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ORIGINAL ARTICLE

Mapping QTL for seedling morphological and physiological traits under normal and salt treatments in a RIL wheat population

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Abstract

Key message **The genetic basis of 27 seedling traits under normal and salt treatments was fully analyzed in a RIL wheat population, and seven QTL intervals were validated in two other genetic populations.**

Abstract Soil salinity seriously constrains wheat (*Triticum aestivum* L.) production globally by influencing its growth and development. To explore the genetic basis of salt tolerance in wheat, a recombinant inbred line (RIL) population derived from a cross between high-yield wheat cultivar Zhongmai 175 (ZM175) and salt-tolerant cultivar Xiaoyan 60 (XY60) was used to map QTL for seedling traits under normal and salt treatments based on a high-density genetic linkage map. A total of 158 stable additive QTL for 27 morphological and physiological traits were identified and distributed on all wheat chromosomes except 3A and 4D. They explained 2.35–46.43% of the phenotypic variation with a LOD score range of 2.61–40.38. The alleles from XY60 increased corresponding traits for 100 QTL, while the alleles from ZM175 had positive effects for the other 58 QTL. Nearly half of the QTL (78/158) were mapped in nine QTL clusters on chromosomes 2A, 2B, 2D, 4B, 5A, 5B, 5D, and 7D (2), respectively. To prove the reliability and potentiality in molecular marker-assisted selection (MAS), seven QTL intervals were validated in two other genetic populations. Besides additive QTL, 94 pairs of loci were detected with significant epistatic effect and 20 QTL were found to interact with treatment. This study provides a full elucidation of the genetic basis of seedling traits (especially root system-related traits) associated with salt tolerance in wheat, and the developed kompetitive allele-specific PCR markers closely linked to stable QTL would supply strong supports to MAS in salt-tolerant wheat breeding.

Introduction

Soil salinity is one of the major abiotic stresses that decrease grain production and threaten food security worldwide (Flowers et al. 1997; Munns and Gilliham 2015; Roy et al. 2014). It was estimated that the global salt-affected land area was more than 800 million hectares (equal to 6% of the world's total land area) and it has been continuously increasing year by year due to climate changing, land clearing, and non-sustainable irrigation (Deinlein et al. 2014; Flowers and Yeo 1995; Munns and Tester 2008; Rengasamy 2010; Roy et al. 2014; Tester and Davenport 2003). Bread wheat (*Triticum aestivum* L.) is a moderately salt-tolerant crop (Munns and Tester 2008) and grows most widely throughout the world (Matthew et al. 2011). To meet the food requirement, wheat production must increase by nearly 70% by 2050 (Dias 2015; Foley et al. 2011; Tilman et al. 2002); however, the area of salinized arable land is also estimated to exceed 50% by then (Jamil et al. 2011).

Soil salinity damages plant growth severely by lowering growth rate, reducing tillers, accelerating the senescence of old leaves, decreasing photosynthesis capability, and affecting reproductive development, which leads to significant reduction in final agricultural yield (Munns and Tester 2008; Roy et al. 2014). To resist salt stress, plants have evolved many mechanisms of salinity tolerance, which fall into three categories: osmotic tolerance (the ability to maintain turgor

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by accumulating small molecular substances such as organic acids, inorganic ions, carbohydrates, and amino acids), Na^+ exclusion (the ability to reduce net Na^+ from root to shoot), and tissue tolerance (the ability to maintain tissue function after Na^+ and Cl^- concentrations elevated) (Munns and Tester 2008). Among them, Na^+ exclusion is the most intensively studied mainly because it is relatively straightforward to phenotype (Roy et al. 2014) and coincides with people's common perception. The *high-affinity potassium* transporter (*HKT*) gene family (Ali et al. 2012; Byrt et al. 2007; Davenport et al. 2007; Hauser and Horie 2010; Horie et al. 2009; Huang et al. 2006; Munns and Tester 2008; Platten et al. 2006) and the salt overly sensitive (SOS) signaling pathway (Ji et al. 2013; Mahajan et al. 2008; Qiu et al. 2002; Shi et al. 2003; Weinl and Kudla 2009; Yang et al. 2009) played significant roles in regulating Na^+ transport. *Kna1* was the first major locus for salt tolerance in wheat, which controlled leaf Na^+ content and maintained a high K^+/Na^+ discrimination in leaf blades (Dubcovsky et al. 1996; Gorham et al. 1997, 1987, 1990; Luo et al. 1996). Further studies found that *HKT1;5-D* retrieving Na^+ from the xylem vessels in roots was the candidate gene of *Kna1* (Byrt et al. 2007, 2014; Davenport et al. 2005). *Nax1* and *Nax2*, which were mapped to chromosomes 2AL and 5AL in durum wheat, respectively, contributed to low Na^+ concentration in leaf blades and also belonged to *HKT* family (Byrt et al. 2007; Huang et al. 2006; James et al. 2006; Lindsay et al. 2004; Munns et al. 2012). Various QTL for Na^+ content have been mapped in bread wheat in different studies (Asif et al. 2021, 2018; Devi et al. 2019; Genc et al. 2010, 2019; Hussain et al. 2017; Oyiga et al. 2018; Xu et al. 2013), but fewer of them were co-localized with *Kna1*, *Nax1*, or *Nax2*, even using the genome-wide association method (Genc et al. 2019). Therefore, the benefits of these QTL (genes) in bread wheat breeding were uncertain although *Nax2* could increase grain yield by 25% in durum wheat (Munns et al. 2012). Considering that the ultimate aim of salt tolerance breeding is to increase crops' ability to maintain growth and productivity in saline soils relative to that in non-saline soils (Roy et al. 2014), breeders usually concentrate on morphological and biomass-related traits besides ions (Na^+, K^+, and Cl^-) content in mapping studies of wheat.

As the major organ for water and mineral nutrient absorption, root is the first tissue sensing osmotic stress and ion toxicity. Although the main site of Na^+ toxicity is the leaf blades rather than the roots (Munns and Tester 2008), the initiation of new seminal or lateral roots obviously decreases with time. Compared with the aboveground traits, little is known about the "hidden" root, especially under stresses in bread wheat. Recently, Fan et al. (2018) mapped QTL for root system architecture-related traits (RSATs) under high- and low-nitrogen environments and found some chromosome regions responding to nitrogen deficiency and an interval on chromosome 7B controlling RSATs and thousand kernel weight concurrently. Soriano and Alvaro (2019) found that 35 meta-QTL were related to root architecture and/or drought stress response by meta-analysis with many published articles. For salt tolerance studies in wheat, researchers formerly focused on the QTL for the maximum root length and biomass (Devi et al. 2019; Ma et al. 2007; Xu et al. 2012, 2013), but few noticed the variation of RSATs under salt stress such as root diameter, main and lateral root number, length, and surface area.

Breeding improved varieties adapting to saline soil through molecular marker-assisted selection (MAS) has been lagging behind in bread wheat because of its complex mechanism of salt tolerance and large genome sequence (~ 17 Gb). Although many loci for morphological and physiological traits were detected through QTL mapping (Asif et al. 2021, 2018; De Leon et al. 2011; Devi et al. 2019; Genc et al. 2013, 2010, 2019; Ghaedrahmati et al. 2014; Jahani et al. 2019; Ma et al. 2007; Masoudi et al. 2015; Nezhad et al. 2019; Quarrie et al. 2005; Xu et al. 2012, 2013), only a few of them were reported to have effects on final grain yield in bread wheat (Asif et al. 2018; Devi et al. 2019; Genc et al. 2013, 2019; Nezhad et al. 2019). There is still a huge gap between understanding the genetic basis of salinity tolerance in wheat and applying the available knowledge to delivering salt-resilient varieties subsequently (Mujeeb-Kazi et al. 2019). QTL mapping results for salt tolerance would be diverse in different wheat populations, even in the same population under various environments. Genetic background (GB) affects the expression as well as the detection of QTL (Han et al. 2012; Jahani et al. 2019; Venuprasad et al. 2012; Vikram et al. 2011), thus hindering the universal utilization of QTL found in different backgrounds (Jahani et al. 2019). As important genetic components, epistatic effect and QTL × environment interaction effect affect most quantitative traits greatly (Xu and Crouch 2008). In order to elucidate the identified QTL comprehensively and apply them to breeding program successfully, researchers have been gradually aware of the importance of the epistasis and QTL-by-environment interaction in QTL mapping for salt tolerance in wheat (Jahani et al. 2019; Nezhad et al. 2019; Xu et al. 2012, 2013).

In this study, a recombinant inbred line (RIL) population derived from a cross Zhongmai 175 (ZM175)/Xiaoyan 60 (XY60) was used to map QTL for seedling traits of shoot and root under normal and salt treatments based on a high-density genetic linkage map constructed with a Wheat55K SNP array. Besides additive QTL (*a*), epistasis (*aa*) and QTL-by-environment (*at*) interaction effects were also analyzed. In addition, to identify true and stable QTL, we calculated the simple mean and best linear unbiased estimates (BLUE) data from three trials as phenotype values and validated some QTL in two other genetic populations.

Materials and methods

Plant materials

XY60 (Xiaoyan 54/Lumai 13) is a new derived cultivar of Xiaoyan 6 and possesses a steady drought and salt resistance. As a classic case of distant hybridization between wheat and *Thinopyrum ponticum* ($2n = 10x = 70$), Xiaoyan 6 was characterized with wide adaptability to multiple environments, high yield potential, and excellent bread-making quality (Li et al. 2008). ZM175 (BPM27/Jing 411) is a main high-yield cultivar grown in the Northern Winter Wheat Region and Huanghuai Wheat Region of China with high water and nutrient use efficiency. A total of 254 lines from a recombinant inbred line (RIL) population derived from a cross between ZM175 and XY60 were used in the present study. In addition, a RIL population containing 182 lines derived from a hybrid between Xiaoyan 54 (St2422/464/Xiaoyan 96) and Jing 411 (Fengkang 2/Changfeng 1) (Xu et al. 2012) and a double haploid (DH) line population consisting of 150 lines derived from a cross between Hanxuan 10 (Nongda 16/Huabei 187) and Lumai 14 (C149/F_4-530) (Hao et al. 2003) were involved in this study as well. It is worth noting that Xiaoyan 54 is a parent of XY60 and Jing 411 is a parent of ZM175.

Methods

The experiment was carried out in the greenhouse at Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China. The salt tolerance of 254 "ZM175/XY60" (ZX) RILs and their parents was evaluated in hydroponic culture at two salt concentrations (0 and 150 mM NaCl, designated as the normal (CK) and salt stress (S) treatments, respectively), and three trials (CK1, S1, CK2, S2, CK3, and S3) were conducted. Fifteen plump seeds of each line were surface-sterilized in 10% H_2O_2 for 30 min, rinsed with deionized water, and then germinated on grid net for 7 days. The eight most uniform seedlings of each line were selected and divided into CK and S groups evenly. Then, they were transplanted into opaque plastic boxes (45 cm × 30 cm × 15 cm) and attached to the polyvinyl chloride (PVC) covers using soft sponge rubber after the residual endosperm being removed. One box contained 15 L nutrition solution (Table S1) with 24 holes (4 seedlings per hole) evenly distributed on its cover. From the next day, 50 mM NaCl was added into the solution every day till the final concentration of 150 mM for S treatment. The solution was renewed every 3 days with pH = 5.8–6.0. Plastic boxes were randomly placed and rearranged when the solution was renewed. The greenhouse was maintained under a 16-/8-h light/darkness cycle at 22 °C/18 °C during the growth period. About three weeks later, all plants were harvested after measuring the chlorophyll content of the first leaf.

The phenotypes of Xiaoyan 54/Jing 411 (XJ) RIL population and Hanxuan 10/Lumai 14 (HL) DH population were performed with the same method.

Traits measurement

The chlorophyll content of each plant was measured using a leaf chlorophyll meter (Soil and Plant Analyzer Development, SPAD-502, Minolta, Osaka, Japan). For each plant, the SPAD value was derived from the average of three readings at the base, middle, and tip of the first leaf. Tiller number (TN), leaf number (LN), and yellow leaf number (YLN) were counted. Shoot height (SH) and maximum root length (RL) were measured with a ruler. Fresh weight of shoot (SFW), dry weight of shoot (SDW), and dry weight of root (RDW) were measured with an electronic balance. Shoot water content (SWC) was calculated as SWC = (SFW − SDW)/SFW *100%. Total dry weight (TDW) was calculated as TDW = SDW + RDW. Root system architecture-related traits (RSATs) were analyzed using WinRHIZO software developed by Regent Instruments Canada Inc. (Ottawa, ON, Canada). The root morphological parameters included total root tip number (TRT), total root average diameter (TRAD), total root length (TRL), total root surface area (TRSA), main root tip number (MRT), main root length (MRL), main root surface area (MRSA), lateral root tip number (LRT), lateral root length (LRL), and lateral root surface area (LRSA). Main root means its average diameter was > 0.300 mm and < = 0.850 mm, and lateral root means its average diameter was > 0.060 mm and < = 0.300 mm. The detection method of K^+ and Na^+ concentration was as follows: Mixed and triturated sample (25–30 mg) from four dry plants of each line was dissolved in a nitric acid solution (13 mL HNO_3 and 2 mL H_2O_2) using an advanced microwave digestion system (ETHOS 1, Milestone S.r.l., Shelton, CT, USA). After that, the concentration of K^+ and Na^+ in shoot (sK and sNa) and root (rK and rNa, only in the first trial) was assayed using inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 5300DV, PerkinElmer, Waltham, Massachusetts, USA). K^+/Na^+ ratios in shoot (sK/Na) and root (rK/Na) were calculated based on the concentration of K^+ and Na^+.

