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QTL MAPPING FOR SALINITY TOLERANCE USING AN ELITE RICE (*Oryza sativa*) BREEDING POPULATION

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SUMMARY

A recombinant inbred line (RIL) population was previously developed with two high yielding rice varieties, At354, a salt tolerant parent and Bg352, a salt susceptible parent aiming at identifying salinity tolerant QTLs together with varietal development. In this study we used 100 F5 RILs of At354 x Bg352 to characterize the population and map genes for salt tolerance. Nine morpho-physiological parameters related to salinity tolerance were assessed under hydroponics supplemented with 100 mM NaCl concentration (12 dS/m). Frequency distributions of these 9 morpho-physiological traits, standard evaluation score, salinity survival index, shoot length, root length, shoot dry weight, root dry weight, shoot Na^+ concentration, shoot K^+ concentration and shoot Na^+/K^+ ratio indicated the broad spectrum of genetic variability in the RIL population under salinity stress while correlation coefficients also were significant with each other. Broad sense heritability also proved that the population was suitable for the gene mapping for salinity tolerance. Composite Interval mapping revealed 6 QTLs distributed in chromosome 1 and 4 namely, *qSSI1, qSL1, qSNK1, qSL4, qSNK4* and *qSSI4*, explaining 10.8%, 10%, 8.9%, 15%, 11% and 16% of the phenotypic variations respectively. In all QTLs At354 allele contributed in favour of salinity tolerance. However, closer flanking markers could not be detected due to the low rate of polymorphism in SSR markers which generated low density molecular map. Therefore, further studies incorporating high throughput marker technologies would be necessary for detecting QTLs with narrow marker intervals.

Key words: Elite population**,** *Oryza sativa*, QTL mapping, recombinant inbred lines, salinity tolerance, SSR markers

Key findings: A mapping population derived from 2 elite rice varieties At354 and Bg352 was characterized with morpho-physiological parameters under salinity stress and the results revealed that the population was suitable for mapping genes for salinity tolerance, though it rendered elite agronomic background. A total of six QTLs, namely *qSSI1, qSL1, qSNK1, qSL4, qSNK4* and *qSSI4* were detected on chromosome 1 and 4 explaining 10.8%, 10%, 8.9%, 15%, 11% and 16% phenotypic variation respectively.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important grains in Asian countries and it is consumed by two thirds of the world population as the staple food. With the escalating growth rate of world population the demand for rice is increasing year by year. Therefore, usage of marginal lands like saline affected areas is important to counteract the challenges in rice production. It is estimated that within 30 years world population will increase by 2 more billions (Mohanty *et al*., 2013). Hence, the food production has to be increased accordingly. As the lands accessible for agricultural purpose are also decreasing it is necessary to maximize the utility of available lands.

Salinity is considered as a serious constrain in rice growing areas and among abiotic stress, salinity has been recognized as the second most wide spread problem for reduction in growth and productivity of rice in all over the world (Gregorio, 1997). Millions of hectares in coastal regions face salinity due to marine/brackish water intrusion to the ground while the inland salinity can occur due to many reasons like poor irrigation, digestion of ores, human activities *etc*. Salinity has covered over 7% of land worldwide which can be technically used for the crop cultivation (Szabolcs, 1994). In Sri Lanka approximately 13% of the irrigated lands are affected by salinity stress (Thiruchelvam and Pathmarajah, 1999) and this percentage is gradually increasing in both coastal regions and inlands.

Most of the rice varieties are extremely sensitive to salinity during young seedling stage and early development stage (Heenan *et al*., 1988). Mainly there are two methods by which rice seedlings are affected by excess salt. Effect of excessive amount of salt present in the soil can rapidly damage the plant by disturbing the osmotic balance to result sudden death of leaves. Prolong damages can be occurred due to the accumulation of salts in tissues which can be interfered with the metabolism like protein synthesis of the rice seedling. Transport of excess salts to older tissues, acquired salt responsive stomata and synthesis of osmo-protectants are some of the mechanisms that are involved with salinity tolerance of rice (Munns and Tester, 2008).

