Mapping of quantitative trait loci for seedling salt tolerance in maize

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Abstract Improved salt tolerance is a major goal for maize breeders, but the control of salt tolerance-related traits in maize is poorly understood. A set of 209 doubled haploid (DH) lines derived from the maize hybrid Xianyu335 was genotyped using 1335 single-nucleotide polymorphism (SNP) markers. Biomass-related traits under salt stress were used as salt tolerance indicators, and a quantitative trait loci (QTL) analysis was conducted in hydroponic culture under salt-stress and normal conditions. Fourteen traits showed significant phenotypic differences between parents, and 16 traits showed transgressive segregation in the DH population. We detected 65 QTL: 41 associated with salt tolerance and 24 related to normal growth. Salt tolerance-related QTL clustered on chromosomes 1, 3, 7, and 9, among which 13 major effect QTL on chromosome 1 individually explained more than 21% of the phenotypic variation. Of these large-effect QTL, QTL controlling seven traits upon salt treatment and two salt tolerance indexes were co-located; they extensively overlapped with the remaining four co-localized QTL related to four other salt tolerance indexes. The QTL identified here shed new light on the molecular mechanism of salt tolerance in maize and may provide robust functional markers for marker-assisted selection of salt tolerant maize varieties.

Introduction

Along with rice and wheat, maize is one of the three main cereal crops that account for more than half of worldwide calorie consumption (Schnable 2015). The global demand for maize is rising, and by 2050, maize production may need to increase by 100% to meet the market's requirements (Ray et al. 2013). However, maize is moderately sensitive to salinity stress, and so its supply is threatened by saline soils (Cui et al. 2015a; Farooq et al. 2015; Zhang et al. 2017). More than 6% of the world's land area and 20% of cultivated regions are affected by salinity (Chang et al. 2014; Munns and Tester 2008; Zhu 2001). To meet the sustained demands of maize production, the development and cultivation of salt-tolerant maize varieties have therefore become the main focus of maize breeding programs.

Salinity causes osmotic stress, ion toxicity, and oxidative stress in plants, thereby leading to the dysregulation of biological and physiological processes and substantial losses in crop productivity (Deinlein et al. 2014; Farooq et al. 2015). To survive under salt stress, plants have evolved a number of tolerance and resistance

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strategies (Muchate et al. 2016; Zhu 2016). Considerable intraspecific genetic variation in salt tolerance is present in maize (Cui et al. 2015a; Farooq et al. 2015; Luo et al. 2017a; Zhang et al. 2017). Hence, tremendous efforts have been made to exploit the genetic control of salt tolerance in this species. Several quantitative trait loci (QTL) related to salt tolerance have been identified in different maize populations. With an $F_{2:5}$ recombinant inbred line population and single-nucleotide polymorphism (SNP) markers, 38 salt tolerance-related QTL for the traits of germination rate, salt tolerance ranking, shoot fresh and dry weight, tissue water content, shoot Na^+ and K^+ concentration, and shoot K^+/Na^+ ratio were identified. These QTL were mapped on 7 out of 10 maize chromosomes, except for chromosome 2 (chr2), chr8, and chr10 (Cui et al. 2015b). Using a doubled haploid (DH) population and SNP markers, and taking plant height in saline field as well as the salt tolerance index of plant height (that in a saline field compared with that in a normal field) as evaluation indicators, 10 QTL for salt tolerance were mapped onto chr1, chr3, chr4, chr5, chr9, and chr10 (Luo et al. 2017b).

Additionally, a few genes associated with salt tolerance have been isolated from maize and characterized. Several were transcription factors genes, such as *ZmbZIP72* (Ying et al. 2012), *Zmhdz10* (Zhao et al. 2014), and *ZmWRKY58* (Cai et al. 2014), and some were protein kinase genes, such as *ZmSIMK1* (Gu et al. 2010), *SnRK2* (Ying et al. 2011), and *ZmSnRK2.11* (Zhang et al. 2015). Recently, an HKT-type transporter (*ZmHKT1*) was identified from a major salt-tolerance QTL (*ZmNC1*) and was demonstrated to regulate Na^+ transport across the cell plasma membrane (Zhang et al. 2017). Those studies laid a solid foundation for dissecting the genetic basis of salt tolerance, and for marker-assisted selection (MAS) of salt-tolerant maize varieties. However, few genetic loci underlying variations in salt tolerance in maize have been described.

Maize is more sensitive to salt stress at the early seedling stage than at later developmental stages (Farooq et al. 2015). Compared with mature plants, maize seedlings not only have the advantage of early phenotypic determination but can also be analyzed in a well-conditioned environment, which reduces the effect of external interference. Therefore, QTL analysis for salt tolerance at the maize seedling stage is useful for exploring the molecular mechanism of salt tolerance.

In the present study, we screened a maize DH population in a growth chamber with and without a salinity treatment to detect QTL for a number of salt tolerance sub-traits at the early seedling stage. Our analyses led to the discovery of major QTL for salt tolerance on chromosome 1. This may facilitate the development of potential molecular markers that can be used in marker-assisted selection for salt-tolerant maize varieties.

Materials and methods

Plant materials and treatments

A DH population containing 209 lines derived from the maize hybrid Xianyu335 (PH6WC × PH4CV) was used in this study. Haploid plants were developed by pollinating Xianyu335 with the parthenogenetic-inducing line Jingkeyou006. Then, they were artificially doubled with colchicine to obtain DH maize lines (Shi et al. 2017). Jingkeyou006 was cultivated by the Maize Research Center of Beijing Academy of Agriculture and Forestry Sciences. The parental line PH6WC was salt tolerant, and the other parental line PH4CV was salt sensitive (Luo et al. 2017b). Both PH6WC and PH4CV were developed by DuPont Pioneer (Johnston, IA, USA).

