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Dissection of genetic overlap of salt tolerance QTLs at the seedling and tillering stages using backcross introgression lines in rice

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QTLs for salt-tolerance (ST) related traits at the seedling and tillering stages were identified using 99 BC_2F_8 introgression lines (IL) derived from a cross between IR64 (indica) as a recurrent parent and Binam (japonica) from Iran as the donor parent. Thirteen QTLs affecting survival days of seedlings (SDS), score of salt toxicity of leaves (SST), shoot K^+ concentration (SKC) and shoot Na^+ concentration (SNC) at the seedling stage and 22 QTLs underlying fresh weight of shoots (FW), tiller number per plant (TN) and plant height (PH) at the tillering stage were identified. Most QTLs detected at the tillering stage showed obvious differential expression to salt stress and were classified into three types based on their differential behaviors. Type I included 11 QTLs which were expressed only under the non-stress condition. Type II included five QTLs expressed in the control and the salt stress conditions, and three of them (*QPh5*, *QPh8* and *QTn9*) had similar quantity and the same direction of gene effect, suggesting their expression was less influenced by salt stress. Type III included six QTLs which were detectable only under salt stress, suggesting that these QTLs were apparently induced by the stress. Thirteen QTLs affecting trait difference or trait stability of ILs between the stress and non-stress conditions were identified and the Binam alleles at all loci except *QPh4*, *QTn2* and *QFw2a* decreased trait difference. The three QTLs less influenced by the stress and 13 QTLs affecting trait stability were considered as ST QTLs which contributed to ST. Comparing the distribution of QTLs detected at the seedling and tillering stages, most (69%) of them were genetically independent. Only four were the same or adjacent regions on chromosomes 1, 2, 8 and 11 harboring ST QTLs detected at the two stages, suggesting that partial genetic overlap of ST across the two stages occurs. It is likely, therefore, to develop ST rice variety for both stages by pyramiding of ST QTLs of different stages or selection against the overlapping QTLs between the two stages via marker-assisted selection (MAS).

rice, salt tolerance (ST), quantitative trait loci (QTL), genetic overlap

Salinity is one of the major abiotic stresses in rice production worldwide. Recently, a large quantity of industrial pollution, abuse of chemical fertilizers and unreasonable irrigation increasingly aggravated soil salinization. Accumulation of salt in the soil often results in reduction of yield or total yield loss in some cases. With the rapid increase of the world's population, it is critically important to make full use of the limited land resources for sustainable development of agriculture.

Salt tolerance (ST) of non-halophytes is complex both

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genetically and physiologically[1]. ST in rice is obviously specific to the developmental stage and may have no significant correlation between different developmental stages[2−7]. Rice plants are most susceptible to salt stress during the early seedling stage (2—3 leaves), and progressively gain tolerance with the development of plant, then becomes relatively susceptible again at the reproductive stage[8−11].

Recently, many reports have been involved in QTL mapping of ST-related traits at the seedling stage in rice using recombinant inbred lines, DH population, $F_{2:3}$ and backcross introgression lines[12−19]. But very few studies involved QTL mapping of ST at the tillering and adult stages. As only one case, Takehisa et al.[20] detected QTLs affecting tiller number, seedling height and fresh weight at the vegetative stage in paddy field flooded with salt water. Previous studies on QTL for ST were mostly concentrated on a special stage of rice development, thus it was difficult to systematically and fully understand the expression and genetic relationship of QTLs detected in different developmental stages. To reveal the correlation and genetic relationship of ST and identify main-effect ST QTLs of different growth stages will be able to better understand genetic behaviors of ST and facilitate rice breeding progress by pyramiding of different main-effect ST QTLs using molecular technologies.

In this study, QTLs affecting ST-related traits at the seedling and tillering stages were dissected using IR64/Binam BC_2F_8 introgression lines (ILs) which were derived from the Global Rice Molecular Breeding Program. The objective was to compare the correlation and genetic relationship of ST and identify main-effect ST QTL from different developmental stages, to provide useful information of genes and markers for improvement of cultivar's ST.

