

# A review of WRKY transcription factors in the regulation of abiotic and biotic stress in cotton (*Gossypium* spp.)

Kasahun Amare Finsa\*, Geleta Dugassa

*Department of Applied Biology, School of Applied Natural Science, Adama Science and Technology University, Adama, Ethiopia*

**\*Corresponding Author:** Kasahun Amare Finsa, Department of Applied Biology, School of Applied Natural Science, Adama Science and Technology University, Adama, Ethiopia.

**Received Date:** 18 September 2022; **Accepted Date:** 21 October 2022; **Published date:** 27 December 2022.

**Citation:** Kasahun Amare Finsa, Geleta Dugassa, (2022). A review of WRKY transcription factors in the regulation of abiotic and biotic stress in cotton (*Gossypium* spp.). *Journal of Transplantation Proceedings and Research*. 1(1). DOI: 10.58489/2836-8991/002

**Copyright:** © 2022 Kasahun Amare Finsa, this is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Abstract

Cotton is an important economic crop that is grown in over 100 countries throughout the world. It provides the textile industry raw materials, cottonseed oil for cooking, and edible and protein-rich oil cake remnants for farm animals. Abiotic and biotic stressors limit its production, which is strongly reliant on climatic circumstances. Cotton plants have several built-in mechanisms for dealing with abiotic and biotic stress. These defense systems are controlled by changes in the expression of stress-responsive genes. Several stress response-related genes have been discovered, including WRKY transcription elements. WRKY proteins bind to the W box (TGACC (A/T)) in the promoter regions of genes to activate or reduce the expression of downstream genes, which regulates the stress response. It also works with other transcription factors to control the defense responses of the plant. The function of WRKY transcription factors in cotton was briefly discussed in this review in terms of drought, salinity, temperature, and oxidative and biotic stress reactions. In conclusion, WRKY genes are thought to be important components of gene regulatory networks involved in plant stress responses and developmental plasticity in response to seasonal variations in environmental conditions and pathogenic infections.

**Keywords:** Transcription Factor, WRKY, Biotic Stress, Abiotic Stress, *Gossypium* Species

## Introduction

Cotton (*Gossypium* spp.) is a member of the Malvaceae family's *Gossypium* genus, which includes over 50 species, only four of which are farmed (*Gossypium arboreum*, *Gossypium herbaceum*, *Gossypium hirsutum*, and *Gossypium barbadense*) (Wendel and Grover, 2015). The first two species are diploid ( $2n = 2x = 26$ ) and are thought to have shared an ancestor. After that, the diploid cotton species were divided into eight groups, A-G and K. Both of the latter two species are tetraploid ( $2n = 4x = 52$ ). Polyploidization between A-genome and D-genome cotton species produces all tetraploid cotton species (Boopathi et al. 2015). The A-genome species have undergone a long period of artificial selection for their high-quality staple fibers, while the D-genome species, under natural selection, have retained great genetic diversity and a variety of desirable traits such as fiber quality and salt resistance (Boopathi et al. 2015; Konan et al. 2020).

Cotton is a globally important cash crop that produces raw cotton lint for the textile industry, cottonseed oil for culinary use, and edible and protein-rich oil cake wastes for livestock, as well as benefiting humans through its sticks, fiber, seeds, and oil as a key product (Ahmad and Hasanuzzaman 2020).

Cotton production is influenced by weather conditions. High humidity from rain or irrigation, as well as dry and warm harvest seasons, produce the highest yield (Khan et al. 2020). Cotton is farmed as a principal crop in more than 100 countries, with large proportions in China (6,423,000 tons), India (6,162,000 tons), the United States (3,181,000 tons), and Brazil (2,341,000 tons), and Pakistan (980,000 tons) due to its monetary importance. Cotton is usually grown in moderately hot climates (Shahbandeh 2021).

Cotton production, on the other hand, is hampered by several reasons, including inadequate environmental conditions (abiotic stresses) and biotic stresses, both

## Transplantation Proceedings and Research

of which have been linked to yield declines (Gull et al. 2019). Drought is the most significant abiotic limitation, limiting photosynthesis, glucose metabolism, and the activity of key enzymes involved in fiber production (Ul-Allah et al. 2021). Salinity, on the other hand, is a severe concern for agriculture since it depletes farmland. More than 6% of the world's land (830 million hectares impacted by salinity, 403 million hectares) is affected by salt water, with the rest affected by linked factors like sodicity (Ullah et al. 2018). Even though cotton is a salt-tolerant plant, its salt level can have a major negative influence on its development and productivity (Zhu et al. 2018). Cotton types and hybrids are more susceptible to insect pests such as jassids (leafhoppers), whiteflies, and bollworms, as well as diseases such as bacterial blight, and verticillium wilt, and leaf curl virus (Boopathi et al. 2015).

Plants deploy several internal mechanisms to deal with pathogens during the growth and development process to battle such situations, and it is vital to understand the mechanisms that plants use to deal with varied stresses at the genetic level. Abiotic and biotic stress activates a network of plant gene expression mechanisms, which causes the stress response to reprogramme several physiological and metabolic processes (Naqvi et al. 2017; Gull et al. 2019; Liu et al. 2020). Changes in the expression of stress genes govern these defensive systems, according to the findings of numerous studies. Many of these stress genes are known to be transcriptionally controlled (Heidel-Fisher et al. 2018). Transcriptome profiling identified some stress-responsive genes that were successfully transferred to other plants and were up-or down-regulated in cotton. Single or multiple pressures substantially activate transcription factors, and transcription factors play a key role in the response to biotic and abiotic stresses (Ullah et al. 2018). MYB (myeloblastosis), MADS (minichromosome maintenance agamous deficiens serum response factor), ARF (auxin response factors), EREBP (ethylene-responsive element-binding protein), DRE (drought-responsive element), HB (Homeobox), SBP (squamosa promoter binding protein), bZIP (basic region leucine zipper), GRAS (gibberellic-acid insensitive, repressor (Alves et al. 2013; Kaur et al. 2016).

The WRKY transcription factors are rated ninth in the plant transcription factor families (Tian et al. 2020) and have received a lot of attention (Liu et al. 2020). WRKY[(Trp(W)-Arg(R)-Lys(K)-Try(Y), tryptophan arginine lysine-Tyrosine] proteins are newly discovered transcription factors with either one or two

highly conserved amino acid sequence WRKYGQK motif at their N-terminus and a 60 amino acid zinc finger motif at their C-terminus (Eulgem et al. 2000; Rushton et al. 2010). These plant-specific WRKY transcription factors work in tandem with other proteins or DNA sequences to promote or inhibit gene transcription. It also regulates a variety of physiological processes in plants by acting as a component of signaling networks (Pandy and Somssich 2009; Wang et al. 2019; Jeyasri et al. 2021; Liu et al. 2020). It also controls biotic and abiotic stressors, as well as germination, seed growth, metabolism, phosphate limitation, and senescence (Zhou et al. 2008). As a result, the role of the WRKY TF in *Gossypium* spp. was briefly reviewed in this paper.

### Transcription Factors

Depending on their biological and physiological function, genes show distinct expression patterns in a variety of adverse environmental conditions such as drought, cold, excessive salinity, and pathogen incursion (Sharoni et al. 2011). Gene expression is required for a range of key plant functions, including growth, development, differentiation, metabolism, and environmental adaptability. The transcription of genes, which plays a key role in regulating the process, is the initial stage in gene expression. Numerous transcription factors that mediate the impact of intercellular and extracellular signals appear to govern transcription (Riao-Pachn et al. 2007; Saibo et al. 2009).

Each DNA promoter contains particular groups of short conserved sequences that TFs can bind to. Some of these components and variables are ubiquitous, and can be discovered and utilized in a wide range of promoters; others are unique, and their usage is controlled (Inukai et al. 2017). Transcription factors are classified into three groups based on their mode of activity. There are three types of factors: general factors, upstream factors, and inducible factors, all of which play various regulatory functions in gene transcription (Cooper 2021).

