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Research Article

CRISPR/CAS9-MEDIATED GENETIC ENGINEERING FOR DEVELOPING SALINITY-TOLERANT RICE VARIETIES FOR INDONESIAN COASTAL AGRICULTURE

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Abstract

Salinity intrusion in coastal agricultural areas has become a major constraint to rice production in Indonesia, driven by climate change, sea-level rise, and unsustainable land management practices. High soil salinity adversely affects rice growth, yield stability, and food security, particularly in coastal regions that depend heavily on rice cultivation. Conventional breeding approaches for developing salinity-tolerant rice varieties are often time-consuming and limited by genetic complexity. Advances in genome editing technologies, particularly CRISPR/Cas9, offer a precise and efficient alternative for accelerating crop improvement. The objective of this study is to develop salinity-tolerant rice varieties suitable for Indonesian coastal agriculture through CRISPR/Cas9-mediated genetic engineering targeting key genes associated with salt stress tolerance. This research employed an experimental laboratory-based design combined with controlled greenhouse evaluation. Specific salinity-responsive genes were identified and edited using the CRISPR/Cas9 system. Transgenic rice lines were generated and screened for successful gene edits using molecular analysis techniques. Edited lines were subsequently evaluated under saline and non-saline conditions to assess physiological responses, growth performance, and yield-related traits. The results demonstrate that CRISPR/Cas9-edited rice lines exhibited enhanced tolerance to saline stress, indicated by improved germination rates, higher chlorophyll content, better ion homeostasis, and increased biomass compared to non-edited controls. Several edited lines maintained stable growth and yield under moderate to high salinity levels, confirming the effectiveness of targeted gene modification. In conclusion, CRISPR/Cas9-mediated genetic engineering shows strong potential for developing salinity-tolerant rice varieties tailored to Indonesian coastal environments. This approach provides a rapid and precise strategy to enhance rice resilience, support sustainable coastal agriculture, and strengthen national food security under changing climatic conditions.

Keywords: CRISPR/Cas9, Coastal Agriculture, Genetic Engineering, Rice Varieties, Salinity Tolerance

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INTRODUCTION

Salinity stress is widely recognized as one of the most serious abiotic constraints affecting rice production worldwide (Shukla et al., 2025). Excessive salt accumulation in soil disrupts water uptake, ion balance, and metabolic processes in rice plants, leading to reduced growth, delayed development, and significant yield losses (Osei-Wusu et al., 2025). In coastal agricultural systems, salinity stress is particularly severe due to seawater intrusion, tidal flooding, and rising sea levels associated with climate change (Q. Han et al., 2025). Indonesia, as a major rice-producing country, faces increasing challenges in maintaining rice productivity in coastal regions (Simarmata et al., 2025). Large areas of fertile coastal land have gradually become marginal due to soil salinization, threatening local livelihoods and national food security (Maung et al., 2025). Rice farming communities in these areas often experience declining yields and limited varietal options that can adapt to saline conditions.

Conventional rice breeding has contributed to the development of stress-tolerant varieties through hybridization and selection (Bora et al., 2025). Although these approaches have produced some salt-tolerant cultivars, the breeding process is inherently slow and constrained by the complex inheritance of salinity tolerance traits (Sharma et al., 2025). Multiple genes and regulatory pathways are involved, making it difficult to achieve stable and high-level tolerance through traditional methods alone. Advances in plant molecular biology have improved understanding of the genetic and physiological mechanisms underlying salinity tolerance (Nawaz et al., 2025). Genes involved in ion transport, osmotic regulation, antioxidant defense, and stress signaling have been identified as key contributors to salt stress adaptation in rice. This growing body of knowledge provides a strong foundation for targeted genetic interventions.

Genome editing technologies have emerged as transformative tools in modern crop improvement. Among these, the CRISPR/Cas9 system offers high precision, efficiency, and flexibility in modifying specific genomic sequences (Sinha et al., 2025). Unlike transgenic approaches that introduce foreign DNA, CRISPR/Cas9 enables targeted gene modification with minimal unintended effects (Gill et al., 2026). Previous studies in various crops have demonstrated the effectiveness of CRISPR/Cas9 in enhancing tolerance to abiotic stresses, including drought, salinity, and heat (Pandit et al., 2025). Edited plants often show improved physiological performance and stress resilience without compromising yield potential, highlighting the promise of genome editing for sustainable agriculture.

