Identification and mapping of quantitative trait loci associated with salinity tolerance in rice (*Oryza Sativa*) using SSR markers

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Abstract
Salinity stress is one of the most widespread soil problems next to drought, in rice growing areas. Reducing Sodium (Na^+), while maintaining Potassium (K^+) uptake in rice are traits that would aid in salinity tolerance. Therefore, the identification of quantitative trait loci (QTLs) associated with those for Na^+ and K^+ uptake, will enable breeders to use marker-assisted selection to transfer QTLs into elite lines in rice improvement programs. In view of this, 62 advanced backcross-inbred lines (BILs), at the BC_2F_5 generation, derived from the cross of Tarome-Molaei (salt tolerant) and Tiqing (Salt sensitive), were used to identify the QTLs involved in salinity stress tolerance, using SSR markers. Advanced backcross inbred lines along with their parents were evaluated for six parameters *viz.* Sodium (Na^+) and Potassium (K^+) in roots and shoots and the Na^+/K^+ ratio, using the modified Yoshida's nutrient solution at an electrical conductivity of 6 and 12 dS/m. A total of 114, out of 235 simple sequence repeats (SSRs) markers that showed polymorphism in the parents, were used to genotype the BILs. A linkage map was constructed with an average interval of 15.3 centiMorgan (cM) between the markers, spanning 1747.3 cM across all 12 rice chromosomes. Using the composite interval mapping (CIM) and a minimum logarithm of the odds (LOD) threshold of 3.0, a total of 14 QTLs were detected as follows; on chromosome 1 (5 QTLs), 3 (1QTL), 4 (3 QTLs), 5 (2 QTLs), 6 (1 QTL), and 8 (2 QTLs) for all six traits except, Sodium (Na^+) in the shoot. The phenotypic variation explained by these QTLs ranged from 9 to 30% of the total variation. A QTL (*QKr1.2*) for K^+ content in the root was identified with the highest LOD score (7.8), on chromosome 1. This QTL explicated 30% of the total variation and was identified as a major QTL conferring salt tolerance in rice.
Keywords**:** QTL; Rice; Salinity; SSR

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INTRODUCTION

The salinization of soil and water, places an increasing constraint on crop production in the arid and semi-arid regions of the world. Plant breeding focused on increasing the salt tolerance of crops, could improve the profitability of many of the worlds one billion salt-affected hectares (Szabolcs, 1989). Salt accumulation in Iranian soils is mainly related to a dry climate, and salt-rich components of the soil. According to the recently published soil map, the extent of saline soils in Iran estimated for slightly and moderately saline soils occupies approximately 25.5 million ha and strongly saline soils cover approximately 8.5 million ha (Food and Agriculture Organization (FAO), 2000).

Rice is one of the most important staple foods for more than half the world's population. The rice growing regions of the world are greatly affected by soil salinity. Soil salinity is the second most widespread soil problem, next to drought in rice growing areas (Gregorio *et al*., 1997). Thus breeding for salinity tolerance in rice is one of the most important objectives in rice breeding programs. Salinity tolerance of rice has been found to be associated with its ability to preferentially take up potassium (K^+) ions, but restrict the uptake of potentially toxic ions such as sodium (Na^+). There is higher genetic variation with regard to Na^+ and K^+ uptake among crop species than among genotypes within a crop species (Gregorio *et al*., 1997). Crop genotypes with high salt tolerance, besides main-

taining K^+, can maintain lower Na^+ concentrations under salinity stress condition. On the other hand, the sensitive rice varieties can not effectively prevent accumulation of sodium (Na^+) as well as the depletion of K^+ under stress. Although the uptake of Na^+ and K^+ is entirely independent, but a lower Na^+/K^+ ratio is considered as a desirable trait for the selection of salt tolerant genotypes (Garciadebleas *et al.*, 2003). Na^+ is transported to the shoot usually through apoplastic pathways (passive transport), while K^+ transport takes place through the symplastic pathway (active transport). Younger leaves have relatively lower levels of Na^+ than K^+ ions, when compared to the older leaves, which in turn leads to a higher Na^+/K^+ ratio in the latter case. Thus, the Na^+/K^+ ratio increases abruptly with increasing salt concentration and leaf age, hence, sensitive and tolerance varieties behave differently. Consequently, the tolerant variety keep their leaves and shoots relatively free of the toxic ions besides having an assured supply of K^+ ions (Garciadebleas *et al.*, 2003).