According to the past research studies, QTLs associated with different mechanisms of salinity tolerance located in various chromosomes were identified under different genetic background of rice (Koyama *et al*., 2001; Singh *et al*., 2007; Haq *et al*., 2010; Singh and Flowers, 2010).Of them a major QTL (*Saltol*), was identified on short arm of chromosome 1 using RILs derived from IR29/Pokkali cross accounting 43% of the variation in shoot Na^+/K^+ ratio at the seedling stage (Gregorio, 1997 ; Bonilla *et al*., 2002). Therefore, identification of these QTLs and pyramid them into one line would enable to develop superior cultivars which can be used in marginal lands that are abandoned due to salinity. Considering the broad objective of developing salinity tolerant cultivars by compiling many salinity tolerant QTLs, this study was planned to identify salinity tolerant QTLs using a RIL population derived from two elite rice varieties. This report presents the salinity responsive behavioral variation of the RIL population along with the initiative outcomes of the QTL mapping.

MATERIALS AND METHOD

Genetic background of the RIL population

We used F5 RILs derived fromAt354 and Bg352 developed by Gimhani *et al*. (2014). At354 (*O. sativa) is indica* rice variety derived from Pokkali and Bg94-1 was selected as salinity tolerant parent. Bg352 (*O. sativa) indica* rice variety derived from Bg380/Bg367-4 cross was selected as a salinity susceptible parent. Both of them are recommended varieties by the Department of Agriculture, Sri Lanka as they contain elite agronomical characters including short maturity and high yield (Department of Agriculture, 2013).

Preparation of saline enriched hydroponic system

Out of 281 RILs, 100 RILs were randomly selected to assess the morpho-physiological

traits. Screening for salinity tolerance was conducted under the natural daylight in the planthouse at Wayamba University of Sri Lanka under hydroponic system developed by Gregorio *et al.* (1997) with slight modification. The Experimental set up was designed according to the Randomized Complete Block Design with two blocks that were supplied with 10 individual plants from each RIL line along with the control.

Screening set up was prepared by placing the Styrofoam seedling floats with nylon net bottom consists of 100 holes in the basin with 23 L capacity. Seeds were incubated at the room temperature for 48 h for germination. After 2 days once seedlings developed primary roots about 2-3 cm long, they were transplanted to the seedling floats suspended on basin filled with water. After 3 days when seedlings were well established, floats were transferred to the basins containing Yoshida nutrient solution (Yoshida *et al*., 1976). Initially nutrient solution in each basin was salinized to EC of 6 dS m^{-1} by adding appropriate amount of NaCl except for control. After 2 days salinity was increased upto EC of 12 dS m^{-1} (100 mM) by adding appropriate amount of NaCl. The pH of the solution was maintained at 5.0 on daily basis, by adding either 1M NaOH or HCl. Nutrient solution was renewed in every 8 days. Screening was conducted for 21 days from the date of salinization to EC of 12 dS m^{-1}. Simultaneously control setup was also maintained under the similar experimental conditions without salinization.

Analysis of morpho-physiological traits

RILs were phenotypically assessed using 6 morphological parameters., standard evaluation score (SES), salinity survival index (SSI), shoot length (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW) and 3 physiological parameters, shoot Na^+ concentration (SNC), shoot K^+ concentration (SKC) and shoot Na^+/K^+ ratio (SNK) under hydroponics supplemented with 12 dS/m. SES was assessed as reported by Gregorio *et al.* (1997)

A quantitative parameter called salinity survival index (SSI) was assessed as described by (Wijerathna *et al*., 2014) using the equation:

$$SSI = \sum_{k=1}^{n} D_k S_k \Big/ \left(\sum_{k=1}^{n} D_k\right) 100$$

Where, D is the Day after salinization (DAS), S is the survival percentage of that particular day, n is the total period in DAS (in this experiment n is 21 DAS), D_k is the DAS at k^{th} data collection, S_k is the survival percentage of k^{th} data collection and k = 1, 2, 3…. n.

SL, RL, SDW, RDW, SNC, SKC and SNK were measured 21 days after salinization. For the analysis of shoot Na^+ and K^+ concentrations, extractions were prepared according to the procedure given by Thomson *et al.* (2010) and the samples were measured by the flame emission spectrometry at International Rice Research Institute (IRRI) using a flame photometer (Sherwood Flame photometer, Model 420, Cambridge, UK) at 589 nm.