1 Materials and methods

1.1 Meterials

A set of 99 BC_2F_8 ILs derived from a cross of an indica variety IR64 from IRRI as a recurrent parent and a japonica variety Binam from Iran as a donor parent was used in the study.

1.2 Evaluation of ST related traits at the seedling stage

The seeds were placed at 50℃ for 3 d to break any possible dormancy, then germinated at 35℃ for 48 h after surface-sterilizing seeds with 1% sodium hypochlorite solution for 10 min and rinsing well with distilled water. The most uniform 20 germinated seeds were sown in holes of thin Styrofoam board with a nylon net bottom in a plastic tray, which floated on water up to two leaf stages, then transferred to floating on Yoshida's cultural solution[21] containing 140 mmol/L NaCl. The experiment was conducted in two replications. The seedlings were grown in a phytotron with 29℃/22℃ day/ night temperature and minimum relative humidity of 70%. The solution was changed every 5 d and the pH was maintained at 5.5 every other day. Scores of salt toxicity of leaves (SST) were investigated 10 d after salinization and survival days of seedlings (SDS) were recorded for each individual plant in days from seeding to death according to the standard evaluation system (SES)[22].

To observe physiological traits, such as Na^+ and K^+ concentration in the shoots, the second experiment was performed. The procedure and management of the experiment was the same as the above-mentioned experiment. To minimize environmental errors, the germinated seeds were sown in holes of PCR plate with broken bottom in each well in two replications. Eight days after treating with NaCl, the shoots were harvested and rinsed with distilled water several times. Shoots were dried under 80℃, weighed and extracted in acetic acid (100 mmol/L) at 90℃ for 2 h. The extraction was divided into two groups, and sodium and potassium in each group were determined by the atomic absorption spectrophotometer (S2, Solaar House, United Kingdom). The concentration of the two ions in the shoots was calculated using the data collected.

1.3 Investigation of ST related traits at the tillering stage

The parents and ILs were evaluated in two replicated experiments in the net house of Tianjin Rice Institute located near the coast at the fall of 2007. Seeds were sown in seedbed which was irrigated with freshwater. The seedlings were transplanted into two plots. One plot was irrigated with freshwater (control group) while the other plot was irrigated with salt water (treated group). For salt stress, two wells inside the net house were dug: one was 30 m deep which contained 1.6% concentration of NaCl, while the other was 10 m deep which contained 0.3% NaCl. The salt concentration in the treated group

remained at about 0.8% (equivalent to 140 mmol/L at the seedling stage) during the whole growth stage by proportionally mixing underground water from the two wells. At the tillering stage (60 d after sowing), plant height (PH), tiller number per plant (TN) and fresh weight of shoots (FW) were investigated.

1.4 Genotyping and linkage map construction

A total of 129 polymorphic SSR markers were used to assay the ILs. Marker position and genetic distance among linkage markers followed the Cornell map[23], which covered all 12 rice chromosomes with a total genome size of 1588.7 cM and an average distance of 13.6 cM between adjacent markers.

1.5 Data analyses

Correlation between different traits was determined by the SAS PROC CORR[24]. Phenotypic data of ILs obtained from both seedling and tillering stages and the difference between the stress and non-stress conditions at the tillering stage, including plant height difference of the stress to control (DPH), tiller number difference of the stress to control (DTN) and fresh weight difference of the stress to control (DFW) were used as input data to identify QTL by one way ANOVA using SAS PROC GLM[24]. The probability level of $P<0.005$ was used as the threshold for claiming the presence of QTL. When a QTL was detected by two or more linked markers, the one with the highest F value was presented[25]. To avoid type-II errors in statistics, QTL for the same trait identified in one condition was reexamined using the data from the other condition under the minimum threshold of $P<0.05$, and the test statistics and QTL parameters associated with the QTL were also reported as long as the QTL reached the minimum threshold[25,26].

