



QTLs for seedling traits under salinity stress in hexaploid wheat

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ABSTRACT: Soil salinity limits agricultural production and is a major obstacle for increasing crop yield. Common wheat is one of the most important crops with allohexaploid characteristic and a highly complex genome. QTL mapping is a useful way to identify genes for quantitative traits such as salinity tolerance in hexaploid wheat. In the present study, a hydroponic trial was carried out to identify quantitative trait loci (QTLs) associated with salinity tolerance of wheat under 150mM NaCl concentration using a recombinant inbred line population (Xiaoyan 54×Jing 411). Values of wheat seedling traits including maximum root length (MRL), root dry weight (RDW), shoot dry weight (SDW), total dry weight (TDW) and the ratio of TDW of wheat plants between salt stress and control (TDWR) were evaluated or calculated. A total of 19QTLs for five traits were detected through composite interval mapping method by using QTL Cartographer version 2.5 under normal and salt stress conditions. These QTLs distributed on 12 chromosomes explained the percentage of phenotypic variation by individual QTL varying from 7.9% to 19.0%. Among them, 11 and six QTLs were detected under normal and salt stress conditions, respectively and two QTLs were detected for TDWR. Some salt tolerance related loci may be pleiotropic. Chromosome 1A, 3A and 7A may harbor crucial candidate genes associated with wheat salt tolerance. Our results would be helpful for the marker assisted selection to breed wheat varieties with improved salt tolerance.

Key words: *Triticum aestivum* L., salinity stress, quantitative trait locus (QTL), marker assisted selection.

Caracterização de QTLs por traços de sementes sob o estresse de salinidade em trigo Hexaploid

RESUMO: A salinidade do solo limita a produção agrícola. O trigo mole é uma das culturas mais importantes com característica allohexaploid e genoma altamente complexo. O mapeamento QTL é uma maneira muito útil de identificar genes para traços quantitativos, como a tolerância à salinidade em trigo hexaplóide. No presente estudo realizou-se um ensaio hidropônico para identificar locos de traços quantitativos (QTLs) associados à tolerância à salinidade do trigo sob concentração de NaCl 150 mM, usando uma população de linhagem consanguíneo recombinante (Xiaoyan 54 × Jing 411). Os valores dos traços de mudas de trigo, incluindo comprimento máximo da raiz (MRL), peso seco da raiz (RDW), ponha o peso seco (SDW), peso seco total (TDW) e a proporção das plantas de trigo TDW entre o estresse salgado e o controle (TDWR), foram avaliados ou calculados. Um total de 19QTLs para cinco traços foram detectados através do método de mapeamento de intervalo composto usando a versão 2.5 do cartógrafo QTL sob condições normais e de estresse salino. Estes QTLs distribuídos em 12 cromossomos explicaram a porcentagem de variação fenotípica por QTL individual variando de 7,9% a 19,0%. Entre eles, foram detectados 11 e 6 QTLs em condições de estresse normal e sal, respectivamente, e dois QTLs foram detectados para TDWR. Cromossoma 1A, 3A e 7A podem conter genes que são candidatos cruciais associados à tolerância ao sal de trigo. Nossos resultados seriam úteis para a seleção assistida por marcadores para produzir variedades de trigo com tolerância salina melhorada.

Palavras-chave: *Triticum aestivum* L., Stress de salinidade, QTL, Seleção assistida por marcador.

