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ORIGINAL PAPER

Identification of QTLs with main, epistatic and QTL × environment interaction effects for salt tolerance in rice seedlings under different salinity conditions

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Abstract Salt tolerance of rice (*Oryza sativa* L.) at the seedling stage is one of the major determinants of its stable establishment in saline soil. One population of recombinant inbred lines (RILs, $F_{2:9}$) derived from a cross between the salt-tolerant variety Jiucaiqing and the salt-sensitive variety IR26 was used to determine the genetic mechanism of four salt tolerance indices, seedling height (SH), dry shoot weight (DSW), dry root weight (DRW) and Na/K ratios (Na/K) in roots after 10 days in three salt concentrations (0.0, 0.5 and 0.7 % NaCl). The main effect QTLs (M-QTLs) and epistatic QTLs (E-QTLs) were detected by QTL IciMapping program using single environment phenotypic values. Eleven M-QTLs and 11 E-QTLs were identified for the salt tolerance indices. There were six M-QTLs and two E-QTLs identified for SH, three M-QTLs and five E-QTLs identified for DSW, two M-QTLs and one E-QTL identified for DRW, and three E-QTLs identified for Na/K. The phenotypic variation explained by each M-QTL and E-QTL ranged from 7.8 to 23.9 % and 13.3 to 73.7 %, respectively. The QTL-by-environment interactions were detected by QTLNetwork program in the joint analyses of multi-environment phenotypic values. Six M-QTLs and five E-QTLs were identified. The phenotypic variation explained by each QTL and QTL × environment interaction ranged from 0.95 to 6.90 % and 0.02 to 0.50 %, respectively. By comparing chromosomal positions of these M-QTLs with those previously identified, five M-QTLs *qSH1.3*, *qSH12.1*, *qSH12.2*, *qDSW12.1* and *qDRW11* might represent novel salt tolerance genes. Five selected RILs with high salt tolerance had six to eight positive alleles of the M-QTLs, indicating that pyramiding by marker-assisted selection (MAS) of M-QTLs can be applied in rice salt tolerance breeding programs.

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Introduction

Soil salinity is one of the key abiotic stresses affecting crop growth and productivity worldwide (Zhu 2001). It is estimated that 20 % of the earth's land mass and 50 % of irrigated land are affected by salinity (Yan et al. 2005). Rice is one of the most important food crops in the world, and salinity is the most widespread soil problem limiting rice production. Approximately 30 % of rice-growing area in the world is affected by salinity (Prasad et al. 2000; Takehisa et al. 2004). High salinity induces injury, inhibits seed germination, reduces seedling growth and decreases rice yield (Ruan et al. 2011). Therefore, improving salt tolerance is one of the most important objectives of rice breeding programs in coastal areas (Lin et al. 2004). However, the development of salt-tolerant rice varieties through conventional breeding is time consuming and labor intensive due to the quantitative nature of salt tolerance. The identification of genomic regions associated with salt tolerance would enable breeders to develop salt-tolerant varieties using marker-assisted selection (MAS), which helps increase the breeding efficiency and power of selection (Lin et al. 2004; Lee et al. 2006; Wang et al. 2011c).

To identify the genomic regions suitable for marker-assisted breeding strategies for rice salt tolerance, the most crucial step in a QTL mapping project is to establish accurate phenotyping methods (Wang et al. 2011c). At the seedling stage, characters such as shoot length, Na^+ and K^+ concentration in the shoots, dry weights of shoots and roots and salt tolerance rating have been used to evaluate salt tolerance (Prasad et al. 2000; Flowers et al. 2000; Koyama et al. 2001; Lee et al. 2003; Lin et al. 2004; Lee et al. 2006). In these studies, different levels of salinity and durations of exposure were evaluated. For example, Koyama et al. (2001) analyzed the traits of Na^+ uptake, K^+ uptake, Na^+:K^+ ratio, dry mass production and concentrations of Na^+ and K^+ ions of seedlings in 50 mM NaCl for 12 days, after which the concentration was increased to 100 mM for another period of 8 days. However, Lin et al. (2004) made similar physiological measurements at 10 days after treatment with 140 mM NaCl. Lee et al. (2003) analyzed the salt tolerance indices after 4 days in NaCl with an electrical conductivity (EC) = 6 dS/m (approx. 66 mM) and then after 2 weeks in NaCl, EC = 12 dS/m (approx. 132 mM). However, Lee et al. (2006) determined the salinity tolerance rating after 4 days in 0.3 % NaCl (approx. 51 mM) and after a subsequent 2 weeks in 0.7 % NaCl (approx. 120 mM).