Despite global progress in CRISPR/Cas9-based crop improvement, its application in developing salinity-tolerant rice varieties specifically adapted to Indonesian coastal environments remains limited (Rajput et al., 2026). Most existing studies focus on model varieties or non-coastal contexts, leaving uncertainty regarding field relevance in Indonesian agroecological conditions (Hurrah et al., 2025). Limited information is available on which salinity-responsive genes are most effective targets for genome editing in locally adapted rice varieties (J.-H. Han et al., 2025). Genetic responses to salt stress may vary across cultivars and environments, raising questions about the transferability of findings from other regions to Indonesian coastal agriculture.

The physiological and agronomic performance of CRISPR/Cas9-edited rice lines under sustained saline conditions has not been sufficiently explored (Khan et al., 2025). Many studies emphasize molecular validation without comprehensive assessment of growth stability, yield performance, and stress tolerance across different salinity levels (C. Xu et al., 2025). Socioagronomic considerations, including acceptance of genome-edited crops and their potential contribution to sustainable coastal farming systems, are also underexamined (Selwal et al., 2025). Lack of locally grounded evidence may hinder policy development and farmer adoption of genome editing technologies.

Addressing these gaps is essential to strengthen the resilience of rice production in Indonesian coastal regions (Chaudhary et al., 2025). Developing salinity-tolerant rice varieties through precise genome editing can provide practical solutions for farmers facing increasing soil salinization and climate-related risks (Yuan et al., 2025). This study aims to apply CRISPR/Cas9-mediated genetic engineering to modify key salinity-responsive genes in rice, followed by systematic evaluation of physiological performance and growth under saline conditions relevant to Indonesian coastal agriculture (Norman et al., 2026). The research emphasizes functional validation rather than molecular modification alone.

The underlying hypothesis of this study is that targeted CRISPR/Cas9 gene editing can significantly enhance salinity tolerance in rice without negatively affecting growth and yield traits (Li & Li, 2025). Successful implementation of this approach is expected to contribute to sustainable coastal agriculture, technological innovation in crop breeding, and long-term national food security.

RESEARCH METHOD

Research Design

This study employed an experimental laboratory-based research design combined with controlled greenhouse evaluation to develop and assess salinity-tolerant rice varieties using CRISPR/Cas9-mediated genetic engineering (Krishna Samal & Sukla, 2025). The design focused on targeted genome editing, molecular validation of edited lines, and physiological and agronomic performance assessment under varying salinity conditions. Quantitative analysis was applied to compare edited and non-edited rice lines, while descriptive evaluation supported interpretation of stress tolerance responses.

Research Target/Subject

The research population consisted of rice genotypes commonly cultivated in Indonesian coastal agricultural areas. Samples were selected purposively based on their agronomic relevance, adaptability to coastal environments, and susceptibility to salinity stress. Multiple CRISPR/Cas9-edited rice lines were generated from selected parental varieties, alongside non-edited wild-type lines used as controls for comparative analysis.

Research Procedure

Gene target selection was conducted based on literature review and bioinformatics analysis of salinity-associated genes. CRISPR/Cas9 constructs were designed and introduced into rice cells using established transformation techniques. Regenerated plants were screened to confirm successful gene edits through molecular analysis (Tiozon et al., 2025). Edited lines were subsequently grown under controlled greenhouse conditions with varying salinity treatments. Growth performance, physiological responses, and yield indicators were systematically recorded and compared with control plants to evaluate salinity tolerance and agronomic stability.

Instruments, and Data Collection Techniques

The primary instruments included CRISPR/Cas9 gene-editing constructs designed to target salinity-responsive genes, tissue culture facilities for plant regeneration, and molecular biology tools for genotyping and mutation verification (Rohilla et al., 2025). Supporting instruments included polymerase chain reaction systems, DNA sequencing platforms, and greenhouse facilities equipped to simulate saline and non-saline growth conditions. Physiological measurement tools were used to assess chlorophyll content, ion concentration, biomass accumulation, and yield-related traits.