INTRODUCTION

Dryland salinity is a major limitation to crop production. About 20% of irrigated agricultural land in the world is affected by salinity (BOYER, 1982; FLOWERS et al., 1995). Soil salinity inhibits plant growth for not only osmotic stress but ion-excess stress. The presence of salt in the soil solution

reduces the ability of plants to take up water, and then affects growth. This is the osmotic effect of salinity. Moreover, uptake excessive amounts of salt will eventually injure cells in the transpiring leaves and this may further reduce growth. This is the ion-excess effect of salinity (MUNNS, 1993; MUNNS, 2006). To survive, plants have evolved complex salt tolerance mechanisms (ZHU et al., 2002; HANIN et

al., 2016). For example, the salt overly sensitive (SOS) pathway of salt tolerance is crucial for maintaining ion homeostasis under salt stress in model plant *Arabidopsis*. SOS pathway was also more active in salt tolerant cultivar PI365967 than in salt sensitive cultivar in tomato indicating that SOS pathway may be conserved across diverse plant species. Besides, salicylic acid (SA), abscisic acid (ABA), brassinosteroids (BRs) pathway and detoxification system may also be involved in salt perception or salt responses (KRISHNA et al., 2003; MARTINEZ-ATIENZA et al., 2007; SUN et al., 2010; SUN et al., 2015; CHEN et al., 2017).

Wheat is one of the most important food crops. Improving salt tolerance of wheat is needed to sustain food production in many regions in the world. To exploit variation in salt tolerance of wheat, large international collections have been screened in hydroponic or sand culture (KINGSBURY et al., 1984; SAYED, 1985; JAFARI-SHABESTARI et al., 1995). These researches laid a solid foundation for breeding wheat varieties with improved salt tolerance and provided us with research materials for probing into the mechanisms of wheat salt tolerance. In recent years, genome-wide transcriptomic and proteomic analysis for identification of salt-responsive genes in common wheat provided many useful clues (KAWAURA et al., 2008; GUO et al., 2012; CAPRIOTTI et al., 2014; GOYAL et al., 2016; JIANG et al., 2017). The differential expressed proteins/genes were involved primarily in carbon metabolism, detoxification and defense, chaperon and signal transduction. A number of salt tolerant related genes (FEKI et al., 2014; MAKHLOUFI et al., 2014; SUN et al., 2015; TOUNSI et al., 2016; GOYAL et al., 2016; CHEN et al., 2017) and QTLs (MEGAN et al., 2004; HUANG et al., 2006; WU et al., 2007; GENC et al., 2010; REN et al., 2012a; XU et al., 2013; MASOUDI et al., 2015; TOUNSI et al., 2016; OYIGA et al., 2017) have been identified in wheat in recent years. These provided useful information for genetic improvement of salt tolerance in wheat. However, for the intricate character of wheat salt tolerance, the underlying genetic basis was still unclear. Therefore, further exploiting QTLs that contribute to natural variation in salt tolerance would be helpful in understanding the mechanisms of wheat salt tolerance.

In wheat, salt tolerance is associated with low rates of transport of Na^+ to shoots and high selectivity for K^+ over Na^+ which were controlled by *Kna1* located on chromosome 4D (GORHAM et al., 1987; GORHAM et al., 1990; DUBCOVSKY

et al., 1996). Correlations between grain yield and Na^+ exclusion from leaves, along with the associated enhanced K^+/Na^+ discrimination, have also been shown in wheat (CHHIPA et al., 1995; ASHRAF et al., 1997; XU et al., 2013; MASOUDI et al., 2015). Growth performance of seedlings of one specific wheat cultivar grown under salt stress can reflect its salt tolerance to a great extent. Root length, root fresh and dry weights, and shoot fresh and dry weights of wheat seedlings are associated with salt tolerance and could be used as selection criteria in wheat (SHAHZAD et al., 2012). In this paper, we mapped QTLs for seedling traits under normal and salt stress conditions using a recombinant inbred line (RIL) population derived from two Chinese wheat varieties Xiaoyan 54 and Jing 411. These results may provide useful information for molecular design of wheat varieties with improved salt tolerance.

MATERIALS AND METHODS

One recombinant inbred line (RIL) population was used in this study. The RIL population contained 142 RILs derived from two Chinese winter wheat varieties Xiaoyan 54 and Jing 411.