2 Results

2.1 Performance of ST-related traits for parents and ILs

Table 1 shows the summary of phenotypic performance of the parents and ILs for SST, SDS, SNC and SKC at the seedling stage and PH, TN and FW in control and salt stress conditions as well as traits difference of stress to control at the tillering stage. The two parents were similar in all traits except PH under control and salt stress conditions. ILs showed transgressive segregation for all traits and showed continuous variation which was suitable for QTL mapping.

2.2 Correlations between ST related traits

SDS showed significantly negative correlation with SST but positive correlation with SKC (Table 2), indicating plants with less injury of leaves or high K^+ content in the shoots can survive longer under salt stress. CPH had negative correlation with CTN, and CFW had positive correlations with CPH and CTN, suggesting taller plants

Table 1 Performance of salt tolerance-related traits of IR64/Binam BC_2F_8 introgression lines (ILs) at the seedling and tillering stages

Treatment	Trait[a)]	Parents			ILs	
		IR64 (P_1)	Binam (P_2)	P_1-P_2[b)]	mean ± SD	range
Salt stress at the seedling stage	SDS	12	12.5	−0.5	12.46±2.76	8.0－19.5
	SST	5	5	0	5.51±1.69	2.0－9.0
	SNC	2.77	2.95	−0.18	2.8308±0.43	2.0818－5.0871
	SKC	0.39	0.4	−0.01	0.3749±0.06	0.2425－0.6453
Control at the tillering stage	CPH	72.4	85	−12.6*	76.06±8.60	63.5－97.17
	CTN	15	12	3	15.75±2.61	9.67－22.67
	CFW	121.32	114.7	6.62	116.71±22.17	62.65－172.76
Salt stress at the tillering stage	SPH	66.23	77.36	−11.13*	69.58±9.36	49.5－108.0
	STN	8	7	1	10.20±2.97	3.7－18.0
	SFW	61.17	49.78	11.39	64.28±20.91	19.7－118.1
Difference of the stress to control	DPH	−6.17	−7.64	1.47	−6.50±6.95	−26.67－9.33
	DTN	−7	−5	−2	−5.54±3.85	−15.83－3.17
	DFW	−60.15	−64.92	4.77	−52.02±28.99	−123.80－8.27

a) SDS, survival days of seedlings; SST, score of salt toxicity of leaves; SKC, shoot K^+ concentration; SNC, shoot Na^+ concentration; CPH, CTN and CFW, plant height, tiller number and fresh weight under the control; SPH, STN and SFW, plant height, tiller number and fresh weight under salt stress; DPH, DTN and DFW, differences of plant height, tiller number and fresh weight between the stress and non-stress conditions. b) * represents the significant level at $P<0.05$.

Table 2 Correlation among salt tolerance-related traits of IR64/Binam BC_2F_8 introgression lines at the seedling and tillering stages

	SDS	SST	SNC	SKC	CPH	CTN	CFW	SPH	STN	SFW	DPH	DTN
SST	**–0.798**											
SNC	–0.091	0.127										
SKC	***0.203***	–0.169	–0.012									
CPH	0.055	0.066	–0.043	0.017								
CTN	–0.134	0	0.092	0.101	***–0.250***							
CFW	–0.019	0.002	0.044	0.136	**0.480**	**0.524**						
SPH	0.184	–0.083	***–0.213***	0.005	**0.704**	**–0.379**	0.176					
STN	0.136	–0.163	–0.137	0.036	–0.013	0.055	–0.039	0.150				
SFW	0.117	–0.080	0.080	–0.058	–0.019	***0.223***	0.115	–0.065	0.087			
DPH	0.180	–0.194	***–0.232***	–0.015	–0.289	***–0.201***	**–0.357**	**0.477**	***0.219***	–0.060		
DTN	0.196	–0.126	–0.169	–0.041	0.160	**–0.635**	**–0.386**	**0.373**	**0.737**	–0.095	0.305	
DFW	0.059	–0.037	0.048	–0.145	**–0.443**	***–0.231***	**–0.698**	–0.281	0.001	**0.632**	***0.224***	0.174

