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Genome-Wide Association Mapping and CRISPR-Based Validation of Salt Tolerance Genes for Climate-Resilient Rice (*Oryza sativa* L.) Production

Ayman Javed *

Department of Botany, University of Agriculture Faisalabad. aymanjaved421@gmail.com

Noor Novishta

Post graduate college mansehra department of botany mansehra. noornovishta@gmail.com

Shazia Parveen

Institute of botany, Bahauddin Zakariya University, Multan. shaziabzu88@gmail.com

Muhammad Ahmad Mazhar

MNS University of Agriculture Multan. ahmadmazhar386@gmail.com

Muhammad Shehryar

Department of Botany, Abdul Wali Khan University Mardan. Shehryar5519@gmail.com OCID ID: 0009-0000-6985-5697

Waqas Mushtaq

Jiangxi Agricultural University, Key Laboratory of Crop Physiology, Ecology, and Genetic Breeding, Ministry of Education College of Agronomy, 1101 Fang Zhimin Avenue, Economic and technological Development Zone, Nanchang 330045, China. waqasuaf123@stu.jxau.edu.cn waqasuaf123@gmail.com ORCID. ID :0009-0004-6396-4155

Ziming Wu

Jiangxi Agricultural University, Key Laboratory of Crop Physiology, Ecology, and Genetic Breeding, Ministry of Education/College of Agronomy, 1101 Fang Zhimin Avenue, Economic and technological Development Zone, Nanchang 330045, China
wuzmjxau@163.com

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ABSTRACT

Soil salinization poses a critical threat to global agricultural sustainability, causing 30% to 50% annual yield reductions in rice (*Oryza sativa* L.), a primary staple for over half the global population. This review synthesizes recent advancements in climate-resilient rice production, focusing on the integration of genome-wide association studies (GWAS) and CRISPR-Cas9 genome editing. Salinity stress exerts a complex, multi-tiered physiological onslaught beginning with osmotic constraints, followed by ionic toxicity and secondary oxidative stress. Critically, salinity stress must be distinguished from alkaline stress, as their underlying genetic and transporter tolerance mechanisms differ fundamentally. To overcome the narrow genetic bases of conventional breeding, modern pipelines utilize multi-stage GWAS mapping across germination, seedling, and reproductive phases to uncover natural alleles from diverse germplasm and multi-parent populations. This genetic discovery is increasingly accelerated by intersecting association mapping with linkage analysis and transcriptomic profiling. Concurrently, CRISPR-Cas9 technologies have revolutionized functional validation by shifting focus from energetically costly overexpressions to the targeted knockout of negative regulatory genes such as *OsRR22*, *OsHHLH024*, and *OsNAC113*. Multiplex editing strategies further bypass functional redundancy and engineer broad-spectrum resilience by targeting intersecting metabolic and signaling networks. The commercial release of varieties such as DRR Rice 100 (Kamala) and Pusa DST Rice 1 marks a major milestone, though it highlights persistent socio-political and cultural friction. Addressing technical bottlenecks such as genotype-dependent transformation recalcitrance and field-level epigenetic drift remains essential to fully realize the translational potential of these biotechnological innovations.

Keywords: CRISPR-Cas9, genome-wide association studies (GWAS), *Oryza sativa*, salinity stress, climate-resilient breeding, multiplex editing, transcriptomics.

1. Introduction

1.1 The Global Threat of Soil Salinization

Soil salinization has emerged as a critical threat to global agricultural sustainability, directly undermining crop productivity and food security in coastal and arid regions alike. Globally, over 800 million hectares of arable land, representing approximately 6% of the world's total cropping area, suffer from varying degrees of salinity (Wu et al., 2015). This crisis is exacerbated by the progressive impacts of climate change, including rising sea levels that drive seawater intrusion into low-lying freshwater agricultural zones, and erratic precipitation patterns that force farmers to irrigate fields with poor-quality, saline, or alkaline groundwater (Kumar & Sharma, 2020).

1.2 Vulnerability of Rice (*Oryza sativa* L.)

Rice (*Oryza sativa* L.), which serves as the primary dietary staple for more than half of the global population, is classified as a salt-sensitive crop. Salinity stress is estimated to cause annual yield reductions ranging from 30% to 50% in rice-producing regions, with severe salinization events occasionally leading to complete crop failure (Nguyen, 2009).

1.3 The Multi-Tiered Physiological Onslaught of Salinity

Salinity stress subjects rice plants to a complex, multi-tiered physiological onslaught:

- **Osmotic Constraints:** The stress begins with osmotic constraints, where high salt concentration in the soil lowers the osmotic potential of the soil solution, thereby inhibiting water uptake by roots and inducing physiological drought

(Hoang et al., 2016).