Analysis of variance

Significant differences among the mapping population for the parameters SL, RL, RDW, SDW, SSI, SNC, SKC and SNK were detected by Analysis of variance (ANOVA) using SPSS 16.0 (SPSS, 2007). All the measured phenotypic parameters of parents were compared with Student's t-test. Correlation coefficients also were calculated and frequency distributions were drawn using MINITAB V17.0 (Minitab17, 2010).

Broad sense heritability

Broad sense heritability (BSH) was calculated for all the traits except SSI and SES using the following formula described by Falconer and Mackay (1996) and Hosseini *et al*. (2012).The genotypic variance (V_G) was obtained by calculating the difference between the total phenotypic variance (V_P) and the environmental variance (V_E). The mean phenotypic variance observed within parental genotypes (At354 and Bg352) was considered as the environmental variance while the variance showed by the 100 F5 RIL population was taken as the total phenotypic variation of each trait.

$$BSH\ (H^2) = (V_P\text{-}V_E)/V_P$$

Genotyping of RILs

Genomic DNA was extracted from the 100 RILs according to the method presented in http://rgp.dna.affrc.go.jp/rgp/protocols/QTL.pdf. At354 and Bg352 parents were screened for the polymorphism with 158 SSR/InDel markers using polymerase chain reaction (PCR). The primer sequence information was obtained from the published sequence database (IRGSP, 2005 andwww.gramene.org). Amplified PCR products were analyzed using 3% agarose and 8% polyacrylamide gel electrophoresis (PAGE). Using the gel profiles, respective alleles were manually scored.

QTL analysis

As reported by Thomson *et al*. (2010), Sandhu *et al*. (2014) and Ye *et al*. (2011) marker distances were approximately estimated in centiMorgan (cM) by multiplying the physical position (Mb) of DNA markers by factor of 4. These cM positions were used for QTL analysis by composite interval mapping (CIM) using QGene 4.3.10. Experiment-wise LOD threshold values at the 0.05 and 0.01 significance level for each trait were estimated based on the 1000 times permutation test (Churchill and Doerge, 1994; Doerge and Churchill, 1996). The coefficient of determination (R^2) was estimated (McCouch *et al*., 1997) and QTLs were named following the standard rice QTL nomenclature (McCouch and Committee on Gene Symbolization, Nomenclature and Linkage, Rice Genetics Cooperative, 2008). Accordingly, the name of the each QTL was italicized and initialized with the letter "q" to represent the QTL, followed by two or three letters to symbolize the trait name. After the trait name, the chromosome number was given to indicate the location of the particular QTL. In order to confirm the results obtained from QGene 4.3.10 mapping was repeated with Windows QTL Cartographer v2.5 (Wang *et al*., 2012).

RESULTS

Salinity responsive behavioral variation of the RIL population

In this study 100 RILs were evaluated for salinity tolerance phenotypically along with 2 parents At354 and Bg352 using 9 morpho-physiological parameters. Mean performances of the parents and the RIL population for all the measured morpho-physiological parameters under saline stress are summarized in the Table 1. Accordingly, selected both parents were significantly different for all the measured parameters indicating their divergent performances under saline stress condition. Results of the analysis of variance revealed that differences among RILs were highly significant ($P < 0.01$) for all the quantitative traits (Data not shown). Higher coefficient of variation (CV) was obtained for all traits ranging from 19.32% to 47.48% (Table 1) showing comparatively wider variation in the RIL population.

According to the frequency distributions, all the morpho-physiological parameters except SES (as SES was assigned as categorical basis) showed continuous and normal distribution which lay down within the acceptable range of skewness below +1.5 and above -1.5 (Tabachnick and Fidell, 2013), exhibiting quantitative nature of the traits (Figure 1). Transgressive segregants were also observed across all the measured traits in both directions indicating superior and inferior performances of certain RILs beyond the tolerant and susceptible parents.

The highest BSH for the studied F_5 mapping population was observed for SNC (99%) followed by RDW (98%). The lowest BSH was 57% which was shown by SL. BSH of RL, SDW, SKC and SNK were 74%, 89%, 83% and 81% respectively.

