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Dynamic QTL analysis of the Na^+ content, K^+ content, and Na^+/K^+ ratio in rice roots during the field growth under salt stress

J. SUN[1], D.T. ZOU[1]*, F.S. LUAN[2]*, H.W. ZHAO[1], J.G. WANG[1], H.L. LIU[1], D.W. XIE[1], D.Q. SU[3], J. MA[1], and Z.L. LIU[1]

College of Agriculture, Northeast Agricultural University, Harbin 150030, P.R. China[1]
College of Horticulture, Northeast Agricultural University, Harbin 150030, P.R. China[2]
Seed Supervise Bureau of Heilongjiang Province, Harbin 150030, P.R. China[3]

Abstract

Rice (*Oryza sativa* L.) is seriously impacted by global soil salinization. To determine the quantitative trait loci (QTLs) related to salt tolerance in rice roots, $F_{2:3}$ and $BC_1F_{2:3}$ populations derived from a cross between the cv. Dongnong 425 of high quality and yield and the salt-tolerant cv. Changbai 10, were studied at different development stages. Two genetic linkage maps of $F_{2:3}$ and $BC_1F_{2:3}$ populations were constructed. A 66 mM NaCl solution was used to irrigate the field and to analyze the dynamic QTL of some rice root traits. Using unconditional and conditional QTL mapping methods, 30 unconditional QTLs and 16 conditional QTLs related to the 6 root traits were detected on the 9 rice chromosomes during different developmental stages. Fourteen pairs of unconditional and conditional QTLs were detected at the identical developmental stage in the identical population. A number of QTLs were detected at different developmental stages, however, many did not appear at the last stage. Remarkably, qRKC1 appeared continuously at multiple stages in both the populations suggesting its key role in regulating the salt tolerance of rice roots.

Additional key words: developmental stages, genetic linkage map, *Oryza sativa*.

Introduction

Na^+ is beneficial in trace amounts for most plants, however, the excess of Na^+ is toxic. Excessive Na^+ restrains the absorption of K^+ and other minerals which leads to an ion imbalance and series of physiological alterations that seriously affect the growth and development of plants and even lead to death (Munns 2005, Munns and Tester 2008). Intracellular Na^+ and K^+ homeostasis plays a vital role in the growth and development of higher plants (Clarkson and Hanson 1980). A low Na^+ content and a low Na^+/K^+ ratio are usually beneficial for plant cells (Barakat *et al.* 2013, Saleh *et al.* 2014). The root is the gate for the ion absorption by plants. The plant root has a remarkable ability to control its Na^+ and K^+ content and to regulate their uptake and transport to the shoot (Munns 2005). However, this ability is variable in different plant species and cultivars, and the ability to maintain a low Na^+/K^+ ratio is a key feature of the salt tolerance (Ahmadi *et al.* 2011).

Rice is a staple food crop for more than a half of the world’s population. However, this crop is salt susceptible (Shankhdhar *et al.* 2000) and salinity decreases its yield up to 30 % (Wu and Garg 2003). The breeding of salt-tolerant cultivars is an effective method to develop rice able to growth in saline land (Moniruzzaman *et al.* 2012). Many quantitative trait loci (QTLs) that are associated with salt-tolerance have been reported in rice (Gregorio *et al.* 2002), including the rate of emergence (Prasad *et al.* 2000, Wang *et al.* 2011), Na^+ and K^+ content in stems and leaves of seedlings (Lin *et al.* 2004, Thomson *et al.* 2010, Wang *et al.* 2012b), the salt damage in seedlings (Yao *et al.* 2005, Lee *et al.* 2007, Sabouri *et al.* 2009), the height of seedlings (Kim *et al.* 2009, Wang *et al.* 2012a), and the survival ability of seedlings (Prasad *et al.* 2000,

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Abbreviations: CTAB - cetyltrimethylammonium bromide; QTLs - quantitative trait loci.

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* Corresponding authors; fax: (+86) 451 55190447, e-mail: zoudt@163.com; fax: (+86) 451 55190243, e-mail: luanfeishi@sina.com.

Thomson *et al.* 2010). Previous studies concerning QTLs for salt-tolerance have primarily focused on the rice seedling stage, and fewer studies concerning QTLs for salt-tolerance in the rice field growth stage have been reported. One study has shown that the salt-tolerance in rice seedlings has little connection with that in the field grown adult rice (Chai *et al.* 2013), whereas the salt-tolerance in the field grown rice has a high influence on the rice yield (Zaidem *et al.* 2004). Therefore, the studies concerning mapping the QTLs related to salt-tolerance in the field grown rice are required.