Statistical and QTL mapping

Correlation analysis was performed by SPSS Statistics software (IBM SPSS Statistics 23.0, Chicago, IL, USA). Analysis of variance (ANOVA) and narrow-sense heritability (h^2) for all traits under CK and S treatments were analyzed in

the IciMapping 4.1 (http://www.isbreeding.net/software/?type=detail&id=18) with the ANOVA function. A total of ten phenotype datasets, which contained the average of each trial (CK1, CK2, CK3, S1, S2, and S3) as well as the simple mean (CKMean and SMean) and BLUE (CKBlue and SBlue) of three trials, were used to map QTL. The high-density genetic linkage map of the "ZM175/XY60" RIL population was constructed utilizing a Wheat55K SNP array, spanning 3250.71 cM and including 2437 bin markers from 16,008 SNPs distributed on 21 chromosomes (Luo et al. 2021). The chromosome length ranged from 85.99 cM (chromosome 4B) to 198.45 cM (chromosome 5D), and the average length was 154.80 cM. The density of bin markers was 1.33 cM with 116 bins on each chromosome averagely. Two kinds of software IciMapping 4.1 and WinQTLCart 2.5 (https://brcwebportal.cos.ncsu.edu/qtlcart/WQTLCart.htm) were used to map QTL with different methods. Pre-adjusted mapping parameters for Icimapping 4.1 were set: method = inclusive composite interval mapping (ICIM), step = 1.0 cM, PIN = 0.001, and logarithm of the odds (LOD) ≥ 2.5. For WinQTLCart 2.5, parameters setting were as follows: method = composite interval mapping (CIM), walk speed = 0.1 cM, and threshold = 11.5. The epistatic effect *aa* and the interaction effect between QTL and treatment (*at*) were also analyzed with IciMapping 4.1. Additive QTL was named as "*Q*" plus trait name along with the chromosome information at the end, and "*c*" was added in the front of QTL for those with environment interaction effect.

High-density genetic linkage maps of the XJ RIL population (unpublished) and HL DH population (Li et al. 2019) were constructed based on Wheat660K SNP array. QTL mapping in these two populations was performed utilizing IciMapping 4.1.

Conversion of SNPs to kompetitive allele-specific PCR (KASP) markers

Based on the sequences, key SNPs linked to major QTL were successfully converted into KASP markers, the specific technology for SNP genotyping. According to the manufacturer's instructions, the designed KASP markers were evaluated for their polymorphisms. KASP reactions were performed in a StepOnePlus Real-time PCR System (Applied Biosystems, USA), and fluorescence was analyzed using corresponding StepOne Software v2.3.

Result

Phenotypic variation, performance, and correlation

Phenotypic characters (27 traits) of the "ZM175/XY60" (ZX) RIL population and their parents were investigated under CK and S treatments in three trials. Based on the correlation analysis, significant positive relationships were found among three trials (Table S2). For most traits, correlation coefficients were about 0.4–0.7 under CK treatments and 0.3–0.6 under S treatments. The coefficients for the ionic traits (K^+, Na^+ and K^+/Na^+ ratio) were less than 0.4, indicating their vulnerability to environmental influences. The phenotypes of 254 RILs and their parents and narrow-sense heritability (h^2) of all traits are summarized in Table 1. According to our experiments, the parental lines ZM175 and XY60 were significantly different in root-related traits (RL, TRL, TRSA, TRT, TRAD, LRL, LRSA, LRT, MRL, and MRT), SPAD, and the cation contents. Although all root-related traits were significantly inhibited under S treatments, XY60 had a more developed root system than ZM175 regardless of salt levels. After suffering salt stress, XY60's old leaves still stayed green, while those of ZM175 became yellow or even died. The K^+ concentration and K^+/Na^+ ratio in XY60's shoot were higher than those in ZM175, whereas the opposite result occurred for Na^+ concentration. Under salt stress, the maximum, minimum, and average values of the RILs for all seedling traits except YLN and Na^+ concentration decreased distinctly compared with those under CK treatment. The h^2 for all measured traits was also obviously declined when the plants were treated with salt stress. For most traits, the skewness and kurtosis were small (less than 1.0), which demonstrated that the phenotype values followed normal distribution. In conclusion, ANOVA indicated that treatments, genotypes, and genotype × treatment interaction significantly affected all of the traits related to seedling growth.

Correlation analysis was also carried out among different traits (Table S3). SFW, SDW, and RDW presented significant and positive correlations with TDW, and the correlation coefficients were more than 0.8 under CK and S treatments. SH, TN, LN, SPAD, and RSATs were also positively correlated with TDW, while YLN and sNa were negatively correlated with TDW. It was reasonable that there existed a positive correlation between sK and TDW under S treatment, but it was unexpected that they were negatively related under CK treatment. The correlation between SH, RL, SPAD, TRT, LRT, RDW, and TDW became higher under S treatment compared with those under CK treatment. In addition, obviously positive correlations were observed between sNa and YLN under S treatment and between sK and SWC under CK treatment.

QTL mapping

In this study, two kinds of mapping software (IciMapping and WinQTLCart) were first used to detect the QTL for 27 seedling traits with the simple mean and BLUE values of three trials. It was shown that about 70% of the QTL detected by two kinds of software were the same, and the

Table 1 Phenotypic performance and heritability (h^2) values for all 27 seedling traits of ZX RILs and their parents determined under normal (CK) and salt (S) treatments

Trait	Treatment	Parents		RILs					
		XY60	ZM175	Max	Min	Mean ± SD	Skewness	Kurtosis	h^2 (%)
SH (cm)	CK	35.11 ± 0.61	35.53 ± 4.12	45.15	28.00	35.41 ± 3.12	0.03	− 0.47	70.9
	S	24.74 ± 3.26	24.31 ± 3.68	29.80	17.61	23.34 ± 1.80	0.14	0.53	56.5
TN	CK	3.0 ± 0.9	2.7 ± 0.6	4.0	1.4	2.7 ± 0.5	− 0.22	− 0.12	48.3
	S	1.6 ± 0.5	1.4 ± 0.5	2.6	1.0	1.4 ± 0.3	0.98	0.50	41.5
LN	CK	5.6 ± 1.7	5.9 ± 1.4	7.8	3.5	5.4 ± 0.7	0.28	0.48	52.1
	S	3.6 ± 1.0	4.3 ± 0.7	5.1	3.0	3.8 ± 0.3	0.41	0.33	47.8
YLN	CK	0.05 ± 0.08	0.21 ± 0.20	1.47	0.00	0.20 ± 0.26	2.36	6.23	66.2
	S	0.44 ± 0.47*	1.15 ± 0.21*	1.31	0.33	0.82 ± 0.18	− 0.12	− 0.26	31.4
RL (cm)	CK	22.29 ± 2.68*	15.80 ± 3.33*	28.26	12.85	20.22 ± 3.22	− 0.14	− 0.60	71.5
	S	19.19 ± 3.93*	11.97 ± 2.94*	21.34	10.18	15.74 ± 2.01	0.03	0.08	61.5
SPAD	CK	39.8 ± 3.9	39.8 ± 1.6	51.7	9.8	40.2 ± 5.2	− 2.50	10.58	56.1
	S	38.1 ± 3.9*	16.2 ± 4.7*	41.7	0.0	30.6 ± 5.8	− 1.02	2.86	45.2
TRL (cm)	CK	682.7 ± 166.7*	493.1 ± 91.2*	1015.3	308.6	599.3 ± 133.6	0.29	− 0.07	60.0
	S	287.7 ± 93.2*	147.7 ± 43.6*	449.5	101.0	237.7 ± 56.6	0.13	0.21	46.6
TRSA (cm^2)	CK	53.4 ± 13.7*	46.9 ± 9.2*	75.3	27.2	50.1 ± 8.7	0.11	− 0.22	59.7
	S	27.1 ± 7.9*	19.0 ± 5.4*	40.4	11.1	25.0 ± 5.2	− 0.19	− 0.08	45.4
TRT	CK	1781 ± 493*	985 ± 265*	3145	656	1525 ± 480	0.72	0.58	41.1
	S	744 ± 343*	461 ± 199*	1204	311	634 ± 149	0.47	0.81	32.4
TRAD (mm)	CK	0.25 ± 0.02*	0.30 ± 0.02*	0.34	0.21	0.27 ± 0.02	0.29	− 0.16	52.1
	S	0.31 ± 0.03*	0.41 ± 0.03*	0.49	0.28	0.34 ± 0.03	1.21	4.75	15.9
LRL (cm)	CK	515.4 ± 130.3*	349.6 ± 70.7*	822.0	207.3	447.3 ± 114.7	0.41	0.14	56.2
	S	187.6 ± 73.4*	66.5 ± 26.1*	302.1	50.7	140.8 ± 41.0	0.39	0.54	39.9
LRSA (cm^2)	CK	20.3 ± 5.0*	15.8 ± 3.1*	30.6	9.6	18.4 ± 4.1	0.26	− 0.19	55.1
	S	8.5 ± 3.1*	3.4 ± 1.3*	13.3	2.5	6.7 ± 1.8	0.26	0.22	39.5
LRT	CK	1753 ± 491*	953 ± 264*	3118	629	1494 ± 479	0.72	0.60	41.1
	S	715 ± 336*	423 ± 191*	1174	289	602 ± 147	0.52	0.93	32.4
MRL (cm)	CK	162.4 ± 42.3*	133.6 ± 22.8*	213.0	76.1	145.6 ± 25.2	0.15	0.03	60.8
	S	98.1 ± 28.4*	79.0 ± 20.0*	144.4	33.8	94.6 ± 18.9	− 0.29	0.16	49.8
MRSA (cm^2)	CK	24.5 ± 6.7	22.1 ± 4.0	32.8	11.9	22.6 ± 3.9	0.17	0.11	57.6
	S	13.5 ± 4.0	12.1 ± 3.3	20.0	4.9	13.6 ± 2.8	− 0.34	0.18	46.5
MRT	CK	26.0 ± 5.4*	28.6 ± 5.3*	36.3	16.8	27.3 ± 3.8	0.05	− 0.22	8.8
	S	27.7 ± 9.7*	35.8 ± 10.9*	49.6	14.9	30.5 ± 5.5	0.32	0.47	36.2
SFW (g)	CK	2.137 ± 0.595	2.126 ± 0.686	2.684	1.071	1.867 ± 0.298	− 0.01	0.05	49.4
	S	0.709 ± 0.256	0.750 ± 0.236	0.948	0.322	0.653 ± 0.110	− 0.18	− 0.11	50.4
SDW (g)	CK	0.250 ± 0.067	0.244 ± 0.079	0.343	0.125	0.230 ± 0.038	0.13	− 0.08	48.8
	S	0.121 ± 0.053	0.125 ± 0.052	0.159	0.059	0.114 ± 0.019	− 0.26	− 0.18	47.3
SWC (%)	CK	88.3 ± 0.3	88.5 ± 0.4	89.9	84.7	87.7 ± 0.8	− 0.34	0.10	73.3
	S	83.2 ± 1.5	83.7 ± 1.8	84.7	81.1	82.6 ± 0.7	0.13	0.08	45.5
RDW (g)	CK	0.053 ± 0.033	0.055 ± 0.034	0.070	0.018	0.050 ± 0.008	− 0.23	0.84	43.5
	S	0.028 ± 0.015	0.028 ± 0.016	0.041	0.011	0.028 ± 0.006	− 0.37	− 0.02	46.3
TDW (g)	CK	0.304 ± 0.099	0.300 ± 0.113	0.402	0.121	0.278 ± 0.045	− 0.05	0.17	46.6
	S	0.148 ± 0.069	0.153 ± 0.068	0.196	0.058	0.140 ± 0.026	− 0.55	0.56	47.4
sK (mg/g)	CK	38.81 ± 3.66	41.31 ± 6.04	51.03	29.41	38.97 ± 3.91	0.11	− 0.02	26.9
	S	25.19 ± 0.97*	22.17 ± 0.90*	29.64	17.01	24.06 ± 2.26	− 0.20	0.21	24.8
sNa (mg/g)	CK	0.20 ± 0.07	0.23 ± 0.12	0.35	0.15	0.21 ± 0.04	0.65	0.37	1.3
	S	21.28 ± 2.31*	28.40 ± 3.66*	42.27	16.77	24.08 ± 3.87	0.92	1.83	24.6

Table 1 (continued)

Trait	Treatment	Parents		RILs					
		XY60	ZM175	Max	Min	Mean ± SD	Skewness	Kurtosis	h^2 (%)
sK/Na	CK	217.0 ± 90.0	222.8 ± 120.4	428.5	126.3	213.0 ± 45.6	1.20	2.59	9.3
	S	1.2 ± 0.1*	0.8 ± 0.1*	1.7	0.5	1.1 ± 0.2	0.11	− 0.03	26.3
rK (mg/g)	CK	24.90	29.00	8.49	50.15	27.37 ± 8.08	− 0.33	− 0.42	–
	S	9.02	9.23	3.85	33.20	9.37 ± 3.00	3.00	20.43	–
rNa (mg/g)	CK	1.17	0.63	0.37	2.97	0.83 ± 0.54	2.93	7.54	–
	S	22.88	23.75	12.21	31.23	20.97 ± 3.75	0.12	− 0.37	–
rK/Na	CK	21.3	46.3	4.2	73.3	40.9 ± 16.6	− 0.57	− 0.22	–
	S	0.4	0.4	0.2	1.2	0.5 ± 0.1	0.92	6.02	–

All data were derived from three trials. SD is short for standard deviation. h^2 represents the narrow-sense heritability. "-" represents missing value. Significant differences between two parental lines ZM175 and XY60 are marked by *, which were determined by the Student's *t*-test at $P < 0.05$

SH, shoot height; TN, tiller number; LN, leaf number; YLN, yellow leaf number; RL, maximum root length; SPAD, soil and plant analyzer development value; TRL, total root length; TRSA, total root surface area; TRT, total root tip number; TRAD, total root average diameter; LRL, lateral root length; LRSA, lateral root surface area; LRT, lateral root tip number; MRL, main root length; MRSA, main root surface area; MRT, main root tip number; SFW, fresh weight of shoot; SDW, dry weight of shoot; SWC, shoot water content; RDW, dry weight of root; TDW, total dry weight; sK, K^+ concentration in shoot; sNa, Na^+ concentration in shoot; sK/Na, K^+/Na^+ ratio in shoot; rK, K^+ concentration in root; rNa, Na^+ concentration in root; rK/Na, K^+/Na^+ ratio in root

major QTL were hardly different (not shown). Then, QTL for all traits were detected by IciMapping with datasets of each trial (CK1, CK2, CK3, S1, S2, and S3). It was found that 153 repeatable QTL (detected with two or more datasets) and 5 QTL for rK, rNa, and rK/Na (rK and rNa were assayed only in the first trial) were distributed on all wheat chromosomes but 3A and 4D (Table 2). These loci individually explained 2.35–46.43% of the total phenotypic variation with LOD scores ranging from 2.61 to 40.38. Among them, 39 QTL were detected under both CK and S treatments, while 80 and another 39 QTL were detected under only CK and S treatment, respectively. A total of 12 QTL could explain more than 10% of the phenotypic variation and 80 QTL explained 5–10% of the phenotypic variation. The additive effects of 100 QTL were derived from XY60 alleles, whereas the effects of the other 58 QTL were from ZM175 alleles. Epistatic effect analysis showed that a total of 94 pairs of loci mainly for YLN, SPAD, TRAD, and MRT were detected but none was co-localized with the additive QTL. In particular, most of them just explained little phenotypic variation, and only ten pairs of loci explained more than 2% of the phenotypic variation (Table S4). Here, a total of 20 QTL were found to interact with treatment (Table 3), and 19 of them were major additive QTL in Table 2. Among them, the interaction effects at five loci explained over 10% of the phenotypic variation, especially the interactions between *cQRl-2B* and treatment (25.98%) and between *cQSh-4B* and treatment (20.11%).

