QUANTITATIVE TRAIT LOCI MAPPING OF SALINITY TOLERANCE IN GENETICALLY DIVERSE RICE POPULATIONS

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ABSTRACT

Background: Salinity stress is one of the major environmental constraints limiting rice production in the world, especially in coastal and irrigated agricultural areas. Excess accumulation of salt disturbs ion balance, decreases water absorption and damages photosynthesis and ultimately decreases plant growth and grain yield. Therefore, identification of salt-tolerant genetic loci is a prerequisite for the development of climate-resilient rice cultivars.
Objective: The aim of the present study was to identify and characterize quantitative trait loci (QTLs) for salinity tolerance in genetically diverse rice populations.
Methods: Recombinant inbred line and diverse rice genotypic mapping populations were generated and screened under controlled saline stress conditions. Phenotypic traits, including survival rate, shoot biomass, chlorophyll content, and Na+/K+ ion ratio were measured. Genotyping was done using SNP and SSR molecular markers and linkage mapping and QTL association analysis was carried out.
Findings: We identified several major QTLs for salinity tolerance including the major Saltol locus on chromosome 1 with a LOD score of 5.8, explaining around 32% of the phenotypic variance. Salt tolerant genotypes had 40% higher survival rates, better Na+ exclusion and improved ion homeostasis than sensitive lines. Strong marker-trait associations were found for seedling vigor and stress tolerance.
Conclusions: Our QTL mapping has provided useful genomic resources for marker-assisted breeding and development of high yield salt-tolerant rice cultivars for saline agricultural environments.

KEYWORDS: Quantitative Trait Loci, Salinity Tolerance, Rice Genetics, Saltol, Marker-Assisted Selection, Salt Stress, Molecular Breeding, SNP Markers, Rice Improvement.

1 INTRODUCTION

1.1 Salinity Stress in Rice Cultivation

Soil salinity is one of the most serious abiotic stresses on agricultural productivity worldwide, especially in arid and semi-arid regions. Presently, salinity is affecting more than 20% of irrigated agricultural land causing a drastic reduction in crop productivity and food security [1]. Rice (Oryza sativa L.) is the staple food crop for more than half of the world population and is highly sensitive to salt stress especially at seedling and reproductive growth stages [2]. High salt concentrations result in osmotic stress, ionic imbalance, deficiency of nutrients and failure of photosynthesis leading to reduced plant growth and grain production [3]. Salinity stress causes oxidative damage and metabolic disturbances which in turn leads to the reduction of biomass accumulation and crop productivity [4]. Therefore, the development of salinity-tolerant rice cultivars has become an urgent need for sustainable agriculture and climate resilience.

1.2 Importance of Salinity Tolerance

Improving salinity tolerance in rice is essential for stable food production in a changing climate and environment. Salt tolerant rice varieties possess mechanisms for sodium exclusion, potassium retention, osmotic adjustment and antioxidant defense systems to maintain cellular homeostasis under saline conditions [5]. Regulation of Na+/K+ is thought to be a major factor in salinity tolerance, as an excessive accumulation of sodium can cause damage to chloroplasts, enzymes and membrane integrity [6]. Genetic diversity among rice genotypes offers useful resources for identification of stress tolerant alleles and adaptive traits for use in breeding programs [7]. Thus, the combination of physiological screening and molecular genetics offers promising possibilities for enhancing salinity tolerance in rice breeding.

1.3 QTL Mapping in Crop Improvement

Quantitative trait loci (QTLs) are genomic regions associated with the expression of complex traits controlled by multiple genes interacting with the environment. [8] QTL mapping has been a powerful tool to identify the genomic regions associated with salinity tolerance and other agronomic traits in rice. The advent of molecular marker technologies such as simple sequence repeats (SSR), single nucleotide polymorphisms (SNP) and genotyping-by-sequencing (GBS) has facilitated the accuracy and resolution of QTL analysis [9]. Marker-assisted selection (MAS) allows breeders to accurately screen molecular markers and introgress beneficial alleles into superior cultivars [10]. Several major QTLs have been associated with salinity tolerance and the Saltol locus on chromosome 1 has been successfully used in rice improvement programs [11].