TFs are also divided into groups according to the structure of their DNA-binding domains (Gonzalez 2016). Some TF families are found in all eukaryotes, while others are only found in certain lineages (Yamasaki 2016). TFs have additional domains that are important in gene activation or repression, dimerization, and the development of protein-protein interactions in addition to the DNA-binding domain (Konishi 2019). TFs are encoded by around 10% of genes in plants at various stages to regulate distinct signaling functions (Gonzalez 2016). TFs are

frequently found in several copies or isoforms, which allow them to carry out their regulatory functions (Jazayeri et al. 2020). According to the Plant Transcription Factor Database, there are now 58 transcription factor families engaged in various plant biological functions (Tian et al. 2020).

WRKY TFs are one of the largest groups of transcriptional regulators that are only found in plants (Wani et al. 2021). Numerous studies, ranging from model plants to crops and other species, have looked at the role and mechanism of members of the WRKY family in recent years (Li et al. 2020). WRKY-TFs are involved in plant disease resistance (Shi et al. 2014; Sun et al. 2015), abiotic stress responses (drought, saline, alkali, temperature, and ultraviolet radiation) (Jiang et al. 2016; Guo et al. 2019), nutrient deficiency, senescence, seed and trichome development embryogenesis and development (Rushton et al. 2010). WRKYs can act as transcription activators or repressors in various homo- and heterodimer combinations (Wani et al. 2021).

### Origin, Structure, and Domains of WRKY TFs

WRKY genes are prevalent in plants and have recently been recognized as important family regulators of genes. They appear to have originated in early eukaryotes and have recently been identified as major family regulators of genes (Zhang and Wang 2005). SPF1, the first cDNA encoding a WRKY protein, was discovered in sweet potato (*Ipomoea batatas*) and binds to the SP8a (ACTGTGTA) and SP8b (TACTATT) sequences in the 5' upstream regions of three distinct genes expressing sporamine and tuber root amylase (Ishiguro and Nakamura).

WRKY protein genes have since been found in a variety of plant species, including wild oats (*Avena fatua*) (Rushton et al. 1995), parsley (*Petroselinum crispum*) (Rushton et al. 1996), *Arabidopsis thaliana* (de Pater et al. 1996), barley (*Hordeum vulgare*) (Sun et al. 2003), cotton (*Gossypium arboreum*) (Xu et al. 2004), etc. Plants have WRKY-TF gene families, and the number of WRKY-TFs varies by species. For example, *Solanum lycopersicum* (81), *Arabidopsis thaliana* (90), *Gossypium arboreum* (111), *Oryza sativa* (128), *Sorghum bicolor* (134), *Zea mays* (161), *Triticum aestivum* (171), *Gossypium raimondi* (Tian et al. 2020).

The WRKY TFs' structures are highly diverse in general; the structure is divided into two parts: the N-terminal DNA binding domain (DBD), which contains the WRKYGQR amino acid sequence, and the C-terminal zinc finger motif (Li et al. 2020). The DBD

sequence of WRKY is based on the heptapeptide WRKYGQK; however other WRKY proteins feature W-R-R-Y, W-S-K-Y, and W-K-R-Y, W-V-K-Y, or W-K-K-Y motifs in place of the WRKY amino acid sequences (Finatto et al. 2018). This domain is a four-stranded beta-sheet with a zinc-binding pocket, forming a unique zinc and DNA binding structure, according to structural investigations. The WRKYGQK residues are found in the N-terminal beta-strand, which provides for a wide range of hydrophobic contacts and adds to the beta-structural sheet's stability (Yamasaki et al. 2005).

WRKY-TFs from the *Arabidopsis* genome can be classified into three groups and types of WRKY domains based on the number of WRKY domains and the layout of the zinc finger motif, which may reflect their various activities (Rahaie et al. 2013). The varied binding ability of the WRKY-TFs could be related to a different amount of DBDs and zinc finger-like motifs, despite a substantially conserved W-box. WRKY proteins are divided into four classes based on these characteristics: I (two WRKY DBDs), II (single DBD with various C<sub>2</sub>H<sub>2</sub> zinc fingers), III (single DBD with C<sub>2</sub>HC zinc fingers), and IV (incomplete WRKY domain/without zinc fingers) (Finatto et al. 2018). Most proteins with two WRKY domains belong to group I, while most proteins with one WRKY domain belong to group II. Although Group III proteins contain a single WRKY domain, the zinc finger motif layout is distinct or varied. According to the phylogenetic study of WRKY domains, Group II was further separated into five subgroups (Eulgem et al. 2000; Li et al. 2020). GhWRKY19, contrary to popular belief, has two WRKY domains and an inserted zinc finger motif, but it lacks the zinc-finger structure at the C-terminus (Zhou and Lu 2014).

A glutamate accumulation domain, a proline accumulation domain, and a leucine zipper structure are also seen in several WRKY proteins (Chen et al. 2012). Gu et al. (2018) also discovered 34 genes for group IId WRKY-TFs and divided them into four groups (clade I-IV). Other studies on the WRKY subfamily of group IId in cotton have also been published. In *Gossypium raimondii*, Cai et al. (2014) found 15 WRKY members of group IId, while Fan et al. (2015) found 15 in *Gossypium iridium*. Due to the presence of two WRKY domains, evolutionary study implies that group I is the oldest, and groups II and III evolved from group I. (Jimmy and Babu 2019). In general, the WRKY domains of Group I and Group II members have the same type of C<sub>2</sub>-H<sub>2</sub> zinc finger motif, which has a unique pattern of possible zinc ligands (CX<sub>4</sub>-5CX<sub>22</sub>-23-HXH) (Fan et al. 2015).

### WRKY Transcription Factors in *Gossypium* spp

The plant TF database was used to construct transcription factors for three *Gossypium* species. More TFs (5022) is found in *Gossypium hirsutum*, followed by *Gossypium raimondii* (4894) and *Gossypium arboreum* (2532). WRKY is responsible for 4.7 percent (238), 4.5 percent (219), and 4.4 percent (111) of the identified TFs, respectively (Tian et al. 2020). In total, 58 WRKY TFs have been identified in the three species, accounting for 4.8 percent of all documented TF families. Similarly, 116 WRKY TFs in *Gossypium raimondii* had the entire genome sequence (Dou et al. 2014) and roughly 239 GhWRKY TFs in *Gossypium hirsutum* and three genes with partial WRKY domain structures (GhWRKY27, GhWRKY238, and GhWRKY239) were classed as group IV in *Gossypium hirsutum*. A total of 15 GhWRKY group IIa members were discovered, mostly on chromosomes 5, 6, and 7 (Gu et al. 2018).

### Roles Of Wrky Tfs in Stress Response

The process of plant adaptation to environmental stress is controlled through the orchestration of complex molecular networks regulated by TF proteins. WRKY proteins represent a large family of these TFs along with bHLH, MYB, ERF, NAC, C2H2, bZIP, and MYB are related, and many reports have shown that the WRKY protein family has multiple developmental and physiological functions that are in response to external stimuli (Eulgem et al. 2000; Rushton et al. 2010). TF expression patterns are generally divided into constitutive and inducible expression, and therefore WRKYs-TFs protein expression is inducible in response to various stresses. WRKY-TFs regulate the expression of pathogen-initiated, senescence-initiated, abscisic acid-induced, gibberellic acid-induced, and salicylic acid-induced genes and play important roles in the regulation of plant growth and development, and their response to numerous types of abiotic stress (Singh et al. 2019) (Table 1). Furthermore, its expression is rapid, immediate, tissue-specific (roots, stems, leaves, and embryos) (Dou et al. 2014), and involved in various physiological processes in plants (Cheng et al. 2021). GhWRKY17 expression in cotton can be induced by drought, salt and H<sub>2</sub>O<sub>2</sub> (Yan et al. 2014). Ultraviolet irradiation induces the expression of three AtWRKYs in *Arabidopsis* and OsWRKY89 in rice, resulting in the production of a thick waxy substance on the leaf surface and improving high-temperature tolerance (Cheng et al. 2021). Expression analysis showed that most group II and III GhWRKY genes are highly expressed under different strains (Dou et al. 2014).