Trait abbreviation is the same as in Table 1. Bold and italic, underlined and bold numbers stand for significant levels of trait correlation at $P<0.05$, 0.01 and 0.001, respectively.

had less tiller number and taller plants or plants with more tillers had heavier fresh weight of the shoots under control condition. DPH was positively correlated with DTN and DFW, respectively, indicating the same trends existed between DPH and DTN, and between DPH and DFW. SPH showed negative correlation with SNC, suggesting plants containing high concentration of Na^+ in the shoot at the seedling stage would become shorter in PH at the tillering stage under salt stress. Positive correlations were detected between SPH and CPH, and between SFW and CTN while negative correlation was found between SPH and CTN, indicating higher plants or plants with less TN in the control condition had relatively high PH under salt stress, and plants with more TN in the control had relatively heavy FW under salt stress. DPH, DTN and DFW all showed negative correlations with CPH, CTN and CFW except that between DTN and CPH, and most trait difference showed positive correlations with traits under salt stress with an exception of negative correlation between DFW and SPH.

2.3 QTL mapping of ST-related traits at the seedling stage

A total of 13 QTLs affecting ST were detected, including three for SST, four for SDS, four for SKC and two for SNC, and distributed on chromosomes 1—3, 6, 8 and 11 (Table 3 and Figure 1).

Three QTLs (*QSst2*, *QSst3* and *QSst8*) affecting SST were identified on chromosomes 2, 3 and 8, and the Binam alleles at all loci decreased SST or increased ST. Four QTLs affecting SDS including *QSds2a*, *QSds2b*, *QSds3* and *QSds8* were identified and mapped on chromosomes 2, 3 and 8 and the Binam alleles at all loci except *QSds2a* prolonged SDS. Four QTLs for SKC

Table 3 QTLs affecting salt tolerance-related traits detected in IR64/Binam BC_2F_8 introgression lines at the seedling stage

	QTL	Chromosome	Marker interval [a)]	*F* value	*A* [b)]
SST	*QSst2*	2	RM250—RM208	8.64	–0.84
	QSst3	3	RM231—RM175	11.96	–1.78
	QSst8	8	RM38—RM25	7.67	–0.64
SDS	*QSds2a*	2	OSR17—RM211	8.95	–0.83
	QSds2b	2	RM530—RM250	9.46	1.15
	QSds3	3	RM231—RM175	8.05	3.57
	QSds8	8	RM38—RM25	7.52	1.09
SKC	*QSkc1*	1	RM562—RM9	10.33	0.03
	QSkc3	3	RM81B—RM22	10.99	0.04
	QSkc6	6	RM50—RM539	9.56	0.02
	QSkc11	11	RM120—RM181	7.68	0.03
SNC	*QSnc3*	3	RM231—RM175	14.21	–0.55
	QSnc6	6	RM527—RM3	19.99	0.54

Trait abbreviation is the same as in Table 1; a) the underlined markers are those closer to the true QTL positions; b) *A* represents the additive effect, estimated as the substitution effect of IR64 allele by Binam allele.

