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Full Length Research Paper

New evidence of QTLs attributed to salinity tolerance in rice

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An $F_{2:3}$ population derived from the cross between Tarommahalli (*indica*) and Khazar (*indica*) was used to mapping salt tolerance in rice. The linkage map constructed by 74 simple sequence repeat (SSR) molecular markers covered a total of about 1231.50 cM rice genome. Plant stand, chlorophyll content, root and shoot length, fresh weight of root and shoot, dry weight of root and shoot, Na^+ uptake, K^+ uptake, Na^+/K^+ ratio related to uptake ions and green leaf area were mapped. Four QTLs for root length under salt stress were detected on chromosomes 1, 4, 7 and 9. Also, two QTLs (on chromosome 9) for dry weight root and three QTLs for ion exchanges (on chromosome 3 and 10) were identified. Tarommahalli alleles in these loci increased salt tolerance. Of these QTLs, the five major QTLs with the very large effect, qRL-7 for root length, qDWRO-9a and qDWRO-9b for dry weight root, qBI-1a and qBI-1b for biomass explained 16.21, 27.43, 25.50, 22.24 and 26.83% of the total phenotypic variance, respectively. All these results reinforced the idea that, new QTLs of this study play an important role in the growth of rice at seedling in Iranian local population under salinity condition.

Key words: Composite interval mapping, ion exchange, molecular markers, salt tolerance, simple sequence repeat.

INTRODUCTION

Rice (*Oryza sativa* L.) is salt sensitive, particularly at a seedling stage. It is important to breed new varieties with raised tolerance to salinity thus the normal approach is to expose a wide range of the crop to the stress and identify tolerant individuals for use in a breeding program. Salt-tolerance related traits are complex and to facilitate the development of new varieties with a high level of salinity tolerance, it will be required to understand the genetic control mechanisms for salt tolerance. This can be done by using molecular markers technology and mapping QTLs controlling salt tolerance-related traits.

QTL analysis of salt tolerance has been conducted by several researchers (Zhang et al., 1995; Koyama et al., 2001; Lin et al., 2004; Ming et al., 2005; Lee et al., 2007). Flowers et al. (2000) detected 16 QTLs related to ion concentration of sodium and potassium in rice shoots using amplified fragment length polymorphism (AFLP) analysis and RIL mapping population. They found that 12 QTLs affected shoot concentration, dry weight production on chromosome 6, one QTL for high sodium uptake on chromosome 1, two QTLs for potassium uptake found on chromosome 9 and 6 and another on chromosome 4 which is responsible for Na^+/K^+ discrimination. Prasad et al. (2000) using a double haploid (DH) mapping population, found seven QTLs for seedling traits, two QTLs for seed germination, one for seedling root length, three for seedling dry matter and one for seedling vigor. Of the total seven QTLs, four QTLs were located on chromosome 6. Lang et al. (2001a, b) identified single sequence repeat (SSR) and restriction fragment length polymorphism (RFLP) markers associated to QTLs for seedling survival in saline condition, shoot and root dry mass, Na^+ and K^+ absorption and for Na^+/K^+ ratio 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.

