

CRISPR/ Cas9 Mediated Salinity Tolerance in Rice Crop

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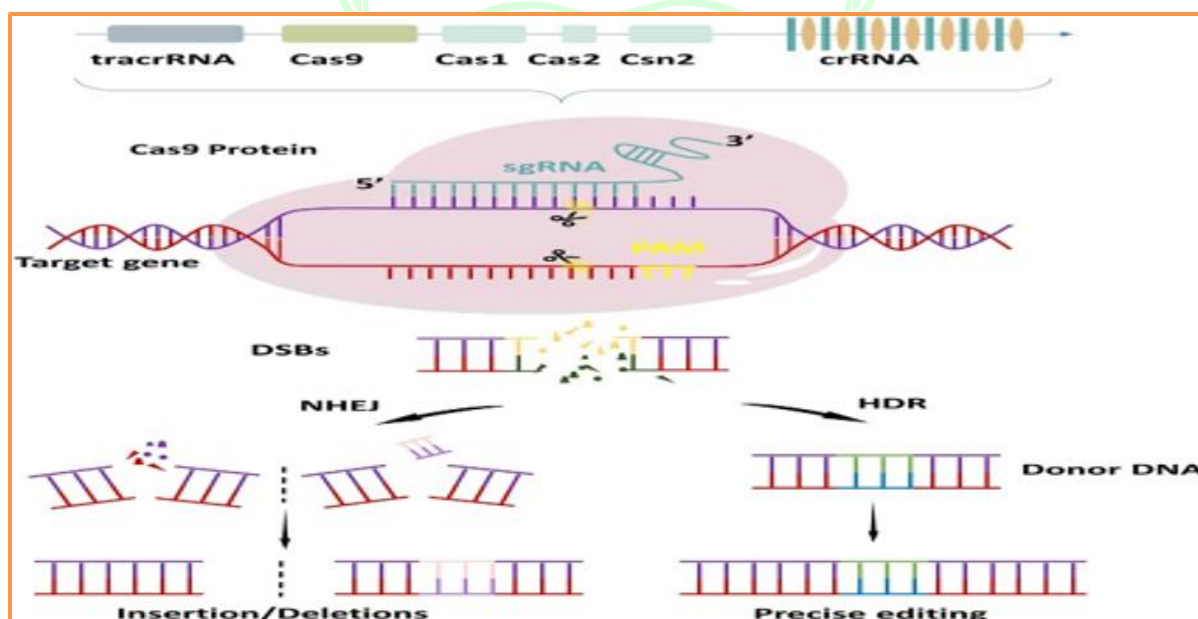
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In recent climate change phenomenon causes abiotic stress mainly drought and salinity stress. Salinity stress remains a major limiting factor crop productivity and sustainability, particularly in coastal areas. Increasing saline soils due to high evaporation in dry humid areas, seawater intrusion, aquaculture, wrong irrigation practices. In recent studies by the time of 2050 year half of the world's arable land could be salinized that threatening food security for increasing population about to exceed 9.7 billion. The physiological point of view, salinity causes ionic and osmotic stresses on plants. The excessive accumulation of Na^+ and Cl^- ions disrupt membrane stability, impairs enzymatic activity, and detrimental to photosynthetic capacity. Simultaneously, osmotic stress impairs water uptake and causes oxidative damage from reactive oxygen species (ROS) and nutrient imbalances leads to growth inhibition. These physiological damage of crops such as rice, wheat, and maize result in yield losses of 30–50% in salt-affected areas.

Mechanism of CRISPR

CRISPR/Cas9-mediated genome editing involves on targeted DNA cleavage followed by cellular repair mechanisms, including homology-directed repair (HDR) and non-homologous end joining (NHEJ), enabling precise genetic modification of stress-responsive genes associated with salinity adaptation. CRISPR/Cas9 can directly cause gene knockout (silencing) by insertion or deletion of a couple of nucleotides and repaired by non-homologous end join (EHEJ) and also CRISPR/Cas9 genome editing can be used to replace an undesirable gene or over expressed gene and an individual gene(knock in). Thus, CRISPR/Cas9 can be used to precisely insert a foreign gene into a specific location within a genome without interrupting other genes shown the mechanism from fig 1



