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QTLs mapping of physiological traits related to salt tolerance in young rice seedlings

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Abstract

Oryza sativa L. F_2 population and $F_{2:3}$ derived from a cross between salt tolerance cv. Tarommahali and salt sensitive cv. Khazar were used in this study. A linkage map based on F_2 population was constructed (74 SSR markers on 192 individuals), which covered a total of 1231.50 cM with an average two locus interval of 19.83 cM. Two QTLs related to Na^+/K^+ ratio were found on chromosome 3 and 6. qDM-3 and qDM-8 (for dry mass of shoot) are major QTLs with very large effects explained 20.90 and 17.72 % of the total phenotypic variance, respectively. Major locus for DM (qDM-3) was bracketed by RM1022 - RM6283 spread over 13.6 cM on chromosome 3. Major part of the variability for standard tolerance ranking (STR) was explained by the qSTR-6 flanked by RM3727 - RM340 on chromosome 6, which exhibited phenotypic variance of 17.25 % and peak likelihood ratio (LR) of 17.51. The length of this QTL is 8.8 cM and identification of any tightly linked markers in this region will serve as a candidate gene for fine-mapping. qSTR-3 overlapped with qNA-3 and qNAK-3. The qSTR-3 may contain a new major gene for salt stress tolerance at seedling stage in rice. Major QTLs identified in this paper, after fine-mapping, could be used for marker assisted selection.

Additional key words: marker-assisted selection, *Oryza sativa*, SSR.

Introduction

To facilitate the development of new rice cultivars with a high salinity tolerance, it is required to understand the genetic control mechanisms for salt tolerance (Yeo and Flowers 1984, Flowers *et al*. 1977, Silva *et al*. 2008). Yeo and Flowers (1984) and Gregorio *et al*. (1993) tried to dissect traits of salt tolerance in rice such as shoot sodium and potassium content, genotype and dry mass of seedlings. Modern techniques of molecular biology (Bassam *et al*. 1991, Saghi Maroof *et al*. 1994, Creste *et al*. 2001) and the mapping of rice genome (Chen *et al*. 1997, Temnykh *et al*. 2000, McCouch *et al*. 2002) allow plant scientists to locate the DNA determining the physiological trait that dictate salt tolerance in rice. QTL analysis of physiological traits related to abiotic stresses 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, Balint *et al*. 2007). Lin *et al*. (2004) reported two major QTLs with very large effects on shoot Na^+ content (qNA-7) and shoot K^+ content (qK-1). These QTLs explained 48.5 and 40.1 % of the total phenotypic variance, 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. Six QTLs for Na^+ and K^+ content and Na^+/K^+ ratio (two QTLs for each trait) in shoot were identified by Koyama *et al.* (2001) in rice. Ming *et al*. (2005) located two QTLs for DM on chromosomes 8 and 9 and two QTLs for Na^+/K^+ on chromosomes 2 and 6. Takehisha *et al*. (2004) detected QTLs associated with salt tolerance in paddy field flooded with salt water. Although there have been extensive studies on QTL mapping for salinity tolerance

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Abbreviations: CIM - composite interval mapping; LOD - logarithmic odds; LR - likelihood ratio; MAS - marker assisted selection; QTL - quantitative trait locus.
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in rice, little or no information has been reported on the mapping of salinity tolerance in local populations. The aim of the present study is to identify QTLs related to salt tolerance by using an Iranian rice population.

