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RESEARCH PAPER

Co-expression of *ZmVPP1*, *ZmNAC111*, and *ZmTIP1* confers enhanced drought tolerance in maize (*Zea mays*)

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Abstract

Maize plants are adversely affected by abiotic stresses, among which drought is one of the most challenging hazards to production. Abiotic stress significantly affects plant development and production, necessitating the identification of stress-responsive genes for crop improvement. In the current study, drought stress was applied on maize cultivar C. Pak and we analyzed their effects on their phytohormones as compared to control. Their results illustrated that significant variations in Indole-3-acetic acid (IAA), Abscisic acid (ABA), and Salicylic acid (SA) levels among the studied treatments were achieved as compared to control. Moreover, we also checked the expression pattern of the targeted 3 genes in maize under stress conditions through semi quantitative real time PCR (qPCR) in roots and shoots. The expression patterns of *ZmVPP1*, *ZmNAC111* and *ZmTIP1* in maize roots and shoots were upregulated as compared to control. The expression of *ZmNAC111* was higher in both root and shoot in maize as compared to *ZmVPP1* and *ZmTIP1*, respectively. These findings highlight the differential roles of *ZmVPP1*, *ZmNAC111*, and *ZmTIP1* in drought stress tolerance, with particular emphasis on *ZmNAC111*, providing valuable insights into their potential applications in maize breeding for enhanced drought resilience.

Keywords: Abscisic acid, Drought stress, Maize, Salicylic acid, *ZmNAC111*, *ZmTIP1*, *ZmVPP1*

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Introduction

Maize (*Zea mays* L.) is one of the world's most important cereal crops, serving as a staple food, animal feed, and a key raw material for industrial products (Ahmad & Ahmad, 2018; Rubab et al., 2020; Mehmood et al., 2022). Its global significance lies not only in its high productivity but also in its wide adaptability to diverse agro-ecological zones (Azam et al., 2023). As demand for food and bio-based resources continues to rise, maize production plays a critical role in ensuring food security, particularly in developing countries (Jamilah et al., 2024; Kekere et al., 2024). However, increasing environmental stresses such as drought, heat, and poor soil conditions pose significant threats to maize yield and stability (Zia et al., 2023). Understanding and enhancing the resilience of maize to such stress has become a major focus in crop improvement research.

Drought is a prevalent ecological condition that significantly reduces maize growth and production (Daryanto et al., 2016). Therefore, the creation of drought-resistant cultivars can assist to confirm that maize is formed in adequate numbers for nutrition, animal nourish, and energy, even in the face of stochastic drought episodes and rising consumer demand (Kloppers, 2024). Growing maize under drought stress is particularly challenging due to the low heritability of traits related to stress resistance (Liu et al., 2022; Tang et al., 2025). Drought confrontation is generally established to be a polygenic characteristic influenced by several genes and additional variables (Ma et al., 2024). In grain yield farming, the degree of injury caused by water deficiency is determined by soil water potential gradients and varies by crop varieties and developmental stage (Bhattacharya et al., 2021). Drought tolerance studies in maize are primarily concerned with plantlet subsistence taxes following drought stress, yield-related kernels features, origin attributes, and photosynthetic effectiveness, amongst other things (Engida et al., 2022). Droughts have been increasingly frequent from spring to summer and hence significant maize growing region, since the early twenty-first century (Jia et al., 2022). As a result, water scarcity poses a hazard to spring germination and seedling development. As a result, better seedling stage is critical in maize study, and occurrences during this period have a thorough influence on advanced developing phases, possibly affecting production (Kim et al., 2023).

Because the drought-tolerant phenotype is governed by several genes, enhancing this characteristic might be additional successful if various genes are pyramided in a single variety (Tao et al., 2024). The transgenic plants characterized by beta gene overexpression displayed elevated transpiration rates, stomatal conductance, and net photosynthesis in comparison to their wild-type counterparts (Shah et al., 2019). For example, stacking genes in maize promotes vegetative development and increases yields more than each transgene alone (Liu et al., 2022). In light of previous research demonstrating that stacking genes can improve the drought tolerance discussed by separate genes or piling *ZmVPP1* through *ZmNAC111* might additionally enhance the belongings of enlarged sapling existence, up controlled drought receptive gene appearance, root growth, or produce below drought situations perceived in transgenic A188 plants articulating whichever gene only (Liu et al., 2022). It is hypothesized that pyramiding these genes would increase drought-responsive gene expression while also stimulating root growth under low water supply, subsequent in a great produce, drought-resistant line (Liu et al., 2023).