Hydroponic culture was used to investigate the seedling traits of wheat. Methods for seed sterilization, germination and the growth conditions of wheat plants were described previously (REN et al., 2012b). The growth chamber was set at 22°C in the day time and 18°C in the night time, 60% relative humidity and a 15-hour day length. The seedling traits of the “Xiaoyan 54 × Jing 411” RIL population were investigated after these lines were grown in nutrition solution in the growth chamber for 25 days under normal (CK) and salt stress (ST, 150mM NaCl) conditions. The maximum root length (MRL) of plant roots were measured using a ruler and then the shoot dry weight (SDW), root dry weight (RDW) and total dry weight (TDW) were measured after oven-drying at 80°C for 48h. The ratio of TDW (TDWR, ST/CK) of each line was also calculated.

The genetic map of the “Xiaoyan 54 × Jing 411” RIL population was described by REN et al., (2012b). Detection of QTLs for wheat seedling traits under normal and salt stress conditions in this RIL population was conducted by composite interval mapping (ZENG, 1994). Analyses of QTL location, additive effect and 95% confidence intervals of QTLs were performed using WinQTLCart 2.5 software (WANG et al., 2012). The stand model was employed and the parameters of forward regression analysis

were set according to the method described by SU et al. (2009) and WANG et al. (2012). In brief, the walk speed and window size were set as 2cM and 10cm, respectively, with five control markers. Phenotypic variation explained by a single QTL was determined by the square of the partial correlation coefficient (R^2). Threshold of LOD value for QTL detection was set as 3.0.

RESULTS

Evaluation of phenotypes

We measured root dry weight (RDW), maximum root length (MRL), shoot dry weight (SDW) and total dry weight (TDW) under normal (CK) and salt stress (ST) conditions. Under CK condition, the male parent Jing 411 had significant higher RDW, SDW and TDW than the female parent Xiaoyan 54, but it had shorter MRL than Xiaoyan 54 (Table 1). Salt stress significantly reduced the values of all these four traits in Xiaoyan 54 and Jing 411 compared to CK treatment. Jing 411 and Xiaoyan 54 had similar RDW and MRL, but Jing 411 had higher SDW and TDW than Xiaoyan 54 under ST condition (Table 1). These two parents also had similar relative TDW (TDWR, the ratio of TDW under ST and CK conditions), indicating that the plant growth of these two parents had similar sensitivity to salt stress.

The RIL lines showed large variations in all the investigated traits (Table 1). There existed RILs with values that were higher or lower than both parents among all these traits, indicating potential transgressive variations and the presence of positive and negative alleles in both parents.

QTLs Identification

We totally detected three QTLs for RDW, three for MRL, five for SDW, six for TDW and two for TDWR. These 19QTLs located on 12 chromosomes, and the percentage of phenotypic variation explained by individual QTL varied from 7.9% to 19.0% (Figure 1 and 2, Table 2).

We detected two and one QTLs for RDW under normal and 150mM NaCl salt stress condition respectively. *qRDW.ST-4A*, the QTL detected under ST condition located on chromosome 4A and explained 19.0% of phenotypic variation in RDW (Figure 1 and 2, Table 2).

One and two QTLs for MRL were detected under CK and ST conditions respectively (Figure 1 and 2, Table 2). *qMRL.CK-2B* for MRL were detected in CK which located on the short arm of chromosome 2B and the other two QTLs were detected under ST condition. *qMRL.ST-4D* located on chromosome 4D and explained 8.3% MRL variation. *qMRL.ST-6B* located on chromosome 6B and explained 16% MRL variation (Table 2).

