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Salinity tolerance QTL mapping from Coastal Aus Landrace Boilam.

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Abstract: *There is a dearth of known donors for salt tolerance traits in rice breeding programs other than Pokkali and Nona Bokra. These landraces are photoperiod sensitive and flower once a year, so that they are poorly compatible with breeding cultivars. Bangladeshi coastal landraces have been found to be biodiverse and salt tolerant accessions are still widely cultivated by farmers. These landraces may be potential donors for novel salt tolerance traits. One of these coastal varieties, Boilam, is both early-maturing as well as photoperiod insensitive and popularly grown as an Aus variety in Noakhali, in the southeast of Bangladesh. Boilam was therefore characterized and analyzed for QTLs linked to salt stress tolerance in a backcross population with a modern Aus variety BRRI dhan27. In order to identify and map salinity tolerance traits, a backcross population was generated between Boilam, and BRRI dhan27, a high yielding Aus variety. A total of 94 polymorphic SSR markers were used to construct a linkage map. 96 BC_2F_2 progeny were selected from both extremes of the population of 200 at the seedling stage with respect to their response to salinity stress at 12 dS/m. Composite interval mapping (CIM) using the linkage map revealed that the salinity tolerance loci were located on chromosomes 1, 4, 9 and 12. The identified QTLs were above the threshold LOD of 3.0 with significant R^2 values ranging from 18.10% - 22.40% as expression of phenotypic variance. Similar results were obtained using single marker regression (SMR), simple interval mapping (SIM) as well as composite interval mapping (ICIM) by QGene v-4.0. Both phenotypic and genotypic data revealed that Boilam can be used as a novel salt tolerance donor at seeding stage. Tolerant BC_2F_3 progenies having the background genotype of BRRI dhan27 will be developed further for release or use as parents with the help of the markers-linked to the salt tolerance QTLs for rice breeding programs aimed at increasing tolerance of rice to salt stress.*

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I. Background

Soil salinity is a constraint to rice production in both inland irrigated areas as well as coastal regions. Despite its high sensitivity to salinity, considerable variation in tolerance was observed in rice (Akbar *et. al.*1972). Salinity affects rice growth in varying degrees at all stages starting from germination to maturation. The genetic complexity of salt tolerance and the strength of genotype and environment interactions make the trait very complex. Thus breeding for salt tolerance has been slow for all crop plants all over the world. Pokkali, a well known and popular Indian salt tolerant donor has been widely used for the development of salt tolerant rice varieties. However, success has been limited principally due to the difficulty of recovering elite genotypes with the salt tolerance trait (Gregorio *et. al.* 2002). Due to shrinking land resources, the production of rice is also being pushed to unfavorable environments (coastal regions, flood-prone, as well as low-lying areas). Due to the prevalent abiotic soil stresses, there is a strong need to combine high yield with salinity tolerance in rice. Therefore the most economic and sustained way to overcome problems of food scarcity will be to develop durable salt tolerant high-yielding rice varieties suitable for growth in coastal areas, particularly for the Southern region of Bangladesh. Due to the high sensitivity of modern rice to the effects of salinity, farmers in the Southern regions are forced to continue growing of traditional landraces which have low yields. These landraces may however be a good source as donor of salt tolerance traits. The traditional Bangladeshi rice landrace Boilam is early-maturing and photo-insensitive in contrast to Pokkali and shows both seedling and reproductive stage tolerance (A.K.Kaul. 1982). It is popularly grown in salt-affected areas in the South coast of Bangladesh and gives average yields of ~1.2 t/h. Boilam is characterized by its red pericarp color. Breeders in Bangladesh have earlier tried unsuccessfully to develop salt tolerant rice varieties by using Boilam as a donor. Molecular breeding therefore may prove to be an appropriate solution for producing salt tolerant high yielding rice. This strategy would involve QTL discovery in the donor, fine-mapping of the most promising loci providing tolerance, and use of Marker-assisted Backcrossing (MABC) for transfer to popular varieties (Thomson *et. al.* 2003). The study of natural variation in diverse donors enhances the functional analysis of plant genomes (Alonso-Blanco and Koornneef 2000; Yano 2001), which in turn increases the potential use of such variation in