The expression of a quantitative trait gene is specific during individual developmental stages, and gene expression at different developmental stages is diverse (Bradshaw and Stettler 1995). The above mentioned studies concerning rice salt-tolerance mostly ignored distinct gene expression at different developmental stages. Recently, dynamic QTL mapping has been used to dissect the genetic architecture of important quantitative traits in rice, such as plant height (Yan *et al.* 1998), root morphology (Qu *et al.* 2008), tiller number (Liu *et al.* 2010), protein content (Zheng *et al.* 2011), *etc.* Using this method, the net effect of a QTL that is expressed at each time interval from time (t - 1) to time t can be estimated, and dynamic gene expression for the development of quantitative traits may be revealed (Zhu 1995, Atchley and Zhu 1997).

In this study, two rice populations ($F_{2:3}$ and $BC_1F_{2:3}$) were used to detect QTLs for salt-tolerance in the field grown rice using conditional and unconditional QTL mapping. The objective of our study was to investigate the developmental changes in the absorption and distribution of Na^+ and K^+ under a salt stress and to identify both unconditional and conditional QTLs in different genetic populations and in various developmental stages.

Materials and methods

Plants and treatments: The two *Oryza sativa* L. populations for the QTL analysis were composed of 180 $F_{2:3}$ and 118 $BC_1F_{2:3}$ plants which were derived from a Dongnong 425 × Changbai 10 cross. The cv. Dongnong 425 is a *japonica* variety of high quality and yield that was obtained from the Northeast Agriculture University and is widely cultivated in the northeast region of China. The cv. Changbai 10 is also from the *japonica* variety and is salt tolerant and so widely cultivated in saline-alkali soils in the Jilin Province of China.

Field experiments were performed on the experimental farm of the Northeast Agricultural University, Harbin, China, in 2012. The parents, and $F_{2:3}$ and $BC_1F_{2:3}$ populations were grown in a randomized block design with three replications of single row plots, with a single plant per hill (2 m row length, 30 cm row spacing, and 10 cm hill spacing). A salt stress was performed after the rice turned green. The rice was irrigated with a 66 mM NaCl solution and a control was irrigated with water. The concentration of the NaCl solution was monitored in the morning, noon, and evening using a conductivity meter (*DDB-303A*, *Shanghai Precise Instrument Company*, Shanghai, China). Water was drained off and irrigated anew after rain to ensure the stability of the concentration of NaCl.

Determination of the root Na^+ and K^+ content: Sampling was done from June 22 to July 20 at five developmental stages: initial tillering (t1), advanced tillering (t2), elongation (t3), booting (t4), and heading (t5). After sampling, the root was cut, washed with distilled water and then wiped dry. The clean roots were put in an oven at 105 °C for 30 min and then dried at 80 °C until their mass was stable. The dried samples were ground to powder and sieved using 100 mesh sieves. The samples (0.1 g of each plant) were put in test tubes with boiling water for 2 h. Then, water was added to reach a total volume of 10 cm^3, precipitated for 6 h, and the supernatant was used to determine the Na^+ and K^+ content (Matsushita and Matoh 1991) using a flame photometer (*M410*, Sherwood Scientific, Cambridge, UK). The traits that were used for QTL mapping were as follows: the root Na^+ content (RNC), root K^+ content (RKC), root Na^+/K^+ ratio (RN/K), root relative Na^+ content (RRNC), root relative K^+ content (RRKC), and root relative Na^+/K^+ ratio (RRN/K). Relative trait value = stressed trait value/controlled trait value. The basic statistical analysis was implemented by the *SPSS16.0* software (*SPSS Inc.*, Chicago, IL, USA), and the traits of the parents were compared by the Student's *t*-test at the 5 and 1 % levels of probability. Averaged phenotypic data for the traits over the three replications were used for QTL analysis.

Genetic linkage map construction and QTL mapping: DNA of the parents and individual F_2 and BC_1F_2 plants was isolated using the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980). PCR was performed using the procedure of Chen *et al.* (1997), and the PCR products were then separated on a 6 % (m/v) polyacrylamide gel. The SSR linkage maps of the F_2 and BC_1F_2 populations were constructed using the MAP function in the software *QTL IciMapping 3.0* (Wang *et al.* 2010), and the genetic distances [cM] were calculated from recombination values using the Kosambi's mapping function (Kosambi 1944).

