



QTL analysis for rice salinity tolerance and fine mapping of a candidate locus *qSL7* for shoot length under salt stress

Noushin Jahan¹ · Yu Zhang² · Yang Lv¹ · Mengqiu Song¹ · Chunyan Zhao¹ · Haitao Hu¹ · Yongtao Cui¹ · Zhongwei Wang¹ · Shenglong Yang¹ · Anpeng Zhang¹ · Jiang Hu¹ · Guoyou Ye³ · Qian Qian¹ · Zhenyu Gao¹ · Longbiao Guo¹

Received: 18 October 2019 / Accepted: 3 December 2019 / Published online: 10 December 2019
© The Author(s) 2019

Abstract

Salinity is one of the major abiotic stresses limiting rice production worldwide. Understanding the genetic basis of salinity tolerance is key for rice breeding. In this study, a recombinant inbred line (RIL) population derived from a super hybrid rice Liang–You–Pei–Jiu (LYP9) parents 93-11 and PA64s, exhibited variation in phenotypes including shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW) under 50 and 100 mM NaCl stress. QTL analysis identified a total of 38 QTLs for these 6 traits under two distinct concentrations of salt, distributing on chromosomes 1, 2, 3, 4, 5, 6, 7 and 10. A total of 21 QTLs were found in 6 stable loci. A novel major QTL, *qSL7* for shoot length on chromosome 7 was identified in two distinct concentrations. A chromosome segment substitution line (CSSL) harboring the *qSL7* locus from PA64s with 93-11 background was developed and exhibited higher SL value, higher K⁺ concentration, and lower Na⁺ concentration compared to 93-11. With BC₅F_{2,3} derived from CSSL-*qSL7*/93-11, the *qSL7* was fine mapped within a 252.9 kb region on chromosome 7 where 40 annotated genes located including, *LOC_Os07g43530*, which encodes a DNA-binding domain containing protein reported previously as a transcription factor playing a positive role in salt stress tolerance. Our study provides new genetic resources for improvement of salinity tolerance in rice breeding.

Keywords Salinity tolerance · QTL analysis · Fine mapping · *qSL7* · Rice

Introduction

The soil salinity is the critical abiotic stress that limiting crop production all over the world. It is the major problem of rice based farming systems in the coastal areas. Also,

inland is affected by salinity due to improper irrigation and drainage system. Approximately 20% irrigated and 8% of rainfed agricultural land is affected by salinity (Asif et al. 2018). About one-third of the irrigated rice growing areas are affected by salinity (Prasad et al. 2000). Salinity stress impacts on rice may vary depending upon its different growth and development stages (Yamaguchi and Blumwald 2005). Rice can tolerate salt stress at late vegetative and maturity stages and susceptible at early the vegetative stage. In general, germination and seedling are the two most sensitive stages of salinity. Salinity tolerance in rice is a physiologically complex trait and attributed to multiple mechanisms. The effect of salinity stress arises as a result of the relationship between the physiological and molecular responses of plants (Zhang et al. 2018). Understanding the salt stress tolerance mechanism of rice is crucial for identifying the responsible genetic material. Progress in rice breeding with salt tolerance is still slow because of genetic complexity (Flowers and Flowers 2005). Besides, salt tolerance

Noushin Jahan and Yu Zhang have contributed equally to this work.

✉ Zhenyu Gao
gaozhenyu@caas.cn

✉ Longbiao Guo
guolongbiao@caas.cn

¹ State Key Lab for Rice Biology, China National Rice Research Institute, Hangzhou 310006, China

² Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao 266101, China

³ Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China

screening is difficult because the phenotypic response of rice to salt stress is highly affected by other confounding environmental factors (Flowers 2004). The genetics behind salinity tolerance governed by many genes exhibiting the quantitative or polygenic nature of the trait can be revealed by using quantitative trait locus (QTLs) analysis (Gimhani et al. 2016; Negrão et al. 2011). Mapping of QTLs by using molecular markers can aid the genetic dissection of the salt tolerant genes. Since the advent of molecular markers, QTL analyses for salinity tolerance were conducted using different populations and most of them reported salinity tolerance is a polygenetic trait (De Leon et al. 2016; Gregorio et al. 2002). Therefore, uncovering the genes behind salt tolerant traits remains difficult and vital for the marker-assisted plant breeding approach.

There were lots of studies have been done on salt tolerant QTL mapping but due to lack of enough molecular marker coverage the genomic interval was so large to identify candidate genes like, previously the promising QTL designated as *Saltol* was mapped in large genomic region of 10.7–12.2 Mb on the short arm of the chromosome 1 (Bonilla et al. 2002; Gregorio et al. 1997). Recently advanced high-density molecular marker technology has been developed for rapid high-throughput genotyping. High-throughput SNP genotyping facilities a number of advantages over previous marker systems providing sufficiently dense genome coverage for the dissection of complex traits (Gimhani et al. 2016; Takehisa et al. 2004; Thomson et al. 2011). Bimpong et al. (2013) mapped salt tolerant QTL by using 384-plex SNP markers. More recently De Leon (De Leon et al. 2016) used 9303 SNP markers generated by genotyping-by-sequencing (GBS) were mapped to 2817 recombination points for salt tolerant QTL identification. In addition, several studies of salinity-tolerant QTLs mapping has been reported although few of them were fine mapped. The large chromosomal intervals delimited the attribution of those QTLs. Finally, the identification of candidate genes become more challenging.

Fine mapping is one of the commonly used approaches to identifying genes underlying the quantitative traits. Number of QTLs have been identified for salt stress related traits in the past among them only *qSKC1* was successfully isolated by map based cloning (Ren et al. 2005) and others need to be cloned.

In this study recombinant inbred lines developed from the cross between 93-11 and PA64s were used for QTL analysis. Parent 93-11 is an *indica* variety widely grown in China and PA64s is a thermosensitive *indica* variety. A high-density SNP linkage map was used for identification of QTLs associated with 6 important traits under salt stress. A novel QTL, *qSL7* for shoot length was fine mapped and delimited into a 252.9 kb region on chromosome 7, which will be helpful for further cloning of it and understanding the genetic basis of rice salinity tolerance.

Materials and methods

Plant materials and growing conditions

A total of 132 RILs derived from the cross between super hybrid rice LYP9's parents 93-11 and PA64s were used in this study for salinity tolerance screening. Susceptible parent 93-11 is an elite *indica* variety and tolerant parent PA64s a thermosensitive *indica* variety. Phenotyping for salt tolerance parameters was commenced on RILs along with two parents (Gao et al. 2013).

Phenotypic evaluation of seedling under salt stress

The parents and RILs were assessed at seedling stage by measuring 6 salinity responsive traits viz. SL, RL, SFW, RFW, SDW, and RDW. The treatments were comprised of three NaCl levels: 0 (control, CK), 50 mM NaCl (moderate salt stress) and 100 mM NaCl (high salt stress). To grow seedlings under hydroponic culture, the grains were surface-sterilized and allowed to germinate in water for 3 days. The germinated seedlings were transferred to nutrient solution containing 1.425 mM NH_4NO_3 , 0.42 mM NaH_2PO_4 , 0.510 mM K_2SO_4 , 0.998 mM CaCl_2 , 1.643 mM MgSO_4 , 0.168 mM Na_2SiO_3 , 0.125 mM Fe-EDTA, 0.019 mM H_3BO_3 , 0.009 mM MnCl_2 , 0.155 mM CuSO_4 , 0.152 mM ZnSO_4 , and 0.075 mM Na_2MoO_4 (Li et al. 2016) and exposed to a 14 h photoperiod, under a temperature regime of 30°C/25°C and a relative humidity level of – 70%. The pH was adjusted to 5.5–5.8. The nutrient solution was replaced every 2 days interval to adjust the volume and pH of nutrient solution. At the 7th day after planting, the solution was salinized by the addition of NaCl, to which the seedlings were exposed for 2 weeks. The whole experiment was conducted in randomized complete block design with two biological replications. Fifteen plants per line of uniform growth were evaluated for traits related to salinity tolerance. Shoot length and root length were measured in centimetre. Shoot length was measured from the base of the culm to the tip of the tallest leaf and root length was measured from the base of the culm to the tip of the longest root. For dry weight, five plants per line per replication were collected and dried at 65°C oven for 5 days prior to weighing.

