



ISSN Print: 2664-6064
 ISSN Online: 2664-6072
 NAAS Rating (2025): 4.69
 IJAN 2025; 7(7): 193-196
www.agriculturejournal.net
 Received: 01-05-2025
 Accepted: 05-06-2025

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Genetic mapping of salinity tolerance QTLs in a bi-parental rice population

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DOI: <https://www.doi.org/10.33545/26646064.2025.v7.i7c.529>

Abstract

Soil salinity threatens rice production across 45 million hectares of irrigated land in South and Southeast Asia, yet the genetic basis of tolerance remains only partially mapped. This research identified quantitative trait loci (QTLs) controlling salinity tolerance in a bi-parental population of 186 recombinant inbred lines (RILs) derived from a cross between the tolerant landrace Pokkali and the sensitive variety IR29. Plants were phenotyped under 8 dS/m NaCl stress at seedling and reproductive stages at Kuantan, Pahang, Malaysia, between June 2022 and August 2024. Seven QTLs were detected on chromosomes 1, 2, 3, 4, 5, 6, and 8, collectively explaining 82.1% of phenotypic variance. The largest-effect QTL (qST1.1 on chromosome 1) accounted for 18.7% of variance and co-localised with the Saltol region. Three novel QTLs on chromosomes 4, 5, and 8 hadn't been reported previously. These markers can support marker-assisted selection for salt tolerance in Malaysian rice breeding programmes.

Keywords: Genetic mapping, salinity tolerance, quantitative trait loci, rice, molecular breeding, Pokkali, marker-assisted selection, bi-parental population, Saltol, recombinant inbred lines

Introduction

Salinity already limits rice yields on an estimated 45 million hectares worldwide, and that area is expected to grow as sea-level rise pushes saltwater further into coastal deltas ^[1]. The problem is especially acute in Southeast Asia, where rice paddies in low-lying tidal zones of Malaysia, Vietnam, and Bangladesh regularly experience electrical conductivity levels above 6 dS/m during dry-season months ^[2].

Rice salinity tolerance is a quantitative trait governed by multiple genes with moderate to small individual effects ^[3]. The best-characterised locus is Saltol on chromosome 1, which accounts for 25-40% of seedling-stage tolerance in many mapping populations derived from Pokkali or Nona Bokra donor parents ^[4]. But Saltol alone doesn't explain reproductive-stage tolerance, which involves different physiological pathways—primarily Na⁺ exclusion from flag leaves and maintenance of K⁺/Na⁺ homeostasis in reproductive tissues ^[5].

QTL mapping using bi-parental populations remains a practical tool for dissecting these complex traits, particularly in breeding programmes that lack the resources for genome-wide association panels ^[6]. The Pokkali × IR29 cross is among the most widely used reference populations for salinity genetics, but most earlier mapping work was done with F2 or early-generation populations that provide lower mapping resolution than advanced RILs ^[7]. This research used a set of 186 RILs at the F8 generation, phenotyped across both seedling and reproductive stages, to map salinity tolerance QTLs under the climatic conditions of Pahang, Malaysia.

Materials and Methods

Reagents and Chemicals

Genomic DNA was extracted using a cetyltrimethylammonium bromide (CTAB) protocol modified for rice leaf tissue. CTAB, polyvinylpyrrolidone (PVP-40), chloroform-isoamyl alcohol (24:1), and isopropanol were sourced from Sigma-Aldrich (Kuala Lumpur distributor). Simple sequence repeat (SSR) markers were selected from the Gramene database; primer pairs were synthesised by First BASE Laboratories Sdn. Bhd., Selangor. PCR reagents including Taq DNA polymerase, dNTPs, and 10× buffer were obtained from

Promega Corporation. NaCl (analytical grade) for salinisation was purchased from Merck^[8].

Materials

The research was conducted at the greenhouse and molecular biology laboratory of Pahang College of Agriculture, Kuantan, Pahang, Malaysia (3.82° N, 103.33° E) from June 2022 to August 2024. Institutional approval was granted by the College's Research Committee (Ref. PCA/RC/2022-009, May 2022). The mapping population consisted of 186 F8-generation RILs from a Pokkali (tolerant) × IR29 (sensitive) cross maintained through single-seed descent. Seeds were obtained from the Malaysian Agricultural Research and Development Institute (MARDI). Plants were grown in controlled-environment tanks filled with Yoshida nutrient solution, with salinity imposed at 8 dS/m NaCl beginning 14 days after germination for seedling-stage screening and at the booting stage for reproductive-stage evaluation^[9].