Materials and methods

In order to selecting isodirectional parents, Iranian rice germplasm were screened during 2004 - 2005 for salt tolerance (Sabouri *et al.* 2007a,b,c). *Oryza sativa* L. F_2 population derived from a cross between a salt tolerant cv. Tarommahali (TAM) and a salt sensitive cv. Khazar (KHZ) were used in this study. The genetic material involved 192 F_3 families, each derived from bagged seeds of a single F_2 plant. F_3 lines (80 plants in each family) were used to evaluate the salt tolerance. The seeds were placed at 50 °C for 3 d to break dormancy, then germinated at 35 °C for 48 h and finally, the germinated seeds were sown in holes of the *Styrofoam* board with a nylon net bottom, which floated on water for 3 d, then transferred to floating on Yoshida's nutrient solution (Yoshida *et al*. 1976) for 11 d. Two weeks after sowing, the seedling were transferred to nutrient solution containing 51.19 mM NaCl (electrical conductivity 6 dS m^{-1}) for 7 d, then NaCl concentration increased to 163.8 mM (12 dS m^{-1}) for further 7 d. This experiment was conducted in controlled conditions with 16-h photoperiod, irradiance of 1 500 μmol m^{-2} s^{-1}, day/night temperature of 29/21 °C and minimum relative humidity of 70 %. The culture solution was renewed weekly and the pH was adjusted daily to 5.5 by adding either NaOH or HCl. After two weeks of salt stress, the first Standard Tolerance Ranking (STR) test was recorded according to Gregorio *et al*. (1997), then the shoots were harvested, dried, weighed and extracted in acetic acid (100 mM) at 90 °C for 2 h, respectively. The extract was divided into four groups, and sodium and potassium in each groups was determined by the flame photometer (*Sherwood 410,* Cambridge, UK) according to Waling *et al.* (1989) at soil laboratory of Rice Research Institute of Iran.

Leaves from the main stem of each F_2 plant were sampled, and genomic DNA was extracted according to Saghi Maroof *et al*. (1994). A total of 365 SSR primer pairs were 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. The 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). 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 mmol dm^{-3} $MgCl_2$, 19.6 unit of *Taq polymerase*, and 0.098 cm^3 of 10× PCR buffer. PCR amplification was performed on a thermal cycler (*Biometra Uno II,* Göttingen, Germany) in biotechnology laboratory of Rice Research Institute of Iran.

PCR products were separated on 6 % (m/v) polyacrylamide gels (38:2 acrylamide:bisacrylamide). Genotypic data of 74 SSR markers were used for QTL analysis. This data set was used for mapping the quantitative trait loci correlated with salt-tolerance of $F_{2:3}$ population. A χ^2 test ($P < 0.005$) was performed on each marker to verify the expected 1:2:1 segregation ratio. Map distances between primers were presented in cM derived using the Kosambi (1944) function of the mapping program. A logarithmic odds (LOD) score of 2.5 was used to determine both the linkage groups and the order of markers. The initial linkage map was constructed based on segregation at 74 microsatellite markers loci using *Map Manager QTXb17* software (Manly and Olson 1999). *QTL cartographer* (Basten *et al*. 2001) used to identify QTLs affecting salt tolerance on the basis of composite interval mapping (Zeng 1994) analysis. The percentage of total phenotypic variation explained by each QTL, and the additive effects were estimated by this software. Test performed at 2-cM interval, and cofactors were selected by forward/backward stepwise regression (*Model 6*) with QTL *Cartographer v 2.5* (Basten *et al*. 2001). Significance threshold for composite interval mapping (CIM) were determined at likelihood ratio (LR) 11.5 (LOD = 2.5). The phenotypic variation explained by a QTL (r^2) conditioned by the CIM cofactors included in the model was calculated at the most likely QTL position. The additive effect of an allelic substitution at each QTL was also obtained. The LOD peak of each significant QTL was considered as the QTL location on the linkage map.

Results

Transgressive segregation was found in F_2 population. Of the 365 SSR markers pairs tested, 85 produced polymorphic bands between the genomic DNAs of parents and 74 primers amplified clear and scorable bands for F_2 individuals. A linkage map based on F_2 population was constructed.

Single marker analysis was employed to determine whether these molecular markers were significantly related to STR, DM, Na^+ content, K^+ content and Na^+/K^+ ratio. 34 markers scattered on chromosomes 1, 2, 3, 5, 8, 9, 10 and 11 were found to be associated with the traits of salt tolerance. Among them, 1, 3, 7, 11 and 12 markers were linked to Na^+, Na^+/K^+, K^+, STR and DM, respectively. Interestingly, either two markers (RM1022 and RM421) on the chromosomes 3 and 5 were found associated with all the traits (except Na^+ content).