Tolerance to salinity is a quantitative or multigenic trait. DNA markers could be important in plant breeding if they are used to aid in the selection of quantitative traits. Different molecular markers are available for use in the genetic dissection of traits including Restriction Fragment Length Polymorphism (RLFP), Amplified Fragment Length Polymorphism (AFLP), Random Amplification of Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR). Amongst these, microsatellite markers have emerged as the markers of choice in rice, since they are abundant and are informative in many types of genetic crosses. Microsatellite markers have been used in several genetic studies of rice with regard to various traits (Temnykh *et al.*, 2000; Chen *et al.*, 1997; Akagi *et al.*, 1996; Panaud *et al.*, 1996; Tanksley, 1993). A total of 2414 new SSR primer pairs, representing 2240 unique marker loci have been developed and experimentally validated for rice (McCouch *et al.*, 2002). At the completion of the draft sequence of the rice genome, with more than 99% accuracy, 18838 new SSRs have been reported on its physical map (International Rice Genome Sequencing Project (IRGSP), 2005). Microsatellites have been used effectively to map QTLs associated with salinity tolerance (Singh *et al.*, 2007; Bonilla *et al.*, 2002). The objective of QTL mapping is to identify the loci that are responsible for variation in quantitative traits, such as salt tolerance. Therefore, determination of the number, location and the interaction of these loci is of prime importance; however, the identification of the actual genes and their functions is the ultimate goal of such studies. For example, plant molecular breeders have attempted to identify the loci that improve the yield or quality of crops, and then bring the favorable alleles together into elite lines (Mauricio, 2001). Therefore, the identification of genomic regions that carry QTLs, allows breeders to use marker-aided selection to precisely move beneficial QTLs into elite lines for crop improvement in breeding programs. The mapping of QTLs also helps quantitative and population geneticists to define the genetic architecture of growth traits (Mauricio, 2001). A major gene for salinity tolerance in rice was mapped on chromosome 7, using RFLP markers (Zhang *et al.*, 1995). Another major QTL for salt tolerance was identified on chromosome 1 by using recombinant inbred lines (F_8) from the Pokkali×IR29 cross (Gregorio *et al.*, 1997). This QTL governed the Na^+/K^+ uptake ratio and accounted for 64.3 to 80.2% of the phenotypic variation in this trait. This segment of chromosome 1 was further saturated by SSR and RFLP markers using recombinant inbred lines (RILs) and the QTLs associated with Na^+, K^+ and Na^+/K^+ uptake ratio, which accounted for 39.2, 43.9 and 43.2% of the total phenotypic variation, respectively (Bonilla *et al.*, 2002). Similarly, in another study, near isogenic lines (NILs) having alleles from Pokkali at the Saltol region in the IR29 background, were used to fine-map the Saltol segment of chromosome 1 (Niones, 2004). Several QTLs for traits associated with salinity tolerance were identified on rice chromosome 5, 6, 7 and 10 (Prasad *et al.*, 2000). Lang *et al.* (2001) reported that a microsatellite, RM223, was linked to QTLs associated with salt tolerance at the vegetative stage. Based on RFLP markers, Koyama *et al.* (2001) identified 11 QTLs on 4 different chromosomes, 1, 4, 6 and 9, for different component traits related to salinity. These consisted of 3 QTLs on chromosome 1 for Na^+ uptake, K^+ concentration, Na^+/K^+ ratio, 4 QTLs on chromosome 4 for Na^+ uptake, K^+ concentration and Na^+/K^+ ratio, 3 QTLs on chromosome 6 for K^+ uptake and Na^+ concentration and 1 QTL on chromosome 9 for K^+ uptake. Several other QTLs for salinity tolerance have been detected using 108 SSR and RFLP markers in the RIL population derived from the cross between Tenasai×CB (Lang *et al.*, 2000). By using F_2 RILs from the cross between Nonabokra×Koshikari, 8 QTLs responsible for the K^+ and Na^+ content were mapped in rice. The QTL, *SKC1*, was mapped as a major QTL for shoot K^+ content on the rice chromosome 1 (Lin *et al*., 2004). Ming *et al*. (2005), reported

that two QTLs for Na^+/K^+ on chromosome 2 and 6, one on each chromosome, control salt tolerance. Sabouri *et al.* (2009), by using SSR markers on the Iranian rice population (F_2 population derived from a cross between a salt tolerant cv. Tarommahali and a salt sensitive cv. Khazar), reported 4 QTLs for shoot Na^+ content on chromosomes 2, 3 and 6, 3 QTLs for shoot K^+ content on chromosomes 5 and 6, and 2 QTLs for shoot Na^+/K^+ ratio on chromosomes 3 and 6. The aim of this study was to determine the response of a backcross population of rice with regard to salinity tolerance under greenhouse conditions, and to identify and map QTLs associated with salinity tolerance, using microsatellite markers.

MATERIALS AND METHODS

Plant materials: Two rice genotypes differing in their salt stress responses, namely, Tarome-Molaei (salt-tolerant) and Tiqing (salt-sensitive), along with a set of 62 backcross-inbred lines (BC_2F_5) derived from the cross of these parents, were used in the present study. The BC_2F_5 population was developed at the International Rice Research Institute (IRRI), Philippines. Tiqing (TQ), as a recurrent parent, was crossed with Tarome-Molaei (TM) as the donor parent, and F_1 plants were backcrossed with Tiqing to produce the BC_2F_1 progeny. Sixty-two advanced backcross-inbred lines (BC_2F_5) were developed by self-fertilization from BC_2F_1 (TQ / Tarome-Molaei // TQ /// TQ) plants by the single-seed descent method.