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Three QTLs on chromosome 1 for Na^+ uptake, K^+ concentration, Na^+/K^+ ratio, four QTLs on chromosome 4 for K^+ uptake, K^+ concentration and Na^+/K^+ ratio, three QTLs on chromosome 6 for dry mass, K^+ uptake and Na^+ concentration and one QTL on chromosome 9 for K^+ uptake were found. K^+ uptake had QTLs on different chromosomes which indicate the regulation of salinity tolerance by multiple set of genes. Lin et al. (2004) reported two major QTLs with very large effects; qSNC-7 for shoot Na^+ concentration and qSKC-1 for shoot K^+ concentration. These QTLs explained 48.5 and 40.1% of the total phenotypic variance, respectively. Ammar (2004) detected 18 QTLs on chromosomes 1, 2, 3, 8 and 9. The Na^+ content was controlled by 6 QTLs, which explained 47.59 - 55.18% of the total variation. QTLs related to Na^+ concentration in shoot at vegetative stage and chloride uptake were detected on chromosome 1. The Na^+/K^+ ratio was controlled by 7 QTLs in the shoot at vegetative and reproductive stage. Takehisha et al. (2004) detected QTLs associated with salt tolerance in paddy field flooded with salt water. Jian et al. (2005) detected an association between SSR marker (RM209, RM287) and relative growth rate and detected markers linked to Na^+/K^+ on chromosome 6 and chromosomes 1 and 11, respectively. Ming et al. (2005) reported that two QTLs for dry weight of shoot 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 chromosome 1 and 3, respectively. QTL found on chromosome 1 that explained 27.8% of the total variation identified as major QTL. Finally, QTL mapping for salinity tolerance was reviewed by Singh et al. (2007).

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 (*O. sativa* L.) segregating for the traits of interest was developed. The parental line, Tarommahalli (TAM) is a salt tolerant variety, and other parental line, Khazar (KHZ) is a salt-susceptible variety that was screened from Iranian rice germplasm during 2004-2007 (Sabouri et al., 2007 a, b, c; Sabouri et al., 2008 a, b).

DNA was extracted from main culm at tillering stage by Saghi Maroof et al. (1994) method. 365 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). Genotype data of 74 polymorphic SSR markers were used for QTL analysis. Polymerase Chain Reaction (PCR) was carried out in a total volume of 0.01 cm^3 containing 2 ng of template DNA, 39.2 µmol dm^{-3} of each primer, 117.6 mmol dm^{-3} of each dNTP, 156.8 $MgCl_2$, 19.6 unit of *Taq polymerase*, 98 of 10 x PCR buffer for 98 PCR reaction. PCR amplification was performed on a thermal cycler (Biometra Uno II, Germany) in the Biotechnology Laboratory of Rice Research Institute of Iran, using the touchdown conditions. An example was primer with 55ºC annealing, as follow: first denaturing (94ºC) for 240 s, denaturing (94ºC), primer annealing (65ºC) and extension (72ºC) for 45, 45 and 60 s, respectively. Annealing temperature was decreased in subsequent cycles by 1ºC. Thereafter amplification was continued with annealing at 55ºC for 26 cycles. Finally, PCR amplification products were extended at 72ºC. The amplification products were electrophoresed for 2 h on 6% polyacrylamide gels and detected by silver staining as described by Bassam et al. (1991) and Creste et al. (2001). Using *Mapmanager QTbX17*, 12 linkage groups were constructed with a minimum LOD score 2.5. 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, which dormancy was broken, were used to evaluate salt tolerance for seedling stage in 2006. This experiment was conducted in controlled conditions with 16/8 day/night length, irradiance of 1500 µmol m^{-2} s^{-1}, day/night temperature of 29/21°C and minimum relative humidity of 70% (Gregorio et al., 1997). 192 F_3 families (eighty germinated seeds of each family were placed in small holes on Styrofoam plates at the bottom) were used to evaluate the salt tolerance. The seeds were sown in holes of the Styrofoam board with a nylon net bottom, which floated on water for three days, then transferred to floating on Yoshida's cultural solution (Yoshida et al., 1976) for 11 days. At 14 days after sowing, the seedling were transferred to cultural solution containing of NaCl (6 $dS.m^{-1}$) for 7 days, then salinity increased to 12 $dS.m^{-1}$ for 7 days. After two weeks of salt stress, plants were harvested and chlorophyll content (CHLC), root length (RL) and shoot length (SHL), fresh weight of root (FWRO) and fresh weight of shoot (FWSH) were recorded. F_3 families were washed with deionized water, dried in a forced-air oven (70°C) and then measured for dry weight of root (DWRO) and dry weight of shoot (DWSH). Survival leaves of plants from each family were sampled from living plants at after two weeks of salt stress. Green Leaf Area (GLA) was calculated as the total area per family (not including fallen leaves). For this, plants were measured individually for total green leaf area per each family, using LI-3100 Area Meter (LI-COR, Inc., Lincoln, Nebraska). To observe physiological traits such as Na^+ and K^+ uptake in the shoot was performed. At 14 days after treating with NaCl, The shoots and root were harvested. They were dried, weighed and extracted with acetic acid (100 mM) At 90°C for 2 h. The extract was divided into four groups, and Na^+ uptake (NAUP), K^+ uptake (KUP), Na^+/K^+ uptake ratio (NAKUP) for each family in each group was determined by flame photometer (Sherwood 410, UK) according to Waling et al. (1989) at the Soil Laboratory of Rice Research Institute of Iran. Data were averaged for sub samples. Plant stand (PS) was conducted 14 days after salinization as number of vital plant.