However, WRKY can also act as a negative regulator of gene expression. Heterologous expression of *Gossypium hirsutum* GhWRKY33 gene reduced drought tolerance of transgenic *Arabidopsis* plants (Cao et al. 2019). Several WRKY33-interacting proteins containing the VQ motif have been discovered that affect the expression of defense genes. VQ proteins appear to act as suppressors of defense genes through their interaction with WRKY-TFs. Indeed, WRKY33, SIB1, and SIB2 were significantly induced by *Botrytis cinerea* infection and showed similar expression patterns (Liu et al. 2015). Overall, WRKY TF is thought to act as transcriptional regulator by binding to the W box, a promoter element common to numerous defense-associated genes (Dang et al. 2013). These include resistance to plant diseases (bacteria, fungi, and viruses). For example, GhWRKY11, GhWRKY15, and GhWRKY39, which are three structurally related groups IIId WRKY members, interact physically and functionally in a complex to confer resistance to the various pathogens in plants (Yu et al. 2012; Sun et al. 2012; Shi et al. 2014), abiotic stress responses (drought, cold, wounding, nutrient deprivation from high salinity and UV exposure, and aging) (Rahaie et al. 2013; Cheng et al. 2021). Microarray, expression profiling, and qRT-PCR data showed that WRKY genes in *Gossypium hirsutum* regulate the development of fibers, anthers, tissues (roots, stems, leaves, and embryos) and are involved in stress response (Dou et al. 2014). In addition, some members of the WRKY family play a role in hormone signaling (Rahaie et al. 2013). For example, GhWRKY3 expression upregulates SA, MeJA, GA, ABA, and ET biosynthesis in response to pathogen infection, drought, salt, and cold stress (Guo et al. 2011) (Table 1). WRKY TFs families directly or indirectly control the plant defense response by altering the biosynthesis of the secondary metabolite (Schlutenhofer and Yuan 2015). All of these benefits have been considered by growers in crops because of the intrinsic role TF plays in these bio-molecular processes.

### The Roles of WRKY TFs in Abiotic Stress Responses

Abiotic factors in the environment are non-living chemical and physical elements that have an impact on both individual organisms and ecosystems. Low or high temperatures, insufficient or excessive water, high salt, heavy metals, and UV radiation are all abiotic stressors that affect plant growth and development, resulting in large crop losses around the world. Plants have developed a more general

defense against abiotic stress through a complex regulatory network that includes upstream signaling molecules like stress hormones, reactive oxygen species, polyamines, phytochromes, and calcium, as well as downstream gene regulation elements, particularly transcription factors (TFs) (Jiang et al. 2016; He et al. 2018).

### Drought Response

Drought is a continuous water scarcity that plants must adjust to as one of the principal abiotic environmental stresses, and it can cause significant economic losses in agriculture (Cominelli and Tonelli 2010; He et al. 2018). Water for irrigating crops is becoming increasingly scarce as a result of global warming and water shortages; hence the creation of drought-resistant agricultural species is critical (Ullah et al. 2017). Drought is a major limiting factor in cotton production, as more than half of the world's cotton is farmed in areas with high water stress (Li et al. 2017). Drought stress has a significant impact on cotton output. Drought stress has been shown to cause a 42 percent drop in seed production and a 55 percent drop in cotton biological yield (Wang et al. 2019). Drought tolerance is a complicated feature mediated by a variety of genes, transcription factors, microRNAs, hormones, proteins, cofactors, ions, and metabolites. Cotton stomata, root development, cellular adaptations, photosynthesis, generation of abscisic acid (ABA) and jasmonic acid (JA), and scavenging of reactive oxygen species (ROS) have all been recognized as significant physiological responses to drought stress (Ullah et al. 2017). Several stress-responsive genes and transcription factors (TFs) responsible for establishing stress tolerance in crop plants have been found through molecular and biochemical research during the last decade. Drought resistance in plants is a complicated process that is generally controlled by several genes, including those encoding WRKY-TFs (Dou et al. 2014; He et al. 2018).

This protein is involved in several processes, including organogenesis and development, as well as stress and defense pathways and other abiotic stressors. Drought stress activates several drought-related signaling pathways by inducing the production of stress-related transcription factors and genes such as ROS scavenging, ABA, or mitogen-activated protein kinase (MAPK) signaling genes (Ullah et al. 2017). WRKY family members play a significant role in various stress responses among numerous stress-related TFs (Li et al. 2020).

Plants have WRKY-TF gene families, and the number

of WRKY-TFs varies by species. Overexpression of AtWRKY57 in Arabidopsis plants has been demonstrated to improve drought tolerance (Jiang et al. 2016). GhWRKY17 was discovered to be triggered by dryness, salt, H<sub>2</sub>O<sub>2</sub>, and ABA (Yan et al. 2014). Physiological analyses of germination rate, root growth, survival rate, leaf water loss, and chlorophyll content revealed that constitutive expression of GhWRKY17 in *Nicotiana benthamiana* significantly lowered plant tolerance to drought and salt stress. GhWRKY17 transgenic plants are more responsive to ABA-mediated seed germination and root development. Overexpression of GhWRKY17 in *Nicotiana benthamiana*, on the other hand, prevented ABA-induced stomata blockage. Furthermore, GhWRKY17 reduced ABA levels, which reduced plant susceptibility to drought, and transcript levels of ABA-inducible genes such as AREB, DREB, NCED, ERD, and LEA were significantly decreased under drought and salt stress conditions (Yan et al. 2014). Drought, salt, ABA, and H<sub>2</sub>O<sub>2</sub> treatment increased glucuronidase activity mediated by the GhWRKY68 promoter (Jia et al. 2015). By altering ABA concentration and boosting transcript levels of ABA-responsive genes, GhWRKY68 can influence salt and drought responses (Jia et al. 2015). GhWRKY33 is a leaf-specific gene that is activated by drought. The transgenic Arabidopsis plant's drought sensitivity is increased by overexpression of GhWRKY33, and the transcription levels of certain genes implicated in drought stress are also altered during drought stress (Wang et al. 2019). However, when subjected to drought, the GhWRKY33 transgenic plants maintained larger stomata opening than the wild-type, resulting in a higher rate of water loss. In the transgenic plants' leaves, both chlorophyll content and proline accumulation were significantly reduced.

This circumstance suggested that GhWRKY33 may have a role in drought responses by modulating stomatal mobility (Wang et al., 2019) (Table 1). Furthermore, according to Li et al. (2017), GhWRKY59, a WRKY TF, is phosphorylated by a MAPK cascade composed of GhMAP3K15-GhMCK4-GhMPK6 and plays a significant role in the drought stress response in cotton. GhWRKY59 regulates MAPK activation via feedback regulation of GhMAP3K expression, which is interesting. GhWRKY59 controls drought-responsive gene expression by binding directly to the promoter of GhDREB2.

Hu et al. (2021) found and reported that WRKY has a positive regulatory role in drought intolerance, which directly manipulates ABA biosynthesis, using the

## Transplantation Proceedings and Research

GhWRKY1-like from highland cotton. GhWRKY1-like overexpression in *Arabidopsis* activated ABA biosynthetic genes, signaling genes, responsive genes, and drought-related marker genes, resulting in improved drought tolerance (Hu et al. 2021). Group IId WRKY gene families were highly expressed during drought treatment circumstances, according to Gu et al. (2018). Drought tolerance was greatly boosted when GhWRKY21 was silenced, whereas ectopic GhWRKY21 overexpression in *Nicotiana benthamiana* lowered drought tolerance (Wang et al. 2021). GhWRKY91 may inhibit natural and stress-induced leaf senescence, laying the groundwork for more functional research into leaf senescence and stress response in cotton, as well as improving drought tolerance in transgenic *Arabidopsis* plants. Furthermore, in cotton plants, virus-induced gene silencing (VIGS) of Gh A11G1801 could lower drought tolerance (Gu et al. 2018; Yang et al., 2021). GhWRKY91, on the other hand, stimulates the expression of its target gene, GhWRKY17 (Gu et al. 2019).