- **Ionic Toxicity:** This is quickly followed by ionic toxicity, which is primarily driven by the excessive intracellular accumulation of sodium (Na^+) and chloride (Cl^-) ions. Elevated intracellular Na^+ levels disrupt enzyme function, inhibit protein synthesis, and interfere with potassium (K^+) uptake, leading to cellular dehydration and metabolic arrest (Hasanuzzaman et al., 2021).
- **Secondary Oxidative Stress:** These primary stresses trigger secondary oxidative stress through the rapid accumulation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide radicals ($\text{O}_2^{\bullet-}$), hydroxyl radicals ($\text{OH}^\bullet$), and singlet oxygen ($^1\text{O}_2$). Excess ROS induces extensive lipid peroxidation of cellular membranes commonly measured via malondialdehyde (MDA) accumulation thereby disrupting membrane integrity, causing electrolyte leakage, and ultimately triggering programmed cell death (AbdElgawad et al., 2016).

1.4 Distinguishing Salinity Stress from Alkaline Stress

It is critical to distinguish salinity stress from alkaline stress, although they frequently coexist in saline-alkaline soil complexes.

Salinity stress is primarily driven by neutral salts such as NaCl and Na_2SO_4 , resulting in ion toxicity and osmotic imbalance.

Alkaline stress is characterized by the presence of bicarbonate (NaHCO_3) and carbonate (Na_2CO_3) salts, which significantly raise soil pH, often above 9.0 (Kumar et al., 2024). This elevated pH severely restricts nutrient availability, causing phosphorus (P) to precipitate as insoluble calcium phosphate complexes and reducing iron (Fe) solubility, which leads to iron chlorosis symptoms (Pérez-Labrada et al., 2024).

Consequently, the genetic and transporter mechanisms required to combat these stresses differ: salinity tolerance relies heavily on Na^+ exclusion, root-to-shoot partitioning, and vacuolar sequestration, whereas alkaline tolerance requires specialized iron and phosphate acquisition pathways. For instance, the basic helix-loop-helix (bHLH) transcription factor OsIRO3 has been characterized as a critical negative regulator of the iron-deficiency response under alkaline stress (Li & Yang, 2023).

1.5 The Modern Breeding Paradigm Shift

To mitigate these losses, classical forward breeding approaches have historically relied on phenotypic screening and the mapping of quantitative trait loci (QTLs) using biparental linkage populations. However, the success of conventional breeding is limited by narrow genetic bases and the high cost of multi-generation backcrossing (Kumar & Sharma, 2020).

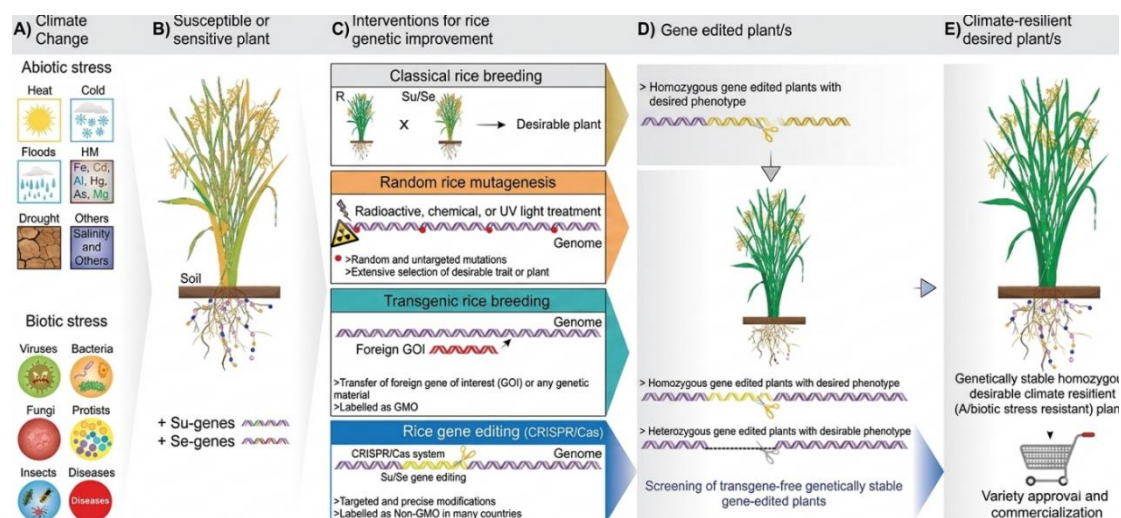
The transition to a highly integrated, climate-adaptive breeding pipeline combines the discovery of favorable natural alleles from diverse germplasm panels using Genome-Wide Association Studies (GWAS) with precise reverse-genetics validation and trait integration using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 gene editing (Sackey, 2025).

