Euphytica (2015) 201:441–452
DOI 10.1007/s10681-014-1236-8

Identification of QTLs associated with salt or alkaline tolerance at the seedling stage in rice under salt or alkaline stress

Jing-long Liang · Ying-ping Qu · Chun-gang Yang · Xiao-ding Ma · Gui-lan Cao · Zheng-wu Zhao · San-yuan Zhang · Tao Zhang · Long-zhi Han

Received: 19 February 2014 / Accepted: 5 August 2014 / Published online: 31 August 2014

Abstract The QTL analysis of dead leaf rate (DLR) and dead seedling rate (DSR) during the seedling stage under salt or alkaline stress were conducted, in order to provide the scientific basis for the fine mapping and cloning of QTLs associated with salt or alkaline tolerance, and for the salt or alkaline tolerance of SSR marker assisted rice breeding. The recombinant inbred line (RIL) population F_8 including 200 lines derived from the cross "Yiai 1 × Lishuinuo" were used in the study. The DLR and DSR of RIL and its parents were evaluated under 1.5 % NaCl of salt stress and pH8.7 to pH8.9 of alkaline stress, respectively. The results showed that DLR was a quantitative trait controlled by multiple genes, and DSR was a quantitative trait controlled by a few major genes and many other minor genes together under salt stress; DLR and DSR under alkaline stress were quantitative trait controlled by multiple genes. The genetic linkage map with 155 SSR markers which overlay the whole rice genome of 1541.5 cM and with the average distance of 9.95 cM between each two markers was constructed. Seven additive QTLs and three pairs of AA epistatic QTLs associated with DLR and DSR under salt or alkaline stress were identified, Of them, ***qDSRs8-1*** with LOD of 6.54 and observed phenotypic variance of 15.96 % under salt stress, and ***qDLRa5-3*** with LOD of 3.51 and observed phenotypic variance of 8.32 % under alkaline stress were new detected QTLs, which can be used in the breeding program in rice to get salt or alkaline tolerance rice cultivars in the future. The results also showed that excellent gene resource could be detected from any one rice germplasm; mechanisms for salt tolerance and alkaline tolerance in rice was different; additive QTLs were closely related with the resistance to salt injured in rice but epistatic effects of AA were closely related with the resistance to alkaline injured in rice.

Keywords Rice · Salt stress · Alkaline stress · Quantitative trait locus (QTL) · Simple repetitive sequence (SSR)

Jing-long Liang and Ying-ping Qu have contributed equally to this work.

J. Liang · Z. Zhao · T. Zhang
College of life science, Chongqing Normal University, Chongqing 400047, People's Republic of China
e-mail: zht2188@126.com

J. Liang · Y. Qu · X. Ma · G. Cao · L. Han (✉)
Institute of Crop Science, Chinese Academy of Agricultural Sciences/The National Key Facility for Crop Gene Resources and Genetic Improvement, NFCRI/Key Laboratory of Crop Germplasm Resources and Biotechnology, Ministry of Agriculture, Beijing 100081, People's Republic of China
e-mail: hanlongzhi@caas.cn

C. Yang · S. Zhang
Institute of Rice Research, Jilin Academy of Agricultural Science, Jilin Gongzhuling 136100, People's Republic of China

Introduction

Rice as a moderately salt sensitive plant is one of the most valuable crop in the world and showed suffer symptoms when the concentration of soluble salt in soil reached at 0.3 %, and lead to yield declines (Akbar et al. 1972; Korbe and Abdel 1974; Maas and Hoffman 1977; Grover and Pental 2003).One-third of the global population use rice as their staple food, and cultivated area of rice in China is 30 million hm^2 and its yield is more than 40 percent of the national production of alimentary crop. Salt-alkaline is one of the main abiotic stresses for agricultural production in the world. About 400–950 million hectares covered by salt-alkaline soil of the earth surface (Chandan et al. 2006). As abiotic stresses salt-alkaline soil is an important restriction factor for the reduction of rice production and growing in Asia and Africa. The trend of salt-alkaline soil was becoming worse with the global warming, improper irrigation and improper fertilization. In order to reduce the effect of salt-alkaline land to rice production, people improve soil condition with a high cost and not a through method; on the other hand they breed high salt-alkaline tolerance rice cultivars through traditional approach, which is a useful but spend time. In view of these, to understand the genetic mechanism of salt-alkaline tolerance and discover the major genes or QTL in rice and then improving superior salt-alkaline tolerance cultivar to increase the soil production would be an economic and effective method to deal with this agricultural problem.

The genetic variation of salt-alkaline tolerance of rice was very plenty, and showed different salt-alkaline tolerance at the different physiological stage (Ikehashi and Ponnamperuma 1978; Gregorio et al. 2002). According to the preceding reports, salt-alkaline tolerance of rice was a quantitative trait controlled by multiple genes, except in some mutants or transgenic strains which were controlled by single major gene (Lin et al. 2004; Qi et al. 2008; Kim et al. 2009; Tanveer et al. 2008). So, identification of QTL associated with salt-alkaline tolerance and then polymerize them to improve salt-alkaline tolerance of rice would be an effective method in the program of breeding salt-alkaline tolerance cultivars (Yeo and Flowers 1986; Flowers et al. 2000). Causse et al. (1994), Cho et al.(1998) and Mccouch et al. (1988) reported that use of molecular marker technique in mapping of rice greatly promoted studies of quantitative trait in rice. Yano et al. (1997) detected QTLs associated with heading stage and yield in rice. Kim et al. (2009) used BC_3F_5 which derived from the cross between Ilpumbyeo and Moroberekan identified 8 QTLs under controlled and salinity conditions at seedling stage, and of them two for reduction rate of dry weight, three for reduction rate of fresh weigh, two for reduction of leaf area, which were located on chromosomes 1, 6, 7, respectively. Islam et al. (2011) used F2 population which derived from the cross between BRRIdhan40 and IR61920-3B-22-2-1 identified 3 major QTL associated with salt damage scale in rice at seedling stage, and these 3 QTL located on chromosomes 1, 8, and 10 respectively. Hossein and Atefeh (2008) used $F_{2:3}$ population which derived from the cross between Tarommahalli and Khazar identified four QTLs associated root length, two QTLs for dry root weigh and three QTLs for ion exchange under salt stress. Lin et al. (2004) used $F_{2:3}$ population derived from the cross between Nona Bokra and Koshihikari detected three QTLs associated seedling survival days of rice under salt stress with the total phenotypic variation from 13.9 to 18.0 %. Jafar and Fotokian (2011) used the BILs population which derived from the cross between Tarome-Molaei and Tiqing indentified 14 QTL, and of them the ***QKr1.2*** for K^+ content was detected on chromosome 1 which with 7.8 of LOD and 30 % of the total phenotypic variation was a major QTL. All studies above show that most QTLs which were detected recently are of little phenotypic variation, and indicated that complex physiological trait as salt-alkaline tolerance may be regulated by several main genes and multiple minor genes. Otherwise, according to the classic genetic research, salt-alkaline tolerance of rice was an integrated performance of several physiological reaction which controlled by many quantitative trait loci, and also to be present epistatic effects (Flower 2004; Moeljopawiro and Ikehashi 1981; Jones 1985). The identification of major QTL will help to understand the mechanism of quantitative trait loci which only based on the single gene model, but according to the knowledge of Biochemistry and Developmental Genetic, there were would be lots of interaction among gene product (Wright, 1980). In the past few years people paid more attention to the research of epistatic loci and there were proof to show that epistatic effects would be an

