IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 12, Issue 3 Series 1 (May. – June. 2026), PP 66-72
www.iosrjournals.org

Genome Editing Tools for Enhancing Stress Tolerance in Plants: Advances, Applications, and Future Perspectives

Priti kushwaha[1], Moumita Majumdar[2], Abin Mani[3], Mohd Arshan[4]
(Centre for Scientific Research and Development, People's University, Bhopal, 462037, India)
Corresponding author, Email address: pritikush77@gmail.com

Abstract: *Globally, agriculture yields have been impacted by climate change and rising environmental challenges. Plants are frequently exposed to various abiotic stresses, such as drought, salinity, extreme temperatures, and heavy metal toxicity, as well as biotic stresses caused by various pathogens and insects. The development of stress tolerant crops through traditional breeding methods is frequently time consuming and constrained by genetic variability. Genome editing technologies have emerged as powerful tools for precise and targeted modification of plant genomes, enabling rapid improvement of stress tolerance traits. Plant biotechnology has been transformed by a number of technologies, including Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated systems. Crop improvement have been further expanded by recent development such as base editing, prime editing, and multiplex genome editing. This review summarizes the major genome editing tools, their mechanisms of action, and their applications in enhancing tolerance to abiotic and biotic stresses. Moreover, the challenges, regulatory aspects, and future prospects of genome editing for the development of climate-resilient crops are also discussed in this review.*

Key Word*: Genome editing, CRISPR-Cas9, TALENs, ZFNs, Base editing, Prime editing, Abiotic stress, Biotic stress, Crop improvement, Climate resilience*

Date of Submission: 08-06-2026 Date of Acceptance: 19-06-2026

I. Introduction

Plants are essential to human civilization, as the main source of food, fodder, fiber, fuel, and several other industrial and pharmaceutical products. Yet, their growth, development, and productivity are continuously hampered by various biotic and abiotic stresses. Abiotic stresses include unfavorable environmental conditions like drought, salinity, extreme temperatures, and heavy metal toxicity [fig.1], while Biotic stresses are caused by living organisms like bacteria, fungi, viruses, pests, nematodes, and weeds. Crop yield and quality are decreased as a result of these stressors' effects on plant physiology, biochemistry, and molecular processes. As the population increases, climate change, and degradation of agricultural land, understanding and mitigating the effects of stress on plant has become an important focus of agricultural research to ensure sustainable food production and global food security [1].

Crop improvement and development of stress-tolerant varieties have benefited from traditional plant breeding, but its effectiveness is limited by breeding cycles, complicated genetic factors, and a lack of desirable genetic variation. Alternative approaches for introducing advantageous traits have been made possible by genetic engineering, which allows the transfer of specific genes linked with stress tolerance. However, its broad adoption has been constrained by worries regarding gene integration, biosafety concerns, public acceptance, and regulation approval. Genome editing technologies, which enable targeted modification of endogenous genes without introducing foreign DNA, have emerged as powerful and precise tools for crop improvement in recent years. [2].

The development of engineered nuclease-based systems such as Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based technologies, has revolutionized the area of plant biotechnology [fig.2]. These tools enable precise gene knockout, insertion, replacement, and transcriptional regulation, facilitating the development of crops with enhanced resistance to biotic stresses and improved tolerance to abiotic stresses such as drought, salinity, heat, cold, and heavy metal toxicity. This review provides a comprehensive overview of major genome editing tools and highlights their applications in developing stress-resilient crops for sustainable agriculture [3, 4].

Genome Editing for Abiotic Stress Tolerance in Plants

Drought Stress

Drought stress triggers osmotic stress, oxidative damage, and the loss of cellular homeostasis through water deficit. At the molecular level, these effects abscisic acid (ABA)-dependent and ABA-independent signaling cascades which regulate stomatal closure, root architecture, osmotic adjustment, and detoxification of reactive oxygen species (ROS) [5]. Major regulatory networks include DREB/CBF transcription factors, AREB/ABF proteins, late-embryogenesis abundant (LEA) proteins, and several type 2C protein phosphatases (PP2Cs) that act as negative regulators of ABA signaling [6]. The earliest genome-editing work on drought tolerance used zinc-finger nucleases (ZFNs), establishing proof-of-concept that stress-responsive loci could be altered at specific sites. In tobacco, ZFN-driven targeted integration at ABA-responsive regulatory sequences showed that specific DNA modification could change stress-gene expression, setting the conceptual groundwork for subsequent development. TALEN platforms then made drought-associated loci more accessible, disrupting negative regulators of stomatal closure with TALENs in Arabidopsis and maize improved water-use efficiency (WUE) through targeted gene knockout, providing a clear functional rationale that CRISPR-based tools would later exploit at scale [7].