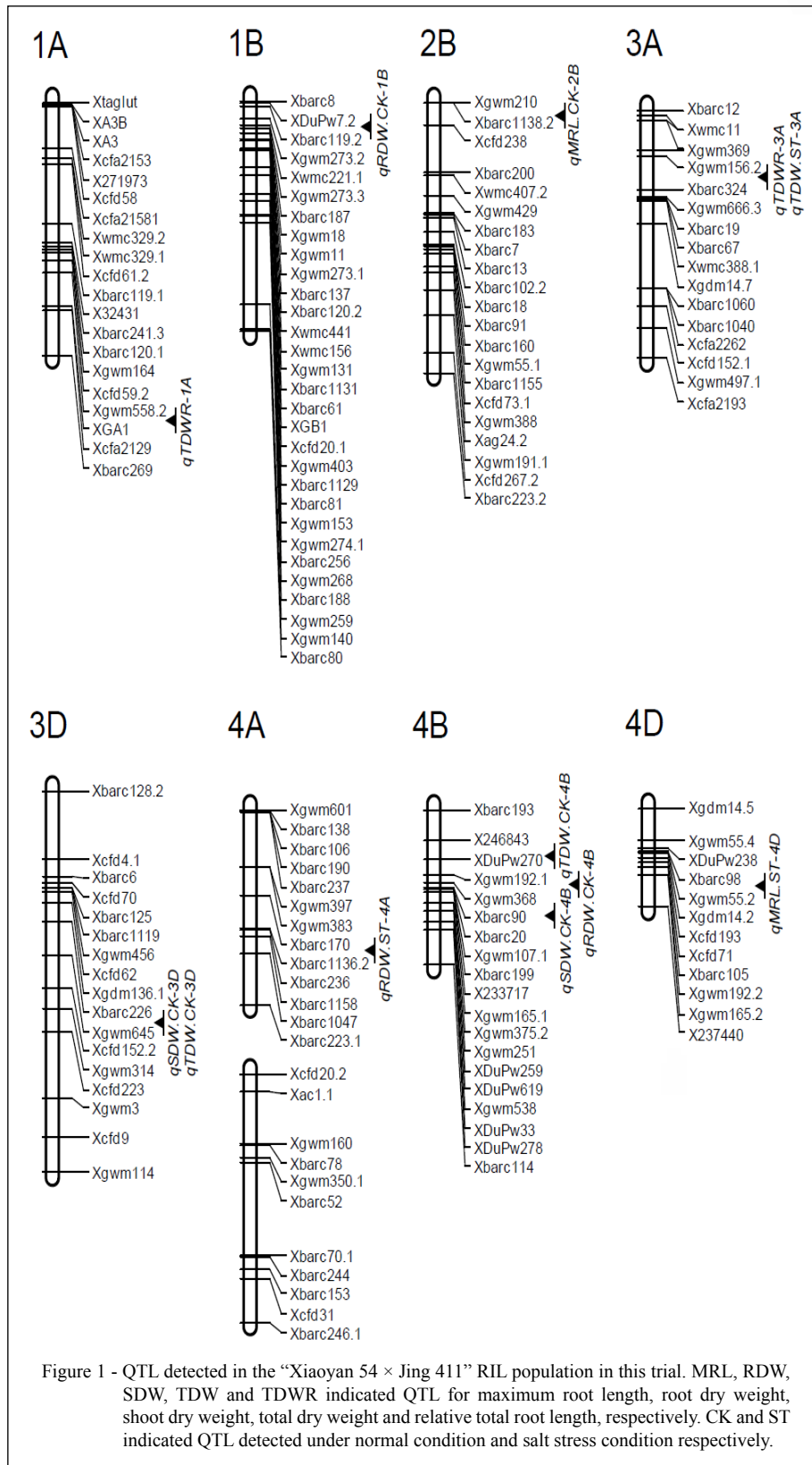
Four and one QTLs for SDW were detected under CK and ST conditions respectively. The only QTL for SDW detected under ST condition, *qSDW.ST-7A*, located on chromosome 7A and explained 12.4% SDW phenotypic variation (Figure 1 and 2, Table 2). We detected four and two QTLs for TDW under CK and ST conditions respectively. (Figure 1 and 2, Table 2). The six QTLs for TDW explained phenotypic variations varying from 9.3% to 15.3%. *qTDW.ST-3A* and *qTDW.ST-7A* were detected under ST condition and explained 12.3% and 12.0% TDW phenotypic variations respectively (Figure 1 and 2, Table 2).