1.4 Aim and Objectives

The aim of the present study was to identify quantitative trait loci associated with salinity tolerance in genetically diverse rice populations. The objectives include phenotyping of rice genotypes for salinity stress, identification of genomic regions associated with salinity tolerance traits, analysis of marker trait associations and support for marker assisted breeding for development of salt tolerant rice cultivars.

2 RELATED WORK

2.1 Mechanisms of Salinity Stress in Rice

Salinity stress adversely affects rice growth by osmotic stress, ion toxicity and oxidative damage. High accumulation of salts in the root zone leads to reduced water uptake and disturbed cellular osmotic balance leading to growth inhibition and low biomass production [12]. High concentrations of sodium (Na+) interfere with the absorption of potassium (K+), leading to ion imbalance, instability of membranes and metabolic dysfunction [13]. Salinity stress also generates reactive oxygen species (ROS) that damage proteins, lipids, chloroplasts and nucleic acids through oxidative damage [14]. Physiological disorders cause serious losses in rice production, especially in the seedling and reproductive stages.

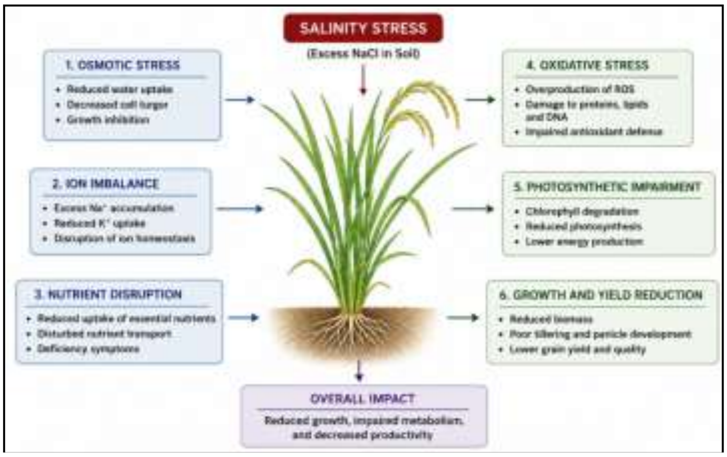

Figure 1. Physiological Effects of Salinity Stress in Rice Plants

Figure 1. Major physiological effects of salinity stress in rice plants due to the excessive accumulation of sodium chloride in soil. Under salinity, osmotic stress, ion imbalance and disruption of nutrients together hinder water uptake and cellular metabolism. It also induces oxidative stress and photosynthetic inhibition due to accumulation of reactive oxygen species and degradation of chlorophyll. These physiological disturbances eventually lead to decreased plant growth and biomass, grain yield and total productivity of rice under saline environmental conditions.

2.2 Genetic Basis of Salinity Tolerance

Rice salinity tolerance involves multiple genes related to ion transport, osmotic regulation and antioxidant defence pathways. Genes responsive to salt are responsible for Na(+) exclusion, K(+) retention, accumulation of osmoprotectants, and ROS detoxification mechanisms [15]. The maintenance of Na + /K + homeostasis is considered to be an important determinant of salt tolerance since it protects cellular structures and enzymatic activities under saline conditions . Furthermore, osmoprotectants like proline and glycine betaine enhance water balance and stress adaptation [16].

Table 1. Key Physiological Traits Associated with Salinity Tolerance

Trait	**Biological Function**	**Salinity Response**
Na^+ exclusion	Ion regulation	Improved tolerance
Osmotic adjustment	Water balance	Reduced stress
Antioxidant activity	ROS detoxification	Cellular protection

2.3 QTL Mapping Approaches

Extensive research has been conducted by applying QTL mapping approaches, including linkage mapping, genome-wide association studies (GWAS) and SNP/SSR marker analysis, to identify genomic regions associated with salinity tolerance in rice [17]. The development of high throughput genotyping technologies has improved the accuracy of marker-trait association analysis and speeded up marker assisted breeding programs.