Unconditional and conditional QTLs were analyzed based on the development theory that was proposed by Zhu (1995). The unconditional QTL indicates the cumulative effect of QTL from the initial time to time *t*, and the conditional QTL indicates the cumulative effects of QTL from time (t - 1) to time t. The conditional phenotypic values at time t and given phenotypic values at time (t-1) were predicted using the software *QGA Station 1.0* (http://ibi.zju.edu.cn/software/qga/). Both the unconditional and conditional QTLs were detected using the software *QTL IciMapping 3.0* with an inclusive composite interval mapping (ICIM) module. The threshold of the LOD score for declaring the presence of a significant QTL was determined by 1 000 permutations at $P < 0.05$. However, the QTL with a LOD value > 2.5 was chosen to make the QTL reported herein authentic and reliable. The QTL nomenclature followed that of McCouch *et al*. (1997).

Results

The root Na^+ content of the parents and of the two populations increased significantly after the salt stress already at the t1 stage. The peak value of the root Na^+ content appeared at the t2 stage and then it decreased gradually until reaching its minimum value at the t5 stage. This result indicates that excessive Na^+ was passively absorbed into the rice root at the beginning of the salt stress and then excreted continuously from the root. The K^+ content under the salt stress was lower than that of the control, and it decreased gradually from the t1 to the t5 stage of both the treatment and control (Tables 1 and 2).

The Na^+ content under control conditions was not significantly different in the two parents during the experiment except for the t5 stage, however, more Na^+ was absorbed by the Changbai 10 roots after the salt stress. Thus, the Na^+ content of Changbai 10 roots was higher than that of Dongnong 425 under the salt stress, and the maximum difference was in the t2 stage. With the exception of the t1 stage, the K^+ content of the Changbai 10 roots was lower than that of Dongnong 425 at all other stages under the salt stress. The Na^+/K^+ ratio in the Changbai 10 roots was higher than that in the Dongnong 425 roots at all the stages under the salt stress. The absolute values of skewness and kurtosis for most traits were less than 1 which indicate that the data for the Na^+ and K^+ content were suitable for the QTL analysis.

In total, 123 SSR markers that exhibited polymorphisms between Dongnong 425 and Changbai 10 were used to construct the genetic linkage maps of the two genetic populations. The genetic linkage maps of the F_2 and BC_1F_2 populations covered 1616.53 and 1728.05 cM, respectively, and the average distances between markers were 13.14 and 14.05 cM, respectively. The order of most markers was consistent with the International Rice Genome Sequencing Project (IRGSP 2005).

In total, 30 unconditional and 16 conditional QTLs for the root Na^+ content, the K^+ content, and the Na^+/K^+ ratio were detected (Fig. 1 and Tables 1, 2, 3 Suppl.). Six

Table 1. The Na^+ content, the K^+ content, and the Na^+/K^+ ratio in rice roots of parents, and $F_{2:3}$ and $BC_1F_{2:3}$ populations at different developmental stages under the salt stress. Means ± SE ($F_{2:3}$ population $n = 180$, $BC_1F_{2:3}$ population $n = 118$, replications $r = 3$; *,** significant at the level of probability 5 and 1 %, respectively, between parents according to Student's t-test; skew. - skewness, kurt. - kurtosis).