Na^+/K^+ Ion Measurement

For the determination of sodium (Na^+) and potassium (K^+) concentration, 28 days after salinization, the shoot was collected from 93-11 and CSSL-*qSL7* (three plants from each genotype). After three times distilled water washing samples were dried at 70 °C for 3 days. Further, 10 mg fine grounded

sample was extracted in 10 ml of 0.1 N acetic acid then set in a 90 °C water bath for at least 2 h until the sample was completely digested. Extracted solution was cooled at room temperature and filtered using Whatman 1 filter paper and diluted ten times. Na⁺ and K⁺ were measured by flame emission spectrometry using a flame photometer at 589 nm (Sherwood flame photometer, Model 420, Cambridge, UK).

DNA isolation and PCR analysis

The parents and BC₅F₂ individuals' genomic DNA was extracted from fresh leaves using the CTAB method described by Luo et al. (2001). The PCR protocol was performed in a 15 µL reaction mix including 25 ng genomic DNA, 2 µL of each primer, 1.0 µL 10× PCR buffer, 0.1 mmol/L dNTP, 0.2 µL 5 U/µL Taq DNA polymerase (Tiangen Biotech, Beijing, China) and 1.5 µL ddH₂O. Amplification conditions consisted of an initial denaturation at 94 °C for 5 min, 40 cycles of 94 °C for 30 s, 55–60 °C for 30 s, and 72 °C for 30 s. The reaction were completed with a final extension at 72 °C for 10 min, and saving at 15 °C forever.

Genetic map construction and QTL analysis

The SNP map covered a total of 1381.9 cM of rice genome (Gao et al. 2013). QTL analysis was conducted with the MultiQTL package (www.multiqtl.com) using the maximum likelihood interval mapping approach for the RILs. The Logarithm of the odds (LOD) threshold and major effect QTLs were obtained based on a permutation test (1000 permutations, $P=0.05$) for each dataset. We followed the suggestions by McCouch for the QTL nomenclature (McCouch 2008). New SSR and Indel markers in the targeted genomic region were designed according to genome sequence differences between the *indica* cv. 93-11 and the *indica* cv. PA64s. The sequences of these newly developed markers are listed in Table 1.

Construction of fine mapping population

To develop the chromosome segment substitution line (CSSL), a RIL containing PA64s genotype at the *qSL7* flanking region on chromosome 7 was selected to backcross with recurrent parent 93-11. Then, a CSSL at the *qSL7* locus, CSSL-*qSL7* was established and crossed with 93-11. A total of 1300 BC₅F_{2:3} seedlings were evaluated under high (100 mM NaCl) salt stress as above mentioned method to fine map of *qSL7* with 8 markers.

Statistical analysis of data

Mean phenotypic values for seedling root traits were compared using the Student's *t* test. Phenotypic correlations were calculated using a generalized linear model implemented within the SAS (Statistical Analysis System) v8.01.

Results

Evaluation of phenotypic characteristics under salt stress

The parents and the RILs population were evaluated under control/0 mM L⁻¹ (L0), moderate/50 mM (L50) and high/100 mM (L100) NaCl salt stress for SL, RL, SFW, SDW, RFW, and RDW. There was no significant difference in all traits except RFW and RDW between two parents under control (0 mM) condition (Fig. 1 a, d–i). Therefore, the treatment of 0 mM NaCl was not conducted for further QTL analysis. Further, the parents and RILs showed varying levels of tolerance in both (L50 and L100) stress levels. Finally, 50 and 100 mM NaCl treatment was conducted for further study among RILs to fully demonstrate their phenotypic variance. The phenotypic measurement showed that the frequency distributions of all traits in RIL populations accorded with the normal distribution and the acceptable range of skewness below + 1.5 and above – 1.5 (Table 2).

Table 1 Primers for fine mapping of *qSL7*

Marker	Marker forward primer (5' → 3')	Reverse primer (5' → 3')
RM21930	TAGCTGTTGTGCATGATGTTCC	GCTGGACTCCTCTTGATCTCTCC
RM22105	AAGTGGAGGCGTACCTTGTTCC	CGTATCGCGTTCGTAATGTTGC
Indel 7-1	GCATCGACGATTTACCGTA	GCATCGACGATTTACCGTA
Indel 7-2	CACCTGGGAAATGCAATGTG	GCGACGATGGATGGAACGA
Indel 7-3	GCTGATTTGGTTTCATGCC	CAAACTTGCCCTACCAAATAGT
Indel 7-4	AATACCATGAGGATACCGC	AAGAGATCGAGGCTATCCG
Indel 7-5	ACAAGTGCACCATTGTCTGTAT	ACAAGTGCACCATTGTCTGTAT
Indel 7-6	AGCACACTCAATCTGTTGGT	GATCGTTCTGAAGCACTCGT

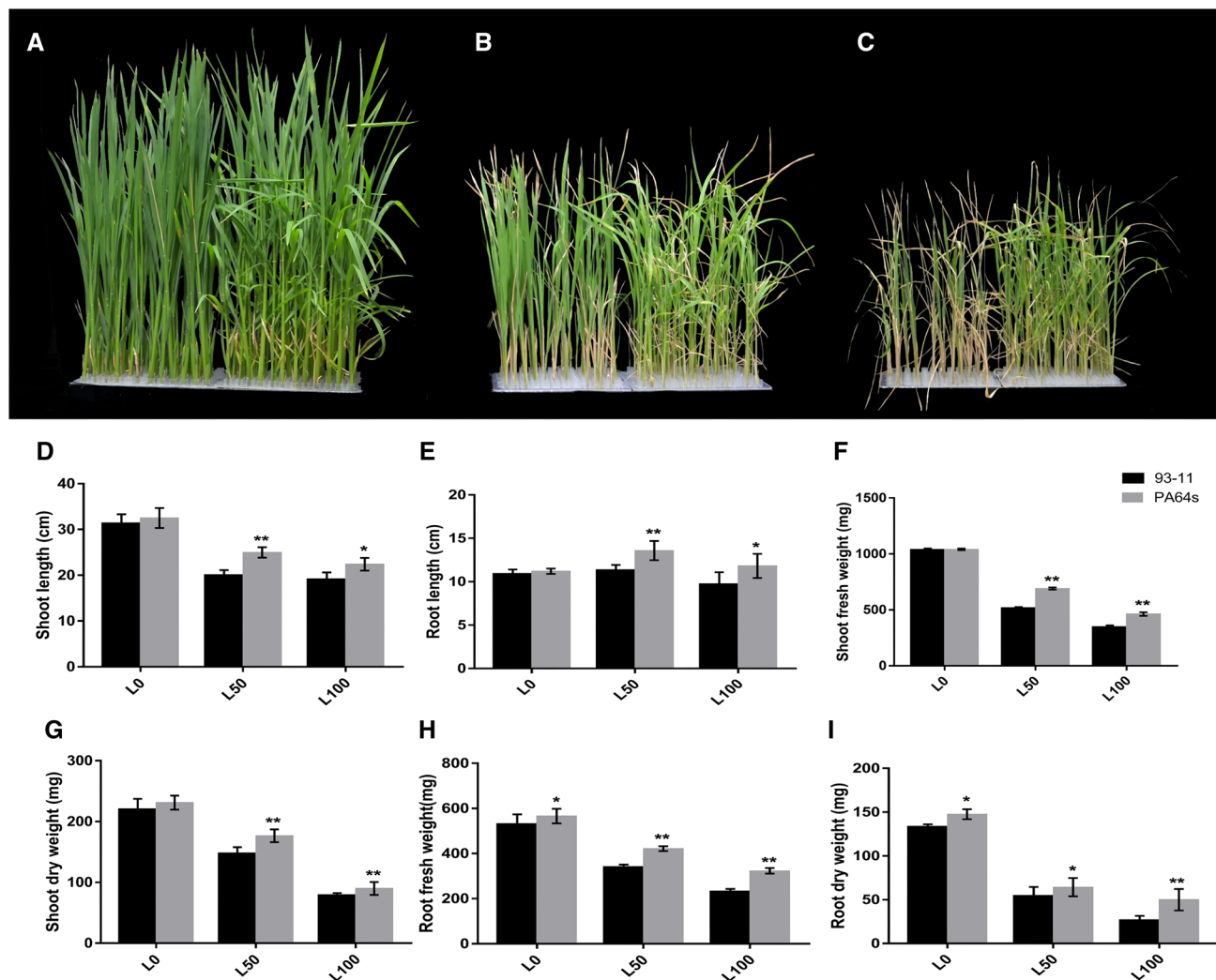


Fig. 1 Phenotypes comparison between two parental lines. **a–c** Morphological responses of 93-11 and PA64s under 0, 50 and 100 mM NaCl stress, respectively; 93-11 is in left and PA64s in right side. **d–i** SL shoot length, RL root length, SFW shoot fresh weight, SDW shoot

dry weight, RFW root fresh weight and RDW root dry weight of parents 93-11 and PA64s. Data represent the mean \pm SD of two biological replicates (Student's *t* test: * $P < 0.05$, ** $P < 0.01$) respectively