### Salt Stress Response

Salt is an environmental component that limits crop development and yields, and it is becoming a major concern around the world. Plants are affected by salt stress in a variety of ways, including osmotic stress, ionic stress, and, most importantly, oxidative damage (Yang and Guo 2018). Salt stress has a major impact on the expression levels of genes involved in a variety of biological processes and signalling pathways.

It's critical to find salt-resistance genes to boost cotton production in salt-stressed areas. Cotton has a low diversity of salt tolerance genes when compared to other model plants (Wang et al. 2020).

Activation of numerous transcription factors such as MYB, WRKY, AP2, and cell wall modification are complex molecular mechanisms of salt stress response in cotton and are very active in response to salt stress (Wang et al. 2020). Understanding the expression patterns of these important genes implicated in the response to salinity could provide insight into the molecular mechanisms behind salt stress. In recent decades, a substantial number of salt-sensitive WRKY genes in cotton have been found and described (Shi et al. 2014; Yan et al. 2014; Chu et al. 2016).

Wild-type (WT) and overexpressing (OE) plants were irrigated with saline water (200 mM) for one month to assess the effect of salinity on seed germination and if GhWRKY68 affects the salt tolerance of transgenic plants in the vegetative stage. In comparison to the

WT plants, the OE plants showed substantial growth retardation, leaf curling, and chlorosis after NaCl treatment. Only around 40% of the OE plants survived the high salinity conditions, and their survival rate was lower than the WT plants. These findings imply that overexpression of GhWRKY68 in transgenic plants during seed germination and vegetative state may result in decreased salt tolerance (Jia et al. 2015). Yan et al. published a paper that was similar to this one in 2014.

Based on overexpression of GarWRKY5 in *Arabidopsis* and virus-induced gene silencing of GarWRKY5 in cotton, Guo et al. (2019) investigated the role of the GarWRKY5 gene family in the response to salt stress in *Gossypium aridum* and found that GarWRKY5 is involved in the response to salt stress through the JA or SA signalling pathway. Under salt stress, GarWRKY5 overexpressors had greater superoxide dismutase and peroxidase activity. As a result, GarWRKY5 may operate as a positive transcriptional regulator in response to excessive salt stress by stimulating the expression of glutathione-S-transferase genes via the ROS scavenging mechanism. Similarly, virus-induced GhWRKY6 gene silencing increases salt tolerance in salt-sensitive cotton cultivars. At different developmental stages, the transgenic *Arabidopsis* lines GarWRKY17 and GarWRKY104 can improve salt tolerance (Fan et al. 2015).

GhWRKY6 downregulation promotes salt tolerance in cotton, presumably due to GhWRKY6 binding to the promoter region of the RAV1 gene, which has three W boxes within a 1 kb region upstream of the gene (Li et al. 2019). Overexpression of WRKY proteins in the upland cotton cultivars LMY37 and ZM12 resulted in robust responses to salt stress. At the transcriptional level, this protein alters the downstream expression patterns of targeted functional genes involved in ROS production and scavenging. At the transcriptional level, this protein alters the downstream expression patterns of targeted functional genes involved in ROS production and scavenging. Differential expression of signaling elements and downstream functional genes can change salinity tolerances directly or indirectly (Zhang et al. 2021). In general, GhWRKY TFs help researchers better understand how cotton copes with salt and osmotic stress (Ullah et al. 2018).

### Temperature Stress Response

Significant abiotic stress is defined as temperatures that are outside of an organism's optimal tolerance range. Extremely hot or low temperatures result in significant crop losses. As a result, to enhance

agricultural production, techniques to protect plant cells from harm caused by severe temperature swings must be developed (Ohama et al. 2017). When plants are exposed to high-temperature stress, the WRKY family is known to be implicated in the high-temperature response, controlling gene expression through many pathways and at different levels, and therefore influencing plant stress response signaling (Zhou et al. 2008).

WRKY TFs have been found to respond positively to plant tolerance to high temperatures in a growing number of studies. CaWRKY40, for example, is implicated in the response of plants to high-temperature stress in pepper, and overexpression of this TF reduces tobacco's susceptibility to high-temperature treatment, whereas deletion of this TF reduces tolerance to high-temperature treatment (Dang et al. 2013). GhWRKY39 expressed in upland cotton had a similar outcome (Shi et al. 2014).

In Arabidopsis, high-temperature treatment inhibited AtWRKY33 expression while inducing AtWRKY25 and AtWRKY26 expression, and constitutive overexpression of AtWRKY25 and AtWRKY26 increased resistance to high-temperature stress, while inhibition of AtWRKY41 expression reduced inhibition of dormant and dormant seed high-temperature inhibition (Fu and Yu 2015). Overexpression of TaWRKY30 in wheat resulted in a boost in high-temperature tolerance (Zhu et al. 2013). However, some WRKYs in plants hurt high-temperature tolerance; for example, heterologous expression of sunflower HaWRKY6 in Arabidopsis could drastically impair the plant's tolerance to high temperatures (Raineri et al. 2015).

In conclusion, it can be concluded that WRKY-TFs can improve plant tolerance to high temperatures via regulating transcription. Furthermore, these findings suggest that WRKY25, WRKY26, and WRKY33 functionally interact and play overlapping and synergistic roles in plant thermo-tolerance by positively regulating the collaboration between the ethylene-activated and heat shock protein-related signaling pathways that mediate responses to heat stress; and that these three proteins functionally interact and play overlapping and synergistic roles in plant thermo-tolerance (Li et al. 2020).

### Oxidative Stress Response

Oxidative stress is a complicated chemical and physiological phenomenon that occurs in higher plants in response to the overproduction and buildup of reactive oxygen species (ROS) as a result of the disruption of multiple metabolic pathways (i.e.,

respiration and photosynthesis) (Demidchik 2015; Pessaraki 2019). ROS regulates a variety of biological processes, including cell death, biotic and abiotic stress responses, and plant growth and development (Xu et al. 2019). ROS, which was once thought to be a poisonous and unwanted chemical, is now widely acknowledged as an essential component for plants (Demidchik 2015; Mittler 2017; He et al. 2018).

Plant stress reactions are frequently linked to the production of reactive oxygen species (ROS). As a result, plants protect themselves by activating numerous stress-related genes linked to the antioxidant defense system (Priya et al. 2019) to preserve ROS scavenging capabilities and maintain intracellular ROS homeostasis (Juszczuk et al. 2012). These are regulated by a variety of genes, including: 1) genes involved in the direct defense of cell membranes and proteins, such as osmotic protectants, radical scavengers, and detoxification enzymes; 2) genes involved in signaling cascades and transcription control, such as Mitogen-activated protein kinase (MAPK), phospholipases, calcium-dependent protein kinase (CDPK), SOS kinase, phospholipases (Ciarmiello et al. 2011). In Arabidopsis, a complicated network containing at least 152 genes regulates ROS levels (Fu et al. 2017).

Recent cotton research revealed that *Gossypium hirsutum* has 515 ROS genes, *Gossypium raimondii* has 261 ROS genes, and *Gossypium arboreum* has 260 ROS genes. *Gossypium*'s high number of ROS genes suggested a more complicated ROS network for fiber formation and abiotic stress tolerance (Xu et al. 2019).

WRKY-TFs have been demonstrated to inhibit ROS generation in cells, and they are involved in several ROS signaling-dependent responses, including senescence progression (Bakshi and Oelmüller 2014). ROS such as superoxide, hydroxyl radicals, and hydrogen peroxide have a significant detrimental impact on the cell wall of the affected cell, resulting in lipid peroxidation, cell damage, and oxidative stress (Banerjee and Roychoudhury 2015). As a result, WRKY proteins indirectly help to reduce oxidative stress by scavenging ROS. Transgenic tobacco plants overexpressing GhWRKY17 demonstrated increased oxidative stress sensitivity. In the transgenic lines, the expression of genes for ROS scavenging enzymes such as APX, catalase, and SOD was decreased (Yan et al. 2014).