QTL cartographer v. 11.5 (Basten et al., 2001) was used to identify QTLs affecting salt tolerance on the basis of composite interval mapping analysis. An 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.

RESULTS

Genetic linkage map

Of the 365 SSR markers pairs tested, 85 produced polymorphic bands between the genomic DNAs of parents and 74 primers were amplified with clear and scorable bands for F_2 individuals. A linkage map based

Table 1. Putative QTLs for salt tolerance in seedling stage the F_2 population derived from Tarommahalli (TAM; a salt tolerant variety) and Khazar (KHZ; a salt-susceptible variety).

Traits	QTL	Chr.	Flanking markers	LR	a	d	PEV	Dpe
Plant stand	qPL-2	2	RM5699-RM262	13.74	-6.30	-1.42	16.45	KHZ
Chlorophyll content	qCHLC-3	3	RM1022-RM6283	20.35	-1.50	-0.07	14.50	KHZ
	qRL-1	1	RM8068-RM8231	11.79	0.48	0.65	13.63	TAM
	qRL-4	4	RM5473-RM551	12.69	0.67	-0.91	11.56	TAM
	qRL-5	5	RM421-RM480	15.99	-0.84	0.46	14.80	KHZ
Root length	qRL-7	7	RM1048-RM11	18.49	0.73	-1.20	16.21	TAM
	qRL-9a	9	RM1553-RM5702	19.51	-0.06	-1.09	14.12	KHZ
	qRL-9b	9	RM7424-RM5702	15.59	0.05	-1.03	11.51	TAM
Shoot length	qSHL-3	3	RM7389-RM7000	11.75	-1.39	0.41	23.57	KHZ
	qSHL-10	10	RM7545-RM4455	23.11	-2.01	1.49	19.19	KHZ
Green leaf area	qGLA-3	3	RM1022-RM6283	24.14	-32.28	-9.55	12.81	KHZ
Fresh weight shoot	qFWSH-1	1	RM8235-RM8144	14.65	-1.01	0.62	22.44	KHZ
	qFWSH-3	3	RM1022-RM6283	21.83	-1.04	-0.29	22.97	KHZ
Fresh weight root	qFWRO-3a	3	RM1022-RM6283	20.50	-0.23	-0.17	20.91	KHZ
	qFWRO-3b	3	RM6283-RM6832	20.24	-0.19	0.20	17.72	KHZ
Dry weight shoot	qDWSH-3	3	RM1022-RM6283	19.44	-0.17	-0.03	23.21	KHZ
	qDWSH-7	7	RM5481-RM11	12.39	-0.10	-0.01	23.17	KHZ
Dry weight root	qDWRO-3	3	RM1022-RM6283	15.63	-0.01	0.01	21.41	KHZ
	qDWRO-5a	5	RM421-RM480	11.55	-0.02	0.01	21.74	KHZ
	qDWRO-5b	5	RM480-RM440	12.53	-0.02	0.01	22.55	KHZ
	qDWRO-9a	9	RM1553-RM7424	14.12	0.01	-0.02	27.43	TAM
	qDWRO-9b	9	RM7424-RM5702	12.88	0.01	-0.02	25.50	TAM
Na^+ uptake	qNAUP-1a	1	RM562-RM543	12.39	0.01	0.00	13.03	KHZ
	qNAUP-1b	1	RM8068-RM8231	24.40	0.01	0.00	22.17	KHZ
	qNAUP-3	3	RM416-RM5626	12.26	-0.01	0.01	13.62	TAM
	qNAUP-9a	9	RM1553-RM7424	21.65	0.01	-0.01	17.71	KHZ
	qNAUP-9b	9	RM7424-RM5702	19.33	0.01	-0.01	16.95	KHZ
	qNAUP-10	10	RM7545-RM4455	16.57	-0.01	-0.01	13.84	TAM
K^+ uptake	qKUP-3	3	RM1022-RM6283	19.53	-0.01	-0.001	22.15	KHZ
	qKUP-8	8	RM4955-RM152	22.49	-0.01	0.001	38.22	KHZ
Na^+/K^+ uptake ratio	qNAKUP-6	6	RM3827-RM340	16.68	0.14	-0.26	12.35	KHZ
	qNAKUP-3	3	RM6832-RM7389	18.52	-0.03	0.21	9.03	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, and [Dpe]direction of phenotypic effect.