At both stress conditions (L50 and L100), two parents 93-11 and PA64s showed significant different responses in all traits (Fig. 1b–c, d–i; Table 2). PA64s showed consistently higher SL, RL, SFW, SDW, RFW, and RDW than 93-11 in both stress levels. Among the RILs, differences were found for all traits, indicating a wide range of variation and the mean values of RILs range between the parental means for all 6 traits. As indicated in the frequency distribution (Fig. 2) and the range of RIL values for each trait (Table 2), several lines showed phenotypically superior to the parents. The six traits in the RIL population exhibited different degrees of transgressive segregation. The results indicated that the recurrent parent 93-11 was more susceptible to salt stress and that the PA64s genome might harbour beneficial alleles associated with enhanced salt tolerance.

Correlation analysis of six traits

The correlation analysis showed that there were positive significant correlations among SL, RL, SFW, SDW, RFW, and RDW under two different salinity conditions (Table 3). According to the correlation analysis, the SL is significantly correlated with RL, SFW, SDW, RFW, and RDW. Although, there was no significant correlation between RL and SFW, and SL and RDW in L50 where coefficient were 0.11 and 0.16, respectively. Moreover, SL was highly significant with all the traits in L100 level. Thus, the association among all morphological traits with each other indicated the co-dependence of phenotypic responses for salinity tolerance and presence of QTL clusters in those regions.

Table 2 Phenotypic response of parents and RIL population for salt tolerance related traits at seedling stage in three stress levels

Traits	Stress Level	Parents(Mean ± SD)		RILs			
		93-11	PA64s	(Mean ± SD)	Range	Skewness	Kurtosis
SL	L0	31.3 ± 2.0	32.5 ± 2.1				
	L50	20.01 ± 1.1	24.99 ± 1.1**	24.18 ± 2.2	10.8	- 0.04	- 0.32
	L100	19.10 ± 1.5	22.40 ± 1.4*	22.45 ± 2.5	12.7	0.45	0.41
RL	L0	10.9 ± 0.5	11.2 ± 0.3				
	L50	11.33 ± 0.6	13.58 ± 1.11**	11.35 ± 1.8	11.8	0.36	1.45
	L100	9.71 ± 1.4	11.81 ± 1.39*	10.22 ± 2.1	12.5	- 0.43	1.12
SFW	L0	1036.3 ± 12.5	1040.7 ± 7				
	L50	515.13 ± 10	690.00 ± 10.0**	516.67 ± 120.4	519.8	0.16	- 0.98
	L100	346.66 ± 15.1	463.33 ± 16.3**	460.84 ± 105.9	447.7	0.21	- 0.71
SDW	L0	220 ± 17.3	231 ± 11.5				
	L50	147.5 ± 10.3	176.66 ± 10.6**	149.56 ± 42.5	184.2	0.57	- 0.26
	L100	78.75 ± 3.5	90.00 ± 10.7**	90.34 ± 22.6	123.8	0.29	0.23
RFW	L0	530 ± 43.5	566 ± 32.1*				
	L50	340.33 ± 10.6	421.66 ± 10.6**	341.85 ± 52.7	263.8	0.28	0.11
	L100	232.22 ± 10.9	323.33 ± 12.2**	308.09 ± 59.2	297.0	- 0.53	0.46
RDW	L0	133.3 ± 2.8	147.6 ± 5.7*				
	L50	54.33 ± 10.3	64.33 ± 10.3*	66.88 ± 20.2	80.1	- 0.24	- 1.15
	L100	36.66 ± 5.0	50.00 ± 12.2**	36.08 ± 9.5	46.1	0.04	- 0.28

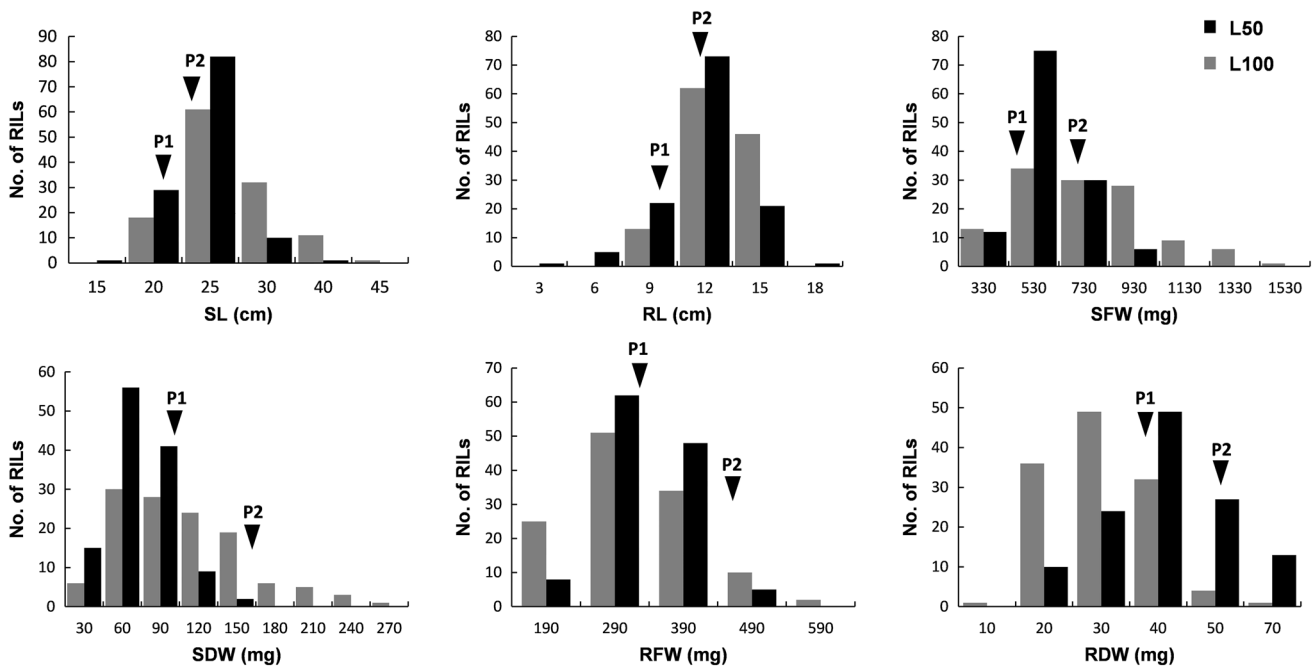


Fig. 2 Phenotypic distribution of six traits of the 93-11/PA64s RIL population across two stress levels. L50: 50 mM NaCl, L100:100 mM NaCl, SL, RL, SFW, SDW, RFW and RDW. P1 and P2 is 93-11 and PA64s, respectively

Identification of QTLs salt tolerance traits at seedling stage

A total of 38 QTLs affecting SL, RL, SFW, SDW, RFW, and RDW were detected across two stress levels and were

distributed on chromosomes 1, 2, 3, 4, 5, 6, 7 and 10 (Table 4, Fig. 3). QTLs, with either negative or positive additive effect were found, which indicated that both parents contributed favorable alleles. More favourable alleles come from tolerant parent PA64s. Ten QTLs were associated

