

# Marker assisted backcrossing of *Saltol* and mapping novel QTLs associated with salinity tolerance in rice by selective genotyping

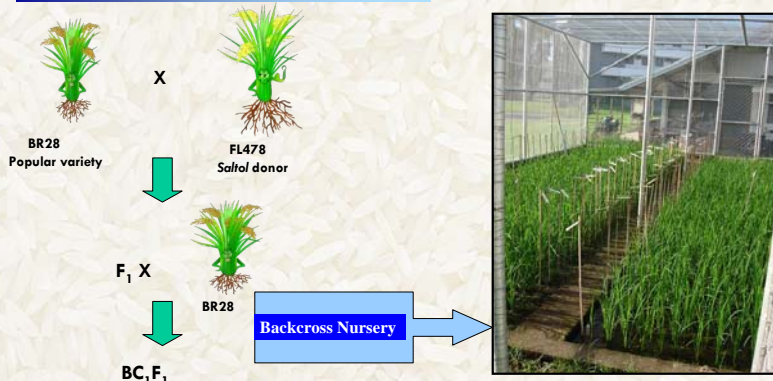
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## Introduction

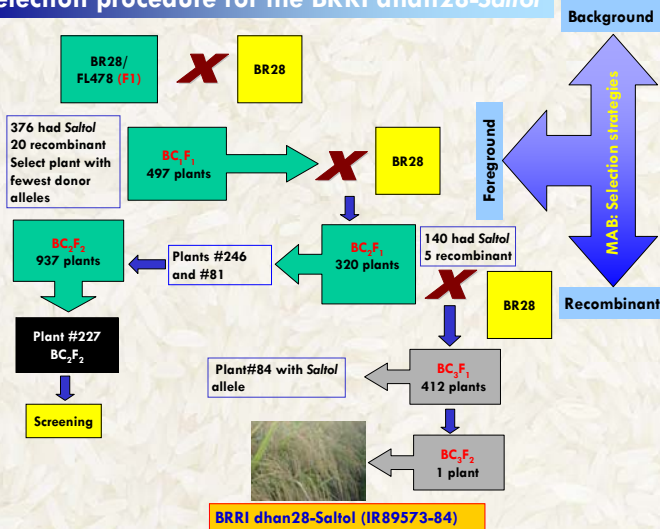
Salinity is a serious abiotic stress in rice growing areas in Asia. Salinity tolerance in rice varies with growth stages; being relatively tolerant during germination, but sensitive during early seedling stage. Improved varieties are needed that combine high grain yield with salinity tolerance. QTLs with relatively large effect can be transferred into mega varieties through marker assisted backcrossing (MABC), to improve their tolerance. If mega varieties like BRRI dhan28 (BR28) a popular dry season variety in Bangladesh, can be converted into salt tolerant types, a significant portion of the rice supply in Bangladesh can be met in the future from increased production in saline coastal areas. We introgressed *Saltol*, a major QTL associated with salinity tolerance in rice, into BR28 through MABC. In addition, Capsule, a salt-tolerant, widely adapted Bangladeshi *indica* landrace, was used to identify new additional QTLs for salinity tolerance. A molecular linkage map was constructed using 105 SSR and insertion/deletion (Indel) markers. QTLs were identified on the long arm of chromosome 1 (in a different region than *Saltol*) for Na<sup>+</sup> uptake, Na<sup>+</sup>/K<sup>+</sup> ratio and survival; on chromosome 3 for Na<sup>+</sup> uptake, survival and visual tolerance scores (SES); and chromosome 5 for K<sup>+</sup> uptake and SES scores. Thus Capsule provides an alternative source of novel salinity tolerance QTLs, aside from Pokkali, and this will be useful for future QTL pyramiding.

## Materials and methods

### Generation of backcross population



### Selection procedure for the BRRI dhan28-*Saltol*



### QTL analysis

- An F<sub>2:3</sub> mapping population comprising two extreme tail 47 tolerant and 47 sensitive individuals was evaluated for 9 physiological and agronomic traits
- DNA extraction was achieved using miniprep protocol
- About 400 SSR and Indel markers were used to survey parental polymorphism
- Genotyping of the population using identified SSR markers and construction of molecular linkage map using QGene version 3.9.1.1 (Nelson, 1997).
- Simple marker analysis, simple interval mapping and composite interval mapping were performed to detect QTLs

## Results and discussion

### Introgression of *Saltol* locus into BRRI dhan28



Graphical genotype of the BR28-*Saltol* (BC3F2) plant homozygous for the recipient genome except for the *Saltol* region on chr. 1. The short arm of chr. 1 containing *Saltol* introgressed from FL478 is shown in blue.