international breeding programs. QTL analyses of salt tolerance traits in rice, has been conducted by several other research groups, such as Zhang et al. 1995, Gong et al. 1999 and 2001, Prasad et al. 2000, Lang et al. 2001a and b, Koyama et al. 2001, Bonilla et al. 2002, Lin et al. 1998 and 2004, Takehisa et al. 2004, Ammar 2004, Ren et al. 2005, Ming et al. 2005, Lee et al. 2007, Zang et al. 2008, Sabouri and Sabouri 2008, out of which three reports used Pokkali as a donor, one, Nona Bokra and the rest were from uncharacterized ones. Nona Bokra is similar to Pokkali in that it is also a photosensitive, long duration variety. In addition to use of diffrent donors, multiple traits as well as marker-types were used for these analyses, making correlation difficult. Moreover, Boilam is a novel donor, due to its being an early-maturing and photo-insensitive cultivar. Therefore this study was undertaken to properly characterize its tolerance at seedling, vegetative and reproductive stages and to identify major quantitative trait loci (QTL) linked to the seedling salt tolerance trait. A backcross mapping population between Boilam and BRRI dhan27 was generated and the BC_2F_2 population analyzed by molecular marker technology for identification of new traits/genes that can be used for breeding purposes.

II. Materials and Methods

Plant material and DNA extraction: Screening and scoring of 200 BC_2F_2 progenies were accomplished in the IRRI Phytotron according to IRRI SES procedure (Glenn B. Gregorio, 1997). We took the advantage of selective genotyping as it can markedly decrease the number of individuals genotyped for a given power at the expense of an increase in the number of individuals phenotyped. 96 BC_2F_2 progenies which were selected from both extremes of the population (200) with respect to the response to salinity at 12 dS/m for seedling stage. Young leaves were collected in liquid nitrogen, crushed to powder and total DNA was isolated using the CTAB method (Doyle,1990) and quantified by Nano drop spectrometer.

Construction of linkage map: From the preliminary screening of 550 SSR markers on two parental DNA Boilam and BRRI Dhan-27, 94 polymorphic SSR markers were selected to genotype the mapping population. By combining the SSR markers with selective genotyping, we constructed a linkage map and assigned each linkage group to a corresponding chromosome.

Polymerase chain reaction (PCR) and genotyping: PCR mixture was prepared and amplified using the PCR settings as initial denaturing at 94°C for 5 min, followed by 35 to 40 cycles of denaturizing at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, with an extension at 72°C for 7 min in a PCR thermal cycler. Polyacrylamide gel electrophoresis (PAGE) was used because it has much higher resolution and reveals unambiguous polymorphic bands among the amplified DNA than agarose gel electrophoresis. The PCR products are separated on non-denaturing polyacrylamide gels with a thickness of 1.5 mm (8% Polyacrylamide gel: 10 ml of 40% polyacrylamide (Invitrogen), 4 ml 25x TBE, 500 µL 10% APS, 42.5 µL TEMED, ddH20 up to 100 ml; pH 8.0; 1x TBE buffer; PH 8.0). Depending on the Molecular weight of the amplified products, 6 to 10% Polyacrylamide gel is used. The gel is stained by immersing it in electrophoresis buffer containing SYBR-Safe stain for 10 minutes at room temperature and then photographed by AlphaImager HP (Alpha Innotech).

Statistical analysis: QTL analysis was performed with QGene v-4.0 by applying a general interval mapping. The hypotheses was that a single locus or two linked loci have an effect on tolerance to salinity were evaluated. Permutation tests were performed 1000 times on the hypothesis that one locus on a chromosome has an effect on the SES (H1) versus the null hypothesis (H0) that the locus has no effect on the SES. The model with the highest LOD score was fitted to the QTL and when the models did not differ significantly the simpler model was chosen. LOD threshold was selected at 3.0. Single marker regression (SMR), Simple interval mapping (SIM) and Composite Interval Mapping (CIM) and Inclusive Composite Interval Mapping (ICIM) were done by QGene v-4.0.

