QTL Mapping and Marker-assisted Backcrossing for Improved Salinity Tolerance in Rice

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Introduction

Salt stress is a major constraint across many rice producing areas because of the high sensitivity of modern rice varieties. Tolerance to salinity is complex, involving a number of different physiological mechanisms, such as sodium exclusion from roots, controlled sodium transport between root and shoot, and sequestering of sodium in older tissues and in the vacuoles. Previously, 80 RILs from a cross between IR29 and the landrace Pokkali were used to map several salt tolerance QTLs, including *Saltol*, a major QTL on the short arm of chromosome 1. Fine-mapping of the *Saltol* QTL is in progress and indel markers based on candidate genes in this region have been developed. A precision marker-assisted backcrossing system is also being employed to efficiently transfer the Pokkali *Saltol* QTL into popular varieties such as IR64, BR28, and Swarna. The long term goal is to identify and combine genes and QTLs controlling different physiological mechanisms to achieve a higher level of salt tolerance in high yielding rice varieties.

Project objectives

- Complete the *Saltol* fine-map and validate candidate genes in the QTL region based on converging positional and functional data
- Develop a precision marker-aided backcrossing (MAB) system to efficiently transfer the *Saltol* allele into popular varieties in collaboration with National Agricultural Research and Extension System (NARES) partners
- Better understand the physiological bases of salinity tolerance
- Enhance capacity of NARES through degree and non degree training

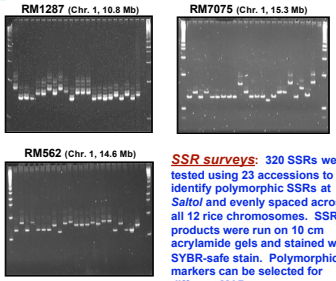
Specific activities

- Identify polymorphic simple sequence repeats (SSRs) and develop gene-based markers across the *Saltol* region to increase marker resolution
- Genotype larger near-isogenic line (NIL) populations to identify additional recombinants and increase the fine-map resolution of the *Saltol* QTL
- Determine if the cloned *SKC1* QTL derived from Nona Bokra is allelic to *Saltol* or if these represent closely linked QTLs in the same region
- Identify an optimal set of polymorphic SSRs for foreground and background MAB system using a set of potential recipient varieties
- Characterize a diverse set of tolerant and susceptible varieties for physiological traits underlying salinity tolerance
- Develop NILs targeting additional salinity tolerance QTLs to prepare for future efforts at pyramiding multiple QTLs

Identifying polymorphic SSRs

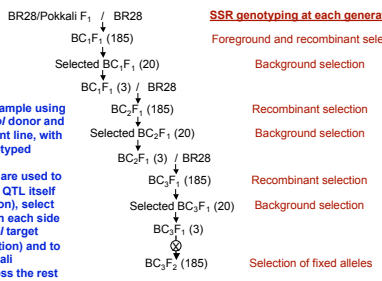
23 accessions in the SSR survey:

1. FL378
2. FL478
3. FL496
4. IR29
5. Pokkali (G. Gregorio)
6. Pokkali (IRGC 8948)
7. Pokkali (IRGC 26869)
8. Nona Bokra
9. Cheriviruppu
10. Kala Rata 1-24
11. IR64
12. BR28
13. BR29
14. BR11
15. Swarna
16. Samba Mahsuri
17. Vijetha
18. Cisadane
19. Gasmal 72-1
20. NPT (IR71700-247-1-1-2)
21. Azucena
22. IR74
23. Jalmagna
24. no template control



