

Engineering Maize for Drought and Salinity Tolerance Traits Through Genome Editing Approaches

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Abstract

Abiotic stresses, particularly drought and salinity, significantly impact maize growth and yield, posing a threat to food security under changing climatic conditions. Genetic variability in maize offers the potential for developing stress-tolerant varieties. Key genes such as *ZmSOS1*, *ZmSRP*, *ZmHKT1*, *ZmABH2*, *ZmDHN*, *ZmDREB*, *ZmDST*, *ZmLEA*, *ZmNAC*, and *ZmP5CS* play crucial roles in mitigating these stresses. Modern genome editing tools, especially CRISPR/Cas9, provide opportunities to enhance maize resilience by targeting and making novel alleles for drought and salinity traits. This article discusses the impact of drought and salinity on maize, the genetic mechanisms involved, and the potential of CRISPR/Cas9 technology in developing drought and salinity-tolerant maize genotypes that in turn contribute to climate-resilient and sustainable agriculture.

Introduction

Maize is a source of feed, food, industrial raw material, and livelihood for millions of farmers in India. Its demand is likely to increase due to the ever-increasing population and to attain the 20% bioethanol blending target envisaged by the Government of India (GOI). The average maize productivity in India (~3.2 t/ha) is still much below the global average (~5.7 t/ha) (FAOSTAT 2021).

Drought and salinity are major abiotic stresses that severely affect crop health and agricultural production. Further, unpredictable and long dry spells leading to drought stress cause huge yield penalties often in the range of 35-66% (Sah et al., 2020). Besides, salinity stress, mainly in parts of Indo-Gangetic plains and upland rice growing areas, also adversely affects maize productivity. Therefore, salt-tolerant maize is the need of the hour to make crop diversification successful in such areas. Taken together, it is imperative to develop high-yielding as well as climate-resilient maize genotypes for yield enhancement and sustainability. Such genotypes could be developed by loss-of-function/gain-of-

function for the major regulator (s) of these traits using genome editing tools. To fulfill all the above requirements, nowadays researchers are using various genome editing tools, in which CRISPR is most prevalent. This article discusses about drought and salinity stress and genes responsible for salt and drought tolerance in maize and how the CRISPR tool can be utilized for editing genes.

Drought and Salinity stress

Among abiotic stresses, drought, and salinity affect crop health and yield significantly (Golldack et al., 2014). It is a major concern in the current global climate change scenario that limits agricultural productivity. Drought affects the plant growth and development during its life cycle. The plant responds to drought stress through several physiological and morphological adaptations like reduced photosynthesis, transpiration, shoot growth, curling of leaves, enhanced production of reactive oxygen species (ROS), activation of stress signalling pathway, and senescence. These alterations in plant metabolism can cause permanent damage and severe yield losses (Ahluwalia et al., 2021).

The salt stress causes disturbance in the osmolarity of the cells, necrosis, and death of mature leaves, premature death of old leaves, and severe disturbance of ions in cells. Also, plants adapt against these stresses through morphological and physiological changes. Moreover, major shifts take place in gene expression and cascades of signaling processes under drought and abiotic stress. The cellular and biochemical response seen inside plants includes rise of secondary signal molecules like reactive oxygen species (ROS), sudden change in calcium ions and changes in activity of Ca^{2+} /calmodulin-dependent kinase, modulation in abscisic acid level and enhancement in activity of salt overly sensitive (SOS) homeostatic signaling pathways genes (Julkowska and Testerink 2015).

Genes tangled in salinity & drought stress Response

In maize, several key genes play crucial roles in mitigating salinity and drought stress. For

providing salinity tolerance, the gene *ZmSOS1* encodes a Na^+/H^+ exchanger that helps in expelling excess sodium from the cells, thereby alleviating salt-induced stress. Another important gene is *ZmSRP*, which contributes to maintaining cellular stability under high salinity conditions. (Iqbal et al., 2021). Moreover, *ZmHKT1* is involved in managing potassium levels within the plant, which is vital for countering the adverse effects of elevated sodium concentrations. Additionally, *ZmABH2*, which encodes an abscisic acid (ABA)-induced protein, plays a role in enhancing salt tolerance by participating in the ABA signaling pathway. When it comes to drought stress, the gene *ZmDHN* encodes dehydrins, proteins that protect plant cells from dehydration. Also, *ZmDST*, which encodes a drought stress transcription factor, is critical in regulating the plant's response to water scarcity by modulating various stress-related genes. Additionally, *ZmLEA* proteins are crucial for protecting cells during water scarcity by stabilizing cellular structures.