Traits	Stage	Dongnong 425	Changbai 10	$F_{2:3}$ population				$BC_1F_{2:3}$ population			
				means ± SE	range	skew.	kurt.	means ± SE	range	skew.	kurt.
Na^+ content [μmol g^{-1}(d.m.)]	t1	748 ± 16.11*	826 ± 3.57	817 ± 18.07	217~1528	0.27	0.08	846 ± 22.42	209~1585	0.37	0.51
	t2	1565 ± 30.25**	2161 ± 11.52	1708 ± 39.08	374~3565	0.56	0.91	2123 ± 41.23	826~3211	0.03	-0.25
	t3	1011 ± 15.60*	1593 ± 104.10	1288 ± 20.39	472~2254	-0.09	1.05	1693 ± 21.27	617~1820	-0.33	0.49
	t4	1198 ± 11.05**	1389 ± 25.33	1151 ± 19.67	172~1796	-0.40	0.69	1163 ± 29.57	296~2078	0.01	0.55
	t5	1015 ± 36.14	965 ± 20.69	1023 ± 25.50	93~2067	0.34	-0.10	1031 ± 31.64	404~2165	0.66	0.62
K^+ content [μmol g^{-1}(d.m.)]	t1	383 ± 7.69	399 ± 13.67	388 ± 11.39	47~1218	0.31	1.85	350 ± 16.58	171~1077	0.60	0.50
	t2	359 ± 22.18**	315 ± 6.22	274 ± 9.08	71~1242	0.99	1.27	318 ± 9.17	76~621	0.40	0.28
	t3	350 ± 15.27	342 ± 16.30	360 ± 12.38	67~1150	0.45	0.61	267 ± 14.75	35~885	0.27	0.75
	t4	356 ± 29.69*	253 ± 16.11	219 ± 5.53	56~477	0.30	0.51	308 ± 10.99	74~883	0.31	1.02
	t5	226 ± 3.20*	149 ± 11.45	197 ± 6.35	62~758	1.18	2.58	159 ± 10.05	81~1049	0.63	0.97
Na^+/K^+ ratio	t1	1.95 ± 0.12	2.07 ± 0.25	2.27 ± 0.05	0.58~4.58	0.69	0.37	2.42 ± 0.05	0.63~4.32	1.03	1.86
	t2	4.36 ± 0.53**	6.86 ± 0.17	7.34 ± 0.30	0.30~28.52	0.93	1.79	7.33 ± 0.25	1.38~17.52	0.53	1.26
	t3	2.89 ± 0.39**	4.66 ± 0.62	4.19 ± 0.14	0.95~15.88	0.48	0.88	6.34 ± 0.58	1.36~7.90	0.31	0.95
	t4	3.37 ± 0.20*	5.49 ± 0.86	5.76 ± 0.17	2.42~27.13	0.68	0.92	5.07 ± 0.11	1.29~9.30	0.43	0.90
	t5	4.49 ± 0.21*	6.48 ± 0.30	5.64 ± 0.16	1.00~15.49	0.25	1.37	6.49 ± 0.18	1.38~13.95	0.57	0.91

Table 2. The Na^+ content, the K^+ content, and the Na^+/K^+ ratio in rice roots of parents, and $F_{2:3}$ and $BC_1F_{2:3}$ populations at different developmental stages under control conditions. Means ± SE ($F_{2:3}$ population $n = 180$, $BC_1F_{2:3}$ population $n = 118$, replications $r = 3$; *,** significant at the level of probability 5 and 1 % between parents according to Student's t-test; skew. - skewness, kurt. - kurtosis)

Traits	Stage	Dongnong 425	Changbai 10	$F_{2:3}$ population				$BC_1F_{2:3}$ population			
				means ± SE	range	skew.	kurt.	means ± SE	range	skew.	kurt.
Na^+ content [μmol g^{-1}(d.m.)]	t1	609 ± 10.39	672 ± 26.37	674 ± 17.47	176~1533	0.82	0.90	584 ± 18.47	87~1276	0.50	0.36
	t2	759 ± 30.02	793 ± 22.54	714 ± 16.77	170~1426	0.17	0.45	734 ± 19.11	170~1298	-0.46	0.25
	t3	809 ± 6.84	596 ± 11.25	737 ± 16.31	304~1461	0.66	0.49	594 ± 19.79	33~1165	0.04	0.15
	t4	628 ± 17.34	652 ± 32.18	616 ± 17.03	15~1585	0.53	0.95	693 ± 19.90	172~1182	-0.05	-0.82
	t5	600 ± 15.44*	376 ± 6.00	598 ± 14.32	50~1193	0.29	0.30	586 ± 21.73	68~1215	-0.05	2.63
K^+ content [μmol g^{-1}(d.m.)]	t1	597 ± 33.93	624 ± 25.41	730 ± 18.25	213~1436	0.40	-0.21	631 ± 18.86	74~1228	0.45	0.58
	t2	524 ± 6.65	556 ± 11.19	556 ± 14.73	103~1158	0.41	0.29	602 ± 16.59	182~1121	0.19	0.33
	t3	435 ± 15.88	367 ± 27.53	445 ± 14.96	127~1331	0.58	0.31	424 ± 21.11	67~1182	0.57	0.25
	t4	427 ± 28.86*	541 ± 13.04	378 ± 10.82	36~941	0.49	0.81	481 ± 14.55	118~1140	0.12	1.11
	t5	286 ± 12.92	244 ± 6.01	249 ± 11.27	38~1305	1.24	1.64	272 ± 14.09	41~929	1.05	2.40
Na^+/K^+ ratio	t1	1.02 ± 0.25	1.56 ± 0.13	0.97 ± 0.02	0.45~2.18	0.91	1.06	0.97 ± 0.06	0.34~2.38	0.87	1.50
	t2	1.45 ± 0.24	1.43 ± 0.03	1.38 ± 0.04	0.49~3.78	0.87	1.79	1.27 ± 0.12	0.44~2.73	0.03	0.75
	t3	1.86 ± 0.25**	1.62 ± 0.39	1.87 ± 0.05	0.23~5.16	0.38	0.79	1.64 ± 0.03	0.03~3.55	0.52	0.26
	t4	1.47 ± 0.08*	1.21 ± 0.09	1.65 ± 0.05	0.04~8.71	1.04	1.90	1.55 ± 0.08	0.38~3.78	0.31	0.37
	t5	2.10 ± 0.22	1.54 ± 0.09	2.79 ± 0.11	0.18~15.6	0.62	0.86	2.64 ± 0.10	0.03~12.86	1.10	2.31