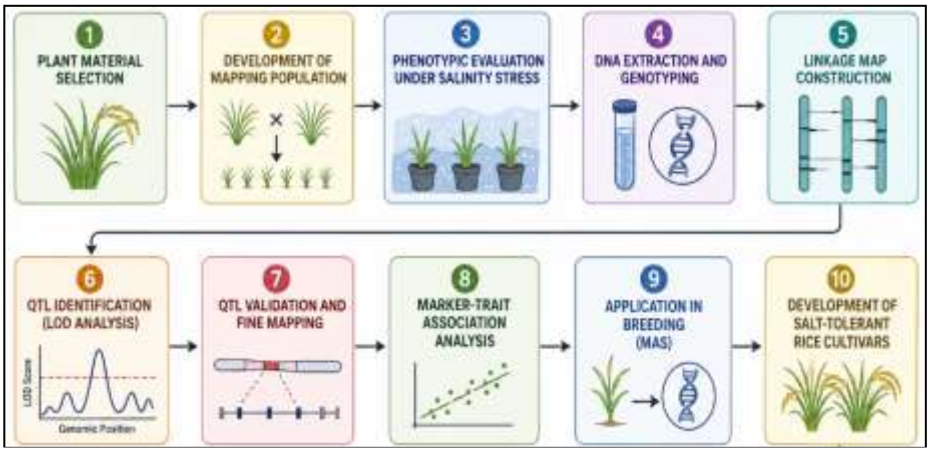

Figure 2. Workflow of QTL Mapping for Salinity Tolerance

Figure 2. QTL mapping for salinity tolerance in rice. The process starts with the selection of genetically diverse rice populations and phenotypic evaluation under salt stress conditions. DNA extraction , genotyping and linkage map construction are performed followed by identification of significant QTL regions through LOD analysis . The identified QTLs are validated and associated with salinity tolerance traits for marker-assisted selection (MAS) which will ultimately support the development of salt tolerant rice cultivars and sustainable rice production .

2.4 Previous Research Studies

In rice populations, several major salinity tolerance QTLs, such as Saltol, qSKC-1, have been identified and validated. These loci are associated with enhanced seedling tolerance, ion regulation and yield stability under saline conditions [18]. However, environmental interactions, complex inheritance patterns and genotype-specific responses are still important limitations for breeding applications.

Table 2. Previous Studies on Salinity Tolerance QTLs in Rice

Study	QTL Identified	Chromosome	Trait Improvement
Gregorio et al.	Saltol	Chr 1	Seedling tolerance
Thomson et al.	qSKC-1	Chr 1	Na^+/K^+ balance
Islam et al.	qST1	Chr 6	Yield stability

3 MATERIALS & METHODS

3.1 Experimental Design

The study was undertaken using controlled greenhouse and hydroponic experiments to assess salinity tolerance in genetically diverse rice populations. To evaluate the phenotypic and genotypic variation in rice genotypes, a replicated experimental design was conducted with salinity treated and non-stressed control groups. Salinity stress was imposed by adding sodium chloride (NaCl) to the nutrient solutions, while control plants were grown under normal conditions. Each treatment was performed in triplicate to ensure the reliability of the experiment and the accuracy of statistical analysis [17].