Table 3 Pearson correlation matrix of traits measured in response to salt stress at 50 and 100 mM NaCl in 93-11/PA64s RIL population at seedling stage

Traits	Stress level	SL (cm)	RL (cm)	SFW (mg)	SDW (mg)	RFW (mg)
RL(cm)	L50	0.1817				
	L100	0.3646 **				
SFW(mg)	L50	0.4005**	0.1176			
	L100	0.2268 *	0.2399*			
SDW(mg)	L50	0.3123**	0.1730	0.5601**		
	L100	0.4732**	0.2975**	0.3278**		
RFW(mg)	L50	0.2750*	0.3506**	0.2654*	0.3517**	
	L100	0.2531*	0.2656*	0.3818**	0.3604**	
RDW(mg)	L50	0.1612	0.2618*	0.2317*	0.3220**	0.5321**
	L100	0.2033*	0.2487*	0.3691**	0.4840**	0.5762 **

* and ** indicate at 5% and 1% significant level, respectively

with SL detected on chromosomes 1, 3, 6, 7 and 10 and each accounted for 6.8–18.20% of the phenotypic variation with LOD values ranging from 2.6 to 5.9. Eleven QTLs for RL were identified on chromosomes 2, 3, 4, 5, 6, 9 and 10 and each accounted for 5.9–23.7% of phenotypic variation. Five QTLs related to SFW on chromosome 1, 6 and 7, each explained 9.8–18.8% phenotypic variation. Six QTLs associated with SDW were detected on chromosomes 6, 7 and 10 were found with 11.2–16.3% phenotypic variation and LOD values ranges from 3.23 to 4.93. Only two QTLs detected on chromosomes 1 and 6 associated with RFW and each explained 7.50% and 10.8% phenotypic variation. Four QTLs were identified for RDW on chromosomes 3, 6 and 7 and each associated with 9.8–12.1% phenotypic variation.

Six QTL loci with five QTLs clusters were repeatedly detected across the two stress levels on chromosomes 1, 3, 6 and 7 (Fig. 3). Thus, 21 QTLs were identified as stable QTLs (Table 5). Stable QTLs were defined based on three criteria, including overlapped QTLs associated with multiple traits in a single stress level, QTLs repeatedly detected in the two stress levels and locus found overlapped with previously reported QTLs. Except for locus 4, all loci of cluster were found overlapped with previous studies (Bimpong et al. 2013; De Leon et al. 2016; Ghomi et al. 2013; Kim et al. 2009; Kumar et al. 2015; Lekklar et al. 2019). Locus 4 consisted of a novel QTL *qSL7* (L50 and L100), and explained 7.5% (L50) and 6.8% (L100) phenotypic variation (Table 4).

Development and characterization of CSSL-*qSL7* under salinity

In order to confirm the effect of *qSL7* for seedling height under salt stress, one chromosomal segment substitution line (CSSL) CSSL-*qSL7*, harboring the segment of *qSL7* from PA64s between the two SSR markers RM21930 and

RM22105 (Fig. 4c, d), was developed. The parent 93-11 and CSSL-*qSL7* were evaluated under control and salt stress conditions (Fig. 4a, b), the seedling height of the CSSL-*qSL7* was significantly higher than that of 93-11 (Fig. 4e). Ion analysis showed that the concentration of K⁺ and Na⁺ were significantly higher and lower, respectively in tolerant CSSL-*qSL7* than 93-11 (Fig. 4f, g), which indicated CSSL-*qSL7* was significantly tolerant to salt stress than 93-11. Therefore, the CSSL-*qSL7* was backcrossed with the parent 93-11 and a total of 1300 (BC₅F_{2,3}) seedlings obtained from the cross were screened for fine mapping of *qSL7* under salt stress.

Fine mapping of *qSL7*

For fine mapping, a total of 1300 BC₅F_{2,3} individual plants derived from CSSL-*qSL7*/93-11 were screened with SSR markers RM21930 and RM22105 flanking *qSL7*. Based on the resequencing data of 93-11 and PA64s, several Insertion/Deletion markers were developed to screen the population (Table 1). According to the SL value, only homozygous recombinant lines with SL > 24 cm or SL < 20 cm were selected for fine mapping. Combined phenotype and genotype analysis, *qSL7* was finally delimited to a 252.9 kb region between markers Indel 7-2 and Indel 7-3 on chromosome 7 (Fig. 5). According to the Rice Genome Annotation Project Database (<http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/>), there were 40 annotated genes in the target region (Table 6), including 33 expressed proteins, a hypothetical protein, and 6 retrotransposons. Among them, *LOC_Os07g43530*, which encodes the DNA-binding domain-containing protein, was reported previously as a major transcription factor regulating genes for potassium ion transporters and thereby controlling potassium homeostasis during salt stress.

Table 4 QTLs for traits related to seedling-stage salt tolerance in 93-11/PA64s RIL population across two stress levels

Traits	QTL	Chr.	Interval	LOD	Var %	Add.
<i>L50</i>						
SL	<i>qSL1</i>	1	SNP1-16–SNP1-29	4.81	13.1	2.65
	<i>qSL6</i>	6	SNP6-99–SNP6-106	5.61	17.3	– 3.05
	<i>qSL7</i>	7	SNP7-191–SNP7-226	2.81	7.5	2.00
	<i>qSL7.1</i>	7	SNP7-131–SNP7-170	2.80	9.9	2.59
RL	<i>qRL2</i>	2	SNP2-265–SNP2-292	2.93	6.7	– 0.96
	<i>qRL3</i>	3	SNP3-167–SNP3-183	5.02	11.6	1.26
	<i>qRL4</i>	4	SNP4-159–SNP4-198	4.97	11.5	– 1.26
	<i>qRL4.1</i>	4	SNP4-333–SNP4-379	4.07	11.2	2.21
	<i>qRL6</i>	6	SNP6-73–SNP6-81	3.76	10.2	– 1.18
	<i>qRL10</i>	10	SNP10-112–SNP10-149	2.74	9.1	1.98
	<i>qRL10.1</i>	10	SNP10-153–SNP10-176	2.62	5.9	0.90
	SFW	<i>qSFW1</i>	1	SNP1-17–SNP1-36	2.86	9.8
<i>qSFW6</i>		6	SNP6-45–SNP6-81	5.70	18.8	– 238.77
<i>qSFW7</i>		7	SNP7-133–SNP7-149	3.11	10.0	174.33
SDW	<i>qSDW6</i>	6	SNP6-45–SNP6-78	4.93	16.3	– 40.03
	<i>qSDW7</i>	7	SNP7-133–SNP7-155	3.45	11.2	33.17
RDW	<i>qRDW3</i>	3	SNP3-165–SNP3-184	2.95	9.8	4.81
	<i>qRDW6</i>	6	SNP6-57–SNP6-82	3.22	11.2	– 5.15
<i>L100</i>						
SL	<i>qSL1</i>	1	SNP1-16–SNP1-28	4.70	12.6	2.60
	<i>qSL3</i>	3	SNP3-139–SNP3-163	3.67	10.7	2.23
	<i>qSL6</i>	6	SNP6-44–SNP6-66	5.16	17.9	– 2.90
	<i>qSL6.1</i>	6	SNP6-99–SNP6-106	5.90	18.2	– 3.12
	<i>qSL7</i>	7	SNP7-198–SNP7-226	2.60	6.8	1.91
	<i>qSL10</i>	10	SNP10-99–SNP10-107	3.63	13.5	2.52
RL	<i>qRL2</i>	2	SNP2-339–SNP2-357	3.20	12.0	1.14
	<i>qRL5</i>	5	SNP5-269–SNP5-289	6.18	23.7	2.08
	<i>qRL6</i>	6	SNP6-1–SNP6-8	3.24	11.2	– 1.43
SFW	<i>qSFW6</i>	6	SNP6-45–SNP6-81	5.20	16.8	– 229.87
	<i>qSFW7</i>	7	SNP7-133–SNP7-171	3.60	11.9	193.13
	<i>qSDW4</i>	4	SNP4-32–SNP4-51	3.32	10.8	– 25.97
SDW	<i>qSDW6</i>	6	SNP6-45–SNP6-78	4.80	15.6	– 39.30
	<i>qSDW6.1</i>	6	SNP6-3–SNP6-35	3.23	12.0	– 27.30
	<i>qSDW7</i>	7	SNP7-134–SNP7-174	3.90	13.0	35.84
	<i>qSDW10</i>	10	SNP10-48–SNP10-75	3.63	12.5	27.90
RFW	<i>qRFW1</i>	1	SNP1-16–SNP1-33	2.50	7.50	49.20
	<i>qRFW6</i>	6	SNP6-99–SNP6-109	2.80	10.8	– 58.88
RDW	<i>qRDW6</i>	6	SNP6-48–SNP6-81	4.00	12.1	– 5.60
	<i>qRDW7</i>	7	SNP7-133–SNP7-155	3.90	11.6	5.48