CRISPR/Cas9 has been deployed most extensively and with the greatest functional impact for engineering drought tolerance across major crops. In maize, CRISPR-mediated promoter insertion of the GOS2 promoter driving overexpression of *ARGOS8* improved grain yield under drought without penalizing yield under well-watered conditions. This study stands as a landmark application of CRISPR for drought tolerance in a major cereal crop [8]. In rice, CRISPR-mediated knockout of *OsNAC45* resulted in enhanced root length and increased drought survival, while targeted editing of *DRO1* (Deep Rooting 1) using base editing approaches enabled fine-tuning of root growth angles to improve water uptake from deeper soil layers. Multiplex editing of negative regulators *OsPP2C06, OsPP2C09,* and *OsPP2C66* enhanced drought tolerance in rice by constitutively activating ABA response pathways [Table-1] [9]. Beyond simple knockout strategies, cytosine base editors (CBEs) and adenine base editors (ABEs) have been used to introduce single-nucleotide changes in *AREB1* and *DREB2A* that replicate naturally occurring drought-tolerant alleles from wild relatives. Epigenome editing with dCas9 fused to the histone acetyltransferase p300 has also been deployed to upregulate drought-responsive genes such as *RD29A* without modifying the DNA sequence itself. Together, this trajectory from ZFN-based proof-of-concept to TALEN-mediated targeted disruption to CRISPR-enabled multiplex and precision editing illustrates the continuously growing toolkit for drought tolerance engineering [10].

Salinity Stress

Plants are exposed to both ionic toxicity and osmotic stress due to salinity, which is primarily caused by excess sodium chloride (NaCl). The ionic component results from the disruption of protein folding and enzyme function, as well as from Na^+ competitively displacing essential K^+ at membrane transporters [11]. The plasma membrane Na^+/H^+ antiporter SOS1, the kinase SOS2, and the calcium sensor SOS3 are all part of the Salt Overly Sensitive (SOS) pathway, which is the primary pathway for Na+ extrusion. The vacuolar NHX1 antiporter, which restores Na^+ away from the cytoplasm and High-Affinity Potassium Transporters (HKTs) that recover Na^+ from the xylem, support this [12]. ZFNs were among the earliest tools used to explore and modify salinity-associated loci. In Arabidopsis, mutagenesis by ZFN in the SOS1 regulatory region provide early evidence that site-specific modification of the ion transport gene could modify Na^+ homeostasis, but the low editing efficiency and unfavorable off-target profiles limited the practical use of the crop. The TALENs have expanded this through their modular, designable repeat-variable diresidue (RVD) architecture. Editing vacuolar antiporter genes with TALENs in tobacco and Arabidopsis improves Na^+ compartmentalization and reduces Na^+ buildup in shoots. The platform was particularly effective in targeting the repetitive and GC-rich promoter sequences common to salinity-responsive genes [13, 14].

CRISPR/Cas9 has led to the most extensive and significant progress in salt tolerance. Knockout of *OsRR22* a type-A response regulator which negatively controls cytokinin signaling and salt stress responses, resulted in a

significantly improvement in salt tolerance in rice without apparent loss of yield. In tomato, disrupting *SlHKT12* alters the distribution of Na^+ between roots and shoots, which significantly reduce the Na^+ toxicity. In wheat, multiplex modification of all the *TaNHX2* homologs results in significantly increased grain yield in saline field conditions [15]. Cytosine base editing of the *ANN4* promoter has enabled fine-tuned modulation of salt-stress gene expression in rice. Prime editing has been used to introduce gain-of-function mutations in *BADH* (betaine aldehyde dehydrogenase) in maize, a species naturally lacking in this suitable solute thereby increasing the synthesis of glycine betaine. CRISPR-based promoter editing of *P5CS1* also improved proline accumulation under salt stress [Table-1]. Across the three generations, salinity tolerance improvement shows a clear pattern: ZFN and TALEN research confirmed mechanistic target validation, while CRISPR tools provided the size and precision required for deployment at the crop level [16].

