

## Role of WRKY Transcription Factor to Molecular Switches to Tolerate the Drought, Temperature and Salinity Stress in Plants

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### ABSTRACT

*One of the main plant protein superfamilies is comprised of the WRKY transcription factor (TF). Plant stress responses are linked to transcriptional reprogramming, which is regulated in part by the WRKY TF gene family. Alterations in the ways that WRKY genes express themselves or alter how they function; take part in the development of several signaling pathways and regulatory networks. The development of gametes, seed germination, post-germination growth, stem elongation, root hair growth, leaf senescence, flowering period, and plant height are all influenced by WRKY proteins. Furthermore, they are important in a variety of environmental signals, such as those related to biotic stressors, temperature, salinity, drought, and cold. This review will refine reader knowledge about the role of the WRKY transcription factor that switches to tolerate the drought, temperature, and salinity stress in plants.*

**Keywords:** Transcriptomics analysis, metabolomic regulations, overexpression, stress signaling, tolerance

### INTRODUCTION

A family of transcription factors (TFs) known as the WRKY family is extensively distributed in plants and is involved in both biotic and abiotic stress management as well as plant growth and development. A global hazard to

important crops, the increased exposure of plants to diverse stresses including severe temperatures, droughts, and salinity has a substantial impact on plant/crop growth and output.

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Numerous TF genes aid in a plant's ability to endure harsh environments and continue to be viable genetic candidates for extensive application in crop development. WRKY TFs are significant molecular switches that assess how plants develop and control how they react to different types of stress (Kapoor et al., 2020).

Genetic engineering has significantly altered the agronomic characteristics of crops and is thought to be an alternative to enhancing stress tolerance. Genes responding to abiotic stressors have been identified as many genes encoding TFs, functional proteins, and proteins involved in signal transduction pathways (Cohen et al., 2021). Several TF families, which are exclusive to plants and have significant and distinct roles, including WRKY, AP2 (APETLA2)/ERF (ethylene responsive factor), and NAC (NAM, ATAF1/3, and CUC1/2) (Jiang et al., 2017).

#### **Drought stress linked with WRKY TFs**

When plants are exposed to different stressors or defense signals, such as salicylic acid (SA) or other chemicals, the expression of WRKY TF is stimulated. Apart from the rapid, transient, and tissue-specific expression of WRKY TF, WRKY proteins perform a variety of roles in plant defenses against various stresses, such as drought, plant growth, development, metabolism, trichome and embryonic morphogenesis, senescence, biosynthesis, and hormonal signal regulation (Wei et al., 2017; Zaib et al., 2023a, 2023b).

Significant roles that the WRKY TFs play in responding to and adapting to drought stress. In *A. thaliana*, overexpression of AtWRKY57 led to an increase in drought tolerance. Research has indicated that transgenic rice *O. sativa* plants may be able to withstand drought conditions thanks to the Arabidopsis WRKY57 transcription factor. Drought, salinity, and polyethylene glycol (PEG) tolerance were all improved in rice when AtWRKY57 was overexpressed, suggesting a potential function for AtWRKY57 in crop growth (Jiang et al., 2016). MaWRKY80 was shown to be a transcription factor (TF) that could bind to the

W-box in *A. thaliana* and was found to be up-regulated under drought-stressed circumstances. Under drought stress, MaWRKY80 overexpression demonstrates better phenotypic morphology, improved survival, a lower rate of water loss, and a lower level of malondialdehyde (MDA) than WT (wild-type).

#### **Temperature stress linked with WRKY TFs**

A precise balance between production and clearance controls ROS-mediated signaling, which is controlled by oxidative stress, a severe stressor brought on by a range of stressors (Salvucci et al., 2001; Zaib et al., 2023c, 2023d, 2023e; Raza et al., 2023). In plants, reactive oxygen species come in four different forms: superoxide anions, hydroxyl radicals, hydrogen peroxide, and oxygen. When *A. thaliana* is treated with hydrogen peroxide, several WRKY TFs (WRKY6, WRKY30, WRKY22, WRKY8, WRKY53, WRKY48, WRKY39, and WRKY75) become activated (Jiang et al., 2017). Research has shown that in *A. thaliana*, treatment with H<sub>2</sub>O<sub>2</sub> induced higher expression of genes that could react to a higher temperature (WRKY6, WRKY8, WRKY22, WRKY30, WRKY39, WRKY48, WRKY53, and WRKY75) (Chen et al., 2010).

