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QTL mapping for salinity tolerance at seedling stage in rice

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Abstract: Salinity tolerance in rice is a quantitative trait. Rice is sensitive at seedling and reproductive stages; however, the tolerance at seedling stage is crucial for better crop establishment in the field. In the present study, we report the detection of QTLs for salinity tolerance at the seedling stage identified in a F_2 breeding population derived from the cross between BRRI dhan40, a moderately tolerant female parent with IR61920-3B-22-2-1 (NSIC Rc106), a highly tolerant male parent. Out of total 300 F_2 segregating plants, 93 plants with extreme phenotype for salinity stress response, i.e. tolerant and sensitive, were used for selective genotyping based on of visual seedling stage salt tolerance symptom. A total of 260 SSR and two EST markers evenly spread throughout the whole rice genome at 5 Mb intervals were used for parental polymorphism survey. The 90 polymorphic makers were used for QTL mapping for salinity tolerance at seedling stage. QTL analysis using single marker, interval mapping and composite interval mapping detected three major QTLs on chromosome 1, 8 and 10 with phenotypic variances (R^2) of 12.50, 29.0 and 20.20%, respectively. The position of QTL on chromosome 1 was flanked by RM8094 and RM3412 marker which is in the same region as a previously identified major QTL designated as *SalTol*. However, two other QTLs with relatively large effects were flanked by RM25 and RM210 on chromosome 8, and RM25092 and RM25519 on chromosome 10, and appear to be novel QTLs. The markers flanking these QTLs should be useful for molecular marker assisted breeding for salinity tolerance.

Keywords: QTL mapping, salinity tolerance, seedling, rice.

خرطنة مواقع الصفات الكمية(QTL) لتحمل الملوحة في مرحلة البادرة لنبات الأرز

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الملخص: صفة تحمل الملوحة في الأرز هي صفة كمية. نبات الأرزحساس للملوحة في مرحلتي البادرات والتكاثر؛إلا أن التحمل في مرحلة البادرة يعد مهماً لتحسين الثبات في الحقل. في الدراسة الحالية، نعلن اكتشاف مواقع صفات الكمية (QTL) لتحمل الملوحة في مرحلة البادرة تم تعريفها في الجيل الثاني لعشيرة مستمدة من هجين بين سلالة BRRI dhan40 وهي متوسطة التحمل مثلت الأم، وسلالة IR61920-3B-22-2-1 (NSIC Rc 106) وهي عالية التحمل ممثلة الأب. من 300 نبات جيل ثاني انعزالي كان هناك 93 نبات شكلها الظاهري عالي الحساسية لضغط الملوحة، أي متحمل أو حساس، استخدمت لانتخاب الطرز الوراثية بناءً على رؤية الأعراض على البادرات. كان هناك 260 واسم SSR وواسمين EST موزعين بالتساوي على طول البناء الوراثي Genome للأرز على مسافات 5 ميجا بايت (5Mb) لخرطنة QTL لتحمل الملوحة في مرحلة البادرات. كشف تحليل QTL باستخدام واسم واحد، خرطنة المسافات وخطنة المسافات المركبة، ثلاث مواقع على QTL الكروموسومات 1و 8 و10 مع تباينات مظهرية (R^2) بنسبة 12.5 و29.0 و 20.20%، على التوالي. كان موقع QTL على كروموسوم 1 محاطاً بالواسم RM 8094 وRM3412 الذي يوجد في نفس الموقع المعرف سابقاً كموقع QTL أساسي وسمي SalTol. إلا أنه وجد أثنين QT L ذوي تأثيرات كبيرة نوعاً محاطين بين RM25 وRM210 على كروموسوم 8، وRM25092 و RM25519 على كروموسوم 10و يبدوان كموقعين جديدين. الواسمات المحيطة بهذه المواقع تعد مفيدة في تقنية تربية النبات بمساعدة الواسمات جزيئية لتحمل الملوحة.