Yan et al. (2014) described the effect of H<sub>2</sub>O<sub>2</sub> on GhWRKY17 transcription, stating that the expression

## Transplantation Proceedings and Research

of GhWRKY17 transcript was down-regulated at 2 hours and up-regulated by H<sub>2</sub>O<sub>2</sub> from 4 to 6 hours, with a substantial reduction (1.5-fold induction) 8 hours after treatment. Drought and salt stress both raised leaf proline levels in WT and OE plants, but the OE lines accumulated 11% and 15% less proline during drought and salt stress, respectively. These findings suggest that GhWRKY17 is important for controlling cellular ROS levels.

GhWRKY6-like were also examined in transgenic and WT plants in the presence of oxidative stressors such as malondialdehyde and H<sub>2</sub>O<sub>2</sub>, with WT plants accumulating more malondialdehyde and H<sub>2</sub>O<sub>2</sub> than transgenic lines. As a result of the findings, it was shown that WT plants were badly harmed by ROS, whereas the overexpressing GhWRKY6-like transgenic lines were protected in comparison to WT plants (Ullah et al. 2018).

In cotton, however, GhWRKY68 transcripts influenced drought stress response by modulating the expression of ABA-responsive genes and ROS detoxification (Jia et al. 2015). Increased activity of antioxidant enzymes to scavenge ROS was observed in Arabidopsis plants overexpressing the GarWRKY5 TF (Guo et al. 2019). Pathogen invasion frequently results in the generation of reactive oxygen species (ROS), which are important in defense responses. GhWRKY39-1 and GhWRKY44 overexpression improved resistance by preventing the buildup of pathogen-induced ROS in cotton (Shi et al. 2014).

### Biotic Stress Response

All phonological phases of plant development are affected by biotic stress. Pathogenic bacteria, fungi, and viruses all-cause biotic stress (Jiang et al. 2016). Plants have evolved a variety of defense systems to combat microbial infestations. In this process, transcription factors play a crucial function. During the response to pathogen infection, the transcriptional expression of several defense-related plant genes is controlled (Eulgem and Somssich 2007; Jiang et al. 2016). The most significant proteins in the WRKY family interact with other transcription factors to govern plant defensive responses (Eulgem and Somssich 2007; Jiang et al. 2016).

Multiple WRKYs can confer resistance to multiple bacterial or fungal pathogens. AtWRKY52, which contains a TIRNBSLRR domain (Toll/interleukin-1 receptor nucleotide-binding site leucine-rich repeat), works in combination with RPS4 to confer resistance to the fungal pathogen *Colletotrichum higginsianum* and the bacterial pathogen *Pseudomonas syringae* (Narusaka et al. 2009). It also displays a nuclear

interaction with the bacterial effector PopP2 and confers immunity to the bacterial pathogen *Ralstonia solanacearum* (Deslandes et al. 2002). Others like AtWRKY16 and AtWRKY19 also share a similar TIRNBSLRR domain, suggesting that these proteins are involved in defense-related ETI signaling (Chi et al. 2013).

WRKY70 and WRKY33 mutations increased Arabidopsis susceptibility to *Botrytis cinerea* necrotrophic fungal infection (Birkenbihl and Somssich 2011). In Arabidopsis, wounding causes the expression of WRKY11, WRKY15, WRKY22, WRKY33, WRKY40, WRKY53, and WRKY60 (Cheong et al. 2002). During fungal infections, about 28 WRKY-related transcripts were differently expressed in pericarp and cottonseed. In response to *Aspergillus flavus* infection, WRKY75 and WRKY72 were induced in the pericarp and seed, respectively.

Under toxigenic strain infection, WRKY75 and WRKY40 were specifically up-regulated in both pericarp and seed. In the pericarp, WRKY6, WRKY41, WRKY53, and WRKY50 were down-regulated (Bedre 2016).

Wang et al. (2014) found that wounding, infection with a bacterial pathogen, stress hormones (SA, MJ, and ET), and contact with the MAPK kinase GhMPK20 raised the expression of GhWRKY40 transcript. GhWRKY40 overexpression decreased defense gene expression and enhanced vulnerability to *Ralstonia solanacearum*. In *Nicotiana benthamiana*, however, constitutive overexpression of GhWRKY39 conferred higher resistance to bacterial and fungal pathogen infections, as well as enhanced expression of numerous pathogen-related genes (Shi et al. 2014).

GhWRKY70 expression was induced by *Verticillium dahliae* infection, SA, and MJ therapy, according to Xion et al. (2019). In GhWRKY70 virus-silenced cotton plants, genes linked with the JA response were increased, while genes related to the SA response were downregulated. GbWRKY1 is also controlled by the SA, JA, and ET pathways (Zhang et al. 2019). In Arabidopsis thaliana, overexpression of GhWRKY70 lowered resistance to *Verticillium dahliae*. SA-associated genes were expressed more frequently in transgenic Arabidopsis plants, while JA response-associated genes were expressed less frequently. This could be because the promoters of GhWRKY70 genes contain at least one hormone-responsive region.

Downregulating GhWRKY70D13 enhanced cotton resistance to *Verticillium dahliae*, which promoted the accumulation of 1-aminocyclopropane-1-carboxylic



acid, JA, and JA-Ile and impeded SA biosynthesis. Cotton's response to *Verticillium dahliae* infection is inhibited by GhWRKY70D13, which blocks the ET and JA signaling pathways (Xiong et al. 2020). Biological activities mediated by SA and JA are involved in many AtWRKY genes. In basal plant defenses, however, AtWRKY46, WRKY70, and WRKY53 all have overlapping and synergistic roles, favorably influencing basal resistance to *Pseudomonas syringae* and SA stress.

GhWRKY7, GhWRKY27, GhWRKY31, GhWRKY50, GhWRKY56, GhWRKY59, GhWRKY60, and GhWRKY102 were all significantly elevated in response to SA and JA, and these genes were linked to probable roles in the SA and JA signaling pathways (Dou et al. 2016). Overexpression of Group I, GhWRKY44, in *Nicotiana benthamiana* improved its resistance to bacterial and fungal diseases. Increased resistance may be linked to defense processes mediated by SA, JA, and ROS. This suggests that GhWRKY resistance to pathogenic pathogens has positive regulatory roles (Li et al. 2015).

### Conclusion

To protect future generations from potential crises, it is critical to strengthen cotton's biotic and abiotic tolerance capabilities. This enables the development of biotic and abiotic resistant transgenic lines using a variety of technologies utilizing transgenic procedures. WRKY TFs are thought to play a role in a variety of stress signaling pathways. Every day, no fewer than 58 TF families implicated in plant stress responses are discovered. The WRKY TFs have been found as molecular stress regulators in *Gossypium* spp. to fight biotic stress (bacteria, fungi, viruses, etc.). Some GhWRKY-TFs, on the other hand, is negatively regulated by pathogen infections, while others are positively regulated by abiotic (drought, salinity, temperature, oxidation, and so on) stresses. In general, WRKY-TFs play a role in both biotic and abiotic processes. However, future research should focus on how these transcription factors interact with other regulatory components to resist environmental stimuli and to better understand their role in metabolic processes. This allows the researchers to better understand how WRKYs regulate metabolism and, as a result, not only improve plant stress tolerance but also increase the synthesis of vital natural plant compounds.

### Declarations

Conflict of interest the authors declare that they have no conflict of interest.