It has been demonstrated that OsWRKY42 functions as a significant negative regulator of oxidative stress; in rice, overexpression of OsWRKY42 leads to an increase in the buildup of ROS (Han et al., 2014). Malonaldehyde (MDA) accumulation was reduced in wheat when TaWRKY10 was overexpressed, and low MDA was linked to a low rate of lipid peroxidation. This shows that because of the enhanced expression of TaWRKY10, which resists decreased heat damage, the transgenic seedlings displayed great resistance to oxidative stress. When *A. thaliana* is subjected to oxidative stress, it has been discovered that the AtWRKY28 controls the expression of downstream-associated genes through ROS (Niu et al., 2012) CIWRKY20 is a member of the WRKY family's third group, and it has been

discovered that its intracellular location is in the nucleus.

#### Cold stress linked with WRKY TFs

Cold stress, defined as temperatures below 20°C and freezing below 0°C, hurts plant growth and development and significantly reduces agricultural output (Ali et al., 2021, 2022a, 2022b). Plants use a variety of physiological, defensive, and molecular response mechanisms to adjust their tolerance to cold stress, chilling, and freezing. Numerous genes that react at the transcriptional level to cold stress have been found through the analysis of regulatory mechanisms in plants (Ritonga et al., 2021). Numerous WRKY TFs are known to

significantly impact different species' ability to withstand cold stress. According to recent research, seedling viability is increased in transgenic lines of *Arabidopsis* overexpressing CsWRKY46 and cucumber WRKY when frozen at 4°C.

Under -20°C, overexpression lines showed superior surveillance compared to WT after 80 minutes and up to 72 hours. Compared to WT plants, the over-expressing plant lines exhibited reduced ion content leakage. It is possible to infer from this that overexpression lines have greater membrane stability (Yokotani et al., 2013; Ali et al., 2022c, 2022d).

**Table 1. Impact of WRKY Transcription Factors on Cold Stress Tolerance in Plants**

WRKY TF	Species	Impact on Cold Stress Tolerance	Reference
CsWRKY46	<i>Arabidopsis</i>	Increased seedling viability when overexpressed	Yokotani et al., 2013
Cucumber WRKY	<i>Cucumber</i>	Enhanced tolerance to freezing at 4°C in transgenic lines	Yokotani et al., 2013
		Superior surveillance under -20°C for overexpression lines	Yokotani et al., 2013
		Reduced ion content leakage compared to wild-type plants	Yokotani et al., 2013

#### Salinity stress linked with WRKY TFs

Salinity refers to the concentration of dissolved salts in water, typically measured in parts per thousand (ppt). According to recent research, overexpressing AtWRKY46 in *Arabidopsis* during salt stress promotes root growth by ABA signaling regulation. Furthermore, transgenic *Arabidopsis* plants' leaves and roots exhibit greater resistance to salt stress when GhWRKY34 (*G. hirsutum*) is overexpressed because it improves the plant's capacity to selectively absorb Na<sup>+</sup> and K<sup>+</sup> and maintain low Na<sup>+</sup>/K<sup>+</sup> levels (Dai et al., 2016). Transgenic *Arabidopsis* plants expressing GmWRKY54 (WRKY soybean) exhibit salt tolerance; Zhou et al. (2008) reported that

over-expressing lines exhibited 70% survival under 180 mM NaCl treatment, compared to 25% survival for WT plants.

In *A. thaliana*, overexpression of MxWRKY55 resulted in elevated proline and chlorophyll levels along with enhanced activity of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). Malondialdehyde (MDA) levels were also decreased in *A. thaliana* when MxWRKY55 was present, especially in response to salt stress. Furthermore, Han et al. (2020) observed that transgenic *A. thaliana* with overexpression of MxWRKY55 exhibited increased root mass, length, chlorophyll, and iron content in comparison to WT. These characteristics have

shown that MxWRKY55 can contribute favorably to the processes of salt resistance, high Fe resistance, and low Fe content. According to another study, salt and iron had an impact on the growth and development of *M. xiaojinesis*, a Chinese semi-dwarf apple (Zubair et al., 2023a, 2023b).