Table 1. Salinity responsive morpho-physiological variation in two parents and RILs.

Trait	Means of Parents		RIL population						BSH
	At354	Bg352	Mean	Min	Max	SD[a]	CV[b] %	Skewness	
SES	3	7	7.07	4.3	9	1.366	19.32	-	
SL (cm)	26.67***	14.78***	21.44	7.92	34.45	6.098	28.44	0.10	0.57
RL (cm)	16.28***	13.53***	14.86	8.500	26.05	2.989	20.12	0.69	0.74
SDW (g)	0.200***	0.056***	0.226	0.056	0.450	0.079	34.99	0.38	0.89
RDW (g)	0.075 ***	0.0375***	0.074	0.016	0.204	0.035	47.48	0.90	0.98
SSI	0.7634	0.2232	0.5405	0.1036	0.9375	0.2191	40.54	-0.21	
SNC (mmol/g)	0.3202***	1.8195***	1.0725	0.3542	1.9360	0.3604	33.61	0.55	0.99
SKC (mmol/g)	0.6396***	0.2814***	0.4052	0.2132	0.6580	0.0894	22.06	0.63	0.83
SNK (mmol/g)	0.5005***	6.512***	2.816	0.696	6.722	1.237	43.93	0.82	0.81

Modified standard evaluation score (SES), salinity survival index (SSI), shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW), shoot Na^+ concentration (SNC), shoot K^+ concentration (SKC), shoot Na^+/K^+ ratio (SNK) and broad sense heritability (BSH)

[a]SD-standard deviation

[b]CV- coefficient of variation

*** significant at $P < 0.001$ according to student's t-test

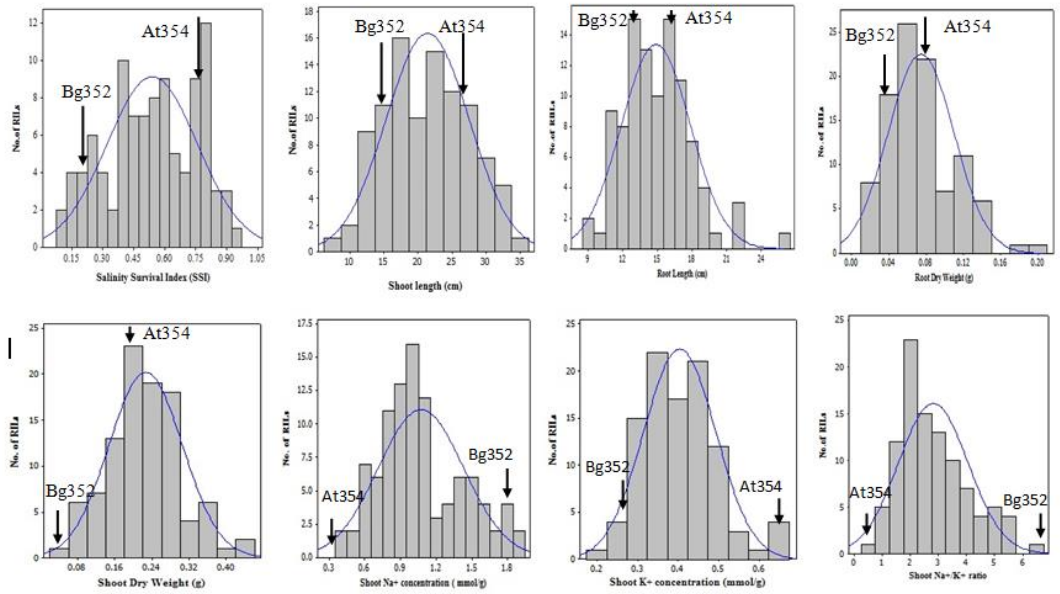


Figure 1. Frequency distributions of salinity survival index, shoot length, root length, root dry weight, shoot dry weight, shoot Na^+ concentration, shoot K^+ concentration and shoot Na^+/K^+ ratio in RIL population derived from At354 x Bg352. Parental means are indicated by arrow.