Evaluation for salinity tolerance: Sixty-two advanced backcross-inbred lines (BC_2F_5) along with their parents were evaluated for salt tolerance in a Phytotron, which was set with a day/night temperature of 29/22ºC. Two rice genotypes, IR29 and FL478, which previously were identified as salt- sensitive and -tolerant were used as check varieties. Surface-sterilized seeds were treated with fungicide and rinsed throughly with distilled water. Sterilized seeds were placed on moistened filter papers in Petri dishes and incubated at 30ºC for 48 h. 10 pre-germinated seeds from each parent variety and the BIL population was placed in the holes of a thin Styrofoam sheet with a nylon net bottom, which floated on distilled water. After 4 days, when seedlings were well established, distilled water was replaced with modified Yoshida's nutrient solution. Initial salinity was kept at an electrical conductivity (EC) of 6 dS/m for one week. Salinity was then increased to 12 dS/m by adding NaCl, and seedlings were exposed to this salinity for 2 weeks. This experiment was conducted in a Phytotron at day and night temperatures of 29/22°C with a relative humidity of 70%. The pH of the solution was maintained at 5.5 on a daily basis, by adding either 1M KOH or HCl. This experiment was repeated 3 times and data were recorded for root and shoot Na^+ and K^+ concentrations. The Na^+/K^+ ratios in the root and shoot were also computed. To determine physiological traits, such as Na^+ and K^+ uptake in the shoot and root, at 21 days following treatment with NaCl, the shoots and roots of 62 BC_2F_5 families (thirty plantlets of each family) were harvested. They were dried, weighed and their saps were extracted with acetic acid (100 mM) at 90ºC for 2 h. The extract was separated and its Na^+, K^+ content, and the Na^+/K^+ uptake ratio were determined by a spectrophotometer (Model 3100, Norwalk, USA).

DNA extraction and quantification: DNA was extracted from leaves of 3 weeks old seedlings (10 seedlings) using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980), and dissolved in TE buffer. DNA quantity was estimated spectrophotometrically and the concentration was adjusted to 10 ng/µl for use in polymerase chain reaction (PCR). PCR amplification was carried out in 10 µl reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 50 µM each of dNTP, 0.01% (w/v) Gelatin, 0.5 µM of forward and reverse primers, 0.5 U of *Taq* DNA polymerase and 10 ng of DNA template per sample. The PCR program involved initial denaturation at 94ºC for 5 min, followed by 35 cycles of 1 min denaturation at 94ºC, 1 min annealing (between 55 and 67ºC), and 2 min extension at 72ºC, plus a final extension of 7 min at 72ºC, on a thermal cycler (MJ Research, USA). The PCR products were separated by electrophoresis on a 3% (w/v) agarose gel and 5% (w/v) polyacrylamide gels.

Simple sequence repeats (SSR) assay and linkage analysis: For the simple sequence repeats assay, a total of 235 microsatellite markers (SSRs), derived from the Cornell SSR linkage map (McCouch *et al*., 2002) were used in a polymorphism survey of Tiqing and Tarome-molaei. A total of 114 SSR primer pairs that were polymorphic between the two parents, were used in map construction and QTL analysis. Linkage groups and the positions of markers were determined by using the Kosambi mapping function (Kosambi, 1944) of MAP-

MAKER/EXP 3.0 (Lander *et al*., 1987) to span 1747.3 cM of the rice genome, with genetic distances expressed in centiMorgan.

Statistical analysis and quantitative trait loci (QTL) mapping: Analysis of variance (ANOVA) and correlation analysis were performed using the SPSS (Ver. 14, 2006) software. The logarithm of the odds (LOD) score for the association between the genotype and trait data was calculated from composite interval mapping (CIM) (Zeng, 1994) that extends the regression equation to include more markers as cofactors, in order to remove the effects of multiple QTLs. Composite interval mapping was performed by using the Windows QTL Cartographer ver. 2.5 program (Wang *et al.*, 2004). Permutation tests (1000 times) were used to establish an experiment-wise significance value at the 0.05 confidence level defined as a minimum LOD threshold for each trait in CIM (Doerge and Churchill, 1996; Churchill and Doerge, 1994). A minimum LOD threshold of 3.0 was selected for the declration of the putative QTL. The proportion of the observed phenotypic variance explained by each QTL was estimated by the coefficient of determination (R^2) (McCouch *et al*., 1997).