Modern approaches like genomic selection and QTL mapping, alongside gene-editing technologies such as CRISPR/Cas9, are being utilized to enhance maize's resilience to these environmental stresses by targeting and modifying abiotic and salinity stress-responsive genes.

Signaling pathways involved for salinity and drought tolerance

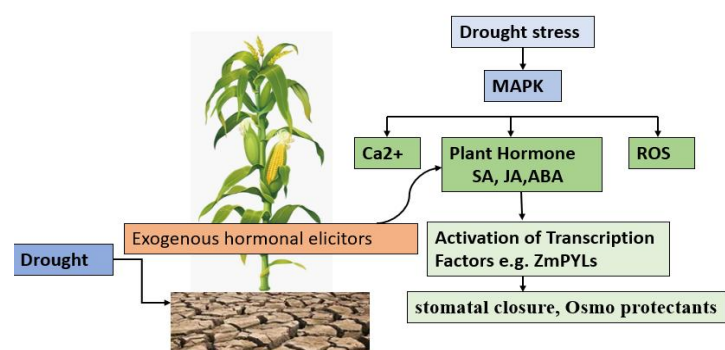


Fig. 1 Plant response in drought conditions

The drought response in maize is significantly mediated through the abscisic acid (ABA) signaling pathway, which is crucial for enhancing the plant's resilience to water scarcity. When maize experiences drought stress, the plant's cells produce ABA, a plant hormone that orchestrates a complex network of responses to mitigate the adverse effects of water shortage. ABA synthesis begins in response to reduced water availability. This ABA is perceived by specific receptors, such as the PYR/PYL/RCAR family, which bind to ABA and inhibit protein phosphatases like PP2C, thus releasing the repression of stress-responsive pathways.

In drought conditions, ABA activates transcription factors such as *ZmDREB* (Dehydration-Responsive Element Binding) and *ZmABF* (ABA-Responsive Element Binding Factor), which bind to dehydration-responsive elements (DRE) and ABA-responsive elements (ABRE) in the promoters of target genes, leading to their expression. These genes include those encoding dehydrins (*ZmDHN*) and late embryogenesis abundant (LEA) proteins (*ZmLEA*), which protect cellular structures and maintain hydration. Additionally, genes involved in osmotic adjustment, such as *ZmP5CS*, which produces proline, are upregulated to help the plant cope with reduced water availability.

ABA signaling also regulates stomatal closure, a critical physiological response that helps conserve water by reducing transpiration. Furthermore, ABA influences adaptive metabolic changes, promoting the accumulation of Osmo protectants like proline and soluble sugars, which stabilize proteins and cellular structures under stress.

In the context of salinity stress, ABA signaling also plays a pivotal role. High salinity conditions trigger ABA accumulation, which similarly activates the ABA signaling pathway to manage ionic and osmotic stress. In response to salinity, *ZmSOS1* helps by encoding a Na^+/H^+ exchanger that expels excess sodium from cells, while *ZmHKT1* manages potassium levels to counteract the toxic effects of sodium. Additionally, *ZmABH2*, an ABA-induced protein, enhances salt tolerance by participating in the ABA signaling network.