### **WRKY TFs are considered as key regulators for plant growth and development**

One of the biggest TF families in plants, the WRKY TF plays a vital role in the growth and development of plants in addition to regulating defense and stress response. Different plant species have been shown to have multiple WRKY genes that support growth and development (Zhang et al., 2017). The genes AtWRKY28, AtWRKY2, and AtWRKY34 are involved in seed germination, early growth following germination, pollen tube expansion, macrospore destiny, and pollen production. A key factor in seed germination is AtWRKY2, a knockout mutant with high ABA sensitivity (Jiang and Yu, 2009). By controlling ROS clearance and osmotic buildup, VvWRKY30 overexpression in *Arabidopsis* enhanced resistance to salt stress at different growth stages (Zhu et al., 2019; Ali et al., 2023a, 2023b).

Based on stress-related WRKY in rubber trees, a novel WRKY TF known as HbWRKY82 was discovered. Nuclear proteins encode this TF, which has a significant role as a transcriptional activator. HbWRK82 transcriptional activity is induced by exogenous ethrel and ABA stimulation. These genes are significant transcriptional regulators in three and in response to abiotic stress and ABA-mediated leaf senescence (Kang et al., 2021). In *Arabidopsis* and several other plant species, the WRKY70 has a negative role in abiotic stress signaling and is implicated in biological stress as a positive regulator. By analyzing MfWRK70 from *M. flabellifolia* in *Arabidopsis* model plants, the nucleus was found to be the location of MfWRKY70

(Zeeshan et al., 2023; Riaz et al., 2022a, 2022b, 2022c).

### **CONCLUSION**

In conclusion, the role of WRKY transcription factors (TFs) in molecular switches for tolerating drought, temperature, and salinity stress in plants is crucial for understanding plant stress responses and developing strategies for crop improvement. The WRKY TF gene family is a key player in transcriptional reprogramming, regulating various signaling pathways and regulatory networks involved in plant growth, development, and stress tolerance. WRKY TFs have been implicated in numerous physiological processes, including gamete development, seed germination, growth, senescence, and flowering, and are crucial for environmental stress responses such as drought, temperature extremes, and salinity. Through transcriptomics analysis, metabolomic regulations, and overexpression studies, WRKY TFs have been shown to modulate stress signaling pathways and enhance stress tolerance in plants. Their involvement in responding to drought, temperature, and salinity stress is well-documented, with instances of WRKY TF overexpression leading to improved stress tolerance in various plant species. Additionally, WRKY TFs play significant roles in regulating ROS-mediated signaling under temperature stress and have been implicated in enhancing cold tolerance in plants. Understanding the multifaceted roles of WRKY TFs in stress responses and plant development is essential for developing crop varieties resilient to environmental challenges, ultimately contributing to global food security and agricultural sustainability.

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**Conflict of Interest:**

There is no such evidence of conflict of interest.

**Author Contribution**

All authors have participated in critically revising of the entire manuscript and approval of the final manuscript.