2. Genome-Wide Association Mapping Across Developmental Stages

2.1 Polygenic and Stage-Specific Nature of Salt Tolerance

The genetic architecture governing salinity tolerance in rice is highly complex, polygenic, and stage-specific, meaning that tolerance mechanisms at one growth stage do not necessarily correlate with those at other developmental phases. Consequently, molecular mapping must be performed systematically across the crop's life cycle, focusing on the germination, seedling, and reproductive stages (Akhlasur Rahman et al., 2021).

Figure 1: Comparative pathways of genetic improvement in rice: From classical breeding and mutagenesis to CRISPR/Cas-mediated gene editing for climate resilience.



2.2 Germination Stage Mapping

At the seed germination stage, high salinity impedes water imbibition, thereby delaying germination or reducing overall germination percentage, which significantly decreases plant stand density and final yield in direct-seeded rice systems. Shi et al. performed a germination-stage genome-wide association study (GWAS) utilizing approximately 6 million single nucleotide polymorphisms (SNPs), identifying 11 loci containing 22 significant SNPs associated with stress susceptibility indices for germination time and vigor index. The strongest association peak was localized on chromosome 1, close to a known seedling-stage Na^+ uptake quantitative trait locus (QTL) (Haque et al., 2021). In a subsequent germination-stage GWAS, researchers re-sequenced a collection of 295 rice accessions (comprising a heuristic core set of 137 accessions and 158 bred varieties) to generate 1.65 million SNPs and indels. This study characterized 17 candidate genes, highlighting *OsMADS31*, a MADS-box family transcription factor, which exhibited significant downregulation under salt stress (Yu et al., 2018).

Crucially, principal component analysis (PCA) based on germination phenotypes showed no clear clustering of accessions along subspecific *indica* and *japonica* lines, despite genotypic PCA separating them cleanly. This indicates that germination-stage salt tolerance is not strongly correlated with traditional rice subgroups, presenting a contrast to seedling-stage tolerance where *indica* cultivars are generally more tolerant than *japonica* (Huang et al., 2024).

2.3 Seedling Stage Mapping

At the early vegetative or seedling stage, young plants are highly vulnerable to salt-induced ionic toxicity. Using more than 33,000 SNP markers on a diverse rice panel, a seedling-stage GWAS evaluated eight primary morphological traits under stress, including shoot length (SL), root length (RL), root dry weight (RDW), root fresh weight (RFW), shoot fresh weight (SFW), shoot dry weight (SDW), relative water content (RWC), and turgor weight (TW), alongside four derived relative traits (SL-R, RL-R, RDW-R, and RFW-R) (Chen et al., 2020).

2.4 Reproductive Stage Mapping

Reproductive-stage salinity stress directly impacts spikelet fertility, pollen viability, and grain filling, resulting in severe yield losses. To dissect this stage, a genome-wide association study (GWAS) was performed using a mixed linear model on reproductive traits, including the salt susceptibility index of net photosynthetic rate, panicle number,

and the ratio of filled to unfilled grains per plant (Yadav et al., 2021). This analysis identified 200 loci containing 448 exonic SNPs, with approximately 73% of the loci co-localizing with previously documented vegetative salt-stress QTLs. The highest concentration of significant reproductive-stage SNPs was mapped to chromosomes 8, 12, 1, and 2 (Lekklar et al., 2019). Furthermore, a reproductive-stage GWAS utilizing 6,000 SNPs across 220 accessions identified 20 loci associated with the shoot Na^+/K^+ ratio and 44 loci linked to other yield-determination traits. Notably, 12 of the associations with the Na^+/K^+ ratio mapped to chromosome 1 within the *Saltol* QTL, demonstrating that seedling-stage ion transport mechanisms remain active and critical through the reproductive stage (Kumar et al., 2015).

3. Genetic Diversity, Multi-Parent Populations, and Transcriptome Integration

3.1 Overcoming Population Structure Confounders

While genome-wide association studies (GWAS) using natural germplasm panels leverage historical recombination to achieve high mapping resolution, they can suffer from confounding population structure and a high false discovery rate. To circumvent these limitations, researchers utilize multi-parent populations, such as multi-parent advanced generation intercross (MAGIC) lines, and integrate association mapping with linkage analysis and transcriptomic profiling (RNA-seq) to accelerate gene discovery.

3.2 Quantitative Trait Loci (QTL) Discovery via MAGIC and Wild Populations

A seedling-stage GWAS using a *japonica* MAGIC population identified four major QTLs (*qDTS1-1*, *qDTS1-2*, *qDTS2*, and *qDTS9*) associated with salt tolerance. In another study, Zhang et al. mapped seven QTLs on chromosomes 1, 2, 5, and 9 using a MAGIC population, explaining 7.42% to 9.38% of the total phenotypic variation (Padmavathi et al., 2024). Similarly, researchers mapped 16 QTLs derived from wild rice (*Oryza rufipogon*) on chromosomes 1, 5, 7, and 9–12, explaining 2% to 8% of the phenotypic variation.

