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Toward the Mapping of Agronomic Characters on a Rice Genetic Map: Quantitative Trait Loci Analysis under Saline Condition

H. Sabouri and A. Biabani
Gonbad High Education Center, Department of Plant Production,
Gorgan University of Agriculture Science and Natural Resource

Abstract: Improvement of rice (*Oryza sativa* L.) yields in saline condition through breeding require a good understanding of genetic factors that control component traits, such as grain yield, number of filled grain, flowering date and other agronomic traits. The objective of this study was to identify and characterize Quantitative Trait Loci (QTLs) salt tolerance at reproductive stage in saline condition using $F_{2:3}$ populations derived from the cross between Tarommahalli (*indica*) and Khazar (*indica*). The linkage map constructed by 74 SSR molecular markers covered a total of about 1231.50 cM. Three QTLs were detected for number of primary branches under salt stress on chromosomes 1, 2 and 6. Also, Two QTLs (on chromosome 1) and two QTLs (on chromosome 3) were identified for biomass and plant height, respectively. Tarommahalli alleles (except qNB-6) increased salt tolerance in these loci. In this study, the three major QTLs with the very large effect, qUFG-1b for number of unfilled grain, qBI-1a and qBI-1b for biomass explained 22.58, 22.24 and 26.83% of the total phenotypic variance, respectively. In this study, new QTLs play essential roles in the growth of rice at reproductive stage in Iranian local population under salt stress and provide a rich source of information about the natural genetic variation underlying breeding rice in saline condition.

Key words: Rice (*Oryza sativa* L.), quantitative trait loci, salt tolerance, SSR

INTRODUCTION

The soil salinity of reclaimed paddy fields is one of the important stresses to limit rice growth and yield in Asia and Africa. Improving the salt tolerance is the most efficient way to dominate with salinity problem because use of non-saline water for irrigation, soil reclamation and leaching brings its own problems. Additionally relatively small increase in salt tolerance may greatly reduce the leaching requirement in areas that need to be reclaimed by leaching (Shannon, 1997). As rice (*Oryza sativa* L.) is salt sensitive, at reproductive stage. For the reason that salt-tolerance related traits are complex to facilities a development of new varieties with a high level of salinity tolerance, it will be required to understand the genetic control mechanisms for salt tolerance and using advanced methods as molecular markers technology. Using of DNA markers and the development of molecular maps (Bassam *et al*., 1991; Saghi Maroof *et al*., 1994; Chen *et al*., 1997; Creste *et al*., 2001; McCouch *et al*., 2002) had possible molecular investigation and mapping QTLs controlling salt tolerance-related traits and allow plant scientists to locate the DNA that is determining the physiological trait that dictate salt tolerance. The recent advances in QTL mapping have made available useful tools for plant breeders in field of elucidating the genetic basis of complex traits (Causse *et al*., 1994). QTL analysis of salt tolerance has been conducted by Zhang *et al*. (1995), Flowers *et al*. (2000), Koyama *et al*. (2001), Lin *et al*. (2004), Ming-Zhe *et al*. (2005) and Lee *et al*. (2007). Prasad *et al*. (2000) using a Double Haploid (DH) mapping population, found seven QTLs for seedling traits. Lang *et al*. (2001a, b) identified SSR and RFLP markers associated to QTLs for seedling survival in salinity condition, shoot and root dry mass and ion exchange on chromosomes 1, 2, 3, 7, 9, 11 and 12. Koyama *et al*. (2001) identified 11 QTLs on 4 different chromosomes, 1, 4, 6 and 9 for different component trait related to salinity (Na^+ uptake, K^+ concentration, Na^+/K^+ ratio tolerance, K^+ uptake and K^+ concentration). Takehisa *et al*. (2004) were detected QTLs associated with salt tolerance in paddy field flooded with salt water. Ming-Zhe *et al*. (2005) reported that, two QTLs for dry mass on chromosome 8 and 9 and two QTLs for Na^+/K^+ on chromosome 2 and 6 one on each chromosome control salt tolerance, respectively. In the study of Lee *et al*. (2007) two QTLs (qST1 and qST3) conferring salt tolerance at young seedling stage were mapped on

Corresponding Author: Abbas Biabani, Gonbad High Education Center, Department of Plant Production,
Gorgan University of Agriculture Science and Natural Resource

chromosome 1 and 3, respectively. QTL mapping for salinity tolerance was reviewed by Singh *et al.* (2007), exactly. Although there have been extensive studies on QTL mapping salinity tolerance in rice, little or no information has been reported on the mapping of salinity tolerance in local population in the all world. The aim of the present study is to identify QTLs related to salt tolerance by using an Iranian rice population.