Maize seeds of the DH population and their parents were surface-sterilized with 1% NaClO solution for 10 min and then were rinsed three times with sterile water. Afterwards, seeds were sown in the grooves of a maize seedling identification instrument (Chinese patent number: ZL200920177285.0) according to the manufacturer's operation guidelines (Luo et al. 2017a). Briefly, seeds were fixed between two sheets of vertically placed filter papers, and the bottom of filter papers was submerged in a nutrient solution to provide nutrients. For planting experiments, 10 seeds were planted per row, with a row length of 18 cm and 3 cm space between rows. The seed planting analyses had three biological replicates, and every DH line was planted in a row in each replicate. A randomized complete block design was applied. The culture apparatuses were placed in a growth chamber under a constant 26 °C, a 12-h/12-h light/dark cycle (150–180 µmol m^{-2} s^{-1}), and 60% relative humidity. For phenotypic analyses, maize seeds were hydroponically grown in sterile water without (control) or with (salt treatment) 100 mM NaCl for 10 days. The culture solution was renewed every 2 days during seedling culture.

Trait measurements

After hydroponic culture for 10 days, seeds were removed from maize seedlings and then the seedlings were cut at the coleoptilar nodes with scissors. Then, shoot length (SL) of seedlings was measured from the top of the longest leaf to the coleoptilar node, and root length (RL) was calculated from the coleoptilar node to the end of the longest roots (Yu et al. 2018). The shoot fresh weight (SF) and root fresh weight (RF) of each genotype per replicate were measured, and then seedling tissues were oven-dried at 80 °C for 3 days to obtain values for seedling shoot dry weight (SD) and root dry weight (RD). The salt tolerance index (R) of each trait was calculated by comparing the phenotypic value under salt-stress treatment (S) with that in the control (e.g., RLR = RLS/RL) (Luo et al. 2017c).

Linkage map construction and QTL identification

Total genomic DNA was extracted from the seedling of the DH lines using the CTAB method, and the MaizeSNP3072 chip (Tian et al. 2015) was used for genotype determination. The comparative linkage map of the DH population was constructed using Kosambi's regression function of the JoinMap4 software (Van Ooijen 2006) with a minimum logarithm of odds (LOD) of 2.0. For QTL analysis, the average value of three biological replicates for each trait was used as input data. Composite interval mapping was performed using Windows QTL Cartographer software with a walking speed of 1 cM and an LOD threshold of 2.5.

Data analysis

The phenotypic differences between the parental lines PH6WC and PH4CV were determined by Student's t test. Student's t test was performed using the GraphPad Prism program (http://www.graphpad.com/). The means, standard deviations, and coefficients of variation were assessed utilizing the column statistics functional module in the GraphPad Prism program. ANOVA analysis was performed using the aov function in the agricolae package in R version 3.4.4 (http://www.r-project.org/). The repeatability for each trait was calculated using the formula: repeatability = $\sigma_G^2/(\sigma_G^2 + \sigma_e^2)$, where σ_G^2 represents the genetic variance and σ_e^2 represents the error variance (Cui et al. 2015b). Phenotypic frequency distribution, correlation analysis, and linear regression analyses were determined using R.

Results

Phenotypic variation and correlation analysis

The maize seeds were treated without (control) or with 100 mM NaCl for 10 days, and the following nine biomass-related traits were evaluated: RL, SL, full length (FL), RF, SF, full fresh weight (FF), RD, SD, and full dry weight (FD). These traits were used to calculate the salt tolerance indexes of all the materials (Table 1). The RLS, RLR, FLR, RFS, and RDS values were significantly higher for the salt-tolerant female parent PH6WC than for the salt-sensitive male parent PH4CV (Table 1; Fig. S1). Compared with PH6WC, PH4CV had significantly higher values for RL, SLS, SL, FL, SFS, SF, RD, SDS, and SD. The two parents showed no significant differences in SLR, FLS, RF, RFR, SFR, FFS, FF, FFR, RDR, SDR, FDS, FD, and FDR (Table 1). Correlation analysis results indicated the significant correlation among the three biological replicates for all 27 traits, and the Pearson's correlation coefficient (r) was more than 0.52 for all paired comparisons (data not shown). The variance effects of genotype on all 27 traits were extremely significant, and the variance of replicates for 17 traits was also significant. Except for RL, SL, and FL, the repeatability of the other 24 traits was greater than 61%, suggesting that the variance of these traits was mainly determined by genotype (Table 1). The mean values of all traits among the DH lines displayed large phenotypic variations, with the coefficient of variation ranging from 19.48 to 52.60%. The mean values of all traits were higher in the control than in the salt treatment (Table 1). The frequency distributions of all traits showed continuous segregation, indicating the involvement of polygenes (Fig. 1a–b).