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Introduction

Salinity is the most common abiotic problem in rice growing areas of the world (Senadhira, 1987). Millions of hectares in the tropics, arid and semi-arid regions are the potential rice cultivation areas but are left idle or being cultivated with low yielding traditional varieties or land races due to the lack of suitable tolerant high yielding varieties. There are different estimates of the world salt-affected areas ranging from 340 m ha to 1.2 b ha (Mossoud, 1977; Tanji, 1990). However current FAO assessment (2001) indicates that world-wide 831 m ha area falls under saline and sodic soils. More than 54 million hectares of rice lands in Asia are affected by salinity; another 9.5 million hectares of saline soils can be managed by large-scale irrigation and drainage schemes and by chemical treatment of soil, but the scale of problem makes these solutions too costly (Gregorio et al., 2002). The rice plant is one of the most suitable crops for saline soils, although it is considered moderately sensitive to salinity (Mori and Kinoshita, 1987).

Salt tolerance is a complex, quantitative, genetic character controlled by many genes (Shanon, 1985; Yeo and Flowers, 1986, Mishra et al, 1998; Singh et al., 2001). Using conventional breeding methods, plant selection for salt tolerance is not easy because of the large environmental effects and low heritability of salt tolerance (Gregorio and Senadhira, 1993; Gregorio et al., 2002). In view of this, repeatable molecular markers with environmental interference could be the best choice, hence are widely accepted as valuable tools for rice breeding, especially abiotic stresses (Mackill et al., 1999).

QTLs for salinity tolerance in rice have been mapped using Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), and microsatellite or simple sequence repeat (SSR) markers in different populations (Zhang et al., 1995; Gregorio et al., 2002; Lin et al., 1998; Gong et al., 1999; Prasad et al., 2000; Koyama et al., 2001; Bonilla et al., 2002, Lin et al., 2004; Lee et al., 2006). Microsatellite markers have been useful for tagging and mapping of genes/QTLs associated with salinity tolerance (Lang et al., 2001). Gregorio et al. (2002), Gong et al. (1999), Bonilla et al. (2002) and Lee et al. (2006) detected a major QTL for salt tolerance on Chromosome 1 but their position in chromosome was not exactly the same. Zhang et al. (1995), Lin et al. (1998) and Prasad et al. (2000) also detected QTL in chromosomes 7, 5 and 6, respectively. Koyama et al. (2001) identified ten QTLs for five shoot traits related to salinity tolerance; Na^+ uptake (one), K^+ uptake (two), Na^+ (two) and K^+ (two) concentration, and Na^+:K^+ ratio (two). Lin et al. (2004) detected five QTLs for four traits associated with salinity tolerance in roots, three QTLs for three traits of shoots but they were not in the same map locations. Lee et al. (2006) detected QTLs for salinity tolerance at seedling stage of rice used visual score of leaf injury symptom as phenotypic trait.

Rice is relatively sensitive at seedling and reproductive stage, so being one of the most critical stages for salinity sensitivity, the identification of new large effect QTLs for salinity tolerance is an important focus of breeding efforts. The F_2 population derived from the cross between BRRI dhan40 (moderately tolerant) and IR61920-3B-22-2-1 (NSIC Rc106; highly tolerant) was used as the mapping population for the study. This population was carefully selected from the ongoing salinity breeding program at the International Rice Research Institute (IRRI) because the highly tolerant parent does not have the commonly-used donor parent Pokkali in its pedigree and thus may represent a potentially novel source of salinity tolerance.

Materials and Methods

Plant materials

F_2 populations from the cross BRRI dhan40/ IR61920-3B-22-2-1 where BRRI dhan40 is a popular *indica* rice variety cultivated in Bangladesh and moderately tolerant to salinity at seedling stage and the IR61920-3B-22-2-1 is highly tolerant to salinity and released as NSIC Rc106 as salinity tolerant high yielding rice variety in the Philippines.

Screening for salt tolerance

Screening of 300 F_2 plants was done under controlled environment conditions following the method described by Gregorio et al. (1997) (Figure 1). A nutrient solution was used as described by Yoshida et al. (1976) for plants growth in solution culture. The salinity stress of 12 dSm^{-1} electrical conductivity (EC) was imposed in the nutrient culture solution. The pH of the nutrient solution was maintained at 5.0 everyday and nutrient solution was renewed weekly. The screening test was conducted in IRRI Phytotron maintained at 29°C/21°C day and night temperature, a relative humidity of 70% and natural daylight. IR66946-3R-178-1-1, also known as FL478, was used as tolerant check while IR29 was used as sensitive check. A modified standard evaluation score was used in rating the symptoms of salt damage. Scoring was done three weeks after salinization or after the death of the sensitive check.

