

Graphical Genotyping of Salt-tolerant Lines of Rice and Comparison of Allelic Variation in Different Pokkali Accessions

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Rice is a salt-sensitive crop species, and soil salinity is the single most widespread soil toxicity problem facing rice production. Salinity tolerance is a vital trait for breeding varieties for coastal and inland salt-stressed environments. Previous work at IRRRI employed a mapping population between IR29 and an accession of the salt-tolerant landrace Pokkali to identify several salt tolerance quantitative trait loci (QTLs), and advanced backcross lines were produced from this material. A simple sequence repeat (SSR) analysis of multiple Pokkali accessions showed clear distinction between the different Pokkali accessions and almost no variation within accessions. In addition, graphical genotypes of recombinant inbred lines (RILs) and near isogenic lines (NILs) at the *Saltol* QTL region have been made using a dense set of markers on chromosome 1. This information is being used to identify potentially novel alleles at salt tolerance QTLs, to further map these QTLs, and to identify the best materials for future marker-assisted selection programs.

Objectives

- Genotype a number of RILs and backcross lines from this population with SSR markers to define a graphical genotype for each line to better define the *Saltol* QTL location.
- Conduct DNA fingerprinting analysis of individual plants from eight Pokkali accessions to characterize the genetic diversity within and between landraces.
- Compare allelic variation in Pokkali introgressions in the mapping population with those in different Pokkali accessions.
- Identify the most promising Pokkali accessions and specific introgressions for future use in marker-assisted selection programs for increased salinity tolerance.

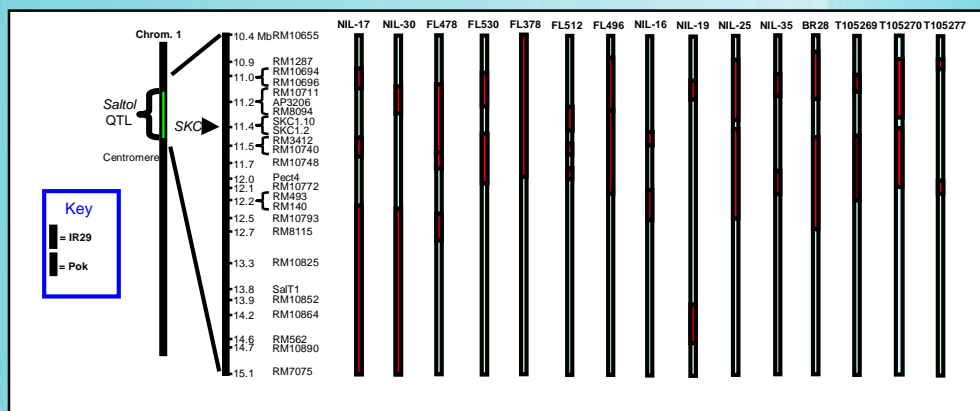
Materials and methods

Seventy-five lines were evaluated for salinity tolerance under controlled conditions (29/21 °C day/night temperature and ~70% RH). Pregerminated seeds were sown on styrofoam floats with a net bottom suspended on trays filled with distilled water. Salt stress was imposed 14 d after germination by adding NaCl to an EC of 12 dS m⁻¹ in Yoshida nutrient solution. IR29 (sensitive) and FL478 (highly tolerant) were used as checks. Entries were scored based on visual symptoms using IRRRI's Standard evaluation system for rice (SES) scores. Screening for shoot sodium concentration, chlorophyll content, leaf to leaf compartmentation, and plant vigor was done. The selected plants were transferred to pots for seed increase. DNA extraction, Polymerase chain reaction, and Polyacrylamide gel electrophoresis were done. The different Pokkali accessions showed varying levels of salinity tolerance ranging from susceptible to highly tolerant (data not shown).



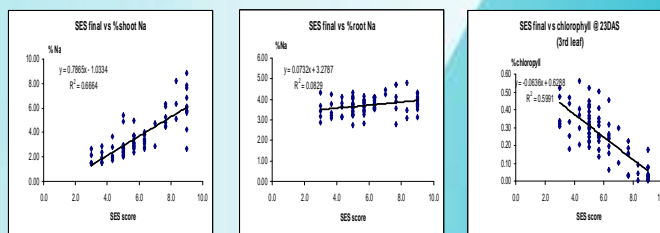
Results

Graphical genotyping

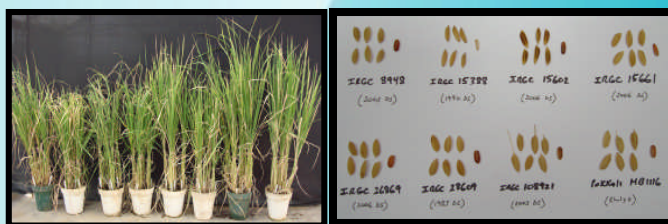


Twenty-five markers across the *Saltol* QTL region on chromosome 1 were used to genotype NILs (NIL-16, NIL-17, NIL-19, NIL-25, NIL-30, and NIL-35) and RILs (FL378, FL478, FL496, FL512, and FL530) from the IR29/Pokkali population. The results showed fragments of Pokkali introgressions across the *Saltol* region in the two NILs used by UC-Davis for physiological and gene expression characterization (NIL-17 and NIL-30) and the highly tolerant RIL FL478, while the RIL FL378 contained a single Pokkali introgression across the region. Different introgressions also had allele sizes corresponding to multiple Pokkali accessions.

Physiological traits across diverse germplasm

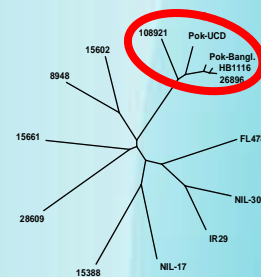
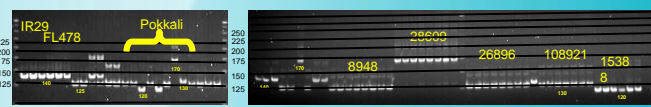


Across 65 diverse lines, the SES had a high correlation with shoot [Na⁺] and % chlorophyll but not with root [Na⁺] under EC 12 dS m⁻¹ in hydroponic solution.



Eight Pokkali accessions used for in-depth physiological and genetic study showed different plant types and grain characters: IRGC8948, IRGC15388, IRGC15602, IRGC15661, IRGC26896, IRGC26809, IRGC108921, and a breeding stock (HB1116).

SSR genotyping



Twenty SSRs evenly spaced across all 12 rice chromosomes were tested to genotype eight individuals from Pokkali accessions. SSR products were run on 10 cm acrylamide gels and stained with SYBR-safe stain. The results showed little variation within the Pokkali landraces, but a large amount of diversity between the accessions. Subsequently, 78 SSRs were used to genotype one representative sample from 10 Pokkali accessions, and a neighbor-joining tree shows a cluster of the highly salt-tolerant Pokkali accessions IRGC108921, IRGC26896, and other accessions studied by UC-Davis, Bangladesh, and IRRRI (HB1116).

Conclusions

- The standard evaluation score across 65 diverse lines was highly significant and correlated well with shoot [Na⁺] and % chlorophyll but not with root [Na⁺].
- An analysis of 20 SSRs distributed across the rice genome showed clear distinction between the different Pokkali accessions and almost no variation within accessions.
- By comparing the position of the Pokkali introgressions in the different genetic materials, we have defined sets of lines with different combinations of the multiple salt tolerance QTLs.