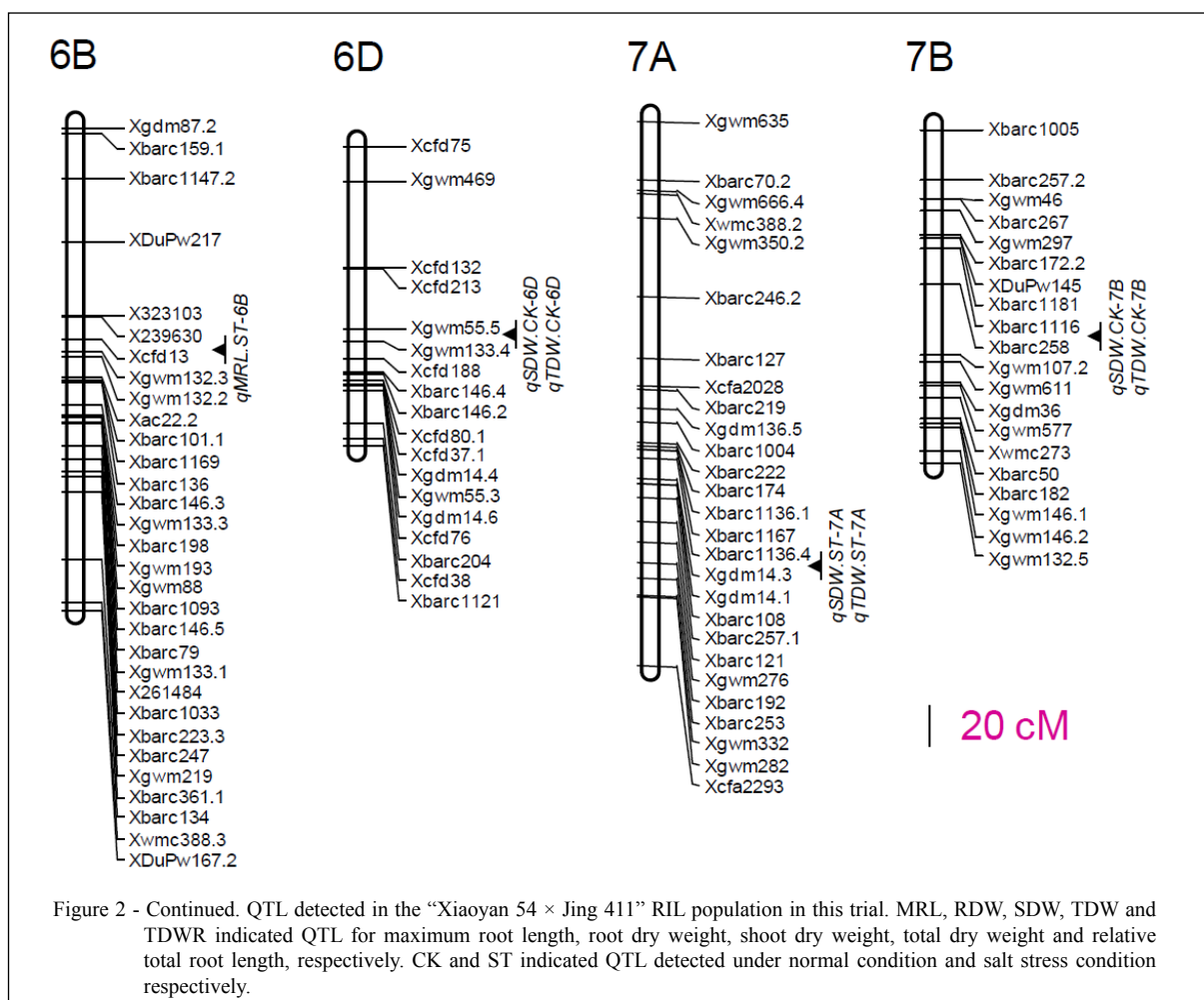
We detected two QTLs for TDWR (Figure 1 and 2, Table 2). *qTDWR-1A* located on chromosome

Table - 1 Mean values and ranges for the investigated traits in the RIL population and their parents at seedling stage in this trial.