Significant correlations were found among traits under both normal ($r = 0.14–0.91$) and salt-treatment conditions ($r = 0.28–0.96$) and also among salt tolerance indexes ($r = 0.35–0.94$) (Fig. 1a–b). As expected, salt tolerance indexes were significantly positively correlated with traits under salt treatments, and negatively correlated with traits under normal treatments. Biomass-related traits under salt treatments were weakly correlated with those in the control, indicating that they may be governed by different genetic mechanisms (Fig. S2).
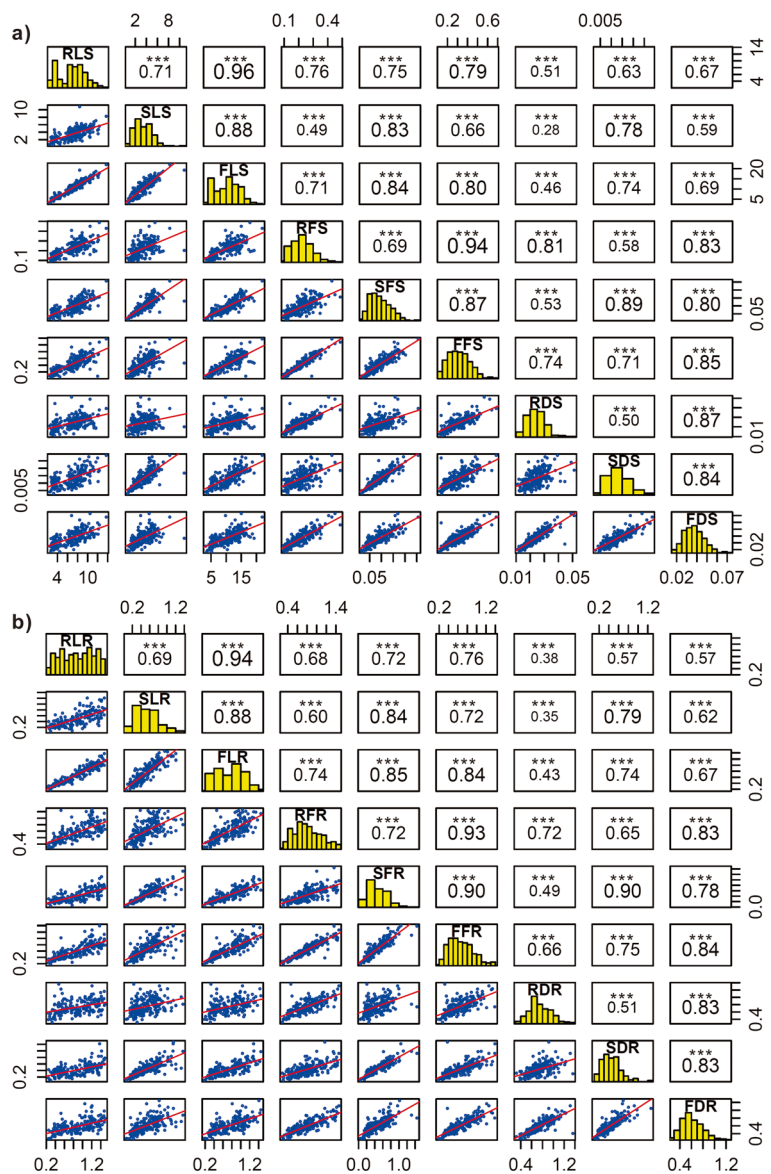
Linkage map construction

The genetic linkage map was constructed using the MaizeSNP3072 chip and 240 DH lines. Of the 3072

Table 1 Statistical summary of phenotypes of 27 traits in doubled haploid (DH) maize lines and their parents

Trait	Treatment	Mean PH6WC ± SD	Mean PH4CV ± SD	t test	DH lines			ANOVA analysis			
					Mean	Range	CV (%)	Genotype	Replication	Error	Repeatability (%)
RL (cm)	Salt	5.80 ± 0.47	4.27 ± 1.02	*	6.87 ± 2.60	2.47–13.72	37.89	19.442***	2.962*	0.900	87.3
	Control	8.21 ± 0.88	12.33 ± 2.06	*	7.26 ± 1.78	2.80–13.75	24.45	8.885***	0.345	2.396	47.4
	Salt tolerance index	0.72 ± 0.12	0.35 ± 0.11	**	0.89 ± 0.36	0.25–1.49	39.93	0.5956***	0.0864*	0.0205	90.3
SL (cm)	Salt	1.35 ± 0.10	3.78 ± 0.92	**	3.43 ± 1.54	0.63–10.84	45.00	7.071***	9.742***	1.226	61.4
	Control	6.51 ± 1.87	17.04 ± 3.67	**	6.67 ± 1.67	2.43–11.18	25.06	8.014***	0.721	1.687	55.6
	Salt tolerance index	0.22 ± 0.07	0.22 ± 0.02	ns	0.54 ± 0.27	0.06–1.37	49.77	0.23809***	0.25116***	0.03012	69.7
FL (cm)	Salt	7.14 ± 0.56	8.05 ± 1.89	ns	10.29 ± 3.85	3.25–21.95	37.44	43.31***	21.50***	3.05	81.5
	Control	14.72 ± 2.67	29.36 ± 5.17	**	13.93 ± 3.03	5.70–23.64	21.73	26.087***	1.543	5.825	53.7
	Salt tolerance index	0.50 ± 0.11	0.27 ± 0.05	**	0.75 ± 0.31	0.19–1.45	41.47	0.3472***	0.1369***	0.0180	85.9
RF (g)	Salt	0.24 ± 0.05	0.14 ± 0.03	*	0.22 ± 0.07	0.10–0.49	33.01	0.015611***	0.019936 ***	0.001238	79.5
	Control	0.42 ± 0.07	0.34 ± 0.03	ns	0.28 ± 0.07	0.10–0.57	26.07	0.015875***	0.007190**	0.001172	80.7
	Salt tolerance index	0.59 ± 0.18	0.42 ± 0.12	ns	0.81 ± 0.28	0.29–1.46	34.62	0.3251***	0.2613***	0.0190	84.3
SF (g)	Salt	0.05 ± 0.01	0.12 ± 0.02	***	0.09 ± 0.04	0.02–0.26	45.73	0.005507***	0.000806	0.000510	76.6
	Control	0.24 ± 0.09	0.45 ± 0.08	*	0.22 ± 0.06	0.07–0.37	27.21	0.010147***	0.003373*	0.000917	77.0
	Salt tolerance index	0.21 ± 0.09	0.26 ± 0.02	ns	0.47 ± 0.25	0.05–1.44	52.60	0.17664***	0.01910	0.01255	81.3
FF (g)	Salt	0.29 ± 0.05	0.26 ± 0.05	ns	0.35 ± 0.11	0.12–0.69	34.36	0.03415***	0.02875***	0.00271	79.5
	Control	0.67 ± 0.16	0.80 ± 0.06	ns	0.50 ± 0.12	0.11–0.86	24.17	0.04138***	0.02041**	0.00327	79.5
	Salt tolerance index	0.46 ± 0.16	0.32 ± 0.05	ns	0.66 ± 0.26	0.19–1.39	38.70	0.10978***	0.04399**	0.00812	80.7
RD (g)	Salt	0.03 ± 0.005	0.02 ± 0.006	*	0.02 ± 0.007	0.01–0.05	27.22	1.27E-04***	2.578e-05	1.619e-05	69.4
	Control	0.04 ± 0.01	0.30 ± 0.002	**	0.03 ± 0.007	0.01–0.06	22.36	1.54E-04***	4.168e-05*	1.212e-05	79.6
	Salt tolerance index	0.76 ± 0.17	0.65 ± 0.21	ns	0.75 ± 0.19	0.32–1.36	25.79	0.23813***	0.02338	0.01789	80.4
SD (g)	Salt	0.009 ± 0.0004	0.02 ± 0.005	**	0.01 ± 0.005	0.003–0.03	38.63	7.60E-05***	4.102e-05**	7.280e-06	75.9
	Control	0.03 ± 0.01	0.05 ± 0.01	*	0.03 ± 0.007	0.01–0.05	21.77	1.28E-04***	3.425e-05	1.254e-05	75.5
	Salt tolerance index	0.29 ± 0.10	0.38 ± 0.03	ns	0.45 ± 0.20	0.10–1.26	43.01	0.10978***	0.04399**	0.00812	80.7
FD (g)	Salt	0.04 ± 0.005	0.04 ± 0.01	ns	0.04 ± 0.01	0.02–0.07	27.11	2.96E-04***	9.085e-05	3.362e-05	72.3
	Control	0.07 ± 0.01	0.08 ± 0.01	ns	0.06 ± 0.01	0.02–0.09	19.48	0.0004211***	0.0001613*	0.0000352	78.5
	Salt tolerance index	0.56 ± 0.16	0.47 ± 0.08	ns	0.61 ± 0.18	0.27–1.25	29.54	0.11542***	0.02496	0.00920	79.4