on F_2 population was constructed, which covered a total of 1231.50 cM with an average of two locus interval of 19.83 cM.

Plants stand (PS)

One QTL was identified for plant stand (PS) on chromosome 2. This QTL located in interval RM8254-RM262 (qPS-2) showed negative effects on the PL with an LR score of 13.74 and exhibiting 16.45% of the total phenotypic variance (Table 1 and Figure 1). In this QTL, alleles from KHZ decreased PL by -6.30. So, this putative QTL showed partial dominance effect for PL.

Chlorophyll content (CHLC)

One QTL was found for chlorophyll content (CHLC) on chromosome 3. The QTL located in interval RM1022-RM6283, showed the large effect on the CHLC with an LR score of 20.34 and explaining 14.5% of the total phenotypic variance. The additive effect of this QTL was negative. This QTL allele from KHZ decreased CHLC by -1.50 and exhibited partial dominance (d/a=-0.04) for decreased CHLC (Table 1).

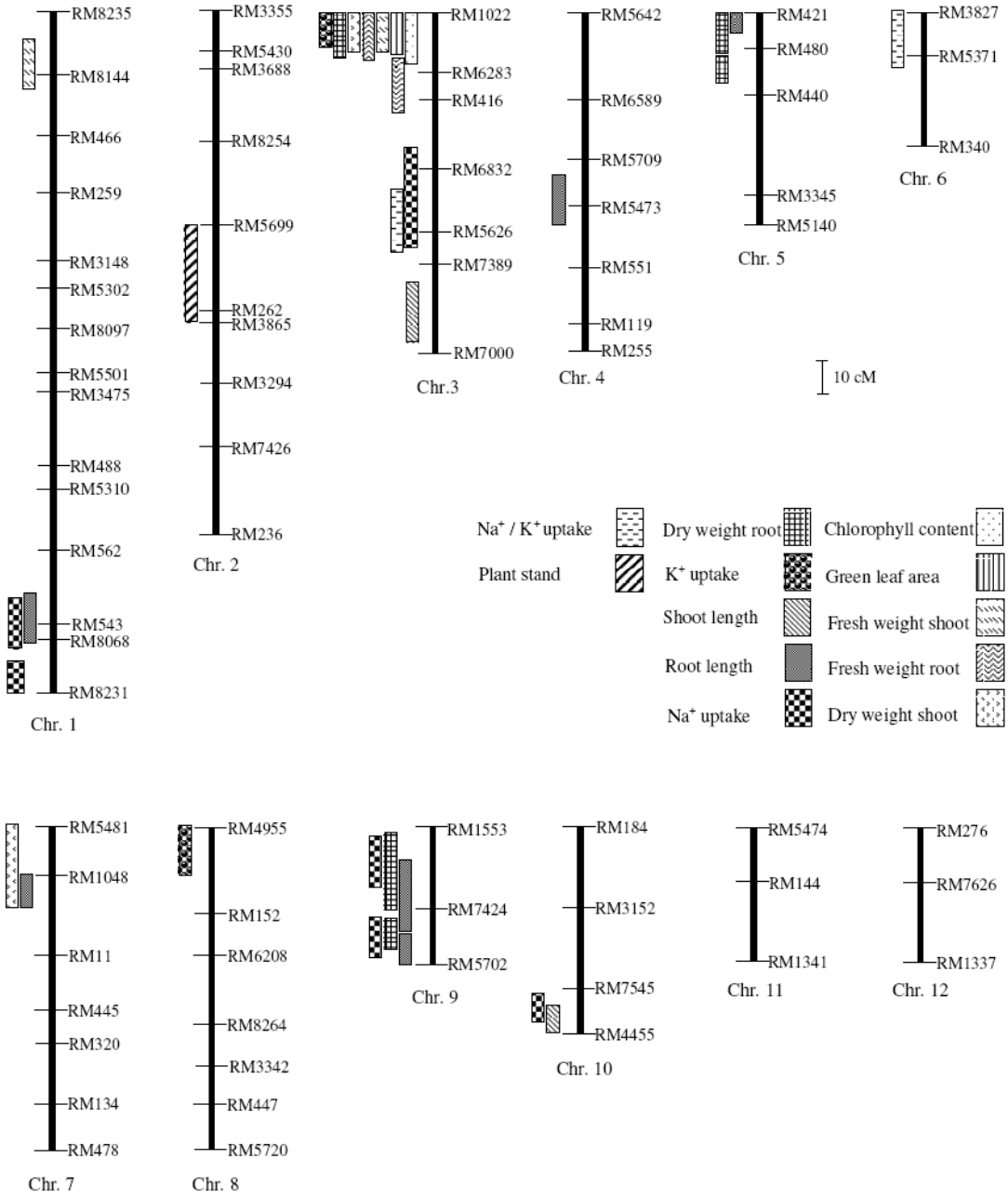

Figure 1. The position of QTL for traits representing salt tolerance of rice in the seedling stage.

Root length (RL)

Six QTLs were mapped for length of root (RL). Two QTLs out of the six QTLs were located on chromosome 9 (Table 1 and Figure 1). Amongst them, three QTLs with the largest effects were qRL-5, qRL-7 and qRL-9a