unconditional QTLs (qRNC1, qRNC1-2, qRNC3, qRNC5, qRNC8-2, and qRNC8-3) for the root Na^+ content under the salt stress were detected in the $F_{2:3}$ and $BC_1F_{2:3}$ populations, and five unconditional QTLs (qRRNC1, qRRNC7, qRRNC8, qRRNC10, and qRRNC12) for the root relative Na^+ content under the salt stress. Three conditional QTLs (qRNC8-1, qRNC8-2, and qRNC8-3) for the root Na^+ content were detected in the two populations, and only one conditional QTL (qRRNC8) for the root relative Na^+ content in the $F_{2:3}$ population.

There were no unconditional or conditional QTLs for the root Na^+ content that were detected at the t1 stage in either the $F_{2:3}$ or the $BC_1F_{2:3}$ population. This result may be connected with a lower accumulation of Na^+ in the rice roots at the t1 stage, and the QTL that controls the Na^+ content was not completely expressed yet. Only one unconditional QTL (qRRNC8) with a smaller phenotypic variation explained (PVE) for the root relative Na^+ content was detected at the t1 stage, and neither unconditional nor conditional QTLs for the root relative Na^+ content were detected at the t5 stage. However, two unconditional QTLs (qRRNC1 and qRRNC7) with a large PVE (14.29 and 18.24 %) were detected at the t2 stage. This result is consistent with the fact that the root Na^+ content under the salt stress rose rapidly at the t2 stage and then decreased gradually, but remained relatively stable in the controls.

Five unconditional QTLs (qRKC1, qRKC5, qRKC8-2, qRKC11-1, and qRKC11-2) for the root K^+ content under the salt stress were detected in the two populations. The qRKC1, which was between RM1321 and RM1360 on chromosome 1, was detected consecutively at the t1, t2, t3, and t4 stages in the $F_{2:3}$ population, and the PVE values were 13.29, 6.85, 13.96, and 11.26 %, respectively. Their additive effects had positive values, and the positive alleles (an allele increasing the trait value) originated from Changbai 10. The qRKC1 was also detected at the t3 and t4 stages in the $BC_1F_{2:3}$ population which indicated its key role for controlling the root K^+ content under the salt stress. Five unconditional QTLs (qRRKC1, qRRKC7, qRRKC8-1, qRRKC8-2, and qRRKC10-2) for the root relative K^+ content were detected in the two populations. The qRRKC7, which was a major QTL, was detected at the t1 and t2 stages in the $F_{2:3}$ population, with PVE values of 23.47 and 27.90 %, respectively, and the positive allele originated from Dongnong 425. The qRRKC8-1 was detected at the t1, t3, and t4 stages in the $F_{2:3}$ population, with PVE values of 7.26, 10.70, and 6.25 %, respectively, and the positive allele originated from Changbai 10. Three conditional QTLs (qRKC1, qRKC5, and qRKC8-1) for the root K^+ content and three conditional QTLs (qRRKC8-1, qRRKC10-1, and qRRKC10-2) for the root relative K^+ content were detected in the two populations.