Targeted gene editing for salinity tolerance

Numerous genes have been targeted using CRISPR/Cas9 to enhance salt tolerance via distinct physiological mechanisms. For example, genes like OsHKT1;5 and TaHKT1;5 D regulate ion homeostasis by reducing Na⁺ accumulation and improving K⁺ retention, respectively. Osmoprotectant synthesis genes including simultaneous editing of P5CS involved in proline biosynthesis and BADH1 responsible for glycine betaine synthesis. Similarly, antioxidant defense genes like OsAPX2 and TaSOD1 mitigate oxidative stress by enhancing ROS scavenging activities. Several successful case studies have demonstrated the potential of CRISPR in conferring salt resilience. In rice, knockout of OsRR22, a known negative regulator of stress signaling, resulted in a 30% biomass increase under salinity stress. CRISPR-Cas9 mediated genome editing of OsDST gene improves drought and high salinity tolerance in rice. The CRISPR-Cas9 nucleotide base deletion was identified in the osbhlh024 mutant resulted in a significant increase in the shoot weight, the total chlorophyll content, and the chlorophyll fluorescence under salt stress. Some of target gene editing by using CRISPR improve salinity tolerance in rice listed from table 1

Table 1. List of target genes for salinity tolerance

Response to salinity stress	Rice crop	Target gene	vector
Salt tolerance	O. sativa	OsDST	Agrobacterium Mediated
Salt tolerance	O. sativa	OsRAV2	Agrobacterium Mediated
Salt tolerance	O. sativa	OsRR22	Agrobacterium Mediated
Salt tolerance	O. sativa	OsNAC45	Agrobacterium Mediated
Salt tolerance	O. sativa	OsBBS1	Agrobacterium Mediated
Salt tolerance	O. sativa	OsAGO2	Agrobacterium Mediated
Salt tolerance	O. sativa	OsVDE	Agrobacterium Mediated
Salt tolerance	O. sativa	OsNAC041	Agrobacterium Mediated
Salt tolerance	O. sativa	OsSAPK2	Agrobacterium Mediated
Salt tolerance	O. sativa	OsPQT3	Agrobacterium Mediated
Salt tolerance	O. sativa	OsPIL14	Agrobacterium Mediated
Salt tolerance	O. sativa	OsBGE3	Agrobacterium Mediated
Salt tolerance	O. sativa	OsSPL10	Agrobacterium Mediated
Salt tolerance	O. sativa	OsDOF15	Agrobacterium Mediated
Salt tolerance	O. sativa	OsFLN2	Agrobacterium Mediated

Targeted modification of genes involved in efficient ion transport, osmotic adjustment, stress oxidative stress tolerance signaling, and cellular protection has substantially improved the salinity adaptation and expanded opportunities for precision trait improvement.

Conclusion

Compared with conventional breeding and earlier genome-editing platforms, modern genome engineering approaches provide improved targeting precision, higher editing efficiency, and broader applicability for crop improvement under abiotic stress conditions. Consequently, integrating genome editing with conventional breeding approaches is essential to ensure trait stability and agronomic performance across diverse environments. Despite these advances, several challenges remain, including off-target effects, functional redundancy among gene families and limited validation under field conditions. Further research needed to overcome challenges.

References

1. Alam, M.S., Kong, J., Tao, R., Ahmed, T., Alamin, M., Alotaibi, S.S., Abdelsalam, N.R. and Xu, J.H., 2022. CRISPR/Cas9 mediated knockout of the OsbHLH024 transcription factor improves salt stress resistance in rice (*Oryza sativa* L.). *Plants*, 11(9), p.1184.
2. Angon, P.B., Mondal, S., Akter, S., Sakil, M.A. and Jalil, M.A., 2023. Roles of CRISPR to mitigate drought and salinity stresses on plants. *Plant Stress*, 8, p.100169.

3. Farooq, M., Khan, A., Hassan, A. and Shah, M.M., 2026. Advancing Drought and Salinity Stress Tolerance in Plants: Mechanisms, Molecular Tools and Breeding Strategies—A Review. *Plant Breeding*.
4. Kumar, M., Prusty, M.R., Pandey, M.K., Singh, P.K., Bohra, A., Guo, B. and Varshney, R.K., 2023. Application of CRISPR/Cas9-mediated gene editing for abiotic stress management in crop plants. *Frontiers in plant science*, 14, p.1157678.
5. Ning, L., Xi, J., Zi, Y., Chen, M., Zou, Q., Zhou, X. and Tang, C., 2023. Prospects and challenges of CRISPR/Cas9 gene-editing technology in cancer research. *Clinical Genetics*, 104(6), pp.613-624.
6. Santosh Kumar, V.V., Verma, R.K., Yadav, S.K., Yadav, P., Watts, A., Rao, M.V. and Chinnusamy, V., 2020. CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. *Physiology and Molecular Biology of Plants*, 26(6), pp.1099-1110.
7. Sun, X., Wu, F., Ma, Z., Liang, G., Chen, S., Hu, X., Fan, S. and Zhao, Y., 2026. Systematic synthesis of CRISPR/Cas applications for enhancing salt tolerance in crops: a decade of progress and challenges. *BMC Plant Biology*, 26(1), p.506.