Figure 1. Phenotypic screening of the population.

Molecular marker analysis

Among the 300 F_2 segregating plants, 93 plants with extreme phenotype for salinity stress response, i.e. tolerant and sensitive, were used for selective genotyping based on of visual seedling stage salt tolerance score on 1 to 9 scales where lower number indicates tolerance. Total two hundred and sixty SSR and two EST markers, selected at 5 Mb intervals within the rice genome were used for parental polymorphism survey (IRGSP, 2005). In *SalTol* QTL region of chromosome 1 (Bonilla et al., 2002) we used 10 additional markers that were tightly-linked to that QTL. Finally 90 makers were found polymorphic and used for QTL mapping of seedling stage salinity tolerance.

Genomic DNA was extracted using the modified CTAB method as described by Zheng et al. (1995) Microsatellite analysis was performed using the methods described by Temnykh et al. (2000). PCR was carrying out in a PTC-100 dyad thermocycler machine (MJ Research) using 384-well plate. Amplification products (2-3 µl) were resolved by polyacrylamide gel electrophoresis (8%, 10% or 12% gels). The gel was run for 2-5 hrs at 100 volts. The gels were stained with SYBR safe staining solution and visualized under UV light. For each marker, allelic bands were scored based on tolerant and sensitive parental

band of the amplified products and were designated as A, B and H (Figure 3) for homozygous tolerant, homozygous sensitive and heterozygote, respectively (Figure 2).

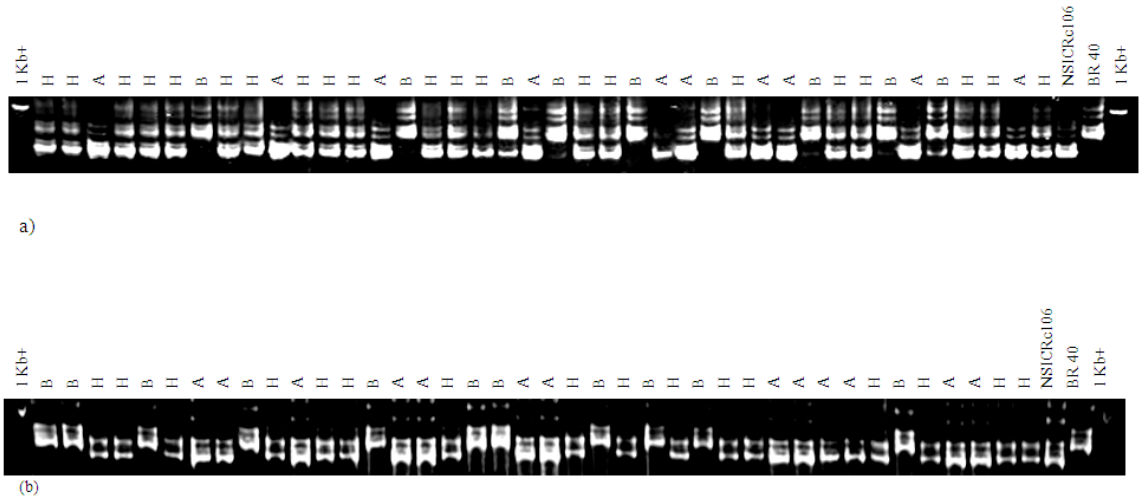

Figure 2. Genotype scoring for marker (a) RM1287 and (b) RM3412 in population 1.

Linkage and QTL Analysis

Linkage analysis was performed using the Map Manager/QTX computer program (Manly et al., 2001) using the Kosambi function and linkage evaluation of P=0.001. The ripple command was used to verify the marker order. QTL analysis was performed using Windows QTL Cartographer version 2.5 (Basten et al., 2001). For interval mapping analysis (IM), a LOD threshold score of 2.5 was selected. The proportion of the total phenotypic variation explained by each QTL was calculated as R^2 value (R^2 = ratio of the sum of squares explained by the QTL to the total sum of squares). For more accurately determining QTL positions, composite interval mapping (CIM) was performed with default parameters (permutation time 300, significance level 0.05, model 6; standard model, method 3; forward and backward method, walk speed 2 cM etc.)