Heat Stress

Heat stress induces protein denaturation, membrane fluidity changes, disruption of photosynthetic apparatus, and activation of the heat shock response (HSR), which is mediated by Heat Shock Factors (HSFs) that bind heat shock elements (HSEs) in the promoters of Heat Shock Protein (HSP) chaperone genes. Photosystem II (PSII) is especially thermolabile, and pollen viability and reproductive performance are among the most heat-sensitive agronomic parameters, making thermotolerance engineering commercially critical [17]. TALEN technology was applied in early studies to dissect HSF-HSP regulatory circuits. In Arabidopsis, TALEN-mediated modification of the HSP promoter regions has shown that modified cis-regulatory architecture could enhance basal thermotolerance and heat shock gene inducibility, confirming these elements as promising engineering targets. ZFN-based methods were used to confirm function of particular transcription factor binding sites in heat-responsive promoters through targeted deletion, providing mechanistic insights later leveraged by CRISPR platforms, though [18,19].

CRISPR/Cas9 has enabled the most comprehensive thermotolerance engineering efforts to date. In tomato, knockout of SlAGO7 (a component of the RNA-induced silencing complex) resulted in enhanced heat stress tolerance with improved pollen viability and fruit set at elevated temperatures. CRISPR-mediated editing of SlMAPK3 in tomato elucidated the role of MAPK cascades in thermotolerance, while in wheat, editing of TaHSP90.1 homeologs modified HSP90 chaperone function [20, 21]. In rice, multiplex editing of OsHsfA2d regulatory elements using base editing introduced enhanced constitutive HSP gene expression, improving basal thermotolerance without a growth penalty [22]. CRISPR-mediated editing of HSFA1 in tomato by removing a repressive domain identified through structural studies resulted in a constitutively active form conferring superior thermotolerance across vegetative and reproductive stages [Table-1] [23]. The TALEN and ZFN studies that preceded CRISPR applications in this domain remain valuable for their identification of regulatory elements and confirmation of gene function, providing the mechanistic framework that has allowed CRISPR-based engineering to achieve its greatest successes.

Cold Stress

Cold stress includes both chilling stress (0–15°C), which slows metabolic enzyme function and causes membrane rigidification, and freezing stress (<0°C), which results in cellular dehydration through extracellular ice crystal formation and direct membrane damage from intracellular ice. Cold acclimation is the process by which plants gradually acquire freezing tolerance following exposure to low non-freezing temperatures which is regulated primarily by the CBF (C-repeat Binding Factor)/DREB1 transcription factor pathway, which activates Cold-Regulated (COR) genes encoding cryoprotective proteins, compatible solute synthesis enzymes, and membrane lipid modifiers [24]. TALEN technology contributed meaningfully to cold stress research through targeted modification of CBF pathway components, TALEN-mediated editing of CBF1 regulatory regions in tomato altered the architecture of cold-responsive promoters and improved chilling tolerance, validating CBF pathway regulation as a viable editing target in a naturally cold-sensitive horticultural crop. ZFN approaches were employed to investigate the cis-regulatory elements controlling COR gene expression in Arabidopsis, with targeted deletions of ICE1-binding motifs confirming the mechanistic basis of CBF-dependent cold acclimation at a precision unavailable through conventional mutagenesis [25]

CRISPR/Cas9 and precision editing tools have since expanded cold tolerance engineering across multiple crops. In rice, knockout of *OsMYB30* is a negative regulator of CBF gene expression which significantly enhanced cold tolerance at the seedling stage, while editing of *OsWRKY76* enhanced cold stress responses by relieving transcriptional repression of *OsDREB1B* [26]. Base editing of *ICE1* to introduce phosphorylation-resistant amino acid variants resulted in constitutive activation of cold-responsive genes and enhanced freezing tolerance in Arabidopsis, demonstrating how base editing can create natural allele analogs without foreign DNA. Multiplex CRISPR editing in wheat targeting all six homologs of *TaCBF14* and *TaCBF15* enhanced frost tolerance in field trials [27]. CRISPR/Cas9-mediated modification of *FAD7* and *FAD8* fatty acid desaturases in tobacco and tomato demonstrated that tuning polyunsaturated fatty acid composition enhances membrane fluidity and reduces electrolyte leakage during cold stress [Table-1]. The history of cold stress editing across three platforms shows a clear progression: ZFNs and TALENs validated mechanistic targets and regulatory elements, CRISPR-based tools, such as base and prime editors, then provided crop-relevant improvements with the efficiency and accuracy required for practical application [28].