At the tillering stage, association mapping on rice exposed to short-term (6 h), medium-term (7 d), and long-term (30 d) salinity identified approximately 1,200 candidate genes associated with vegetative growth parameters and ion accumulation (Ju et al., 2022). Physiologically, these studies established that the visual salt injury score is negatively correlated with Na^+ and K^+ concentrations in the roots, but positively correlated with the Na^+/K^+ ratio in the shoots (De Leon et al., 2017).

3.3 Classic Salinity Loci: *Saltol* and *SKC1*

Historically, the major *Saltol* QTL, which regulates shoot Na^+/K^+ homeostasis, was mapped on chromosome 1 using recombinant inbred lines (RILs) derived from a cross between the salt-tolerant *indica* cultivar Pokkali and the sensitive cultivar IR29 (Thomson et al., 2010). Similarly, crossing the highly tolerant *indica* cultivar Nona Bokra with the sensitive *japonica* cultivar Koshihikari led to the mapping of the essential QTL *SKC1* (shoot K^+ content).

SKC1 encodes *OsHKT1;5*, a high-affinity, Na^+ -selective transporter that facilitates the exclusion of toxic Na^+ from the xylem of roots and shoots, protecting photosynthetic tissues. This transporter is regulated downstream by *OsRR22*, which coordinates the transcription of several salt-responsive genes (Sackey, 2025).

3.4 Multi-Omics Intersection: GWAS, Linkage Mapping, and RNA-seq

To identify the causal genes within large mapped intervals, researchers combine GWAS with RIL linkage analysis and RNA-seq. For example, a seedling-stage study utilized a panel of 295 *japonica* accessions (analyzed with 788,396 SNPs) and a RIL population derived from a cross between CD (salt-sensitive) and WD20342 (salt-tolerant) (Vitiello, 2026). GWAS mapped 15 lead SNPs linked to relative root length (RRL), relative root number (RRN), relative root fresh weight (RRFW), and relative root dry weight

(RRDW). Haplotype, qRT-PCR, and sequence analysis of this interval identified *LOC_Os02g36880* as the causal gene, which acts as a key negative regulator of seedling-stage salinity tolerance (Xu et al., 2024).

Integrating GWAS with transcriptomics has proved highly effective. RNA-seq of contrasting tolerant (PA64s) and sensitive (93-11) cultivars under stress identified 517 co-upregulated and 223 co-downregulated differentially expressed genes (DEGs), primarily enriched in responses to chemical and endogenous stimuli. Intersecting 275 GWAS-identified candidate genes with these DEGs narrowed the causal pool to 30 candidate genes (Lv et al., 2022).

This subset included 13 cloned genes with no prior reported link to salinity tolerance and 17 genes of completely unknown function. Among these, *OsHKT1;1* (*LOC_Os04g51820*) was co-detected in both the global and *indica*-specific panels. *OsHKT1;1* acts to reduce Na⁺ accumulation in shoots under stress and is transcriptionally activated by the OsMYBc transcription factor (Ben Amar et al., 2013). Haplotype analysis of *OsHKT1;1* revealed four main haplotypes:

- **Hap.1:** Exclusively present in *indica*.
- **Hap.2:** Present in both *indica* and *japonica*; accessions carrying this haplotype (e.g., Menjiading and Haobayong1) display a higher visual tolerance score.
- **Hap.3 and Hap.4:** Predominantly restricted to *japonica*.

Similarly, the transcription factor *OMTN3* (*LOC_Os12g41680*, located at candidate interval Chr12_25666325) was identified as a key candidate under strong evolutionary selection during the domestication of *Oryza rufipogon* into *indica* and *japonica* (Shohan et al., 2019).

Table 1: Summary of Key Salinity Tolerance Mapping Intervals and Candidate Genes

Mapping Locus / Interval	Chromosome	Associated Traits	Primary Mapping Method	Identified Candidate Gene	Gene Function / Mechanism	Source
Chr1_36063662	Chromosome 1	Seedling SES score	Natural GWAS (191 lines)	<i>ZFP179</i> and <i>OsDSR2</i>	zinc-finger protein and DUF966 stress-repressive protein	Haque et al., 2021
11.26 Mbp (<i>SalTol1</i>)	Chromosome 1	SL, RL, RDW, RFW, SDW	GWAS (33K SNPs, MLM)	<i>Os01g0304100</i>	Cation chloride cotransporter	Chen et al., 2020
22.241–22.437 Mb	Chromosome 2	RRL, RRN, RRFW, RRDW	GWAS and CD × WD20342 RIL population	<i>LOC_Os02g36880</i>	Negative regulator of seedling salinity tolerance	Xu et al., 2024
Chr4_5182	Chromosome	Na ⁺ .	GWAS	<i>OsHKT1;1</i>	High-affinity	Lv et