RL root length, *SL* shoot length, *FL* full length, *RF* root fresh weight, *SF* shoot fresh weight, *FF* full fresh weight, *RD* root dry weight, *SD* shoot dry weight, *FD* full dry weight. SLS, RLS, FLS, SFS, RFS, FFS, SDS, RDS, and FDS represent traits under salt treatment. SLR, RLR, FLR, SFR, RFR, FFR, SDR, RDR, and FDR represent salt tolerance indexes of traits. *CV* coefficient of variation. *Significant at $p = 0.05$, **significant at $p = 0.01$, ***significant at $p = 0.001$

Fig. 1 Frequency distribution, linear regression, and correlation analyses of phenotypic data. **a** Biomass-related traits under salt treatment. **b** Salt tolerance indexes of biomass-related traits. Graphs on the diagonal position represent the phenotypic frequency distribution. Graphs located on the upper right of the diagonal represent correlation coefficients, while on the lower left of the diagonal represent linear regression of phenotypic data. RLS, SLS, FLS, RFS, SFS, FFS, RDS, SDS, and FDS represent root length (RL), shoot length (SL), full length (FL), root fresh weight (RF), shoot fresh weight (SF), full fresh weight (FF), root dry weight (RD), shoot dry weight (SD), and full dry weight (FD) under salt treatment (S), respectively; SLR, RLR, FLR, SFR, RFR, FFR, SDR, RDR, and FDR represent salt tolerance index (R) of traits. ***Significant at $p = 0.001$

SNPs, a total of 1335 polymorphic SNP markers were detected and were used for genetic map construction. This linkage map covered all 10 chromosomes and spanned 1379.95 cM of the maize genome with an average interval of 1.03 cM between marker loci.

QTL mapping

Using the average values of three biological replicates of each trait as input data and the composite interval mapping method, we were able to identify a total of 65 QTL for all 27 traits. In this study, traits under salt treatment as well as salt tolerance indexes were considered as salt tolerance-related traits, and identified QTL for these traits were regarded as QTL for salt tolerance. In total, 41 QTL for salt tolerance-related traits were obtained and were located on chr1, chr2, chr3, chr4, chr7, and chr9 (Table 2). In this study, any QTL with a phenotypic variation larger than 20% was defined as a major effect QTL. Under salt-treatment conditions, one major QTL cluster for RLS, SLS, FLS, RFS, SFS, FFS, and FDS was identified on chr1. All these QTL were co-located, including *qRLS1* (LOD, 48.28; proportion of phenotypic variance explained, PVE, 63.19%; additive effect, Add, 2.35 cm), *qSLS1–2* (LOD, 16.94; PVE, 24.71%; Add, 0.91 cm), *qFLS1-2* (LOD, 40.46; PVE, 55.21%; Add, 0.91 cm),

Table 2 Summary of quantitative trait loci (QTL) for 27 traits under control and salt-stress conditions