Dual transgenic maize plants by overexpressing of *ZmVPP1* and *ZmNAC111* performed well in drought tolerance as compared to the only transgene and wild-type (WT) plants as well (Mao et al., 2022). It has been found that co-expressing *ZmVPP1* and *ZmNAC111* in the drought-sensitive maize line A188 enhances drought tolerance by improving water use efficiency, increasing antioxidant enzyme activity, reducing the anthesis-silking interval (ASI), and promoting the expression of stress-responsive genes under prolonged water-deficit conditions (Chen et al., 2024). In another scientific discovery based on the functional characterization and validation of *ZmTIP1*, which encodes a functional S-acyltransferase, plays their active role in regulating the root hairs length recovery and eventually perform direct function in drought patience in maize (Zhang et al., 2020). Considering the documented significance of these genes in the literature and their established roles in conferring drought tolerance in maize, we have designed the current study to explore the functions of *ZmVPP1*, *ZmNAC111* and *ZmTIP1* in maize cultivar C. Pak under drought stress conditions as compared to control.

Materials and Methods

Soil preparation and sowing of maize seeds

The seeds of C. Pak cultivar were obtained from Plant Genetic Resources Institute (PGRI), National Agricultural Research Center (NARC), Islamabad, Pakistan. The soil sample was gathered in clean plastic bags from the garden at a depth of 0 to 20 cm. The soil sample was appropriately labeled, sealed, and transported to the glasshouse condition. Four maize seeds were sown randomly per pot, with three biological replicates for each treatment and three technical replicates per biological replicate. A control group (plants grown under normal water conditions without stress) was also included.

Application of drought stress

Drought stress was imposed when maize seedlings reached the two-leaf stage, i.e., 7 days after germination. Watering was withheld for 10 consecutive days until visible drought symptoms appeared. During this period, soil moisture content was monitored and maintained at approximately 35–40% of field capacity using a soil moisture meter. For the control treatment, water was applied regularly to maintain soil moisture at 80–85% of field capacity.

Estimation of chlorophyll

Chlorophyll levels were determined utilizing the method of Lichtenthaler (1987). During the collection of new leaf weights, they were centrifuged for five minutes at 1000 rpm. After that, three times, one milliliter of 80% acetone was used to clear the waste. Subsequently, by employing optical densities, the supernatants were prepared and mutual through acetone to yield a solution with a concluding capacity of 7.0 ml. Following that, the visual concentrations of these solutions at 663, 645 and 480 nm wave lengths were determined.

Determination of indole-3-acetic acid (IAA)

The fluid culture was turned for 2 mins at 1200 rpm tracked by the removal of the fluid since the model leaves behind schedule the dense fragment. In a shaded area, prepare one mL of Salkowski reagent from two mL of supernatant and let it stationary for 30 minutes. The reaction at 540nm was completed using a spectrophotometer.

Determination of abscisic acid (ABA)

To detect abscisic acid (ABA) in plant extract by spectrophotometry, filtrate containing 1% acetic acid incubate the mixture overnight at 4°C, then centrifuge at 10,000 g for 15 minutes to obtain the supernatant. Optionally, purify the extract with a C18 solid-phase extraction cartridge. Create a standard curve using pure ABA dissolved in methanol and diluted to varying amounts. Using a UV spectrophotometer, measure the absorbance of the standards and plant extract at 265 nm, and then compare the sample absorbance to the standard curve to calculate the ABA content.

Determination of salicylic acid (SA)

Salicylic acid substances of designated plants extract were measured by the technique of Warrier et al. (2013) through certain changes. Proceeds 0.1 mL of the plants extracted in a test tube and added 0.1% recently ready ferric chloride to create up the ending capacity (3.0 mL). The Fe+3 ion in combination reacts with salicylic acid and made violet. The OD was dignified at 540 nm in contrast to the blank 0.1 percent $FeCl_3$.

Expression analysis of *ZmVPP1*, *ZmNAC111*, and *ZmTIP1* gene in maize under drought stress conditions

After applying drought stress on maize plant at seedling stage, *ZmVPP1*, *ZmNAC111*, and *ZmTIP1* gene appearance examination was completed in shoot and root of maize plants as compared to the control treatments. For expression analysis the following steps were being carried out:

i. RNA extraction and cDNA synthesis

The shoot and root samples were ground independently in liquid nitrogen. The whole RNA from trials was mined by TRIZOL created technique. The abstraction was surveyed by DNase action using Turbo DNA free kit (Invitrogen, Carlsbad, CA, USA) to remove any genomic DNA contamination in the RNA samples. After, RNA pureness was tested and calculated for the synthesis of the cDNA. 2 µg of total RNA was used for cDNA preparation utilizing superScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). The cDNA quality was discovered by using interior control and was additionally diluted 20X and used for gene expression studies.