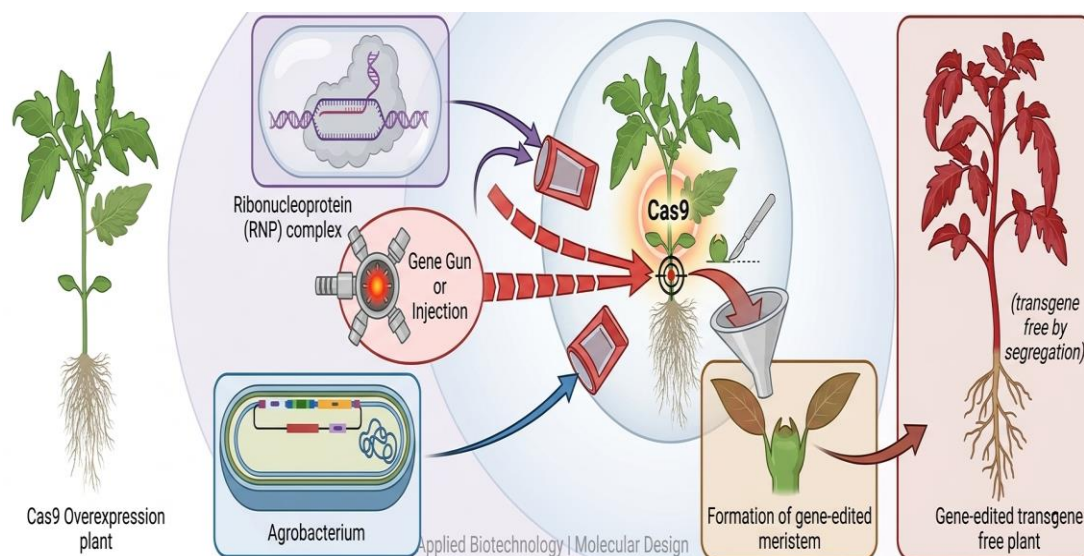
0	ome 4	exclusio n	and NA- intersec tion		Na ⁺ xylem loading/unlo ading transporter	al., 2022
Chr5	Chromos ome 5	N+. sheath- to-blade ratio	GWAS (296 accessio ns)	Sheath candidate group	Thirteen sheath- specific DEGs (5 upregulated and 8 downregulat ed)	Ju et al., 2022
Chr12_256 66325	Chromos ome 12	Endoge nous stimulus respons e	GWAS and DEG intersec tion	<i>OMTN3</i>	No Apical Meristem (NAM) transcription factor under selection	Shoh an et al., 2019

4. CRISPR-Cas9 Functional Validation of Negative Regulatory Genes

4.1 Knocking Out Negative Regulators vs. Overexpression Penalties

The rapid characterization of candidate genes mapped via GWAS has been transformed by CRISPR-Cas9 genome editing. Traditional transgenic approaches overexpressing positive regulators (such as *P5CS* for proline accumulation, *SKC1* or *OsNHX1* for ion sequestration, and *OsHAK1* for potassium transport) often impose severe energetic and pleiotropic penalties on plants under non-stressed conditions. Consequently, modern molecular breeding focuses on the targeted knockout of genes that negatively regulate salinity tolerance (Li et al., 2022).

Figure :1 Agrobacterium and RNP-Mediated Transgene-Free Gene Editing in Cas9-Expressing Plants



4.2 Targeted Mutation of *OsRR22*

The response regulator gene *OsRR22* (also known as *HST1*, located on chromosome 6) is a key target for CRISPR-mediated knockout. Originally identified via MutMap from a population of 6,000 ethyl methanesulfonate (EMS) mutants, *OsRR22* encodes a 696-amino acid B-type response regulator transcription factor that acts in cytokinin signal transduction and metabolism. Under saline stress, the wild-type *OsRR22* protein suppresses downstream stress-tolerance signaling pathways (Wang et al., 2019). Zygosity and inheritance analyses showed that the mutations were transmitted stably to the T1 and T2 generations, allowing researchers to isolate transgene-free, homozygous *osrr22* mutant lines. Under salinity stress, these mutant lines showed significantly enhanced seedling survival without displaying any fitness penalties or changes in plant height, tiller number, grain count, or final yield under normal conditions (Razzaq et al., 2021).