III. Results

From the preliminary screening of 550 SSR markers on the two parental DNA of Boilam and BRRI dhan27, 94 polymorphic SSR markers were selected to genotype the mapping population. 96 BC_2F_2 progeny were selected from both extremes of the population of 200 with respect to their response at 12 dSm^{-1} salinity stress at seedling stage. Of the total population 27% were found to be tolerant which SES score 3 to 4 and 29% were sensitive with SES scores of 8 to 9 (Fig.1). Such selective genotyping, where 25% of the total population is adequate for QTL determination has been reported previously (Darvasi and Soller, 1992; Ronin et al. 2003).

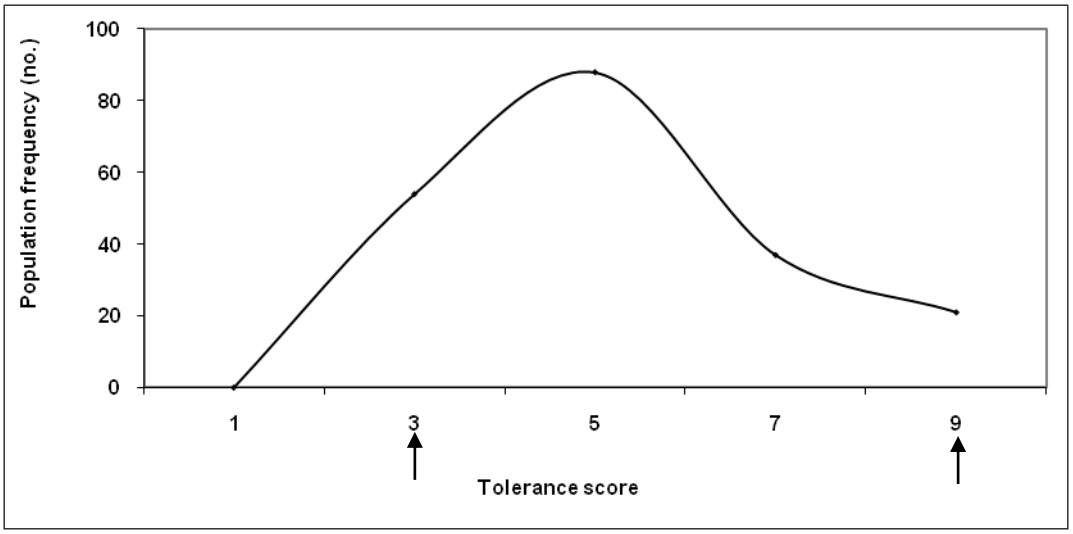

Figure 1: Distribution of BC_2F_2 progeny against salinity stress (EC 12 $dS^{-1}m$) at seedling stage. Black arrows indicate the position of parents.

94 SSR primers were used to construct the genetic linkage map. 8 Qtls were identified by Single Marker Regression (SMR) using Win QGene. Markers linked to QTLS were RM490 (6.7 cM, Chr 1) RM8094 (11.0 cM, Chr 1), RM5749 (20 cM, Chr 4), R9M30 (15 cM, Chr 9) RM257 (18 cM, Chr 9), RM27877 (9.2 cM, Chr 12) RM28746 (26 cM, Chr 12), RM17 (27cM, Chr 12) with LOD threshold level of 3.0 and R^2 ranging from 16.9%-22.3% (Table 3). Analyses using Simple Interval Mapping (SIM), Composite Interval Mapping (CIM) and Inclusive Composite Interval Mapping (ICIM) reduced the number of significant QTLs to 4, namely the ones at RM490, RM5749, RM257 and RM28746 with LOD scores of 5.18, 4.86. 4.56 and 4.07 respectively and R^2 values ranging from 18.1% -22.4% (Table 1 and Fig. 2). At least 1000 Permutations were performed to get the analyzed data. The Shapiro-Wilk normality test confirmed the null hypothesis that the phenotypic data were normally distributed. Alpha level of 0.865 and 0.0 p-value further supported this conclusion.