Correlations among phenotypic characters for saline stress

According to the correlation analysis of the studied traits, SSI was significantly and positively correlated with SL (r = 0.719), RL (r = 0.338), SDW (r = 0.246) and RDW (r = 0.430) above $P < 0.05$ (Table 2). Also it was observed that SNC was inversely correlated with SSI (r = -0.367), at $P < 0.001$. In addition, strong positive correlations were also observed between pair of traits of SL and RL (r = 0.607, $P < 0.001$), SDW and RDW (r = 0.719, $P < 0.001$), SNC and SNK (r = *0.849*, $P < 0.001$) and SKC and SNK (r = *0.626*, $P < 0.001$) (Table 2).

Table 2. Correlations of 8 morpho-physiological traits examined in the RIL population (100 RILs) derived from At354 x Bg352 under salt stress.

	SSI	SL	RL	SDW	RDW	SNC	SKC
SL	0.719***						
RL	0.338**	0.607***					
SDW	0.246*	0.242*	0.168ns				
RDW	0.430***	0.359***	0.244*	0.719***			
SNC	-0.367***	-0.333**	-0.230*	-0.171ns	-0.154ns		
SKC	-0.275**	-0.143ns	0.057ns	-0.040ns	-0.129ns	-0.196ns	
SNK	-0.144ns	-0.213*	-0.205*	-0.091ns	-0.057ns	0.849***	-0.626***

Salinity Survival Index (SSI),shoot length (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW), shoot Na^+ concentration (SNC), shoot K^+ concentration (SKC) and shoot Na^+/K^+ ratio (SNK)
*Significant at $P < 0.05$, **Significant at $P < 0.01$, ***Significant at $P < 0.001$, ns Not significant

SSR/InDel marker polymorphism

Of 158 markers surveyed (149 SSR/9 InDel markers) between two parents 45 markers were found polymorphic. The results based on the marker loci analyzed, revealed 93% overall homozygosity in the RIL population and these data are in line with the expected homozygosity of $F_{5:6}$ RIL populations as reported by Vinod (2006). The highest polymorphism percentage was obtained in chromosome 4 (38.4%) followed by chromosome 1 (31.5%). Therefore, these two chromosomes were selected for the QTL mapping. QTL mapping was not conducted for other chromosomes because reasonable amount of polymorphism could not be obtained.

QTL analysis

QTLs obtained from QGene 4.3.10 and Windows QTL Cartographer v. 2.5 are shown in the Table 3. In chromosome 1, CIM (QGene 4.3.10) revealed 3 QTLs for SSI (*qSSI1*), SL (*qSL1*) and SNK (*qSNK1*) with 2.4, 2.2 and 2.0 LOD scores respectively. The *qSSI1* and *qSL1* were co-located at RM10793 (12.5 Mb) marker position flanked by RM140 and RM10852 markers while *qSNK1*was located at RM10772 (12 Mb) marker position flanked by RM10745 and RM140. Three QTLs, SSI (*qSSI1*), SL (*qSL1*) and SNK (*qSNK1*) were account for substantial proportion of each respective phenotypic variation by 10.8%, 10% and 8.9% respectively. Furthermore, the results obtained from the Windows QTL Cartographer confirmed the co-localization of *qSSI1* and *qSL1* within the same flanking region with the LOD values of 3.3 and 2.7 respectively.

CIM approach based on QGene 4.3.10 revealed another three QTLs for SSI (*qSSI4*), SL (*qSL4*) and SNK (*qSNK4*) on chromosome 4 with 3.3, 2.4 and 3.6 LOD scores respectively. The *qSSI4* and *qSL4* were co-located at 33.5 Mb position flanked by RM3843 and RM280 markers while *qSNK4*was located at 7.5 Mb position flanked by RM518 and RM5749 markers. Three QTLs, *qSSI4*, *qSL4* and *qSNK4*could explain 15%, 11% and 16% of the phenotypic variation observed among the RIL population respectively. Windows QTL Cartographer results confirmed the same flanking marker regions for the three QTLs on chromosome 4. Resulted LOD values for the QTLs were 3.6 (*qSSI4)*, 2.7 *(qSL4)* and4.5 *(qSNK4).*Further, positive additive effect was observed for all QTLs detected on chromosome 1 and 4 indicating the contribution of At354 alleles in favour of salinity tolerance (Table 3). Locations of the QTLs in chromosomes are indicated in the Figure 2.