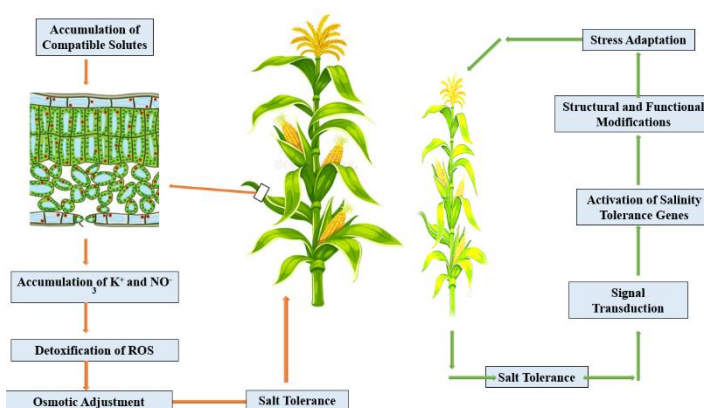


Fig 2 Salt stress activates signals that bind to receptors, starting physiological and molecular processes that help maize plants withstand salinity stress.

Together, the ABA signaling pathway integrates responses to both drought and salinity stresses, coordinating gene expression, physiological adaptations, and metabolic adjustments to improve maize's resilience under varying environmental conditions.

Genome editing tools

Genome editing technologies have revolutionized crop improvement by allowing for precise modifications to plant genomes. When it comes to enhancing drought and salinity tolerance in maize (corn), several genome editing tools are particularly relevant:

1. **CRISPR/Cas9:** This is the most widely used genome editing tool. CRISPR/Cas9 uses a guide RNA (gRNA) to direct the Cas9 protein to a specific location in the genome, where it creates a double-strand break. The cell's natural repair mechanisms then introduce changes, which can be harnessed to knock out or modify genes associated with drought and salinity tolerance. (Svitashev et al., 2016).
2. **Base Editing:** This newer technique allows for precise nucleotide changes without creating double-strand breaks. Base editors convert one DNA base pair into another, potentially correcting mutations or introducing beneficial traits with fewer unintended effects. (Hwang et al., 2018).
3. **Prime Editing:** Prime editing is another recent advancement that enables targeted insertions, deletions, and base substitutions with high precision. This method involves a "search-and-replace" mechanism to edit specific genetic sequences (Anzalone et al., 2019).

CRISPR/Cas9 mediated genome editing in maize for salinity and drought tolerance

CRISPR technology offers a powerful approach to enhancing maize resilience against salt and drought stress by precisely targeting and modifying stress-responsive genes. The CRISPR/Cas9 system operates by introducing targeted breaks in the DNA at specific locations. This process involves the Cas9 protein, which acts as molecular scissors guided by a single-guide RNA (sgRNA) that directs Cas9 to the precise gene of interest. (Hernandes-Lopes et al., 2023). Once the DNA is cut, the plant's natural repair mechanisms are triggered. Researchers can harness these repair processes to either insert beneficial genes, correct mutations, or knock out undesirable genes (Young et al., 2019).

For drought tolerance, CRISPR can target genes such as *ZmCLCg* and *ZmPMP3*, which regulate stress-responsive pathways. (Sheoran et al., 2022). Additionally, *P5CS* and *GBLT* genes, involved in osmotic regulation through proline and glycine betaine synthesis, can be edited to improve stress resilience. To address salt stress, CRISPR can modify

SOS1 and *SOS2* for better ion transport management, and *HKT1* and *AKT1* for maintaining ion balance. (Singh et al., 2023). *ABH2* can be edited to enhance oxidative damage repair, while *DST* can be targeted to improve dehydration tolerance (Rahman et al., 2022). Furthermore, modifying genes like *ARF* and *PXY* can improve root architecture, aiding in better water and nutrient uptake (Jiang et al., 2022).

Despite its potential, CRISPR technology faces challenges including ensuring precision to avoid off-target effects, navigating complex regulatory and biosafety evaluations, and addressing public concerns about genetically modified crops. However, with careful application, CRISPR holds significant promise for developing maize varieties with improved resilience to environmental stresses, thus supporting agricultural stability and productivity (Nuccio et al., 2021).