important genetic mechanism for species adjusting to environment(Allard 1996; Rieseberg et al. 1996). Otherwise, some researchers indicated that QTL in plant showed spatiotemporal expression (Wu et al. 2010; Cui et al. 2011). So, to analyze spatiotemporal expression of QTL associated with salt or alkaline tolerance will give us more information of the genetic mechanism in plants than to analyze QTL at a single stage (Cui et al. 2011; Wang et al. 2010; Wu et al. 2010), and it will be very importance for understanding the mechanism of genetic basis in plants. Most of preceding studies focus on the salt tolerance of rice. There were only a few of preceding studies on the alkaline tolerance of rice (Cheng et al. 2008; Qi et al. 2009;2008). But there were no reports of using the same population to indentify QTL and epistatic loci associated with salt or alkaline tolerance and analyze the difference between them at the same time, and also few reports of spatiotemporal expression of salt or alkaline tolerance at seedling stage in rice. In view of this, RILs population derived from the cross between Yiai1 and Lishuinuo are used in this study, and traits of DLR and DSR at seedling stage would be measured at 10 and 20 days under salt stress, and trait of DLR at seedling stage would be measured at 10 to 60 days, but the trait of DSR would be measured at seedling stage at 55 days after transplanted in alkaline stress. Meanwhile, with the purpose of further understand genetic mechanism of salt or alkaline tolerance in rice, SSR Markers which different between parent strain were used for construction of genetic linkage map, and based on this map QTL and epistatic loci associated with DLR and DSR at seedling stage under salt or alkaline stress were also analyzed.

Materials and methods

Mapping population

An RILs population F_8 containing 200 lines derived from the cross between Yiai 1 (YA) and Lishuinuo (LSN), two rice varieties, and F_1 population was made in Beijing in 2001, and F_2 seeds acquired in 2002 through adding generation, and F_8 generation acquired during 2002–2005, in Beijing and Hainan through adding generation and SSD method. YA was a salt-alkaline tolerant parent and LSN was a salt-alkaline sensitive parent.

Evaluation of the salt tolerance for the mapping population and the two parents

The salt tolerance of the two parents and RILs were evaluated in the greenhouse of the Institute of Crop Science, Chinese Academy of Agricultural Sciences in 2006. The seeds of parents and RILs were soaked in water for one day at the room temperature, and transported to calorstat for accelerating germination. The budding seeds near the same derived from each line were planted in vinyl chamber, each line a row and each row for 20 strains, and the distance between rows and between hills was 5 cm × 1 cm. The salt stress with the NaCl concentration of 0 and 1.5 % was carried out at the stage of 3 leaves of the seedling, respectively, and each treatment was repeated twice. Dead leaf rate (DLR) and dead seedling rate (DSR) of 10 strains of each line was evaluated at 10 and 20 days after transplanted under salt stress, then average DLR and average DSR was accounted, and the statistic unit was the average of that 10 strains. The means were used for data analysis with the following method:

DLR (%) = (the total number of dead leaves of treated strains/the total number of leaves of treated trains) × 100

DSR (%) = (the total number of dead seedlings of treated strains/the total number of seedlings of treated trains) × 100

Evaluation of the alkaline tolerance for the mapping population and the two parents

The DLR and DSR of alkaline tolerance of the two parents and RILs were evaluated in the alkaline tolerance evaluated pool in Rice Research Institute, Jilin Academy of Agricultural Sciences. The soil in the evaluated pool is from heavy salt-alkaline area of Baicheng, Jilin Province, and its pH value was above 9.5. After running water irrigation, the pH value of the soil and water in the pool was adjusted to between 8.7 and 8.9, at 25 °C. The depth of water was kept at 3-5 cm above soil at the seedling stage of rice, 2–3 days checked the water depth level and then irrigated water into the pools so as to kept the water at the lever scale, the water in the pools were also kept still. In order to maintain the stabilization of the water pH value, the open canopy was placed 2 meter above the pools to keep off rain fall. The rice seeds were sown on April 10th, 2006 with the

method of seedlings grown on the flat nursery with dry soil in a greenhouse. These seeds were transplanted into the evaluated pools at the stage of 4 leaves. 20 seeds of each line were planted in a single row 20 cm × 10 cm apart. Each treatment was repeated twice. The DSR of 15 plants was investigated at 55 days after transplanted into the pools, and the DLR was also investigated at 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 days. The means were used for data analysis with the following method:

DLR (%) = (the total number of dead leaves of treated strains/the total number of leaves of treated trains) × 100

DSR (%) = (the total number of dead seedlings of treated strains/the total number of seedlings of treated trains) × 100

Extraction of DNA and SSR markers selection

The total genomic DNA of both parents and RILs was extracted from 4–5 leaves of the plant at the tillering stage and the concentration of the DNA was analyzed in ultraviolet spectrophotometer. Two thousand pairs of SSR primers were selected from the Website of http://www.gramene.org/microsat/microsats.txt . The SSR primers were synthesized by the Saibaisheng Company in Beijing. Among these, 155 pairs of SSR primers selected were polymorphic between the two parents. PCR reaction system as follows: 1 μl (20 ng/μl) DNA; 0.25 μl(5U/μl) Taq polymerase; 1.0 μl (2 μM)SSRprimer; 0.75 μl(2.5 mM) dNTPs; 1 μl 10 × buffer(including $Mg2^{+}$); 6.0 μl ddH_2O. PCR program as follows: 94 °C for 5 min for intial; 94 °C for 30 s; 55 °C for 30 s; 72 °C for 1 min; for 35 cycles. The PCR products were separated in 8 % polyacrylamide gel electrophoresis and visualized by the method of silver staining.