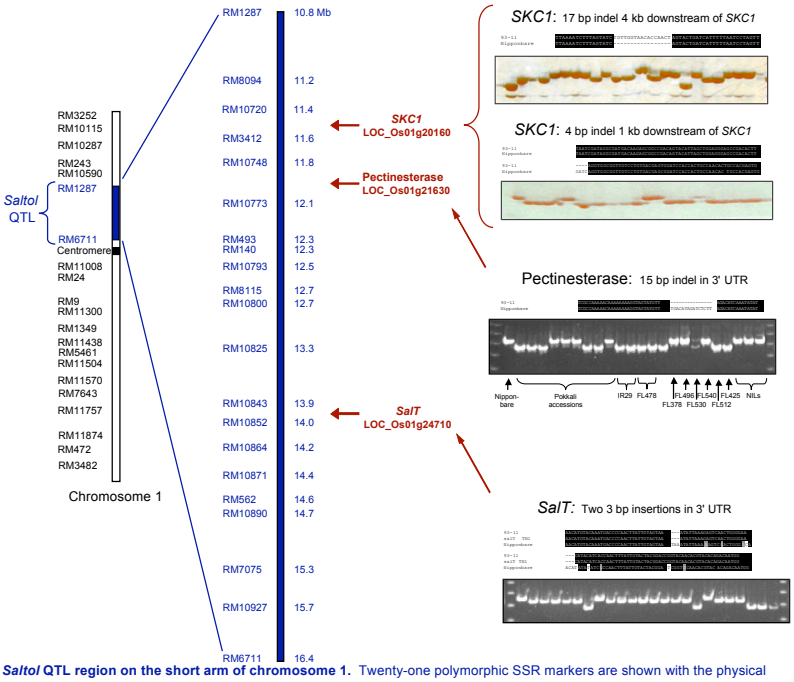
SSR surveys: **320 SSRs were tested using 23 accessions to identify polymorphic SSRs at *Saltol* and evenly spaced across all 12 rice chromosomes. SSR products were run on 10 cm acrylamide gels and stained with SYBR-safe stain. Polymorphic markers can be selected for different MAB parent combinations.**

Marker-assisted backcrossing



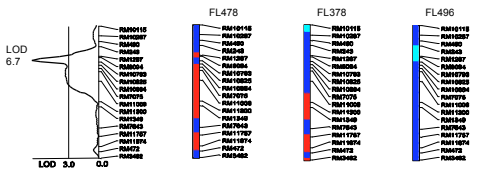
➢***MAB scheme***: **Example using Pokkali as the *Saltol* donor and BR28 as the recipient line, with the number of genotyped individuals given in parentheses. SSRs are used to select for the *Saltol* QTL itself (foreground selection), select for recombinants on each side of the Pokkali *Saltol* target (recombinant selection) and to select against Pokkali introgressions across the rest of the genome (background selection).**

Saltol: a major QTL for salt tolerance



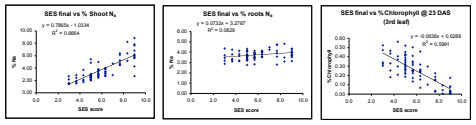
***Saltol* QTL region on the short arm of chromosome 1.** Twenty-one polymorphic SSR markers are shown with the physical map position in megabases (TIGR pseudomolecule version 4). Three candidate gene loci were targeted for developing gene-based markers. Nipponbare by 93-11 sequence alignments were used to identify insertion/deletions (indels), flanking primers were designed, and 1-2 polymorphic indel markers for each locus were selected and used to genotype a set of RILs and NILs for further mapping of *Saltol*.

Graphical genotypes of donor RILs for *Saltol*

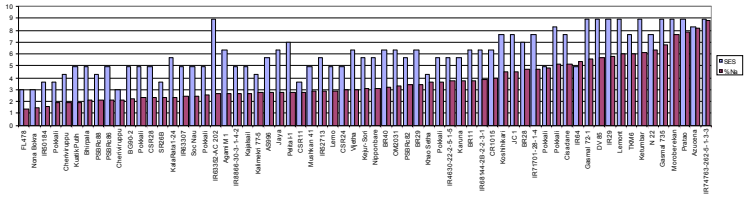


A QTL study using 140 RILs (IR29 x Pokkali) and 100 SSRs confirmed the *Saltol* QTL location on the short arm of chromosome 1. The shoot Na^+/K^+ ratio for seedlings grown under EC 12 dS m^{-1} in a hydroponic system gave a LOD of 6.7 and R^2 of 20% for this QTL. The graphical genotypes of three tolerant RILs are shown at right (Pokkali alleles are dark blue, IR29 alleles are red, and missing data is light blue). FL478 is currently being used as the primary donor for *Saltol* in MAB programs at IRRI.

Physiological traits across diverse germplasm



Across 65 diverse lines, the standard evaluation score (SES) has a high correlation with shoot $[Na^+]$ and % chlorophyll but not with root $[Na^+]$ under EC 12 dS m^{-1} in a hydroponic system. These diverse lines will be used for association testing for salinity tolerance at candidate gene loci.



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