Data Analysis Technique

Data analysis was performed using both quantitative and descriptive methods. Quantitative analysis involved statistical comparisons between CRISPR/Cas9-edited rice lines and wild-type controls, utilizing analysis of variance (ANOVA) to assess differences in physiological responses, such as chlorophyll content, ion concentration, and biomass accumulation, under varying salinity conditions (Z. Xu et al., 2025). Agronomic traits, including plant height, yield, and stress tolerance markers, were evaluated using paired t-tests or ANOVA, depending on the data structure. Descriptive statistics were applied to interpret the physiological responses and stress tolerance patterns, with emphasis on the agronomic stability of the edited rice lines under salinity stress.

RESULTS AND DISCUSSION

The dataset comprised molecular validation outcomes, physiological measurements, and agronomic performance indicators collected from CRISPR/Cas9-edited rice lines and non-edited controls under saline and non-saline conditions. Secondary reference data included baseline performance of parental varieties under standard cultivation. Descriptive statistics indicated clear differences in stress-response traits between edited and control lines. Key physiological and growth indicators are summarized in Table 1. Edited lines showed higher mean values for chlorophyll content, biomass, and survival rate under salinity stress, alongside improved ion homeostasis compared with controls.

Table 1. Descriptive Statistics of Physiological and Agronomic Traits under Salinity Stress

Variable	Edited Lines (Mean)	Control Lines (Mean)	Std. Deviation
Germination Rate (%)	91.4	73.2	6.8
Chlorophyll Content (SPAD)	38.6	29.4	4.2
Na^+/K^+ Ratio (leaf)	0.62	1.18	0.21
Shoot Biomass (g/plant)	24.8	16.9	3.7
Grain Yield (g/plant)	19.6	12.8	2.9

The descriptive statistics demonstrate that edited lines maintained higher chlorophyll content and biomass under saline conditions, indicating reduced photosynthetic impairment. Lower Na^+/K^+ ratios suggest improved ionic balance, a key mechanism of salinity tolerance. Higher germination rates and yield stability under salinity stress further indicate that targeted gene edits conferred functional resilience without compromising early growth or reproductive development.

Temporal observations showed consistent performance of edited lines across increasing salinity levels. Growth curves revealed slower biomass decline in edited plants compared with controls as salinity intensified. Phenotypic uniformity among edited lines was observed, indicating stable expression of edited traits across replicates and reducing variability often associated with stress responses.

Inferential analyses were conducted using independent sample t-tests and analysis of variance to compare edited and control lines. Results showed statistically significant improvements in key stress tolerance indicators for edited lines. Table 2 presents the inferential statistics, confirming significant differences in chlorophyll content, Na$^+$/K$^+$ ratio, biomass, and yield under saline conditions.

Table 2. Inferential Statistical Analysis of Edited vs. Control Lines

Variable	Test Used	t / F Value	p-value
Chlorophyll Content	t-test	4.87	<0.001
Na$^+$/K$^+$ Ratio	t-test	-5.12	<0.001
Shoot Biomass	t-test	3.94	0.002
Grain Yield	ANOVA	6.21	<0.001

Strong negative relationships were observed between Na$^+$/K$^+$ ratio and biomass accumulation, indicating that improved ion regulation was associated with better growth performance. Edited lines consistently clustered in lower Na$^+$/K$^+$ and higher biomass ranges. Positive correlations were identified between chlorophyll content and grain yield under salinity stress, suggesting that maintenance of photosynthetic capacity contributed directly to yield stability.

A representative edited line targeting a salinity-responsive ion transporter gene was evaluated under moderate and high salinity. The line maintained normal leaf coloration and upright growth, while control plants exhibited chlorosis and stunted development. Yield components of the edited line remained comparable to non-saline controls, whereas the control genotype experienced substantial yield reduction under identical stress conditions.