Date analysis

The genetic linkage map and QTL analysis were done by using the software of Mapermaker 3.0, and QTL detected at the critical value of 3.0.The nomenclature of QTL was done as described previously (MaCouch et al. 1997). The genetic linkage map was made as described previously (Liu and Meng 2003). The mean, range of variation, standard deviation and coefficient of variation of the date was analyzed by using the SAS 9.1 ($p < 0.05$).

Results

Phenotypic variation of DLR and DSR of both parents and population of RILs at the seedling stage under salt stress

The results of the evaluation of DLR and DSR of both parents and RILs at the seedling stage under salt stress were shown in Table 1. Under salt stress for 10 days after transplanted, the DLR of Yiai 1 was 32.90 % and the DLR of Lishuinuo was 68.19 %, but the DSR of Yiai 1 was 6.67 %, the DSR of Lishuinuo was 18.33 %. At 20 days after transplanted, the DLR of Yiai 1 was 55.93 %, and DLR of Lishuinuo was 97.87 %, but the DSR of Yiai 1 was 26.67 %, and DSR of Lishuinuo was 87.50 %, and it showed that salt tolerance of Yiai 1 was obviously stronger than Lishuinuo. The range of variation of DLR of RILs under salt stress for 10 days and 20 days were 23.4–66.5 % and 53.0–100 %, respectively; The range of variation of DSR of RILs under salt stress for 10 and 20 days were 0–34.6 % and 12.1–100 %, respectively. When the time of salt stress was increasing, the degree of salt damage was also increased, and the mean of DLR for the RILs at 10 and 20 days were 42.94 and 81.79 %, respectively, and the mean of DSR for the RILs at 10 and 20 days were 7.10 and 63.75 %, respectively.

The distribution of DLR and DSR under salt stress for RILs showed in Figs. 1 and 2, respectively. At 10 and 20 days after transplanted in the salt stress, the DLR of the RILs showed a continuous normal or near normal distribution, it is quantitative trait controlled by multiple genes. The DSR of the RILs showed a continuous distribution with 2–3 peaks and skewed to lower value under salt stress for 10, and 20 days, it is quantitative trait controlled by a few major genes and many other minor genes together.

Phenotypic variation of DLR of both parents and RILs population at the seedling stage under alkaline stress

In general, the results of the evaluation of DLR for both parents and population of RILs at the seedling stage under alkaline stress showed an increasing tendency with the time of alkaline stress increasing (Table 2). At 10–60 days after transplanted in alkaline stress, the range of variation of DLR for Lishuinuo was 18.34–58.24 %, but the range of variation of DLR for

Table 1 Variation of dead leaf rate (DLR) and dead seedling rate (DSR) at the seedling stage for RILs under salt stress

Condition	Item (%)	Parents		RILs population			
		Yiai 1	Lishuinuo	Range of variance	Mean	SD	CV (%)
Under salt stress For 10 days	DLR	32.90	68.19	23.4–66.5	42.94	8.53	19.86
	DSR	6.67	18.33	0.0–34.6	7.10	6.67	93.88
Under salt stress For 20 days	DLR	55.93	97.87	53.0–100	81.79	9.78	11.95
	DSR	26.67	87.50	12.1–100.0	63.75	18.27	28.66

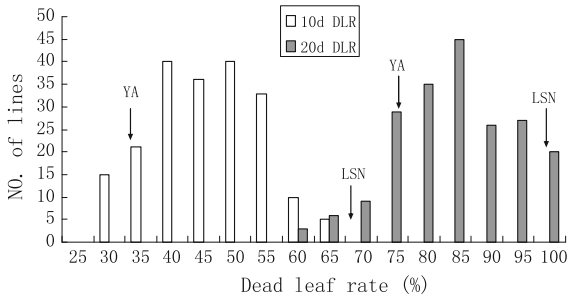

Fig. 1 Distribution of dead leaf rate under salt stress for mapping population (*YA* Yiai 1, *LSN* Lishuinuo)

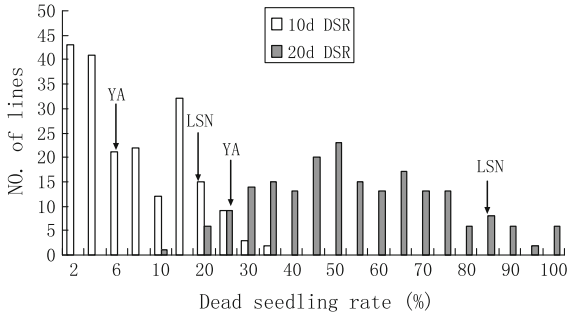

Fig. 2 Distribution of dead seedling rate under salt stress for mapping population (*YA* Yiai 1; LSN: Lishuinuo)

Yiai1 was 10.75–33.88 % and this indicated that the alkaline tolerance of Yiai1 was obviously stranger than Lishuinuo.

The tendency of the mean values (35.29–38.78 %) of DLR for RILs was increasing during the days of 10–20, but it was decreasing at the early time and increasing at the end time during the days of 25–40, and then it was increasing during the days of 45–60, after transplanted in the alkaline stress. The range of variance of the mean values for the DLR of RILs populaion was 22.76–38.78 % at the whole alkaline stress time, but the range of variance of the mean values for the DLR of RILs population was 8.87–12.4 %. According to the distribution of DLR for RILs population (Fig. 3), we known that the DLR of RILs showed a near normal continuous distribution during the days from 10 to 60 under alkaline stress, it was considered that the DLR is a quantitative trait controlled by multiple genes.

Phenotypic variation of DSR of both parents and RILs population at the seedling stage under alkaline stress

At 55 days after transplanted in alkaline stress, the DSR of Yiai 1 and Lishuinuo was 0 and 35.0 %, respectively, it showed that the alkaline tolerance of Yiai 1 was obviously stronger than Lishuinuo. The DSR of the RILs showed a large phenotypic variation with a range of variance from 0 to 88.9 %, and its means was 22.1 %, and its coefficient of variation was 110.8 %. According to the distribution of the DSR when treated 55 days under alkaline stress for RILs population (Fig. 4), it showed a continuous distribution which was secund a low value of phenotypic variation with 2–3 peaks, it indicated that the DSR was a quantitative trait controlled by a few major genes and many other minor genes together.