Conclusion

Maize is a crucial crop in India, vital for food security, livestock feed, and farmers' incomes. However, its productivity is hampered by drought and salinity stress. To tackle these challenges, leveraging CRISPR/Cas9 technology for precise gene editing offers a promising solution. This technique can target specific genes related to stress resistance, such as *ZmSOS1* and *ZmDREB*, to develop maize varieties that better withstand environmental stresses. Combining CRISPR/Cas9 with traditional breeding and genomic selection can accelerate the creation of high-yield, climate-resilient maize. Field trials will be essential to test these new varieties under various conditions before commercialization, which will support farmers and enhance food security.

References

- Ahluwalia, O., Singh, P.C. and Bhatia, R., 2021. A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. *Resources, Environment and Sustainability*, 5, p.100032.
- Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A. and Liu, D.R., 2019. Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 576(7785), pp.149-157.
- FAOSTAT (2021). <https://www.fao.org/faostat/en/#data/QCL>.
- Golldack, D., Li, C., Mohan, H. and Probst, N., 2014. Tolerance to drought and salt stress in plants: unraveling the signaling networks. *Frontiers in plant science*, 5, p.151.

- Hernandes-Lopes, J., Yassitepe, J.E.D.C.T., Koltun, A., Pauwels, L., Silva, V.C.H.D., Dante, R.A., Gerhardt, I.R. and Arruda, P., 2023. Genome editing in maize: Toward improving complex traits in a global crop. *Genetics and molecular biology*, 46, p.e20220217.
- Hwang, G.H., Park, J., Lim, K., Kim, S., Yu, J., Yu, E., Kim, S.T., Eils, R., Kim, J.S. and Bae, S., 2018. Web-based design and analysis tools for CRISPR base editing. *BMC bioinformatics*, 19, pp.1-7.
- Iqbal, S., Hussain, S., Qayyum, M.A., Ashraf, M. and Saifullah, S., 2020. The response of maize physiology under salinity stress and its coping strategies. *Plant stress physiology*, pp.1-25.
- Jiang, Y., Sun, K. and An, X., 2022. CRISPR/Cas system: applications and prospects for maize improvement. *ACS Agricultural Science & Technology*, 2(2), pp.174-183.
- Julkowska, M.M. and Testerink, C., 2015. Tuning plant signaling and growth to survive salt. *Trends in plant science*, 20(9), pp.586-594.
- Nuccio, M.L., Claeys, H. and Heyndrickx, K.S., 2021. CRISPR-Cas technology in corn: a new key to unlock genetic knowledge and create novel products. *Molecular Breeding*, 41(2), p.11.
- Sah RP, Chakraborty M, Prasad K, Pandit M, Tudu VK, Chakravarty MK, Narayan SC, Rana M, Moharana D (2020) Impact of water deficit stress in maize: Phenology and yield components. *Scientific reports*. **10**(1):2944.
- Setten, R.L., Rossi, J.J. and Han, S.P., 2019. The current state and future directions of RNAi-based therapeutics. *Nature reviews Drug discovery*, 18(6), pp.421-446.
- Sheoran, S., Kaur, Y., Kumar, S., Shukla, S., Rakshit, S. and Kumar, R., 2022. Recent advances for drought stress tolerance in maize (*Zea mays* L.): Present status and future prospects. *Frontiers in Plant Science*, 13, p.872566.
- Singh, A., Pandey, H., Pandey, S., Lal, D., Chauhan, D., Aparna, Antre, S.H. and Kumar, A., 2023. Drought stress in maize: stress perception to molecular response and strategies for its improvement. *Functional & Integrative Genomics*, 23(4), p.296.
- Svitashev, S., Schwartz, C., Lenderts, B., Young, J.K. and Mark Cigan, A., 2016. Genome editing in maize directed by CRISPR-Cas9 ribonucleoprotein complexes. *Nature communications*, 7(1), pp.1-7.
- Young, J., Zastrow-Hayes, G., Deschamps, S., Svitashev, S., Zaremba, M., Acharya, A., Paulraj, S., Peterson-Burch, B., Schwartz, C., Djukanovic, V. and Lenderts, B., 2019. CRISPR-Cas9 editing in maize: systematic evaluation of off-target activity and its relevance in crop improvement. *Scientific reports*, 9(1), p.6729.

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