Table 1: QTL statistics for single marker regression and composite interval mapping

Chromosome	Locus name	SMR					CIM			
		QTL Pos (Mbp)	Add effect	F(add)	LOD	R^2	Add effect	F(add)	LOD	R^2
1	RM490	6.7	0.99	17.661	5.162	0.223	1.03	22.605	5.184	0.224
1	RM8094	11	0.998	13.725	3.537	0.159	-	-	-	-
4	RM5749	20	1.23	23.729	4.865	0.212	1.23	23.729	4.865	0.212
9	R9M30	15	1.096	25.716	5.241	0.226	-	-	-	-
9	RM257	18	1.095	20.936	4.566	0.2	1.095	20.936	4.566	0.2
12	RM27877	9.2	0.851	17.98	3.776	0.169	-	-	-	-
12	RM28746	26	0.815	13.466	4.068	0.181	0.815	13.466	4.068	0.181
12	RM17	27	0.938	17.231	4.55	0.2	-	-	-	-

Significance at the level of 0.01%

SIM (Simple interval mapping and ICIM (Inclusive composite interval mapping) also shows the similar results (data not shown). Identified QTLs are presented in Fig 2.

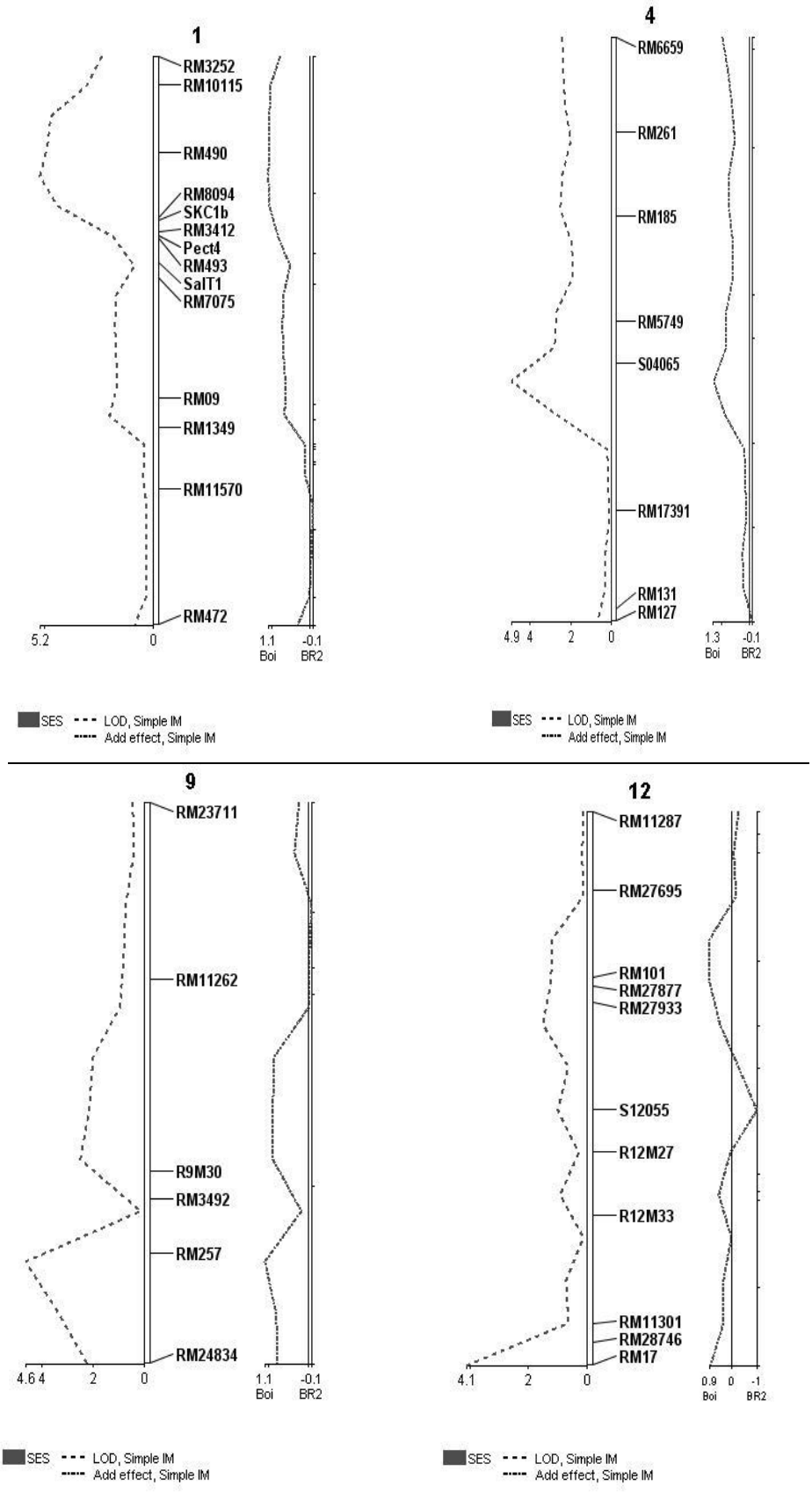

Figure 2: LOD curve

IV. Discussion

The 550 molecular markers tested for polymorphism between the two parental DNA, BRRI dhan 27 and Boilam consisted of 530 SSRs, 10 InDels and 10 gene-based markers. Among them 84 SSRs, 3 InDels and 7 gene based markers were found polymorphic between Boilam and BRRI dhan 27 which are both *indica* cultivars (Supplimentary data table1). SSR marker sequences were collected from Gramene marker database (http://www.gramene.org/db/markers/marker_view). Markers based on InDels (sequences with an insertion/deletion) were collected from the study of Ying group (Ying-Jia Shen, 2004). More polymorphic primers in chromosome 11 were not found and only three SSR polymorphic markers were used,. Out of the 8 QTLs initially detected in the BC_2F_3 population, 4 were reconfirmed using multiple statistical software. An individual QTL may be described as major or minor based on the proportion of the phenotypic variation explained by a QTL (R^2 value) and a value of >10% is considered major. QTL that are stable across the environment are also sometimes referred to as major while sensitive ones called minor (Li *et al.* 2001; Lindhout, 2002; Pilet-Nayel, *et al.