ii. Quantitative-Real Time PCR (qRT-PCR)

To conduct quantitative real time PCR (qRT-PCR), the primers ITS5 5' (GGA AGT AAA AGT CGT AAC AAG G) 3' and ITS4 5' (TCC TCC GCT TAT TGA TAT GC) 3′ were used for the *ZmVPP1*, *ZmNAC111* and *ZmTIP1* genes were developed and utilized. The maize internal regulation (housekeeping) gene, GAPDH, was employed for comparative and authenticity. The qRT-PCR was carried out utilizing an Applied Biosystems Model 7500 Real-Time PCR Instrument utilizing a 1/20 dilute of the relevant cDNAs. All qRT-PCR analyses were carried out using SYBR Green I (QuantiFast® SYBR® Green PCR Kit, Qiagen, Hilden, Germany). The effectiveness of the qRT-PCR was evaluated, and a melt curve analysis was done for each of the results of the PCR in compliance with the instructions. Gene expression studies were performed using three biological and technological duplicates. Gene proportional fold expression was measured using the delta-delta CT technique (2ΔΔCT).

Results

Effect of drought stress on chlorophyll content of maize plant during seedling stage

Under drought stress in maize plants, chlorophyll content varied between control and drought-stressed plants. The chlorophyll content was decreased in drought stress plants as compared to the control; chlorophyll a decreased -13, chlorophyll b -15 and total chlorophyll by -16%, respectively as compared to the control (Fig. 1, 2 and 3).

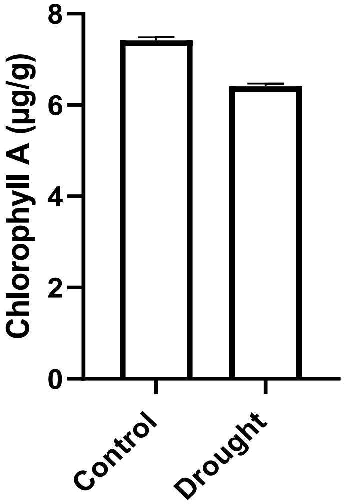

Fig. 1 Effect of drought stress on chlorophyll a in maize seedlings grown under lab conditions. Data was obtained from 3 replicates with their respective error bars.

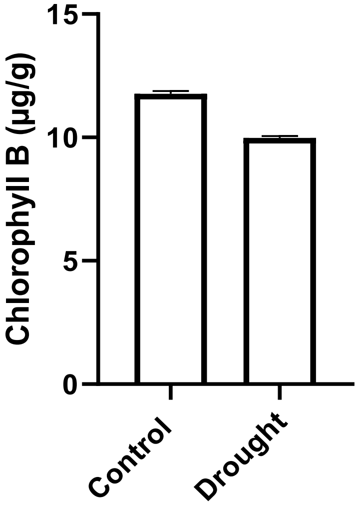

Fig. 2 Effect of drought stress on chlorophyll b in maize seedlings grown under lab conditions. Data was obtained from 3 replicates with their respective error bars.

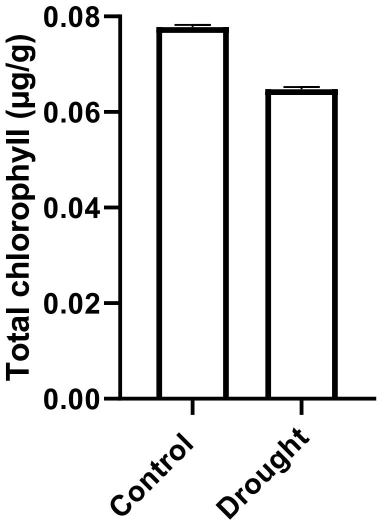

Fig. 3 Effect of drought stress on total chlorophyll in maize seedlings grown under lab conditions. Data was obtained from 3 replicates with their respective error bars.

Effect of drought stress on biochemicals of maize plants during seedling stage

Under drought stress conditions in maize plants, the levels of key phytohormones varied among control and stress plants. All the phytohormones levels were decreased in drought stress plants as compared to the control one. The IAA content was decreased by -20%, ABA was -4% and SA decreased by -15%, respectively as compared to the control (Fig. 4, 5 and 6).