Using the rice seedling salt tolerance indices mentioned above, many QTLs have been detected (Prasad et al. 2000; Koyama et al. 2001; Lee et al. 2003; Lin et al. 2004; Lee et al. 2006), each with small effects scattered throughout the genome. A majority of these QTLs have been reported on chromosome 1, such as QNa for Na uptake (Flowers et al. 2000), QTL for Na^+ uptake, K^+ concentration and Na/K ratio (Koyama et al. 2001), SKC1 or OsHKT8, RNTQ1 and SDS1 (Lin et al. 2004; Ren et al. 2005). There are more reports of other QTLs for contributing traits on other chromosomes, for example, chromosomes 4, 6 and 9 (Flowers et al. 2000; Koyama et al. 2001), chromosomes 4, 6, 7 and 9 (Lin et al. 2004) and chromosome 3 (Lee et al. 2006). Indeed, the salt tolerance of rice is complex and influenced by both genetic and environmental factors. Epistasis, or interaction between non-allelic genes, and interaction between QTLs with environments have been recognized as contributing to the genetic control of quantitative traits (Li et al. 1997; Kubo and Yoshimura 2005; Malmberg et al. 2005; Zeng 2005; Carlborg et al. 2006; Shen et al. 2006; Würschum et al. 2011). However, it is difficult to identify epistatic QTLs (E-QTL) and QTL × environment interaction effects. Some programs, such as the QTLNetwork, genotype matrix mapping (GMM) and the multiple interval mapping (MIM) of QTL Cartographer, have been used to identify E-QTL in several crop species (Xing et al. 2002; Mohan et al. 2009; Yang et al. 2009; Ravi et al. 2011; Wang et al. 2011b, c), and QTLNetwork and GMM have also been used to identify interaction of QTLs with environments (Ravi et al. 2011). Recently, an efficient mapping method, including composite interval mapping (ICIM), for QTL with both additive and epistatic effects (M-QTL; E-QTL) was proposed by Li et al. (2007, 2008). In rice, the complex epistatic and QTL × environment interaction effects are important in controlling the salt tolerance (Wang et al. 2011c), but few studies have been reported previously. Therefore, with the ICIM and QTLNetwork approaches and so on, there is a need to identify E-QTLs and interaction between QTLs with different salinity conditions, in addition to simple M-QTLs, for the salt tolerance of rice.

Salt tolerance is a polygenic trait and is highly influenced by the environments, which makes it difficult to identify the genes or linked markers required for MAS breeding. In this study, one RIL population derived from a cross between *japonica* Jiucaiqing (salt tolerant) and *indica* IR26 (salt sensitive) (Wang et al. 2011c) was exposed to different salinity conditions (0.0, 0.5 and 0.7 % NaCl). With the ICIM and QTLNetwork approaches, the identification of QTLs with main, epistatic and QTL × environment interaction effects for salt tolerance in rice seedlings under different salinity condition was conducted. The results should contribute to understanding the genetic control of salt tolerance in rice. Furthermore, the *japonica* rice, Jiucaiqing, is a good source of gene(s) for salt tolerance, and the QTLs identified could be used to improve salt tolerance by MAS.

Materials and methods

Plant materials

Two rice varieties, Jiucaiqing (*japonica*) and IR26 (*indica*) and their 150 RIL lines ($F_{2:9}$) were used in this study. In our previous experiments, we found that Jiucaiqing was highly salt-tolerant while IR26 was salt-sensitive (Wang et al. 2011c).

Evaluation of salt tolerance

Fifty healthy grains of each parent and RIL were surface-sterilized with 0.1 % mercuric chloride solution for 10 min and then rinsed three times with sterile distilled water. The seeds were soaked in distilled water at 30 °C for 3 days to allow the seeds to germinate. Then, the germinated seeds were sown in a plastic box (40 cm × 30 cm × 18 cm) filled with 1.5 kg of quartz sand and placed in a growth chamber at 30 °C. At the three-leaf seedling stage, the well-established, uniform seedlings were transplanted to 1-cm plugged holes in foam sheets floated over 15 l of nutrient

solution (Yoshida et al. 1976) in the plastic box for 7 days. For salt treatment, the nutrient solution was replaced by a fresh solution containing 0.5 % or 0.7 % NaCl (w/v) for 10 days. The culture solution was renewed every 2 days, and the pH was maintained at 5.6 by adding either 1 M NaOH or 1 M HCl. After 10 days of salinity stress, the seedling height (SH), dry shoot weight (DSW), dry root weight (DRW) and Na/K ratio (Na/K) in roots were analyzed. The Na and K were extracted by liquid nitrogen (Gulati and Jaiwal 1993), and the concentrations were analyzed by atomic absorption methods using a TAS-986 machine (PGENERAL, Beijing, China). The experimental design included both non-saline (control) and saline nutrient solutions (0.5 and 0.7 % NaCl) in a randomized complete block design with three replications and three seedlings per replication.