**REFERENCES**

- Ali, S., Mahmood, T., Ullah, S., Aslam, Z., Nasir, S., Zain, R., & Zain S. (2021). Review: Biofortification of Cereals with Zinc through Agronomic practices. *International Journal of Agricultural and Applied Sciences*, 2(2), 14-19. <https://doi.org/10.52804/ijaas2021.223>
- Ali, S., Riaz, A., Mamtaz, S., & Haider, H. (2023a). Nutrients and Crop Production, *Current Research in Agriculture and Farming*, 4(2), 1-15. <http://dx.doi.org/10.18782/2582-7146.182>
- Ali, S., Riaz, A., Shafaat, S., Sidra, Shakoor, K., Sufyan, M., Huzaifa, M., Imtiaz, S., & Ur Rehman, H. (2022a). Biofortification of Cereals with Iron through Agronomic Practices, *Current Research in Agriculture and Farming*, 3(5), 11-16. <http://dx.doi.org/10.18782/2582-7146.174>
- Ali, S., Ullah, S., Umar, H., Aslam, M. U., Aslam, Z., Akram, M. S., Haider, H., Nasir, S., Hayat, S., & Zain, R. (2022b). Effects of Wastewater use on Soil Physico-chemical Properties and Human Health status, *Indian Journal of Pure and Applied Biosciences*, 10(2), 50-56. <http://dx.doi.org/10.18782/2582-2845.8864>
- Ali, S., Ullah, S., Umar, H., Saghir, A., Nasir, S., Aslam, Z., M. Jabbar, H., ul Aabdeen, Z., & Zain, R. (2022c). Effects of Heavy Metals on Soil Properties and their Biological Remediation, *Indian Journal of Pure and Applied Biosciences*, 10(1), 40-46. <http://dx.doi.org/10.18782/2582-2845.8856>
- Ali, S., Ullah, S., Umar, H., Usama Aslam, M., Saghir, A., Nasir, S., Imran, S., Zain, S., & Zain, R. (2022d). Influence of Slope variation on northern areas on soil physical properties, *Indian Journal of Pure and Applied Biosciences*, 10(2), 38-42. <http://dx.doi.org/10.18782/2582-2845.8881>
- Ali, S., Zaib, M., Zubair, H., Afzal, A. H., Zehra, E., Ashraf, M. Z., Farooq, M. B., Ahmad, M., Asif, H., & Marium, A. (2023b). Ecological Restoration of Degraded Lands, *Current Research in Agriculture and Farming*, 4(4), 25-34. <http://dx.doi.org/10.18782/2582-7146.205>
- Chen, L., Zhang, L., & Yu, D. (2010). Wounding-induced WRKY8 is involved in basal defense in Arabidopsis. *Molecular Plant-Microbe Interactions*, 23(5), 558-565.
- Dai, X., Wang, Y., & Zhang, W. H. (2016). OsWRKY74, a WRKY transcription factor, modulates tolerance to phosphate starvation in rice. *Journal of Experimental Botany*, 67(3), 947-960.
- Han, D., Zhou, Z., Du, M., Li, T., Wu, X., Yu, J., ... & Yang, G. (2020). Overexpression of a Malus xiaojinensis WRKY transcription factor gene (MxWRKY55) increased iron and high salinity stress tolerance in Arabidopsis thaliana. *In Vitro Cellular & Developmental Biology-Plant*, 56, 600-609.
- Han, M., Kim, C. Y., Lee, J., Lee, S. K., & Jeon, J. S. (2014). OsWRKY42 represses OsMT1d and induces reactive oxygen species and leaf senescence in rice. *Molecules and Cells*, 37(7), 532.
- 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.
- Jiang, W., & Yu, D. (2009). Arabidopsis WRKY2 transcription factor mediates seed germination and postgermination arrest of development by abscisic acid. *BMC Plant Biology*, 9(1), 1-14.
- 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.
- Kang, G., Yan, D., Chen, X., Yang, L., & Zeng, R. (2021). HbWRKY82, a novel IIc WRKY transcription factor from *Hevea brasiliensis* associated with abiotic stress tolerance and leaf senescence in *Arabidopsis*. *Physiologia plantarum*, 171(1), 151-160.
- Kapoor, D., Bhardwaj, S., Landi, M., Sharma, A., Ramakrishnan, M., & Sharma, A. (2020). The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. *Applied Sciences*, 10(16), 5692. <https://doi.org/10.3390/app10165692>
- Niu, C. F., Wei, W. E. I., Zhou, Q. Y., Tian, A. G., Hao, Y. J., Zhang, W. K., ... & Chen, S. Y. (2012). Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic *Arabidopsis* plants. *Plant, cell & environment*, 35(6), 1156-1170.
- Raza, I., Zubair, M., Zaib, M., Khalil, M. H., Haidar, A., Sikandar, A., Abbas, M. Q., Javed, A., Liaqat, M., Ain, A., Nafees, M. & Ashfaq, M. (2023). Precision Nutrient Application Techniques to Improve Soil Fertility and Crop Yield: A Review with Future Prospect. *International Research Journal of Education and Technology*. 05(08), 109-123.
- Riaz, A., Ali, S., Arshad, I., Sidra, Taiba., Khan, H. N., Ullah, M., & Ashraf, K. (2022a). Silicon in the environment and their role in the management of abiotic and biotic stresses towards crop production. *International Research Journal of Modernization in Engineering Technology and Science*, 4(11), 70-75. <http://dx.doi.org/10.56726/IRJMETS30959>
- Riaz, A., Ali, S., Inayat, M. S., Safi, A., Imtiaz, M., Shabbir, Q., Yasin, M. T., & Shafiq, M. T. (2022b). Effect of mulching on crop production and soil health in dry land region: An overview, *International Research Journal of Modernization in Engineering Technology and Science*, 4(10), 774-780. <http://dx.doi.org/10.56726/IRJMETS30482>
- Riaz, A., Usman, M., Ali, S., Farooq, U., Mahmood, U., & Ilyas, A. (2022c). Wastewater Use: A Debatable Scenario and their Impacts on crop production, *Current Research in Agriculture and Farming*, 3(6), 1-8. <http://dx.doi.org/10.18782/2582-7146.178>
- Ritonga, F. N., Ngatia, J. N., Wang, Y., Khoso, M. A., Farooq, U., & Chen, S. (2021). AP2/ERF, an important cold stress-related transcription factor family in plants: A review. *Physiology and Molecular Biology of Plants*, 1-16.
- Salvucci, M. E., Osteryoung, K. W., Crafts-Brandner, S. J., & Vierling, E. (2001). Exceptional sensitivity of Rubisco activase to thermal denaturation in vitro and in vivo. *Plant physiology*, 127(3), 1053-1064.
- Wei, S., Ma, X., Pan, L., Miao, J., Fu, J., Bai, L., ... & Chen, M. (2017). Transcriptome analysis of *Taxillus chinensis* (DC.) Danser seeds in response to water loss. *PLoS One*, 12(1), e0169177.
- Yokotani, N., Sato, Y., Tanabe, S., Chujo, T., Shimizu, T., Okada, K., ... & Nishizawa, Y. (2013). WRKY76 is a rice transcriptional repressor playing



