

# Development of near-isogenic lines targeting salinity tolerance

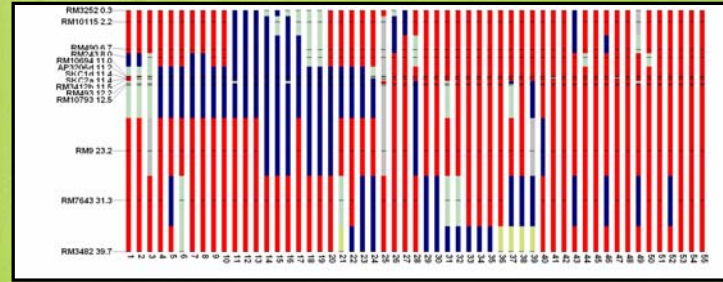
## QTLs derived from salt-tolerant variety Pokkali

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Salinity tolerance is a vital trait for breeding varieties for coastal and inland salt-affected environments. Previous work at IRRI employed a recombinant inbred line (RIL) mapping population between IR29 and an accession of the salt-tolerant landrace Pokkali to identify several salt tolerance quantitative trait loci (QTLs), including the large-effect *Salto1* QTL on chromosome 1. Forty-three IR29/Pokkali BC<sub>4</sub>F<sub>3</sub> lines and 27 BC<sub>2</sub>F<sub>4</sub> lines using the highly tolerant RIL FL 478 as the tolerant donor were genotyped with 44 simple sequence repeat (SSR) markers and were screened for salinity tolerance at the seedling stage using a hydroponic system in the phytotron. A second set of 140 FL 478/IR29 BC<sub>3</sub>F<sub>4</sub> lines were genotyped with 28 SSR markers, and 55 selected lines were screened for tolerance in the phytotron. While many of the tolerant lines contained a Pokkali allele at the *Salto1* QTL, there were a number of highly tolerant lines with allele IR29 at the *Salto1* region but containing Pokkali introgressions at other QTLs in the background. These additional QTLs will be essential for future fine-mapping and pyramiding multiple QTLs to increase the level of salinity tolerance in high-yielding rice varieties.



### Graphical genotyping



**Key**  
■ = IR29  
■ = Pokkali  
■ = Heterozygote  
■ = Missing

Fourteen markers across chromosome 1 were used to genotype the IR29/FL478 NILs to define the Pokkali introgression at the *Salto1* QTL, and were compared with background SSRs and tolerance screening data to confirm the QTLs. While most of the tolerant lines contained the Pokkali *Salto1* allele, several tolerant lines such as # 33, 34 and 35 had an IR29 allele at *Salto1*, but a Pokkali allele at the bottom on chromosome 1 (see above). In addition, data from the IR29/Pokkali NILs revealed two different Pokkali alleles at *Salto1*, and also indicated potential QTLs on chromosomes 1, 2 and 10 (see below; allele sizes indicated in basepairs).

### Objectives

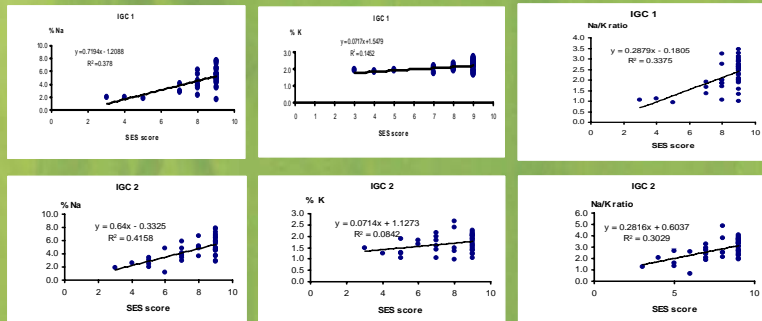
- Develop near-isogenic lines (NILs) that contain a single Pokkali introgression in the background of the recurrent parent IR29
- Genotype 43 IR29/Pokkali BC<sub>4</sub>F<sub>3</sub> lines and 27 FL 478/IR29 BC<sub>2</sub>F<sub>4</sub> lines with 44 SSR markers, and phenotype them for salinity tolerance in the phytotron
- Genotype 140 FL 478/IR29 BC<sub>3</sub>F<sub>4</sub> lines with 28 SSR markers and screen 55 selected lines for salinity tolerance in the phytotron
- Backcross the most promising lines to IR29 to remove any extra background introgressions

### Experimental setup

Forty lines were evaluated for salinity tolerance under controlled conditions (29/21°C day/night temperature and ~70% RH) and higher temperature in indoor growth chamber (IGC). Pregerminated seeds were sown on styrofoam floats with a net bottom suspended on trays filled with distilled water. Salt stress was imposed 5 d after germination by adding NaCl to an EC of 12 and 18 dS m<sup>-1</sup> in Yoshida nutrient solution. IR29 (sensitive) and FL 478 (highly tolerant) were used as checks. The pH of the nutrient solution was adjusted daily at 5.0 and culture solutions were changed weekly. Entries were scored based on visual symptoms using IRR's standard evaluation system (SES) scores.

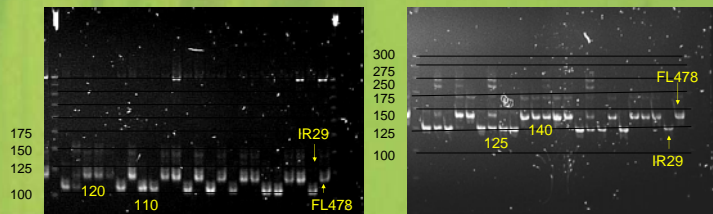


### Physiological traits



Across 40 NIL lines, SES scores positively correlated with % K and Na/K in IGC 1 (high temperature). There was high correlation with % Na in indoor growth chamber IGC 2 (29/21°C day/night temperature and 70% RH) under EC 12 dS m<sup>-1</sup> in hydroponic solution.

### SSR genotyping



Twenty-eight SSRs (for the IR29/FL 478 NILs) and 44 SSRs (for IR29/Pokkali NILs) evenly spaced across all 12 rice chromosomes were tested to genotype the NIL populations. SSR products were run on 10 cm acrylamide gels and stained with SYBR-safe stain (such as RM3867 and SO1160 shown above).

### Conclusions

- The SES across 40 diverse lines was highly significant and correlated well with % Na and % K.
- An analysis of SSRs distributed across the rice genome clearly revealed the positions of the Pokkali introgressions, which provides important data for more precise NIL development.
- There were a number of highly tolerant lines with IR29 at *Salto1* but containing Pokkali introgressions at other QTLs in the background.
- The most promising lines were backcrossed again to IR29 to remove any extra background introgressions to complete the development of a clean NIL for each QTL target.