### References

- Ahmad, S., & Hasanuzzaman, M. (Eds.). (2020). Cotton Production and Uses: Agronomy, Crop Protection, and Postharvest Technologies. Springer Nature.
- Alves, M. S., Dadalto, S. P., Gonçalves, A. B., De Souza, G. B., Barros, V. A., & Fietto, L. G. (2013). Plant bZIP transcription factors responsive to pathogens: a review. *International journal of molecular sciences*, 14(4), 7815-7828.
- Bakshi, M., & Oelmüller, R. (2014). WRKY transcription factors: Jack of many trades in plants. *Plant signaling & behavior*, 9(2), e27700.
- Banerjee, A., & Roychoudhury, A. (2015). WRKY proteins: signaling and regulation of expression during abiotic stress responses. *The Scientific World Journal*, 2015.
- Bedre, R. (2016). *Genome-wide Transcriptome Analysis of Cotton (Gossypium hirsutum L.) to Identify Genes in Response to Aspergillus flavus Infection, and Development of RNA-Seq Data Analysis Pipeline*. Louisiana State University and Agricultural & Mechanical College.
- Birkenbihl, R. P., & Somssich, I. E. (2011). Transcriptional plant responses critical for resistance towards necrotrophic pathogens. *Frontiers in plant science*, 2, 76.
- Boopathi, N. M., Sathish, S., Kavitha, P., Dachinamoorthy, P., & Ravikesavan, R. (2015). Molecular breeding for genetic improvement of cotton (*Gossypium* spp.). *Advances in plant breeding strategies: breeding, biotechnology and molecular tools*, 613-645.
- Cai, C., Niu, E., Du, H., Zhao, L., Feng, Y., & Guo, W. (2014). Genome-wide analysis of the WRKY transcription factor gene family in *Gossypium raimondii* and the expression of orthologs in cultivated tetraploid cotton. *The Crop Journal*, 2(2-3), 87-101.
- Cheng, Z., Luan, Y., Meng, J., Sun, J., Tao, J., & Zhao, D. (2021). WRKY transcription factor response to high - temperature stress. *Plants*, 10(10), 2211.
- Cheong, Y. H., Chang, H. S., Gupta, R., Wang, X., Zhu, T., & Luan, S. (2002). Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis*. *Plant physiology*, 129(2), 661-677.
- Chi, Y., Yang, Y., Zhou, Y., Zhou, J., Fan, B., Yu, J. Q., & Chen, Z. (2013). Protein-protein

## Transplantation Proceedings and Research

- interactions in the regulation of WRKY transcription factors. *Molecular plant*, 6(2), 287-300.
12. Chu, X., Wang, C., Chen, X., Lu, W., Li, H., Wang, X., ... & Guo, X. (2016). Correction: the cotton WRKY gene GhWRKY41 positively regulates salt and drought stress tolerance in transgenic *Nicotiana benthamiana*. *PLoS One*, 11(6), e0157026.
  13. Ciarmiello, L. F., Woodrow, P., Fuggi, A., Pontecorvo, G., & Carillo, P. (2011). Plant genes for abiotic stress. *Abiotic stress in plants—mechanisms and adaptations*, 283-308.
  14. Cominelli, E., & Tonelli, C. (2010). Transgenic crops coping with water scarcity. *New Biotechnology*, 27(5), 473-477.
  15. Cooper, J. A. (2021). Transcription factor. Encyclopedia Britannica. <https://www.britannica.com/science/transcription-factor>. (Accessed 3 December 2021).
  16. DANG, F. F., WANG, Y. N., Yu, L. U., Eulgem, T., Lai, Y. A. N., LIU, Z. Q., ... & HE, S. L. (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.
  17. de Pater, S., Greco, V., Pham, K., Memelink, J., & Kijne, J. (1996). Characterization of a zinc-dependent transcriptional activator from *Arabidopsis*. *Nucleic Acids Research*, 24(23), 4624-4631.
  18. Demidchik, V. (2015). Mechanisms of oxidative stress in plants: from classical chemistry to cell biology. *Environmental and experimental botany*, 109, 212-228.
  19. Deslandes, L., Olivier, J., Theulières, F., Hirsch, J., Feng, D. X., Bittner-Eddy, P., ... & Marco, Y. (2002). Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive RRS1-R gene, a member of a novel family of resistance genes. *Proceedings of the National Academy of Sciences*, 99(4), 2404-2409.
  20. DOU, L. L., GUO, Y. N., Ondati, E., PANG, C. Y., WEI, H. L., SONG, M. Z., ... & YU, S. X. (2016). Identification and expression analysis of group III WRKY transcription factors in cotton. *Journal of Integrative Agriculture*, 15(11), 2469-2480.
  21. Eulgem, T., & Somssich, I. E. (2007). Networks of WRKY transcription factors in defense signaling. *Current opinion in plant biology*, 10(4), 366-371.
  22. 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.
  23. Fan, X., Guo, Q., Xu, P., Gong, Y., Shu, H., Yang, Y., ... & Shen, X. (2015). Transcriptome-wide identification of salt-responsive members of the WRKY gene family in *Gossypium aridum*. *PLOS one*, 10(5), e0126148.
  24. Foyer, C. H., & Noctor, G. (2005). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The plant cell*, 17(7), 1866-1875.
  25. Fu, Q. T., & Yu, D. Q. (2010). Expression profiles of AtWRKY25, AtWRKY26 and AtWRKY33 under abiotic stresses. *Yi chuan= Hereditas*, 32(8), 848-856.
  26. Gonzalez, D. H. (2016). Introduction to transcription factor structure and function. In *Plant transcription factors* (pp. 3-11). Academic Press.
  27. Grunewald, W., De Smet, I., De Rybel, B., Robert, H. S., Van De Cotte, B., Willemsen, V., ... & Beeckman, T. (2013). Tightly controlled WRKY23 expression mediates *Arabidopsis* embryo development. *EMBO reports*, 14(12), 1136-1142.
  28. Gu, L., Ma, Q., Zhang, C., Wang, C., Wei, H., Wang, H., & Yu, S. (2019). The cotton GhWRKY91 transcription factor mediates leaf senescence and responses to drought stress in transgenic *Arabidopsis thaliana*. *Frontiers in Plant Science*, 10, 1352.
  29. Gu, L., Wang, H., Wei, H., Sun, H., Li, L., Chen, P., ... & Yu, S. (2018). Identification, expression, and functional analysis of the group IId WRKY subfamily in upland cotton (*Gossypium hirsutum* L.). *Frontiers in Plant Science*, 9, 1684.
  30. Gull, A., Lone, A. A., & Wani, N. U. I. (2019). Biotic and abiotic stresses in plants. *Abiotic and biotic stress in plants*, 1-19.
  31. Guo, Q., Zhao, L., Fan, X., Xu, P., Xu, Z., Zhang, X., & Shen, X. (2019). GarWRKY5, a Member of the WRKY Transcription Factor Gene Family from a Diploid Cotton Species (*Gossypium aridum* L.), Is Involved in Salt Stress Response.
  32. Guo, R., Yu, F., Gao, Z., An, H., Cao, X., & Guo, X. (2011). GhWRKY3, a novel cotton (*Gossypium*