RESULTS

Phenotypic variation for salinity tolerance: The analysis of variance revealed significant differences ($P < 0.01$) between two parental genotypes for all the traits assessed in the current study, with the exception of Na^+ content in the root. The differences were highly significant ($P<0.01$) among advanced BILs for the traits measured. It could be therefore expected that the BILs population derived from the cross between two parents would be suitable for mapping of the QTLs for salinity tolerance traits. The frequency distribution of the traits measured is given in Figure 1. The BILs showed continuous variation and large transgressive segregation in both directions for traits, such as Na^+ content in the shoot, Na^+ in root and K^+ content in root. The transgressive segregation was observed only in one direction for the Na^+/K^+ ratio in the shoot and the root and for K^+ content in the shoot (Fig. 1).

Correlations among traits: The correlation between traits was computed by regressing phenotypic values of one trait on those of other traits. The Correlations among traits are presented in Table 1. Concentration of Na^+ and K^+ in the root under salt stress virtually showed no relationship with Na^+ and K^+ content in the shoot. Na^+/K^+ ratio in the shoot was significantly and positively correlated with Na^+ in the shoot ($r = 0.72$), however, its correlation was negative for K^+ in the shoot ($r = -0.74$). Na^+/K^+ ratio in the root was significantly and positively correlated with Na^+ in the shoot ($r = 0.47$), whereas correlation between Na^+ in the root and shoot was not significant. The positive correlation between Na^+/K^+ ratio in the root with Na^+ in the shoot is the result of negative correlation between shoot Na^+ and root K^+.

Linkage map using simple sequence repeats (SSR) markers: Out of total 235 SSR markers, 114 showed polymorphism between parental varieties, Tiqing and Tarome-Molaei. A linkage map was constructed from the backcross-inbred lines (BILs) population of 62 individuals using 114 markers, giving an evenly spaced coverage to the rice genome, with an average interval of 15.3 cM between markers spanning 1747.3 cM across all 12 rice chromosomes (Fig. 2). The average intervals of markers were smaller than 20 cM in the genetic map of this study, showing their suitability for QTL mapping (Lander and Botstein, 1989).

Table 1. Correlations between salt tolerance traits in the advanced backcross-inbred lines (BC2F5) population of rice investigated under greenhouse conditions (n = 62).

Salinity Tolerance Traits	K^+ in shoot	Na^+ in shoot	K^+ in root	Na^+ in root	Na^+/K^+ ratio in shoot
Sodium (Na+) in shoot	-0.12^{ns}				
Potassium (K+) in root	-0.03^{ns}	-0.34^{**}			
Sodium (Na+) in root	-0.08^{ns}	0.02^{ns}	0.48^{**}		
Na+/K+ ratio in shoot	-0.74^{**}	0.72^{**}	-0.16^{ns}	0.07^{ns}	
Na+/K+ ratio in root	0.005^{ns}	0.47^{**}	-0.73^{**}	-0.01^{ns}	0.23^{*}

ns: non-significant, *: significant ($p < 0.05$) ; **: significant ($p < 0.01$).