MATERIALS AND METHODS

A mapping of rice (*Oryza sativa* L.) segregating for the traits of interest was identified from an initial screening of potential parents. The parents of Tarommahalli and Khazar showed extreme phenotypes for agronomical traits in saline condition. The parental line, Tarommahalli (TAM) was a salt tolerant variety, and other parental line, Khazar (KHZ) was a salt-susceptible variety that screened from Iranian rice germplasm in during 2004-2005 (Sabouri *et al*., 2007a-c; Sabouri *et al*., 2008a, b). Crosses were carried out with potted plants in screen houses at the experimental site of Rice Research Institute of Iran during 2004. Khazar serves as female parents were castrated using scissors and the emasculator developed, when the panicles were half exerted from the sheath of the flag leaf, which was removed for that purpose. The panicles were then covered with transparent plastic bags, which were removed on several subsequent days between 12:00 and 12:30 h for artificial pollination. Donor for pollen sown at 15-day intervals in order to continuously provide viable pollen. At 25 to 30 days after pollination, F_1 grains were harvested from individual panicles and stored at 48°C until they were used for the experiment. The F_2 *Oryza sativa* L. mapping population (192 genotypes) was derived from self-pollinating an F_1 hybrid obtained in 2005. The seeds of F_2 population and their parental cultivars were imbibed distilled water at 30°C for 2 days, after surface-sterilization with 70% ethanol solution and 1% sodium hypochlorite solution. Germinated seeds were sown on a paddy field. Field experiments were conducted during 2006. Basal fertilizer (N, PO_5 and K) was applied to the paddy fields at 3-7 days before transplanting. Nitrogen, Phosphorus and Potassium fertilizer were applied at the rates of 30, 30 and 30 kg ha^{-1}, respectively. The plants were transplanted into a paddy field with single planting per hill. The space between hills was 25 cm. Leaves from the main stem of each plant examined were sampled, and genomic DNA was extracted according to CTAB method (Saghi Maroof *et al*., 1994). Three hundred and sixty five SSR primer pairs which were appropriately distributed on 12 rice chromosomes were chosen according to Chen *et al.* (1997), Temnykh *et al.* (2000) and McCouch *et al.* (2002). Three hundred and sixty five SSR primer pairs surveyed based on their polymorphism between two parents, and the primers exhibiting polymorphism were used to amplify the DNA of each plant of F_2 population. Seventy four polymorphic SSR markers were used for QTL analysis. PCR amplification was performed on a thermal cycler (Biometra Uno II, Germany) in biotechnology laboratory of Rice Research Institute of Iran. The amplification products were electrophoresed for 1 until 2 h on 6% polyacrylamide gels and detected by silver staining as described by Basam *et al.* (1991) and Creste *et al.* (2001). Using Mapmanager QTbX17, 12 linkage groups were constructed with a minimum LR score 11.5. Map distances between were presented in centi Morgan (cM) derived using the Kosambi function (Kosambi, 1944) of the program.

The genetic material involved 192 F_3 families, each derived from bagged seeds of a single F_2 plant from a cross between TAM and KHZ. The seeds of $F_{2:3}$ populations, whose dormancy was broken, were used to evaluate salt tolerance for seedling stage in 2007. For per family 15 seeds were used for recording traits in saline condition at reproductive stage according to Gregorio *et al.* (1997) in 2007. Three seeds of each family were sown in each pot. When seedlings were 25 old, water was siphoned out and the pots were drained for 24 h, after which they were watered with EC 4 dS m^{-1} using NaCl. The salinity level of the second treatment was increased gradually to 6 dS m^{-1} (for 3 days), 8 dS m^{-1} (for 4 days) and finally stabilized at 12 dS m^{-1} up to repining. Plants were harvested and Plant Height (PH), number of Filled Grain (FG), number of Unfilled Grain (UFG), days to heading (DH), Panicle Length (PL), Number of Tiller (NT), Harvest Index (HI), Number of Branches (NB) and biomass (BI) were recorded.