QTL analysis

Construction of genetic linkage map

In this study 155 SSR markers which were polymorphism between parents were used for the construction of genetic linkage map which with the average distance of 9.95 cM between each two markers was overlay the whole rice genome of 1541.5 cM (Fig. 5)

Table 2 Variation of dead leaf rate (DLR) for parents and RILs population under alkaline stress condition

Days under alkaline stress	Parents		RILs population			
	Yiai 1	Lishuinuo	Range of variation	Mean value	SD	CV (%)
10	10.75	18.34	4.55–83.33	35.29	8.87	25.13
15	20.88	34.21	17.02–100.00	37.68	10.00	26.54
20	32.29	31.82	14.81–100.00	38.78	10.94	28.22
25	27.89	30.87	8.00–100.00	29.22	12.47	42.67
30	21.27	27.50	3.72–81.48	22.76	9.38	41.21
35	17.05	22.86	6.97–82.35	24.41	11.31	46.33
40	12.65	23.68	3.35–80.00	27.87	11.21	40.22
45	14.80	27.69	4.40–50.00	25.50	10.15	39.81
50	16.21	37.87	4.26–100.00	29.13	10.94	37.57
55	26.19	41.13	4.52–87.18	34.86	11.48	32.92
60	33.88	58.24	14.77–77.42	37.25	11.34	30.43

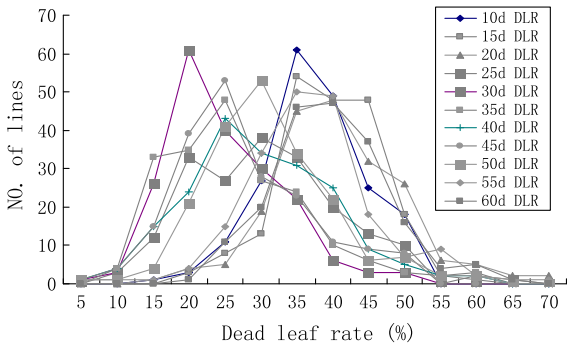

Fig. 3 Distribution of dead leaf rate for RILs population during the days of 10–60 after transplanted into alkaline stress

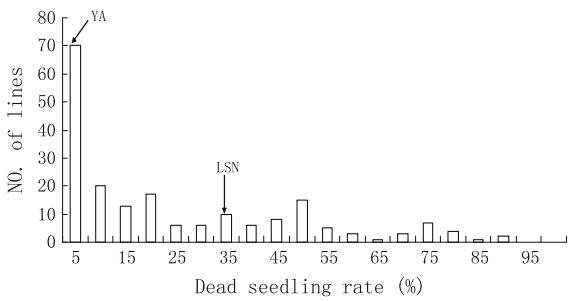

Fig. 4 Distribution of dead seedling rate when treated 55 days under alkaline for RILs population (*YA* Yiai 1, *LSN* Lishuinuo)

The DLR and DSR of rice under salt stress

As shown in Table 3, the one QTL, ***qDLRs4-1*** which associated with the DLR at 10 days after translated in salt stress was detected in RM3471-RM261 on chromosome 4, with the LOD of 3.26, which explained 8.65 % of the observed phenotypic variance. Two QTLs, ***qDLRs4-1*** and ***qDLRs8-1***, which associated with the DLR at 20 days after translated in salt stress were detected in RM307-RM471 on chromosome 4 and in RM22741-RM404 on chromosome 8, Respectively, which explained 11.01 % and 18.28 % of the observed phenotypic variance, respectively, which are major QTLs.

For the DSR, three QTLs, ***qDSRs4-2*** and *qDSRs8-1* which associated with the DSR at 10 days and ***qDSRs5-1*** associated with the DSR at 20 days after translated in salt stress were detected. These QTLs located in RM307-RM471 on chromosome4, RM22741-RM404 on chromosome 8, and RM163-RM5401 on chromosome 5, respectively. Which the observed phenotypic variance were 27.2, 15.96 and 9.99 %, respectively, the *qDSRs4-2* and *qDSRs8-1* are major QTLs. Additive alleles of ***qDLRs4-1***, ***qDLRs8-1***, ***qDSRs4-2***, and ***qDSRs8-1*** originated from Lishuinuo (LSN), but additive alleles of ***qDSRs5-1*** originated from Yiai1 (YA).

The DLR and DSR of rice under alkaline stress

Three QTLs (Table 3), ***qDLRa1-1***, ***qDLRa5-2*** and ***qDLRa5-3*** associated with the DLR at 20 days, 45 days and 50 days after transplanted in alkaline stress were detected located in RM243-RM583 on chromosome 1, RM289-RM413 on chromosome 5, RM7118-RM169 on chromosome 1. The range of variation for LOD value of these three QTLs was