Five unconditional QTLs for the root Na^+/K^+ ratio were detected in the two populations at different developmental stages and localized on chromosomes 1, 4, and 8. The qRNa/K1-1 with the largest PVE were detected at the t1 (18.55 %) and t2 (17.79 %) stages in the $F_{2:3}$ population, and the positive alleles originated from Changbai 10. The qRNa/K1-2 was detected at the t1 (PVE 14.95 %) and t4 (PVE 15.47 %) stages in the $F_{2:3}$ population, and the positive alleles again originated from

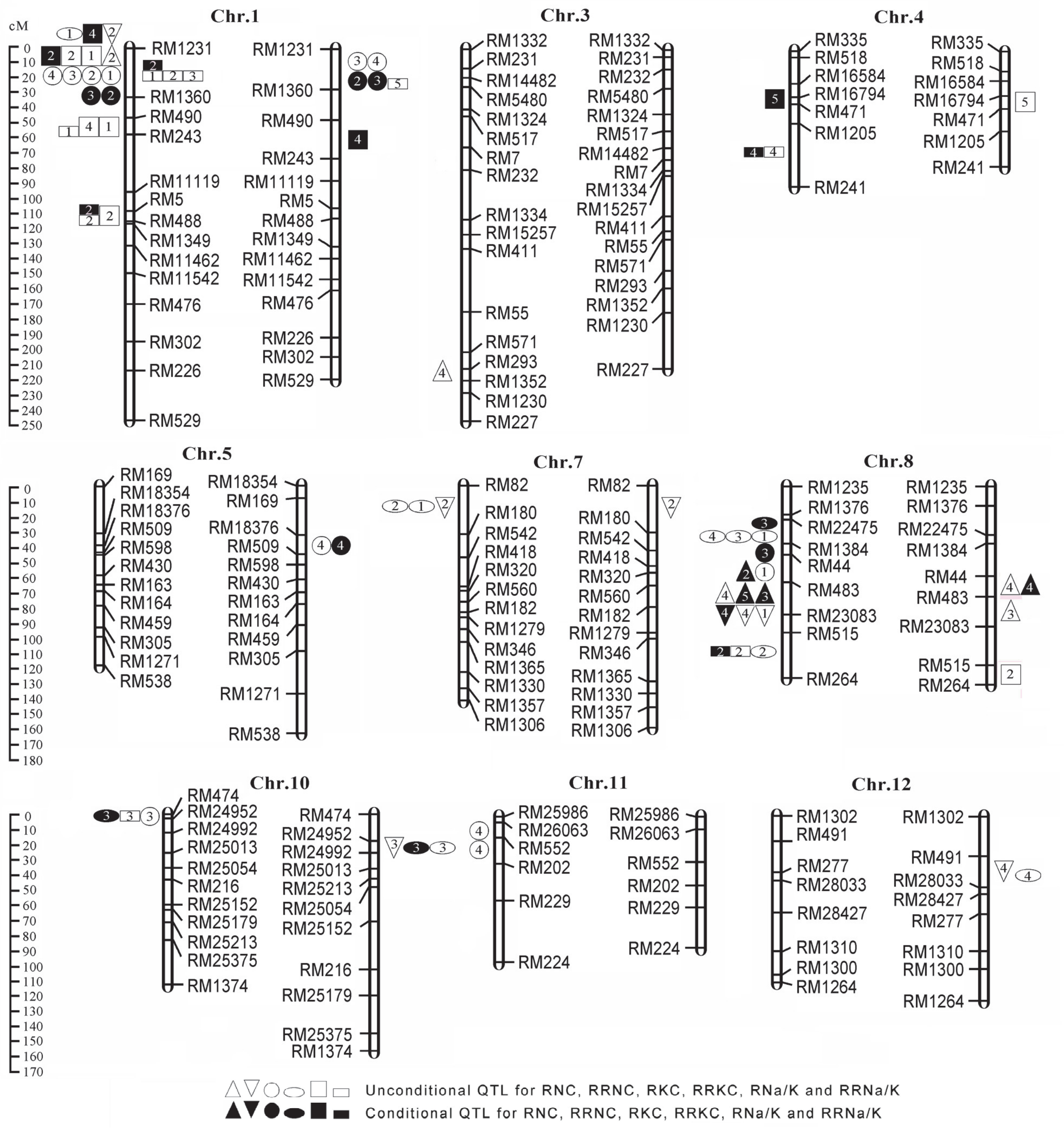

Fig. 1. QTLs of the Na^+ content, K^+ content, and Na^+/K^+ ratio in rice roots under the salt stress. The QTLs were located on rice chromosomes using the $F_{2:3}$ and $BC_1F_{2:3}$ populations. *On the left* of each chromosome is the linkage map that was constructed using the $F_{2\ 3}$ population and *on the right* is the linkage map that was constructed using the $BC_1F_{2\ 3}$ population. Different patterns represent the QTLs of various traits that were mapped using unconditional or conditional methods, and the number in each pattern represents various developmental stages.

Changbai 10. Five unconditional QTLs (qRRNa/K1-1, qRRNa/K1-2, qRRNa/K4, qRRNa/K8, and qRRNa/K10) for the root relative Na^+/K^+ ratio were detected in the two populations. The qRRNa/K1-1 was detected continuously at the t1 (PVE 18.99 %), t2 (PVE 12.20 %), and t3 (PVE 9.05 %) stages in the $F_{2:3}$ population, and the positive alleles originated from both Dongnong 425 and Changbai 10 which indicates its persistent and stable expression under the salt stress (Fig. 1 and Table 3 Suppl.).

Three conditional QTLs (qRNa/K1-1, qRNa/K1-2, and qRNa/K4) for the root Na^+/K^+ ratio were detected in the two populations. The qRNa/K1-1 was detected at the t1 and t2 (PVE 10.37 %) and also at t3 and t4 (PVE 6.47 %) stages in the $F_{2:3}$ population, and the positive alleles originated from Dongnong 425 and Changbai 10, respectively. Only one QTL with a small PVE value (qRNa/K1-2, PVE 4.19 %) was detected in the $BC_1F_{2:3}$ population. Four conditional QTLs (qRRNa/K1-1, qRRNa/K1-2, qRRNa/K4, and qRRNa/K8) for the root relative Na^+/K^+ ratio were detected in the $F_{2:3}$ population, however, no QTLs were detected in the $BC_1F_{2:3}$ population.