Table 2: Agronomic Performance of Wild-Type and *OsRR22*-Edited Lines Under Non-Stressed Field Conditions

Agronomic Parameter (Normal Conditions)	Wild-Type (WPB106)	Edited Line (WPB106-cas-1)	Edited Line (WPB106-cas-2)	Source
Days to 50% Flowering	.87.81 $\pm$ 0.70.	.87.01 $\pm$ 1.19.	.86.51 $\pm$ 0.71.	Razzaq et al., 2021
Plant Height (cm)	.92.82 $\pm$ 3.91.	.95.01 $\pm$ 2.23.	.93.03 $\pm$ 2.86.	Razzaq et al., 2021
Number of Tillers per Plant	.8.49 $\pm$ 0.25.	.8.43 $\pm$ 0.21.	.8.38 $\pm$ 0.23.	Razzaq et al., 2021
Number of Grains per Panicle	.123.51 $\pm$ 3.23.	.124.01 $\pm$ 4.46.	.123.52 $\pm$ 3.05.	Razzaq et al., 2021
Spikelet Fertility (%)	.93.11 $\pm$ 3.09.	.96.45 $\pm$ 1.98.	.94.29 $\pm$ 2.58.	Razzaq et al., 2021
Thousand-Grain Weight (g)	.25.15 $\pm$ 0.79.	.25.05 $\pm$ 0.94.	.25.29 $\pm$ 0.61.	Razzaq et al., 2021
Yield per Plant (g)	.21.19 $\pm$ 0.57.	.20.72 $\pm$ 0.77.	.20.82 $\pm$ 0.64.	Razzaq et al., 2021

4.3 Integration with Heterosis: Third-Generation Hybrid Rice Systems

To integrate this trait with heterosis, CRISPR-Cas9 was also applied to elite *indica* hybrid parental lines to target the third exon of *OsRR22*. This editing generated:

- A genic male-sterile (GMS) line (733Srr22-T1447-1) containing a 110-bp homozygous deletion.
- An elite restorer line (HZrr22-T1349-3) containing a 1-bp homozygous deletion.

Both mutations caused reading frame shifts that produced truncated, non-functional *OsRR22* proteins. Crossing these lines within a "third-generation hybrid rice system" yielded a highly tolerant hybrid (ST1) (Zhao et al., 2022). Under normal field conditions, the homozygous T4 mutant lines exhibited growth and yield parameters identical to their wild-type parents. However, under severe salinity stress, the edited lines exhibited robust physical survival and biomass retention compared to the completely dead parent lines (Haque et al., 2021).

Table 3: Seedling Physiological Performance of Restorer Lines and Edited Mutants Under Salinity Stress

Seedling Parameter (T3)	Restorer Line Parent (HZ-	Mutant Line (HZrr22-	Mutant Line (HZrr22-	Source
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under Salt Stress)	WT)	T1349-3-T3)	T1347-6-T3)	
Seedling Length (cm)	Complete Lethality	.29.53 \pm 0.92.	.24.63 \pm 1.33.	Zhao et al., 2022
Root Length (cm)	Complete Lethality	.10.40 \pm 1.22.	.9.47 \pm 0.90.	Zhao et al., 2022
Fresh Weight (g)	Complete Lethality	.0.73 \pm 0.07.	.0.46 \pm 0.06.	Zhao et al., 2022
Dry Weight (mg)	Complete Lethality	.185.00 \pm 10.82.	.86.00 \pm 8.00.	Zhao et al., 2022
Survival Ratio (%)	.0.00 \pm 0.00.	.61.48 \pm 2.79.	.47.69 \pm 2.28.	Zhao et al., 2022

4.4 Validation of Other Prominent Negative Regulators

Beyond OsRR22, several other negative regulators have been validated using CRISPR-Cas9:

- **OsbHLH024:** Knocking out this bHLH transcription factor generated the A91 mutant line (harboring a 1 bp deletion in target 1, causing a premature stop codon). Under salt stress, the A91 line exhibited increased expression of structural ion transporters, including OsHKT1;3, OsHAK7, and the plasma membrane Na⁺/H⁺ exchanger OsSOS1, which reduced intracellular Na⁺ toxicity (Alam et al., 2022).
- **OsNF-YC5:** Knocking out this nuclear factor gene enhanced salinity tolerance and abscisic acid (ABA) sensitivity. This edit improved the activity of catalase (CAT) enzymes and fine-tuned downstream ABA-responsive genes (Yan et al., 2024).
- **OsNAC113:** Knocking out this stress regulator altered downstream signaling and metabolic pathways, allowing edited plants to maintain higher water content and membrane stability in saline soils.
- **LOC_Os02g36880:** Knocking out this seedling-stage negative regulator produced mutant lines (CR-1 and CR-15) with lower salinity toxicity scores and improved survival (Xu et al., 2024).