Seven and nine QTL were detected for SH and RL, respectively. Among them, *QSh-4B.2* and *QRl-2B.1* were detected with significant additive × treatment (*at*) effects. *QSh-4B.2* explained the maximum phenotypic variation (36.55%) with a LOD score of 29.35. However, it was only detected under CK treatment. Interestingly, *QSh-4B.1* was found under S treatment nearby *QSh-4B.2*. Similarly, *QRl-2B.1* could explain the maximum phenotypic variation (46.43%) with a LOD score of 38.4 under CK treatment, while *QRl-2B.2* was detected 14 cM away from *QRl-2B.1* under both CK and S treatments. Eight and 12 QTL were detected for TN and LN, respectively. Three QTL for TN (*QTn-2A*, *QTn-2D*, *QTn-5B)* and six for LN (*QLn-2A.2*, *QLn-2D.2*, *QLn-3D*, *QLn-5A*, *QLn-5B.1* and *QLn-6A*) were detected under both CK and S treatments, while *QTn-7A*, *QTn-7B*, *QLn-2D.1*, *QLn-5B.2* and *QLn-6D-1* were discovered only under S treatments. *QTn-5B* and *QLn-2B* explained the maximum phenotypic variation for corresponding traits. A total of six QTL (*QTn-5B*, *QLn-2A.2*, *Ln-2D.1*, *QLn-5A*, *QLn-5B.1*, and *QLn-6A*) were found with significant *at* effects, but they only explained a little phenotypic variation (< 3%).

For shoot and root biomass-related traits (SFW, SDW, RDW, and TDW), there were seven, five, nine, and six QTL detected, respectively. Among them, three intervals on chromosomes 2A, 2B, and 5A were found to contribute to all the four biomass-related traits, and two intervals on 1B and 4B were proved to be related to all biomass traits but RDW under both treatments or only under S treatment. For SWC, nine QTL were mapped on chromosomes 1B, 2B (2), 2D, 3B, 4B, 5B, 6B, and 7B. *QSwc-4B* explained the maximum phenotypic variation with a LOD score of 6.94 under CK treatment. *QSwc-6B* was detected under both CK and S treatments and explained 7.20% of the phenotypic variation.

Table 2 QTL with additive effects (*a*) for seedling traits under CK and S treatments in ZX RIL population

TraitName	QTL	Treatment	Chr	Position (cM)	LOD	PVE(%)	Add	LeftCI	RightCI
SH	*QSh-1A*	CK3/CKMean	1A	0/0	3.66	3.24	− 0.667	0.0	1.5
	QSh-2B	CK1/CK2	2B	25/35	2.97	3.37	− 0.695	18.5	39.5
	QSh-2D	CK2/CKBlue/CKMean	2D	122/122/122	3.40	2.77	− 0.582	120.5	123.5
	QSh-4B.1	S1/S2/SBlue/SMean	4B	17/16/17/17	4.82	7.74	0.603	15.5	17.5
	QSh-4B.2	CK1/CK2/CK3/CKBlue/CKMean	4B	30/30/29/30/29	29.35	36.55	2.209	25.5	31.5
	QSh-5B	CK3/CKMean/SBlue/SMean	5B	149/131/127/127	3.59	4.54	0.555	125.5	156.5
	QSh-6B	CK2/CKBlue	6B	46/54	3.62	3.10	0.623	42.5	57.5
TN	*QTn-2A*	CK1/CK2/CK3/CKBlue/CKMean/S1/S3/SBlue/SMean	2A	1/2/0/2/2/2/2/2/2	5.86	7.18	− 0.133	0.0	6.5
	QTn-2B	CK1/CK2/CK3/CKBlue/CKMean	2B	4/4/0/1/1	4.80	5.66	− 0.139	0.0	4.5
	QTn-2D	CK1/CK2/CKBlue/CKMean/S1/SBlue/SMean	2D	122/120/120/120/121/120/121	5.68	7.04	0.139	118.5	122.5
	QTn-5A	CK1/CK2/CK3/CKBlue/CKMean	5A	19/32/21/29/29	4.57	5.37	0.137	18.5	32.5
	QTn-5B	CKBlue/CKMean/S1/S2/S3/SBlue/SMean	5B	44/44/40/44/42/42/40	6.49	8.80	− 0.120	39.5	44.5
	QTn-6D-2	CK1/CKBlue/CKMean	6D-2	8/8/9	3.16	3.86	− 0.109	4.5	10.0
	QTn-7A	SBlue/SMean	7A	55/55	3.43	4.41	0.067	54.5	56.5
	QTn-7B	S2/SBlue/SMean	7B	72/75/75	3.09	4.06	− 0.068	71.5	75.5
LN	*QLn-2A.1*	CK1/CK2/CKBlue/CKMean	2A	3/2/2/2	4.14	5.49	− 0.172	0.0	7.5
	QLn-2A.2	CK3/CKBlue/CKMean/S2/S3/SBlue/SMean	2A	33/33/33/33/44/32/32	4.26	4.66	− 0.101	27.5	44.5
	QLn-2B	CK1/CK2/CK3/CKBlue/CKMean	2B	4/1/0/1/1	5.96	7.95	− 0.201	0.0	4.5
	QLn-2D.1	S2/S3/SBlue/SMean	2D	104/89/89/90	6.35	6.98	0.095	83.5	106.5
	QLn-2D.2	CK1/CK2/CK3/CKBlue/CKMean/SBlue/SMean	2D	121/119/141/120/120/118/118	5.70	7.02	0.165	117.5	141.5
	QLn-3D	CK3/S2/S3/SBlue	3D	106/106/107/106	4.68	4.71	0.094	105.5	107.5
	QLn-4B	CKBlue/CKMean	4B	31/31	4.45	5.73	− 0.156	25.5	31.5
	QLn-5A	CK2/CKMean/S2/S3/SBlue	5A	35/35/34/35/35	3.71	4.04	0.103	33.5	37.5
	QLn-5B.1	CK2/CK3/CKBlue/CKMean/S1/S3/SBlue/SMean	5B	44/52/44/44/40/55/45/45	4.53	5.19	− 0.122	39.5	55.5
	QLn-5B.2	S3/SBlue/SMean	5B	147/137/137	4.26	4.11	− 0.071	136.5	148.5
	QLn-6A	CK3/S3/SBlue/SMean	6A	68/60/59/60	5.32	5.82	0.101	53.5	72.5
	QLn-6D-1	S3/SBlue	6D-1	30/30	3.04	2.65	0.059	29.5	30.5

Table 2 (continued)

TraitName	QTL	Treatment	Chr	Position (cM)	LOD	PVE(%)	Add	LeftCI	RightCI
RL	*QRl-1A.1*	SBlue/SMean	1A	75/75	2.83	3.28	− 0.359	74.5	75.5
	QRl-1A.2	CK3/CKBlue/CKMean/SBlue/SMean	1A	154/155/155/155/155	3.49	3.15	− 0.483	153.5	155.0
	QRl-2A	CK3/CKBlue/CKMean/S1/SBlue/SMean	2A	35/35/35/34/47/47	5.42	5.29	− 0.636	33.5	47.5
	QRl-2B.1	CK1/CK2/CK3/CKBlue/CKMean	2B	4/4/4/4/4	38.40	46.43	− 2.245	3.5	4.5
	QRl-2B.2	CK2/S1/S2/SBlue/SMean	2B	17/18/17/18/18	6.26	7.56	− 0.673	15.5	18.5
	QRl-2D	SBlue/SMean	2D	0/0	4.80	5.59	− 0.468	0.0	0.5
	QRl-3B	S1/SBlue/SMean	3B	89/83/83	5.59	7.27	0.598	80.5	89.5
	QRl-4A	S2/SBlue/SMean	4A	55/55/55	4.36	5.05	− 0.483	53.5	55.5
	QRl-7B	CK2/S2	7B	68/59	5.32	5.57	− 0.677	58.5	68.5
SPAD	*QSpad-1A*	S2/S3/SBlue/SMean	1A	40/38/38/38	4.50	7.60	− 1.728	37.5	40.5
	QSpad-2B	CK2/CK3/CKBlue/CKMean	2B	2/4/4/4	3.70	5.76	− 1.426	0.0	4.5
	QSpad-3D	SBlue/SMean	3D	76/77	3.06	4.87	− 1.167	74.5	79.5
	QSpad-7A.1	CK2/CKBlue/CKMean	7A	68/68/68	3.14	5.08	− 1.348	63.5	71.5
	QSpad-7A.2	CKBlue/CKMean	7A	155/155	4.15	6.30	1.296	154.5	155.5
YLN	***QYln-1A***	S3/SBlue/SMean	1A	40/40/40	5.04	7.24	0.056	38.5	40.5
	QYln-2D	CK1/CKBlue/CKMean	2D	129/129/129	7.30	8.64	0.109	126.5	129.5
	QYln-3D.1	S3/SBlue	3D	79/80	2.86	4.37	0.046	76.5	81.5
	QYln-3D.2	CK2/CK3/CKBlue	3D	92/92/94	4.30	7.29	− 0.055	91.5	97.5
	QYln-5B	CK3/CKBlue	5B	68/62	3.56	5.28	0.044	61.5	68.5
	QYln-7A	S3/SMean	7A	165/163	3.33	5.54	− 0.054	156.5	167.5
SFW	*QSfw-1B*	CKBlue/S2/SBlue/SMean	1B	22/22/22/22	3.81	6.08	− 0.035	18.5	23.5
	QSfw-1D	CK3/CKBlue/CKMean/S3	1D	147/147/146/146	2.85	4.50	0.048	142.5	148.5
	QSfw-2A	CK2/CK3/CKBlue/CKMean	2A	33/34/33/33	4.43	6.15	− 0.077	28.5	34.5
	QSfw-2B	CK2/CK3/CKBlue/CKMean	2B	0/0/0/0	5.83	7.93	− 0.088	0.0	3.5
	QSfw-4B	S2/SBlue/SMean	4B	16/15/15	3.51	6.03	0.026	14.5	16.5
	QSfw-5A	CK1/CK3/CKBlue/CKMean	5A	36/34/34/34	3.74	4.25	0.072	33.5	38.5
	QSfw-6D-1	CK3/CKBlue	6D-1	28/28	6.60	7.84	0.084	20.5	28.5
SDW	*QSdw-1B*	CK2/S2/SBlue/SMean	1B	22/22/22/22	2.82	4.37	− 0.005	19.5	24.5
	QSdw-2A	CK3/CKBlue	2A	33/33	4.31	7.43	− 0.009	29.5	34.5
	QSdw-2B	CK2/CKBlue	2B	0/0	5.25	7.96	− 0.011	0.0	2.5
	QSdw-4B	CK2/CKBlue/CKMean/S2/SBlue	4B	32/32/32/21/20	4.87	7.76	0.009	17.5	33.5
	QSdw-5A	CK1/CKMean	5A	39/36	3.36	5.16	0.011	34.5	39.5

Table 2 (continued)