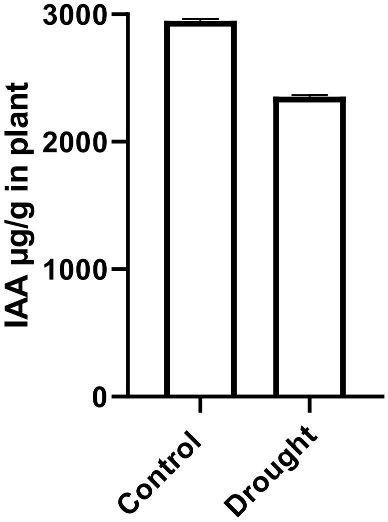

Fig. 4 Effect of drought stress on IAA in maize seedlings grown under lab conditions. Data was calculated from 3 replicates with their respective error bars.

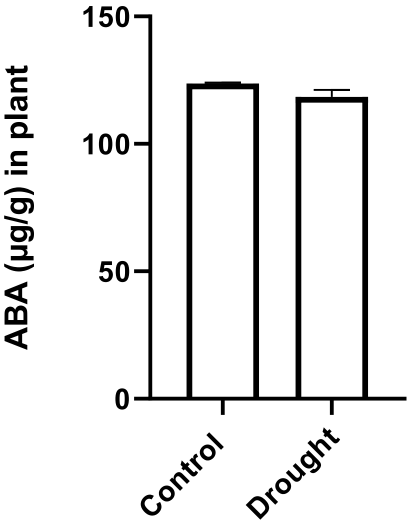

Fig. 5 Effect of drought stress on ABA in maize seedlings grown under lab conditions. Data was calculated from 3 replicates with their respective error bars.

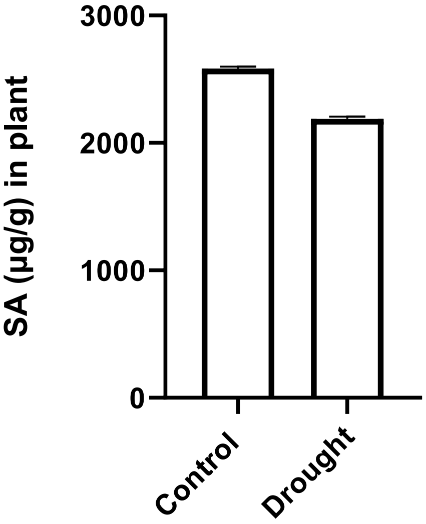

Fig. 6 Effect of drought stress on SA in maize seedlings grown under lab conditions. Data was calculated from 3 replicates with their respective error bars.

Expression of drought tolerant genes in root and shoot

Gene expression profiling of the selected genes (*ZmNAC111*, *ZmVPP1* and *ZmTIP1*) was checked in the root and shoot parts of C. Pak cultivar under drought stress in maize. The results of gene expression levels varied significantly between roots and shoots. *ZmNAC111* exhibited the highest expression in roots 2.7-fold expression followed by *ZmVPP1* 2.3 and *ZmTIP1* 1.8-fold, respectively. While in shoot, *ZmNAC111* also showed the highest 2.4-fold expression whereas *ZmVPP1* had a moderate response 2 followed by *ZmTIP1* 1.4-fold, respectively as shown in (Fig. 7 and 8). These findings indicate that *ZmNAC111* is highly responsive to drought stress conditions in both roots and shoots, suggesting its crucial role in stress tolerance mechanisms in maize (Fig. 9).

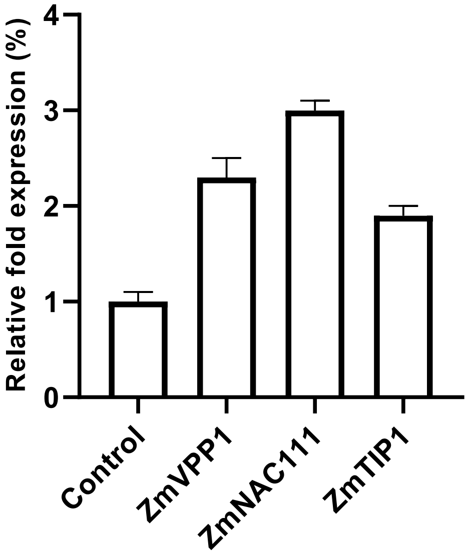

Fig. 7 Effect of drought stress on root relative fold expression in maize seedlings grown under lab conditions. Data was calculated from 3 replicates with their respective error bars.

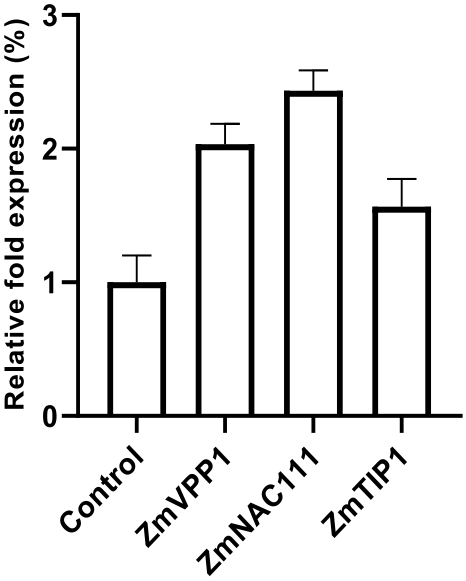

Fig. 8 Effect of drought stress on shoot relative fold expression in maize seedlings grown under lab conditions. Data was calculated from 3 replicates with their respective error bars.