that individually explained 16.21, 14.80 and 14.12% of the total phenotypic variation and had an additive effect of 0.73, -0.84 and -0.06 centimeter, respectively. For two QTLs out of six QTLs the alleles for decreased RL were from KHZ, whereas for other QTLs, alleles for increased RL were from TAM. There was one QTL (qRL-5) that showed partial dominance effects for decreased RL (except for qRL-1 that showed over dominance effect for increased RL).

Shoot length (SHL)

Two QTLs were detected for length of shoot (SHL). Two QTLs with the large effects were qSHL-3 and qSHL-10 that individually explained 23.57 and 19.19% of the total

phenotypic variation and had an additive effect of -1.39 and -2.11 centimeter, respectively, for decreased LS. These QTLs had exhibited a partial dominance effect with induced SHL with d/a ratio of 0.29 and 0.74, respectively (Table 1).

Green Leaf Area (GLA)

One QTL was mapped for green leaf area (LAI) on chromosome 3. This QTL located in interval RM1022-RM6283 (qLAI-3) showed negative effects on the LAI with an LR score of 24.14 and exhibiting 12.81% of the total phenotypic variance (Table 1 and Figure 1). In this QTL, alleles from KHZ decreased LAI by -32.28 cm^2. So, this putative QTL showed partial dominance effect for LAI with d/a ratio of -0.29.