Table 1. Putative QTLs for salt tolerance in the F_2 population derived from TAM × KHZ cross. QTLs are named by abbreviations plus chromosomal number. [b] - additive effect, [c] - dominant effect, [d] - percentage of total phenotypic variance explained by the QTL, [e] - direction of phenotypic effect, TAM and KHZ indicate Tarommahalli and Khazar, respectively.

Traits	QTL	Chr.	Flanking markers	Peak LOD	a[b]	d[c]	PEV[d]	Dpe[e]
STR	qSTR-6	6	RM3727-RM340	17.51	0.56	-0.97	17.25	KHZ
	qSTR-3a	3	RM1022-RM6283	13.44	0.49	0.34	16.15	KHZ
	qSTR-3b	3	RM6832-RM7389	24.51	0.32	0.74	13.07	KHZ
DM	qDM-3	3	RM1022-RM6283	20.50	-0.17	-0.04	20.90	KHZ
	qDM-8	8	RM4955-RM152	20.24	-0.10	-0.01	17.72	KHZ
Na^+	qNA-2a	2	RM8264-RM262	12.59	0.25	0.20	10.55	KHZ
content	qNA-2b	2	RM7426-RM236	12.57	0.35	0.15	12.70	KHZ
	qNA-6	6	RM3827-RM5371	11.92	0.38	-0.49	10.13	KHZ
	qNA-3	3	RM6832-RM7389	16.34	-0.11	0.62	10.92	TAM
K^+	qK-6	6	RM3827-RM340	14.46	-0.01	0.19	10.80	KHZ
content	qK-5a	5	RM421-RM480	17.16	-0.18	0.27	15.58	KHZ
	qK-5b	5	RM480-RM440	12.35	0.02	0.28	9.7	TAM
Na^+/K^+	qNAK-6	6	RM3827-RM340	16.68	0.14	-0.26	12.35	KHZ
ratio	qNAK-3	3	RM6832-RM7389	18.52	-0.03	0.21	9.03	TAM

14 QTLs associated with salt tolerance were detected (Table 1). The explanation for phenotypic variation by a single QTL varied from 9.03 to 20.90 %. Three QTLs for STR were identified on chromosome 6 and 3 (two QTLs). The QTLs qSTR-6 and qSTR-3a with LR score of 17.51 and 13.44, respectively, showed the largest effects on STR and explained 17.25 and 16.15 % of the total phenotypic variance, respectively. The additive effect of a single QTL ranged from 0.32 to 0.56. In all three QTLs, the alleles from KHZ increased STR by 0.46 on average. The QTLs, qSTR-6 and qSTR-3b exhibited over-dominance for increased and decreased STR, respectively, whereas qSTR-3a exhibited partial dominance with dominance to additive ratio of 0.69. Two QTLs were mapped for shoot DM. The QTLs, qDM-3 and qDM-8 with an LR score of 20.50 and 20.24, respectively showed the large effects on DM, explained 20.90 and 17.72 % of the total phenotypic variance and had additive effects of -0.17 and -0.10 for decreased DM. In the two putative QTLs, alleles from KHZ decreased DM. The dominant effects for QTLs were positive and showed incomplete dominance for increased DM. Four QTLs were identified for Na^+ content. Two QTLs out of four QTLs located on chromosome 2 (Table 1, Fig. 1). Four located QTLs, additionally explained 44.25 % of the total phenotypic variance. In three QTLs (qNA-2a, qNA-2b and qNA-6) alleles from KHZ increased Na^+ content by 0.33 on the average, whereas QTL allele from TAM (qNA-3) decreased Na^+ content. The QTLs, qNA-6 and qNA-3 exhibited overdominance for decreased and increased Na^+ content. The dominance to additive ratio for other QTLs was positive and showed incomplete dominance for increased Na^+ content. Three QTLs were mapped for K^+ content. The QTL, qK-5a with an LR score of 17.16 showed the largest effect on the K^+ content and explained 15.58 % of the total phenotypic variance. This QTL located in interval RM421 - RM480. The additive effect of qK-5b was positive. All of the three QTLs exhibited overdominance for increased K^+ content. The QTLs, qNAK-6 and qNAK-3 with an LR score of 16.68 and 18.52 showed large effects on Na^+/K^+ ratio, explained 12.35 and 9.03 % of the total phenotypic variance and had additive and negative effects of 0.14 and -0.03 for increased and decreased Na^+/K^+ ratio, respectively. Two putative QTLs alleles for Na^+/K^+ ratio were from KHZ and TAM, respectively. The over-dominant effects of qNAK-6 and qNAK-6 were negative and positive which decreased and increased Na^+/K^+ ratio, respectively. 38 QTLs were identified for STR, K^+ content, Na^+/K^+ and Na^+ content with interval mapping and composite interval mapping. qSTR-1, qSTR-2, qSTR-3a, qSTR-5, qSTR-8, qK-5c and qNAK-5 were mapped in interval mapping and qNA-2a, qNA-2b and qNA-6 were mapped in composite interval mapping, only. 11 QTLs were identified in both methods.