- opposite roles in blast disease resistance and cold stress tolerance. *Journal of experimental botany*, 64(16), 5085-5097.
- Zaib, M., Abbas, Q., Hussain, M., Mumtaz, S., Khalid, M., Raza, I., Abbas, S., Danish, M., Abbas, R., Muhammad, N. & Bano, S. (2023d). Micronutrients and Their significance in Agriculture: A Mini Review with Future Prospects Corresponding Author address. International Research Journal of Education and Technology. 05(04), 234-252.
- Zaib, M., Aryan, M., Khaliq, A., Haider, K., Ahmad, S., Zeeshan, A., Haq, U., Ahmad, U., Akbar, H. & Zubair, H. (2023e). Essential Insights for Effective Environmental Management and Human Well-being: Strategies for Remediation in Soil-Plant-Environment Systems. *Journal of Asian Development Studies*, 12(03), 1-17.
- Zaib, M., Farooq, U., Adnan, M., Abbas, Z., Haider, K., Khan, N., Abbas, R., Nasir, A., Sidra., Muhay-Ul-Din, M., Farooq, T., & Muhammad, A. (2023b). Optimization water stress in crop plants, implications for sustainable agriculture: current and future prospects. *Journal of Environmental & Agricultural Sciences*. 25 (1&2), 37-50.
- Zaib, M., Zeeshan, A., Akram, H., Amjad, W., Aslam, S. & Qasim, S. (2023c). Impact of Climate Change on Crop Physiology and Adaptation Strategies: A Review. International Research Journal of Education and Technology. 05(08), 15-38.
- Zaib, M., Zeeshan, A., Aslam, S., Bano, S., Ilyas, A., Abbas, Z., Nazar, A. & Mumtaz, S. (2023a). Drought Stress and Plants Production: A Review with Future Prospects. International Journal of Scientific Research and Engineering Development. 06(04), 1278-1293.
- Zeeshan, A., & Zaib, M., Ahmad, S., Akram, H. & Qasim, S. (2023). Diversity of Weeds in Rose Field in District Kasur Punjab Pakistan. *Int. J. Adv. Res. Sci. Technol.* 12(09), 1122-1132.
- Zhang, H., Zhang, J., Lang, Z., Botella, J. R., & Zhu, J. K. (2017). Genome editing—principles and applications for functional genomics research and crop improvement. *Critical Reviews in Plant Sciences*, 36(4), 291-309.
- 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.
- Zhu, D., Hou, L., Xiao, P., Guo, Y., Deyholos, M. K., & Liu, X. (2019). VvWRKY30, a grape WRKY transcription factor, plays a positive regulatory role under salinity stress. *Plant science*, 280, 132-142.
- Zubair, H., Afzal, A., Shahid, M., Ali, U., Iftikhar, H., Abuzar, A., Zaib, M. & Marium, A. (2023a). Historical Overview of the Chinese Agricultural Sciences and Technological Development. *Curr. Res. Agri. Far.* 4(4), 35-45. <http://dx.doi.org/10.18782/2582-7146.206>
- Zubair, M., Raza, I., Batool, Y., Arif, Z., Muneeb, M., Uzair, M., Haidar, A., Zaib, M., Ashfaq, M., Akbar, H., Abbas, Q. & Ali, A. (2023b). A Review of Remediation Strategies against Arsenic (As) Removal from Groundwater by Using Different Filtration Techniques. *Curr. Res. Agri. Far.* 4(3), 1-14. <http://dx.doi.org/10.18782/2582-7146.192>