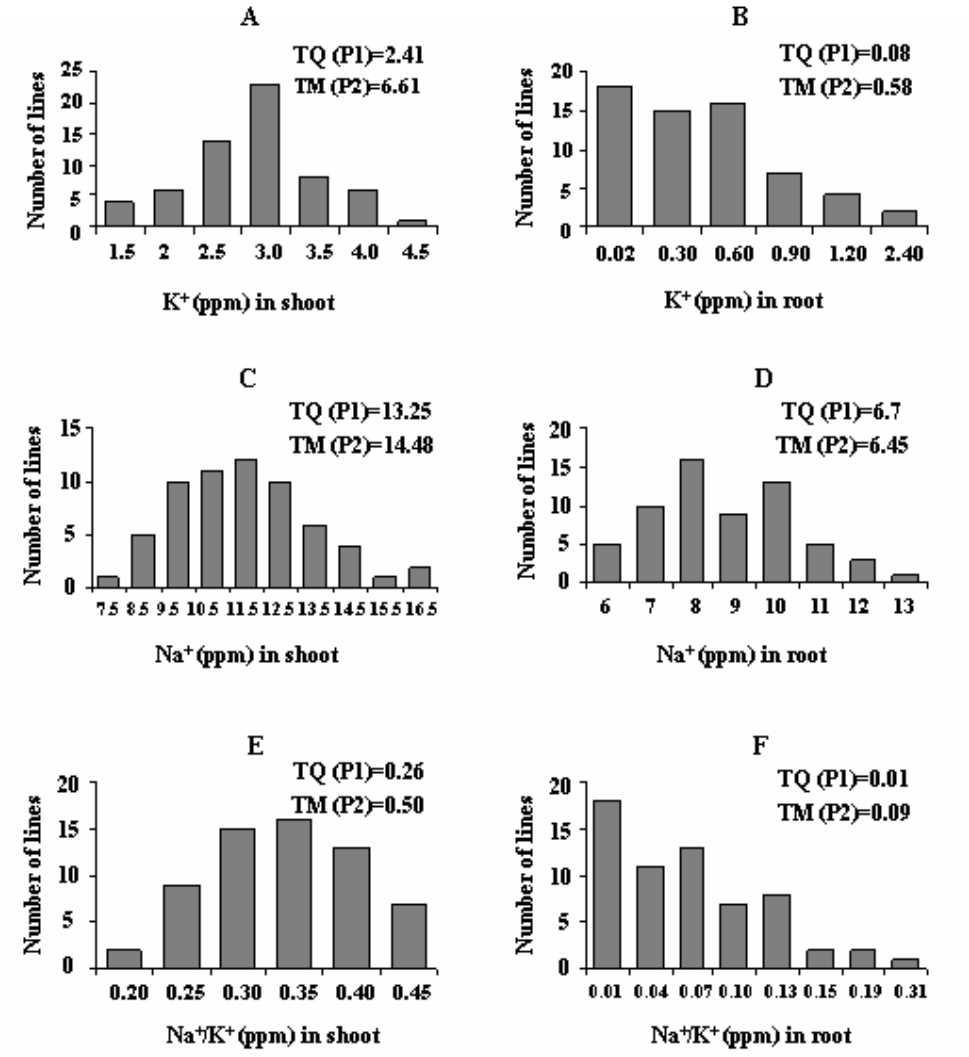

Figure 1. Frequency distribution of traits in the advanced backcross-inbred lines (BC2F5) population of rice. The mean trait values of both parents P1= Tiqing (TQ), P2=Tarome molaei (TM) are indicated.

QTL analysis and association of molecular markers with quantitative traits: Using genotypic data on the segregation of each of the 114 markers and that of the 6 quantitative traits, i.e. K^+ in the Shoot, Na^+ in the Shoot, K^+ in the Root, Na^+ in the Root, Na^+/K^+ ratio in the shoot and root, the chi-square (χ^2) statistic test for the independence of attributes, was carried out to identify molecular markers showing independent association with each of the 6 traits. Fourteen QTLs were detected on chromosomes 1 (5 QTLs), 3 (1 QTL), 4 (3 QTLs), 5 (2 QTLs), 6 (1 QTL) and 8 (2QTLs), for the 6 traits with the exception of Na^+ concentration in the shoot. The location of detected QTLs is shown on the linkage map (Fig. 2).

QTL Mapping

Potassium (K^+) *concentration in root*: Five QTLs for K^+ in the root were positioned on chromosomes 1 (2 QTLs), 3 (1 QTL), 4 (1 QTL) and 8 (1 QTL). The phenotypic variation explained by each QTL localized between RM200-RM220 and RM473A-RM128 on chromosome 1, was 17 and 30% of the total phenotypic variance, respectively. The LOD scores of QTLs, *QKr*1.1 and *QKr*1.2, were 3.5 and 7.8, respectively (Table 2). The QTL *QKr1.2,* for K^+ in the root on chromosome 1 indicated 30% of the variation for this trait and was thus designated as the main QTL. At these QTLs (*QKr*1.1 and *QKr*1.2), the Tarome-Molaei alle-