Fresh weight of root (FWRO)

Two QTLs were identified for fresh weight of root (FWRO) on chromosome of 3 (Table 1). The QTL located in interval RM1022-RM6283 (qFWRO-3a) showed large effect on the FWRO with a 20.50 LR score of and explaining 20.91% of the total phenotypic variance. For the two putative QTLs, the alleles for decreased FWRO were from KHZ.

Fresh weight of shoot (FWSH)

Two QTLs were mapped for fresh weight of shoot (FWSH). Two QTLs with the largest effects were qFWSH-1 and qFWSH-3 that individually explained 22.44 and 22.97% of the total phenotypic variation and had an additive effects -1.00 and -1.04 g, for decreased FWSH, respectively. qFWSH-1 and qFWSH-3 exhibited induced and reduced partial dominance effects, respectively (Table 1).

Dry weight of root (DWRO)

Five QTLs were detected for dry weight root (DWRO). Amongst them, two QTLs with the largest effects were qDWRO-9a and qDWRO-9b that individually explained 27.43 and 25.50% of the total phenotypic variation and had an additive effect of 0.01 g in both QTLs. These QTLs (qDWRO-9a and qDWRO-9b) showed over dominant effect for reduced DWRO while the other QTLs (except qDWRO-3) showed partial dominance for induced DWRO.

Dry weight of shoot (DWSH)

Two QTLs were identified for dry weight of shoot (DWSH) (Table 1 and Figure 1). These QTLs on chromosome 3 and 7, showed large effect on the DWSH with a LR score of 19.44 and 23.17% of the total phenotypic variation, respectively. In two QTLs, alleles from KHZ decreased DWSH by -0.17 (qDWSH-3) and -0.10 (qDWSH-7). There were two QTLs (qDWSH-3 and qDWSH-7) that showed partial dominance effects (with d/a ratio -0.18 and -0.10, respectively) for reduced DWSH.

Na^+ uptake (NAUP)

Six QTLs were mapped for Na^+ uptake (NAUP). Two QTLs out of the six QTLs located on chromosome 9. QTL on chromosome 3 (qNAUP-3) had particularly high LR value (24.40) and explained 22.17% of the phenotypic variation. In two QTLs the TAM alleles were responsible for a decrease in Na^+ uptake, while the other four QTLs individually explained less 20% of the phenotypic variation. KHZ alleles at this QTL caused an increase in Na^+ uptake. There were two QTLs (qNAUP-9a and qNAUP-10) that showed over dominance effects for induced Na^+ uptake (except qNAUP-9b) while other QTLs showed only partial dominance for increased Na^+ uptake (Table 1).

K^+ uptake (KUP)

Two QTLs were identified for K^+ uptake. These QTLs with the large effects were qKUP-3 and qKUP-8 that individually explained 22.15 and 38.22% of the total phenotypic variation, and had additive effects of -0.006 and -0.010, respectively, for decreased K^+ uptake. Theses QTLs showed no dominance effects (with d/a ratio of zero).

Na^+/K^+ uptake ratio (NAKUP)

Two QTLs were found for Na^+/K^+ uptake ratio. These QTLs are located on chromosomes 6 (qNAKUP-6 and qNAKUP-3) with LR score of 16.67 and 13.12, respectively. KHZ alleles at qNAKUP-6 caused an increased in Na^+/K^+ uptake ratio whereas in another QTL, alleles from TAM were responsible for decrease in Na^+/K^+ uptake ratio. Moreover, two QTLs had exhibited an over dominance effects with reduced Na^+/K^+ uptake ratio with d/a=-1.78 in qNAKUP-6 and induced Na^+/K^+ uptake ratio with d/a ratio of 6.67.

DISCUSSION

Salt tolerance in rice is controlled by polygene with the additive and dominant effects (Gregorio and Sinadhira, 1993). Akbar et al. (1986) reported that the dry matter weight of rice seedling under salt stress was affected by at least two groups of genes with additive effect, and no

epistatic effect was detected. Gregorio and Sinadhira (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.