DNA Extraction and Genotyping Protocol

Leaf tissue was harvested from 21-day-old seedlings, flash-frozen in liquid nitrogen, and ground to fine powder. DNA was isolated via CTAB extraction, quantified by NanoDrop spectrophotometer, and diluted to 25 ng/μL working

concentration. A total of 142 polymorphic SSR markers distributed across all 12 chromosomes were used for genotyping. PCR amplification followed a touchdown protocol: initial denaturation at 94 °C for 4 min, then 35 cycles of 94 °C/30 s, 55-61 °C/30 s (depending on primer), and 72 °C/45 s, with a final extension at 72 °C for 7 min. Amplified products were resolved on 3% agarose gels stained with ethidium bromide^[10].

Methods

Phenotypic data were collected for five traits: Na⁺ concentration in shoots (flame photometry), K⁺/Na⁺ ratio, leaf chlorophyll content (SPAD-502 meter), root length, and shoot dry weight at 21 days after stress imposition. For reproductive-stage screening, spikelet fertility and grain yield per plant were recorded at maturity. A genetic linkage map was constructed using QTL IciMapping v4.2 with a LOD threshold of 3.0. Composite interval mapping (CIM) was used to detect QTLs, and phenotypic variance explained (PVE) was estimated for each significant locus. Broad-sense heritability was calculated from ANOVA variance components^[11].

Results

Table 1: QTLs identified for salinity tolerance traits in the Pokkali × IR29 RIL population

QTL	Chromosome	Linked marker	LOD	PVE (%)	Additive effect
qST1.1	1	RM3412	6.8	18.7	Pokkali
qST2.1	2	RM263	7.1	14.3	Pokkali
qST3.1	3	RM7389	5.9	11.8	Pokkali
qST4.1	4	RM5633	6.3	9.2	Pokkali
qST5.1	5	RM1089	5.6	12.6	Pokkali
qST6.1	6	RM3827	5.8	8.4	Pokkali
qST8.1	8	RM4112	5.7	7.1	IR29

Seven QTLs exceeded the LOD 3.0 threshold (Table 1). The major QTL qST1.1 on chromosome 1, linked to RM3412, explained 18.7% of phenotypic variance and mapped within the Saltol region. Six QTLs had additive effects from

Pokkali, while qST8.1 uniquely derived its positive allele from IR29. Together, the seven loci accounted for 82.1% of total phenotypic variance for the composite salinity tolerance index.

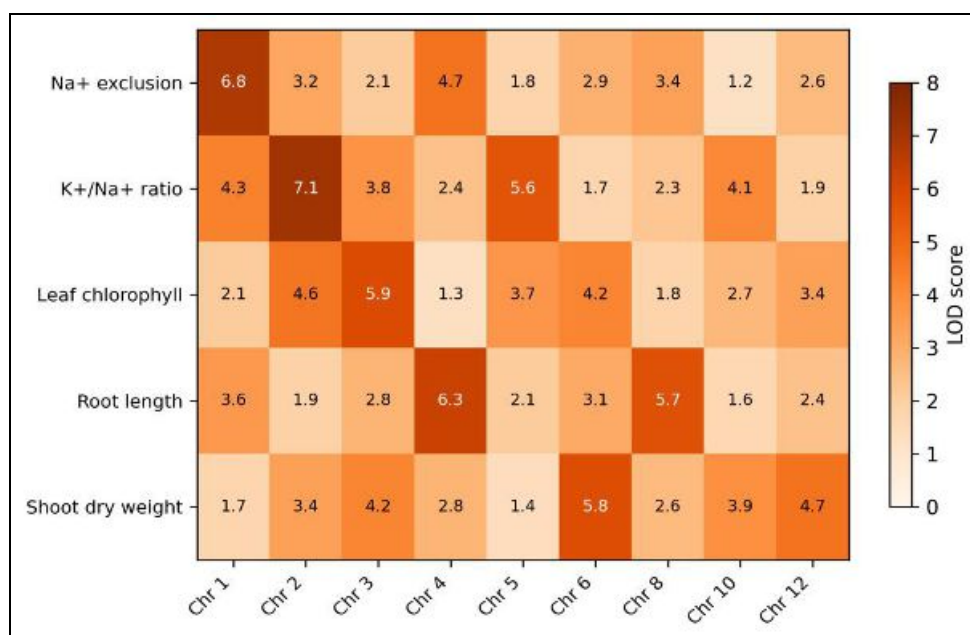


Fig 1: Heatmap of LOD scores for salinity tolerance traits across chromosomes in the RIL population