Results

Phenotypic Scoring

Among the 300 plants (Figure 3), 93 tolerant and sensitive plants were selected for selective genotyping for molecular study. The selected population behaved little skewed in favor of tolerant types. Among 93 plants 3 were scored as 1 (highly tolerant), 42 were scored as 3 (Tolerant), 33 were scored 5 (moderately tolerant), 6 were scored as 7 (susceptible) and 9 were scored as 9 (highly susceptible).

Construction of linkage map

Linkage analysis showed that all of the markers formed 12 linkage groups except RM10287, RM6681 and RM21333 which were unlinked. This map covered whole rice genome and three to eleven markers were found to link to different linkage groups (chromosome).

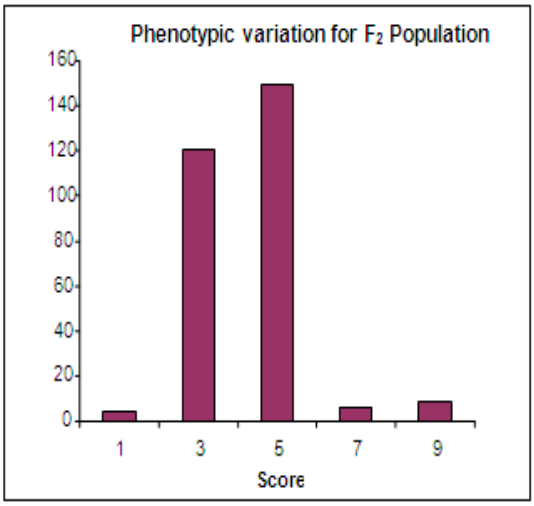

Figure 3. Phenotype variation of salinity tolerance for 300 F_2 plants.

Quantitative trait loci (QTL) analysis

Single marker analysis results for all the chromosomes are summarized in Table 1. In chromosome 1, RM8094 was found to be strongly associated with salinity tolerance (P<0.001). Three more markers RM3412, RM493 and CP03970 were found significantly associated with salinity tolerance (P<0.01) and other four markers RM10665, RM1287, RM10825 and RM11008 also found significantly associated (P<0.05). The graph derived from IM and CIM are shown in Fig.4. The QTL was tightly linked with the marker RM8094 with the LOD score of 2.7 and R^2 value 0.125. A LOD peak was located near RM8094 as depicted in IM and CIM line graph that falls, between the flanking markers RM8094 and RM3412. The overall phenotypic variance was additive type toward the parent BRRI dhan40 allele that had 1.04 additive effects for increased salinity tolerance.

Table 1. Results of Single Marker Analysis for association with salinity tolerance in rice at seedling stage.

Chromosome #	Markers	F-value	P- Value
Chromosome 1	RM10665	4.4	0.038*
	RM1287	4.2	0.044*
	RM8094	12.3	0.001***
	RM3412	8.1	0.005**
	RM493	9.6	0.003**
	CP03970	7.9	0.006**
	RM10825	4.1	0.047*
	RM11008	4.2	0.042*
	RM11438	5.4	0.022*
Chromosome 8	RM25	9.588	0.003**
	RM210	31.149	0.000****
Chromosome 10	RM25092	14.040	0.000***
	RM25217	47.089	0.000****
	RM25519	4.154	0.044*

Second robust QTL was found on chromosome 8. RM210 was found to be strongly associated with salinity tolerance with a probability value of < 0.0001. RM25 marker was also found significantly associated with salinity tolerance (P<0.01) as revealed in the graph derived from IM and CIM (Figure 5). The QTL was tightly linked with the marker RM210 with the LOD score of 7.0 and R^2 value 0.290. The peak was in IM and CIM line graph was located near RM210, and flanked by RM210 and RM25. IR61920-3B-22-2-1 allele had 1.76 additive effects for increased salinity tolerance. Third QTL with high LOD value was fund on linkage group 10. RM25217 was found strongly associated with salinity tolerance (P<0.0001) while markers RM25092 and RM25519 found significantly associated with salinity tolerance with P values <0.001 and <0.05, respectively.