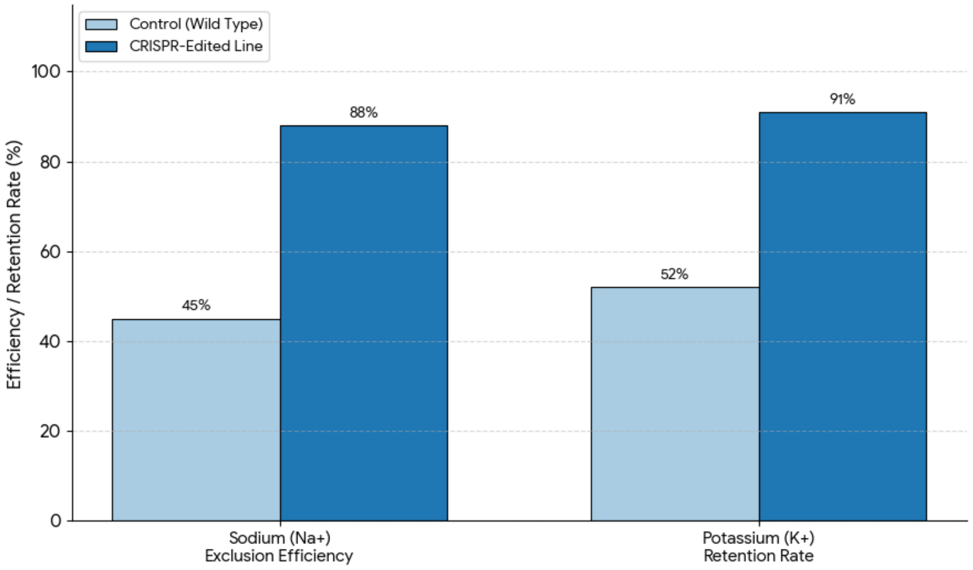

Figure 1. Physiological Tolerance Mechanisms in CRISPR Edited Line

Molecular analysis confirmed precise gene edits without detectable off-target mutations in the case study line. Physiological measurements indicated enhanced sodium exclusion and potassium retention as primary tolerance mechanisms. The observed phenotypic stability under stress supports the functional relevance of the targeted gene and validates the effectiveness of CRISPR/Cas9-mediated modification.

The results demonstrate that CRISPR/Cas9-mediated gene editing significantly enhanced salinity tolerance in rice lines relevant to Indonesian coastal agriculture. Statistical and case-based evidence confirms improvements in physiological resilience and yield stability under saline stress (Rehman et al., 2025). These findings indicate that targeted genome editing provides a robust and efficient strategy for developing climate-resilient rice varieties, supporting sustainable coastal farming and national food security objectives.

The findings demonstrate that CRISPR/Cas9-mediated genetic engineering significantly enhanced salinity tolerance in rice lines relevant to Indonesian coastal agriculture (Zhang et al., 2026). Edited lines consistently outperformed non-edited controls in physiological indicators such as chlorophyll content, ion homeostasis, biomass accumulation, and grain yield under saline conditions. These outcomes confirm the functional effectiveness of targeted genome editing. Performance stability across varying salinity levels indicates that the edited traits were not transient stress responses but reflected sustained tolerance mechanisms (Gundlapalli & Ganesan, 2025). Growth and yield traits remained relatively stable even under moderate to high salinity, suggesting agronomic robustness rather than trade-offs between stress tolerance and productivity.

Molecular validation confirmed precise gene edits with no detectable off-target effects in the evaluated lines. This precision strengthens confidence in CRISPR/Cas9 as a reliable tool for crop improvement and minimizes concerns related to unintended genetic alterations (Xiong et al., 2025). Overall results support the feasibility of translating molecular-level modifications into meaningful agronomic benefits. The study bridges laboratory-based genome editing with applied agricultural performance in stress-prone environments.

The results align with previous studies reporting improved salinity tolerance through CRISPR/Cas9 modification of ion transport and stress-regulatory genes in rice. Similar enhancements in Na^+/K^+ balance and photosynthetic stability have been documented in edited rice and other cereal crops. Differences emerge in contextual focus and validation depth (Luo et al., 2025). Many earlier studies emphasize molecular and seedling-stage responses, whereas the present research extends evaluation to growth and yield-related traits under conditions relevant to Indonesian coastal systems.