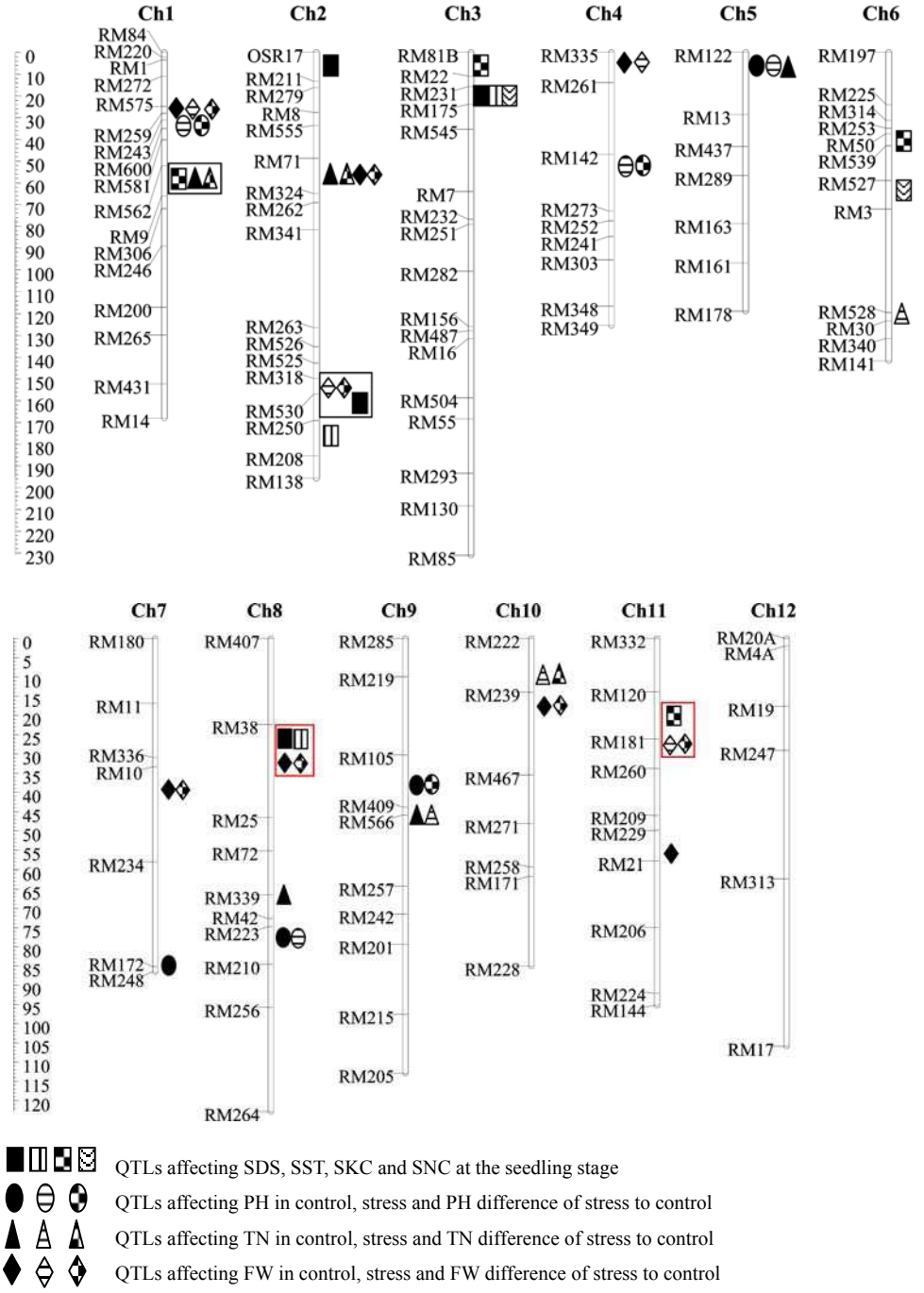

Figure 1 QTLs affecting survival days of seedlings (SDS), score of salt toxicity of leaves (SST), shoot K^+ concentration (SKC) and shoot Na^+ concentration (SNC) at the seedling stage, and plant height (PH), tiller number (TN) and fresh weight of shoots (FW) at the tillering stage detected in IR64/Binam BC_2F_8 introgression lines. QTLs in rectangles mean genetic overlapping QTLs underlying ST of seedling and tillering stages.

including *QSkc1*, *QSkc3*, *QSkc6* and *QSkc11* were detected on chromosomes 1, 3, 6 and 11, and the Binam alleles at all loci were associated with increase of K^+ concentration. Two QTLs affecting SNC were identified on chromosomes 3 and 6, and the Binam allele at *QSnc3* reduced SNC whereas the IR64 allele at *QSnc6* decreased SNC.