Discussion

Advancement in the salt tolerant rice breeding improvement would have a significant impact on food security particularly when the world facing the sea level rising issue due to global warming. However, developing tolerant varieties requires sufficient genetic diversity. QTL mapping is one of the effective ways of breeding programs to discover underlying salt

tolerant genes of quantitative traits. Previously many QTLs related to salt tolerance have been identified most of them covered large chromosomal intervals due to less availability of polymorphic marker, low-throughput, and the uneven genome-wide distribution (De Leon et al. 2016; Gimhani et al. 2016). However, the breakthrough of modern technology in molecular sector facilitated the high-resolution QTL mapping by the implementation of the high-throughput SNP

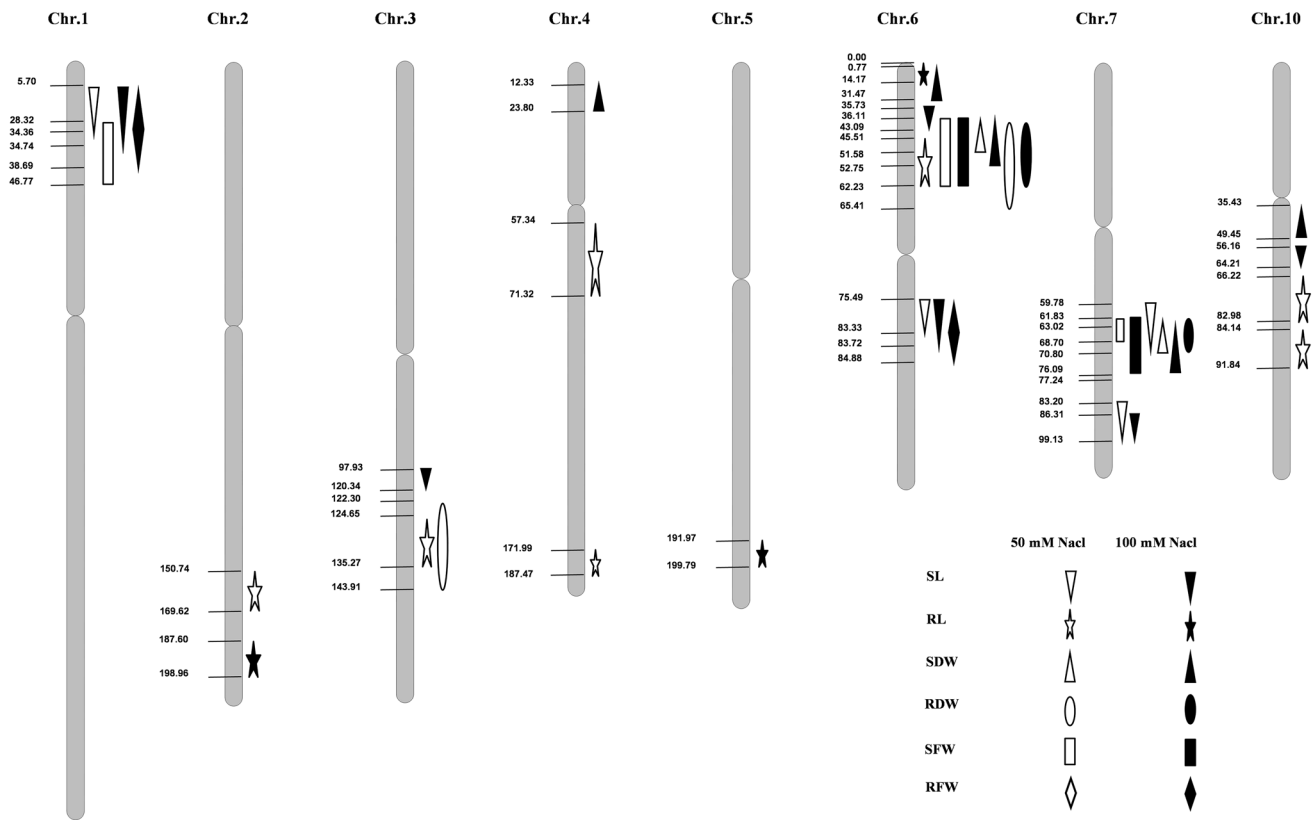


Fig. 3 Molecular linkage map showing the positions of QTLs for six traits investigated across two salt stress levels. Marker name are shown on the left in centimorgan (cM) along each chromosome

Table 5 Information on the 21 stable QTLs in 6 loci

Loci	QTL	Interval	Source of allele	known loci
Locus 1	<i>qSL1(L50)</i> , <i>qSFW1(L50)</i> , <i>qSL1(L100)</i> , <i>qRFW1(L100)</i>	SNP1-16–SNP1-36	PA64s	De Leon et al. (2016), Zang et al. (2008)
Locus 2	<i>qSL6(L50)</i> , <i>qSL6.1(L100)</i> , <i>qRFW6(L100)</i>	SNP6-99–SNP6-106	93-11	De Leon et al. (2016), Sabouri et al. (2009)
Locus 3	<i>qSL7.1(L50)</i> , <i>qSDW7(L50)</i> , <i>qSFW7(L100)</i> , <i>qSDW7(L100)</i> , <i>qRDW7(L100)</i>	SNP7-131–SNP7-170	PA64s	De Leon et al. (2016)
Locus 4	<i>qSL7(L50)</i> , <i>qSL7(L100)</i>	SNP7-198–SNP7-226	PA64s	
Locus 5	<i>qRL3(L50)</i> , <i>qRDW3(L50)</i>	SNP3-165–SNP3-184	PA64s	Kumar et al. (2015), Sabouri and Sabouri (2008)
Locus 6	<i>qRL6(L50)</i> , <i>qSFW6(L50)</i> , <i>qSDW6(L50)</i> , <i>qRDW6(L50)</i> , <i>qSL6(L100)</i> , <i>qSFW6(L100)</i> , <i>qRDW6(L100)</i>	SNP6-45–SNP6-81	93-11	Bimpong et al. (2013), De Leon et al. (2016), Sabouri et al. (2009), Sabouri and Sabouri (2008), Zang et al. (2008)

linkage map. In this study, we utilized a high-throughput genetic linkage map of a RIL population derived from a cross between 93-11 and PA64s to identify QTLs for SL, RL, SFW, SDW, RFW and RDW under moderate and high salt stress at the seedling stage. Rice shows sensitivity to salt stress mostly at the seedling stage of crop development with a threshold as low as an EC of -3 dSm^{-1} (Ahn et al. 2004; Bimpong et al. 2013). Salt stress resulted in a substantial reduction in root and shoot length followed by decreased

fresh and dry weight in RILs across both stress conditions compared to the control condition. Present findings revealed 38 putative QTLs for 6 traits on chromosomes 1, 2, 3, 4, 5, 6, 7 and 10.