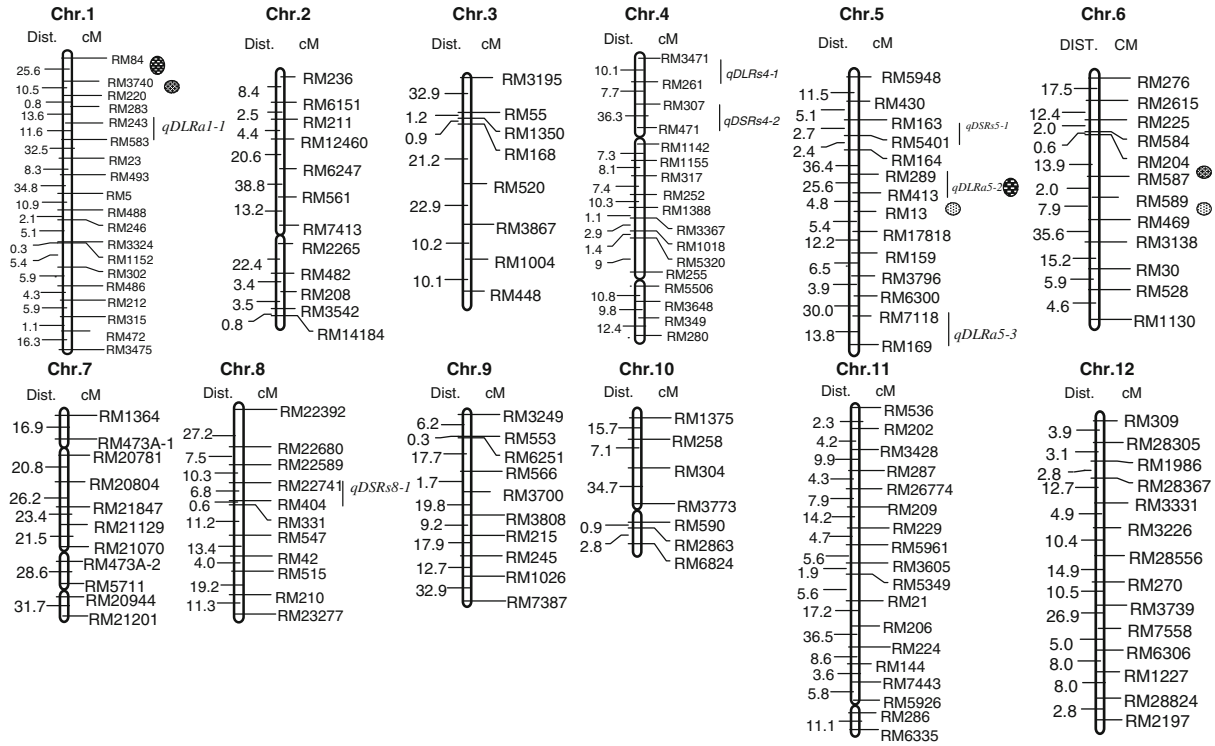

Fig. 5 Likelihood intervals for additive and AA epistatic QTLs of dead leaf rate and dead seedling rate under salt or alkaline stress for mapped RILs derived from the cross between rice variety Yiai 1 and Lishuinuo. Epistetic QTL areas that each two figures with the same shading located in represent interaction interval of Epistetic QTL

Table 3 Additive QTL detection for DLR and DSR under salt stress and alkaline stress respectively in rice

Traits	QTLs	Chr.	Marker flanking	LOD	PVE(%)	Add	Source of alleles
DLR for salt-stress							
10 days	*qDLRs4-1*	4	RM3471-RM261	3.26	8.65	2.51	LSN
20 days	*qDLRs4-1*	4	RM3471-RM261	4.48	11.01	3.25	LSN
	qDLRs8-1	8	RM22741-RM404	7.65	18.28	4.21	LSN
DSR for salt-stress							
10 days	*qDSRs4-2*	4	RM307-RM471	4.46	27.2	0.15	LSN
	qDSRs8-1	8	RM22741-RM404	6.54	15.96	0.11	LSN
20 days	*qDSRs5-1*	5	RM163-RM5401	4.65	9.99	−0.07	YA
DLR for alkaline -stress							
20 days	*qDLRa1-1*	1	RM243-RM583	3.62	8.29	−2.93	YA
45 days	*qDLRa5-2*	5	RM289-RM413	4.41	33.25	5.85	LSN
50 days	*qDLRa5-3*	5	RM7118-RM169	3.51	8.32	2.97	LSN

3.51 to 4.41, and the observed phenotypic variance of these 3 QTLs were 8.29, 33.25 and 8.32 %, respectively. The ***qDLRa5-2*** is major QTL. Additive alleles of ***qDLRa1-1*** originated from Yiai 1, but additive alleles of ***qDLRa5-2*** and ***qDLRa5-3*** originated from Lishuinuo. And there were no QTLs associated with DSR for alkaline stress were detected in this study.

Table 4 Epistatic QTL of AA interaction for DLR and DSR under salt or alkaline stress in rice

Trait	QTLi	Chr.i	Marker flanking	QTLj	Chr.j	Marker flanking	LOD	AA	PVE (%)
DLR for alkaline -stress									
30 days	*qDLRa5-1i*	5	RM413-RM13	*qDLRa6*-1j	6	RM589-RM469	5.5	5.27	13.08
35 days	*qDLRa1-1i*	1	RM3740-RM220	*qDLRa6*-2j	6	RM204-RM587	5.0	−6.67	23.91
DSR for alkaline-stress									
55 days	*qDSRa1*-2i	1	RM84-RM3740	*qDLRa5*-2j	5	RM289-RM413	6.2	13.41	28.84

Epistatic QTL of AA interaction for DLR and DSR under salt or alkaline stress

In the study of epistatic QTL of AA interaction for DLR and DSR under salt or alkaline stress, three pairs of epistatic QTLs were detected (Table 4). Of them, two pairs for DLR were detected at 30 and 35 days after transplanted in alkaline stress, respectively. One pairs for DSR was detected at 55 days after transplanted in alkaline stress. All these QTL located on chromosome 1, 5, 6. The AA values of epistatic QTL for DLR at 30, 35 days were 5.27 and -6.67, respectively; LOD values were 5.5 and 5.0, respectively; and the observed phenotypic variance were 13.08 % and 23.91 %, respectively. The AA value of epistatic QTL for DSR at 55 days was 13.14, LOD value of it was 6.2, and the observed phenotypic variance was 28.84 %.

qDLRa1-1i/qDLRa6-2j was with the negative value which implies that the two-locus genotypes from the same parent Yiai 1 or Lishuinuo take the negative effects, while the two-locus genotypes of recombination from parent Yiai 1 and Lishuinuo take the positive effects, and the case of plus AA values such as the other two pairs of QTL were just the opposite. Otherwise, there was no Epistatic QTL of AA interaction for DLR and DSR under salt stress.

Discussion

Genetic control of salt or alkaline tolerance in rice

Salt and alkaline tolerance in rice is not only a complicated physiological trait, but also a complicated genetic trait (Qi et al. 2008). The common understanding was that rice increases its salt tolerance through discharge of Na^+ and absorption of K^+ to maintain a lower rate of Na^+/K^+. (Gregorio and Senadhira 1993) considered that additive and dominant genes play a role in maintaining the lower rate of Na^+/K^+. The salt tolerance materials can be induced by the incomplete dominance genetic mutation (Zhang et al. 1995). The salt tolerance at the seedling stage in rice was controlled by a few genes (Jones 1985) and genetic variation of under salt stress showed an additive and interactional character (Moeljopawiro and Ikehashi 1981).

In the present study, the DLR of RILs population under salt or alkaline stress showed a continuous distribution, it was controlled by multiple genes, which is the same with the results in the previous studies. The results showed that rice could adapt the salt stress condition at the early stage, but with the time of salt stress increasing, the cellular tissue of rice was continue to be destructed and then the limit of the previous salt tolerance was to be broken, so at 20 days after transplanted in salt stress showed a higher DLR distribution. Under alkaline stress, the RILs population showed an increasing tendency of DLR during the days of 10–20, then showed a decrease of DLR at early stage in a little extend, but a high decrease of DLR at late stage in a little extend during the days of 25–40, and then showed an increasing tendency of DLR again during the days of 45–60. This may be because of that the rice was sensitive to alkaline stress at the early seedling stage and showed an increasing tendency of DLR during the days of 10–20; the rice adjusted itself to adapt the alkaline stress condition during the days of 25–40 and showed a decrease of DLR at early stage in a little extend, but a high decrease of DLR at late stage in a little extent; the rice was sensitive to alkaline stress again during the days of 45–60 and showed an increasing tendency of DLR again. The result was similar to the results of (Flowers and Yeo 1981) and Lutts et al. (1995). In this study, DLR is a quantitative trait controlled by multiple genes, but DSR is a quantitative trait which controlled by a few major

genes and many other minor genes together. The result was similar to the results of Flowers and Flowers (2005) and (Jones 1985) indicated in their studies on barley, rice, citrus, and tomato.