Trait	QTL[a]	Chr	Position (cM)	Marker Interval (bp)[b]	LOD[c]	PVE (%)[d]	Add[e]
RLS (cm)	qRLS1	1	86.68	119,220,296—194,632,379	48.28	63.19	2.35
SLS (cm)	qSLS1-1	1	39.82	12,109,561—19,031,623	3.27	4.08	− 0.35
	qSLS1-2	1	86.65	119,220,296—194,632,379	16.94	24.71	0.91
	qSLS1-3	1	177.87	296,628,427—297,702,831	6.52	8.62	− 0.46
	qSLS3	3	100.82	164,583,401	2.62	3.05	− 0.30
	qSLS4	4	55.92	151,200,735—170,217,401	3.66	4.58	− 0.34
	qSLS9	9	49.15	111,416,088—133,015,204	2.93	3.64	0.30
FLS (cm)	qFLS1-1	1	39.82	17,766,414—19,031,623	2.84	2.47	− 0.70
	qFLS1-2	1	86.65	119,220,296—194,632,379	40.46	55.21	3.36
	qFLS9	9	101.08	148,320,278—149,096,159	2.60	2.40	0.60
RFS (g)	qRFS1	1	86.65	119,220,296—194,632,379	23.92	37.09	0.05
	qRFS7	7	135.84	160,703,574—167,217,528	3.76	4.61	0.02
SFS (g)	qSFS1	1	86.65	119,220,296—194,632,379	16.94	27.90	0.03
	qSFS3	3	100.82	164,583,401—166,846,056	3.34	4.70	− 0.01
FFS (g)	qFFS1	1	86.65	119,220,296—194,632,379	22.83	35.64	0.07
	qFFS7	7	135.84	164,385,525—167,217,528	3.63	4.54	0.02
RDS (g)	qRDS1	1	83.66	172,752,437—194,359,703	7.04	12.48	0.002
	qRDS7	7	109.67	154,072,064—159,010,003	3.01	5.36	0.002
SDS (g)	qSDS1	1	86.65	119,220,296—194,632,379	9.12	15.36	0.002
	qSDS3	3	99.85	18,806,037—168,776,735	5.36	8.65	− 0.002
	qSDS9	9	73.12	139,126,847—146,636,204	3.61	5.72	0.001
FDS (g)	qFDS1	1	86.65	119,220,296—194,632,379	12.12	21.29	0.005
RLR	qRLR1	1	86.68	119,220,296—194,632,379	39.13	58.35	0.29
SLR	qSLR1–1	1	82.67	93,613,290—194,359,703	16.00	25.88	0.18
	qSLR1–2	1	179.87	296,628,427—297,702,831	4.13	5.67	− 0.06
	qSLR9	9	76.76	138,768,805—146,927,858	6.48	9.14	0.08
FLR	qFLR1	1	86.65	119,220,296—194,632,379	32.42	45.88	0.24
	qFLR2	2	73.72	149,695,765—150,300,040	2.82	2.75	0.07
	qFLR9	9	75.43	140,758,224—144,601,527	3.24	3.28	0.06
RFR	qRFR1	1	86.65	93,613,290—194,359,703	17.46	31.47	0.18
SFR	qSFR1–1	1	83.66	93,613,290—194,359,703	13.33	22.19	0.12
	qSFR1–2	1	179.87	297,702,831	3.31	4.89	− 0.05
	qSFR7	7	61.75	30,202,941—96,732,148	2.83	4.17	− 0.05
	qSFR9	9	76.76	142,503,481—144,601,527	2.60	3.81	0.05
FFR	qFFR1	1	83.66	93,613,290—194,359,703	17.84	29.59	0.15
	qFFR7	7	61.78	21,260,827—123,601,649	4.30	6.06	− 0.10
RDR	qRDR1	1	83.66	168,281,620—189,717,290	5.22	10.53	0.09
SDR	qSDR1	1	82.67	93,613,290—194,359,703	5.79	10.48	0.09
	qSDR9	9	76.76	138,768,805—146,927,858	5.90	9.84	0.06
FDR	qFDR1	1	83.66	93,613,290—194,359,703	9.95	18.30	0.11
	qFDR7	7	60.49	18,951,532—21,260,827	2.59	4.31	− 0.04
RL (cm)	qRL1	1	77.84	95,490,996—161,564,373	2.94	5.30	− 0.43
	qRL3	3	120.9	179,218,369—197,457,705	3.82	6.96	− 0.48
SL (cm)	qSL1	1	77.84	60,166,133—181,718,514	6.29	10.93	− 0.58

Table 2 (continued)

Trait	QTL[a]	Chr	Position (cM)	Marker Interval (bp)[b]	LOD[c]	PVE (%)[d]	Add[e]
	qSL2	2	77.11	22,027,898	3.69	6.81	− 1.23
	qSL9	9	42.09	12,461,059—17,342,675	4.61	7.84	− 0.48
FL (cm)	*qFL1*	1	77.84	60,166,133—175,500,322	5.50	9.91	− 1.00
RF (g)	*qRF7–1*	7	68.86	101,321,793—131,527,199	3.03	5.21	0.02
	qRF7-2	7	145.02	164,385,525—169,955,912	4.39	7.73	0.02
	qRF9	9	47.24	25,771,962—130,771,216	3.29	5.68	− 0.02
SF (g)	*qSF1*	1	76.11	93,613,290—168,281,620	3.17	5.44	− 0.01
	qSF2	2	82.77	179,719,121—184,963,993	3.78	6.83	− 0.02
	qSF7	7	70.03	120,108,221—138,923,162	3.58	6.20	0.02
	qSF9	9	49.97	25,771,962—130,771,216	3.45	5.94	− 0.01
FF (g)	*qFF2*	2	82.77	184,963,993	3.93	7.07	− 0.03
	qFF7	7	111.67	154,072,064—160,703,574	3.37	5.89	0.03
	qFF9	9	47.24	21,749,801—133,015,204	5.35	9.4	− 0.04
RD (g)	*qRD2*	2	82.77	184,963,993	2.75	5.08	− 0.002
	qRD7	7	113.84	156,524,408—160,703,574	3.27	6.25	0.002
	qRD9	9	22.02	8,350,986—124,316,134	2.91	5.97	− 0.002
SD (g)	*qSD2*	2	82.77	184,963,993	2.72	5.05	− 0.002
	qSD9	9	76.76	143,618,488—144,601,527	2.79	5.17	− 0.002
	qSD10	10	63.24	133,679,018—141,014,412	3.02	7.34	− 0.002
FD (g)	*qFD2*	2	82.77	179,719,121—184,963,993	3.39	6.08	− 0.003
	qFD7	7	127.98	160,703,574—164,385,525	3.03	5.65	0.003