TraitName	QTL	Treatment	Chr	Position (cM)	LOD	PVE(%)	Add	LeftCI	RightCI
SWC	*QSwc-1B*	S1/SBlue/SMean	1B	14/24/24	4.43	5.02	− 0.002	12.5	25.5
	QSwc-2B.1	SBlue/SMean	2B	52/52	4.82	5.07	0.002	51.5	52.5
	QSwc-2B.2	SBlue/SMean	2B	116/117	3.70	4.22	− 0.001	114.5	117.0
	QSwc-2D	CK1/CKBlue/CKMean	2D	123/123/123	4.28	4.95	0.002	122.5	124.5
	QSwc-3B.1	CK2/CKBlue/S1	3B	38/39/40	3.44	4.80	− 0.002	35.5	40.5
	QSwc-3B.2	S3/SBlue/SMean	3B	71/71/71	3.55	3.98	− 0.002	69.5	75.5
	QSwc-4B	CK1/CKBlue/CKMean	4B	30/31/31	6.94	9.26	− 0.003	22.5	31.5
	QSwc-5B	S3/SMean	5B	106/102	3.78	4.56	− 0.002	100.5	106.5
	QSwc-6B	CK1/CK3/CKBlue/CKMean/S2/S3	6B	87/87/87/87/91/103	5.94	7.20	− 0.003	85.5	106.5
	QSwc-7B	S2/SBlue/SMean	7B	29/24/24	3.03	5.19	0.002	11.5	32.5
RDW	*QRdw-2A*	CK1/CK2/CK3/CKBlue/CKMean/SBlue/SMean	2A	48/43/33/33/33/47/48	3.96	5.55	− 0.002	29.5	48.5
	QRdw-2B	CK2/CK3/CKBlue/CKMean	2B	17/11/3/2	5.49	7.38	− 0.002	0.0	18.5
	QRdw-2D	S2/SBlue	2D	15/15	3.69	5.62	− 0.001	12.5	26.5
	QRdw-5A	CK2/CKBlue/CKMean/S2/SBlue	5A	27/34/26/19/36	3.26	4.43	0.001	18.5	38.5
	QRdw-5B	CKBlue/CKMean	5B	44/44	4.09	5.27	− 0.002	43.5	44.5
	QRdw-5D	S3/SBlue/SMean	5D	125/135/136	4.30	6.23	− 0.001	124.5	136.5
	QRdw-6A	CK3/CKBlue	6A	63/64	3.26	4.76	0.001	62.5	70.5
	QRdw-6D-1	CK2/CK3	6D-1	33/28	3.44	4.98	0.002	18.5	37.5
	QRdw-7D	SBlue/SMean	7D	119/119	3.66	5.01	0.001	118.5	119.5
TDW	*QTdw-1B*	S2/SBlue/SMean	1B	22/22/22	2.81	4.64	− 0.005	19.5	24.5
	QTdw-1D	CKBlue/CKMean	1D	146/146	2.78	4.52	0.009	142.5	147.5
	QTdw-2A	CK1/CK2/CK3/CKBlue/CKMean	2A	48/33/34/33/33	3.98	6.48	− 0.012	27.5	48.5
	QTdw-2B	CK2/CKBlue/CKMean	2B	0/0/0	5.57	8.55	− 0.013	0.0	2.5
	QTdw-4B	CK2/CKBlue/CKMean/S2	4B	32/32/32/21	3.97	6.30	0.010	17.5	33.5
	QTdw-5A	CK1/CK3	5A	36/34	2.96	5.16	0.012	33.5	38.5
rK	*QrK-1D*	CK1	1D	31	2.70	4.61	− 1.810	29.5	33.5
	QrK-3B	S1	3B	29	2.73	5.02	− 0.675	22.5	29.5
	QrK− 5D	CK1	5D	35	3.66	5.53	− 1.983	32.5	36.5
rK/Na	*QrK/Na-5D*	CK1	5D	35	3.09	5.54	− 3.924	31.5	36.5
rNa	*QrNa-6A*	S1	6A	62	2.77	5.05	− 0.887	58.5	62.5
sK	*QsK-1D*	CK2/CKMean	1D	126/127	3.21	4.10	0.999	119.5	133.5
	QsK-2B	CK3/S2	2B	0/3	2.88	4.37	− 0.727	0.0	3.5
	QsK-2D	CK1/CK3/CKMean	2D	124/121/122	3.70	4.99	1.093	118.5	124.5
	QsK-4B	CK1/CK2/CK3/CKMean/SMean	4B	31/31/31/31/34	8.90	12.87	− 1.827	21.5	34.5
	QsK-5D	CK1/CKMean	5D	150/151	3.43	4.54	− 1.131	146.5	152.5
	QsK-6B	CK3/CKMean/SMean	6B	88/89/91	3.57	4.80	− 0.761	87.5	92.5
sK/Na	*QsK/Na-2B*	S2/SMean	2B	3/3	4.54	7.73	− 0.073	0.0	3.5
	QsK/Na-4B	CK2/CKMean	4B	31/31	4.97	8.67	− 15.295	24.5	31.5
	QsK/Na-6A	CK3/CKMean	6A	121/121	2.83	5.02	− 17.874	119.5	121.5

Table 2 (continued)

TraitName	QTL	Treatment	Chr	Position (cM)	LOD	PVE(%)	Add	LeftCI	RightCI
TRL	*QTrl-2A*	CK2/CK3/CKBlue/CKMean	2A	36/34/34/34	7.19	7.48	− 39.07	33.5	37.5
	QTrl-2B	CK2/CK3/CKBlue/CKMean	2B	4/4/4/4	20.34	24.07	− 69.47	3.5	4.5
	QTrl-2D	S2/SBlue/SMean	2D	19/21/21	3.21	6.65	− 20.70	12.5	34.5
	QTrl-5A	CK3/CKBlue/SBlue/SMean	5A	26/27/34/34	3.01	3.35	19.96	24.5	34.5
	QTrl-5D	CK2/CK3/CKBlue/CKMean/S2/S3/SBlue/SMean	5D	153/144/154/154/167/136/137/137	3.14	3.84	− 20.48	135.5	168.5
	QTrl-6D-1	CK2/CK3/CKBlue/CKMean	6D-1	33/31/31/31	4.46	4.54	31.25	30.5	37.5
TRT	*QTrt-2A*	CK3/CKBlue/CKMean	2A	34/35/35	5.66	5.79	− 126.1	33.5	35.5
	QTrt-2B	CK2/CK3/CKBlue/CKMean	2B	4/4/4/4	12.83	9.62	− 206.3	3.5	4.5
	QTrt-2D	S2/SBlue/SMean	2D	22/30/26	3.17	5.26	− 63.1	12.5	39.5
	QTrt-5A	CK3/SBlue/SMean	5A	61/69/63	24.45	7.77	132.7	57.5	74.5
	QTrt-5D	CKBlue/CKMean/S2/SMean	5D	154/153/161/161	3.45	2.51	− 69.4	152.5	164.5
	QTrt-7B	CKBlue/CKMean	7B	69/69	21.89	15.37	− 234.3	67.5	69.5
	QTrt-7D	CK2/CKBlue/CKMean	7D	58/58/58	3.95	2.41	− 107.3	57.5	58.5
TRAD	***QTrad-2B***	CK2/CK3/CKBlue/CKMean	2B	4/4/4/4	15.37	23.05	0.012	3.5	4.5
	QTrad-5A	SBlue/SMean	5A	128/128	2.79	4.92	0.009	126.5	129.5
	QTrad-7B	CK2/CK3/CKBlue/CKMean/SBlue/SMean	7B	55/53/53/53/65/65	3.75	5.49	0.007	52.5	66.5
TRSA	*QTrsa-1D*	SBlue/SMean	1D	146/146	3.02	5.24	1.300	141.5	147.5
	QTrsa-2A	CK2/CK3/CKBlue/CKMean	2A	37/34/34/34	8.66	9.42	− 2.914	31.5	37.5
	QTrsa-2B	CK2/CK3/CKBlue/CKMean	2B	4/2/4/4	13.24	15.14	− 3.702	0.0	4.5
	QTrsa-5A	CK3/CKBlue/CKMean/S2/SBlue/SMean	5A	33/33/33/34/34/34	3.23	4.36	1.619	32.5	34.5
	QTrsa-5D	CKBlue/SBlue	5D	179/167	2.61	3.33	− 1.271	164.5	180.5
	QTrsa-7D	CKBlue/CKMean	7D	117/117	5.43	5.02	2.073	116.5	117.5
MRL	*QMrl-2A*	CK2/CK3/CKBlue/CKMean	2A	40/33/33/33	8.29	10.07	− 8.607	29.5	40.5
	QMrl-2B	CK2/CK3/CKBlue/CKMean	2B	1/0/2/2	11.73	15.20	− 10.572	0.0	3.5
	QMrl-3B	CKBlue/CKMean	3B	155/155	3.98	4.13	− 5.271	145.5	158.5
	QMrl-5D	CKBlue/CKMean	5D	185/183	2.88	3.10	− 4.563	180.5	185.5
	QMrl-7D	CKBlue/CKMean	7D	116/116	2.70	2.82	4.356	115.5	116.5
MRT	*QMrt-7B*	CKBlue/CKMean	7B	80/80	2.73	4.86	0.828	78.5	80.5

Table 2 (continued)

TraitName	QTL	Treatment	Chr	Position (cM)	LOD	PVE(%)	Add	LeftCI	RightCI
MRSA	*QMrsa-1D*	S3/SBlue/SMean	1D	147/146/146	3.62	6.19	0.709	141.5	147.5
	QMrsa-2A	CK2/CK3/CKBlue/CKMean	2A	37/33/34/34	7.92	8.84	− 1.266	30.5	37.5
	QMrsa-2B	CK2/CK3/CKBlue/CKMean	2B	0/1/1/1	8.05	9.09	− 1.278	0.0	3.5
	QMrsa-3B	CK3/CKBlue/CKMean	3B	153/150/151	3.67	5.23	− 0.943	140.5	160.5
	QMrsa-5A	CKBlue/CKMean/S2/SBlue/SMean	5A	33/33/34/34/34	3.16	4.02	0.679	32.5	34.5
	QMrsa-5B	CK3/CKBlue/CKMean	5B	44/44/44	3.95	3.94	− 0.836	43.5	44.5
	QMrsa-5D	S2/S3/SBlue/SMean	5D	168/160/169/169	2.81	4.51	− 0.649	158.5	174.5
	QMrsa-6A	CK3/CKBlue/CKMean	6A	63/63/63	3.05	3.09	0.738	62.5	68.5
	QMrsa-7D	CK3/CKBlue/CKMean	7D	117/117/116	3.44	3.54	0.790	115.5	117.5
LRL	*QLrl-2A*	CK2/CK3/CKBlue/CKMean	2A	36/34/34/34	6.35	6.22	− 30.72	33.5	37.5
	QLrl-2B	CK2/CK3/CKBlue/CKMean	2B	4/4/4/4	20.60	23.09	− 58.70	3.5	4.5
	QLrl-2D	S2/SBlue/SMean	2D	19/20/20	3.01	3.89	− 13.74	12.5	34.5
	QLrl-5A	SBlue/SMean	5A	68/63	40.38	8.92	40.30	62.5	75.5
	QLrl-5D	CK2/CK3/CKBlue/CKMean/S2/S3/SBlue/SMean	5D	153/144/154/154/168/136/137/160	3.33	3.38	− 16.42	135.5	173.5
	QLrl-6D-1	CK3/CKBlue/CKMean	6D-1	31/32/34	4.16	4.02	25.85	30.5	37.5
	QLrl-7D.1	CK2/CKBlue/CKMean	7D	58/58/58	4.29	4.23	− 24.02	57.5	58.5
	QLrl-7D.2	CKBlue/CKMean	7D	118/118	2.67	2.35	18.02	117.5	118.5
	QLrl-7D.3	CKBlue/CKMean	7D	172/172	3.31	3.85	− 23.08	162.5	178.5
LRT	*QLrt-2A*	CK3/CKBlue/CKMean	2A	34/35/35	5.62	5.74	− 125.3	33.5	35.5
	QLrt-2B	CK2/CK3/CKBlue/CKMean	2B	4/4/4/4	12.89	9.68	− 206.4	3.5	4.5
	QLrt-2D	S2/SBlue/SMean	2D	22/30/26	2.97	5.15	− 59.1	11.5	39.5
	QLrt-5A	CK3/SBlue/SMean	5A	61/69/63	25.20	7.80	132.7	57.5	74.5
	QLrt-5D	CKBlue/CKMean/S2/SMean	5D	154/153/161/161	3.67	2.55	− 69.9	152.5	164.5
	QLrt-7B	CKBlue/CKMean	7B	69/69	21.97	15.45	− 234.5	67.5	69.5
	QLrt-7D	CK2/CKBlue/CKMean	7D	58/58/58	3.96	2.42	− 107.3	57.5	58.5
LRSA	*QLrsa-2A*	CK2/CK3/CKBlue/CKMean/S3	2A	37/34/34/34/35	5.70	6.05	− 1.022	33.5	37.5
	QLrsa-2B	CK2/CK3/CKBlue/CKMean	2B	4/4/4/4	19.05	22.26	− 2.122	3.5	4.5
	QLrsa-2D	SBlue/SMean	2D	16/20	2.86	2.53	− 0.480	12.5	33.5
	QLrsa-5A	CK3/CKBlue	5A	26/27	3.65	3.74	0.930	24.5	29.5
	QLrsa-5D	CK3/S3/SBlue/SMean	5D	144/137/137/137	3.66	3.75	− 0.575	135.5	144.5
	QLrsa-6D-1	CK3/CKBlue/CKMean	6D-1	31/31/31	5.04	4.88	1.037	30.5	32.5
	QLrsa-7D	CK2/CKMean	7D	58/58	4.03	4.25	− 0.891	57.5	58.5

PVE is short for phenotypic variation explained; Add represents the additive effect; LeftCI and RightCI represent the left and right boundaries of the confidence interval, respectively. LOD, PVE (%), and Add are an average of detected values under different treatments. Positive additive effects indicate that alleles from ZM175 enhance corresponding trait values, and negative additive effects indicate that alleles from XY60 enhance corresponding trait values. QTL with bold and underlined font are detected with significant additive × treatment interaction effect (*at*)

Table 3 QTL with additive × treatment interaction effect (*at*) detected at seedling stage under CK and S treatments in ZX RIL population