Detection of QTLs for salt tolerance in rice

Zhang et al. (1999) showed that ***OSA3*** gene on rice chromosome 12 expresses PM H^+-ATPase; based on the relative abundance of PM H^+ -ATPase gene transcripts in M-20 shoots, they indicated that the active role of this gene in the strict control of Na^+ and Cl^+ uptake into root symplast and apoplast and further translocation into the shoots and leads to the reduced gene expression in M-20 shoots under salt stress condition to adjust its salt tolerance. Michael et al. (2010) indicated that the ***Salto*** gene controlled the balance of Na^+/K^+ in rice cells to increase its salt tolerance. Zhang et al. (2006) indicated that the ***Mangrin*** increased the alkaline tolerance in transgenic rice. Eight QTLs associated with dry weight, fresh weight, leaf area, seedling length, and survival rate of seedlings at the seedling stage in rice were detected on chromosomes 1, 6 and 7, respectively (Kim et al. 2009). Lin et al. (2004) used $F_{2:3}$ population to detect two QTLs for survival days of seedlings under salt stress. Of these QTLs, the two major QTLs with the higher effect, ***qSNC-7*** for shoot Na^+ concentration and ***qSKC-1*** for shoot K^+ concentration, explained 48.5 and 40.1 % of the total phenotypic variance, respectively, and were major genes. Takehisa et al. (2004) detected 27 QTLs associated with saline tolerance use the BILs population. According to all the studies above, we know that using different mapping populations always got the different results when identified QTLs associated with salt tolerance under salt stress in rice.

Contrasting the result in this study with the preceding researches, ***qDSRs5-1*** was located in the same marker flanking as ***QSst5a*** (Qian et al. 2009); ***qDLRs4-1*** for 10 days**, and** ***qDLRs4-1*** for 20 days in salt stress were located in the same marker interval as ***qSH4*** (Chen et al.2008), ***QSst4, QKc4b***(Yang et al.2009); ***qDSRs4-2*** was located in the same marker interval as ***QSw4,QGw4a*** (Chen et al.2008).These marker intervals of QTLs associated with different salt tolerance trait were detected in different mapping populations show that these marker intervals were important areas of rice genome, and would be was closely related with salt tolerance in rice, and worth to be used in the molecular marker-assisted breeding program in rice to get salt tolerance cultivars. In chromosome 8, ***qDLRs8-1*** for DLR of rice, and the ***qDSRs8-1*** for DSR of rice, were detected on the same marker interval, and the distance of this maker interval is 6.8 cM, which was likely to be the same QTL, or one gene with multifunction or two QTLs with compact linkage.This QTL was a new detected one, so can be used in the breeding program in rice to get salt tolerance rice cultivars in the future.

Detection of QTLs for alkaline tolerance in rice

Qi et al.(2008) used $F_{2:3}$ population to detect 13 QTLs for DLR of rice at the seedling stage of the days of during 20–62 under alkaline tolerance; of them, the alleles of ***qDLR9-2*** and ***qDLR4*** originated from alkaline sensitive parent "Gaochang 106", but the alleles of ***qDLR7-1*** and ***qDLR6-2*** originated from alkaline tolerance parent "Changbai 9". Cheng et al. (2008) used two DH populations (DH1: ZYQ8 × J-X17and DH2: CJ06 × TN1) identified 10 additive QTLs in H1, 14 additive QTLs in DH2 at the germination and early seedling stage under alkaline stress. At present, there were only a few of studies for alkaline tolerance in rice. In this study three QTLs associated with DLR in rice under alkaline stress were detected, among them, the observed phenotypic variance of ***qDLRa1 -1*** for 20 days, ***qDLRa5-2*** for 45 days and ***qDLRa5-3*** for 50 days, with the observed phenotypic variance of 8.29, 33.25 and 8.32 %, respectively.

Contrasting the result in this study with the preceding researches for salt or alkaline tolerance, *qDLRa1-1* was located in the same marker interval as ***qSKc1*** for shoot K^+ concentration (Lin et al.2004), ***Std*** for major salt-tolerance gene (Gong et al.1998), ***TS1*** of cDNA clone for salt response (Qian et al. 2003), and ***QSt1a*** (Qian et al. 2009). ***qDLRa5-2*** was located in the same marker interval as ***QDss5, QSf5, QGW5*** (Chen et al.2008). The observed phenotypic variance of ***qDLRa5-2*** was 33.25 %. The reason of it may because of the distance between two SSR markers were very large, which of 25.6 cM. The same marker interval could be detected QTLs for salt an alkaline tolerance by using the different mapping population, and indicates that people could cultivate new rice cultivars

associated with several resistant traits by pyramiding useful QTLs. Otherwise, ***qDLRa5-3*** was a new detected QTL located in chromosome 5, which with the observed phenotypic variance of 8.32 %, the distance between two markers of 13.8 cM.

Detection of epistatic QTLs of AA interaction for salt or alkaline tolerance in rice

Quantitative trait loci were controlled by multiple genes, epictatic effects plays an important role in quantitative trait (Phillips 1998). Epictatic effects were the no additive genetic effects between genelocis. Cheng et al. (2008) used two DH populations (DH1: ZYQ8 × JX17and DH2: CJ06 × TN1) identified 15 epictatic QTLs in H1, 15 epictatic QTLs in DH2 at the germination and early seedling stage under alkaline stress. Zhao et al. (2011) used 16 single segment substitution lines (SSSL) and 15 double segment substitution lines (DSSL) to detected seven pairs of epictatic interactions for the grain shape. Lei and Che (2010) used a population of 284 recombinant inbred lines (RIL) from the indica-japonica rice cross Lemont × Teqing to detected nine pairs of epistasis for heading date. Jiang et al. (2009) used a RIL population derived from the—rice cross Minghui86 Jiafuzhan identified 15 pairs of epistatic QTLs with significant additive-by-additive (AA) interaction effects for three traits. Identification for epistatic QTLs associated with salt or alkaline tolerance was scarce at present.