Heavy Metal Stress

Heavy metal pollution in agricultural soils due to mining, smelting, industrial waste, and the use of phosphate fertilizers creates significant risks to crop development and food security. Metals like cadmium (Cd), arsenic (As), lead (Pb), mercury (Hg), and chromium (Cr) are harmful to plants. These metals can damage enzymes, induce stress from harmful reactive compounds, and hinder the absorption of essential nutrients. Plants naturally deal with these metals by binding them with special proteins like phytochelatins and metallothioneins, storing them in specific cell compartments, and actively eliminating them from the plant body [29]. ZFNs were historically played a significant role in laying the groundwork the for targeted gene editing in plants. A key achievement was the first successful demonstration of using ZFNs to alter the tobacco acetolactate synthase (ALS) gene, which demonstrated that precise editing of genes inside their natural location was possible. Although this work did not exactly focus on heavy metal tolerance. This idea was subsequently extended to genes involved in metal transport. Later research using ZFNs to study metal-responsive DNA regions in model plants enabled researchers to understand how genes related to metal detoxification are controlled and expressed. [30]. TALENs, with their improved target design flexibility, were applied to disrupt silicon/arsenic co-transporter genes in rice, with TALEN-mediated editing of OsLsi1 and OsLsi2 reducing arsenite accumulation in grain tissues and demonstrating that transporter gene knockout is a viable strategy for reducing dietary arsenic exposure through plant-based intervention [31].

CRISPR/Cas9 has enabled the most impactful advances in this domain, most notably the knockout of *OsNramp5* in rice, which reduced Cd^{2+} accumulation in polished rice grain by more than 90% without significant yield penalty an advance with direct and immediate food safety implications. CRISPR-mediated disruption of the competing silicon/arsenite uptake function of *OsLsi1* further reduced as accumulation, though the challenge of pleiotropic effects on beneficial silicon uptake has highlighted the need for allele-specific approaches achievable only through base or prime editing [31]. In poplar, knockout of *PtNramp5* significantly reduced Cd^{2+} accumulation while *PtHMA4* editing enhanced shoot translocation of Zn^{2+} and Cd^{2+} for improved phytoextraction efficiency [32]. CRISPR-mediated activation of *AtPCS1* (phytochelatin synthase) and editing of vacuolar transporters *AtABCC1/AtABCC2* in Arabidopsis demonstrated enhanced Cd^{2+} and Hg^{2+} tolerance and accumulation [Table-1] [33]. The successive contributions of ZFNs (mechanistic validation), TALENs (transporter gene disruption), and CRISPR tools (high-efficiency crop-level application) collectively define a comprehensive genome editing strategy for heavy metal stress management in plants.

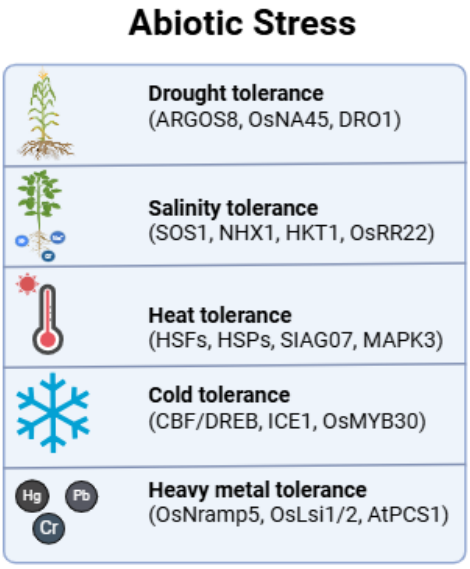

Fig.1 Abiotic Stresses and Their Stress-Responsive Genes (Created in https://BioRender.com)