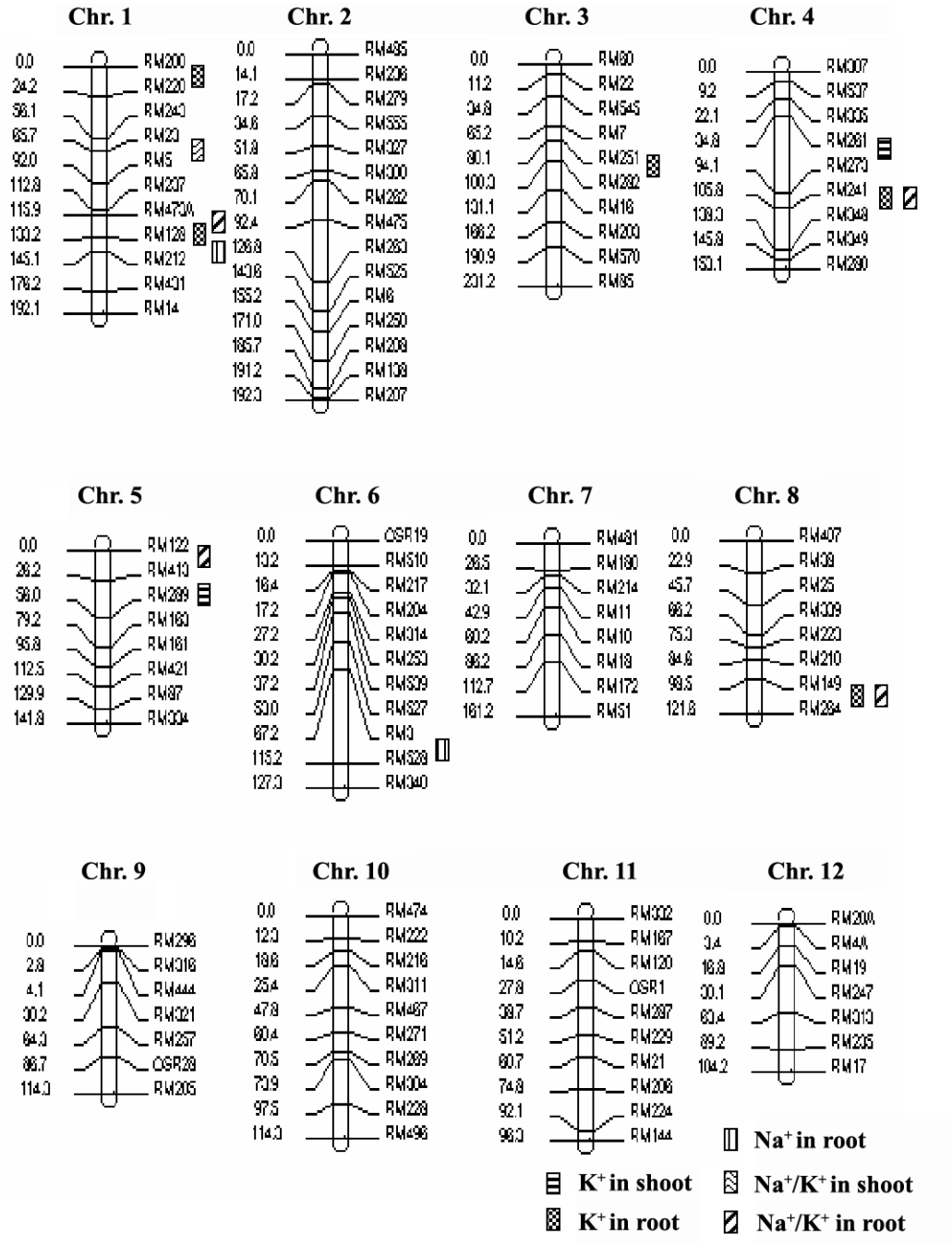

Figure 2. SSR linkage map of rice based on 62 advanced backcross-inbred lines (BC2F5) populations and their respective QTL positions. RM represnets rice microsatellite marker, as developed by McCouch et al. (1997a).

les, as the donor parent, increased the concentration of K^+ in the root up to the range of 13-16%. One QTL (*QKr*3) was found between markers RM251-RM282 on chromosome 3, with an LOD score of 4, explaining 14% of the total phenotypic variation. At this QTL, the Tarome-Molaei allele increased K^+ concentration in the root by 14%. Similarly, the interval between RM241-RM348 on chromosome 4 contained one QTL for root K^+ and explained 9% of the total phenotypic variance. This QTL (*QKr*4) was detected with an LOD of 3, at which the Tiqing allele, as a recurrent parent, increased K^+ content in the root by 7%. The fifth QTL (*QKr*8) for K^+ concentration in the root, was located on chromosome 8, and accounted for 20% of the total

Table 2. Putative QTLs and SSR marker loci associated with Na+, K+ concentration and Na+/K+ uptake ratio detected in an advanced backcross-inbred lines (BC2F5) population of rice (n = 62) by CIM.

Traits	Chr. No.[e]	Marker Intervals	QTL[f]	Additive Effect[b]	R^{2c}	LOD[a]
Potassium in Shoot (K^+S)	4	RM261d-RM273	*QKs*4	-8	19	4.1
	5	RM413-RM289	*QKs*5	13.7	22	5.7
Potassium in Root(K^+R)	1	RM200-RM220	*QKr*1.1	-13	17	3.5
	1	RM473A-RM128	*QKr*1.2	-16	30	7.8
	3	RM251-RM282	*QKr*3	-14	14	4
	4	RM241-RM348	*QKr*4	7	9	3
	8	RM149-RM264	*QKr*8	-10	20	3.5
Sodium in Root (Na^+R)	1	RM128-RM212	*QNas*1	-3.6	15	3.8
	6	RM3-RM528	*QNas*6	-2	24	4
Na^+/K^+ ratio in shoot	1	RM23-RM5	*QNas/Ks*1	-7.4	19	4
Na^+/K^+ ratio in root	1	RM473A-RM128	*QNar/Kr*1	-12	18.4	4
	4	RM241-RM348	*QNar/Kr*4	7.3	9	3
	5	RM122-RM413	*QNar/Kr*5	-8.7	27.6	5
	8	RM149-M264	*QNar/Kr*8	-8.7	16.7	3.7

a: logarithm of the odds (LOD) score (threshold 3.0). b: Estimated effect of replacing TM allele by TQ alleles. c: Coefficient of determination (Proportion of the phenotypic variation explained by the individual QTL). d: underlined markers are those found significant for the putative locus. e: Chromosome number. f: Quantitative trait loci.

phenotypic variation, with an LOD score of 3.5. This QTL (*QKr*8) had a negative additive effect on K^+ content in the root, Thus at this locus, the Tarome-Molaei allele contributed to increasing K^+ concentration in the root by 10%.