[a] QTL were named "q" followed by trait name and chromosome number on which each QTL was mapped. Multiple QTL on the same chromosome were distinguished with − 1, − 2, and so on. See Table 1 caption for abbreviations of traits

[b] Coordinates were according to B73 reference sequence, version 2

[c] Logarithm of odds

[d] Phenotypic variation explained by QTL

[e] Additive effect, where positive values suggest that favorable alleles are contributed by PH6WC and negative values suggest that favorable alleles are contributed by PH4CV

qRFS1 (LOD, 23.92; PVE, 37.09%; Add, 0.91 g), *qSFS1* (LOD, 16.94; PVE, 27.90%; Add, 3.36 g), *qFFS1* (LOD, 22.83; PVE, 35.64%; Add, 0.07 g), and *qFDS1* (LOD, 12.12; PVE, 21.29%; Add, 0.005 g) (Table 2; Fig. 2a). All seven of these major QTL exhibited positive additive effects, thus indicating that the maternal allele (PH6WC) at these loci could increase maize salt tolerance at the early seedling stage while it would be decreased by the paternal allele (PH4CV) at these loci. The LOD peak of these major QTL was mapped at 86.65 cM on chr1 and they covered the genomic region of 76.67–101.06 cM between SNP markers *MC0202* and *MC0277*. For the traits of RDS and SDS, two minor effect QTL, *qRDS1* and *qSDS1*, respectively, were identified at the same location as those major QTL (Table 2; Fig. 2a). The simultaneous detection of these QTL based on nine traits indicated that the chromosomal region of these major QTL was stable under salt-stress conditions. In contrast, no major effect QTL controlling biomass-related traits under normal conditions were discovered. A total of 24 minor effect QTL were mapped on chr1, chr2, chr3, chr7, chr9, and chr10, with PVE ranging from 5.05 to 10.93% and the LOD scores ranging from 2.72 to 6.29 (Table 2; Fig. 3c). Taken together, these results indicated that these major QTL were responsible for the salt tolerance of maize seedlings.

Salt tolerance index has been used as the main criterion in salt tolerance evaluation of cowpea (Win and Oo 2015), rice (Ali et al. 2013), and maize (Cui et al. 2015b; Luo et al. 2017b). QTL mapping using a salt tolerance index has the advantage of identifying the specific loci
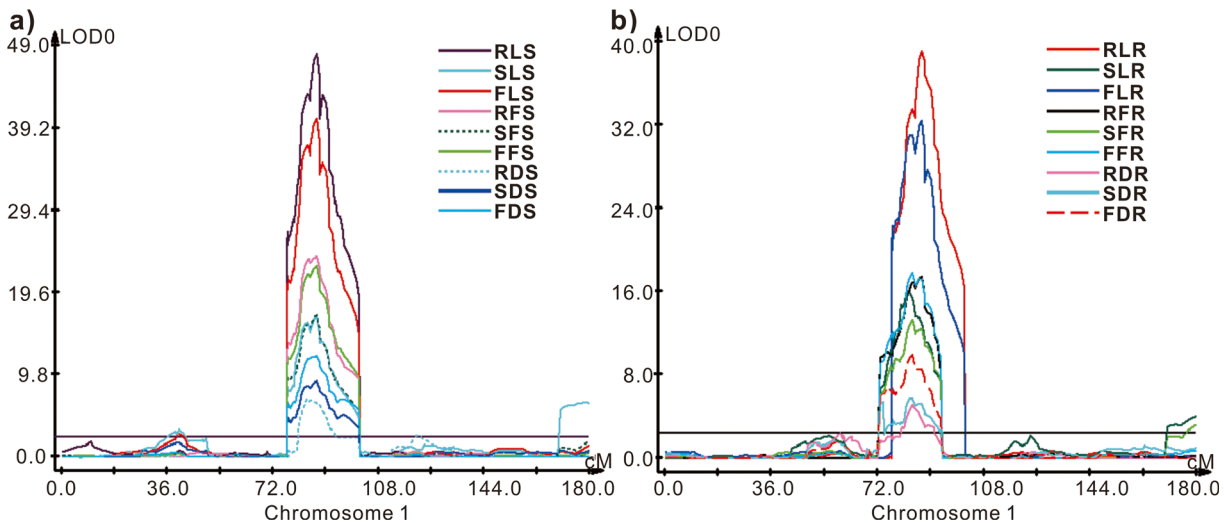
Fig. 2 Chromosomal locations of QTL for salt tolerance-related traits on chromosome 1. **a** Traits under salt treatment. **b** Salt tolerance indexes of traits. See Fig. 1 caption for abbreviations

for salt tolerance by eliminating the effects of traits under normal treatment. To exclude the influence of normal traits, the salt tolerance index (phenotypic value under salt treatment/phenotypic value in control) was calculated for each trait and then used for QTL mapping. One major QTL for each of RLR and FLR was detected on chr1 (*qRLR1* and *qFLR1*, PVE of 58.35% and 45.88%, Add of 0.29 and 0.24, and the LOD scores of 39.13 and 32.42, respectively) (Table 2; Fig. 2b). These two QTL were co-located and were located at the same chromosomal region as *qRLS1*, *qSLS1-2*, *qFLS1-2*, *qRFS1*, *qSFS1*, *qFFS1*, and *qFDS1*, further indicating that this chromosomal region containing these major QTL conferred salt tolerance in maize seedlings.