Data analysis

The experimental data were analyzed using the statistical analysis system (SAS) software, and the indices of the parents were compared by Student's *t*-test at the 5 and 1 % levels of probability. The correlations of indices were computed using PROC CORR by SAS software (Wang et al. 2010b).

Construction of linkage map

DNA was extracted from rice seedlings by the SDS method (Dellaporta et al. 1983). PCR was performed using the procedure of Chen et al. (1997); the PCR products were then separated on an 8 % non-denaturing polyacrylamide gel and visualized by the silver staining method of Sanguinetti et al. (1994). The Mapmaker/EXP 3.0 program was used to construct a complete linkage map (Lander et al. 1987). Finally, a set of 135 SSR markers covering most of the rice genome at an average interval of 16.5 cM was constructed (Wang et al. 2011c).

QTL mapping

The M-QTLs and E-QTLs were identified by the QTL IciMapping program ver. 3.1 (Li et al. 2008) using single environment phenotypic values (Wang et al. 2011a). Briefly, for the M-QTL, the *P* values for entering variables (PIN) and removing variables (POUT) were set at 0.01 and 0.02, and the scanning step was 2 cM; for the E-QTL, the PIN and POUT were set at 0.0001 and 0.0002, respectively, and the scanning step was 5 cM. The QTLNetwork program ver. 2.0, based on a mixed linear model (Yang et al. 2005) was used to identify QTL × environment interactions for salt tolerance indices in joint analyses of multi-environment phenotypic values. The LOD thresholds for each index of QTL were determined by 1,000 permutation test at 95 % confidence level. The proportion of observed phenotypic variance explained by each M-QTL or E-QTL and the corresponding additive effects were also estimated. The QTL nomenclature followed the method of McCouch and CGSNL (2008).

Results

Salt tolerance phenotyping

There were significant differences in the SH, DSW, DRW and the Na/K ratio between two parents under different salinity conditions (Table 1). The salt-tolerant Jiucaiqing had higher SH, DSW and DRW than the sensitive IR26, whereas Jiucaiqing had a lower Na/K ratio than IR26. There was a continuous frequency distribution and transgressive segregation in the SH, DSW, DRW and Na/K ratio among the RILs population under different salinity conditions. The values of SH, DSW and DRW of parents and lines were decreased with the increase of NaCl concentration, and the case of Na/K ratio just opposite. The heritabilities of SH, DSW, DRW and Na/K were different under three NaCl concentration (Table 1).

The correlation analyses showed that there were significant positive correlations between SH and DSW, SH and DRW, and DSW and DRW under three different salinity conditions (Table 2). Although there were no significant correlations ($r < 0.2$) between Na/K and SH, DSW or DRW under the control, there were significant negative correlations between Na/K and SH, Na/K and DSW, and Na/K and DRW under 0.5 and 0.7 % NaCl conditions. These results showed that SH, DSW, DRW and Na/K are good indices representing salt tolerance.

M-QTLs for seedling salt tolerance

A total of 11 putative M-QTLs for the indices of SH, DSW and DRW assessed under 0.0, 0.5 and 0.7 % NaCl conditions were identified (Table 3; Fig. 1), while no M-QTLs identified for Na/K ratios.

There were two M-QTLs *qSH1.2* and *qSH4*, two M-QTLs *qSH1.3* and *qSH12.1*, and three M-QTLs *qSH1.1*, *qSH1.3* and *qSH12.2* controlling the SH under 0.0, 0.5 and 0.7 % NaCl condition, respectively. Among these M-QTLs only *qSH1.3* was identified under both 0.5 and 0.7 % NaCl conditions. Phenotypic variance explained by each M-QTL ranged from 9.3 to 20.5 %, and the total phenotypic variance explained by these M-QTLs was 25.9, 22.6 and 30.6 % under 0.0, 0.5 and 0.7 % NaCl conditions, respectively. The positive alleles of four M-QTLs (*qSH1.2*, *qSH1.3*, *qSH12.1* and *qSH12.2*) from Jiucaiqing and two

Table 1 Phenotypic values of salt tolerance indices among parents and RILs population under control (CK), 0.5 and 0.7 % NaCl conditions