Discussion

Transgressive segregation might be attributed to the gathering of some QTLs associated with salt tolerance and showed that transgressive breeding for salt tolerance in rice could be achieved *via* MAS. Linkage map covered a total of 1231.50 cM with an average two locus interval of 19.83 cM. The position of most SSR markers on chromosomes was identical with the previous reports (Chen *et al.* 1997, Temnykh *et al*. 2000, McCouch *et al*.

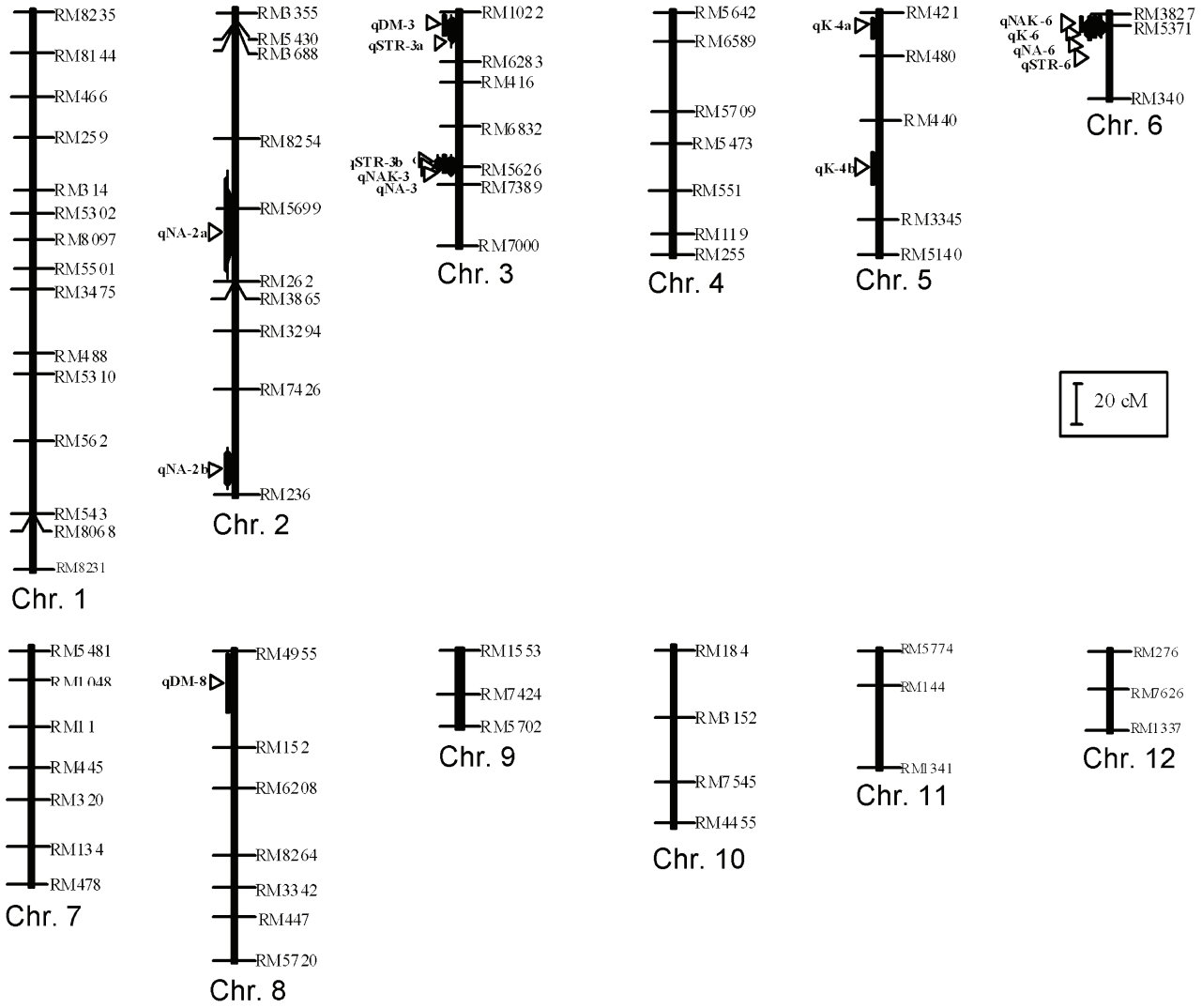

Fig. 1. Genetic linkage map showing the location of QTLs for standard tolerance ranking (STR) under salt stress in the Tarommahalli × Khazar F_2 population. The markers are signed *on the right side* of the chromosomes. *On the left*, the QTLs detected by composite interval mapping analysis are indicated. Abbreviations for the QTLs: qNA - Na^+ content, qK - K^+ content, qNA/K - Na^+/ K^+ ratio and qDM - dry matter of shoot.

2002) but its order was not identical. However, 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.

The previous research showed that salt tolerance in rice was controlled by polygene with the additive and dominant effects, the former playing a major role (Moeljopawiro and Ikehashi 1981, Gregorio and Senadhira 1993). Koyama *et al*. (2001) have identified some QTLs associated with Na^+/K^+ ratio on chromosomes 1 and 4. In our study no QTLs associated with salt tolerance were detected in this region. It is probably due to the low density of SSR linkage map. However, in this study, two QTLs on chromosome 1 for STR and