TraitName	QTL	Chr	Position (cM)	LeftMarker	RightMarker	LOD	LOD (A)	LOD (AbyE)	PVE	PVE (A)	PVE (AbyE)	Add	AbyE_01	AbyE_02	LeftCI	RightCI
SH	*cQSh-4B*	4B	30	AX-109968140	AX-110928817	37.63	31.57	6.06	48.28	28.17	20.11	1.13	0.96	− 0.96	28.5	31.5
TN	*cQTn-5B*	5B	40	AX-110576186	AX-111178999	9.48	3.10	6.38	5.09	2.28	2.81	− 0.06	0.07	− 0.07	39.5	40.5
LN	*cQLn-2A*	2A	32	AX-110514157	AX-94419084	9.97	7.19	2.78	5.59	5.37	0.21	− 0.11	− 0.02	0.02	27.5	33.5
LN	*cQLn-2D*	2D	89	AX-110638095	AX-109853546	6.52	2.62	3.90	2.01	1.87	0.14	0.06	− 0.02	0.02	84.5	94.5
LN	*cQLn-5A*	5A	34	AX-110939747	AX-111028216	9.19	6.68	2.51	5.16	4.95	0.20	0.10	0.02	− 0.02	33.5	34.5
LN	*cQLn-5B*	5B	44	AX-108891185	AX-111183263	9.13	6.14	2.99	4.36	4.31	0.05	− 0.09	− 0.01	0.01	43.5	44.5
LN	*cQLn-6A*	6A	61	AX-111624010	AX-110676003	9.79	5.56	4.23	3.99	3.99	0.00	0.09	0.00	0.00	57.5	62.5
YLN	*cQYln-1A*	1A	40	AX-111028882	AX-108873053	6.45	3.24	3.21	5.58	3.63	1.95	0.03	− 0.02	0.02	38.5	40.5
RL	*cQRl-2B*	2B	4	AX-109521051	AX-109470204	47.64	41.84	5.80	60.83	34.85	25.98	− 1.24	-1.07	1.07	3.5	4.5
sK	*cQSk-4B*	4B	31	AX-109968140	AX-110928817	15.23	10.92	4.31	23.20	11.06	12.14	− 0.89	− 0.94	0.94	28.5	31.5
TRSA	*cQTrsa-2A*	2A	36	AX-111699117	AX-108741112	11.64	8.23	3.41	10.14	6.20	3.94	− 1.60	− 1.27	1.27	35.5	37.5
TRSA	*cQTrsa-2B*	2B	4	AX-109521051	AX-109470204	14.48	9.96	4.53	13.50	8.03	5.47	− 1.82	− 1.50	1.50	3.5	4.5
TRT	*cQTrt-5A*	5A	63	AX-108969605	AX-109440091	69.70	25.01	44.69	12.51	9.16	3.35	147.56	− 89.24	89.24	62.5	63.5
TRT	*cQTrt-5D*	5D	160	AX-111670747	AX-109241094	6.19	3.48	2.71	1.07	1.07	0.01	− 50.14	− 4.29	4.29	158.5	163.5
TRAD	*cQTrad-2B*	2B	4	AX-109521051	AX-109470204	18.21	2.73	15.48	11.65	3.70	7.96	0.01	0.01	-0.01	3.5	4.5
MRL	*cQMrl-2B*	2B	1	AX-109851387	AX-110913047	10.41	5.95	4.46	9.98	5.58	4.40	− 4.98	− 4.42	4.42	0.0	2.5
LRL	*cQLrl-5A*	5A	63	AX-108969605	AX-109440091	77.96	31.91	46.05	30.63	20.20	10.43	41.13	− 29.56	29.56	62.5	63.5
LRSA	*cQLrsa-5A*	5A	63	AX-108969605	AX-109440091	72.06	38.30	33.76	35.50	22.54	12.96	1.72	− 1.31	1.31	62.5	63.5
LRT	*cQLrt-5A*	5A	63	AX-108969605	AX-109440091	70.89	24.93	45.96	12.84	9.40	3.43	146.79	− 88.72	88.72	62.5	63.5

Table 3 (continued)

TraitName	QTL	Chr	Position (cM)	LeftMarker	RightMarker	LOD	LOD (A)	LOD (AbyE)	PVE	PVE (A)	PVE (AbyE)	Add	AbyE_01	AbyE_02	LeftCI	RightCI
LRT	*cQLrt-5D*	5D	161	AX-111670747	AX-109241094	6.24	3.48	2.76	1.09	1.08	0.01	− 49.47	− 4.39	4.39	158.5	163.5

A is short for additive QTL; E is short for environment; E_01 and E_02 represent CK treatment and S treatment, respectively. PVE is short for phenotypic variation explained; Add represents the additive effect; LeftCI and RightCI represent the left and right boundaries of the confidence interval, respectively

Under salt treatment, Na^+ content in root and shoot tissues increased rapidly, while K^+ absorbing capacity decreased. It was previously verified that K^+ and Na^+ concentrations and K^+/Na^+ discrimination were very important to wheat salt tolerance (Byrt et al. 2014; Dubcovsky et al. 1996; Dvorak and Gorham 1992; Gorham et al. 1987; Lindsay et al. 2004; Munns et al. 2012; Shah et al. 1987). Here, cation (K^+ and Na^+) contents in root and shoot were assayed in one and three trials, respectively. We found that the QTL (on chromosomes 1D, 3B, 5D and 6A) for K^+, Na^+ and K^+/Na^+ ratio in root were completely different from those (on chromosomes 1D, 2B, 2D, 4B, 5D, 6A, and 6B) in shoot. Although QTL for Na^+ in shoot were discovered with only one dataset, two QTL (*QsNa-2B* and *QsNa-5A*) could be detected with the mean value by both kinds of mapping software (Fig. S1). Interestingly, *QsNa-2B*, *QsK-2B*, and *QsK/Na-2B* were mapped to the same interval of 0–3.5-cM on chromosome 2B under CK and S treatments or just S treatment. *QsK-4B* and *QsK/Na-4B* were mapped to the same interval (21.5–34.5 cM) on the chromosome 4B, and they could explain the maximum phenotypic variation (12.87% and 8.67%). It has been shown that sNa was positively correlated with YLN, and they had a significantly negative relationship with SPAD under S treatment (Table S3). Here, *QSpad-1A* and *QYln-1A* were detected in the same interval 37.5–40.5 cM on chromosome 1A and *QSpad-3D* and *QYln-3D.1* were co-located in 74.5–81.5 cM on chromosome 3D. Coincidently, the additive effects of *QSpad-1A* and *QSpad-3D* were derived from XY60 alleles, while the additive effects came from ZM175 alleles at *QYln-1A* and *QYln-3D.1*. In addition, *QSpad-1A* explained the maximum phenotypic variation under S treatment and *QYln-1A* had significant *at* effects.

For ten RSATs, a total of 60 QTL were detected. Among them, 13 QTL were discovered under both CK and S treatments and 37 and 10 QTL were under only CK and S treatment, respectively. Besides, nine (*QTrt-5A*, *QTrt-5D*, *QTrad-2B*, *QTrsa-2A*, *QTrsa-2B*, *QMrl-2B*, *QLrl-5A*, *QLrt-5A*, and *QLrt-5D*) of all 60 QTL had significant *at* effects. The *at* effects of *cQLrl-5A* and *cQLrsa-5A* explained more than 10% of the phenotypic variation. Significantly, two chromosome intervals (i.e., 0–4.5 cM on chromosome 2B and 29.5–40.5 cM on chromosome 2A) were significantly important for root-related traits. The interval on chromosome 2B contributed to all the root-related traits except for MRT, and it could explain the maximum phenotypic variation for all the traits but TRT and LRT. The interval on chromosome 2A was related to all the root traits except for TRAD and MRT, and it could stably explain 5%–10% of the phenotypic variation. In addition, chromosome 7B was important for root tip number. *QTrt-7B* and *QLrt-7B* were mapped in the same interval (67.5–69.5 cM), and *QMrt-7B* was close to them in 78.5–80.5 cM.

QTL clusters

QTL for different traits could cluster together in one interval on a certain chromosome, which was usually pleiotropic and important. In the present study, nearly half of the QTL (78/158) were identified to gather on group-2 and 5 chromosomes, as well as chromosomes 4B and 7D (Fig. 1), which were designated as C2A, C2B, C2D, C5A, C5B, C5D, C4B, C7D-1, and C7D-2, respectively. In C2A, there were 14 QTL for LN, RL, SFW, SDW, RDW, TDW, and RSATs (TRL, TRT, TRSA, MRL, MRSA, LRL, LRT, and LRSA) in the interval of 27.5–48.5 cM.

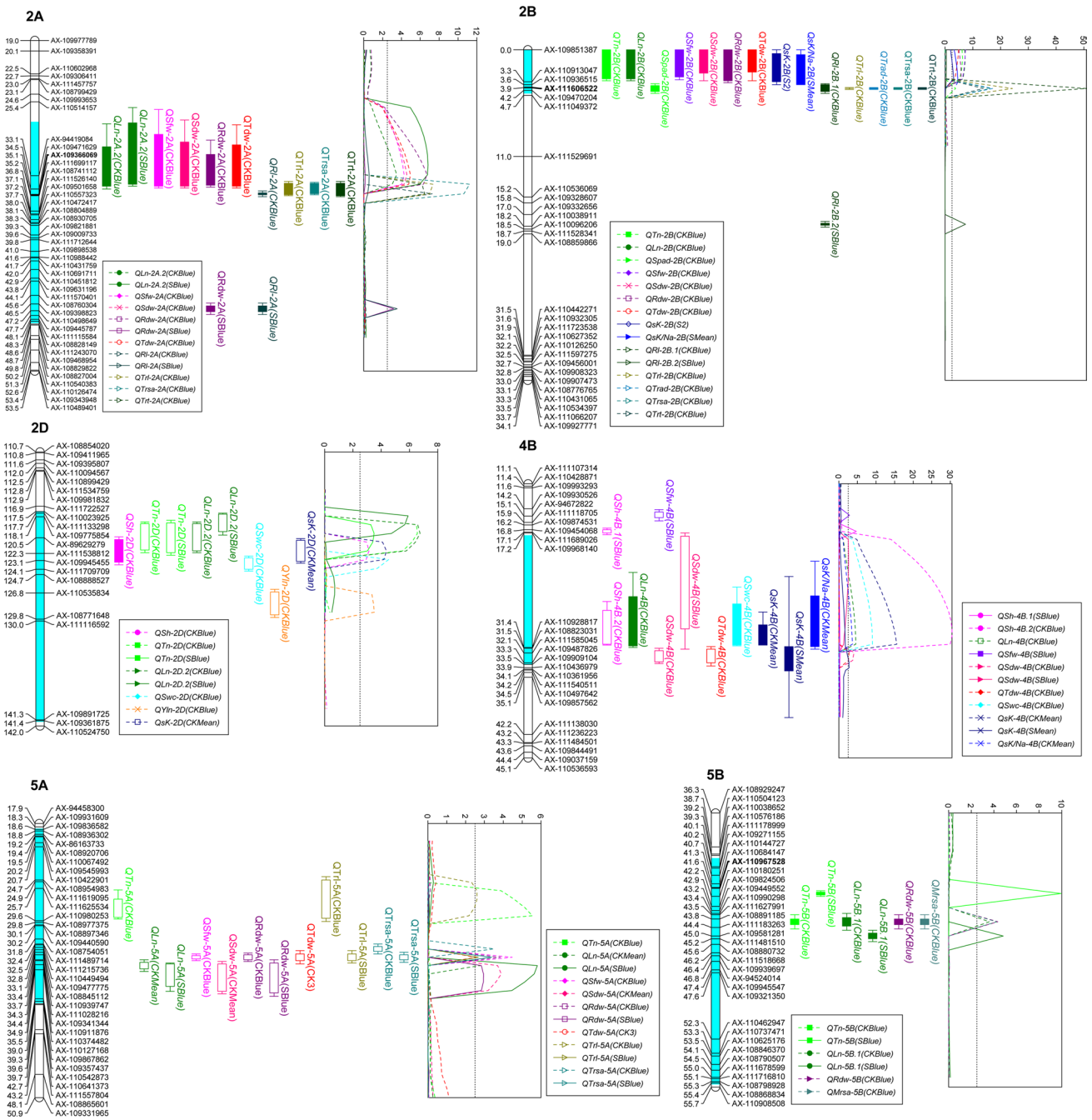

Fig. 1 Six QTL clusters with LOD curves and their involved QTL and SNP markers. The segments in cyan of the six chromosomes indicate the intervals of the QTL clusters. The solid rectangles indicate that the alleles from XY60 increase the corresponding traits; the blank rectangles indicate that the alleles from ZM175 increase the corresponding traits. The solid lines on LOD graph denote the QTL detected under salt treatment; the dashed lines denote the QTL detected under normal treatment. The SNPs in bold font were converted into KASP markers

The additive effects of them were all from XY60 alleles. Only two QTL had significant *at* effects, which just could explain 3.94% (*QTrsa-2A*) and 0.21% (*QLn-2A*) of the phenotypic variation. In C2B, a total of 19 QTL for TN, LN, SPAD, RL, sK, sK/Na, SFW, SDW, RDW, TDW, and RSATs (TRL, TRT, TRAD, TRSA, MRL, MRSA, LRL, LRT, and LRSA) were in the region of 0–4.5 cM, and their additive effects were all derived from XY60 alleles, too. The *at* effects of *cQRl-2B.1*, *cQTrsa-2B*, *cQTrad-2B*, and *cQMrl-2B* explained 25.98%, 5.47%, 7.96%, and 4.40% of the phenotypic variation, respectively. In the interval 117.5–141.5 cM, six QTL for SH, TN, LN, YLN, sK, and SWC assembled to form C2D. None of them had significant *at* effect, and the additive effects of them except for *QSh-2D* were derived from ZM175 alleles. Seven QTL for SH, LN, sK, sK/Na, SDW, SWC, and TDW were located in C4B (17.5–33.5 cM). *QSh-4B.2* and *QsK-4B* had significant *at* effects (20.11% and 12.14%) as well as high additive effects (36.55% and 12.87%). There were ten QTL for TN, LN, SFW, SDW, RDW, TDW, and RSATs (TRL, TRSA, MRSA, and LRSA) in the block of 18.5–39.5 cM on chromosome 5A (C5A), at which the ZM175-derived alleles had positive effects on corresponding traits. Only *QLn-5A* was observed with significant *at* effect explaining 0.2% of the phenotypic variation. In C5B, four QTL for TN, LN, RDW, and MRSA clustered in the region of 39.5–55.5 cM, and the alleles from XY60 expressed positive effects on the corresponding traits. Among them, *QTn-5B* was detected under both CK and S treatments, and its additive effect could explain 8.80% of the phenotypic variation, while its *at* effect explained 2.81%. *QLn-5B.1* was also detected under both treatments, and it contributed 5.19% to the phenotypic variation with just 0.05% of the *at* effects. The positions of ten QTL (*QRdw-5D*, *QsK-5D*, *QTrl-5D*, *QTrt-5D*, *QTrsa-5D*, *QMrl-5D*, *QMrsa-5D*, *QLrl-5D*, *QLrt-5D*, and *QLrsa-5D*) on chromosome 5D were not very consistent for different datasets, which led to a wide physical distance (124.5–185.5 cM). But their additive effects were all from XY60 alleles. Two QTL clusters (C7D-1 and C7D-2) were found in 57.5–58.5 cM and 115.5–119.5 cM on chromosome 7D, respectively. All QTL in them were for RSATs, and they only explained 2–5% of the phenotypic variation with no significant *at* effects. The alleles from XY60 at all four QTL (*QTrt-7D*, *QLrl-7D.1*, *QLrt-7D*, and *QLrsa-7D*) in the cluster C7D-1 could increase the corresponding traits values, while the alleles from ZM175 at all five QTL (*QRdw-7D*, *QTrsa-7D*, *QMrl-7D*, *QMrsa-7D*, and *QLrl-7D.2*) in the cluster C7D-2 showed positive effects. Fortunately, the additive effects of QTL above in one cluster are usually derived from a same parent's alleles, which would promote their effective utilization.