The graph derived from IM and CIM are shown in Figure 6. The QTL was tightly linked with the marker RM25217 with the LOD score of 4.5 and R^2 value 0.202. The LOD peak was located near RM25217, between the flanking markers RM25092 and RM25519. BRRI dhan40 allele had 1.44 additive effects for increased salinity tolerance.

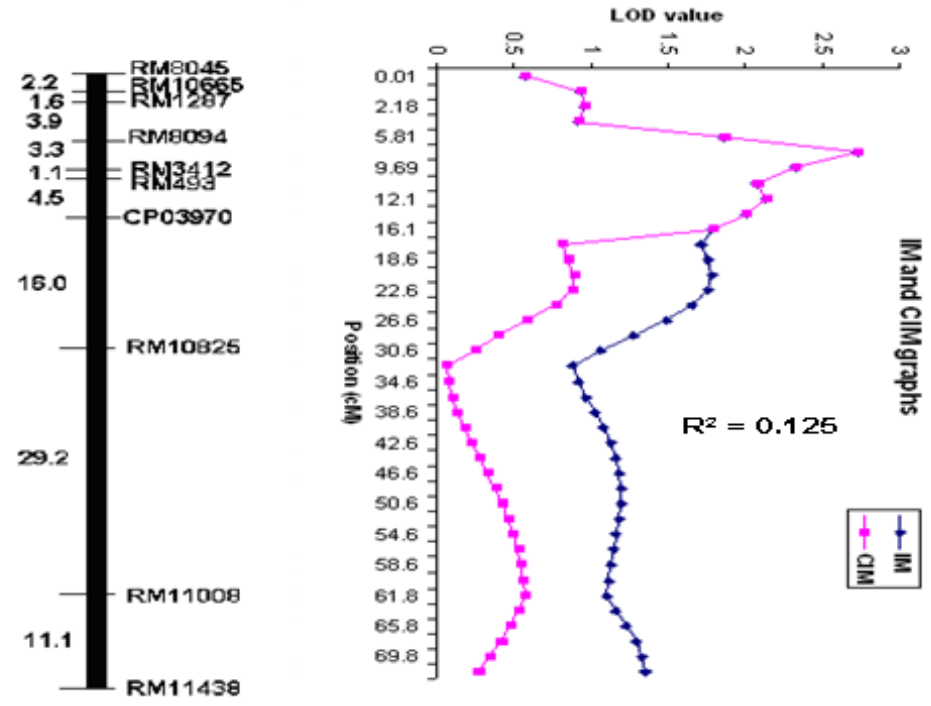

Figure 4. IM and CIM graphs for Chromosome 1 of F_2 population from the cross of BR40/NSIC Rc106.

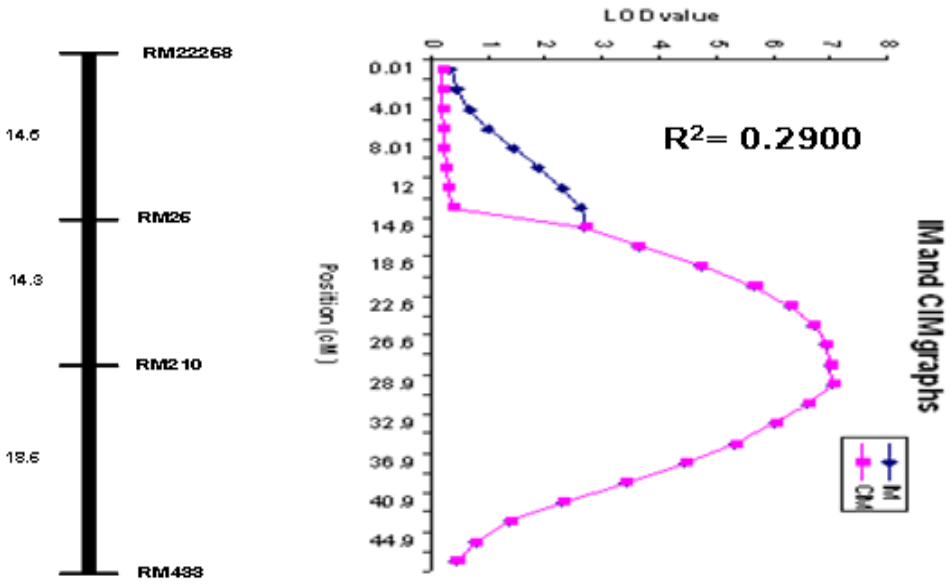

Figure 5. IM and CIM graphs for Chromosome 8 of F_2 population from the cross of BR40/NSIC Rc106.