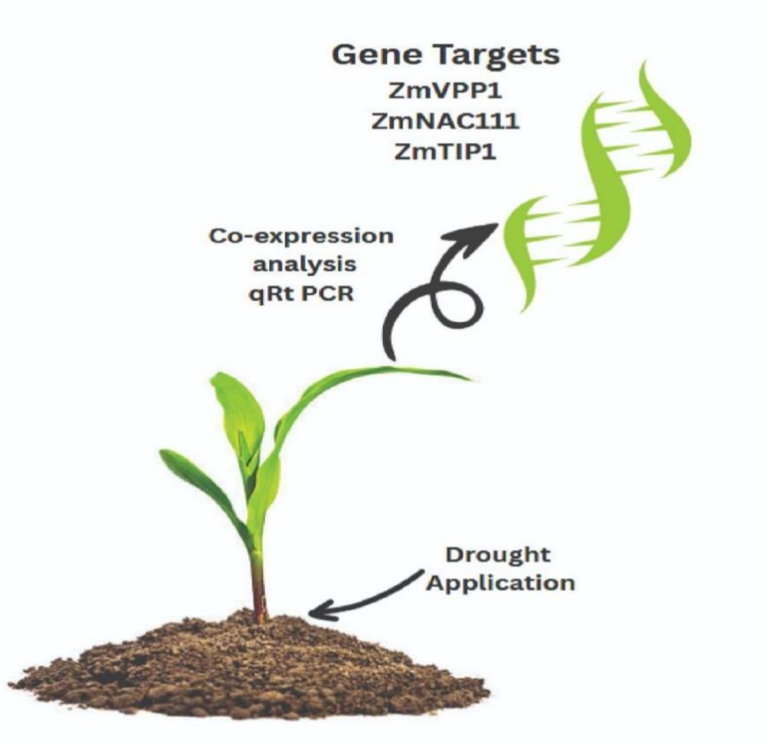

Fig. 9 Expression analysis of drought tolerant genes *ZmVPP1*, *ZmNAC111* and *ZmTIP1* under drought stress

Discussion

Low water availability induced environmental stress continues to represent a significant threat to maize along with other major grain cultivation in growing locations across the world. Consequently, drought responses and crop restoration ability following drought mitigation has long been the subject of strong agronomic research interest (Wang et al., 2016). Plants' resilience to drought circumstances allows them to survive, if not flourish amid water shortages and quickly resume their regular physiological activities post rehydration. In the current study, we applied drought stress on C. Pak cultivar of maize and then Chlorophyll contents (a, b and total), IAA, SA, ABA were checked in these plants as compared to control. Our results showed that the level of IAA, ABA and SA decreased in drought stress treatment as compared to control plants. The plant hormone analysis revealed significant variations in IAA, ABA, and SA levels, which are critical regulators of stress responses. Indole-3-acetic acid (IAA), a key auxin, exhibited notable changes, which align with previous studies suggesting that auxin homeostasis is essential for root growth and shoot development under stress conditions (Tian et al., 2014). The fluctuations in IAA levels among different two treatments indicate their potential role in modulating root architecture and growth responses to environmental cues. Similar to our results, Abscisic acid (ABA)**,** known as the "stress hormone," was significantly increased, particularly in control plants (Cutler et al., 2010). SA levels in maize plants under drought stress suggest that this gene may influence systemic acquired resistance and oxidative stress mitigation (Vlot et al., 2009).