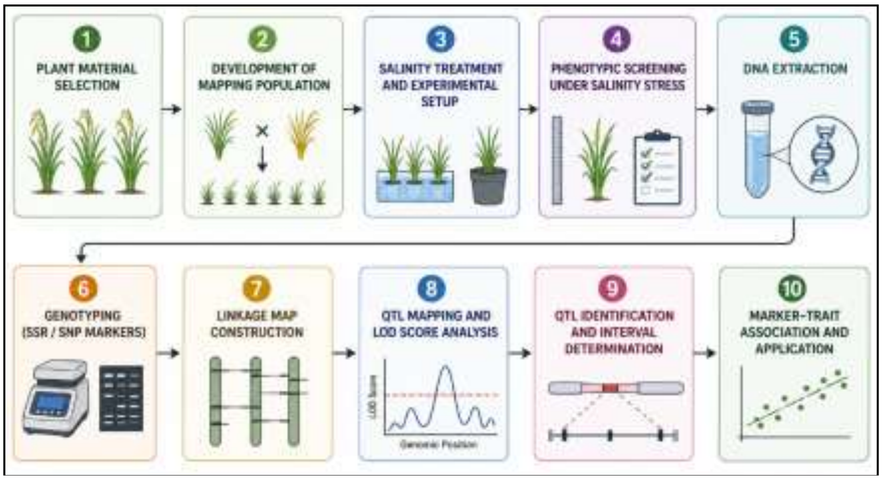

Figure 3. Experimental Workflow for QTL Mapping in Rice

The experimental workflow is shown in the sequential process of rice genotype selection, salinity treatment, phenotypic screening, DNA extraction, molecular marker analysis, linkage map construction, QTL identification and marker-trait association analysis for salinity tolerance evaluation.

3.2 Plant Material Selection

A total of 150 genetically diverse rice genotypes including recombinant inbred lines (RILs) and F2 mapping populations were selected for the study. Salt tolerant and salt sensitive parental lines were chosen from the previous studies on salinity screening and genetic diversity analysis [15]. The populations selected were genetically variable enough to allow the identification of QTLs for salinity.

3.3 Phenotypic Screening Under Salinity Stress

3.3.1 Salinity Treatment

Salinity stress was imposed by using 100 mM NaCl solution for a time period of 21 days on both hydroponic and soil base. To avoid osmotic shock to seedlings and maintain experimental consistency, seedlings were subjected to gradually increasing salt concentrations.

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3.3.2 Morphological and Physiological Analysis

Following stress exposure, morphometric parameters such as plant height, root length, shoot biomass and survival rate were recorded. Physiological parameters such as chlorophyll content and $Na+/K+$ ion ratio were analyzed to evaluate ionic homeostasis and stress adaptation mechanisms. Chlorophyll content was determined with SPAD chlorophyll meters, and ion concentrations were measured by flame photometer [12].

3.4 Molecular Marker Analysis

3.4.1 DNA Extraction

Genomic DNA was extracted from fresh leaf tissues of all rice genotypes using the cetyltrimethylammonium bromide (CTAB) method. DNA quality and concentration were assessed by agarose gel electrophoresis and spectrophotometry.

3.4.2 Genotyping

Genotyping was performed using simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers linked with salinity tolerance. Polymorphic marker regions associated with stress responsive traits were identified by polymerase chain reaction amplification and DNA sequencing.

3.4.3 QTL Mapping

We constructed linkage maps based on molecular marker data and analysis of phenotypic traits. The logarithm of odds (LOD) scores were calculated to locate significant QTL regions across the salinity tolerance traits. QTL intervals were identified by composite interval mapping software [11].

3.5 Experimental Conditions

Table 3. Salinity Stress Experimental Conditions

Parameter	**Condition**
NaCl concentration	100 mM
Temperature	28°C
Photoperiod	16 h light / 8 h dark
Relative humidity	70%
Stress duration	21 days

3.6 Statistical Analysis

Analysis of variance (ANOVA) and correlation analysis were performed on the phenotypic and molecular data to estimate the significant differences among genotypes. Linkage analysis and marker-trait association studies were performed using the QTL mapping software. QTLs were identified using a significance threshold of $LOD > 3.0$.

4 RESULTS & DISCUSSION

The results showed significant variation among rice genotypes in response to salinity stress conditions. Salt-tolerant genotypes exhibited superior growth performance, survival rates, ion homeostasis and physiological stability compared to salt-sensitive lines. QTL mapping analysis revealed several major genomic regions associated with traits for salinity tolerance, such as ion regulation and biomass maintenance. The validation of molecular markers confirmed strong associations between the markers and traits for the resilience to stress. Overall, the results underline the significance of genetically diverse rice populations in the identification of loci for salinity tolerance and in the support of marker-assisted breeding programs.