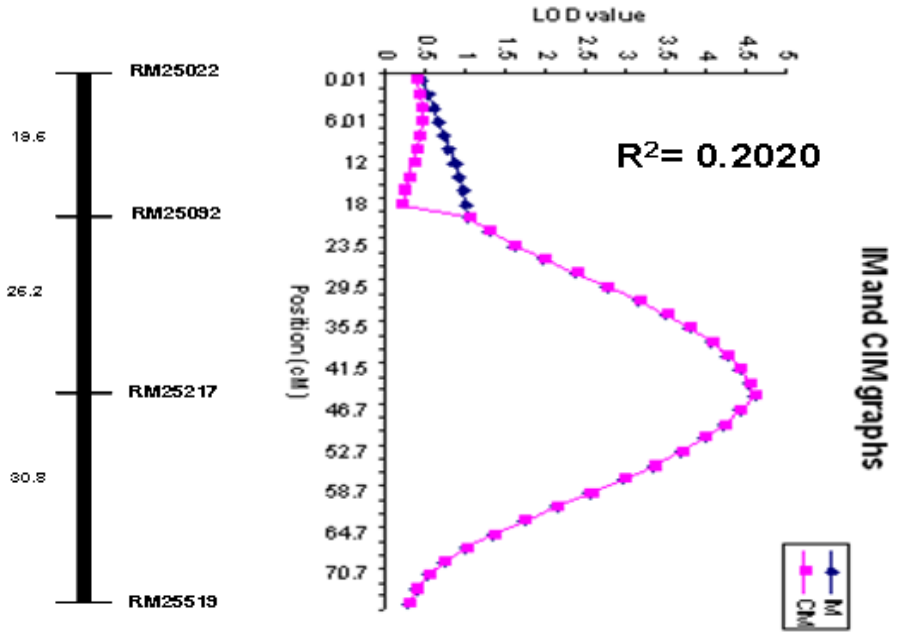

Figure 6. IM and CIM graphs for Chromosome 10 of F_2 population from the cross of BR40/NSIC Rc106.

The summary results from QTL analysis is given in Table 2. As we found only three major QTLs on Chromosome 1, 8 and 10, they are named as *SalTol*1-1, *SalTol*8-1 and *SalTol*10-1. No other marker was found significantly associated with salinity tolerance on any other chromosomes.

Discussion

One problem often associated with the application of QTLs identified from preliminary mapping studies is that QTLs may not be effective in actual breeding material. Often parents for QTL mapping studies that represent the extremes of a trait are used for mapping, which may not provide a realistic indication of the QTL effect in actual breeding programs because elite varieties may already possess desirable alleles (Charcosset and Moreau, 2004; Collard and Mackill, 2006). For this reason, a 'real' breeding population was used in this study. Furthermore, the best lines identified in this study could be advanced to the next generation within the breeding program, thus bridging the gap between QTL discovery (basic research) and breeding (applied research).

Table 2. Putative QTLs for rice salt tolerance at seedling stage in F_2 population derived from BRRI dhan40 and IR61920-3B-22-2-1 (NSIC Rc106).

QTL[a]	Chr.	Markers bordering QTL	Peak LOD	Additive effect	PVE[b]	DPE[c]
*SalTol*1-1	1	RM8094-RM493	2.7	-1.04	12.5	BR
*SalTol*8-1	8	RM25-RM210	7.0	1.76	29.0	IR
*SalTol*10-1	10	RM25092-RM25519	4.5	-1.44	20.2	BR

a QTLs are named by abbreviations plus chromosomal number and serial

b Percentage of total phenotypic variance explained by the QTL

c Direction of phenotypic effect: BR and IR indicate BRRI dhan40 and IR61920-3B-22- 2-1, respectively.