Potassium (K^+) *concentration in the shoot*: A total of 2 QTLs for K^+ concentration in the shoot was detected on chromosomes 4 and 5, which significantly influenced K^+ concentration (Table 2). The locus *QKs*4 on chromosome 4 with an LOD score of 4.1 accounted for 19% of the total K^+ with regard to shoot variation. The other QTL (*QKs*5) with an LOD score of 5.7 explained 22% of the total K^+ in shoot variation. At these loci, the Tiqing allele increased K^+ concentration in the shoot at *QKs*5 by 13.7%, while the Tarome-Molaei allele increased K^+ in shoot at *QKs*4 by 8%.

Sodium (Na^+) *concentration in the root*: A total of 2 QTLs associated with Na^+ content in root was found on chromosomes 1 (*QNas*1) and 6 (*QNas*6), significantly influencing the Na^+ uptake (Table 2). The phenotypic variations explained by these QTLs, were 15 and 24% of the total variation in root Na^+. The position of *QNas*1 was between RM128-RM212, whereas *QNas*6 was located between markers RM3-RM528 on chromosomes 1 and 6, respectively. The additive effect of TQ alleles was negative on Na^+ content in the root at both QTLs. Therefore, TM alleles led to an increase in root Na^+ concentration.

Na^+/K^+ ratio in root: A total of 4 QTLs associated with the Na^+/K^+ ratio in the root was detected on chromosomes 1 (*QNar/Kr*1), 4 (*QNar/Kr*4), 5 (*QNar/Kr*5) and 8 (*QNar/Kr*8) (Table 2). These QTLs were located at marker intervals RM473A-RM128 on chromosome 1, RM241-RM348 on chromosome 4, RM122-RM413 on chromosome 5 and RM149-M264 on chromosome 8, respectively. The total phenotypic variation explained by each of the 4 QTLs ranged from 9 to 27.6%. These QTLs cumulatively accounted for 71.7% of the total variation in the Na^+/K^+ ratios in the roots. At the QTLs located on chromosome 4 (*QNar/Kr*4), the alleles from TQ contributed to an increase in the Na^+/K^+ ratio in the root. The QTLs on chromosomes 1, 5 and 8 of the TQ parent had a negative additive effect on the Na^+/K^+ ratio. Therefore, the Tarome-Molaei alleles (*QNar/Kr*1, *QNar/Kr*5, *QNar/Kr*8) increased the Na^+/K^+ ratio in the root by 29.4. Among the 4 QTLs for Na^+/K^+ ratio in the root, the *QNar/Kr5* with $R^2 = 27.6$ was selected as the main QTL.

Na^+/K^+ ratio in shoot: Only one QTL associated with the Na^+/K^+ ratio in the shoot was detected on chromosome 1 (Table 2). The QTL, *QNas/Ks1*, was detected with an LOD score of 4 and accounted for 19% of the total variation in the Na^+/K^+ ratio of the shoot. The additive effect of TQ alleles was negative on Na^+/K^+ ratio in the shoot. The TM alleles contributed to increasing the Na^+/K^+ ratio in the shoot by 7.4.

DISCUSSION

Generally, studies of QTL mapping conducted on data collected from a relatively small population size under a single environment are likely to detect the loci with large effects and fail to identify those with small effects (Tanksley, 1993; Edwards *et al*., 1992). Therefore, the number of putative QTLs detected in this study should be considered as a minimum of those in the segregating population. Transgressive segregation was observed for various traits in this population (BC_2F_5). Transgressive segregation is defined as the appearance of individuals in segregating populations that exceed those of the parental phenotypes (Mauricio, 2001). For traits in which two or more significant QTLs were detected (K^+ in the root and shoot and Na^+/K^+ ratio in the root), both parents were found to possess the QTL alleles which increased phenotypic values. Thus both parents possess QTL alleles that confer salt tolrance. Veldboom *et al.* (1994) and Xiao *et al.* (1996) showed that correlated traits often have QTLs that map to the same chromosomal region. This result was also observed in the current study. For example, K^+ and Na^+ concentrations in the root were significantly correlated (r = + 0.48) and had QTLs with large effect, which were mapped at similar intervals and map positions (RM128-RM212 and RM473A-RM128 on chromosome 1). Similarly, K^+ in the root was negatively correlated with Na^+/K^+ ratio in the root (r = -0.73), and also mapped at the same interval (RM473A-RM128) on chromosome 1. The correlation between traits may result from the linkage of several genes controlling the traits or pleiotropic effects of single genes. In order to differentiate between these causes it is necessary to fine-map the intervals where QTLs for the different traits are co-localized.