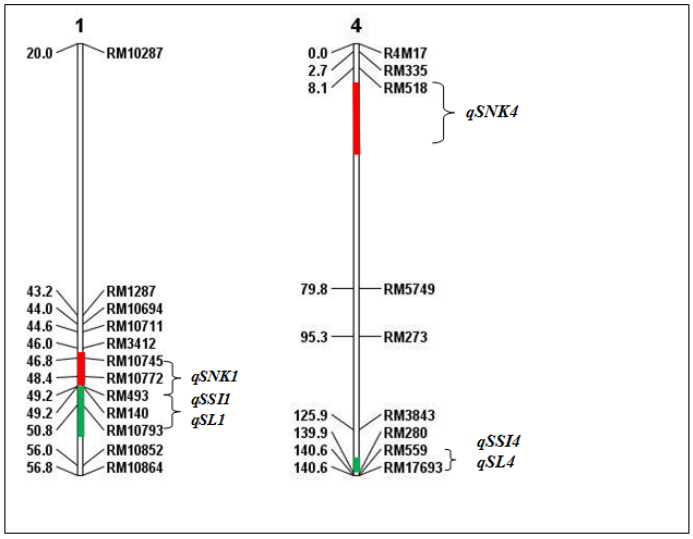
DISCUSSION

Salinity tolerance is resulted by cumulative effect of many morpho-physiological mechanisms. Our study showed many correlations among morpho-physiological parameters, probably may be due to their inter-related salinity tolerance mechanisms. Results of this study revealed significant differences between two parental genotypes, At354 and

Table 3. Putative QTLs identified for salinity tolerance in F_5 RIL population by composite interval mapping method.

Trait[a]	QTL	Chr	Flanking markers		Peak position (cM)	Estimated peak Position (Mb)	Add. effect[b]	LOD	R^2 (%)	LOD threshold		Salt tolerant allele Donor[c]	LOD*	R^{2*} (%)
										α = 0.05	α = 0.01			
SSI	*qSSI1*	1	RM140	RM10852	50	12.5	0.146	2.4	10.8	1.536	2.319	At354	3.3	15
	qSSI4	4	RM3843	RM280	134	33.5	0.140	3.3	15.0	1.834	2.461	At354	3.6	47
SL	*qSL1*	1	RM140	RM10852	50	12.5	6.711	2.2	10.0	1.643	2.395	At354	2.7	12
	qSL4	4	RM3843	RM280	134	33.5	3.300	2.4	11.0	1.812	2.624	At354	2.7	52
SNK	*qSNK1*	1	RM10745	RM140	48	12	-0.391	2.0	8.9	1.595	2.378	At354	2.1	9
	qSNK4	4	RM518	RM5749	30	7.5	-2.250	3.6	16.0	1.775	2.458	At354	4.5	51

[a]Salinity survival index (SSI), shoot length (SL) and shoot Na^+/K^+ ratio (SNK) Chr - chromosome
[b] Positive value means At354 allele contributes to increase effect of respective trait, negative value means Bg352 allele contributes to increase effect of respective trait
[c]Allele donor in favour of salt tolerance
*LOD and R^2 values resulted from Windows QTL Cartographer v2.5



qSSI1- QTL identified for salinity survival index; *qSL1*- QTL identified for shoot length; *qSNK1* - QTL identified for shoot Na+/K+ ratio in chromosome 1; *qSSI4* - QTL identified for salinity survival index; *qSL4* - QTL identified for shoot length; *qSNK*4 - QTL identified for shoot Na+/K+ ratio in chromosome 4

Figure 2. Putative QTLs identified on chromosome 1 and 4using composite interval mapping. Distances are indicated in Kosambi centimorgans.

Bg352 for all the traits assessed exhibiting divergent performances under salinity stress proving their suitability to use as a mapping population. Although size of the selected population was small, wider variation of frequency distributions was revealed for all measured traits indicating the presence of considerable number of recombination events which would facilitate the QTL mapping for salt tolerance. Also, transgressive segregants observed beyond favourable parent, indicates the possibility of utilizing them in varietal improvement for salinity tolerance.