Discussion

There is a close correlation between the Na^+ allocation in different rice organs under salt stress and the salt-tolerance of a certain rice cultivar. In salt-tolerant cultivars, the root/shoot Na^+ ratio is higher due to restriction of Na^+ transport to shoot under salt stress, whereas the shoots of salt-sensitive rice cultivar has greater Na^+ accumulation. The restriction of Na^+ transport to the shoot is important for the normal growth of the plant under salt stress. Maintaining the intracellular equilibrium of Na^+ and K^+ is important for the normal physiological metabolism of rice. The intrinsic equilibrium system of Na^+ and K^+ is destroyed by excessive Na^+, which is absorbed by rice after salt stress. This excess can lead to variations in the Na^+/K^+ ratio; therefore, the Na^+/K^+ ratio is a key index for measuring the salt tolerance of rice. The result of this study show that a considerable amount of Na^+ that was absorbed by the salt-tolerant cv. Changbai 10 under the salt stress conditions but it was stored in the roots, which might be a reason for the higher salt-tolerance of Changbai 10 than that of Dongnong 425.

Since the conditional QTL analysis was introduced by Zhu (1995), a dynamic QTL mapping strategy, which is based on unconditional and conditional QTL analyses, has generally been utilized in various crops (Han *et al.* 2011, Li *et al.* 2012, Zhang *et al.* 2013). In this study, we used unconditional and conditional QTL analyses to map dynamic QTLs that are associated with six traits in rice roots. Unconditional and conditional QTLs were detected for all the traits, however, some QTLs could be detected only using the unconditional method, such as qRNC1-1, qRNC1-2, qRNC5, *etc.*, and some QTLs could be detected only using the conditional method, such as qRNC8-1, qRKC8-1, qRRKC10-1, *etc.* The above results show that the unconditional QTL analysis method could only detect the QTLs that had a cumulative effect from the initiation of the experiment to the measured moment, and more QTLs could be detected using the conjoined strategy of unconditional and conditional QTLs. Thus, not only the final result of QTL expression but also the concrete time interval and the expression from time t0 to time t could be identified.