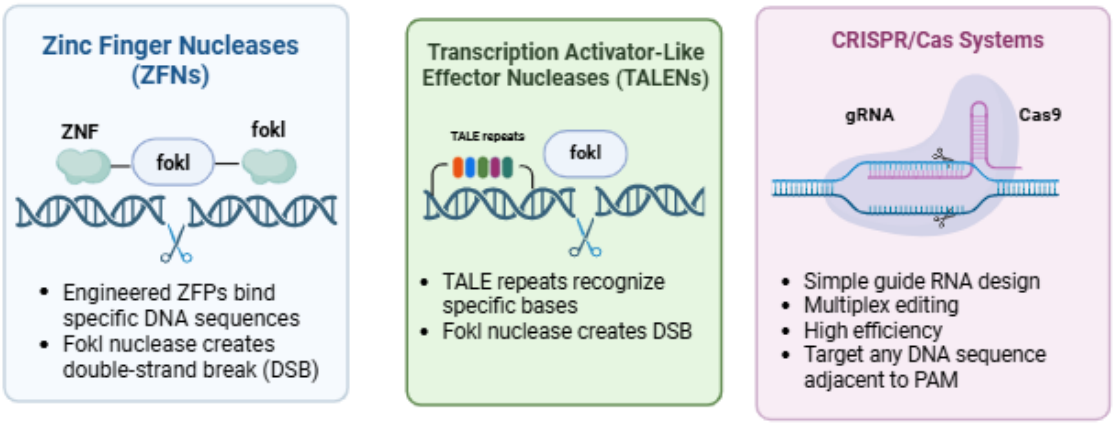

Fig.2 Genome Editing Technologies for Crop Improvement and Stress Tolerance (Created in https://BioRender.com)

Table 1: Overview of genome editing applications for major abiotic stresses in crop plants.

stress	tool	crop	Target gene	outcome	reference
Drought	ZFN	Tobacco	ABA-responsive loci	Early proof-of-concept; targeted integration at stress loci	[7]
	CRISPR/Cas9	Maize	ARGOS8	Improved grain yield under water deficit	[8]
		Rice	OsNAC4	Enhanced root growth & drought survival	[9]
Salinity	ZFN	Arabidopsis	SOS1 locus	Targeted mutagenesis at Na^+ extrusion locus	[13]
	CRISPR/Cas9	Rice	OsRR22	Improved salinity tolerance at seedling stage	[15]
		Tomato	SlHKT1;2	Reduced Na^+ accumulation in leaves	
Heat	TALEN	Arabidopsis	HSP loci	Targeted activation of thermotolerance pathway	[18]

	CRISPR/Cas9	Tomato	SlAGO7	Thermotolerance; improved fruit set	[20]
Cold	CRISPR/Cas9	Rice	OsMYB30	Improved cold tolerance at seedling stage	[26]
	TALEN	tomato	CBF1/DREB1	Chilling tolerance; altered CBF pathway regulation	[25]
Heavy metal	ZFN	Tobacco	ALS locus	Pioneer ZFN editing; regulatory framework for metal loci	[30]
	CRISPR/Cas9	Rice	OsNramp5	Drastically reduced Cd^{2+} in grain (>90%)	[31]
	TALEN		OsLsi1/OsLsi2	Reduced arsenie uptake in rice grain	

II. Conclusion

This review describes how the engineering of stress tolerance in crop plants has been radically changed by genome editing. From the early proof-of-concept work with ZFNs and the enhanced targeting flexibility of TALENs, to the precision and throughput of CRISPR/Cas9 and its derivatives, the field has followed a clear path. While CRISPR-based technology produced crop-relevant result at scale, earlier platforms confirmed mechanistic targets, Landmark achievements which includes ARGOS8 editing for drought-resilient maize, OsRR22 knockout for salinity tolerance in rice, SlAGO7 disruption for thermotolerance in tomato, and over 90% reduction of cadmium in rice grain via OsNramp5 editing shows that genome editing has progressed far beyond the laboratory. The toolkit has been further improved by the introduction of base editing, prime editing, and epigenome editing, which allow for single-nucleotide precision and gene expression modulation without the integration foreign of DNA. Together, these advances mark a genuine paradigm shift from conventional breeding toward targeted, knowledge-driven crop improvement. When these requirement are satisfied, genome editing offers a viable and effective route toward climate-resilient crops capable of maintaining global food security.