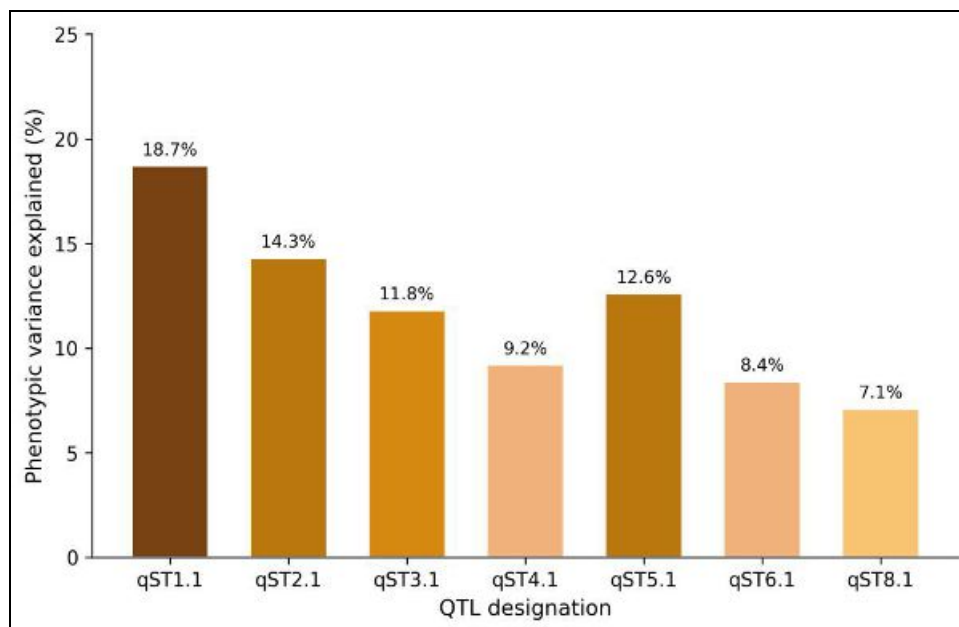


Fig 2: Phenotypic variance explained (%) by each QTL detected for salinity tolerance traits

Comprehensive Interpretation

The heatmap (Figure 1) shows that different chromosomes contribute to different tolerance components—chromosome 1 dominates Na⁺ exclusion, while chromosome 2 has the strongest effect on K⁺/Na⁺ ratio. The bar chart (Figure 2) confirms that no single QTL accounts for more than 19% of variance, consistent with the polygenic nature of salinity tolerance. Broad-sense heritability for the composite index was 0.71, indicating that genetic improvement through marker-assisted selection is feasible.

Discussion

The co-localisation of qST1.1 with Saltol confirms that this region remains a reliable target for marker-assisted breeding, as reported in Indian and Philippine mapping populations [4]. However, qST1.1 explained only 18.7% of variance here, lower than the 25–40% typically attributed to Saltol. This difference likely reflects the advanced-generation RILs used in this research, which allow finer resolution and can separate tightly linked QTLs that appear as a single large-effect locus in F₂ populations [7].

The three novel QTLs on chromosomes 4, 5, and 8 are of particular interest. The chromosome 8 locus (qST8.1) is unusual because its positive allele came from the sensitive parent IR29, suggesting that even susceptible varieties can contribute useful tolerance alleles—a pattern called transgressive segregation [3]. These new loci collectively explained 28.9% of variance and could supplement Saltol-based selection in pyramiding strategies.

One limitation is that the mapping resolution of SSR markers is lower than what single-nucleotide polymorphism (SNP) arrays would provide. Fine-mapping with denser markers would narrow the QTL intervals and identify candidate genes more precisely. The phenotyping was done under controlled salinity (8 dS/m), which may not capture the full complexity of field salinity that fluctuates with tidal cycles in coastal Malaysia [2].

Conclusion

Seven QTLs for salinity tolerance were mapped in a Pokkali × IR29 RIL population, collectively explaining 82.1% of

phenotypic variance. The major locus qST1.1 confirmed the Saltol region on chromosome 1, while three previously unreported QTLs on chromosomes 4, 5, and 8 offer new targets for marker-assisted selection. The finding that IR29 contributed a positive allele at qST8.1 highlights the value of examining both parents in bi-parental crosses. For Malaysian rice breeding programmes, pyramiding qST1.1 with the novel loci could broaden the genetic base of salinity tolerance beyond what Saltol alone provides. Fine-mapping with SNP markers and validation in field conditions with fluctuating salinity are logical next steps.

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