### Phenotypic evaluation of BRRI dhan28-*Saltol* (IR89573-84)

Effects of salinity on BR28-*Saltol* lines (NIL-84 and NIL-163) and checks in a phytotron experiment. Data are means of three replications with 5 subsamples per replication.

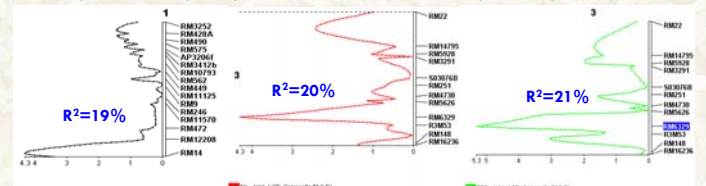
Genotype	SES score <sup>1</sup>		% Survival		Concentration (mmol g <sup>-1</sup> dwt)		Na-K ratio
	a	b	a	b	[Na <sup>+</sup> ]	[K <sup>+</sup> ]	
BR28- <i>Saltol</i>	4.2	5.3	93.5	58.3	0.015	0.492	0.0179
NIL-163	5.7	5.8	82.1	48.6	0.031	0.484	0.0383
BR28	7.0	9.0	63.0	0.0	0.123	0.490	0.1479
IR29	8.3	9.0	59.8	0.0	0.196	0.462	0.2499
FL 478	3.3	4.3	100.0	91.7	0.008	0.443	0.0113
Mean	5.7	-	-	-	0.075	0.474	-
Significance	***	-	-	-	***	***	-
LSD <sub>0.05</sub>	0.69	-	-	-	0.0018	0.008	-
CV	6.4	-	-	-	1.6	1.2	-

<sup>1</sup>SES scale 1-9 (1 = most tolerant, 9 = most sensitive)

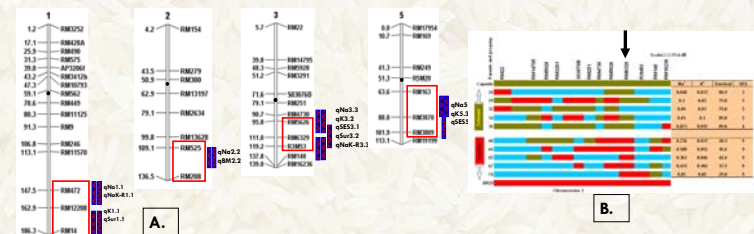
<sup>2</sup>Salinization starts at 2 weeks after seeding (EC=12 dS/M)

\*\*\* Significant at the 0.001 probability level

<sup>3</sup>Salinization starts at 5 days after seeding (EC=12 dS/M)



QTL likelihood curves of the LOD score of a) Na<sup>+</sup>-K<sup>+</sup> ratio on chromosome 1, b) Na<sup>+</sup> uptake and c) overall phenotypic performance (SES) for chromosome 3, that were above the significance threshold of LOD = 3.0 and explained approximately 20% of the phenotypic variance for each QTL.



A) Locations of large effect QTLs in the linkage map of chr. 1, 2, 3 and 5. The blue bars represent novel regions of QTLs with allelic effect. B) Chromosomal constitution of 5 salt-tolerant and 5 salt-sensitive F<sub>2</sub> individuals for chromosome 3. The sky blue indicates heterozygous genotypes at the marker loci, the green indicates homozygous donor genotype and red color indicates sensitive parent BR28 alleles. The names of markers are listed at the top, with an arrow at the QTL position at RM6329. Apparently salt tolerant individuals had the favorable QTL alleles (homozygous or heterozygote alleles from Capsule) and the salt sensitive individuals had opposite QTL alleles at RM6329.

## Conclusions

- BR28, a mega variety was converted to salt tolerance type using MABC. Seeds of BR28-*Saltol* (IR89573-84) were delivered to NARES partners in Bangladesh for field trials.
- Twenty nine QTLs with significant threshold of P < 0.001 (corresponding to an interval mapping or a composite interval mapping LOD > 3.0) were identified. The LOD of these QTLs ranged from 3.0- 5.3 with phenotypic variation explained by those QTLs ranging from 12- 26%.
- qNa1.1, qNaK-R1.1, qK1.1 qSur1.1 and qNa3.3, qK3.2, qNaK-R3.1, qSur3.2 and qSES3.1 were co-localized for 4 traits i.e. Na<sup>+</sup> uptake, K<sup>+</sup> uptake, Na<sup>+</sup>-K<sup>+</sup> ratio and survival (%) on long arm of chr 1 and on chr 3, indicating the functional relatedness among the traits and salinity tolerance.

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## Reference

Nelson, J. C. 1997. QGene: software for marker-based genomic analysis and breeding. *Mol. Breed.* 3: 239-245