III. Future Perspective

Future developments in genome editing are expected to focus on improving editing efficiency, decreasing off-target impacts, and expanding editing capabilities. Combining genome editing with genomics, transcriptomics, proteomics, metabolomics, and artificial intelligence-based target identification will accelerate the development of climate-resilient crops. Advanced technologies such as prime editing, epigenome editing, and multiplex editing are likely to play important roles in sustainable agriculture.

Acknowledgment

The authors thankfully acknowledge the Department of Biotechnology and Microbiology, Centre for Scientific Research and Development, People's University, Bhopal, for providing the necessary facilities to conduct this review.

References

[1]. Du, B., Haensch, R., Alfarraj, S., & Rennenberg, H. (2024). Strategies of plants to overcome abiotic and biotic stresses. *Biological Reviews*, *99*(4), 1524-1536.
[2]. Mansi, M., & Danai, P. (2026). The emerging impact of CRISPR and gene editing on global crop improvement. *Transgenic Research*, *35*(1), 8.
[3]. Gaj, T., Gersbach, C. A., & Barbas, C. F. (2013). ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in biotechnology*, *31*(7), 397-405.
[4]. Khalil, A. M. (2020). The genome editing revolution. *Journal of genetic engineering and biotechnology*, *18*(1), 68.
[5]. Fang, Y., & Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and molecular life sciences*, *72*(4), 673-689.
[6]. Jung, C., Nguyen, N. H., & Cheong, J. J. (2020). Transcriptional regulation of protein phosphatase 2C genes to modulate abscisic acid signaling. *International journal of molecular sciences*, *21*(24), 9517.
[7]. Mushtaq, M., Sakina, A., Wani, S. H., Shikari, A. B., Tripathi, P., Zaid, A., ... & Salgotra, R. K. (2019). Harnessing genome editing techniques to engineer disease resistance in plants. *Frontiers in plant science*, *10*, 550.
[8]. Shi, J., Gao, H., Wang, H., Lafitte, H. R., Archibald, R. L., Yang, M., ... & Habben, J. E. (2017). ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant biotechnology journal*, *15*(2), 207-216.
[9]. Zhang, X., Long, Y., Huang, J., & Xia, J. (2020). OsNAC45 is involved in ABA response and salt tolerance in rice. *Rice*, *13*(1), 79.
[10]. Roca Paixão, J. F., Gillet, F. X., Ribeiro, T. P., Bournaud, C., Lourenço-Tessutti, I. T., Noriega, D. D., ... & Grossi-de-Sa, M. F. (2019). Improved drought stress tolerance in Arabidopsis by CRISPR/dCas9 fusion with a Histone AcetylTransferase. *Scientific Reports*, *9*(1), 8080.

[11]. Mohamed, I. A., Foda, M. F., Khan, I. U., Batool, M., Awad-Allah, E. F., Fan, C., ... & Wu, H. (2025). Nano-improved plant salinity tolerance: The importance of K+/Na+ homeostasis and crosstalk between Ca2+ and hormones. *Plant Nano Biology*, 100196.

[12]. Hu, S., Jiang, P., & Guo, Q. (2025). Characterization of the Salt Overly Sensitive 1 (SOS1) Pathway Genes in Tea Plant (Cameliia sinensis) Under Environmental Stress. *Horticulturae*, *11*(7), 855.

[13]. Zhang, F., & Voytas, D. F. (2010). Targeted mutagenesis in Arabidopsis using zinc-finger nucleases. In *Plant chromosome engineering: methods and protocols* (pp. 167-177). Totowa, NJ: Humana Press.

[14]. Bhardwaj, A., & Nain, V. (2021). TALENs—an indispensable tool in the era of CRISPR: a mini review. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 125.

[15]. Zhang, A., Liu, Y., Wang, F., Li, T., Chen, Z., Kong, D., ... & Luo, L. (2019). Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Molecular breeding*, *39*(3), 47.

[16]. Feng, X. J., Li, J. R., Qi, S. L., Lin, Q. F., Jin, J. B., & Hua, X. J. (2016). Light affects salt stress-induced transcriptional memory of P5CS1 in Arabidopsis. *Proceedings of the National Academy of Sciences*, *113*(51), E8335-E8343.

[17]. Mondal, S., Karmakar, S., Panda, D., Pramanik, K., Bose, B., & Singhal, R. K. (2023). Crucial plant processes under heat stress and tolerance through heat shock proteins. *Plant Stress*, *10*, 100227.