5. Multiplex Editing, Intersecting Networks, and Broad-Spectrum Resilience

5.1 Circumventing Genetic Redundancy

Because salinity tolerance is a complex polygenic trait, single-gene editing strategies can be limited by functional redundancy and compensatory upregulation of paralogous genes. For example, knocking out *OsHKT1;5* can trigger compensatory transcription of *OsHKT1;4* or other HKT family members to limit Na⁺ transport (Wang et al., 2025). To resolve this, investigators edited targets in six distinct backgrounds across both *indica* (*xian*) and *japonica* (*geng*) germplasms to generate two-gene mutant combinations (*hst1-lrrk1*, *hst1-strk2*, and *hst1-pcl*) (Cui et al., 2022). All three double-knockout combinations significantly outperformed single-gene knockout lines in salinity tolerance across both backgrounds.

5.2 Evaluating Epigenetic and Background Effects

However, this study revealed a significant genetic background effect:

- In the Chun199S (C199S) sterile line, *hst1-pcl* showed excellent salinity tolerance, matching the other combinations.
- In the Changhui 70 (CH70) and 8TX23 restorer lines, the *hst1-pcl* combination showed much lower tolerance and a reduced seed-setting rate.
- The *hst1-strk2* combination was identified as the most stable across all six backgrounds, yielding the highest seedling survival and maintaining grain yield

under normal conditions (Ding et al., 2022).

5.3 Engineering Cross-Tolerance: Abiotic and Biotic Integration

Multiplex editing can also be used to engineer broad-spectrum disease resistance alongside abiotic stress tolerance. By simultaneously knocking out four negative regulators *OsMads26*, *OsBsr-d1*, *OsELF3-2*, and *OsERF922* investigators generated quadruple knockout mutants in the *japonica* variety ZG616. These quadruple mutants showed enhanced resistance to both rice blast (*Magnaporthe oryzae*) and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*), while also displaying improved drought and salinity tolerance (Sen et al., 2024).

6. Commercial Deployments and the Indian CRISPR Revolution of 2025

6.1 Policy and Financial Architecture

The translation of molecular research into commercial, field-ready agriculture reached a major milestone on May 4, 2025. India's Union Minister for Agriculture launched the country's first two genome-edited, climate-resilient rice varieties: **DRR Rice 100 (Kamala)** and **Pusa DST Rice 1**. This development was supported by an INR 500 crore allocation in the Indian national budget specifically for genome editing in agriculture, and was regulated under the relaxed 2022 biosafety guidelines for transgene-free SDN-1 and SDN-2 crops (Kumawat, 2025).

6.2 DRR Rice 100 (Kamala): Cytokinin-Driven Productivity Enhancements

DRR Rice 100 (Kamala) was developed by the ICAR-Indian Institute of Rice Research (IIRR) in Hyderabad, using the premium, high-yielding *indica* cultivar Samba Mahsuri (BPT 5204) as the recipient background (Kumar, 2026). Researchers used CRISPR-Cas9 to edit the cytokinin oxidase gene (*OsCKX2*), creating a novel loss-of-function allele. By knocking out this gene, the plant accumulated higher levels of cytokinin in its inflorescence meristems, which increased reproductive organ branching and grain number per panicle (Pasion et al., 2021).

This edit resulted in a 19% to 25% increase in grain yield, while also shortening the crop's maturity cycle by up to 20 days (approximately 130 days total duration). The shorter growth cycle reduces seasonal irrigation water and fertilizer demands, while lowering methane emissions from flooded paddies. Agronomically, the variety features a rigid, lodging-resistant stalk, with grain quality identical to the original Samba Mahsuri (Ahmed, 2021).

6.3 Pusa DST Rice 1: Stomatal Regulation Under Abiotic Extremes

Pusa DST Rice 1 was developed by the ICAR-Indian Agricultural Research Institute (IARI) in New Delhi, using the widely cultivated *indica* variety MTU 1010 as the genetic background (Yadav et al., 2023). Scientists targeted the drought and salt tolerance (*DST*) gene, which acts as a negative regulator of stress tolerance by maintaining open stomata under water deficit. By knocking out *DST*, the edited plants exhibited reduced stomatal density and enhanced stomatal closure, minimizing transpirational water loss and improving survival in dry and saline soils (Kaur et al., 2022).

This variety can boost grain yields by 9.66% to 30.4% in highly saline and alkaline soils, and features improved tillering and grain quality. It is recommended for rainfed, salt-affected agricultural zones in Eastern India, including Bihar, Jharkhand, and Eastern Uttar Pradesh (Chhabra, 2022).