2.4 QTL mapping of ST-related traits at the tillering stage

Six QTLs for PH were detected on chromosomes 1, 4, 5,and 7–9, including 4 QTLs detected under the

non-stress condition, 4 under salt stress and 3 by trait difference between the stress and control conditions (Table 4 and Figure 1). These QTLs could be classified into three types based on their differential behaviors. Type I included *QPh7* and *QPh9* which were expressed only in the control but not under stress, and the Binam alleles at the two loci increased PH. Type II QTLs included *QPh5* and *QPh8* which were detectable both in the stress and non-stress conditions, and the Binam allele at *QPh5* decreased PH but its allele at *QPh8* increased PH under the two conditions. Type III QTLs included *QPh1* and *QPh4*, which were detectable only under salt stress, suggesting they were apparently induced by the stress. At the former locus, the Binam allele increased PH while at the latter locus IR64 allele increased PH. Three QTLs (*QPh1*, *QPh4* and *QPh9*) which contributed to PH differences of the ILs between salt stress and non-stress conditions were identified, and the Binam alleles at all loci except *QPh4* reduced the trait difference, or improved trait stability under salt stress.

Seven QTLs affecting TN were identified and

Table 4 QTLs affecting salt tolerance-related traits detected in IR64/Binam BC_2F_8 introgression lines at the tillering stage

Trait	QTL	Chromosome	Marker interval[a)]	Parameter	Control[b)]	Stress	Difference
PH	*QPh1*	1	RM243－RM600	*F* value		10.4	4.27
				A		5	–1.85
	QPh4	4	RM142－RM273	*F* value		4.45	9.28
				A		–2.12	4.58
	QPh5	5	RM122－RM13	*F* value	7.06	5.6	
				A	–2.43	–2.37	
	QPh7	7	RM172－RM248	*F* value	8.78		
				A	2.87		
	QPh8	8	RM223－RM210	*F* value	13.75	7.29	
				A	5.56	4.53	
	QPh9	9	RM105－RM409	*F* value	4.65		7.26
				A	2.44		–2.25
TN	*QTn1*	1	RM562－RM9	*F* value	7.01		5.8
				A	0.98		–1.04
	QTn2	2	RM71－RM324	*F* value	7.49		7.31
				A	–0.95		4.26
	QTn5	5	RM122－RM13	*F* value	9.39		
				A	0.84		
	QTn6	6	RM528－RM30	*F* value		7.6	
				A		3.94	
	QTn8	8	RM339－RM42	*F* value	7.49		
				A	1.35		
	QTn9	9	RM409－RM566	*F* value	8.45	7.67	
				A	–1.1	–1.13	
	QTn10	10	RM222－RM239	*F* value		4.35	9.38
				A		0.64	–3.38
FW	*QFw1*	1	RM575－RM259	*F* value	13.53	9.8	6.91
				A	11.26	17.58	–11.39
	QFw2a	2	RM71－RM324	*F* value	7.07		7.4
				A	–10.04		13.34
	QFw2b	2	RM318－RM530	*F* value		5.1	7.43
				A		3.56	–10.74
	QFw4	4	RM335－RM261	*F* value	4.04	9.24	
				A	–12.99	–7.76	
	QFw7	7	RM10－RM234	*F* value	9.33		4.63
				A	14.91		–15.36
	QFw8	8	RM38－RM25	*F* value	10.07		8.75
				A	9.44		–11.91
	QFw10	10	RM239－RM467	*F* value	10.36		7.3
				A	10.5		–12.29
	QFw11a	11	RM181－RM260	*F* value		8.38	4.5
				A		6.57	–9.38
	QFw11b	11	RM229－RM21	*F* value	10.21		
				A	9.46		

a) The underlined markers are those closer to the true QTL positions; b) the underlined numbers indicate that these marker-trait associations were detected at the subthresholds of $0.005<P<0.05$.

mapped to chromosomes 1, 2, 5, 6 and 8－10, including five QTLs detected under the control, three under the stress and three by trait difference of stress and non-stress conditions (Table 4 and Figure 1). These included *QTn1*, *QTn2*, *QTn5* and *QTn8* of type I detected under the control condition. The Binam alleles at all loci except *QTn2* were associated with increased TN. *QTn9* was associated with Type II which could be detected both in the stress and non-stress conditions, and the Binam allele at this locus decreased TN in both conditions. Type III included 2 QTLs (*QTn6* and *QTn10*) which were induced only by the stress, and the Binam alleles at these two loci increased TN. In addition, 3 QTLs (*QTn1*, *QTn2* and *QTn10*) which contributed to TN differences between the stress and non-stress conditions were also detected, and the Binam alleles at all loci except *QTn2* reduced the trait difference.