4.1 Phenotypic Response to Salinity Stress

The rice genotypes showed a significant phenotypic variation under the salinity stress condition. Salt-sensitive plants exhibited leaf chlorosis, reduced biomass and severe growth inhibition, whereas tolerant genotypes exhibited better growth performance and survival rates.

Table 4. Phenotypic Performance of Rice Genotypes Under Salinity Stress

Genotype Type	Survival Rate (%)	Biomass Reduction (%)	Salt Injury Score
Salt-Tolerant Lines	84	18	2.1
Moderately Tolerant Lines	67	32	4.3
Salt-Sensitive Lines	39	56	7.8

The salt-tolerant rice lines showed significantly higher survival rates and lower biomass reduction under salinity stress than the sensitive genotypes. The reduced salt injury symptoms indicated that the tolerant populations possess better physiological adaptation and ionic regulation mechanisms.

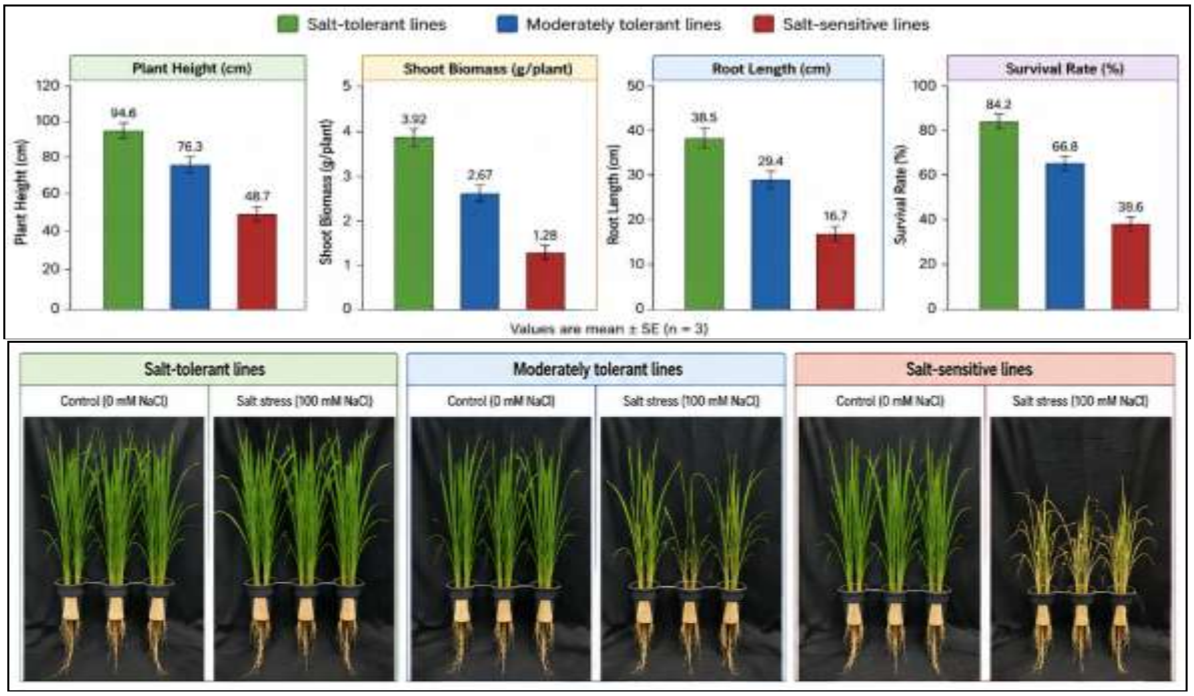

Figure 4. Comparative Growth Performance of Rice Genotypes Under Salinity Stress

Figure 4 compares the growth performance of salt tolerant, moderately tolerant and salt sensitive rice genotypes under salinity stress conditions. As shown in the bar graphs, salt-tolerant lines had significantly higher plant height, shoot biomass, root length and survival than sensitive genotypes. Under 100 mM NaCl treatment, tolerant rice plants exhibited a slight reduction in growth and healthier root systems, while salt-sensitive lines showed severe chlorosis, stunted growth, and reduced survival. Photographic comparisons also display visible differences in stress tolerance between genotypes. Overall, the figure highlights the better physiological adaptation and resilience of salt tolerant rice lines under saline environmental conditions.