Treatments	Indices	Parents[a]		RILs[b]				
		Jiucaiqing	IR26	Min	Max	Mean	Coefficient of variance (%)	Heritability (%)
CK	SH (cm)	37.04 ± 1.21**	23.58 ± 0.85	15.78	44.99	32.23	16.7	93.27
	DSW (g)	0.135 ± 0.043*	0.091 ± 0.032	0.049	0.208	0.127	26.4	77.88
	DRW (g)	0.040 ± 0.011	0.038 ± 0.009	0.014	0.063	0.037	27.1	63.58
	Na/K	0.076 ± 0.021	0.099 ± 0.012	0.062	0.578	0.135	63.5	82.60
0.5 % NaCl	SH (cm)	34.45 ± 1.48**	19.34 ± 0.47	14.77	35.54	25.68	17.3	36.93
	DSW (g)	0.124 ± 0.068*	0.080 ± 0.036	0.030	0.181	0.090	32.8	71.77
	DRW (g)	0.038 ± 0.012*	0.026 ± 0.017	0.007	0.052	0.028	33.7	60.55
	Na/K	0.368 ± 0.018**	0.604 ± 0.041	0.052	1.192	0.638	32.8	58.54
0.7 % NaCl	SH (cm)	31.11 ± 1.11**	17.69 ± 0.94	14.60	33.26	23.09	17.1	49.22
	DSW (g)	0.116 ± 0.054**	0.066 ± 0.006	0.025	0.152	0.079	32.8	35.80
	DRW (g)	0.038 ± 0.002*	0.026 ± 0.001	0.010	0.051	0.029	29.6	40.83
	Na/K	0.539 ± 0.031**	1.124 ± 0.060	0.473	1.618	0.855	23.5	51.25

SH seedling height, *DSW* dry shoot weight, *DRW* dry root weight, *Na/K* Na/K ratio

*,** significance at the level of 5 and 1 %, respectively, according to Student's *t*-test

[a] Means ± SD (standard deviation)

[b] RILs sample size $n = 150$, replications $r = 3$

Table 2 The correlation of different salt tolerance indices under control (CK), 0.5 and 0.7 % NaCl conditions

Indices	CK				0.5 % NaCl				0.7 % NaCl			
	SH	DSW	DRW	Na/K	SH	DSW	DRW	Na/K	SH	DSW	DRW	Na/K
SH	1				1				1			
DSW	0.6839**	1			0.7477**	1			0.7844**	1		
DRW	0.5426**	0.7879**	1		0.6885**	0.8984**	1		0.6590**	0.8879**	1	
Na/K	0.1524	0.0995	0.1817	1	−0.3784**	−0.3786**	−0.2773**	1	−0.2284*	−0.2697**	−0.2160*	1

SH seedling height, *DSW* dry shoot weight, *DRW* dry root weight, *Na/K* Na/K ratio in roots

*,** significance at the level of 5 and 1 %, respectively

M-QTLs (*qSH1.1* and *qSH4*) from IR26 contributed to the increase of SH.

There were three M-QTLs *qDSW6.1, qDSW6.2* and *qDSW12.1* controlling the DSW under 0.5 % NaCl condition, but not any M-QTLs identified under 0.0 and 0.7 % NaCl conditions (Table 3; Fig. 1). Under 0.5 % NaCl condition the phenotypic variance explained by each QTL ranged from 17.1 to 23.9 %; the total phenotypic variance explained by the M-QTLs was 38.0 %. The positive alleles of two M-QTLs (*qDSW6.1* and *qDSW12.1*) from Jiucaiqing and one M-QTL (*qDSW6.2*) from IR26 contributed to the increase of DSW.

There were two M-QTLs *qDRW6* and *qDRW11* controlling the DRW under 0.7 % NaCl condition, but not any M-QTLs identified under 0.0 and 0.5 % NaCl conditions (Table 3; Fig. 1). The phenotypic variance explained by *qDRW6* and *qDRW11* was 7.8 and 9.6 %, respectively; the total phenotypic variance explained by the two M-QTLs was 18.3 %. The positive alleles of one M-QTL (*qDRW6*) from Jiucaiqing and one M-QTL (*qDRW11*) from IR26 contributed to the increase of DRW.

Compared with the heritabilities of SH, DSW, DRW and Na/K, the total phenotypic variances explained by the M-QTLs were relatively lower under NaCl conditions, indicating there might be E-QTLs controlling SH, DSW, DRW and Na/K.

E-QTLs for seedling salt tolerance

With the ICIM approach epistatic interaction analysis was further conducted using single environment phenotypic values under three different NaCl conditions. A total of 11 E-QTLs were identified for the indices of SH, DSW, DRW and Na/K (Table 4).

There were two E-QTLs controlling the SH under the control, but none for SH identified under 0.5 and 0.7 %

Table 3 The M-QTLs for salt tolerance indices under control (CK), 0.5 and 0.7 % NaCl conditions