Other major QTL for SLR, RFR, SFR, and FFR, designated as *qSLR1-1*, *qRFR1*, *qSFR1-1*, and *qFFR1*, respectively (PVE of 25.88%, 31.47%, 22.19%, and 29.59%; Add of 0.18, 0.18, 0.12, and 0.15, respectively), were also detected on chr1. These four QTL were co-located and their chromosomal position was the same as that of *qRDR1* (PVE, 10.53%; Add, 0.09), *qSDR1* (PVE, 10.48%; Add, 0.09), and *qFDR1* (PVE, 18.3%; Add, 0.11) identified based on the traits of RDR, SDR, and FDR (Table 2; Fig. 2b). The chromosomal regions of these QTL were extensively overlapped with that of *qRLR1* and *qFLR1* (Table 2; Fig. 2b), suggesting that the genomic region harboring all of these major QTL involved a single gene with pleiotropic effects or contained a block of closely linked genes controlling different salt tolerance-related traits.

Aside from the abovementioned QTL, another 23 minor effect QTL related to salt tolerance were identified on chr2, chr3, chr4, chr7, and chr9, as well as on chr1. These QTL exhibited small PVE values of 2.40 to 9.84% and LOD scores of 2.59 to 6.52, indicating that they were sensitive to the environments (Table 2).

Discussion

Effects of salt stress on growth of maize seedlings

Maize is more sensitive to salt stress at the early seedling stage than at the adult stage. Salinity has been shown to significantly reduce the growth of maize roots and shoots by suppressing organ initiation and expansion (Farooq et al. 2015; Luo et al. 2017a). For maize, biomass-related traits under stress treatment at the seedling stage are largely correlated with seedling vigor and later, with grain yield (Yu et al. 2018). Therefore, biomass-related traits, such as length and weight, which show high heritability (Khan et al. 2003) and can be measured at an early stage, are of great importance for the evaluation and mapping of salt tolerance in maize (Giaveno et al. 2007). In this study, all biomass-related traits were greatly affected by the salt treatment, and significant phenotypic differences were found among the DH lines (coefficient of variation > 25.8%), suggesting that there are large variations in salt tolerance in maize.
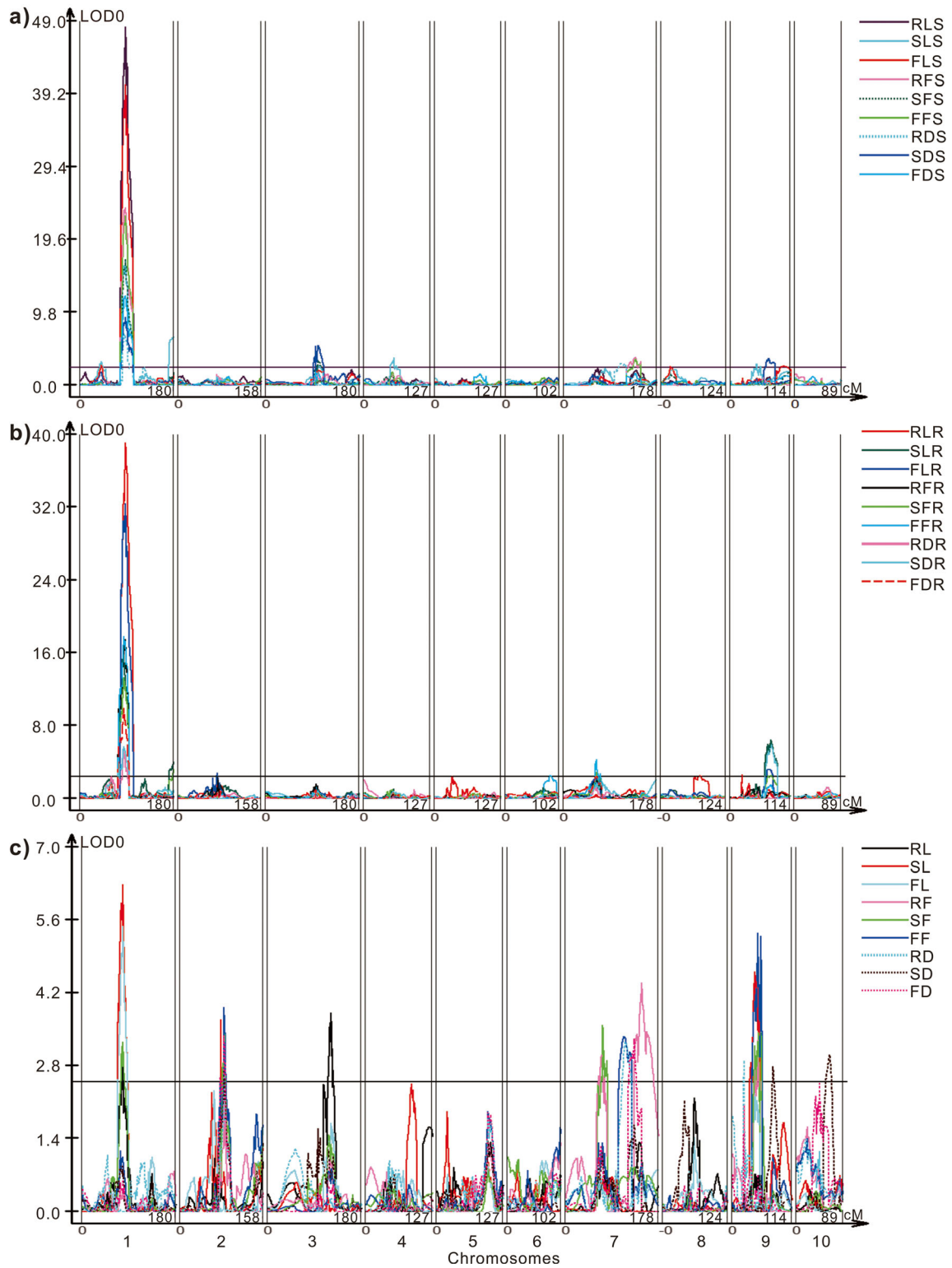
Fig. 3 Chromosomal locations of QTL for biomass-related traits under control and salt-stress conditions. **a** Biomass-related traits under salt treatment. **b** Salt tolerance indexes of traits. **c** Traits in control. See Fig. 1 caption for abbreviations