The three QTL analysis methods single marker analysis, interval mapping (IM) and composite interval mapping (CIM) were used in order to confirm QTL results because some differences in results are sometimes obtained using different methods. From single marker analysis in chromosome 1, RM8094 was found to be strongly associated with salinity tolerance with significant on $P<0.001$. Other three markers RM3412, RM493 and CP03970 found significantly associated with salinity tolerance ($P<0.01$) and other four markers RM10665, RM1287, RM10825 and RM11008 were also significantly associated ($P<0.05$). These results revealed that there was important QTL for salinity tolerance in this region of the chromosome 1 segment.

To more precisely determine the location of the identified QTL for salt tolerance by single marker analysis, IM and CIM analysis was performed. The LOD plot of IM and CIM was similar. A LOD peak was found from IM and CIM line graph which was located at RM8094 and flanking between RM8094 and RM3412. From this result it was assumed that a QTL was tightly linked with the marker RM8094 with the LOD score of 2.7 and R^2 value was 0.125. Niones et al. (2005) also reported that a QTL for salinity tolerance was present in this region of chromosome 1 segment and the position of QTL was between the marker loci CP6224 and RM8094 (1.5 cM) by using NILs (BC_3F_4) of the cross of Pokkali/ IR29. Bonilla et al. (2002) saturated this segment of chromosome 1 with RFLP and microsatellite markers using the RIL population and reported that two microsatellite markers, RM23 and RM140 flanked the *SalTol* QTL with 16.4 and 10.1 cM distance, respectively. This suggests that the distal region of chromosome 1 is an important region of the rice genome for salinity tolerance and may indicate the presence of different QTL alleles or the presence of a gene cluster.

On chromosome 8, from single marker analysis, RM210 was found to be strongly associated with salinity tolerance with significant on $P<0.0001$ while marker RM25 found significantly associated with salinity tolerance ($P<0.01$). These results revealed that there was important QTL for salinity tolerance in this region of the chromosome 8 segment. A LOD peak was found from IM and CIM line graph which was located near RM210 and flanking between RM25 and RM210. From this result it was assumed that a major QTL was tightly linked with this region with the LOD score of 7.0 and R^2 value was 0.2900. This QTL represents a potential candidate for marker assisted selection because of the high LOD score and relatively large effects. This is the first report of QTL identification from the IR61920-3B-22-2-1 parent, despite the high salinity tolerance of this genotype. Ammar (2004) also detected 4 QTLs with LOD scores ranging from 3.95 to 4,84 on chromosome 8 for the traits of Na^+ concentration in the leaf tissue at vegetative stage, Na^+ concentration in the leaf tissue at reproductive stage, Na^+ concentration in the stem tissue at vegetative stage, and Na^+/K^+ ratio in the stem tissue at reproductive stage and their positions were in between of RM3395 and RM281 in chromosome 8 by using SSR markers in F_2 populations of CSR27/ MI48. The position of the QTL detected in our study and Ammar (2004) is in the same region of chromosome 8.

On chromosome 10, from single marker analysis, RM25217 was found to be strongly associated with salinity tolerance with significant on $P<0.0001$ while marker RM25092 and RM25519 found significantly associated with salinity tolerance $P<0.001$ and $P>0.05$, respectively. These results revealed that there was large QTL for salinity tolerance in this region of the chromosome 10 segment. The LOD peak was located near RM25217 and flanking between RM25092 and RM25519. The results suggested that a major QTL was tightly linked with this region with the LOD score of 4.5 and R^2 value was 0.2020. Gregorio (1997) also detected a major QTL contributing 86.1 % phenotypic variation of the trait of Na^+/K^+ ratio in chromosome 10 by using AFLP markers in F_8 recombinant inbred lines (RILs) of Pokkali/IR29 cross.

In the conclusion, the detection of new QTLs associated with salinity tolerance should be useful for rice improvement in the future, especially if these QTLs appear to have relatively large effects. Ideally fine mapping of

the regions of chromosomes 8 and 10 where the new major QTLs were found should be performed in order to more accurately resolve the QTL positions and validate their effects; fine mapping of *SalTol*1 region in chromosome 1 is currently in progress. After validation of all tightly linked flanking markers for these QTLs, these markers could potentially be routinely used by breeders for marker assisted selection for salinity tolerance rice breeding programs.

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