In this study, one QTL on chromosome 1 for root length, fresh weight root and two QTLs were found for Na^+ uptake. Koyama et al. (2001) have mapped QTLs related to Na^+ uptake on chromosome 1 by trait based QTL method. Bonilla et al. (2002) have reported *Saltol* gene for Na^+ uptake in RM140-C1733S interval on chromosome 1.

We found QTLs related to Na^+/K^+ uptake ratio on chromosome 3 and 6. QTLs associated to Na^+/K^+ uptake ratio in other reports were found on chromosomes 1 (Grigorio, 1997; Lang et al., 2001b; Koyama et al., 2001; Bonilla et al., 2002), 2 (Lang et al., 2001b), 3 (Ammar, 2004 for reproductive stage), 4 (Koyama et al., 2001), 7(Lang et al., 2001b), 8 (Ammar, 2004 for vegetative stage), 10 and 12 (Grigorio, 1997). We detected QTLs associated to Na^+/K^+ uptake ratio only on chromosomes 3 and 6. It is probably due to the low density of SSR linkage map. Sabouri et al. (2008b,c) mapped QTLs Na^+/K^+ ratio related to Na^+ and K^+ concentration in these same region and chromosomes.

Lin et al. (1998, 2004) have reported some QTLs for surviving days of rice seedling on chromosomes 1 (qSDS-1), 6 (qSDS-6) and 7 (qSDS-7). Here we explored that QTL associated with plant stand was located on chromosomes 2.

In this study, one QTL was identified for fresh weight shoot on chromosome 1, one QTL was identified for dry weight shoot on chromosome 7, two QTLs were mapped for dry weight root on chromosome 3, and two QTLs were detected for dry weight root on chromosome 9. QTLs related to biomass dry matter on chromosome 3 (Koyama et al., 2001) and 5 (Prasad et al., 2000), QTLs related to shoot weight on chromosome 11 (Lang et al., 2001b), QTLs related to root weight on chromosome 3 and 9 have been mapped. Most of the QTLs related to biomass matter were detected on chromosome 3 (qFWSH-3, qFWRO-3a, qFWRO-3b, qDWSH-3 and qDWRO-3).

For the first time, we detected QTLs related to root and shoot length in seedling stage. These QTLs were mapped on chromosomes 1, 3, 4, 5, 7, 9 and 10. qRL-7, qSL-3 and qSL-10 explained most of the total phenotypic variation, 16.21, 23.57 and 19.19%, respectively.

The QTLs associated with chlorophyll content, green leaf area, fresh weight root, fresh weight shoot, dry weight shoot, dry weight root and K^+ uptake in the region of RM1022 - RM6283 on chromosome 3 overlapped. It is concluded that there is a relationship between these traits, which may be controlled by the same gene or linked genes. We detected QTLs related to root length, dry weight root and Na^+ uptake in the same region on chromosome 9. Region of RM1022 - RM6283 reduced salt tolerance while two QTLs in RM6832 - RM7389 interval increased salt tolerance. The phenotype of salt tolerance in rice is a general expression of some physiological factors. 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 (Zhang et al., 1995; Lin et al., 1998; Koyama et al., 2001). Most of these QTL analyses were conducted by using RFLP or AFLP markers except for the study of Parasad et al. (2000) and Ming 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. qKUP-8, qKUP-3, qNAUP-1b, qDWR9a, qDWRO9b, qDWSH-3, qDWSH-7, qFWRO-3a, qFWSH-1, qFWSH-3 and qSHL-3 as major QTLs with very large effect explained 38.22, 22.15, 22.17, 27.43, 25.50, 23.21, 23.17, 20.91, 22.44, 22.97 and 23.57% of the total phenotypic variance, respectively.