Some QTLs associated with salinity tolerance has been reported in rice. Gong *et al*. (1999) has reported a major QTL for salinity tolerance in rice on chromosome 1, but it is very difficult to correlate the positions of the QTLs reported in this study with chromosome 1, as different molecular markers and genetic materials have been used in our study. Prasad *et al*. (2000) have detected a QTL on chromosome 6 related to salinity tolerance, which may be related to the QTL found in this study (*QNas6* on chromosome 6) for Na^+ concentration in the root. In agreement with the results of this research regarding the detected QTL (*QNas/Ks1*) for Na^+/K^+ ratio in the shoot, on chromosome 1 (Table 2), Lang *et al*. (2001) have also reported a QTL for Na^+/K^+ ratio in the rice shooton chromosome 1. Koyama and colleagues (2001) using RFLP markers, have detected a QTL involved in K^+ concentration in the shoot, on chromosome 1. They have also mapped a QTL related to the Na^+/K^+ ratio using an RM5 marker on chromosome 1, and a QTL related to K^+ uptake using RM261 marker on chromosome 4. Similarly, in this study, the QTL of the Na^+/K^+ ratio linked to the RM5 marker (Table 2) was mapped on chromosome 1 and the QTL for K^+ uptake linked to the RM261 marker (Table 2) was mapped on chromosome 4 with an LOD score of 4 that accounted for 19% of the total variation. Flowers *et al*. (2000), using RFLP markers, have reported a QTL for Na^+ uptake in rice on chromosome 1 and a QTL for the Na^+/K^+ ratio on chromosome 4. In this study, 2 QTLs for Na^+ uptake (*QNas*1) and theNa^+/K^+ ratio (*QNar/Kr*4) were also mapped on chromosomes 1 and 4, respectively. Similarly, Koyama and coworkers (2001) have mapped QTLs related to Na^+ uptake on chromosome 1 by a trait-based QTL method. Bonilla *et al.* (2002) have also reported the *Saltol* gene for Na^+ uptake at the RM140-C1733S interval on chromosome 1. In this study, QTLs were found to be related to the Na^+/K^+ uptake ratio on chromosomes 1, 4, 5. Eight QTLs associated with the Na^+/K^+ uptake ratio, as reported by other researchers were found on chromosomes 1 (Bonilla *et al*., 2002; Lang *et al*., 2001; Koyama *et al*., 2001; Grigorio, 1997), 2 (Ming *et al*., 2005; Lang *et al*., 2001), 3 (Sabouri *et al*., 2009), 4 (Koyama *et al*., 2001), 6 (Sabouri *et al*., 2009; Ming *et al*., 2005), 7 (Lang *et al*., 2001), 10 and 12 (Grigorio, 1997).

In this research, QTLs associated with the Na^+/K^+ uptake ratio were detected only on chromosomes 1, 4, 5 and 8, which is probably due to the low density of the SSR linkage map. In fact this study reports for the first time the detection of QTLs related to the Na^+/K^+ ratio in the root. These new QTLs are mapped on chromosomes 5 (*QNar/Kr*5) and 8 (*QNar/Kr*8). *QNar/Kr*5 and *QNar/Kr*8 explain most of the total phenotypic variations, 27.6 and 16.7%, respectively. The QTL of *QNar/Kr*5 on chromosome 5, because of high pheno-

typic variation (27.6%), could be a major QTL. This study also reports for the first time the detection of QTLs related to K^+ uptake in the root. These QTLs are mapped on chromosomes 3 (*QKr*3) and 8 (*QKr*8). *QKr*3 and *QKr*8 genes explain most of the total phenotypic variation, which are 14 and 20%, respectively. The QTL associated with Potassium (K^+) in the root (*QKr*1) found on chromosome 1 coincides with the estimated location of a QTL affecting Na^+/ K^+ ratio in the root (*QNar/Kr*1) (Table 2). Both of these QTLs are co-localized at the marker interval M473A-RM128, and explain a large amount of variation for each trait. A question arises as to whether there is one QTL affecting both traits or whether there are QTLs affecting two separate traits, but located adjacent to each other. This is one of the major problems in QTL analysis. The QTL for Sodium (Na^+) in the root (*QNas*1 between RM128-RM212) is also found on chromosome 1,overlapping those of the QTLs associated with the K^+ uptake and Na^+/K^+ ratio in the root (Table 2). In agreement with the results of this study, Sabouri *et al*. (2009) has also mapped QTLs Na^+/K^+ ratio related to Na^+ and K^+ concentrations on the same region and chromosomes.

The comparison between the chromosomal positions of QTLs related to K^+, Na^+ concentrations and Na^+/K^+ ratio in the root is difficult to assess; QTLs in these regions may be located at the same loci or are at different tightly linked loci. Further analysis, including verifying the mapped QTLs, fine-mapping of both QTLs using common markers, and cloning and sequence comparisons of these QTLs, will be required to answer these questions.

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