- hirsutum L.) WRKY gene, is involved in diverse stress responses. *Molecular biology reports*, 38, 49-58.
33. Han, Y., Fan, T., Zhu, X., Wu, X., Ouyang, J., Jiang, L., & Cao, S. (2019). WRKY12 represses GSH1 expression to negatively regulate cadmium tolerance in Arabidopsis. *Plant Molecular Biology*, 99, 149-159.
  34. He, M., He, C. Q., & Ding, N. Z. (2018). Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance. *Frontiers in plant science*, 9, 1771.
  35. Heidel-Fischer, H. M., Musser, R. O., & Vogel, H. (2014). Plant transcriptomic responses to herbivory. *Annual Plant Reviews: Insect-Plant Interactions*, 47, 155-196.
  36. Inukai, S., Kock, K. H., & Bulyk, M. L. (2017). Transcription factor–DNA binding: beyond binding site motifs. *Current opinion in genetics & development*, 43, 110-119.
  37. Ishiguro, S., & Nakamura, K. (1994). Characterization of a cDNA encoding a novel DNA-binding protein, SPF1, that recognizes SP8 sequences in the 5' upstream regions of genes coding for sporamin and  $\beta$ -amylase from sweet potato. *Molecular and General Genetics MGG*, 244, 563-571.
  38. Jazayeri, S. M., Villamar-Torres, R. O., Zambrano-Vega, C., Cruzatty, L. C. G., Oviedo-Bayas, B., Santos, M. D. A., ... & Viot, C. (2020). Transcription factors and molecular markers revealed asymmetric contributions between allotetraploid Upland cotton and its two diploid ancestors. *Bragantia*, 79, 30-46.
  39. Jeyasri, Rajendran, Pandiyan Muthuramalingam, Lakkakula Satish, Sivakumar Adarshan, Muthukannan Aishwarya Lakshmi, Shunmugiah Karutha Pandian, Jen-Tsung Chen et al. "The role of OsWRKY genes in rice when faced with single and multiple abiotic stresses." *Agronomy* 11, no. 7 (2021): 1301.
  40. Jia, H., Wang, C., Wang, F., Liu, S., Li, G., & Guo, X. (2015). GhWRKY68 reduces resistance to salt and drought in transgenic *Nicotiana benthamiana*. *PLoS one*, 10(3), e0120646.
  41. Jiang, J., Ma, S., Ye, N., Jiang, M., Cao, J., & Zhang, J. (2017). WRKY transcription factors in plant responses to stresses. *Journal of integrative plant biology*, 59(2), 86-101.
  42. Jiang, Y., Qiu, Y., Hu, Y., & Yu, D. (2016). Heterologous expression of AtWRKY57 confers drought tolerance in *Oryza sativa*. *Frontiers in Plant Science*, 7, 145.
  43. Jimmy, J. L., & Babu, S. (2019). Variations in the structure and evolution of rice WRKY genes in indica and japonica genotypes and their co-expression network in mediating disease resistance. *Evolutionary Bioinformatics*, 15, 1176934319857720.
  44. Juszczuk, I. M., Szal, B., & Rychter, A. M. (2012). Oxidation–reduction and reactive oxygen species homeostasis in mutant plants with respiratory chain complex I dysfunction. *Plant, cell & environment*, 35(2), 296-307.
  45. Kaur, S., Iquebal, M. A., Jaiswal, S., Tandon, G., Sundaram, R. M., Gautam, R. K., ... & Kumar, D. (2016). A meta-analysis of potential candidate genes associated with salinity stress tolerance in rice. *Agri Gene*, 1, 126-134.
  46. Khan, M. A., Wahid, A., Ahmad, M., Tahir, M. T., Ahmed, M., Ahmad, S., & Hasanuzzaman, M. (2020). World cotton production and consumption: An overview. *Cotton production and uses: Agronomy, crop protection, and postharvest technologies*, 1-7.
  47. Konan, N., Baudoin, J. P., & Mergeai, G. (2020). Potential of ten wild diploid cotton species for the improvement of fiber fineness of upland cotton through interspecific hybridisation. *Journal of Plant Breeding and Crop Science*, 12(2).
  48. Konishi, M., & Yanagisawa, S. (2019). The role of protein-protein interactions mediated by the PB1 domain of NLP transcription factors in nitrate-inducible gene expression. *BMC plant biology*, 19, 1-12.
  49. Li, F., Li, M., Wang, P., Cox Jr, K. L., Duan, L., Dever, J. K., ... & He, P. (2017). Regulation of cotton (*Gossypium hirsutum*) drought responses by mitogen-activated protein (MAP) kinase cascade-mediated phosphorylation of Gh WRKY 59. *New Phytologist*, 215(4), 1462-1475.
  50. Li, W., Pang, S., Lu, Z., & Jin, B. (2020). Function and mechanism of WRKY transcription factors in abiotic stress responses of plants. *Plants*, 9(11), 1515.
  51. Li, Z., Li, L., Zhou, K., Zhang, Y., Han, X., Din, Y., ... & Yang, Z. (2019). GhWRKY6 acts as a negative regulator in both transgenic Arabidopsis and cotton during drought and salt stress. *Frontiers in Genetics*, 10, 392.
  52. Liu, C., Yuan, J., & Xiang, L. (2020). Literature Review of Plant WRKY Transcription

## Transplantation Proceedings and Research

- Factors. *Hans J. Agric. Sci*, 10, 628-633.
53. Liu, S., Kracher, B., Ziegler, J., Birkenbihl, R. P., & Somssich, I. E. (2015). Negative regulation of ABA signaling by WRKY33 is critical for Arabidopsis immunity towards *Botrytis cinerea* 2100. *elife*, 4, e07295.
54. Mittler, R. (2017). ROS are good. *Trends in plant science*, 22(1), 11-19.
55. Narusaka, M., Shirasu, K., Noutoshi, Y., Kubo, Y., Shiraishi, T., Iwabuchi, M., & Narusaka, Y. (2009). RRS1 and RPS4 provide a dual Resistance-gene system against fungal and bacterial pathogens. *The Plant Journal*, 60(2), 218-226.
56. Nimchuk, Z., Eulgem, T., Holt Iii, B. F., & Dangl, J. L. (2003). Recognition and response in the plant immune system. *Annual review of genetics*, 37(1), 579-609.
57. Pandey, S. P., & Somssich, I. E. (2009). The role of WRKY transcription factors in plant immunity. *Plant physiology*, 150(4), 1648-1655.
58. Priya, M., Dhanker, O. P., Siddique, K. H., HanumanthaRao, B., Nair, R. M., Pandey, S., ... & Nayyar, H. (2019). Drought and heat stress-related proteins: an update about their functional relevance in imparting stress tolerance in agricultural crops. *Theoretical and Applied Genetics*, 132, 1607-1638.
59. Rahaie, M., Xue, G. P., & Schenk, P. M. (2013). The role of transcription factors in wheat under different abiotic stresses. *Abiotic stress-plant responses and applications in agriculture*, 2, 367-385.
60. Raineri, J., Ribichich, K. F., & Chan, R. L. (2015). The sunflower transcription factor HaWRKY76 confers drought and flood tolerance to Arabidopsis thaliana plants without yield penalty. *Plant Cell Reports*, 34, 2065-2080.
61. Riaño-Pachón, D. M., Ruzicic, S., Dreyer, I., & Mueller-Roeber, B. (2007). PlnTFDB: an integrative plant transcription factor database. *BMC bioinformatics*, 8(1), 1-10.
62. Rushton, P. J., Macdonald, H., Huttly, A. K., Lazarus, C. M., & Hooley, R. (1995). Members of a new family of DNA-binding proteins bind to a conserved cis-element in the promoters of a-Amy2 genes. *Plant molecular biology*, 29(4), 691-702.
63. Rushton, P. J., Somssich, I. E., Ringler, P., & Shen, Q. J. (2010). WRKY transcription factors. *Trends in plant science*, 15(5), 247-258.
64. 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.
65. Saibo, N. J., Lourenço, T., & Oliveira, M. M. (2009). Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. *Annals of botany*, 103(4), 609-623.
66. Schluttenhofer, C., & Yuan, L. (2015). Regulation of specialized metabolism by WRKY transcription factors. *Plant Physiology*, 167(2), 295-306.
67. Shahbandeh, M. (2021). World cotton production by country 2019. Statista. Retrieved December 21, 2021, from <https://www.statista.com/statistics/263055/cotton-production-worldwide-by-top-countries/>
68. Sharoni, A. M., Nuruzzaman, M., Satoh, K., Shimizu, T., Kondoh, H., Sasaya, T., ... & Kikuchi, S. (2011). Gene structures, classification and expression models of the AP2/EREBP transcription factor family in rice. *Plant and cell physiology*, 52(2), 344-360.
69. Shi, W., Hao, L., Li, J., Liu, D., Guo, X., & Li, H. (2014). The *Gossypium hirsutum* WRKY gene GhWRKY39-1 promotes pathogen infection defense responses and mediates salt stress tolerance in transgenic *Nicotiana benthamiana*. *Plant cell reports*, 33, 483-498.
70. Shi, W., Liu, D., Hao, L., Wu, C. A., Guo, X., & Li, H. (2014). GhWRKY39, a member of the WRKY transcription factor family in cotton, has a positive role in disease resistance and salt stress tolerance. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 118, 17-32.
71. Singh, A., Singh, P. K., Sharma, A. K., Singh, N. K., Sonah, H., Deshmukh, R., & Sharma, T. R. (2019). Understanding the role of the WRKY gene family under stress conditions in pigeonpea (*Cajanus cajan* L.). *Plants*, 8(7), 214.
72. Sun, C., Palmqvist, S., Olsson, H., Borén, M., Ahlandsberg, S., & Jansson, C. (2003). A novel WRKY transcription factor, SUSIBA2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the iso1 promoter. *The Plant Cell*, 15(9), 2076-2092.
73. Sun, J., An, H., Shi, W., Guo, X., & Li, H. (2012). Molecular cloning and characterization of GhWRKY11, a gene implicated in pathogen responses from cotton. *South African Journal of*