In this study, 3 pairs of AA epistatic QTLs (Table 4) associated with alkaline tolerance. The observed phenotypic variances of them were larger than additive QTLs under alkaline stress,and showed that AA epistatic QTLs was closely related with the resistance to alkaline injured in rice, and these results were similar as Lei et al. (2008) which indicated that epistatic effects was closely related with the inheritance about grain shape and chalkiness degree in rice. But the observed phenotypic variances of AA epistatic QTLs were smaller than additive QTLs under salt stress in rice. The results above showed that the mechanism for salt tolerance was difference to alkaline tolerance in rice. How to use these results in the molecular marker-assisted breeding program in rice to get salt or alkaline tolerance cultivars should be further researched on the base of the preceding studies.

The origin of additive alleles for QTLs of salt or alkaline tolerance in rice

Three QTLs associated with DLR of rice under salt or alkaline stress were detected, of them, the additive alleles for two QTLs originated from the salt-alkaline tolerance parent YA, and the additive alleles for the other seven QTLs originated from the salt-alkaline sensitive parent LSN. The results showed that salt-alkaline tolerance alleles were not only contained in salt-alkaline tolerance variety, but also were contained in salt-alkaline sensitive variety. The phenomenon was similar to some early reports, such as the alleles of over half of the numbers of QTLs for cold tolerance at seedling stage and stooling stage originated from cold sensitive parent "Miyang23" (Han et al. 2005), and such as the alleles of six QTLs for alkaline tolerance at seedling stage in rice originated from alkaline sensitive parent "Gaochan106" (Qi et al. 2008), and such as the alleles of two QTLs associated with high yields originated from lower yield wild rice (Li et al. 2002). Based on the results in this study and the early reports above, we know that excellent gene resource could be detected from any one crop germplasm and should never be eliminated because of poor phynotype.

Acknowledgments We thank the Chinese National Germplasm Bank for providing the improved japonica rice seeds. This work was supported by the National Key Technology Research and Development Program of China (2013BAD01B02-2), the Project of 973 (2010CB125904-5), Science and Technology Innovation Project of CAAS and The platform of National Crop Germplasm Resources, the Protective Program of Crop Germplasm of China (NB2013-2130135-25-01), the Basic Work Project of the Ministry of Science and Technology (2007FY110500-12), the International Cooperation Project from National Institute of Crop Science, RDA (PJ00868505), the National Institute of Crop Science, RDA (PJ00871702).

References

Akbar M, Yabuno T, Nakao S (1972) Breeding for saline resistant varieties of rice, variability for salt-tolerance among some rice varieties. Jpn J Breed 22:277–284

Allard RW (1996) Genetic basis of the evaluation of adaptedness in plants. Euphytica 92:1–11

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu J, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Chandan S, Amanjot S, Krishan K, Eduardo B, Anil G (2006) Salt stress response in rice: genetics, molecular biology, and comparative genomics. Funct Integr Genomics 6:263–284

Chen HT, Jiang H, Yan MX, Dong GJ, Qian Q, Guo LB (2008) QTL-mapping comparison of tolerance to alkali at germination period and early seeding stage between two different double haploid populations in rice. Mol Plant Breed 6(3):439–450 (in Chinese)

Cheng HT, Jiang H, Xue DW, Guo LB, Zeng DL, Zhang GH, Qian Q (2008) Mapping of QTLs underlying tolerance to alkali at germination and early seedling stages in rice. Acta Agron Sin 34(10):1719–1727 (in Chinese)

Cho YG, McCouch SR, Kuiper M, Kang MR, Pot J, Groenen JTM, Eun MY (1998) Integrated map of AFLP, SSLP and RFLP markers using a recombinant inbred population of rice(Oryza sativa L.). Theor Appl Genet 97:370–380

Cui F, Li J, Ding A, Zhao C, Wang L, Wang X, Li S, Bao Y, Li X, Feng D, Kong L, Wang H (2011) Conditional QTL mapping for plant height with respect to the length of the spike and internode in two mapping populations of wheat. Theor Appl Genet 122(8):1517–1536

Flower TJ (2004) Improving crop salt tolerance. J Exp Bot 396:399–409

Flowers TJ, Flowers SA (2005) Why does salinity pose such a difficult problem for plant breeders? Agric Water Manag 78:15–24

Flowers TJ, Yeo AR (1981) Variability in the resistance of sodium chloride salinity within rice(Oryza sativa L.) varieties. New Phytol 88:363–373

Flowers TJ, Koyama ML, Flowers SA, Sudhakar C, Singh KP, Yeo AR (2000) QTL: their place in engineering tolerance of rice to salinity. J Exp Bot 51:99–106

Gong JM, He P, Qian Q, Shen LS, Zhu LH, Chen SY (1998) Identification of QTLs for salt tolerance in rice. Chin Sci Bull Publ 43(17):1847–1850 (in Chinese)

Gregorio GB, Senadhira D (1993) Genetic analysis of salinity tolerance in rice (Oryza sativa L.). Theor Appl Genet 86:333–338

Gregorio GB, Senadhira D, Mendoza RD, Manigbas NL, Roxas JP, Guerta CQ (2002) Progress in breeding for salinity tolerance and associated abiotic stresses in rice. Field Crops Res 76:91–101

Grover A, Pental D (2003) Breeding objectives and requirements for producing transgenic for the major field crops of India. Curr Sci 84:310–320

Han LZ, Qiao YL, Cao GL, Zhang YY, An YP, Yea JD, Koh HJ (2005) QTL analysis on cold tolerance during early growth period in rice. Chin J Rice Sci 19:122–126 (in Chinese)

Hossein S, Atefeh S (2008) New evidence of QTLs attributed to salinity tolerance in rice. Afr J Biotechnol 7(24):4376–4383 17 December

Ikehashi H, Ponnamperuma FN (1978) Varietal tolerance of rice for adverse soils. Soils and Rice. IRRI, Los Banos, pp 801–823

Islam MR, Salam MA, Hassan L, Collard BCY, Singh RK, Gregorio GB (2011) QTL mapping for salinity tolerance at seedling stage in rice. Emir J Food Agric 23(2):137–146

Jafar A, Fotokian MH (2011) Identification and mapping of quantitative trait loci associated with salinity tolerance in rice (Oryza Sativa) using SSR markers. Iran J Biotechnol 9:1 January 2