Validation of the QTL

Although most QTL were simultaneously detected by different kinds of mapping software, we evaluated them in "Hanxuan 10/Lumai 14" (HL) DH population and "Xiaoyan 54/Jing 411" (XJ) RIL population. Here, the additive effects of seven QTL intervals were verified (Fig. 2, Fig. S2, and Table S5). QTL for SFW, SDW, and TDW on chromosome 1B were detected in the same interval in HL population as well, explaining the phenotypic variation by 2.56%, 6.46%, and 8.88%, respectively (Table S5). Moreover, two common SNP markers (AX-109819289 and AX-108785293) linked to these QTL were found in ZX and HL populations (Fig. 2). *QLn-6A* was found to be linked to five common SNPs in ZX and HL populations. On chromosome 2B, QTL for TDW was detected in 13.45–14.15 cM in HL population and QTL for SPAD and root traits were found in 70.5–77.5 cM in XJ population, which sharing many SNPs with those in ZX population. It was worth mentioning that *QRl-2B*(XJCK), *QTrl-2B*(XJCK), and *QTrsa-2B*(XJCK) explained extensive phenotypic variation (42.20%, 24.56%, and 14.46%, respectively) in XJ population, which was similar to those in ZX population. QTL for SH, SDW, TDW, and SWC on chromosome 4B were discovered to be linked to eight identical SNP markers between ZX and XJ populations. In particular, *QSh-4B*(XJCK) could explain remarkable phenotypic variation (31.74%) in XJ population as *QSh-4B.2* (36.55%) in ZX population. On chromosome 5B, QTL for TN was detected under S treatment in both ZX and XJ populations, and two common SNPs (AX-109928742 and AX-89400290) were linked to it. QTL for SWC detected under both CK and S treatments were found in ZX and XJ populations and linked to 12 same SNP markers (Fig. S2).

KASP markers development

To apply important QTL associated with salt tolerance to wheat breeding, six SNPs, i.e., AX-109383322 (1A) linked to *QSpad-1A* and *QYln-1A*, AX-109819289 (1B) linked to QTL for biomass (*QSfw-1B*, *QSdw-1B*, and *QTdw-1B*), AX-109366069 (2A) linked to QTL for RSATs (*QRl-2A*, *QTrl-2A*, *QTrsa-2A*, and *QTrt-2A*), AX-111606522 (2B) linked to QTL for root-related traits (*QRl-2B.1*, *QTrl-2B*, *QTrad-2B, QTrsa-2B*, and *QTrt-2B*), AX-110967528 (5B) linked to *QTn-5B*, and AX-109593935 (6A) linked to *QLn-6A* were successfully converted to KASP markers (Fig. S4 and Table S6), which would also play a role in the process of gene cloning.

Discussion

Seedling stage is a very sensitive period to salt stress in the whole life of wheat. From late February to March, seedlings at spring greenup stage (Feekes 4) need to rapidly grow to

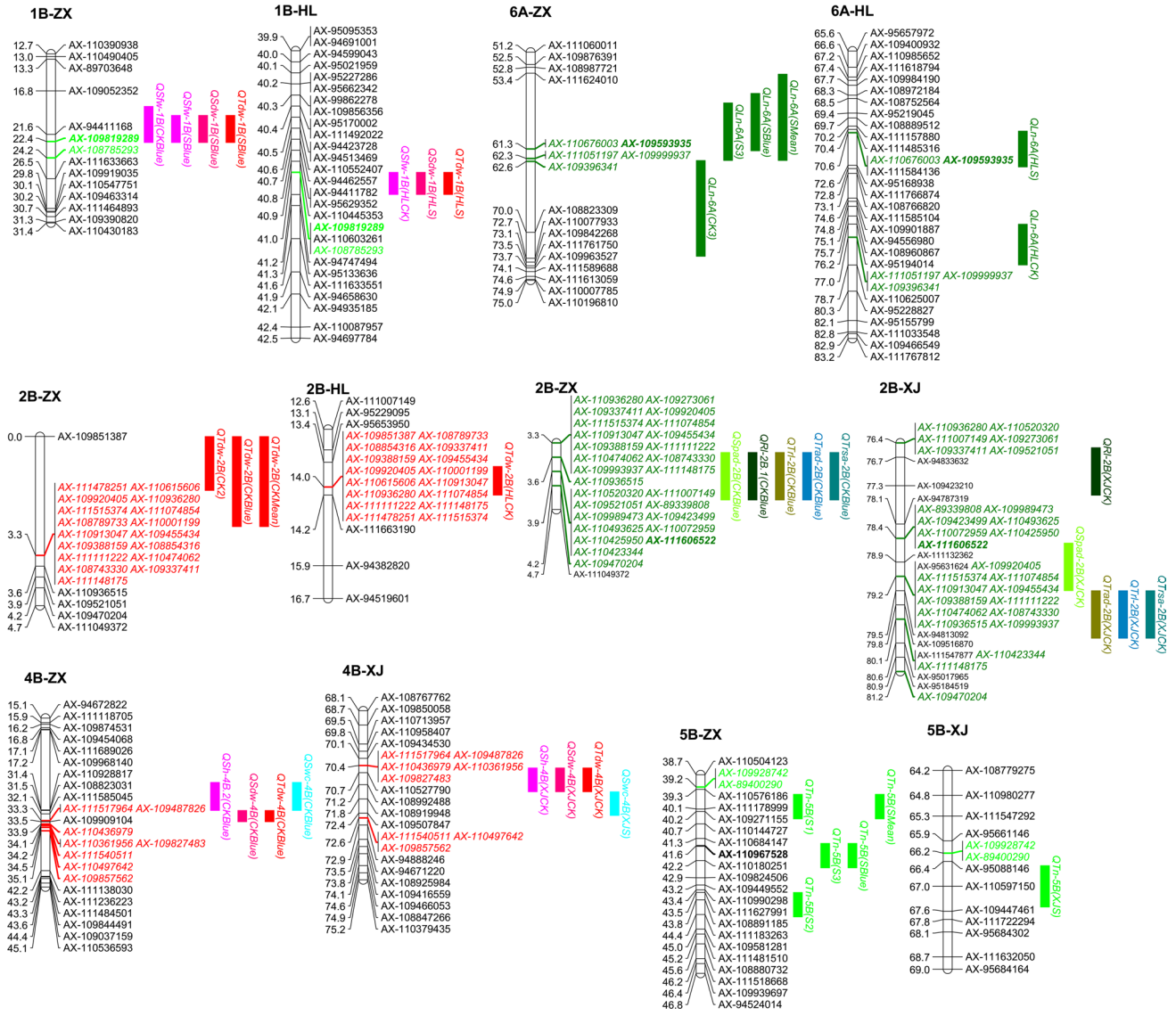

Fig. 2 Validate six QTL intervals in HL and XJ populations. Comparison of the common QTL detected in ZX and HL populations or in ZX and XJ populations. The colorful markers along the chromosomes were the same SNPs linked to the common QTL in two populations. The SNPs in bold font were converted into KASP markers

enter stem elongation stage (Feekes 5) (https://bookstore.ksre.ksu.edu/pubs/MF3300.pdf); at the same time, they have to face the high salinity of surface soil due to water evaporation and saline accumulation in monsoon climate region. Thus, as the major part of breeding salt-tolerant wheat cultivar, screening plants with high salt tolerance at seedling stage is a critical step.

It is known that K^+, Na^+ concentration and their ratio are very important for the salinity tolerance. Na^+ inhibits K^+ uptake and competes its binding sites in enzymes due to their physicochemical similarity. As a major gene enhancing K^+/Na^+ ratio in wheat, *Kna1* was found to be located at the long arm terminal of chromosome 4D (Byrt et al. 2014; Davenport et al. 2007; Dubcovsky et al. 1996; Dvorak and Gorham 1992; Gorham et al. 1997, 1987) and it could be a critical reason why hexaploid wheat is more tolerant to salinity than durum wheat (Colmer et al. 2006; Gorham et al. 1987). In this study, no QTL was detected on chromosome 4D, probably because there was no difference in *Kna1* between two parental lines. Furthermore, to our knowledge, it has not been reported in any hexaploid wheat populations previously (Do et al. 2018; Genc et al. 2010, 2019; Ilyas et al. 2020; Jahani et al. 2019; Nezhad et al. 2019; Xu et al. 2012, 2013). *Kna1* likely has come from Aegilops tauschii (Huang et al. 2008), which may lead to little or no allelic variation in hexaploid wheat. Although K^+ and Na^+ concentrations in XY60 and ZM175 were significantly different, no QTL for Na^+ exclusion was co-localized with *Nax1* (Huang et al.

2006; James et al. 2006; Lindsay et al. 2004) and *Nax2* (Byrt et al. 2007; James et al. 2006; Munns et al. 2012) in our study. However, we found a cation transporter gene *TraesCS2D02G428300* annotated as *HKT7* in the QTL cluster C2D (52,662115–613348655). Besides, as recent study pointed out, ion accumulation (Na^+, Cl^-) and biomass-related QTL could be mapped to a same region (Asif et al. 2021), *QsK-2B*, *QsNa-2B*, and *QsK/Na-2B* were co-located with QTL for TN, LN, SFW, SDW, RDW, and TDW. Similarly, *QsK-4B* and *QsK/Na-4B* were also co-localized with QTL for SH, LN, SDW, and TDW, and *TraesCS4B01G043100.1* in the interval of *QSh-4B* was annotated as *Rht-B1* by UniProt. Significantly, this region also mapped multiple QTL in dry salinity field, which contained QTL for plant height (PH), spike number per plant (SN), spikelet number per spike (SPS), kernel number per spike (KPS), thousand kernel weight (TKW), grain number per plant (GN), and harvest index (HI) (Luo et al. 2021). In addition, *QrNa-6A* for Na^+ concentration in root tissue could be the same locus as *Q.Na6A* (*cfd080–barc171*) (Genc et al. 2010) based on their physical positions. Their smaller correlation coefficients among three trials and lower h^2 proved that K^+ and Na^+ contents in seedling shoot were easily affected by environments, which may be one reason why major stable genes such as *Nax1* and *Nax2* were not detected in this study. The increased senescence rate of old leaves could be considered as Na^+-specific toxicity symptom due to either high leaf Na^+ or low tolerance to the accumulated Na^+ (Munns and Tester 2008). Consistently, YLN was positively correlated with sNa, while SPAD of the first leaf was negatively correlated with sNa, which was accordant with previous study (Masoudi et al. 2015). In ZX population, YLN and SPAD were more stable than sK and sNa according to their higher correlation coefficients among three trials and narrow-sense heritability. Thus, YLN and SPAD could be good indicators of sNa, and the regions 37.5–40.5 cM (*QYln-1A* and *QSpad-1A*) on chromosome 1A and 74.5–81.5 cM (*QYln-3D* and *QSpad-3D.1*) on chromosome 3D deserved further study. Specifically, gene prediction and functional annotation showed that *QSpad-1A* contained some genes such as potassium transporter, H-ATPase 3, calcium-transporting ATPase, and glutathione S-transferase according to IWGSC RefSeq v1.0 (Table S7).

Root plays an important role in seedling biomass under salt stress, but there is a lack of systemic study on the morphological characters of root after wheat suffering salt treatment. In this study, we noticed that root-related traits (RL, TRL, MRL, LRL, TRSA, MRSA, and LRSA) had high correlation coefficients (more than 0.5) with TDW under S treatment, and RL, TRT, LRT, and RDW even had higher correlation coefficients with TDW under S treatment than under CK treatment (Table S3). It was also demonstrated that QTL for root traits could be co-localized with QTL for biomass-related traits. For example, QTL for RSATs and biomass traits were co-located at the short arm terminal of chromosome 2B, which consisted of genes such as WRKY transcription factor, ethylene receptor, jasmonate-induced protein, and defensin (Table S7). Based on the physical positions of the linked markers, we determined that this region was previously reported (Cao et al. 2014; Ren et al. 2012) to benefit phosphate nutrient uptake and biomass accumulation and contribute to root length significantly but hinder root diameter under N deficiency. On chromosome 2D, QTL for root-related traits (RDW, TRL, TRT, LRL, LRSA, and LRT) were detected in 11.5–39.5 cM under S treatment in this study. Similarly, Xu et al. (2012) found QTL for root, shoot, and total dry weight under both CK and S treatments in the interval *Xcfd53–Xwmc112* on chromosome 2D. Additionally, it was noticed that QTL for K^+, proline content, tiller number (TN), TKW, days to heading (DTH), and days to anthesis (DTA) were also detected under sodic stress in this region (Devi et al. 2019). Moreover, it was analyzed that the QTL region on chromosome 2D in the present study matched with those in the above two studies based on the physical positions of linked markers. Notably, compared with our previous study (Luo et al. 2021), QTL for "hidden" underground traits at seedling stage were mapped to the same chromosome regions with some QTL for observable aboveground traits at adult stage. Examples are as follows: *QTrt-5A* (57.5–74.5 cM), *QLrt-5A* (57.5–74.5 cM), and *QLrl-5A* (62.5–75.5 cM) were mapped to the similar interval on chromosome 5A as *QSps-5A* (54.5–61.5 cM) and *QGn-5A* (59.5–80.5 cM). Like QTL for RSATs and seedling biomass traits, *QHi-5A* (33.5–35.5 cM) in dry salinity field was also detected in the region of C5A (18.5–39.5 cM). QTL for RSATs (*QTrt-5D*, *QTrl-5D*, *QTrsa-5D*, *QMrsa-5D*, *QLrt-5D*, and *QLrl-5D*) on chromosome 5D were co-localized with *QSps-5D* (146.5–173.5 cM). *QRdw-6A* and *QMrsa-6A* were mapped to the same region as QTL for PH, spike length (SL), SPS, KPS, TKW, and kernel-related traits (kernel length, kernel width, and perimeter of kernel). Besides, *QRl-7B*, *QTrt-7B*, *QTrad-7B*, and *QLrt-7B* were also co-localized with QTL for kernel length. Fan et al. (2018) found that QTL for RSATs were clustered in the 82.50–97.50-cM interval of chromosome 7B, which also had a significant effect on TKW. Based on our analysis, three chromosome regions on chromosome 5A, 5D, and 6A, respectively, controlled both RSATs and SPS. Thus, root traits could not only improve the seedling biomass under salt treatment, but also contribute to yield-related traits in saline soil. Accordingly, selecting plants with favorable alleles for seedling growth traits especially RSATs under salt treatment could be useful for the final grain yield in salinity field.