DM were found *via* interval mapping (unreported data); two QTLs related to Na^+/K^+ ratio were found on chromosome 3 and 6. The *Kna* gene related to salt tolerance in wheat (controlling Na^+/K^+ discrimination) has been mapped on chromosome arm 4DL (Dubcovsky *et al*. 1996) and this region is probably equivalent to the tip of chromosome 3S in rice. One QTL associated with Na^+/ K^+ ratio was detected in this region. We also, found other QTL (qDM-3) in this region. Gu *et al*. (1999) detected three QTLs for STR on chromosomes 1, 5 and 9. Here we explored that QTLs associated with STR were located on chromosomes 3 and 6. Lin *et al*. (1998) have reported some QTLs for surviving days of rice seedling on chromosome 1. QTLs related to biomass dry matter has been mapped on chromosome 3 and 8 (Prasad *et al*. 2000, Koyama *et al*.

2001). The QTLs associated with Na^+, Na^+/K^+ ratio and STR in the region of RM6832 - RM7389 on chromosome 3 was overlapped. It is concluded that there is a relationship between Na^+, Na^+/K^+ ratio and STR, which may be controlled by the same gene or linked genes. We detected four QTLs (qSTR-6, qNA-6, qK-6 and qNAK-6) in the same region. This region reduced salt tolerance while chromosome 3 increased salt tolerance. The phenotype of salt tolerance in rice is a general expression of some physiological factors. This study reveal that alleles of QTL enhancing salt tolerance were not only from salt-tolerant parent but also from salt sensitive parent (Table 1), which supported other report (Gu *et al*. 1999).