Several studies have been reported that usually QTL cluster responsible for correlated traits lie in the same genomic region confirming stable QTL (Gimhani et al. 2016; Marathi et al. 2012; Yang et al. 2019). This QTLs cluster of different traits may be due to the presence of pleiotropy or

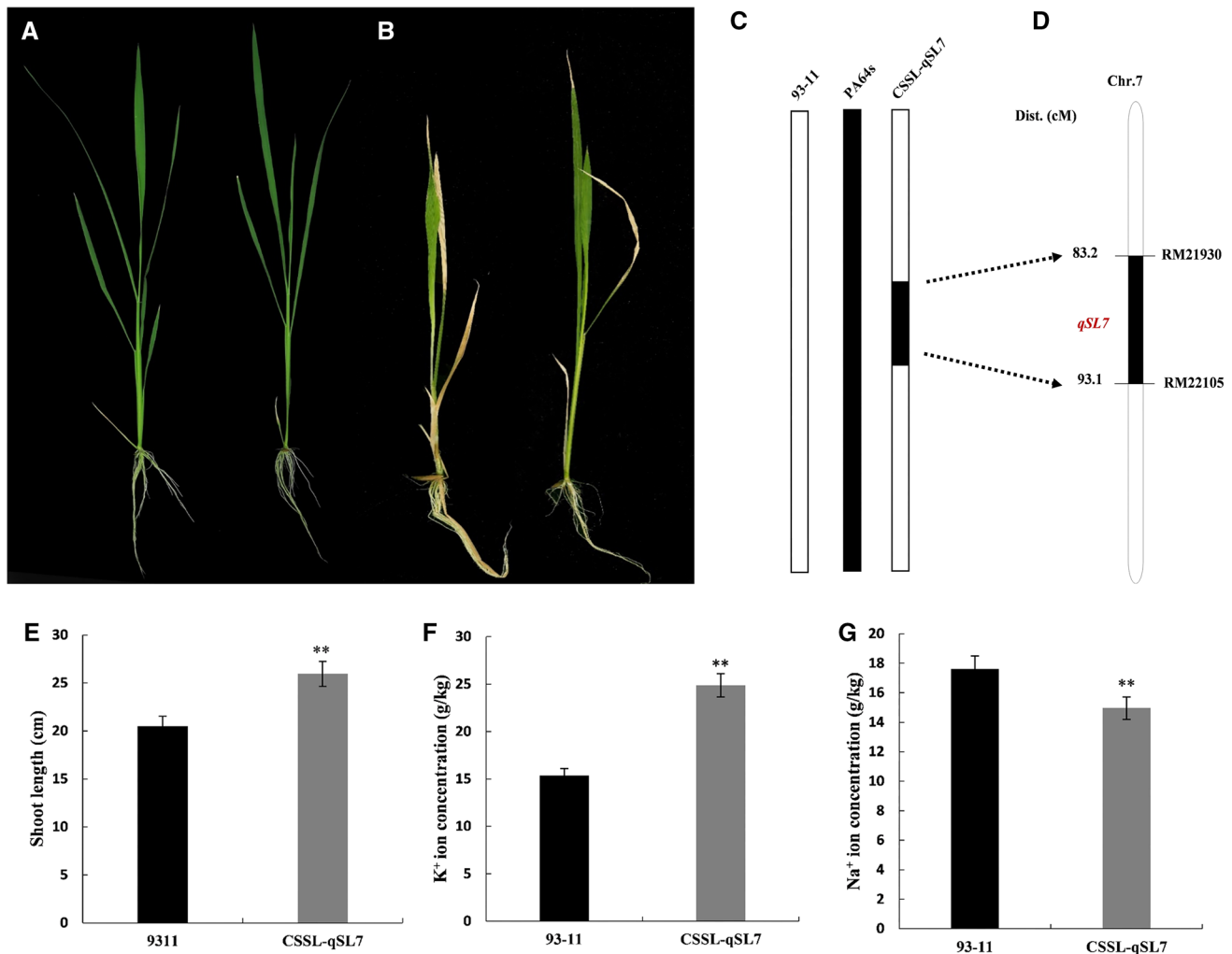


Fig. 4 Phenotypic and genotypic performance of the parent and CSSL-*qSL7*. **a, b** Shoot morphology of 93-11 (left) and CSSL-*qSL7* (right) under control and stress condition. **c** Graphical genotype of CSSL-*qSL7* compared to the parental lines (chromosomal segments

derived from PA64s in black). **d** Genetic location of *qSL7* on chromosome 7. **e–g** Shoot length, shoot K⁺ and Na⁺ concentration of 93-11 and CSSL-*qSL7*, respectively

tight linkage of genes controlling the traits (Gao et al. 2013; Gimhani et al. 2016). Therefore, co-located chromosomal regions responsible for different salinity tolerant traits may have an opportunity to introgress them together as a unit into rice varieties through MAS/MAB and to develop resilient salt-tolerant cultivars (Gimhani et al. 2016). In this study, the traits are significantly correlated to each other, confirming that QTLs responsible for different traits were co-localized at the specific genomic regions across chromosomes 1, 3, 6 and 7 forming strong QTL clusters. Six stable loci with five QTL clusters associated with salt tolerance were obtained, and each QTL accounted for 6.8–18.8% of the corresponding phenotypic variation. Further, cluster 1, 2 and 3 contain 4, 3 and 5 QTLs, respectively and locus 4 and 5 contains 2 QTLs each. Cluster 1 contains 4 QTLs across both stress levels which affects SL, SFW and RFW

shared a similar chromosomal region with previously identified QTL in between marker S1_5501756 and S1_5792183 by De Leon et al. (2016) using the SNP linkage map with *indica* derived mapping population. Also, a QTL cluster was found in the same location with cluster 1 in salt tolerant introgression lines in rice (Zang et al. 2008). In cluster 3 between SNP7-131 and SNP7-170 markers, 5 co-localized QTLs were found for 4 traits (SL, SFW, SDW, and RDW), which is also overlapped with reported QTLs for salt injury score and root length (De Leon et al. 2016; Sabouri et al. 2009). Moreover, in cluster 5 we identified 1 QTL cluster of 2 traits (RL and RDW) under moderate salt stress although it could not withstand under high salt stress level. These results suggested that root is highly affected by high salt concentration in the field. A similar finding was showed by Gimhani et al. (2016). The cluster 6 contains 7 stable QTLs