QTL cartographer v. 2.5 used to identify QTLs affecting salt tolerance on the basis of composite interval mapping analysis. A LR score 11.5 was used to declare the presence of putative QTL in a genomic region. The percentage of total phenotypic variation explained by each QTL, and the additive effects were estimated by QTL cartographer v. 2.5 (Basten, 2001).

RESULTS

A linkage map based on F_2 population was constructed, which covered a total of 1231.50 cM with an average two locus interval of 19.83 cM (Fig. 1). The position of most SSR markers on chromosomes was identical with the previous reported by Chen *et al.* (1997), Temnykh *et al.* (2000) and McCouch *et al.* (2002).

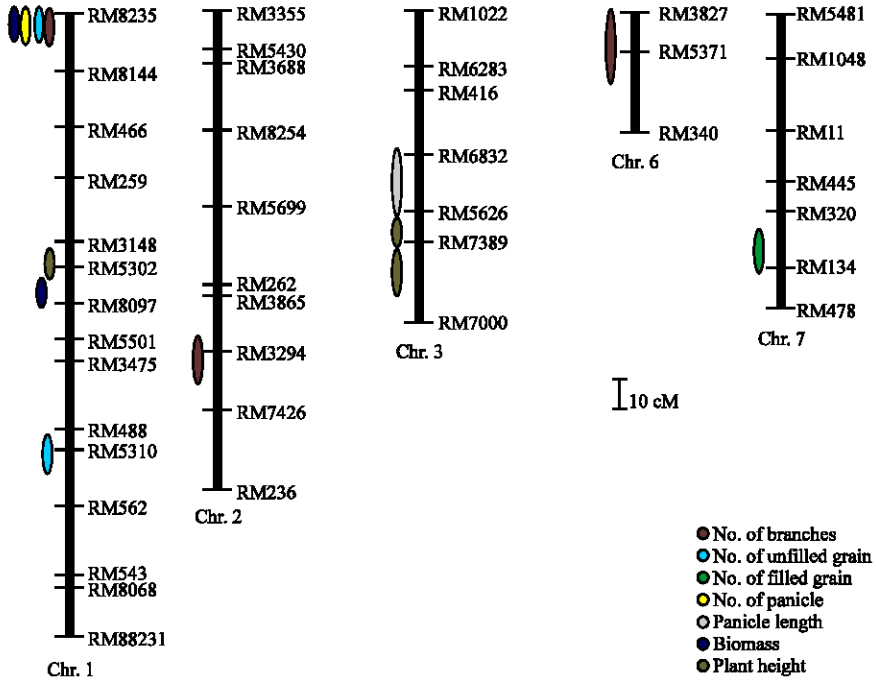

Fig. 1: The position of QTL for traits representing salt tolerance of rice in the reproductive stage

Table 1: Putative QTLs for salt tolerance in reproductive stage the F_2 population derived from TAM and KHZ

Traits	QTL	Chr.	Flanking markers	LR	a	d	PEV	Dpe
No. of branches	qNB-2	2	RM3294-RM7420	12.920	0.67	0.17	19.26	KHZ
	qNB-1	1	RM8235-RM8122	17.010	0.99	-0.17	18.69	KHZ
	qNB-6	6	RM3827-RM5371	14.030	-0.84	-0.05	19.77	TAM
No. of panicle	qNP-1	1	RM8235-RM8144	20.610	0.60	-0.36	11.53	TAM
Panicle length	qPL-3	3	RM6832-RM5626	14.460	3.72	-1.43	12.95	TAM
Biomass	qBI-1a	1	RM8235-RM8144	4.583	5.99	-4.16	22.24	TAM
	qBI-1b	1	RM5302-RM8097	13.340	8.04	-2.32	26.83	TAM
Plant height	qPH-3a	3	RM5626-RM7389	13.230	11.77	-10.42	12.95	TAM
	qPH-3b	3	RM7389-RM7000	12.230	11.43	-10.79	13.50	TAM
No. of unfilled grain	qUFG-1a	1	RM8235-RM8144	27.860	24.06	-11.91	9.78	KHZ
	qUFG-1b	1	RM488-RM3310	16.540	21.50	-24.45	22.58	KHZ
No. of filled grain	qFG-7	7	RM134-RM478	18.130	7.71	2.95	8.76	TAM

QTL: QTLs are named by abbreviations plus chromosomal number, a: Additive effect, d: Dominant effect, PEV: Percentage of total phenotypic variance explained by the QTL, Dpe: Direction of phenotypic effect, TAM and KHZ indicate Tarommahalli and Khazar, respectively

QTL mapping

No. of Branches (NB): Three QTLs were mapped for No. of Branches (NB). Amongst them, two QTLs with the largest effects were qNB-2 and qNB-6 that individually explained 19.26 and 19.77% of the total phenotypic variation and had an additive effect of 0.67 and a negative effect of -0.84, respectively. One QTLs (qNB-2) showed partial dominant effect for induced NB while the other QTLs showed partial dominance for reduced NB (Table 1).