Treatments	Indices	QTL	Chr[a]	Marker interval	LOD	A[b]	r^2 [c] (%)	r^2 [d] (%)
CK	SH	*qSH1.2*	1	RM5389–RM5759	7.3	2.6249	20.5	
		qSH4	4	RM518–RM16535	3.1	−1.7290	9.9	25.9
0.5 % NaCl	SH	*qSH1.3*	1	RM3482–RM3362	3.5	1.7146	14.4	
		qSH12.1	12	RM5609–RM7376	3.0	1.5704	11.9	22.6
	DSW	*qDSW6.1*	6	RM6818–RM6811	4.1	0.0138	20.1	
		qDSW6.2	6	RM340–RM3509	6.9	−0.0146	23.9	
		qDSW12.1	12	RM5609–RM7376	4.7	0.0123	17.1	38.0
0.7 % NaCl	SH	*qSH1.1*	1	RM294–RM11179	4.1	−1.2070	9.3	
		qSH1.3	1	RM3482–RM3362	4.2	1.5162	14.7	
		qSH12.2	12	RM7376–RM6953	4.6	1.6509	17.2	30.6
	DRW	*qDRW6*	6	RM5531–RM3183	3.0	0.0029	7.8	
		qDRW11	11	RM6091–RM229	2.9	−0.0027	9.6	18.3

SH seedling height, *DSW* dry shoot weight, *DRW* dry root weight, *Na/K* Na/K ratio

[a] Chromosome on which the QTL was located

[b] Additive effect is the effect of substituting a Jiucaiqing allele for an IR26 allele; Its positive value indicates that Jiucaiqing has the positive allele; The case of negative values is just the opposite

[c] Variation explained by each putative QTL

[d] The total phenotypic variance explained by the QTLs

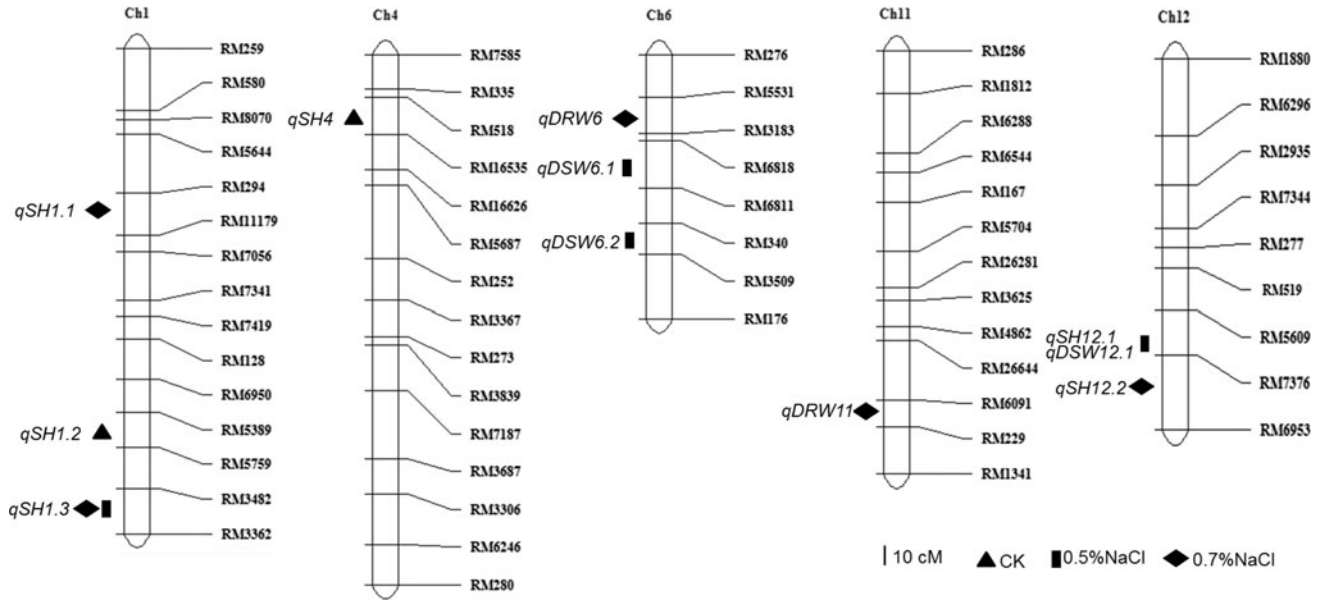

Fig. 1 Location of M-QTLs for salt tolerance indices under control (CK), 0.5 and 0.7 % NaCl conditions on linkage groups

NaCl conditions (Table 4). There were two, one and two E-QTLs controlling the DSW under 0.0, 0.5 and 0.7 % NaCl conditions, respectively. Only one E-QTL was identified for the DRW under 0.5 % NaCl condition, but none for DRW identified under 0.0 and 0.7 % NaCl conditions. There was each one E-QTL controlling the Na/K under 0.0, 0.5 and 0.7 % NaCl conditions, respectively. The variation explained by these E-QTLs ranged from 13.3 to 73.7 % (Table 4).