[18]. Swindell, W. R., Huebner, M., & Weber, A. P. (2007). Transcriptional profiling of Arabidopsis heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. *BMC genomics*, *8*(1), 125.

[19]. Joung, J. K., & Sander, J. D. (2013). TALENs: a widely applicable technology for targeted genome editing. *Nature reviews Molecular cell biology*, *14*(1), 49-55.

[20]. Yu, W., Wang, L., Zhao, R., Sheng, J., Zhang, S., Li, R., & Shen, L. (2019). Knockout of SlMAPK3 enhances tolerance to heat stress involving ROS homeostasis in tomato plants. *BMC Plant Biology*, *19*(1), 354.

[21]. Wang, G. F., Wei, X., Fan, R., Zhou, H., Wang, X., Yu, C., ... & Zhang, X. (2011). Molecular analysis of common wheat genes encoding three types of cytosolic heat shock protein 90 (Hsp90): functional involvement of cytosolic Hsp90s in the control of wheat seedling growth and disease resistance. *New Phytologist*, *191*(2), 418-431.

[22]. Wu, N., Yao, Y., Xiang, D., Du, H., Geng, Z., Yang, W., ... & Xiong, L. (2022). A MITE variation-associated heat-inducible isoform of a heat-shock factor confers heat tolerance through regulation of JASMONATE ZIM-DOMAIN genes in rice. *New Phytologist*, *234*(4), 1315-1331.

[23]. Tiwari, J. K., Singh, A. K., & Behera, T. K. (2023). CRISPR/Cas genome editing in tomato improvement: Advances and applications. *Frontiers in Plant Science*, *14*, 1121209.

[24]. Kidokoro, S., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2022). Transcriptional regulatory network of plant cold-stress responses. *Trends in plant science*, *27*(9), 922-935.

[25]. Ke, Y., Kong, D., Zhang, W., Zeng, W., Kong, Z., Dang, X., ... & Miki, D. (2025). Precise knock-in of stress-responsive cis-regulatory elements using gene targeting for improving abiotic stress tolerance in plants. *New Phytologist*, *247*(5), 2147-2162.

[26]. Zhang, M., Zhao, R., Huang, K., Huang, S., Wang, H., Wei, Z., ... & Du, X. (2022). The OsWRKY63–OsWRKY76–OsDREB1B module regulates chilling tolerance in rice. *The Plant Journal*, *112*(2), 383-398.

[27]. Soltész, A., Smedley, M., Vashegyi, I., Galiba, G., Harwood, W., & Vágújfalvi, A. (2013). Transgenic barley lines prove the involvement of TaCBF14 and TaCBF15 in the cold acclimation process and in frost tolerance. *Journal of experimental botany*, *64*(7), 1849-1862.

[28]. Ahmadikhah, A., Zarabizadeh, H., Nayeri, S., & Abbasi, M. S. (2025). Advancements in genome editing tools for genetic studies and crop improvement. *Frontiers in Plant Science*, *15*, 1370675.

[29]. Mohamed, H. I., Ullah, I., Toor, M. D., Tanveer, N. A., Din, M. M. U., Basit, A., ... & Rehman, M. U. (2025). Heavy metals toxicity in plants: Understanding mechanisms and developing coping strategies for remediation: A review. *Bioresources and Bioprocessing*, *12*(1), 95.

[30]. Petolino, J. F. (2015). Genome editing in plants via designed zinc finger nucleases. *In Vitro Cellular & Developmental Biology*, *51*(1), 1.

[31]. Xu, X., Sun, S. K., Zhang, W., Tang, Z., & Zhao, F. J. (2024). Editing Silicon Transporter Genes to Reduce Arsenic Accumulation in Rice. *Environmental science & technology*, *58*(4), 1976–1985. https://doi.org/10.1021/acs.est.3c10763

[32]. Shan, Q., Guan, J., Yang, Y., Chai, T., Gong, S., Wang, J., & Qiao, K. (2024). Cadmium-induced protein AS8: A protein to improve Cd accumulation and transport via Cd uptake in poplar. *Plant physiology and biochemistry*, *216*, 109199.

[33]. Park, J., Song, W. Y., Ko, D., Eom, Y., Hansen, T. H., Schiller, M., ... & Lee, Y. (2012). The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. *The Plant Journal*, *69*(2), 278-288.