We checked the expression pattern of *ZmNAC111*, *ZmVPP1* and *ZmTIP1* in maize root and shoot under drought stress. Their results revealed that the expression patterns of *ZmVPP1*, *ZmNAC111* and *ZmTIP1* in maize roots and shoots were upregulated as compared to control. The expression of *ZmNAC111* was higher in both root and shoot in maize as compared to *ZmVPP1* and *ZmTIP1*. According to our results, earlier findings have proven that drought resilience is a polygenic feature governed by an intricate gene system, and that raising *ZmVPP1* or *ZmNAC111* expression can improve the reaction of maize to water deprivation (Zhang et al., 2014). Plants developed several complicated methods to endure water scars conditions, including the production of stress hormones, the upregulation of antioxidant enzymes and metabolites, and the stimulation of root expansion. These systems assist in regulating stomatal conductivity, eliminating reactive oxygen compounds, and getting water from lower in the soil (Bhat et al., 2021). Plant roots are phenotypically malleable and very responsive to drought stress; therefore, a brief water scarcity can stimulate root development (Hu & Xiong, 2014). It is probable that growing a reasonably long and robust root system may improve water intake from deeper in the soil, thereby enhancing seedlings rate of survival (Lynch et al., 2015). In line with our findings, activation of the root developing regulators *ZmVPP1*, *ZmTIP1* or *ZmLRL5* can boost root growth, leading in higher root biomass, root hair lengthening, and improved drought tolerance (Xiang et al., 2017).

Root systems in plants exhibit significant phenotypic plasticity and are highly responsive to drought stress. Even short-term water scarcity can stimulate root elongation and development (Hu & Xiong, 2014). Consequently, the formation of deeper, more robust root architectures is believed to improve access to water stored in lower soil layers, which may enhance seedling survival during early growth stages (Lynch & Wojciechowski, 2015). Genetic overexpression of key regulators such as *ZmVPP1*, *ZmTIP1*, and *ZmLRL5* have been shown to enhance root biomass and promote root hair elongation, thereby improving drought tolerance (Wang et al., 2016; Wang et al., 2019; Zhang et al., 2020). In the pyramid lines developed in this study, both root development and the root-to-shoot ratio were significantly enhanced compared to wild-type (WT) plants. These modifications likely contribute to the improved drought resilience observed at the seedling stage and may support robust recovery following rehydration. Drought stress also disrupts reproductive development, particularly by limiting the transport of assimilates required for pollination and kernel formation. During such stress, the distribution of photosynthetically derived carbon becomes tightly regulated. One critical metabolite, trehalose 6-phosphate, plays a central role in controlling carbon allocation and source-sink relationships during the reproductive phase. Modulating its levels has been proposed as a strategy to optimize assimilates distribution and maintain grain filling under water-limited conditions (Oszvald et al., 2018). Interestingly, our study demonstrates that combining *ZmVPP1* and *ZmNAC111* in pyramided lines reduces the anthesis-silking interval and significantly improves grain yield under drought in field trials. Collectively, these findings provide a valuable foundation for molecular breeding strategies aimed at enhancing drought tolerance in maize.

Conclusion

This study highlights the differential expression of *ZmVPP1*, *ZmNAC111* and *ZmTIP1* in maize under stress conditions, providing insights into their potential roles in stress tolerance. Among the studied genes, *ZmNAC111* exhibited the highest expression in both roots and shoots, indicating its strong involvement in stress adaptation. *ZmVPP1* showed moderate expression, while *ZmTIP1* had the lowest response, suggesting gene-specific variations in stress resilience. Additionally, phytohormonal analysis revealed significant changes in IAA, ABA, and SA levels, further supporting the regulatory role of these genes in stress responses. These findings contribute to a better understanding of maize stress physiology and offer valuable targets for genetic improvement programs aimed at enhancing crop resilience to abiotic stress conditions.

Declarations

i. Ethics approval and consent to participate

Ethical approval and informed consent were not required for this study as it did not involve human participants, human data, or animals.

ii. Consent for publication

Consent for publication is not applicable.

iii. Data availability

All data generated or analyzed during this study are included in this article.

iv. Competing interests

Authors have declared that no competing interests exist.

v. Authors' contributions

A. carried out the laboratory experiments and contributed to data collection; M.A.Z supervised the project; S.S. contributed to experimental design, and performed statistical analyses; S.A. assisted with experimental work, data interpretation, and literature review; I.U.K. contributed to methodology development; A.K. provided technical support and helped in data validation; H.M. wrote the initial draft of the manuscript; S.Z. participated in critical revisions of the manuscript.

vi. Funding

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vii. Acknowledgement

Not applicable.

viii. SDGs addressed

Zero Hunger, Climate Action, Life on Land, Responsible Consumption and Production, Industry, Innovation and Infrastructure

References

Ahmad, M., & Ahmad, S. (2018). Evaluation of insect pest infestation and yield losses in maize crop in Maina, district Malakand. *Advances in Agriculture and Biology, 1*(1), 34-39. https://doi.org/10.63072/aab.18005

Azam, M., Saeed, B., Khan, S., & Zafar, D. (2023). Exploring the effect of nitrogen levels on yield and yield attributes of diverse open-pollinated varieties (OPVs) of maize (*Zea mays*). *Advances in Agriculture and Biology, 6*(1), 61-68. https://doi.org/10.63072/aab.23008

Bhat, M. A., Mir, R. A., Kumar, V., Shah, A. A., Zargar, S. M., Rahman, S., & Jan, A. T. (2021). Mechanistic insights of CRISPR/Cas-mediated genome editing towards enhancing abiotic stress tolerance in plants. *Physiologia Plantarum*, *172*(2), 1255-1268.