QTL × environment interactions for seedling salt tolerance

In order to identify the interaction between QTLs and environments for rice salt tolerance, the joint analyses of multi-environment phenotypic values for the indices of SH, DSW, DRW and Na/K under three different NaCl conditions by QTLNETwork approach was conducted. Six M-QTLs and five E-QTLs were identified (Table 5). It was found that five M-QTLs and one E-QTL for SH, one

Table 4 The E-QTLs for salt tolerance indices under control (CK), 0.5 and 0.7 % NaCl conditions

Treatments	Indices	Loci (*i*)		Loci (*j*)		LOD	AA[b]	r^2 [c] (%)
		Chr[a]	Marker interval	Chr[a]	Marker interval			
CK	SH	5	RM249–RM7568	12	RM519–RM5609	5.1	2.7482	26.4
		7	RM427–RM6872	12	RM1880–RM6296	6.3	−2.9197	29.3
	DSW	2	RM5631–RM3685	12	RM519–RM5609	7.2	−0.0166	22.0
		5	RM3777–RM249	7	RM1306–RM172	5.0	0.0123	13.3
	Na/K	4	RM6246–RM280	12	RM519–RM5609	17.9	−0.0628	73.7
0.5 % NaCl	DSW	3	RM130–RM3684	11	RM3625–RM4862	5.2	0.0138	19.3
	DRW	4	RM16535–RM16626	8	RM210–RM223	5.2	0.0044	19.0
	Na/K	5	RM7302–RM5874	5	RM3777–RM249	5.2	0.0830	43.5
0.7 % NaCl	DSW	3	RM130–RM3684	11	RM26281–RM3625	5.8	0.0107	15.6
		4	RM3306–RM6246	5	RM7568–RM305	5.4	0.0114	19.6
	Na/K	4	RM6246–RM280	12	RM277–RM519	5.6	−0.1093	25.6

SH seedling height, *DSW* dry shoot weight, *DRW* dry root weight, *Na/K* Na/K ratio

[a] Chromosome on which the QTL was located

[b] AA is the effect of additive by additive interaction between two points: its positive value indicates that two loci genotypes being the same as those in parent Jiucaiqing (or IR26) take the positive effects, while the two-loci recombinants take the negative effects. The case of negative values is just the opposite

[c] Variation explained by each pair of epistatic loci

Table 5 The QTL × environment interaction effects of QTLs for salt tolerance indices under control (CK), 0.5 and 0.7 % NaCl conditions

Indices	Chr[a]	Marker interval	Chr[a]	Marker interval	A/AA[b]	AE1/ AAE1[b]	AE2/ AAE2[c]	AE3/ AAE3[c]	r^2(A/AA) (%)[d]	r^2(AE/AAE) (%)[d]
SH	1	RM5759–RM3482			2.1994	0.6416	−0.3390	−0.2919	5.34	0.50
	3	RM49–RM6712			1.0363	0.2105	−0.1036	−0.1032	0.95	0.17
	4	RM518–RM16535			−1.3028	−0.0002	0.0001	0.0002	2.99	0.14
	5	RM1366–RM7302			1.3134	0.0001	−0.0001	−0.0001	3.05	0.05
	6	RM340–RM3509			−0.6521	−0.0001	0.0001	0.0001	1.38	0.05
	4	RM7187–RM3687	8	RM223–RM3459	−1.4920	−0.0002	0.0001	0.0002	2.68	0.23
DSW	5	RM1366–RM7302			0.0070	0.0001	−0.0001	0.0001	3.64	0.12
	1	RM259–RM580	4	RM16626–RM5687	0.0101	0.0001	−0.0001	0.0001	4.18	0.07
	2	RM5804–RM5631	2	RM6312–RM3850	0.0078	0.0001	−0.0001	0.0001	4.51	0.02
	3	RM130–RM3684	11	RM26281–RM3625	0.0098	0.0001	0.0001	−0.0001	5.15	0.05
DRW	4	RM16535–RM16626	8	RM22491–RM72	0.0029	0.0002	0.0001	−0.0003	6.90	0.46

SH seedling height, *DSW* dry shoot weight, *DRW* dry root weight

[a] Chromosome on which the QTL was located

[b] A represents the estimated additive effect of M-QTL; its positive value indicates that Jiucaiqing has the positive allele and the case of negative values is just the opposite; AA represent the estimated additive effects of E-QTL; its positive value indicates that two loci genotypes being the same as those in parent Jiucaiqing (or IR26) take the positive effects, while the two-loci recombinants take the negative effects

[c] AE1, AE2 and AE3 represent the additive effects of M-QTL under control (CK), 0.5 and 0.7 % NaCl conditions, respectively; its positive value indicates that Jiucaiqing has the positive allele and the case of negative values is just the opposite; AAE1, AAE2 and AAE3 represent the additive effects of E-QTL under control (CK), 0.5 and 0.7 % NaCl conditions, respectively; its positive value indicates that two loci genotypes being the same as those in parent Jiucaiqing (or IR26) take the positive effects, while the two-loci recombinants take the negative effects

[d] r^2(A), r^2(AA), r^2(AE) and r^2(AAE) represent the phenotypic variation explained by the M-QTL, E-QTL, M-QTL × environment interactions and the E-QTL × environment interactions, respectively

M-QTL and three E-QTLs for DSW and one E-QTL for DRW were identified. The phenotypic variance explained by each QTL ranged from 0.95 to 6.90 %, and the phenotypic variation explained by QTL × environment interaction ranged from 0.02 to 0.50 % (Table 5).