In this study, more than half (87/158) of the QTL were located on group-2 and 5 chromosomes (Fig. S3). It has been reported that group-5 chromosomes were regarded to carry

genes for abiotic stress resistance, including salt tolerance in wheat (Cattivelli et al. 2002; Quarrie et al. 2005). *Nax1* (*HKT7*) (Huang et al. 2006; Lindsay et al. 2004) and *Nax2* (*HKT8*) (Byrt et al. 2007; Munns et al. 2012) were located at the long arm terminals of chromosomes 2A and 5A, respectively. Interestingly, a homologous gene of *HKT7* was found in the cluster C2D in the present study. Significantly, the cluster C2D (117.5–141.5 cM) contained not only QTL at seedling stage such as *QSh-2D*, *QTn-2D*, *QLn-2D*, *QSwc-2D*, and *QsK-2D* under salt stress, but also QTL for SPS, TKW, yield per plant (YPP), aboveground biomass per plant (BM), HI, and kernel-related characters in dry salinity field (Luo et al. 2021). In cereals, salinity would mainly reduce the tiller number to decrease the total leaf area (Munns and Tester 2008). *QTn-5B* was stably detected in both ZX and XJ populations under S treatment, which could be a potential locus to improve salt tolerance. Furthermore, based on the wheat reference genome (IWGSC RefSeq v1.0), *QTn-5B* contained genes related to gibberellin-regulated family protein, ERD (early-responsive to dehydration stress) family protein, potassium transporter, calcium-binding protein, and so on (Table S7).

In wheat, strongly influenced by chromosome positions, recombination rate was markedly higher toward the distal ends of the chromosomes than in the interstitial and proximal regions (Ramirez-Gonzalez et al. 2018). Most QTL were distributed on distal ends of both chromosome arms, which could increase the adaptive plasticity of wheat and was verified in present and previous studies. As a result of the allopolyploid nature of the wheat genome, quantitative variation for many agronomic traits is modulated by genetic interactions between multiple sets of homoeologs in A, B, and D subgenomes (Borrill et al. 2015). Moreover, QTL for seedling and grain yield traits associated with salt tolerance were detected in homoeologous regions (Ma et al. 2007; Quarrie et al. 2005; Xu et al. 2012, 2013). Based on 850 wheat RNA-sequencing datasets from different tissues, developmental stages, and cultivars, it was found that about 70% of triads (A, B, and D homoeologs) showed balanced expression among homoeologs, whereas 30% showed non-balanced expression patterns with higher or lower expression from a single homoeolog with respect to the other two (Ramirez-Gonzalez et al. 2018). In our results, QTL for root-related traits (RL, RDW, TRL, TRT, LRL, LRT, and LRSA) were found on the short arm distal ends of group-2 chromosomes but with distinctly different phenotypic variation explained (PVE). A typical example is that *QRl-2B* (3.5–4.5 cM, PVE = 46.43%) significantly explained more phenotypic variation than *QRl-2A* (33.5–47.5 cM, PVE = 5.29%) and *QRl-2D* (0–0.5 cM, PVE = 5.59%). Transcriptome analysis also demonstrated that syntenic triads in the balanced category were overrepresented in the low-recombination regions, while homoeolog-dominant and homoeolog-suppressed triads were overrepresented toward the high-recombination distal ends of chromosomes (Ramirez-Gonzalez et al. 2018). This could be the reason why three homoeologous genes were rarely detected at the same time in mapping studies and the possible homoeologous QTL explained different phenotypic variation.

Early, epistatic effect (*aa*) was found to play an important role in maize (Doebley and Stec 1995) and rice (Yu et al. 1997). Later, researchers discovered that in wheat *aa* was also significant for coleoptile growth (Rebetzke et al. 2007), water-soluble carbohydrates (Yang et al. 2007), plant height (Zhang et al. 2008), heading (Ashraf and Foolad 2013), and kernel morphometric traits (Prashant et al. 2012). Based on various studies (Azadi et al. 2015; Ilyas et al. 2020; Jahani et al. 2019; Ma et al. 2007; Nezhad et al. 2019; Quarrie et al. 2005; Villalta et al. 2007; Xu et al. 2012, 2013; Xue et al. 2009), the detected QTL would be inconsistent in a same genetic population, and the magnitude and direction of QTL effects, as well as LOD scores, could also be changed in different environments. Here, about a quarter of the QTL (39/158) were stable under both CK and S treatments, while about half of them (80/158) were observed only under CK conditions. QTL differed with environments, indicating significant QTL-by-environment effect (*at*) (Genc et al. 2013). Epistatic effect and QTL-by-environment effect had been reported for salt tolerance in wheat (Genc et al. 2013; Jahani et al. 2019; Masoudi et al. 2015; Nezhad et al. 2019; Xu et al. 2012, 2013). In the present study, although 94 pairs of *aa* were detected, only one for SWC was near to an additive QTL (*QSwc-2B.1*), which was consistent with other reports that the majority of the interacting loci had no significant main additive effect in wheat (Jahani et al. 2019; Reif et al. 2011), barley (Xu and Jia 2007), and rice (Li et al. 1997). Stable QTL across multiple environments are vital to MAS in wheat breeding. Hence, it is very necessary to figure out *at* effect under salinity stress in mapping studies. In this paper, QTL for TN (*QTn-2A* and *QTn-2D*), LN (*QLn-2D.2*), RL (*QRl-2B.2*), and SDW (*QSdw-4B*) were all stably detected under different treatments without significant *at* effect, and they could explain nearly 10% of the phenotypic variation. *QSh-4B.2* and *QRl-2B.1* discovered only under CK treatment could explain the maximum phenotypic variation (36.55% and 46.43%, respectively). Even though higher *at* effects were found at these two loci, they were very stable due to their validation in XJ population as well. Besides, *QSh-4B.2* should play a vital role during the growing period because it could also be detected at maturity stage. Consequently, the above loci should be on the useful list of MAS in salt-tolerant wheat breeding.

Besides *aa* and *at*, genetic background (GB) also influences the QTL detection and MAS utilization in breeding. For examples, Cui et al. (2014) and Jahani et al. (2019) found that only a few of QTL were shared across 2–3

wheat GBs. Here, the parental lines of ZX and XJ population have a definite genetic relationship, while there is no direct relationship among the parents of ZX and HL populations. Verification experiments demonstrated that four stable major QTL were concurrently detected in both ZX and XJ populations and three QTL were shared by HL and ZX populations (Fig. 2). Since genetic positions on different genetic linkage maps were greatly different, same markers or consistent physical positions were the most credible information to decide if two QTL were the same one. With better wheat reference sequence and deeper mapping study, we would search out more reliable loci for MAS in salt-tolerant wheat breeding based on big data analysis.

In conclusion, this paper identified 158 stable additive QTL for 27 morphological and physiological traits at seedling stage of wheat. Among them, 19 QTL were detected with significant QTL × treatment effects (*at*), but none was found with epistatic effects (*aa*). About half of the QTL (78/158) were mapped in nine QTL clusters mainly on group-2 and 5 chromosomes as well as 4B and 7D. Seven QTL intervals were further validated in the other two genetic populations. In addition, six SNPs linked to important QTL were successfully converted to KASP markers, which will benefit the MAS breeding and future gene cloning. Our results fully explored the genetic basis of seedling traits (especially root system-related traits) associated with salt tolerance in wheat and will provide important information for MAS in salt-tolerant wheat breeding.

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Author Contribution statement ZSL and QZ supervised the research. QZ and QLL designed the experiment. QLL and QZ performed the phenotypes of ZX RIL population. PH and QLL performed the phenotypes of XJ and HL population. QLL performed data analysis and QTL mapping, confirmed the QTL effects and wrote the manuscript. QZ also put forward many constructive suggestions and revised the manuscript. GTY, HWL, LQL, ZSW, and BL provided a lot of help in the phenotype identification and materials preparation. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare no conflicts of interest.

References

Ali Z, Park HC, Ali A, Oh D-H, Aman R, Kropornicka A, Hong H, Choi W, Chung WS, Kim W-Y, Bressan RA, Bohnert HJ, Lee SY, Yun D-J (2012) *TsHKT1;2*, a HKT1 homolog from the extremophile Arabidopsis relative *Thellungiella salsuginea*, shows K^+ specificity in the presence of NaCl. Plant Physiol 158:1463–1474

Ashraf M, Foolad MR (2013) Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection. Plant Breed 132:10–20

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, Gilliham M, Fleury D, Tester M, Roy SJ, Pearson AS (2018) Mapping of novel salt tolerance QTL in an Excalibur × Kukri doubled haploid wheat population. Theor Appl Genet 131:2179–2196

Asif MA, Garcia M, Tilbrook J, Brien C, Dowling K, Berger B, Schilling RK, Short L, Trittermann C, Gilliham M, Fleury D, Roy SJ, Pearson AS (2021) Identification of salt tolerance QTL in a wheat RIL mapping population using destructive and non-destructive phenotyping. Funct Plant Biol 48:131–140

Azadi A, Mardi M, Hervan EM, Mohammadi SA, Moradi F, Tabatabaee MT, Pirseyedi SM, Ebrahimi M, Fayaz F, Kazemi M, Ashkani S, Nakhoda B, Mohammadi-Nejad G (2015) QTL mapping of yield and yield components under normal and salt-stress conditions in bread wheat (*Triticum aestivum* L.). Plant Mol Biol Rep 33:102–120

Borrill P, Adamski N, Uauy C (2015) Genomics as the key to unlocking the polyploid potential of wheat. New Phytol 208:1008–1022

Byrt CS, Platten JD, Spielmeyer W, James RA, Lagudah ES, Dennis ES, Tester M, Munns R (2007) *HKT1;5*-like cation transporters linked to Na^+ exclusion loci in wheat, *Nax2* and *Kna1*. Plant Physiol 143:1918–1928

Byrt CS, Xu B, Krishnan M, Lightfoot DJ, Athman A, Jacobs AK, Watson-Haigh NS, Plett D, Munns R, Tester M, Gilliham M (2014) The Na^+ transporter, *TaHKT1;5-D*, limits shoot Na^+ accumulation in bread wheat. Plant J 80:516–526

Cao P, Ren Y, Zhang K, Teng W, Zhao X, Dong Z, Liu X, Qin H, Li Z, Wang D, Tong Y (2014) Further genetic analysis of a major quantitative trait locus controlling root length and related traits in common wheat. Mol Breed 33:975–985

Cattivelli L, Baldi P, Crosatti C, Fonzo ND, Faccioli P, Grossi M, Mastrangelo AM, Pecchioni N, Stanca AM (2002) Chromosome regions and stress-related sequences involved in resistance to abiotic stress in *Triticeae*. Plant Mol Biol 48:649–665

Colmer TD, Flowers TJ, Munns R (2006) Use of wild relatives to improve salt tolerance in wheat. J Exp Bot 57:1059–1078

Cui F, Zhao C, Ding A, Li J, Wang L, Li X, Bao Y, Li J, Wang H (2014) Construction of an integrative linkage map and QTL mapping of grain yield-related traits using three related wheat RIL populations. Theor Appl Genet 127:659–675

Davenport R, James RA, Zakrisson-Plogander A, Tester M, Munns R (2005) Control of sodium transport in durum wheat. Plant Physiol 137:807–818

Davenport RJ, Munoz-Mayor A, Jha D, Essah PA, Rus A, Tester M (2007) The Na^+ transporter AtHKT1;1 controls retrieval of Na^+ from the xylem in Arabidopsis. Plant Cell Environ 30:497–507

De Leon JLD, Escoppinichi R, Geraldo N, Castellanos T, Mujeeb-Kazi A, Roder MS (2011) Quantitative trait loci associated with salinity tolerance in field grown bread wheat. Euphytica 181:371–383

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI (2014) Plant salt-tolerance mechanisms. Trends Plant Sci 19:371–379

Devi R, Ram S, Rana V, Malik VK, Pande V, Singh GP (2019) QTL mapping for salt tolerance associated traits in wheat (*Triticum aestivum* L.). Euphytica 215:23

Dias J (2015) Plant breeding for harmony between modern agriculture production and the environment. Agric Sci 6:87–116

Do TD, Vuong TD, Dunn D, Smothers S, Patil G, Yungbluth DC, Chen P, Scaboo A, Xu D, Carter TE, Nguyen HT, Grover Shannon J (2018) Mapping and confirmation of loci for salt tolerance in a novel soybean germplasm, Fiskeby III. Theor Appl Genet 131:513–524

Doebley J, Stec A (1995) Teosinte branched1 and the origin of maize: evidence for epistasis and the evolution of dominance. Genetics 141:333–346

Dubcovsky J, María GS, Epstein E, Luo MC, Dvořák J (1996) Mapping of the K^+/Na^+ discrimination locus *Kna1* in wheat. Theor Appl Genet 92:448–454

Dvorak J, Gorham J (1992) Methodology of gene transfer by homoeologous recombination into *Triticum turgidum*: transfer of K^+/Na^+ discrimination from *Triticum aestivum*. Genome 35:639–646