Bhattacharya, A. (2021). Effect of soil water deficit on growth and development of plants: A review. In *Soil water deficit and physiological issues in plants* (pp. 393–488). Springer. https://doi.org/10.1007/978-981-33-6276-5_5

Chen, F., Chen, L., Yan, Z., Xu, J., Feng, L., He, N., Guo, M., Zhao, J., Chen, Z., Chen, H., Yao, G., & Liu, C. (2024). Recent advances of CRISPR-based genome editing for enhancing staple crops. *Frontiers in Plant Science, 15*, 1478398. https://doi.org/10.3389/fpls.2024.1478398

Cutler, S. R., Rodriguez, P. L., Finkelstein, R. R., & Abrams, S. R. (2010). Abscisic acid: Emergence of a core signaling network. *Annual Review of Plant Biology*, *61*(1), 651-679.

Daryanto, S., Wang, L., & Jacinthe, P. A. (2016). Global synthesis of drought effects on maize and wheat production. *PLOS ONE*, *11*(5), e0156362.

Engida, B. T. (2022). *Abiotic stress tolerance and nutritional traits of newly developed quality protein maize hybrids in sub-Saharan Africa* (Doctoral dissertation, University of the Free State).

Hu, H., & Xiong, L. (2014). Genetic engineering and breeding of drought-resistant crops. *Annual Review of Plant Biology*, *65*(1), 715-741.

Jamilah, Suryani, I., Thesiwati, A. S., & Diyanti, A. (2024). Addition of urea fertilizer to crocober plus liquid organic fertilizer improves growth and yield of corn plants (*Zea mays* L.). *Advances in Agriculture and Biology, 7*(1), 1-7. https://doi.org/10.63072/aab.24001

Jia, H., Chen, F., Zhang, C., Dong, J., Du, E., & Wang, L. (2022). High emissions could increase the future risk of maize drought in China by 60–70%. *Science of The Total Environment*, *852*, 158474.

Kekere, O., Adeleke, G. P., Ajayi, O. M., Bulu, Y. I., Ekundayo, T. O., & Akinbuwa, O. (2024). Assessing the effect of carbide waste-contaminated soil on the growth and yield of maize (*Zea mays* L.). *Advances in Agriculture and Biology, 7*(1), 50-57. https://doi.org/10.63072/aab.24006

Kim, K. H., & Lee, B. M. (2023). Effects of climate change and drought tolerance on maize growth. *Plants*, *12*(20), 3548.

Kloppers, D. (2024). *Developing a managerial framework to manage the production implications of grain shortages in central South Africa* (Doctoral dissertation, North-West University (South Africa).

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In *Methods in enzymology* (Vol. 148, pp. 350-382).

Liu, S., Liu, X., Zhang, X., Chang, S., Ma, C., & Qin, F. (2022). Co-expression of *ZmVPP1* with *ZmNAC111* confers robust drought resistance in maize. *Genes, 14*(1), 8. https://doi.org/10.3390/genes14010008

Liu, S., Wang, H., & Qin, F. (2023). Genetic dissection of drought resistance for trait improvement in crops. *The Crop Journal*, *11*(4), 975-985.

Lynch, J. P., & Wojciechowski, T. (2015). Opportunities and challenges in the subsoil: Pathways to deeper rooted crops.

Journal of Experimental Botany, 66(8), 2199–2210. https://doi.org/10.1093/jxb/erv041

Lynch, J. P., & Wojciechowski, T. (2015). Opportunities and challenges in the subsoil: Pathways to deeper rooted crops. *Journal of Experimental Botany, 66*(8), 2199–2210.

Ma, Y., & Li, Y. (2024). The research progress on genomic selection and breeding for drought resistance in wheat: From genetic analysis to smart breeding applications. *Geographical Research Bulletin*, *3*, 625-646.