The phenotypes and linked M-QTLs in selected RILs

Five selected RILs with relatively high SH, DSW and DRW and low Na/K were found in this study (Table 6). The SH of selected RILs was less affected by salinity stress

Table 6 Phenotype of salt tolerance indices under control (CK), 0.5 and 0.7 % NaCl conditions in selected RILs and their related M-QTLs

Selected RILs	CK				0.5 % NaCl				0.7 % NaCl				Linked M-QTLs
	SH (cm)	DSW (g)	DRW (g)	Na/K	SH (cm)	DSW (g)	DRW (g)	Na/K	SH (cm)	DSW (g)	DRW (g)	Na/K	
8	36.78	0.1543	0.0418	0.1022	33.97	0.1388	0.0378	0.0528	31.97	0.1368	0.0457	0.7883	*qSH1.1 qSH12.1 qSH12.2 qDSW6.2 qDSW12.1 qDRW6 qDRW11*
39	39.48	0.1455	0.0358	0.1173	34.09	0.1358	0.0432	0.6851	32.60	0.1395	0.0372	0.6480	*qSH1.1 qSH1.3 qSH12.1 qSH12.2 qDSW6.1 qDSW6.2 qDSW12.1 qDRW6*
57	34.11	0.1035	0.0288	0.1088	34.12	0.1067	0.0360	0.6861	27.16	0.1013	0.0315	1.1133	*qSH1.1 qSH1.3 qSH12.1 qSH12.2 qDSW6.1 qDSW6.2 qDSW12.1*
64	36.70	0.1682	0.0403	0.0955	33.37	0.1447	0.0403	0.4965	27.12	0.1067	0.0320	0.8563	*qSH1.1 qSH12.1 qSH12.2 qDSW6.1 qDSW6.2 qDSW12.1 qDRW6 qDRW11*
86	41.74	0.1715	0.0468	0.0859	35.54	0.1808	0.0480	0.4677	30.98	0.1357	0.0512	0.7470	*qSH1.1 qSH1.3 qDSW6.1 qDSW6.2 qDRW6 qDRW11*
Jiucaiqing	37.04	0.1350	0.0400	0.0764	34.45	0.1242	0.0377	0.3684	31.11	0.1164	0.0379	0.5385	*qSH1.2 qSH1.3 qSH12.1 qSH12.2 qDSW6.1 qDSW12.1 qDRW6*
IR26	23.58	0.0910	0.038	0.0988	0.6035	0.6035	0.6035	0.6035	17.69	0.0661	0.0256	1.1242	*qSH1.1 qSH4 qDSW6.2 qDRW11*

SH seedling height, *DSW* dry shoot weight, *DRW* dry root weight, *Na/K* Na/K ratio

under 0.5 % NaCl condition compared with the control. The DSW and DRW of selected RILs were similar with the control, and the scores of Na/K in selected RILs were relative lower under 0.5 and 0.7 % NaCl conditions. Analysis revealed that these RILs had six to eight positive alleles of the M-QTLs (Table 6).

Discussion

The most crucial step of QTL mapping for rice salt tolerance is the evaluation of salt tolerance (Wang et al. 2011c). In the present study, it was found that the SH, DSW, DRW and Na/K were strongly affected by salinity stress, and the SH, DSW and DRW decreased and Na/K increased to different extents under 0.5 and 0.7 % NaCl conditions. These results were very similar with the previous reports on salt tolerance researches in rice (Prasad et al. 2000; Flowers et al. 2000; Koyama et al. 2001; Lee et al. 2003; Lin et al. 2004; Lee et al. 2006). As the correlations among the SH, DSW, DRW and Na/K were also found significant under salinity stress, it was appropriate to jointly estimate the QTL for rice seedling salt tolerance with these indices in this study.