No. of Panicle (NP): One QTL was identified for No. of Panicle (NP) on chromosome 1. The QTL located in interval RM8235-RM8144, showed the large effect on the CHL with an LR score of 20.61 and explaining 11.53% of the total phenotypic variance. The additive effect of this QTL was positive. This QTL allele from TAM increased NP by 0.60 and exhibited partial dominance for decreased NP (Table 1).

Panicle Length (PL): One QTL was identified for Panicle Length (PL) on chromosome 3. This QTL located in interval RM6832-RM5626 (qPL-3) showed positive effects on the PL with an LR score of 12.23 and exhibiting 12.95% of the total phenotypic variance. In this QTL, alleles from TAM increased PL by 3.72 cm. So, this putative QTL showed partial dominance effect for PL with d/a ratio of -0.38 (Table 1).

Biomass (BI): Two QTLs were identified for biomass (BI). Two QTLs with the large effects were qBI-1a and qBI-1b that individually explained 22.26 and 26.83% of the total phenotypic variation and had an additive effect of 5.99 and 8.34 g, respectively, for increased BI. Theses QTLs had exhibited a partial dominance effect with reduced BI with d/a ratio of 0.69 and 0.47, respectively (Table 1).

Plant Height (PH): Two QTLs were mapped for Plant Height (PH) on chromosome of 3. Two QTL located showed the small effect on the PH with a explaining 12.95 (qPH-3a) and 13.50% (qPH-3b) of the total phenotypic variance. For two the putative QTLs the alleles for decreased PJH were from KHZ (Table 1).

No. of Filled Grain (FG): One QTL was identified for No. of Filled Grain (FG) on chromosome 7. This QTL located in interval RM134-RM478 (qFG-7) showed positive effects on the FG with an LR score of 18.13 and exhibiting 8.76% of the total phenotypic variance. In this QTL, alleles from TAM increased FG by 7.71. This putative QTL showed partial dominance effect for FG (Table 1).

No. of Unfilled Grain (UFG): Two QTLs were mapped for No. of unfilled grain (NFG) on chromosome of 1. The QTL located in interval RM488-RM3310 (qUFG-1b) showed the large effect on the UFG with a 22.58% of the total phenotypic variance. For two the putative QTLs the alleles for increased UFG were from KHZ (Table 1).

DISCUSSION

Salt tolerance in rice was controlled by polygene with the additive and dominant effects (Gregorio and Sendhira, 1993). Gregorio and Sendhira (1993) observed that there were two groups of genes involved in sodium and potassium uptake in rice, one group for sodium exclusion and the other for potassium absorption.

We detected QTLs related to number of unfilled grain, number of primary branches, number of panicle and biomass in the same region in RM8235-RM8144 interval on chromosome 1. Brondani *et al.* (2002) reported QTLs related to spikelet per panicle, grain yield per panicle in this region, in normal condition. Region of RM8235-RM8144 increased salt tolerance while one QTLs in RM3827-RM5371 interval reduced salt tolerance. This study revealed that alleles of QTL enhancing salt tolerance were not only from salt-tolerant parent but also from salt sensitive parent.

There have been several other reports on QTL analysis of rice salt-tolerance in seedling stage (Zhang *et al.*, 1995; Lin *et al.*, 1998; Koyama *et al.*, 2001) but little or no information has been reported on the mapping of salinity tolerance in reproductive stage. Most of these QTL analyses were conducted by using RFLP or AFLP markers except for the study of Prasad *et al.* (2000) and Ming-Zhe *et al.* (2005) which used an SSR linkage map. However SSR markers were used in the present study. Use of SSR markers allowed us to roughly compare the QTLs detected in different groups. A chromosomal segment on chromosome 3 (RM6832-RM5626) explains the major part of the variation for PH. Hittalmani *et al.* (2002) and Ishimaru *et al.* (2001) also detected one QTL for plant length in the same region on chromosome 3 in non saline condition. The comparison between the chromosomal positions of QTLs related to number of unfilled grain, number of primary branches, number of panicle and biomass on chromosome 1 is difficult to determine; whether QTLs in these regions are at the same loci or are different tightly linked loci. Further analysis, including the verifying mapped QTLs, fine mapping of both QTLs using common markers, and cloning and the sequence comparison of these QTLs, will be required to answer these questions. Identification of any tightly linked markers in this region will serve as a candidate gene for fine-mapping and further use in Marker-Assisted Selection (MAS).