Table 4: Technical and Agronomic Specifications of Commercialized Genome-Edited Rice Varieties

Agricultural Parameter	DRR Rice 100 (Kamala)	Pusa DST Rice 1
Recipient	Samba Mahsuri (BPT 5204)	MTU 1010 / Pusa 44

Genetic Background		
Gene Target & Mechanism	<i>OsCKX2</i> (Cytokinin Oxidase) knockout	<i>DST</i> (Zinc-Finger Stress Suppressor) knockout
Yield Advantage	19%–25% increase	9.66%–30.4% increase in saline soils
Maturity Reduction	Approximately 20 days earlier (~130 days total duration)	Early maturing; suitable for rapid crop rotation
Primary Abiotic Tolerance	Drought and low-fertilizer tolerance	Salinity, alkalinity, and severe drought tolerance
Key Agronomic Features	Strong, lodging-resistant stalks; lower methane emissions	Reduced stomatal density, increased tillering, and high survival rate
Target Growing Regions	Irrigated regions of Southern India (Andhra Pradesh, Telangana, Tamil Nadu, Karnataka)	Rainfed regions of Eastern India (Bihar, Jharkhand, Eastern Uttar Pradesh)

7. Socio-Political Controversies, Cultural Friction, and Economic Paradoxes

7.1 Off-Target Mutations and Ecological Gene Flow Concerns

The commercial release of DRR Rice 100 (Kamala) and Pusa DST Rice 1 triggered significant debate in India, highlighting the friction between biotechnology and traditional agricultural values. On May 5, 2025, the Coalition for a GM-Free India demanded the immediate withdrawal of both varieties, raising biological, ecological, and socio-economic concerns (Wang et al., 2019).

Biologically, opponents argue that SDN-1 gene editing can produce off-target mutations during the cellular repair of double-strand breaks. These unintended edits could lead to structural changes in proteins, potentially generating novel allergens or toxins that could affect consumers or enter the food chain (Shohan et al., 2019). From an ecological perspective, critics warn of gene flow. Because cultivated rice can outcross with wild rice (*Oryza rufipogon*), the unmonitored spread of engineered traits could alter the evolutionary fitness of wild populations, potentially leading to the loss of pure, native germplasm (Li et al., 2022).

7.2 Cultural Resistance: The Concept of *Akshat* vs. Molecular Deletions

Furthermore, the release ran into unique cultural opposition related to the role of rice in Indian heritage. In many Indian communities, rice is used as *akshat* in religious and cultural rituals (Yang et al., 2023). The Sanskrit word *akshat* translates to "unbroken, whole, and undamaged" (Razzaq et al., 2021).

Opponents argued that deleting 366 base pairs to create Pusa DST Rice 1 and 33 base pairs to develop DRR Rice 100 structurally "broke" the genetic integrity of the crop. This molecular alteration was viewed as a violation of the grain's traditional purity, demonstrating how modern crop biotechnology can clash with deeply rooted cultural and sacred beliefs (Sackey, 2025).

7.3 Macroeconomic Grain Surpluses and Storage Logistics

Socio-economically, critics questioned the government's claim that higher-yielding, salt-tolerant rice would allow farmers to reduce their rice acreage and diversify into pulses and oilseeds. As of April 1, 2025, India's public rice stocks totaled a record 63.09 million tonnes, far exceeding the government's buffer target of 13.6 million tonnes (Nguyen, 2009). Under conditions of structural grain surplus, critics argued that introducing yield-enhancing varieties without addressing pricing support or grain storage logistics could depress market prices, reducing farm income despite improved

crop resilience (Wu et al., 2015).

8. Conclusion and Future Directions

The transition from classical forward breeding to an integrated molecular pipeline combining GWAS mapping, transcriptomics, and CRISPR-Cas9 reverse genetics represents a paradigm shift in engineering climate-resilient rice. Dissecting the stage-specific, polygenic architecture of salinity tolerance has revealed key candidate loci and transporters such as the *SalTol1* locus and the HKT transporter family while successfully distinguishing it from the independent physiological mechanisms required for alkaline stress tolerance. Functional validation has shifted productively toward the targeted knockout of negative regulatory genes and feedback loops, avoiding the severe energetic and vegetative fitness penalties historically associated with constitutive overexpression.

The commercial deployment of pioneering genome-edited varieties such as DRR Rice 100 (Kamala) and Pusa DST Rice 1 demonstrates the immense potential of biotechnology to secure food production under worsening environmental constraints. However, the socio-political debates surrounding genetic integrity, public grain surpluses, and international regulatory divergence underscore that biological success must be paired with nuanced market logistics and cultural awareness.

Moving forward, overcoming key technical bottlenecks specifically genotype-dependent transformation recalcitrance in elite *indica* cultivars and unpredictable gene-by-environment (G $\times$ E) interactions in variable field conditions is imperative. Future research focusing on stress-inducible promoters, single-cell transcriptomics, and precise base or prime editing promises to fine-tune endogenous networks, ultimately delivering stable, high-yielding, and broad-spectrum resilient crops to meet global demand.

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