Comparison between QTL identified in this study and previously identified QTL

Genetic analyses frequently use SNP markers because of their high density, ease of use, and high-throughput operation. Using the MaizeSNP3072 chip, we constructed a high-density genetic linkage map with a total length of 1379.95 cM and an average marker interval of 1.03 cM. This allowed for high-resolution QTL localization. Based on this map, 41 QTL related to salt tolerance at the seedling stage were identified in maize. Among them, 13 QTL including *qRLS1*, *qSLS1-2*, *qFLS1-2*, *qRFS1*, *qSFS1*, *qFFS1*, *qFDS1*, *qRLR1*, *qFLR1*, *qSLR1-1*, *qRFR1*, *qSFR1-1*, and *qFFR1* independently explained > 21% of the phenotypic variation in their respective traits (Table 2). Nine major QTL (*qRLS1*, *qSLS1-2*, *qFLS1-2*, *qRFS1*, *qSFS1*, *qFFS1*, *qFDS1*, *qRLR1*, and *qFLR1*) co-located to the same chromosomal region, suggesting that this QTL region had pleiotropic effects and played an essential role in regulating salt tolerance in maize seedlings. These nine QTL overlapped with another four co-located large-effect QTL (*qSLR1-1*, *qRFR1*, *qSFR1-1*, and *qFFR1*) that explained > 22% of the phenotypic variation in their respective traits. These results suggested that these QTL may function as closely linked genes or as a single gene.

There have been two other reports on QTL analysis of salt tolerance in maize. Using SNP markers and an F$_{2:5}$ population, two co-located major QTL for field germination rate and field salt tolerance ranking, designated as *QFgr1* and *QFstr1*, respectively, were identified in a saline field trial (Cui et al. 2015b). Luo et al. (2017b) analyzed a DH population using SNP markers and identified two co-located major QTL, named *qSPH1* and *qPHI*, for the height of mature plants in a saline field trial and a plant height-based salt tolerance index, respectively. A comparison of our data with previous mapping results revealed that the chromosomal position of the major effect QTL (*qRLS1*, *qSLS1-2*, *qFLS1-2*, *qRFS1*, *qSFS1*, *qFFS1*, *qFDS1*, *qRLR1*, *qFLR1*, *qSLR1-1*, *qRFR1*, *qSFR1-1*, and *qFFR1*) reported here was similar to that of the previously reported QTL *QFgr1* (Cui et al. 2015b), *QFstr1* (Cui et al. 2015b), *qSPH1* (Luo et al. 2017b), and *qPHI* (Luo et al. 2017b). The consistent detection of this chromosomal position in different populations and at different developmental stages (both seedling and adult stages), as well as the ability of these QTL to account for a large proportion of phenotypic variation, suggested that this

chromosomal region, was important for maize salt tolerance and was a good target for fine mapping of genes associated with maize salt tolerance. Notably, the chromosomal positions of *QFgr1* and *QFstr1* could be detected in a saline field trial but not under hydroponic culture conditions in a previous study (Cui et al. 2015b), while 13 major QTL at a similar position to that of *QFgr1* and *QFstr1* were detected under hydroponic culture conditions in this study. This discrepancy may be due to differences in experimental conditions. For example, the salt treatment was carried out at the three-leaf seedling stage in the previous study, whereas maize seeds were sown and grown in saline solution in the present study.

We identified 65 QTL, of which 52 were minor effect QTL, and 46 had PVE values of less than 10%. Eight minor effect QTL were located in similar regions as QTL detected in other studies (Burton et al. 2014; Cai et al. 2012; Cui et al. 2015b; Luo et al. 2017b). These results indicated that the multiple minor effect QTL also played a role in the determination of salt- and growth-related traits of maize seedlings.

QTL clusters and trait correlations

Many studies have reported the clustering of QTL for multiple traits (Cui et al. 2015b; Lin et al. 2004; Song et al. 2016). In the current study, nine clusters affecting more than one trait were observed, with three clusters on chr1, two on chr7, two on chr9, one on chr2, and one on chr3 (Table 2; Fig. 3a–c). Consistently, the two QTL clusters for salt-related traits spanned the genomic regions of 93,613,290–194,632,379 bp on chr1 and 18,806,037–168,776,735 bp on chr3, respectively, which were similar to the regions identified as being related to salt tolerance in maize seedlings under a salt treatment in a previous study (Cui et al. 2015b). Markers in these genomic regions are thus important for marker-assisted breeding of salt-tolerant maize varieties. In agreement with previous findings (Huang et al. 2006; Li et al. 2010; Lin et al. 2004; Shehzad and Okuno 2016), most traits with QTL in the same cluster were highly correlated in the current study. For example, RLS, SLS, FLS, RFS, SFS, FFS, SDS, and FDS were strongly positively correlated (*r* = 0.49–0.96), and their controlling QTL (*qRLS1*, *qSLS1–2*, *qFLS1–2*, *qRFS1*, *qSFS1*, *qFFS1*, *qSDS1*, and *qFDS1*) were located at the same map position on chr1 (Fig. 1a and 2a). This trend

may be related to the pleiotropic effects and/or indicate close linkage of genes.

Compliance with ethical standards

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