Frequency distributions of all the measured traits except SES exhibited continuous and normal distribution within the acceptable range of skewness indicating the appropriateness of the traits for QTL mapping. SES was not included in the QTL analysis as it was measured as categorical basis. It was realized the importance of assessing survival potential of RILs under salt stress with a quantitative parameter as such parameter is convenient for the analysis, mapping and independent from personal skills. As a solution SSI parameter was used and results of SSI proved its applicability in analysis such as frequency distribution, correlation and QTL mapping. In this study multiple phenotypic traits, salinity survival index, shoot length, root length, shoot dry weight, root dry weight, shoot Na^+ concentration, shoot K^+ concentration and shoot Na^+/K^+ ratio were used for the QTL analysis. Out of these traits salinity survival index appears to be the most suitable trait for sanity tolerance screening experiments as it significantly correlates with most of other traits assessed in this experiment.

Results obtained by BSH analysis revealed that except SL and RL other traits showed more than 80% BSH for the population indicating there appropriateness for the breeding programmes. According to Singh (2001) and Amare *et al*. (2015) selection of traits having a high heritability (≥ 80%) would provide an efficient transfer of that particular trait to the progeny due to the lower involvement of the environment for the trait performance. Therefore, it can be predicted that the traits having extremely high BSH values are governed by major genes or QTLs. However, traits having low heritability (≤ 40%) would be unfeasible in selection for breeding programmes because of the masking effect of the environmental interaction (Singh, 2001). This fact suggests the unsuitability of usage of traits having low heritability for molecular mapping studies. Nevertheless, the lowest BSHs observed for this mapping population were 57% and 74% and they were beyond the threshold value given by Singh (2001).

In this study, the major problem encountered was the presence of low polymorphism in SSR loci of the two parents. This may be due to the fact that both parents have similar genetic background derived from elite parents with regards to the agronomical characters in their pedigree, even though they exhibited divergent performances under salinity stress. Total polymorphism observed in At354 and Bg352 cross was 28.4% which was less compared with the studies reported by Lang *et al*. (2008) and Islam *et al*. (2011). However, study conducted by Luu *et al*. (2012) reported that the total polymorphic markers appeared in AS996/FL478 cross was only 12.6%. It seems unlikely to get a saturated linkage map with SSR markers even though they produce a few hundreds of polymorphism (Mammadov *et al*., 2012). As a solution to this, scientists have developed high throughput SNP technology which usually produces polymorphism with more than 1000 markers. Therefore, as an alternative for SSR markers this study was extended with SNP markers for high resolution QTL mapping as a further study.

In this study linkage map was not used for QTL mapping in chromosome 1 and 4 as all polymorphic markers were not linked in expected linkage groups. This may be due to low level of polymorphic markers and small size of the selected population. Also Semagn *et al*. (2006) reported that complete linkage groups could not be obtained due to the low level of polymorphic markers and small size of the population. As a solution, molecular map was constructed using the physical position of the SSR markers based on the Nipponbare genome following the studies reported by Thomson *et al*. (2010), Sandhu *et al*. (2014) and Ye *et al*. (2011).

Previously, several mapping studies have identified QTLs associated with salinity tolerance in rice (Ammar *et al*., 2007; Bonilla *et al*., 2002; Flowers *et al*., 2000; Gregorio., 1997; Haq *et al*., 2010; Kim *et al*., 2009; Koyama *et al*., 2001; Lee *et al*., 2007; Lin *et al*., 2004; Prasad *et al*., 1999; Sabouri *et al*., 2009; Singh *et al*., 2007; Singh and Flowers, 2010; Takehisa *et al*., 2004). A study employing an $F_{2:3}$ population between the tolerant *indica* landrace Nona Bokra with the susceptible *japonica* Koshihikari identified several QTLs controlling tolerance traits, including major QTLs for shoot K^+ concentration on chromosome 1 (*qSKC-1*) and

shoot Na^+ concentration on chromosome 7 (*qSNC-7*) (Lin *et al*., 2004). Similarly, a recombinant inbred line population between the highly tolerant landrace Pokkali and sensitive IR29 identified a major QTL designated as *Saltol*, on chromosome 1 at the 10.8 - 16.4 Mb, in the same region as *qSKC1*, explaining 43% of the variation for shoot Na^+/K^+ ratio (Gregorio, 1997; Bonilla *et al.*, 2002). As the RIL population of this study was also derived from the parents with the pedigree of Pokkali it was expected to have similar QTL within this region. Proving this fact, all 3 putative QTLs identified in chromosome 1 were located within the region of *Saltol* QTL indicating the probable inheritance of *Saltol* region from the Pokkali background.