The growth and its related traits included BI, NB and NP. A total of four QTL have been identified for these traits in RM8235-RM8144 region. Major loci for BI (qBI-1a and qBI-91b) were bracketed by RM8235-RM8144 and RM5302-RM8097 that spread over 15 and 12 cM on chromosome 1, respectively. A complex trait (e.g., salt tolerance) is regulated by a number of elementary factors, but it is likely that the factors are not equally effective in determining the trait. Comparing the location of QTLs makes it possible to determine the genetic relations among traits and the genetic limiting factors for a complex trait. QTL for BI overlapped with NB, NP and. These multiple effect of QTL on the same chromosomal region could be due to the fact that salt tolerance performance is derived from number of panicle, number of primary branches and number of unfilled grains. It is concluded that there is a relationship these traits, which may be controlled by the same gene or linked genes. The QTL on chromosome 1 may contain a new major gene for salt stress tolerance at reproductive stage in rice. This needs to be confirmed by conducting several field trials in saline soils for two or three seasons to test if the QTL are stable across seasons and growth phases of the crop. Rice breeders are resorting to molecular marker technology for developing salt-tolerant varieties, as traditional breeding practices, turned out to be difficult exercise in tackling complex

Table 2: Correlation coefficients among traits studied in 192 $F_{2:3}$ families

Parameters	1	2	3	4	5	6	7
1	1.000						
2	0.321**	1.000					
3	0.477**	0.364**	1.000				
4	0.299**	0.399**	0.183*	1.000			
5	0.504**	0.476**	0.360**	0.561**	1.000		
6	0.437**	0.761**	0.387**	0.295**	0.390**	1.00	
7	0.350	0.437**	0.381**	0.218**	0.450**	0.19	1.00

*and** are probability levels of 0.05 and 0.01, respectively, 1: No. of branches, 2: No. of Panicle, 3: Panicle length, 4: Biomass, 5: Plant height, 6: No. of unfilled grain, 7: No. of filled grain

traits. QTL mapping is the first step in applying marker technology to the molecular breeding program. QTL identified by this technique, after fine-mapping, could be used for indirect selection of salt-tolerant traits to be used in MAS.

Trait correlations and clustering of QTLs for traits correlated were often mapped in the same chromosomal regions (Paterson, 1996). This trend was observed in this study. For example, qNB-1, qNP-1, qBI-1a, qBI-1b, qUFG-1a and qUFG-1b were located on chromosome 1 and were found at approximately the same map locations in chromosome 1. These traits (NB, NP, BI and UFG) showed a high correlation (Table 2). It seems that statistical correlation does necessarily reflect genetic relations, but Ishimaru *et al*. (2001) reported that statistical correlation does not relate with genetic relations. In these cases, the directions of the correlations were consistent with that of the effects of the QTLs on the traits. The regions of the genome that had effects on the multiple traits may have acted through the pleiotropic effects of a single gene or by the chance linkage of multiple, because salinity tolerance is complex physiological trait related to several traits. QTL pyramiding is the method that assembles many genes that work well together and, for a specific trait, assemble the alleles with similar effects from different loci (Lin *et al*., 2004). This process can make the superior genotype to improve the variety.

In this study of 192 F_3 families, seven families showed the highest NB, NP, BI and with high FG, i.e. high salt tolerance. Actually, the alleles of several QTLs from the high salt-tolerance variety TAM were pyramided in these seven families. Also, in the seven families, the TAM alleles of three QTLs (related to NB, NP and BI), were assembled. These results indicate that breeding methods of QTLs pyramiding by using marker-assisted selection are very useful for the development of new varieties with a high level of salt tolerance. In this study, precise detection of QTLs for salt tolerance remained a problem due to less SSR markers and low density linkage map and thus it is suggested that further study should be performed with more SSR markers and perpetual mapping population.

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