The comparison between the chromosomal positions of QTLs related to root length, dry weight root and Na^+ uptake on chromosome 9, dry weight shoot and root length on chromosome 7, root length and Na^+ uptake on chromosome 1, dry weight root and root length on chromosome 5 and shoot length and Na^+ uptake is difficult to determine; QTLs in these regions may be at the same loci or are in 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. A part of the variability for Na^+ uptake and Na^+/K^+ uptake was explained by the QTL qNAUP-3 and qNAKUP-3 flanked by RM6832 - RM7389 on chromosome 3, which exhibited phenotypic variance of 13.62 and 9.03% and peak LR of 12.26 and 18.52, respectively. 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). Major loci for DWR (qDWRO-9a and qDWRO-9b) were bracketed by RM1553 – RM7424 and RM7424 - RM5702 that spread over 20 and 10 cM on chromosome 9, respectively. QTL for Na^+ uptake overlapped with Na^+/K^+ uptake ratio and also QTL for K^+ uptake with QTLs related to biomass matter. These multiple effect of QTL on the same chromosomal region could be due to the fact that salt tolerance performance is derived from exchange of ions. The QTL on chromosome 3 may contain a new major gene for salt stress tolerance at seedling stage in rice. This needs to be confirmed by conducting 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 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

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

		1	2	3	4	5	6	7	8	9	10	11	12
1	Plant stand	1.000											
2	Chlorophyll content	0.264**	1.000										
3	Root length	-0.052	-0.077	1.000									
4	Shoot length	0.127	0.253**	0.076	1.000								
5	Green leaf area	0.523**	0.436**	0.129	0.259**	1.000							
6	Fresh weight shoot	0.454**	0.423**	0.138	0.283**	0.859**	1.000						
7	Fresh weight root	0.349**	0.380**	0.257**	0.236**	0.706**	0.865**	1.000					
8	Dry weight shoot	0.291**	0.354**	0.113	0.357**	0.747**	0.898**	0.832**	1.000				
9	Dry weight root	0.316**	0.290**	0.229**	0.265**	0.719**	0.848**	0.903**	0.908**	1.000			
10	Na^+ uptake	-0.093	0.082	0.128	0.248**	0.362**	0.575**	0.590**	0.765**	0.638**	1.000		
11	K^+ uptake	0.348**	0.338**	0.113	0.297**	0.803**	0.920**	0.821**	0.951**	0.881**	0.716**	1.000	
12	Na^+/K^+uptake	-0.609*	-0.392**	-0.004	-0.110	-0.525**	-0.455**	-0.334**	-0.277**	-0.329*	0.276**	-0.393**	1.000

*,**Probability levels at 0.05 and 0.01, respectively.

traits to be used in MAS.

Trait correlations and clustering of QTLs for traits correlated were often mapped in the same chromosomal regions (Paterson et al., 1996). This trend was observed in this study. For example, qFWSH-3, qFWRO-3a, qFWRO-3b, qDWSH-3 and qDWRO-3 were located on chromosome 3 and were found at approximately the same map locations in chromosome 3. These traits showed a high correlation (Table 2). In these cases, the directions of the correlations were consistent with that of the effects of the QTLs on the traits. These results supported the fact that the trait correlation may be attributed to the effect of pleiotropy or to the very close linkage of genes, because salinity tolerance is complex physiological trait related to ion concentration. Salinity affects almost all processes of the plant, because of the osmotic effects by high ionic concentrations, and because of competitive interference with nutrient uptake and of toxic effects within the plant tissue (Yeo and Flowers, 1984). In this study, FWSH, CHLC, RL and SHL were correlated with shoot Na^+ and K^+ concentration. 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 lead to superior genotype to improve the variety. In this study of 192 F_3 families, five families showed the lowest NA^+/K^+ ratio uptake of the seedlings with high PS, i.e. high salt tolerance. Actually, the alleles of several QTLs from the high salt-tolerance variety TAM were pyramided in these five families. Also, in the three families, the TAM alleles of two QTLs (related to NAUP and NAKUP), 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 insufficient 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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