Thomson *et al.* (2010) reported a QTL in chromosome 4 for plant height closer to the region reported in this study. The peak of the QTL reported by Thomson *et al*. (2010) located on RM3843 marker (31.5 Mb) region and in our study peak region was detected at 33.5 Mb flanked by RM3843 and RM280 (Table 3) which are somewhat closer to each other. From these evidences, it can be speculated that these two instances refer to the same QTL.

Previous studies showed that the QTL mapping performed with a comparatively small number of individuals in a single environmental condition show only the QTLs which have major effect and such population is unable to detect QTLs that show minor effects (Tanksley, 1993; Edwards *et al*., 1992). Also, the study conducted by Raghavan and Collard (2012) revealed that the accuracy of the location of the QTLs and their effects rely on the size of the mapping population. Accordingly, when small size populations are used false negative QTLs can be detected and R^2 values can be overestimated or underestimated. The number of putative QTLs identified in this study was comparatively low and it might be mainly due to use of small size population and prevalence of low polymorphism. Therefore, the resulted QTLs of this study and their properties need to be further studied and confirmed before employing breeding techniques for varietal development. With regards to the size of the population, it has been reported that minimum number of individuals in a segregating population should not be less than 50 for QTL mapping (Collard *et al*., 2005; Mohan *et al*., 1997), but for fine mapping a larger population is required. However, Raghavan and Collard (2012) recommended at least 190 lines for a QTL mapping study. In this study we were able to proceed the project with 100 individuals following the recommendation by Collard *et al*. (2005) and Mohan *et al*. (1997). In this study we selected 100 RILs randomly. However, if we had obtained 100 RILs representing extreme ends of the population we would have obtained many QTLs as reported by Collard *et al*. (2005), but probably with overestimated values for QTL effects.

Usually, after mapping QTLs, such QTLs need to be validated and confirmed by repeating the mapping study in different environments and seasons. Then promising QTLs are used to detect candidate genes or to backcross breeding. Usually, QTL mapping studies are performed with divergent parents where trait-donor -parent derived from not elite genetic background. Therefore, scientists used to backcross the QTL-donor parent to a recurrent parent which possess other favourable agronomic traits to get the fewest donor alleles and maximum recurrent alleles by using marker assisted selection. In this study, the both parents, At354 and Bg352 are high yielding varieties and possess elite genetic background. Therefore, RILs that show high salinity tolerance by accumulating investigated QTLs can be released directly as cultivars if they contain other favorable agronomic traits. However, it is necessary to locate the precise QTLs with narrow marker intervals before selection of the RILs. The extended study of this experiment with SNP markers would enable to identify tightly linked markers to the QTLs with narrow intervals. These tightly linked markers would be the forerunners in marker assisted selection in the development of salt tolerant rice varieties.

In this study, a mapping population derived from 2 elite rice varieties At354 and Bg352, was characterized for the traits related to salinity tolerance. The broad sense heritability, co-efficient of variance and frequency distributions of morpho-physiological parameters revealed that the population was suitable for mapping genes for salinity tolerance,

though it rendered elite agronomic background. Six QTLs, namely *qSSI1, qSL1, qSNK1, qSL4, qSNK4* and *qSSI4* were detected on chromosome 1 and 4 explaining 10.8%, 10%, 8.9%, 15%, 11% and 16% phenotypic variation respectively, under salinity stress. However, prevalence of low level of polymorphism suggested that the population was not suitable to be genotyped by SSR markers. Further studies incorporating high throughput marker technologies would be necessary to develop high density molecular map and for detecting QTLs with narrow marker intervals.

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