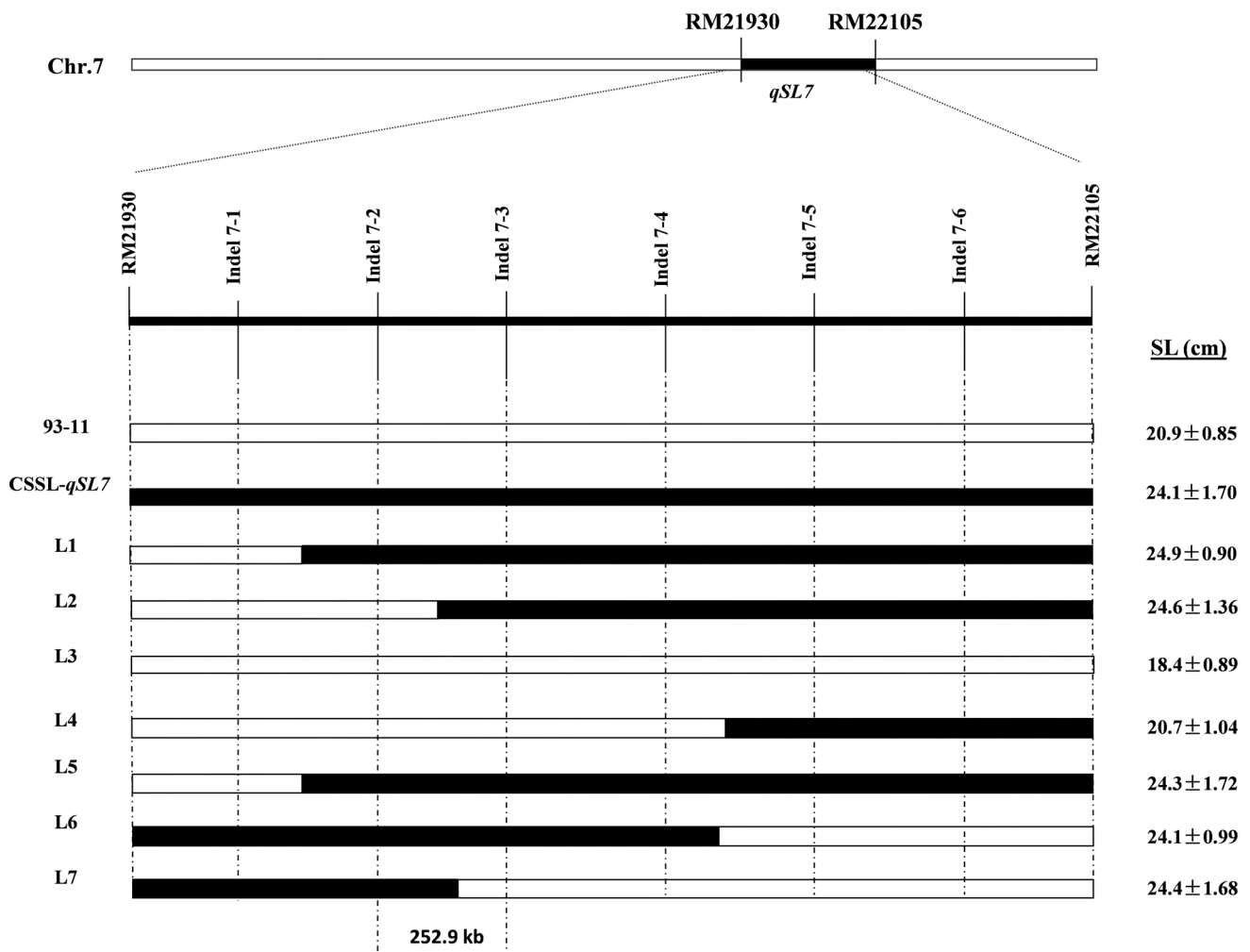


Fig. 5 Fine mapping of *qSL7* on chromosome 7. Locus *qSL7* was narrowed down to a 252.9 kb interval defined by markers Indel 7-2 and Indel 7-3. Values represent the mean \pm SD of SL

in between marker SNP6-45–SNP6-81 is overlapped with previously reported marker S6_5848568—S6_5905669, RM10-RM234, RM3027-RM5371 and RM586-RM253 (Bimpong et al. 2013; De Leon et al. 2016; Sabouri et al. 2009; Sabouri and Sabouri 2008; Zang et al. 2008).

One major potential QTL in cluster 4, *qSL7* accounted for 7.5 and 6.8% phenotypic variation, respectively and the allele contributed from tolerant parents PA64s was identified in both moderate and high salt stress conditions. This locus was stable and not previously reported for shoot length under salt stress condition thus, we selected *qSL7* to narrow down further.

Furthermore, one CSSL, CSSL-*qSL7*, which produced significantly higher shoot length was selected for further study and crossed with the parent 93-11 and a total of 1300 ($BC_5F_{2,3}$) seedlings obtained from the cross were screened for fine mapping of *qSL7* under high salt stress condition. Besides, CSSL-*qSL7* harboring *qSL7* locus was performed significantly

better than 93-11 in terms of shoot length and Na/K ion accumulation under high salt stress level, indicating *qSL7* could withstand under high salt stress condition. The gene *qSL7* was finally fine mapped to a region of 252.9 kb on the long arm of rice chromosome 7. Among 40 predicted genes in this region, only one gene *LOC_Os07g43530* DNA-binding domain containing protein belongs to basic helix-loop-helix (bHLH) family has been reported previously as a transcription factor that plays a positive role in salt stress tolerance by binds to the promoter of a potassium ion transporter gene to regulate potassium homeostasis during salt stress (Wu et al. 2015). A number of transcription factor of the bHLH gene family are important for regulating plant growth and plant responses to salt stress, such as *OsbHLH094*, *OsbHLH062*, and *OsbHLH035* already reported to take part in salt tolerance by DNA-binding in the promoter region of some ion transporter genes (Blumwald 2000; Chen et al. 2018; Toda et al.

Table 6 Annotated genes at the *qSL7* locus

Gene ID	Annotation
LOC_Os07g43360	MYST-like histone acetyltransferase 1, putative, expressed
LOC_Os07g43370	Amine oxidase family protein, putative, expressed
LOC_Os07g43380	Zinc finger, C3HC4 type domain containing protein, expressed
LOC_Os07g43390	4-alpha-glucanotransferase, chloroplast precursor, putative, expressed
LOC_Os07g43400	SWIM zinc finger family protein, putative, expressed
LOC_Os07g43410	Expressed protein
LOC_Os07g43420	MYB family transcription factor, putative, expressed
LOC_Os07g43430	Expressed protein
LOC_Os07g43440	Expressed protein
LOC_Os07g43450	Expressed protein
LOC_Os07g43460	Sphingolipid C4-hydroxylase SUR2, putative, expressed
LOC_Os07g43470	GTP-binding protein, putative, expressed
LOC_Os07g43480	Expressed protein
LOC_Os07g43490	Expressed protein
LOC_Os07g43500	Expressed protein
LOC_Os07g43510	40S ribosomal protein S9, putative, expressed
LOC_Os07g43530	Helix-loop-helix DNA-binding domain containing protein, expressed
LOC_Os07g43540	ORC6—Putative origin recognition complex subunit 6, expressed
LOC_Os07g43560	TKL_IRAK_DUF26-lc.24—DUF26 kinases have homology to DUF26 containing loci, expressed
LOC_Os07g43570	TKL_IRAK_DUF26-lc.25—DUF26 kinases have homology to DUF26 containing loci, expressed
LOC_Os07g43580	MYB family transcription factor, putative, expressed
LOC_Os07g43600	Ribonuclease T2 family domain containing protein, expressed
LOC_Os07g43604	Expressed protein
LOC_Os07g43610	Hypothetical protein
LOC_Os07g43630	Retrotransposon protein, putative, Ty3-gypsy subclass, expressed
LOC_Os07g43640	Ribonuclease T2 family domain containing protein, expressed
LOC_Os07g43650	Retrotransposon protein, putative, Ty3-gypsy subclass, expressed
LOC_Os07g43660	Expressed protein
LOC_Os07g43670	Ribonuclease T2 family domain containing protein, expressed
LOC_Os07g43690	Expressed protein
LOC_Os07g43700	Lactate/malate dehydrogenase, putative, expressed
LOC_Os07g43710	CSLA7—cellulose synthase-like family A; mannan synthase, expressed
LOC_Os07g43720	DCN1, putative, expressed
LOC_Os07g43730	Transcription elongation factor 1, putative, expressed
LOC_Os07g43740	Zinc finger, C3HC4 type domain containing protein, expressed
LOC_Os07g43750	Retrotransposon protein, putative, unclassified, expressed
LOC_Os07g43760	Retrotransposon protein, putative, unclassified
LOC_Os07g43770	Expressed protein
LOC_Os07g43780	Retrotransposon protein, putative, Ty3-gypsy subclass
LOC_Os07g43790	Retrotransposon protein, putative, unclassified, expressed

2013). Hence, *LOC_Os07g43530* may be the candidate gene for the *qSL7* locus associated with shoot length.

Conclusion

In this study, an *indica* derived population with a high-density genetic map was used to identify QTLs for rice salt tolerance at the seedling stage. A total of 38 QTLs were detected across two stress levels. Six clusters were identified with 21 stable QTLs. A novel major QTL for SL, *qSL7* was identified on chromosome 7. With the BC₅F_{2,3} population

derived from the CSSL-*qSL7*, it was delimited to a 252.9 kb region. Among 40 annotated genes identified in the target region, *LOC_Os07g43530* was previously reported as a transcription factor of the potassium ion transporter gene and might play an important role in regulating shoot length of rice under salt stress. Further study is needed for cloning of *qSL7* and understanding of its molecular and biological functions. Finally, these recombinant lines derived from CSSL-*qSL7* will provide genetic resources for improvement of rice salinity tolerance.