- Botany*, 81, 113-123.
74. Sun, X. C., Gao, Y. F., Li, H. R., Yang, S. Z., & Liu, Y. S. (2015). Over-expression of SIWRKY39 leads to enhanced resistance to multiple stress factors in tomato. *Journal of Plant Biology*, 58, 52-60.
  75. Tian, F., Yang, D. C., Meng, Y. Q., Jin, J., & Gao, G. (2020). PlantRegMap: charting functional regulatory maps in plants. *Nucleic acids research*, 48(D1), D1104-D1113.
  76. Ul-Allah, S., Rehman, A., Hussain, M., & Farooq, M. (2021). Fiber yield and quality in cotton under drought: Effects and management. *Agricultural Water Management*, 255, 106994.
  77. Ullah, A., Sun, H., Yang, X., & Zhang, X. (2017). Drought coping strategies in cotton: increased crop per drop. *Plant biotechnology journal*, 15(3), 271-284.
  78. Ullah, A., Sun, H., Hakim, Yang, X., & Zhang, X. (2018). A novel cotton WRKY gene, GhWRKY6-like, improves salt tolerance by activating the ABA signaling pathway and scavenging of reactive oxygen species. *Physiologia Plantarum*, 162(4), 439-454.
  79. Wang, D., Lu, X., Chen, X., Wang, S., Wang, J., Guo, L., ... & Ye, W. (2020). Temporal salt stress-induced transcriptome alterations and regulatory mechanisms revealed by PacBio long-reads RNA sequencing in *Gossypium hirsutum*. *Bmc Genomics*, 21(1), 1-15.
  80. Wang, N. N., Xu, S. W., Sun, Y. L., Liu, D., Zhou, L., Li, Y., & Li, X. B. (2019). The cotton WRKY transcription factor (GhWRKY33) reduces transgenic Arabidopsis resistance to drought stress. *Scientific reports*, 9(1), 724.
  81. Wang, X., Yan, Y., Li, Y., Chu, X., Wu, C., & Guo, X. (2014). GhWRKY40, a multiple stress-responsive cotton WRKY gene, plays an important role in the wounding response and enhances susceptibility to *Ralstonia solanacearum* infection in transgenic *Nicotiana benthamiana*. *PloS one*, 9(4), e93577.
  82. Wang, Y., Li, Y., He, S. P., Gao, Y., Wang, N. N., Lu, R., & Li, X. B. (2019). A cotton (*Gossypium hirsutum*) WRKY transcription factor (GhWRKY22) participates in regulating anther/pollen development. *Plant Physiology and Biochemistry*, 141, 231-239.
  83. Wani, S. H., Anand, S., Singh, B., Bohra, A., & Joshi, R. (2021). WRKY transcription factors and plant defense responses: latest discoveries and future prospects. *Plant Cell Reports*, 40, 1071-1085.
  84. Wendel, J. F., & Grover, C. E. (2015). Taxonomy and evolution of the cotton genus, *Gossypium*. *Cotton*, 57, 25-44.
  85. Xiong, X. P., Sun, S. C., Zhang, X. Y., Li, Y. J., Liu, F., Zhu, Q. H., ... & Sun, J. (2020). GhWRKY70D13 regulates resistance to *Verticillium dahliae* in cotton through the ethylene and jasmonic acid signaling pathways. *Frontiers in Plant Science*, 11, 69.
  86. 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 *Verticillium dahliae*. *The Crop Journal*, 7(3), 393-402.
  87. Xu, Y. H., Wang, J. W., Wang, S., Wang, J. Y., & Chen, X. Y. (2004). Characterization of GaWRKY1, a cotton transcription factor that regulates the sesquiterpene synthase gene (+)- $\delta$ -cadinene synthase-A. *Plant physiology*, 135(1), 507-515.
  88. Xu, Y., Magwanga, R. O., Cai, X., Zhou, Z., Wang, X., Wang, Y., ... & Liu, F. (2019). Deep transcriptome analysis reveals reactive oxygen species (ROS) network evolution, response to abiotic stress, and regulation of fiber development in cotton. *International journal of molecular sciences*, 20(8), 1863.
  89. Yamasaki, K. (2016). Structures, Functions, and Evolutionary Histories of DNA-Binding Domains of Plant-Specific Transcription Factors. In *Plant Transcription Factors* (pp. 57-72). Academic Press.
  90. Yamasaki, K., Kigawa, T., Inoue, M., Tateno, M., Yamasaki, T., Yabuki, T., ... & Yokoyama, S. (2005). Solution structure of an Arabidopsis WRKY DNA binding domain. *The Plant Cell*, 17(3), 944-956.
  91. Yan, H., Jia, H., Chen, X., Hao, L., An, H., & Guo, X. (2014). The cotton WRKY transcription factor GhWRKY17 functions in drought and salt stress in transgenic *Nicotiana benthamiana* through ABA signaling and the modulation of reactive oxygen species production. *Plant and Cell Physiology*, 55(12), 2060-2076.
  92. Yang, D., Liu, Y., Cheng, H., Wang, Q., Lv, L., Zhang, Y., ... & Zuo, D. (2021). Identification of the Group III WRKY Subfamily and the Functional Analysis of GhWRKY53 in *Gossypium hirsutum* L. *Plants*, 10(6), 1235.

## Transplantation Proceedings and Research

93. Zhang, J., Zhang, P., Huo, X., Gao, Y., Chen, Y., Song, Z., ... & Zhang, J. (2021). Comparative phenotypic and transcriptomic analysis reveals key responses of upland cotton to salinity stress during postgermination. *Frontiers in Plant Science*, 12, 639104.
94. Zhang, X., Liu, J., Wu, L., Wang, Z., & Zhang, S. (2019). GbWRKY1, a member of the WRKY transcription factor family identified from *Gossypium barbadense*, is involved in resistance to *Verticillium* wilt. *Biotechnology & Biotechnological Equipment*, 33(1), 1354-1364.
95. Zhang, Y., & Wang, L. (2005). The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. *BMC evolutionary biology*, 5, 1-12.
96. Zhou, Q. Y., Tian, A. G., Zou, H. F., Xie, Z. M., Lei, G., Huang, J., ... & Chen, S. Y. (2008). Soybean WRKY-type transcription factor genes, GmWRKY13, GmWRKY21, and GmWRKY54, confer differential tolerance to abiotic stresses in transgenic *Arabidopsis* plants. *Plant biotechnology journal*, 6(5), 486-503.
97. Zhu, G., Li, W., Zhang, F., & Guo, W. (2018). RNA-seq analysis reveals alternative splicing under salt stress in cotton, *Gossypium davidsonii*. *BMC genomics*, 19(1), 1-15.
98. Zhu, X., Liu, S., Meng, C., Qin, L., Kong, L., & Xia, G. (2013). WRKY transcription factors in wheat and their induction by biotic and abiotic stress. *Plant Molecular Biology Reporter*, 31, 1053-1067.