QTL Mapping, Candidate Gene Analysis, and Marker-assisted Backcrossing for Improved Salinity Tolerance in Rice

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Salt stress is a major constraint across large rice-producing areas because of the high sensitivity of modern rice varieties. Tolerance for salinity is complex, involving a number of different physiological mechanisms, including sodium exclusion from roots, controlled sodium transport between roots and shoots, and sequestering of sodium in older tissues and in the vacuoles. Although many genes likely contribute to salt tolerance, a major quantitative trait locus (QTL) of surprisingly large effect has been identified from the salt-tolerant landrace 'Pokkali'. We are currently fine-mapping the *Saltol* QTL, integrating gene expression data with candidate gene analysis, and developing a marker-assisted backcrossing system to transfer the Pokkali *Saltol* allele into popular varieties. A long-term goal is to identify and combine QTLs/genes controlling different physiological mechanisms to achieve a higher level of salt tolerance in popular rice varieties for a wide range of coastal and inland salt-affected areas.

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 breeding (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

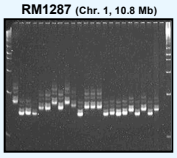
Specific activities

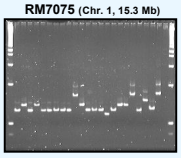
- Identify polymorphic simple sequence repeats (SSRs) and develop gene-based markers across the *Saltol* region to increase marker resolution
- Genotype larger near-isogenic line (NIL) populations to identify additional recombinants and increase the fine-map resolution of the *Saltol* QTL
- Determine 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

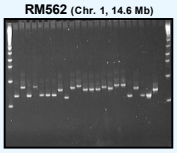
Identifying polymorphic SSRs

23 accessions in the SSR survey:

1. FL478
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>

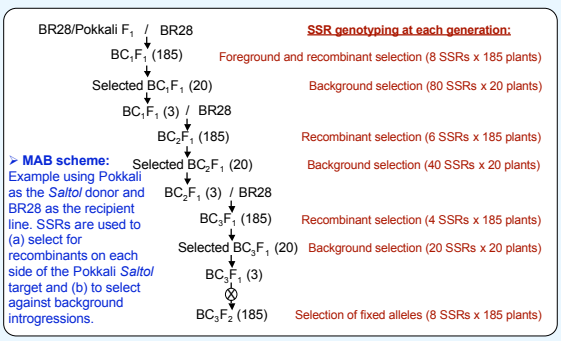






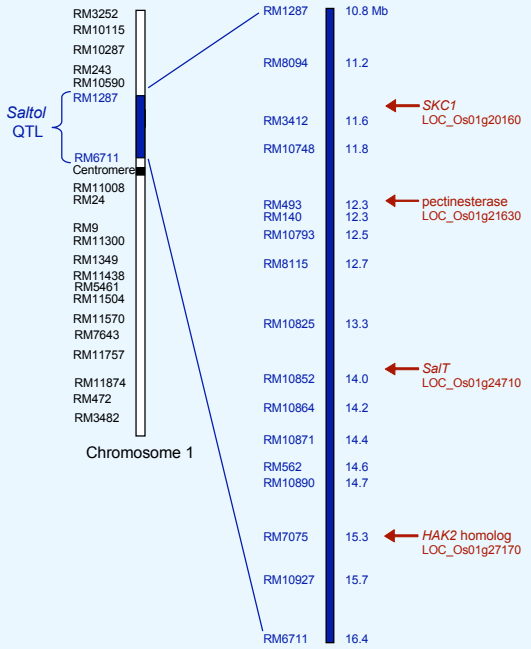
SSR surveys: 320 SSRs were tested using 23 accessions to identify polymorphic SSRs at *Saltol* and evenly spaced across all 12 rice chromosomes. For the Pokkali and IR29 parents, 144 polymorphic SSRs were identified. SSR products were run on 10 cm acrylamide gels and stained with SYBR-safe stain.

Marker-assisted backcrossing



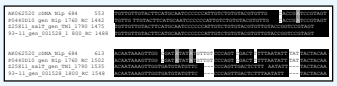
➢ MAB scheme: Example using Pokkali as the *Saltol* donor and BR28 as the recipient line. SSRs are used to (a) select for recombinants on each side of the Pokkali *Saltol* target and (b) to select against background introgressions.

Saltol: a major QTL for salt tolerance



***Saltol* QTL region on the small arm of chromosome 1.** Polymorphic SSR markers are shown with the physical map position in megabases (TIGR pseudomolecule version 4). Four loci targeted for developing gene-based markers are shown on the right with the TIGR gene locus identifiers.

Gene-based markers

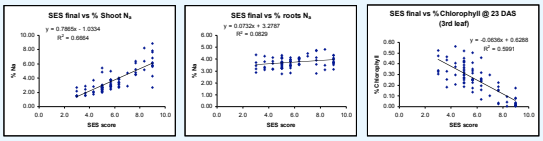


***SalT*:** Alignment of *SalT* sequences from Nipponbare, 93-11, and TN1 showing two 3-bp insertion/deletions in an exon, which can be used to design an indel marker.

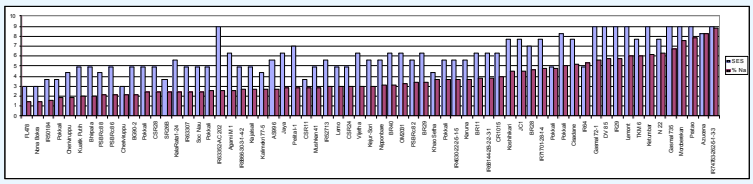
Pectinesterase: Alignment of Nipponbare and 93-11 sequences from a pectinesterase gene showing an 8-bp insertion/deletion in an intron.

***SKC1*:** Alignment of SKC1 sequences from Nipponbare and 93-11 showing a 2-bp and 1-bp insertion/deletion in an intron.

Physiological traits



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 hydroponic solution.



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