Jiang LR, Wang W, Huang JX, Huang RY, Zheng JS, Huang YM, Wang HC (2009) Analysis of epistatic and QE interaction effects of QTLs for grain shape in rice. Mol Plant Breed 4(7):690–698 (in Chinese)

Jones MP (1985) Genetic analysis of salt tolerance in mangrove swamp rice. In Rice Genetics Proceeding of International Rice Genetics Symposium, IRRI 5:41–122

Kim DM, Ju HG, Kwon TR, Oh CS, Ahn SN (2009) Mapping qtls for salt tolerance in an introgression line population between japonica cultivars in rice. J Crop Sci Biotechnol 12(3):121–128

Korbe SA, Abdel ARM (1974) Effect of total salinity and type of salts on rice crop. Agric Res Rev 52:73–78

Lei DY, Xie FM, Xu JL, Chen LY (2008) QTLs mapping and epistasis analysis for grain shape and chalkiness degree of rice. Chin J Rice Sci 22(3):255–260 (in Chinese)

Lei DY, Che LY (2010) Mapping QTLs with epistatic effect and QTL × environmental interaction effect of heading date in rice. J Hunan Agric Univ 36(3):245–249 (in Chinese)

Li DJ, Sun CQ, Fu YC, Li C, Zhu ZF, Chen L, Cai HW, Wang XK (2002) Identification and mapping of genes for improving yield from Chinese common wild rice (O. rufipogon Griff.) using advanced backcross QTL analysis. Chin Sci Bull 47:1533–1537

Lin HX, Zhu MZ, Yano M, Gao JP, Liang ZW, Su WA, Hu XH, Ren ZH, Chao DY (2004) QTLs for Na+ and K+ uptake of the shoots and roots controlling rice salt tolerance. Theor Appl Genet 108:253–260

Liu RH, Meng JL (2003) Mapdraw: a microsoft excel macro for drawing genetic linkage maps based on given genetic *linkage data*. Hereditas 25(3):317–321 (in Chinese)

Lutts S, Kinet JM, Bouharmont J (1995) Changes in plant response to NaCl during development of rice (Oryza sativa L.) varieties differing in salinity resistance. J Exp Bot 46:1843–1852

Maas EV, Hoffman GJ (1977) Crop salt tolerance-current assessment. J Irrig Drain Div 103:115–134 Handb 60

MaCouch SR, Cho YG, Yang M, Paul E, Blinstrub M, Morishima H, Kinoshita T (1997) Report on QTL nomenclature. Rice Genet Newslett 14:11–13

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Moeljopawiro S, Ikehashi H (1981) Inheritance of salt tolerance in rice. Euphytica 30:291–300

Phillips PC (1998) The language of gene interaction. Genetics 149:1167–1171

Qi DL, Guo GZ, Lee MC, Zhang JG, Cao GL, Zhang SY, Suh SC, Zhou QY, Han LZ (2008) Identification of quantitative trait loci for the dead leaf rate and the seedling dead rate under alkaline stress in rice. J Genet Genomics 35:299–305

Qi DL, Guo GZ, Lee MC, Yang CG, Zhang JG, Cao GL, Zhang SY, Zhou QY, Han LZ (2009) Indentification of quantitative trait loci for alkaline tolerance at early seedling stage under alkaline stress in japonica rice. Acta Agron Sin 35(2):301–308 (in Chinese)

Qian Q, Seiji Y, Teng S, Zeng DL, Zhu LH, Chen SY (2003) Isolation, expression characteristics and chromosomal locations of three cDNA fragments under salt stress in rice. Acta Botanica Sinica 45(9):1090–1095

Qian YL, Wang H, Chen MY, Zhang LK, Chen BX, Cui JT, Liu HY, Zhu LH, Shi YY, Gao YM, Li ZK (2009) Detection of salt-tolerance QTL using BC2F3 yield selected introgression lines of rice (Oryza sativa L.). Mol Plant Breed 7(2):224–232 (in Chinese)

Rieseberg LH, Sinervo B, Linder CR, Ungerer MC, Arias DM (1996) Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids. Science 272:741–745

Takehisa H, Shimodate T, Fukuta Y, Ueda T, Yano M, Yamaya T, Kameya T, Sato T (2004) Identification of quantitative trait loci for plant growth of rice in paddy field flooded with salt water. Field Crops Res 89:85–95

Tanveer UH, Javaid A, Jone G, Katherine A, Steeleand MK (2008) Genetic mapping of QTLs, controlling shoot fresh and dry weight under salt stress in rice (oryza satival.) cross between Co39 × moroberekan. Pak J Bot 40(6):2369–2381

Wang Z, Wu X, Ren Q, Chang X, Li R, Jing R (2010) QTL mapping for developmental behavior of plant height in wheat. (Triticum aestivum L.). Euphytica 174(3):447–458

Wright S (1980) Genetic and organismic selection. Evolution 34:825–843

Wu X, Wang Z, Chang X, Jing R (2010) Genetic dissection of the developmental behavious of plant height in wheat under diverse water regimes. J Exp Bot 61(11):2923–2937

Yang J, Sun Y, Cheng LR, Zhou Z, Wang Y, Zhu LH, Cang J, Xu JL, Li ZK (2009) Genetic background effect on QTL mapping for salt tolerance revealed by a set of reciprocal introgression line populations in rice. Acta Agron Sin 35(6):974–982 (in Chinese)

Yano M, Sasaki T (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol Report 35:145–153

Yeo AR, Flowers TJ (1986) Salinity resistance in rice (Oryza sativa L.) and a pyramiding approach to breeding varieties for saline soils. Austr J Plant Physiol 13:161–173

Zhang GY, Guo Y, Chen SL, Chen SY (1995) RFLP tagging of a salt tolerance gene in rice. Plant Sci 110:227–234

Zhang ZS, Xie C, Li ZY, Chen SY (1999) Expression of the plasma membrane H+ -ATPase gene in response to salt stress in a rice salt-tolerant mutant and its original variety. Theor Appl Genet 99:1006–1011

Zhang WH, Lin T, Zhang HX, Chen L, Shan L (2006) Transformed rice with salt tolerance-related genes of bruguiera sexan-gula by agrobacterium meditation. Acta Bot Boreali–Occident Sin 26:1106–1109 (in Chinese)

Zhao FM, Zhang GQ, Zeng RZ, Yang ZL, Ling YH, Sang XC, He GH (2011) Analysis of Epistatic and Additive Effects of QTLs for Grain Shape Using Single Segment Substitution Lines in Rice (Oryza sativa L.). Acta Agron Sin 37(3):469–476 (in Chinese)