A total of nine QTLs for FW were identified and mapped on chromosomes 1, 2, 4, 7, 8, 10 and 11, including 7 QTLs detected under the non-stress condition, 4 under the stress, and 7 by FW differences between the stress and non-stress conditions (Table 4 and Figure 1). Type I QTL included *QFw2a*, *QFw7*, *QFw8*, *QFw10* and *QFw11b* which were expressed only under the control, and the Binam alleles at all loci except *QFw2a* increased FW. *QFw1* and *QFw4* belonged to Type II which was detected both under the stress and non-stress conditions, and the Binam allele at the former locus increased FW while its allele at the latter locus decreased FW under the two conditions. Type III QTL included *QFw2b* and *QFw11a* which were induced by the stress, and the Binam alleles at these two loci increased FW. Seven QTLs contributing to TN difference between the stress and non-stress conditions were identified, and Binam alleles at all loci except *QFw2a* reduced the trait difference between stress and non-stress conditions.

3 Discussion

3.1 ST QTLs at the tillering stage and their genetic overlap with those at the seedling stage

ST of rice is a complicated quantitative trait. PH, TN and FW are much sensitive to salt stress and belong to ST-related traits, which are easy to measure, but their phenotypic values are easily influenced by environmental factors. In the present study, ST-related traits at the tillering stage were investigated in the adjacent cement pools, in which the soil and fertilizer conditions were completely the same except salt water in the treatment group and fresh water in the control group. Moreover, environmental effects on phenotyping were to the maximum extent eliminated by setting replications and planting border lines.

Most QTLs affecting PH, TN and FW detected at the tillering stage showed obvious differential expression to salt stress. Of the 22 QTLs detected in this study, 11 were expressed only under the control, six were induced and expressed under the stress, and five (*QPh5*, *QPh8*, *QTn9*, *QFw1* and *QFw4*) were expressed under both the two conditions with the same direction of gene effect (Table 4 and Figure 1). Among the 13 QTLs detected by trait difference of the stress to the control, the alleles at all loci except *QPh4*, *QTn2* and *QFw2a* which decreased trait difference were associated with Binam, indicating Binam alleles at the most loci can improve trait stability and then increase ST under salt stress. Although QTLs induced only by salt stress may be associated with the mechanism(s) of rice stress response, they may not necessarily contribute to ST. We believe that those QTLs that can reduce trait difference between stress and non-stress conditions should have contributed to ST because of their obvious contribution to trait stability. Therefore, QTLs which contributed to ST were from two types: one included 13 QTLs affecting trait difference as mentioned above, and the other included three QTLs including *QPh5* and *QPh8* for PH and *QTn9* for TN which had similar quantity and the same direction of gene effect under the stress and control conditions, and the IR64 alleles at all loci except *QPh8* increased ST.

Gene expression is specific to the developmental stage[27], so dynamic QTL analysis across different stages will help us to understand gene expression[28]. From the results of correlation analysis, there was no significant correlation of ST-related traits between seedling and tillering stages under the stress condition (Table 2), but QTL analysis indicated that QTLs affecting ST both at the seedling and tillering stages were detected in the four same or neighboring genome regions, including the region of RM562－RM9 on chromosome 1, which affected SKC of the seedling stage and TN of the tillering stage, the region of RM318－RM530 on chromosome 2 affecting FW of the tillering stage and the adjacent region of RM530－RM250 affecting SDS of the seedling stage, the region of RM38－RM25 on chromosome 8 affecting SST and SDS of the seedling stage and FW of the tillering stage, and the region of