We found 14 QTLs including three for STR, two for DM, and nine for three traits related to Na^+, K^+ content and Na^+/K^+ ratio at eight chromosomal regions (Table 1, Fig. 1). qSTR-6, qDM-3 and qDM-8 as major QTLs with very large effects explained 17.25, 20.90 and 17.72 % of the total phenotypic variance, respectively. The chromosomal position of qSTR-1, qSTR-5 and qNAK-6 detected in this study was similar to QTLs reported by Ming *et al*. (2005). The comparison between the chromosomal positions of Na^+ and K^+ some QTLs is difficult to determine; whether both QTLs are at the same loci or are different tightly linked loci. Further analysis, including the fine mapping of both QTLs using common markers, cloning and the sequence comparison of these QTLs, will be required to answer these questions. Major part of the variability for STR was explained by the QTL qSTR-6 flanked by RM3727 - RM340 on chromosome 6, which exhibited phenotypic variance of 17.25 % and peak LR of 17.51. The length of this QTL is 8.8 cM and identification of any tightly linked markers in this region will serve as a candidate gene for fine-mapping and further use in MAS. Major locus for DM (qDM-3) was bracketed by RM1022 - RM6283 spread over 13.6 cM on chromosome 3. QTL for STR (qSTR-3) was overlapped with qNA-3 and qNAK-3. This 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 qSTR-3 may contain a new major gene for salt stress tolerance at seedling stage in rice.

Trait correlations and clustering of QTLs for traits correlated were often mapped in the same chromosomal regions (Abler *et al*. 1991, Paterson *et al*. 1996, Vedboom *et al*. 1994). This trend was observed in this study. For example, qNA-3, qSTR-3 and qNAK-3 were located on chromosome 3 and qNAK-6, qK-6, qNA-6 and qSTR-6 were found at approximately the same map locations in chromosome 6. These traits showed a high correlation. In these cases, the directions of the correlations were consistent with that of the effects of the QTLs on the traits. These results support the fact that the trait correlation may be attributed to the effect of pleiotropy or to the very close linkage of genes. STR is complex physiological trait related to ion concentration or quantity and to osmosis (Yeo and Flowers 1989). In this study, STR was correlated with shoot Na^+ and K^+ content (r^2 = 0.758** and -0.364**, respectively). QTL pyramiding is the process that assembles many genes that work well together and, for a specific trait, assemble the alleles with similar effects from different loci (Xu 1997). This process can create the superior genotype. In this study of 192 F_3 lines, three lines showed the lowest STR of the seedlings with code 1, *i.e.* high salt tolerance. Actually, the alleles of several QTLs from the high salt-tolerant TAM were pyramided in these three lines, respectively. In the three lines, the TAM alleles of three QTLs (qNA-3, qSTR-3 and qNAK-3), were assembled. These results indicate that breeding methods of QTLs pyramiding by using MAS are very useful for the development of new cultivar with a high level of salt tolerance. The processes of Na^+ and K^+ uptake in rice were considered to be independent upon salt stress (Yeo *et al*. 1988, Garcia *et al*. 1997, Yadav *et al*. 1997). Koyama *et al*. (2001) also pointed out that the uptake of Na^+ and K^+ maybe be independent, due to the major pathways of Na^+ and K^+ uptake in rice occur in parallel and not directly in competition. However, based on data in this study, there was a negative correlation between K^+ and Na^+ content (r^2 = -0.250**), suggesting that a competition between Na^+ and K^+ occurred in terms of uptake in the shoots. K^+ is critical for Na^+ tolerance due to the fact that K^+ and Na^+ are chemically very similar. Finally, QTL identified by this technique, after fine-mapping, could be used for indirect selection of salt-tolerant traits to be used in MAS.

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