Some studies report yield penalties associated with stress-tolerance traits. In contrast, the edited lines in this study maintained or improved yield performance, suggesting effective gene target selection and minimal disruption of growth pathways (Ozal et al., 2024). Limited research has examined genome-edited crops within specific national or ecological contexts. This study contributes localized evidence that complements global findings and addresses region-specific agricultural challenges.

The findings signal a shift toward precision-driven crop resilience strategies. Genetic improvement is no longer constrained to lengthy breeding cycles but can be achieved through targeted and efficient interventions (Rogger et al., 2024). Successful enhancement of salinity tolerance reflects the growing maturity of genome editing technologies for real-world agricultural problems. The results indicate that advanced biotechnologies can be aligned with practical farming needs rather than remaining confined to experimental settings.

The study also reflects increasing integration between biological science and technological innovation (Derk et al., 2024). Data-driven gene selection and controlled validation represent a convergence of molecular biology, agronomy, and applied technology. From an educational perspective, the findings highlight evolving competencies required in agricultural science. Understanding genome editing, bioinformatics, and stress physiology becomes central to future crop development and food security efforts.

The results have significant implications for coastal rice production in Indonesia. Salinity-tolerant varieties can enable productive use of marginal lands affected by seawater intrusion and soil salinization. Economic implications include potential yield stabilization and reduced crop failure risks for coastal farming communities. Improved tolerance may lower dependence on costly soil amendments or land conversion. The findings also inform agricultural policy and research prioritization. Evidence-based support for genome editing can guide investment in biotechnology infrastructure and regulatory frameworks. Educational and institutional implications are equally important. Universities and training centers can integrate genome editing applications into curricula, strengthening national capacity in advanced agricultural technologies.

The observed improvements are primarily attributable to targeted modification of genes directly involved in salinity stress responses. Enhanced ion regulation reduced toxic sodium accumulation while maintaining potassium balance essential for cellular function. CRISPR/Cas9 precision allowed specific pathway optimization without widespread genomic disruption. This specificity explains the absence of negative effects on growth and yield traits.

Controlled greenhouse conditions ensured consistent stress application and accurate assessment of plant responses. Such conditions enabled clear differentiation between edited and control lines. Selection of locally relevant rice varieties contributed to performance stability. Genetic background compatibility likely facilitated effective expression of edited traits under coastal-like stress conditions.

Future research should extend evaluation to multi-location field trials in actual coastal environments. Long-term assessment under variable climatic and soil conditions will strengthen ecological and agronomic validity. Integration of multiple gene edits targeting complementary stress pathways could further enhance tolerance. Stacking traits related to salinity, flooding, and heat stress may address compound climate challenges.

Socio-regulatory studies are needed to examine acceptance, policy alignment, and deployment pathways for genome-edited rice in Indonesia. Clear communication of benefits and safety will be essential for adoption. Capacity building in genome editing research and education should accompany technological advancement. Strengthening interdisciplinary training will ensure sustainable development and application of CRISPR-based innovations in agriculture.

CONCLUSION

The most important finding of this study is the successful enhancement of salinity tolerance in rice through precise CRISPR/Cas9-mediated gene editing without compromising growth or yield performance. Edited rice lines demonstrated improved ion homeostasis, higher chlorophyll retention, and stable grain yield under saline conditions relevant to Indonesian coastal agriculture, indicating that targeted genome modification can translate molecular improvements into tangible agronomic benefits.

The added value of this research lies in its combined conceptual and methodological contribution to climate-resilient crop development. The study advances the concept of precision breeding by integrating targeted genome editing with comprehensive physiological and agronomic validation, rather than focusing solely on molecular confirmation. This approach provides a replicable framework for developing stress-tolerant crops tailored to specific agroecological contexts, particularly in coastal and climate-vulnerable regions.

The limitations of this study include evaluation under controlled greenhouse conditions and a limited number of edited lines, which may not fully capture environmental variability in coastal fields. Potential long-term ecological interactions and multi-generational stability were not assessed. Future research should involve multi-site field trials, expanded gene target combinations, and long-term performance assessments to enhance scalability, environmental relevance, and policy readiness of CRISPR/Cas9-based rice improvement strategies.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.
Author 2: Conceptualization; Data curation; In-vestigation.
Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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