To reveal the genetic control of rice salt tolerance at the seedling stage, the identification of QTLs for different salt tolerance indices mentioned above were conducted under different salt concentrations (0, 0.5 and 0.7 % NaCl) in this study. The use of multiple indices related at different salinity stress not only greatly facilitated the detection of QTL, but also allowed the identification of QTL × environment interactions. The QTLs (M-QTL; E-QTL) identified under single environment at different salt concentrations were distributed on the most of rice chromosomes except chromosomes 9 and 10, with M-QTLs mainly on chromosomes 1, 4, 6, 11 and 12. Most of M-QTLs identified were for SH, followed by DSW and DRW, but none for Na/K. Although the indices of SH, DSW, DRW and Na/K were highly correlated (Table 2), the detection of co-localized M-QTLs only occurred on one genomic region RM5609-RM7376 of chromosome 12 for *qSH12.1* and *qDSW12.1* (Fig. 1). These co-localized M-QTLs could be very useful for the simultaneous improvement of more than one trait, as the desirable alleles at these M-QTLs were contributed by a single parent; the positive alleles of *qSH12.1* and *qDSW12.1* originated from Jiucaiqing. Only a common M-QTL for SH (*qSH1.3*) was identified under both 0.5 and 0.7 % NaCl conditions, indicating the *qSH1.3* might play an important role in controlling SH under salinity stress. In summary, majority of the identified M-QTLs did not reveal a high phenotypic variance; the total phenotypic variance explained by the M-QTLs was relative lower compared with the heritabilities of four indices, SH, DSW, DRW and

Na/K (Tables 1, 3), which indicated that the performance of four indices might be affected by E-QTLs or interaction between QTLs and environments; therefore, E-QTL mapping or QTL × environment interaction analysis is needed.

In order to further clarify the genetic control of rice seedling salt tolerance, the E-QTL and QTL × environment interactions were analyzed in the present study. Eleven E-QTLs were detected for different salt tolerance indices under single environment at various salt concentrations by ICIM (Table 4). The most of E-QTLs identified was for DSW, followed by Na/K, SH and DRW. Compared with the M-QTLs, the number and phenotypic effects of E-QTLs for DSW and Na/K were much larger (Tables 3, 4). These results suggested that epistasis as a genetic factor was much more important than M-QTLs for DSW and Na/K in rice. The joint analyses of multi-environment phenotypic values can reveal those QTLs expressed under various environments. Six M-QTLs and five E-QTLs were identified, respectively, using multi-environment phenotypic values in three salt stress conditions by QTLNetwork (Table 5). It indicated that QTL × environment interactions were important components for rice seedling salt tolerance although the degree of interactions was very low.

With the successful application of QTL mapping technology, QTL analyses of salt tolerance have been reported in several plants, such as solanum (Villalta et al. 2007), tomato (Villalta et al. 2008), soybean (Tuyen et al. 2010) and white clover (Wang et al. 2010a). In rice, a number of M-QTLs for salt tolerance have been identified (Prasad et al. 2000; Koyama et al. 2001; Lang et al. 2001; Lin et al. 2004; Takehisa et al. 2004; Ren et al. 2005; Lee et al. 2006; Pandit et al. 2010; Wang et al. 2011c). By comparing chromosomal positions of these M-QTLs, several salt-tolerance-related M-QTLs identified in previous studies were near the M-QTLs identified in this study. For example, we found that *qSH1.1* was near the region of *qSDS-1* for survival days of seedlings (Lin et al. 2004), *qDSW6.1* located at a position that coincided with the region of a QTL for seedling root length (Prasad et al. 2000), and *qDSW6.2* near a QTL *qSTR* for standard tolerance ranking in young rice seedlings (Sabouri et al. 2009). Furthermore, *qDRW6* was located near the region of a QTL *qIR-6* for seed imbibition rate under salinity stress (Wang et al. 2011c). There were no QTLs previously reported to be close to *qSH1.3*, *qSH12.1*, *qSH12.2*, *qDSW12.1* and *qDRW11*, which indicates that these M-QTLs might be novel salt tolerance genes. With the increase of QTL numbers identified for salt tolerance, the genetic control of rice salt tolerance will be described more clearly.

As in other plants, tolerance to salinity stress in rice is based on mechanisms that prevent salt accumulating in the cytoplasm (Ahmadi et al. 2011). For instance, membrane transporter gene families, particularly the K^+ transporter (HKT) family, have been shown to play important roles in Na^+ and K^+ uptake and homeostasis in rice (Golldack et al. 2003; Horie et al. 2007). To date, only one QTL, *SKC1*, has been map-based cloned. This QTL was found to encode an HKT-type transporter, *OsHKT8*, responsible for regulating K^+/Na^+ homeostasis under salinity stress (Ren et al. 2005). The fine mapping of M-QTL *qDSW6.1* identified in this study is now in progress using NILs strategy to cloning functional genes. Those closely linked markers to the M-QTLs identified in this study will be useful for rice breeding by MAS for pyramiding of the M-QTLs involved in salt tolerance and achieving a high level of salt tolerance in rice. In this study, the analysis of five selected RILs with high salt tolerance showed that these RILs had six to eight positive alleles of M-QTLs, indicating that QTL pyramiding by MAS is applicable to rice salt tolerance breeding programs.

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