Fan X, Zhang W, Zhang N, Chen M, Zheng S, Zhao C, Han J, Liu J, Zhang X, Song L, Ji J, Liu X, Ling H, Tong Y, Cui F, Wang T, Li J (2018) Identification of QTL regions for seedling root traits and their effect on nitrogen use efficiency in wheat (*Triticum aestivum* L.). Theor Appl Genet 131:2677–2698

Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants: where next? Aust J Plant Physiol 22:875–884

Flowers TJ, Garcia A, Koyama M, Yeo AR (1997) Breeding for salt tolerance in crop plants—the role of molecular biology. Acta Physiol Plant 19:427–433

Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, Mueller ND, O'Connell C, Ray DK, West PC, Balzer C, Bennett EM, Carpenter SR, Hill J, Monfreda C, Polasky S, Rockstrom J, Sheehan J, Siebert S, Tilman D, Zaks DP (2011) Solutions for a cultivated planet. Nature 478:337–342

Genc Y, Oldach K, Verbyla AP, Lott G, Hassan M, Tester M, Wallwork H, McDonald GK (2010) Sodium exclusion QTL associated with improved seedling growth in bread wheat under salinity stress. Theor Appl Genet 121:877–894

Genc Y, Oldach K, Gogel B, Wallwork H, McDonald GK, Smith AB (2013) Quantitative trait loci for agronomic and physiological traits for a bread wheat population grown in environments with a range of salinity levels. Mol Breed 32:39–59

Genc Y, Taylor J, Lyons G, Li Y, Cheong J, Appelbee M, Oldach K, Sutton T (2019) Bread wheat with high salinity and sodicity tolerance. Front Plant Sci 10:1280

Ghaedrahmati M, Mardi M, Naghavi MR, Haravan EM, Nakhoda B, Azadi A, Kazemi M (2014) Mapping QTLs associated with salt tolerance related traits in seedling stage of wheat (*Triticum aestivum* L.). J Agric Sci Technol 16:1413–1428

Gorham J, Hardy C, Jones RGW, Joppa LR, Law CN (1987) Chromosomal location of a K/Na discrimination character in the D-genome of wheat. Theor Appl Genet 74:584–588

Gorham J, Jones RGW, Bristol A (1990) Partial characterization of the trait for enhanced K^+/Na^+ discrimination in the D genome of wheat. Planta 180:590–597

Gorham J, Bridges J, Dubcovsky J, Dvorak J, Hollington PA, Luo MC, Khan JA (1997) Genetic analysis and physiology of a trait for enhanced K^+/Na^+ discrimination in wheat. New Phytol 137:109–116

Han Y, Li D, Zhu D, Li H, Li X, Teng W, Li W (2012) QTL analysis of soybean seed weight across multi-genetic backgrounds and environments. Theor Appl Genet 125:671–683

Hao ZF, Chang XP, Guo XJ, Jing RL, Jia JZ (2003) QTL mapping for drought tolerance at stages of germination and seedling in wheat (*Triticum aestivum* L.) using a DH population. Agric Sci China 2:943–949

Hauser F, Horie T (2010) A conserved primary salt tolerance mechanism mediated by HKT transporters: a mechanism for sodium exclusion and maintenance of high K^+/Na^+ ratio in leaves during salinity stress. Plant Cell Environ 33:552–565

Horie T, Hauser F, Schroeder JI (2009) HKT transporter-mediated salinity resistance mechanisms in Arabidopsis and monocot crop plants. Trends Plant Sci 14:660–668

Huang SB, Spielmeyer W, Lagudah ES, James RA, Platten JD, Dennis ES, Munns R (2006) A sodium transporter (HKT7) is a candidate for *Nax1*, a gene for salt tolerance in durum wheat. Plant Physiol 142:1718–1727

Huang SB, Spielmeyer W, Lagudah ES, Munns R (2008) Comparative mapping of HKT genes in wheat, barley, and rice, key determinants of Na^+ transport, and salt tolerance. J Exp Bot 59:927–937

Hussain B, Lucas SJ, Ozturk L, Budak H (2017) Mapping QTLs conferring salt tolerance and micronutrient concentrations at seedling stage in wheat. Sci Rep 7:15662

Ilyas N, Amjid MW, Saleem MA, Khan W, Wattoo FM, Rana RM, Maqsood RH, Zahid A, Shah GA, Anwar A, Ahmad MQ, Shaheen M, Riaz H, Ansari MJ (2020) Quantitative trait loci (QTL) mapping for physiological and biochemical attributes in a Pasban90/Frontana recombinant inbred lines (RILs) population of wheat (*Triticum aestivum*) under salt stress condition. Saudi J Biol Sci 27:341–351

Jahani M, Mohammadi-Nejad G, Nakhoda B, Rieseberg LH (2019) Genetic dissection of epistatic and QTL by environment interaction effects in three bread wheat genetic backgrounds for yield-related traits under saline conditions. Euphytica 215:25

James RA, Davenport RJ, Munns R (2006) Physiological characterization of two genes for Na^+ exclusion in durum wheat, *Nax1* and *Nax2*. Plant Physiol 142:1537–1547

Jamil A, Riaz S, Ashraf M, Foolad MR (2011) Gene expression profiling of plants under salt stress. Crit Rev Plant Sci 30:435–458

Ji H, Pardo JM, Batelli G, Van Oosten MJ, Bressan RA, Li X (2013) The salt overly sensitive (SOS) pathway: established and emerging roles. Mol Plant 6:275–286

Li ZK, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997) Epistasis for three grain yield components in Rice (*Oryza Sativa* L.). Genetics 145:453–465

Li Z, Li B, Tong Y (2008) The contribution of distant hybridization with decaploid *Agropyron elongatum* to wheat improvement in China. J Genet Genom 35:451–456

Li L, Peng Z, Mao X, Wang J, Chang X, Reynolds M, Jing R (2019) Genome-wide association study reveals genomic regions controlling root and shoot traits at late growth stages in wheat. Ann Bot 124:993–1006

Lindsay MP, Lagudah ES, Hare RA, Munns R (2004) A locus for sodium exclusion (*Nax1*), a trait for salt tolerance, mapped in durum wheat. Funct Plant Biol 31:1105–1114

Luo MC, Dubcovsky J, Goyal S, Dvorak J (1996) Engineering of interstitial foreign chromosome segments containing the K^+/Na^+ selectivity gene *Kna1* by sequential homoeologous recombination in durum wheat. Theor Appl Genet 93:1180–1184

Luo Q, Zheng Q, Hu P, Liu L, Yang G, Li H, Li B, Li Z (2021) Mapping QTL for agronomic traits under two levels of salt stress in a new constructed RIL wheat population. Theor Appl Genet 134:171–189

Ma LQ, Zhou EF, Huo NX, Zhou RH, Wang GY, Jia JZ (2007) Genetic analysis of salt tolerance in a recombinant inbred population of wheat (*Triticum aestivum* L.). Euphytica 153:109–117

Mahajan S, Pandey GK, Tuteja N (2008) Calcium- and salt-stress signaling in plants: shedding light on SOS pathway. Arch Biochem Biophys 471:146–158

Masoudi B, Mardi M, Hervan EM, Bihamta MR, Naghavi MR, Nakhoda B, Amini A (2015) QTL mapping of salt tolerance traits with different effects at the seedling stage of bread wheat. Plant Mol Biol Rep 33:1790–1803

Matthew R, David B, Chapman SC, Furbank RT, Yann M, Parry MAJ (2011) Raising yield potential of wheat. I. Overview of a consortium approach and breeding strategies. J Exp Bot 62:439–452

Mujeeb-Kazi A, Munns R, Rasheed A, Ogbonnaya F, Ali N, Hollington P, Dundas I, Saeed N, Wang R, Rengasamy P, Saddiq M, León J, Ashraf M, Rajaram S (2019) Breeding strategies for structuring salinity tolerance in wheat. Adv Agron 155:121–187

Munns R, Gilliham M (2015) Salinity tolerance of crops—what is the cost? New Phytol 208:668–673

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Munns R, James RA, Xu B, Athman A, Conn SJ, Jordans C, Byrt CS, Hare RA, Tyerman SD, Tester M (2012) Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. Nat Biotech 30:360–364

Nezhad NM, Kamali MRJ, McIntyre CL, Fakheri BA, Omidi M, Masoudi B (2019) Mapping QTLs with main and epistatic effect on Seri "M82 × Babax" wheat population under salt stress. Euphytica 215:19

Oyiga BC, Sharma RC, Baum M, Ogbonnaya FC, Leon J, Ballvora A (2018) Allelic variations and differential expressions detected at quantitative trait loci for salt stress tolerance in wheat. Plant Cell Environ 41:919–935

Platten JD, Cotsaftis O, Berthomieu P, Bohnert H, Davenport RJ, Fairbairn DJ, Horie T, Leigh RA, Lin H-X, Luan S, Maeser P, Pantoja O, Rodriguez-Navarro A, Schachtman DP, Schroeder JI, Sentenac H, Uozumi N, Very A-A, Zhu J-K, Dennis ES, Tester M (2006) Nomenclature for HKT transporters, key determinants of plant salinity tolerance. Trends Plant Sci 11:372–374

Prashant R, Kadoo N, Desale C, Kore P, Dhaliwal HS, Chhuneja P, Gupta V (2012) Kernel morphometric traits in hexaploid wheat (*Triticum aestivum* L.) are modulated by intricate QTL × QTL and genotype × environment interactions. J Cereal Sci 56:432–439

Qiu QS, Guo Y, Dietrich MA, Schumaker KS, Zhu JK (2002) Regulation of SOS1, a plasma membrane Na^+/H^+ exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. Proc Natl Acad Sci 99:8436–8441

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti MC, Hollington PA, Aragues R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865–880

Ramirez-Gonzalez RH, Borrill P, Lang D, Harrington SA, Brinton J, Venturini L, Davey M, Jacobs J, van Ex F, Pasha A, Khedikar Y, Robinson SJ, Cory AT, Florio T, Concia L, Juery C, Schoonbeek H, Steuernagel B, Xiang D, Ridout CJ, Chalhoub B, Mayer KFX, Benhamed M, Latrasse D, Bendahmane A, International Wheat Genome Sequencing C, Wulff BBH, Appels R, Tiwari V, Datla R, Choulet F, Pozniak CJ, Provart NJ, Sharpe AG, Paux E, Spannagl M, Brautigam A, Uauy C (2018) The transcriptional landscape of polyploid wheat. Science 361:eaar6089

Rebetzke GJ, Ellis MH, Bonnett DG, Richards RA (2007) Molecular mapping of genes for coleoptile growth in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 114:1173–1183

Reif JC, Maurer HP, Korzun V, Ebmeyer E, Miedaner T, Würschum T (2011) Mapping QTLs with main and epistatic effects underlying grain yield and heading time in soft winter wheat. Theor Appl Genet 123:283–292

Ren Y, He X, Liu D, Li J, Zhao X, Li B, Tong Y, Zhang A, Li Z (2012) Major quantitative trait loci for seminal root morphology of wheat seedlings. Mol Breed 30:139–148

Rengasamy P (2010) Soil processes affecting crop production in salt-affected soils. Funct Plant Biol 37:613–620

Roy SJ, Negrao S, Tester M (2014) Salt resistant crop plants. Curr Opin Biotech 26:115–124

Shah SH, Gorham J, Forster BP, Jones RGW (1987) Salt tolerance in the Triticeae: the contribution of the D genome to cation selectivity in hexaploid wheat. J Exp Bot 38:254–269

Shi HZ, Lee BH, Wu SJ, Zhu JK (2003) Overexpression of a plasma membrane Na^+/H^+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. Nat Biotech 21:81–85

Soriano JM, Alvaro F (2019) Discovering consensus genomic regions in wheat for root-related traits by QTL meta-analysis. Sci Rep 9:14

Tester M, Davenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Ann Bot Lond 91:503–527

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677

Venuprasad R, Bool ME, Quiatchon L, Atlin GN (2012) A QTL for rice grain yield in aerobic environments with large effects in three genetic backgrounds. Theor Appl Genet 124:323–332

Vikram P, Swamy MB, Dixit S, Ahmed UH, Cruz MTS, Singh AK, Kumar A (2011) *qDTY1.1*, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. BMC Genet 12:89

Villalta I, Bernet GP, Carbonell EA, Asins MJ (2007) Comparative QTL analysis of salinity tolerance in terms of fruit yield using two solanum populations of F_7 lines. Theor Appl Genet 114:1001–1017

Weinl S, Kudla J (2009) The CBL-CIPK Ca^{2+}-decoding signaling network: function and perspectives. New Phytol 184:517–528

Xu Y, Crouch JH (2008) Marker-assisted selection in plant breeding: from publications to practice. Crop Sci 48:391–407

Xu S, Jia Z (2007) Genomewide analysis of epistatic effects for quantitative traits in barley. Genetics 175:1955–1963

Xu Y, An D, Liu D, Zhang A, Xu H, Li B (2012) Mapping QTLs with epistatic effects and QTL × treatment interactions for salt tolerance at seedling stage of wheat. Euphytica 186:233–245

Xu Y, Li S, Li L, Zhang X, Xu H, An D (2013) Mapping QTLs for salt tolerance with additive, epistatic and QTL × treatment interaction effects at seedling stage in wheat. Plant Breed 132:276–283

Xue D, Huang Y, Zhang X, Wei K, Westcott S, Li C, Chen M, Zhang G, Lance R (2009) Identification of QTLs associated with salinity tolerance at late growth stage in barley. Euphytica 169:187–196

Yang DL, Jing RL, Chang XP, Li W (2007) Identification of quantitative trait loci and environmental interactions for accumulation and remobilization of water-soluble carbohydrates in wheat (*Triticum aestivum* L.) stems. Genetics 176:571–584

Yang Q, Chen ZZ, Zhou XF, Yin HB, Li X, Xin XF, Hong XH, Zhu JK, Gong Z (2009) Overexpression of SOS (Salt Overly Sensitive) genes increases salt tolerance in transgenic Arabidopsis. Mol Plant 2:22–31

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, Maroof MAS (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci 94:9226–9231

Zhang K, Tian J, Zhao L, Wang S (2008) Mapping QTLs with epistatic effects and QTL × environment interactions for plant height using a doubled haploid population in cultivated wheat. J Genet Genom 35:119–127