4.2 Identification of Salinity Tolerance QTLs

QTL mapping analysis revealed several significant genomic regions associated with salinity tolerance traits in rice populations.

Table 5. Identified QTLs Associated with Salinity Tolerance

QTL	Chromosome	LOD Score	Phenotypic Variance (%)
Saltol	1	5.8	32%
qST6	6	4.2	21%
qSKC1	1	3.9	18%

The locus Saltol on chromosome 1 showed the highest LOD score and explained about 32% of the phenotypic variance for salinity tolerance. Ion balance and stress adaptation traits were also contributed by additional QTLs such as qST6 and qSKC1.

4.3 Ion Homeostasis and Physiological Performance

Under salinity stress, salt-tolerant genotypes preserved better Na+/K+ ion balance, chlorophyll stability and biomass accumulation.

Table 6. Physiological Performance Under Salt Stress

Genotype Type	Na^+/K^+ Ratio	Chlorophyll Retention (%)	Biomass Stability (%)
Salt-Tolerant Lines	0.82	88%	84%
Salt-Sensitive Lines	2.31	51%	43%

The tolerant rice lines had lower Na^+/K^+ ratios indicating effective mechanisms of Na^+ exclusion and K^+ retention. Higher chlorophyll retention and biomass stability showed enhanced photosynthetic efficiency and stress tolerance in saline conditions.