* 2002). So the 4 QTLs in this study can be considered as major QTLs for salinity tolerance traits of Boilam. Different normality tests were done to satisfy the normal distribution of the phenotype data as expression of phenotype data has a significant impact QTL mapping. In the normality test the null hypothesis for this test was that the phenotype data were normally distributed. By analyzing different normality tests we found our hypothesis was not rejected i.e. our phenotype data were evenly distributed. The values shown were significant, such as Shapiro-Wilk (0.865, p = 0), Cramer-Von Mises (0.741, p = 0), Anderson-Darling (4.528, p = 0), D'Agostino-Pearson (6.904, p = 0.0317), Jarque-Bera (8.452, p = 0.0146), Shapiro-Francia (0.875, p = 0), Kolmogorov-Smirnov-Lilliefors (0.254, p = 0) (Data are not shown). All data were generated by Win QGene. Prasad et al. (2000) mapped a QTL for salt tolerance on chromosomes 1 and 6. Lin developed an F_2 population derived from a cross between a high salt-tolerance indica variety, Nona Bokra, and a susceptible elite japonica variety, Koshihikari and that mapping population was used for QTL mapping for physiological traits related to rice salt-tolerance. Three QTLs for survival days of seedlings (SDSs) under salt stress were detected on chromosomes 1, 6 and 7, respectively, and explained 13.9% to 18.0% of the total phenotypic variance (H. X. Lin, 2004). *'Saltol'* was mapped on chromosome 1 using a population generated from a cross between the sensitive variety IR29 and a tolerant landrace, Pokkali and it explained more than 70% of the variation in salt uptake in this population (Bonilla, 2002). It was shown proven that Boilam has a significant difference at their salinity mechanism other than Pokkali and Nona Bokra in this current study as we detected four QTLs for seedling stage tolerant on chromosome 1, 4, 9 and 12 respectively and explained 18.10% -22.40% total phenotyping variance. All detected QTLs have additive effect from Boilam.

V. Conclusion

Boilam was found to be a very useful salt tolerant donor landrace since the derived population developed showed some of its superior characteristics. In a targeted approach, emphasis will be given to fine-mapping of the newly discovered QTL at 18.0 cM identified by RM257 on Chr 9 with LOD score of 4.55 as it explained 20% of the total phenotypic variance. Some of the tolerant BC_2F_3 progenies were found to have the background genotype of the agronomically superior BRRI dhan27 in addition to salt tolerance traits. These which will be developed further for release or use as parents in breeding programs with the help of the markers-linked to the salt tolerance QTLs.

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