In this study, 14 pairs of unconditional and conditional QTLs appeared at the same stage in the same population indicating that these QTLs had a cumulative and net effect simultaneously. For example, unconditional and conditional QTLs of qRNC8-2 were detected simultaneously at the t4 stage in the $BC_1F_{2:3}$ population. The unconditional QTL was the accumulation of a gene from sowing to the t4 stage, and the conditional QTL was the net effect from the t3 to t4 stage. The additive effect of unconditional QTL qRNC8-2 was 10.20 and of the conditional QTL was 6.09 which indicated that qRNC8-2 was continuously expressed from sowing to the t4 stage, and the conditional QTL is only a net effect at one stage of its continuous expression. Similar QTLs were qRKC1, qRNa/K1-1, and qRRNa/K8 at the t2 stage, qRKC1 and qRRKC8-1 at the t3 stage, and qRRNC8, qRKC5, and qRRNa/K4 at the t4 stage. Unconditional and conditional QTLs of qRRKC10-2 were detected simultaneously at the t4 stage in the $BC_1F_{2:3}$ population, however, their effects were proximal, at 0.67 and 0.65, respectively. These results indicate that the effect of qRRKC10-2 was primarily the net effect of the conditional QTL at the t2→t3 stage; thus, the expression of qRRKC10-2 detected at the t3 stage was new, and was not the continuous expression from the t0 stage. Similar QTLs were qRRNa/K1-1 and qRRNa/K1-2 at the t2 stage. Therefore, the expression and interaction of QTLs at each stage can be clear-cut, and the molecular mechanism can be explained by using unconditional and conditional QTL mapping methods.

Some QTLs that were detected by this study appeared simultaneously at different stages. Among these QTLs, five (qRNC8-3, qRRNC8, qRRKC7, qRNa/K1-1, and qRNa/K1-2) appeared simultaneously at two stages, and two (qRRKC8-1 and qRRNa/K1-1) appeared simultaneously at three stages. The variation in some traits, such as ion absorption and distribution in the rice roots under the salt stress, was a gradual and continuous process. Its variation was the result of a consecutive regulation by the QTLs that were expressed at different stages. However, many of the QTLs that were detected in this study only appeared in one population at one developmental stage, and no QTLs appeared simultaneously at all developmental stages. This result indicates that the expression of these QTLs varied at the different stages of the rice growth and development, and a QTL could be expressed at one stage and could be absent at other developmental stages. The QTLs of many traits that were detected at t1 did not appear at t5, *e.g.*, qRKC1, which affected the root K^+ content, was detected continuously at the t1, t2, t3, and t4 stages but it was not detected at the t5 stage. The qRRKC8-1, which affected the root relative K^+ content, was detected at the t1, t3, and t4 stages but it was not detected at the t5 stage. Therefore, a QTL analysis should consider the specific expression of a gene at different developmental stages, otherwise this method would not detect some QTLs that are expressed at the beginning of development if the analysis only considers the final phenotypic value when mapping the QTL, and the result would not comprehensively reflect the total number and the mode of action of QTLs controlling the trait.

So far, there have been few studies concerning QTL mapping for root traits under salt stress, and any studies concerning QTL mapping for the root Na^+ content, the K^+ content, and the Na^+/K^+ ratio in field grown rice have not been reported. Yao *et al.* (2005) reported two QTLs that affect the root Na^+/K^+ ratio under salt stress in rice seedlings; however, these QTLs were not distributed on the same chromosome as the QTLs that were detected in this study. Wang *et al.* (2012a, 2012b) reported some QTLs that affect the root Na^+ content, the K^+ content and the Na^+/K^+ ratio under salt stress in rice seedlings; however, these QTLs had a farther chromosome distance than the QTLs that were detected in this study. Thomson *et al.* (2010) reported six QTLs that affect the root K^+ content and the Na^+/K^+ ratio that are located on chromosomes 1, 2, 6 and 9, respectively. Among these QTLs, qRKC1, which affects the root K^+ content and was found in the RM1287-RM11300 interval on chromosome 1, was close to qRNa/K1-3 and qRRNa/K1-2, which affect the root Na^+/K^+ ratio and the root relative Na^+/K^+ ratio in this study; however, the K^+ content and the Na^+/K^+ ratio were not identical traits. Thus it is necessary to study QTLs for salt tolerance under the field conditions since salt tolerance in rice seedlings is different form that in the further growth stages, and the QTLs in seedlings are not completely consistent with the QTLs in the field growth stages.

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