Acknowledgements This study was supported by the National Science-technology Support Projects (Grant No. 2015BAD01B02-02), the Natural Science Foundation of China (Grant Nos. 31461143014, 31701390 and 31801336), the National Key Research and Development Program of China (Grant No. 2016YFD0100902-07).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Ahn SJ, Shin R, Schachtman DP (2004) Expression of KT/KUP genes in Arabidopsis and the role of root hairs in K⁺ uptake. *Plant Physiol* 134:1135–1145
- Asif MA, Schilling RK, Tilbrook J, Brien C, Dowling K, Rabie H, Short L, Trittermann C, Garcia A, Barrett-Lennard EG, Berger B, Mather DE, Gilliam M, Fleury D, Tester M, Roy SJ, Person AS (2018) Mapping of novel salt tolerance QTL in an Excalibur x Kukri doubled haploid wheat population. *Theor Appl Genet* 131:2179–2196
- Bimpong IK, Manneh B, El-Namaky R, Diaw F, Amoah NKA, Saneh B, Ghislain K, Sow A, Singh R, Gregorio GB, Bizimana JB, Wopereis MC (2013) Mapping QTLs related to salt tolerance in rice at the young seedling stage using 384-plex single nucleotide polymorphism SNP marker sets. *Mol Plant Breed* 5:47–63
- Blumwald E (2000) Sodium transport and salt tolerance in plants. *Curr Opin Cell Biol* 12:431–434
- Bonilla P, Mackell D, Deal K, Gregorio G (2002) RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. *Philipp Agric Sci* 85:68–76
- Chen HC, Cheng WH, Hong CY, Chang YS, Chang MC (2018) The transcription factor OsbHLH035 mediates seed germination and enables seedling recovery from salt stress through ABA-dependent and ABA-independent pathways, respectively. *Rice* 11(1):50
- De Leon TB, Linscombe S, Subudhi PK (2016) Molecular dissection of seedling salinity tolerance in rice (*Oryza sativa* L.) using a high-density GBS-based SNP linkage map. *Rice* 9:52
- Flowers TJ (2004) Improving crop salt tolerance. *J Exp Bot* 55:307–319
- Flowers TJ, Flowers SA (2005) Why does salinity pose such a difficult problem for plant breeders? *Agric Water Manag* 78:15–24
- Gao ZY, Zhao SC, He WM, Guo LB, Peng YL, Wang JJ, Guo XS, Zhang XM, Rao YC, Zhang C, Dong GJ, Zheng FY, Lu CX, Hu J, Zhou Q, Liu HJ, Wu HY, Xu J, Ni PX, Zeng DL, Liu DH, Tian P, Gong LH, Ye C, Zhang GH, Wang J, Tian FK, Xue DW, Liao Y, Zhu L, Chen MS, Li JY, Cheng SH, Zhang GY, Wang J, Qian Q (2013) Dissecting yield-associated locus in super hybrid rice by resequencing recombinant inbred lines and improving parental genome sequences. *Proc Natl Acad Sci* 110:14492–14497
- Ghomi K, Rabiei B, Sabouri H, Sabouri A (2013) Mapping QTLs for traits related to salinity tolerance at seedling stage of rice (*Oryza sativa* L.): an agrigenomics study of an Iranian rice population. *OMICS* 17:242–251
- Gimhani DR, Gregorio GB, Kottareachchi NS, Samarasinghe WL (2016) SNP-based discovery of salinity-tolerant QTLs in a biparental population of rice (*Oryza sativa*). *Mol Genet Genomics* 291:2081–2099
- Gregorio GB, Senadhira D, Mendoza RD (1997) Screening rice for salinity tolerance. *IRRI Discussion paper series no. 22*
- 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
- 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* 12:121–128
- Kumar V, Singh A, Mithra SV, Krishnamurthy SL, Parida SK, Jain S, Tiwari KK, Kumar P, Rao AR, Sharma SK, Khurana JP, Singh NK, Mohapatra T (2015) Genome-wide association mapping of salinity tolerance in rice (*Oryza sativa*). *DNA Res* 22:133–145
- Lekklar C, Pongpanich M, Suriya-Arunroj D, Chinpongpanich A, Tsai H, Comai L, Chadchawan S, Buaboocha T (2019) Genome-wide association study for salinity tolerance at the flowering stage in a panel of rice accessions from Thailand. *BMC Genomics* 20:76
- Li Q, Yang A, Zhang WH (2016) Efficient acquisition of iron confers greater tolerance to saline-alkaline stress in rice (*Oryza sativa* L.). *J Exp Bot* 67:6431–6444
- Luo ZY, Zhou G, Chen XH, Lu QH, Hu WX (2001) Isolation of high-quality genomic DNA from plants. *Hunan Yi Ke Da Xue Bao* 26:178–180
- Marathi B, Guleria S, Mohapatra T, Parsad R, Mariappan N, Kurungara VK, Atwal SS, Prabhu KV, Singh NK, Singh AK (2012) QTL analysis of novel genomic regions associated with yield and yield related traits in new plant type based recombinant inbred lines of rice (*Oryza sativa* L.). *BMC Plant Biol* 12:137
- McCouch SR (2008) Gene nomenclature system for rice. *Rice* 1:72–84
- Negrão S, Courtois B, Ahmadi N, Abreu I, Saibo N, Oliveira MM (2011) Recent updates on salinity stress in rice: from physiological to molecular responses. *Crit Rev Plant Sci* 30:329–377
- Prasad SR, Bagali PG, Hittalmani S, Shashidhar HE (2000) Molecular mapping of quantitative trait locus associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.). *Curr Sci* 78:162–164
- Ren ZH, Gao JP, Li GL, Cai XL, Huang W, Chao DY, Zhu MZ, Wang ZY, Luan S, Lin HX (2005) A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nat Genet* 37:1141–1146
- Sabouri H, Sabouri A (2008) New evidence of QTLs attributed to salinity tolerance in rice. *Afr J Biotechnol* 7:4376–4383
- Sabouri H, Rezai AM, Moumeni A, Kavousi A, Katouzi M, Sabouri A (2009) QTLs mapping of physiological traits related to salt tolerance in young rice seedlings. *Biol Plant* 53:657–662
- 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

- Thomson MJ, Zhao K, Wright M, McNally KL, Rey J, Tung CW, Reynolds A, Scheffler B, Eizenga G, McClung A, Kim H, Ismail AM, Ocampo M, Mojica C, Reveche MY (2011) High-throughput single nucleotide polymorphism genotyping for breeding applications in rice using the BeadXpress platform. *Mol Breed* 29:875–886
- Toda Y, Tanaka M, Ogawa D, Kurata K, Kurotani K, Habu Y, Ando T, Sugimoto K, Mitsuda N, Katoh E, Abe K, Miyao A, Hirochika H, Hattori T, Takeda S (2013) Rice salt sensitive 3 forms a ternary complex with JAZ and class-C bHLH factors and regulates Jasmonate-induced gene expression and root cell elongation. *Plant Cell* 25:1709–1725
- Wu H, Ye H, Yao R, Zhang T, Xiong L (2015) OsJAZ9 acts as a transcriptional regulator in Jasmonate signaling and modulates salt stress tolerance in rice. *Plant Sci* 232:1–12
- Yamaguchi T, Blumwald E (2005) Developing salt-tolerant crop plants: challenges and opportunities. *Trends Plant Sci* 10:615–620
- Yang J, Sun K, Li D, Luo L, Liu Y, Huang M, Yang G, Liu H, Wang H, Chen Z, Guo T (2019) Identification of stable QTLs and candidate genes involved in anaerobic germination tolerance in rice via high-density genetic mapping and RNA-Seq. *BMC Genomics* 20:355
- Zang J, Sun Y, Wang Y, Yang J, Li F, Zhou Y, Zhu L, Jessica R, Mohammadhosein F, Xu J, Li Z (2008) Dissection of genetic overlap of salt tolerance QTLs at the seedling and tillering stages using backcross introgression lines in rice. *Sci China Ser C* 51:583–591
- Zhang Y, Lv Y, Jahan N, Chen G, Ren D, Guo L (2018) Sensing of abiotic stress and ionic stress responses in plants. *Int J Mol Sci* 19(11):3298

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.