Mao, H., Jian, C., Cheng, X., Chen, B., Mei, F., Li, F., Zhang, Y., Li, H., Li, C., Jing, R., & Chen, G. (2022). The wheat ABA receptor gene TaPYL1-1B contributes to drought tolerance and grain yield by increasing water-use efficiency. *Plant Biotechnology Journal, 20*(5), 846–861. https://doi.org/10.1111/pbi.13717

Mehmood, K., Nasreen, S., Latif, A., & Shah, A. N. (2022). Growth of maize is improved by the combined use of natural and synthetic nutritional sources of potassium: A review. *Advances in Agriculture and Biology, 5*(1), 8-15. https://doi.org/10.63072/aab.22002

Oszvald, M., Primavesi, L. F., Griffiths, C. A., Cohn, J., Basu, S. S., Nuccio, M. L., & Paul, M. J. (2018). Trehalose 6-phosphate regulates photosynthesis and assimilate partitioning in reproductive tissue. *Plant Physiology, 176*(4), 2623–2638.

Rubab, S., Fayyaz, S., & Asmatullah. (2020). Plant parasitic nematodes associated with wheat and maize. *Advances in Agriculture and Biology, 3*(1), 8-17. https://doi.org/10.63072/aab.20002

Shah, S. H., Ali, S., & Ali, G. M. (2019). Morphological analysis of cold-tolerant tomato (*Solanum lycopersicum* Mill.) plants expressing *CBF3* gene. *Advances in Agriculture and Biology, 2*(1), 14-24. https://doi.org/10.63072/aab.19003

Tang, H., Zhang, L., Xie, X., Wang, Y., Wang, T., & Liu, C. (2025). Resilience of maize to environmental stress: Insights into drought and heat tolerance. *International Journal of Molecular Sciences, 26*(11), 5274. https://doi.org/10.3390/ijms26115274

Tao, X., Wang, Y., & Sheng, H. (2024). The research progress on wheat root system architecture and drought resistance: Morphological characteristics, genetic regulation, and application prospects. *Geographical Research Bulletin*, *3*, 558-576.

Tian, H., De Smet, I., & Ding, Z. (2014). Shaping a root system: Regulating lateral versus primary root growth. *Trends in Plant Science*, *19*(7), 426-431.

Vlot, A. C., Dempsey, D. M. A., & Klessig, D. F. (2009). Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology*, *47*(1), 177-206.

Wang, C.-X., Qi, C.-Y., Luo, J.-H., Liu, L., He, Y., & Chen, L.-Q. (2019). Characterization of LRL5 as a key regulator of root hair growth in maize. *The Plant Journal, 98*(1), 71–82.

Wang, X., Wang, H., Liu, S., Ferjani, A., Li, J., Yan, J., Yang, X., & Qin, F. (2016). Genetic variation in *ZmVPP1* contributes to drought tolerance in maize seedlings. *Nature Genetics, 48*, 1233–1241. https://doi.org/10.1038/ng.3636

Wang, X., Wang, H., Liu, S., Ferjani, A., Li, J., Yan, J., Yang, X., & Qin, F. (2016). Genetic variation in ZmVPP1 contributes to drought tolerance in maize seedlings. *Nature Genetics, 48*(10), 1233–1241.

Warrier, R. R., Paul, M., & Vineetha, M. V. (2013). Estimation of salicylic acid in Eucalyptus leaves using spectrophotometric methods. *Genetics and Plant Physiology, 3*, 90–97.

Xiang, Y., Sun, X., Gao, S., Qin, F., & Dai, M. (2017). Deletion of an endoplasmic reticulum stress response element in a *ZmPP2C-A* gene facilitates drought tolerance of maize seedlings. *Molecular Plant*, *10*(3), 456-469.

Zhang, J.-Y., Cruz de Carvalho, M. H., Torres-Jerez, I., Kang, Y., Allen, S. N., Huhman, D. V., Tang, Y., Murray, J., Sumner, L. W., & Udvardi, M. K. (2014). Global reprogramming of transcription and metabolism in *Medicago truncatula* during progressive drought and after rewatering. *Plant, Cell & Environment, 37*(11), 2553–2576. https://doi.org/10.1111/pce.12328

Zhang, X., Mi, Y., Mao, H., Liu, S., Chen, L., & Qin, F. (2020). Genetic variation in *ZmTIP1* contributes to root hair elongation and drought tolerance in maize. *Plant Biotechnology Journal, 18*(5), 1271–1283.

Zhang, X., Mi, Y., Mao, H., Liu, S., Chen, L., & Qin, F. (2020). Genetic variation in *ZmTIP1* contributes to root hair elongation and drought tolerance in maize. *Plant Biotechnology Journal, 18*(6), 1271–1283.

Zia, M. A., Shoukat, S., Arif, M., Ahmad, B., Nawaz, A. F., Bahadur, A., Zakria, M., Khan, H. S., Khan, S., Suleman, M., & Ali, S. (2023). A discussion on maize transformation during the last two decades (2002–2022): An update on present trends and future prospects. *Advances in Agriculture and Biology, 6*(1), 1-10. https://doi.org/10.63072/aab.23001