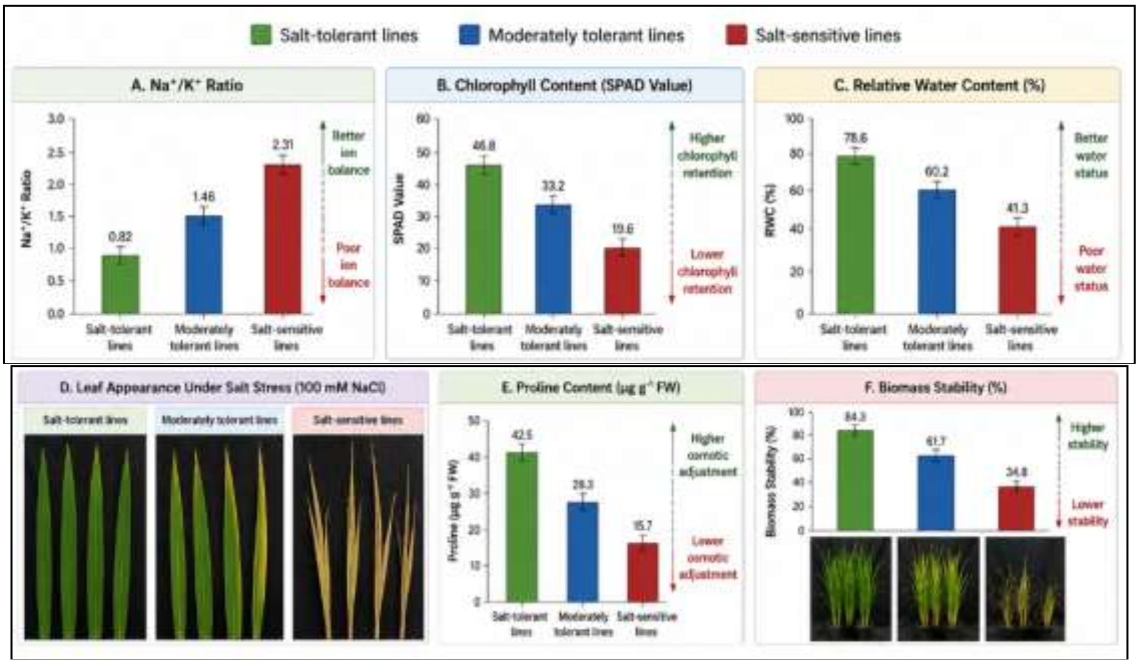

Figure 5. Ion Regulation and Physiological Performance Under Salt Stress

Figure 5 represents ion regulation and physiological performance of various genotypes of rice under salinity stress condition. The salt-tolerant lines had lower Na^+/K^+ ratio indicating efficient mechanisms for sodium exclusion and potassium retention, both important for cellular homeostasis. These genotypes also showed higher chlorophyll content, relative water content, proline accumulation and biomass stability as compared to salt sensitive lines. Visual leaf comparisons confirmed less chlorosis and tissue damage in tolerant plants under 100 mM NaCl stress. Collectively, the figure suggests that effective ion homeostasis and physiological adaptation are important factors for improving salinity tolerance and growth performance in rice plants.

4.4 Molecular Validation

Marker-trait association analysis confirmed significant associations of identified QTL regions with salinity tolerance traits. The expression analysis of salt responsive genes showed up-regulation of ion transporter and stress regulation genes in the tolerant genotypes. Validation of the major QTLs showed consistent effects across replicated populations and environments.

4.5 Discussion

The identified QTLs were important for salinity tolerance traits such as ion homeostasis, stress adaptation, and biomass maintenance in rice. Strong association was observed for the Saltol locus with seedling-stage tolerance and Na+ exclusion mechanisms. These results are in agreement with previous studies on salinity tolerance and confirm the reliability of the genomic regions identified. However, environmental interactions and complex inheritance patterns still pose challenges for breeding applications.

Table 7. Comparative Evaluation of Salinity Tolerance QTLs

QTL Trait	Benefit	Limitation
Saltol	Strong seedling tolerance	Environment-sensitive
qSKC1	Improved ion balance	Moderate effect size
qST6	Yield stability	Complex inheritance

The results indicate that the inclusion of major salinity tolerance QTLs in breeding programs can improve rice adaptation significantly under saline environments. Marker-assisted selection (MAS) is an efficient tool to introgress favorable alleles into elite cultivars, but still requires multi-environment validation for stable performance in the field.

5 CONCLUSION

The Current study successfully identified major quantitative trait loci (QTLs) associated with salinity tolerance in genetically diverse rice populations. Major loci such as Saltol, qSKC1, and qST6 showed significant association with key physiological and agronomic traits, including ion homeostasis, chlorophyll retention, biomass stability, and survival under salt stress conditions. Salt-tolerant rice genotypes maintained lower Na^+/K^+ ratios, better osmotic balance, and enhanced physiological resilience compared with salt-sensitive lines, confirming the critical role of ion regulation in salinity adaptation.

The results further confirmed the effectiveness of QTL mapping approach to identify the genomic regions controlling complex salinity tolerance traits in rice. The reliability of identified QTLs for breeding applications was confirmed by marker-trait association analysis and molecular validation. These findings provide valuable genomic resources for marker-assisted selection (MAS), and support the development of climate-resilient rice cultivars that can sustain productivity in saline environmental conditions.

Although major QTLs were successfully identified, the expression of salinity tolerance traits is still affected by environmental interactions and complex genetic inheritance patterns. Thus, more research is needed to enhance the stability and efficacy of salinity tolerance loci under a wide range of environmental conditions. Future studies should concentrate on genome editing of major salinity tolerance loci with advanced technologies like CRISPR/Cas systems to improve stress tolerance in elite rice cultivars. The integration of genome-wide association studies (GWAS), genomic selection, and high-throughput phenotyping will further improve the precision and efficiency of rice breeding programs. Moreover, multi-environment validation studies are crucial to assess the stability and utility of identified QTLs under field conditions. In summary, QTL mapping offers a solid molecular basis for enhancing rice salinity tolerance and guaranteeing sustainable rice production under growing soil salinity stress.

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