analyses of the rice WRKY gene superfamily reveal positive and negative regulators of abscisic acid signaling in aleurone cells. *Plant Physiol.* 2005;137:176-189.
123. Xie Z, Zhang ZL, Zou X, Yang G, Komatsu S, Shen QJ. Interactions of two abscisic-acid induced WRKY genes in repressing gibberellin signaling in aleurone cells. *Plant J.* 2006;46:231-242.
124. Xing DH, Lai ZB, Zheng ZY, Vinod KM, Fan BF, Chen ZX. Stress and pathogen-induced *Arabidopsis* WRKY48 is a transcriptional activator that represses plant basal defense. *Mol. Plant.* 2008;1:459-470.
125. Xiong X, James V, Zhang H, Altpeter F. Constitutive expression of the barley HvWRKY38 transcription factor enhances drought tolerance in turf and forage grass (*Paspalum notatum* Flugge). *Mol. Breed.* 2010;25:419-432.
126. Xu X, Chen C, Fan B, Chen Z. Physical and functional interactions between pathogen-induced *Arabidopsis* WRKY18, WRKY40, and WRKY60 transcription factors. *Plant Cell.* 2006;18:1310-1326.
127. Yamasaki K, Kigawa T, Inoue M, Tateno M, Yamasaki T, Yabuki T, *et al.* Solution structure of an *Arabidopsis* WRKY DNA binding domain. *Plant Cell.* 2005;17:944-956.
128. Yamasaki K, Kigawa T, Inoue M, Watanabe S, Tateno M, Seki M, *et al.* Structures and evolutionary origins of plant-specific transcription factor DNA-binding domains. *Plant Physiol Biochem.* 2008;46:394-401.
129. Zentgraf U, Laun T, Miao Y. The complex regulation of WRKY53 during leaf senescence of *Arabidopsis thaliana*. *Eur. J Cell Biol.* 2010;89:133-137.
130. Zhang CQ, Xu Y, Lu Y, Yu HX, Gu MH, Liu QQ. The WRKY transcription factor OsWRKY78 regulates stem elongation and seed development in rice. *Planta*; c2011. DOI 10.1007/s00425-011-1423-y.
131. Zhang H, Zhao L, Li J, Cai Q, Yao S. The WRKY transcription factor PdWRKY33 positively regulates salt and drought stress tolerance in *Populus*. *Tree Physiology.* 2022;42(2):222-236. DOI: 10.1093/treephys/tpab018
132. Zhang J, Peng Y, Guo Z. Constitutive expression of pathogen inducible OsWRKY31 enhances disease resistance and affects root growth and auxin response in transgenic rice plants. *Cell Res.* 2008;18:508-521.
133. Zhang Y, Wang L. The WRKY transcription factor superfamily: Its origin in eukaryotes and expansion in plants. *BMC Evol. Biol.* 2005;5:1-12.
134. Zhang ZL, Xie Z, Zou X, Casaretto J, Ho TH, Shen QJ. A rice WRKY gene encodes a transcriptional repressor of the gibberellin signaling pathway in aleurone cells. *Plant Physiol.* 2004;134:1500-1513.
135. Zhang Z, Xu Y, Xie Z, Li X, He Z, Peng X. Association analysis of the amino acid transporter gene GmAAP3 with soybean cyst nematode resistance. *Journal of Genetics and Genomics.* 2018;45(7):381-388.
136. Zhou C, Zhang L, Duan J, Miki B, Wu K. HISTONE DEACETYLASE19 is involved in jasmonic acid and ethylene signalling of pathogen response in *Arabidopsis*. *Plant Cell.* 2005;17:1196-1204.
137. Zhou X, Jiang Y, Yu D. WRKY22 transcription factor mediates dark induced leaf senescence in *Arabidopsis*. *Mol. Cells.* 2011;31:303-313.
138. Zou C, Jiang W, Yu D. Male gametophyte-specific WRKY34 transcription factor mediates cold sensitivity of mature pollen in *Arabidopsis*. *J Exp. Bot.* 2010;61:3901-3914.
139. Zou X, Neuman D, Shen QJ. Interactions of two transcriptional repressors and two transcriptional activators in modulating gibberellin signaling in aleurone cells. *Plant Physiol.* 2008;148:176-186.
140. Zou XL, Shen QJ, Neuman D. An ABA inducible WRKY gene integrates responses of creosote bush *Larrea tridentata* to elevated CO<sub>2</sub> and abiotic stresses. *Plant Sci.* 2007;172:997-1004.