RM120－RM181 on chromosome 11 affecting SKC of the seedling stage and FW of the tillering stage. These QTLs affecting ST at the seedling and tillering stages located in the same or adjacent regions and might be identical or closely linked, indicating partial genetic overlap of ST exists between the seedling and tillering stages. Most QTLs (69%) affecting ST at the two stages were genetically independent. But with some degree of genetic overlap between ST at the two stages and with offset of phenotypic effect which results from inconsistent directions of gene effects at some loci, no significant correlations of ST-related traits were detected between the seedling and tillering stages.

3.2 Comparison of ST QTLs across different populations

Comparison of QTLs affecting ST at the seedling and tillering stages detected in this study with those reported in previous studies was performed by the same SSR markers and comparative linkage maps[23,29,30]. The results showed some QTLs detected located in the same or adjacent regions with QTLs previously reported. For instance, *QPh1* with the flanking markers of RM243 and RM600 on chromosome 1 which affected PH of tillering stage was mapped in the same region with *qSKC-1* for K^+ concentration in the shoots[15], major ST gene *Std*[12], salt responsive cDNA clone *TS1*[31] and QTLs for K^+ and Na^+/K^+[16]. *QFw2b* located in RM318－RM530 on chromosome 2 which affected FW of the tillering stage and *QSds2b* located in the adjacent region of RM530－RM250 which affected SDS of the seedling stage were mapped together with the QTLs affecting tiller number under salt stress[20], K^+ and Na^+ concentration, and SDS[19]. *QSkc3* located in the region of RM81B－RM22 on chromosome 3 which affected SKC of the seedling stage and *QSst3*, *QSds3* and *QSnc3* located in the adjacent region of RM231－RM175 which influenced SST, SDS and SNC, respectively, were mapped together with salt responsive cDNA clone *TS2*[31], *qSV-3* for seedling vigor under salt stress[14], QTLs for K^+, SST and SDS[19], and root weight QTL[32]. *QFw7* located in the region of RM10－RM234 on chromosome 7 which affected FW of the tillering stage was mapped together with salt responsive cDNA clone *TS3*[31], and *qSGM-7* affecting seed germination under salt stress[14], one major gene linked to RFLP marker RG4[17]. The QTL regions for the related traits mentioned above that were detected in different mapping populations and various environments were significant genomic regions for ST of rice. This will play an important role in MAS for development of ST variety.

3.3 Implication in rice breeding for ST

Binam, a landrace introduced from Iran, is suitable to grow under drought and saline conditions in the Middle East Area[33]. Although its ST is not ideal under the experimental condition, it possesses more favorable alleles to increase ST as compared with IR64. Actually, this is a common phenomenon in most germplasms. Many germplasms take some favorable genes affecting the target traits such as high yield[34,35] and ST[36], etc., although the overall agronomic traits of the germplasms look poor. As to this kind of favorable genes 'hidden' in germplasms, it is an effective way to mine them by developing ILs combined with molecular marker technologies[37－39].

Rice is most susceptible to salt stress at the 2－3 leaf stage. So ST of the seedling stage is very important for rice to normally grow under the salt stress condition. In this study, two important regions, RM231－RM175 on chromosome 3 and RM38－RM25 on chromosome 8, affected SST and SDS, respectively, and their favorable alleles for increased ST were from the donor parent, Binam. It is, therefore likely to improve the ST level of the seedling stage by pyramiding these two non-allelic ST QTLs via MAS. Some important QTL regions affecting ST of tillering stage were identified, including RM575－RM600 on chromosome 1 which influenced PH and FW, and RM71－RM324 on chromosome 2 which affected TN and FW, and so on. Because most ST QTLs of the seedling and tillering stages were genetically independent, it is possible to develop ST rice variety for both stages by accumulating important QTLs of the different stages as mentioned above or selection against the overlapping QTLs of the two stages through MAS.

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