Trait	Treatment	-----Parent-----		-----RIL-----		
		Xiaoyan54	Jing 411	Mean \pm SD	Min.	Max.
RDW	CK	9.5 \pm 0.8(a)	11.7 \pm 0.8(b)	11.8 \pm 3.3	6.0	23.0
	ST	8.0 \pm 1.6	8.0 \pm 0.5	10.1 \pm 2.8	3.6	34.0
MRL	CK	26.4 \pm 0.8(A)	19.8 \pm 0.7(B)	23.2 \pm 4.8	13.0	37.5
	ST	16.5 \pm 0.5	16.8 \pm 1.5	15.6 \pm 2.2	9.4	22.4
SDW	CK	61.2 \pm 1.5(A)	76.1 \pm 4.0(B)	71.9 \pm 17.3	39.0	127.7
	ST	41.2 \pm 1.2(a)	52.4 \pm 3.4(b)	42.4 \pm 8.2	21.7	66.0
TDW	CK	70.7 \pm 2.2(a)	87.9 \pm 3.1(b)	83.7 \pm 19.9	46.7	149.6
	ST	49.2 \pm 1.6(a)	60.4 \pm 2.9(b)	52.5 \pm 9.8	25.3	82.7

RDW, root dry weight (mg plant⁻¹); MRL, maximum root length (cm); SDW, shoot dry weight (mg plant⁻¹); TDW, total biomass dry weight (mg plant⁻¹). Statistical difference between the two parents is indicated by different letters after the means. Lower case letters designate significance at P < 0.05. Capital letters designate significance at P < 0.01.





1A and explained 13.7% TDWR variation. the other QTL, *qTDWR-3A*, located on chromosome 3A and explained 15% TDWR variation. (Table 2).

DISCUSSION

Although some single-gene effects for salinity tolerance have been identified in higher plants (for example *AtNHX1* and *RAS1* in *Arabidopsis* (APSE et al., 1999; REN et al., 2010), *OsNHX1* and *SKC1* in rice (FUKUDA et al., 1999; REN et al., 2005)), the tolerance of salinity is genetically and physiologically complex. Large international collections have been screened (KINGSBURY et al., 1984; SAYED, 1985; JAFARI-SHABESTARI et al., 1995), however, the application of salt-tolerant varieties to the improvement of cereal crops such as wheat remains hampered because of the quantitative

nature of the genes involved. Identification of QTLs and some defined regions of chromosome are of crucial importance to enhance wheat salt tolerance. Markers closely associated with major QTLs for salt tolerance might be used for breeding programs in wheat using marker-assisted selection. QTL analyses for salt tolerance in wheat at seedling stage have been conducted in previous studies (MEGAN et al., 2004; HUANG et al., 2006; WU et al., 2007; GENC et al., 2010; REN et al., 2012a; XU et al., 2013; MASOUDI et al., 2015; OYIGA et al., 2017). However, these genes and/or QTLs are not sufficient for understanding the genetic basis and the genetic improvement of salt tolerance in wheat. To further exploit QTLs for wheat salt tolerance, we evaluated the seedling traits of a RIL population under normal and salinity stress conditions in this paper. We found that there exist no significant differences of RDW and MRL between Xiaoyan 54

Table 2 - QTLs detected in this trial using the “Xiaoyan 54×Jing 411” RIL population.

Trait	Treat-ment	QTL	Chr ^a	Marker interval ^b	LOD ^c	R ² × 100	Additive ^d
RDW	CK	<i>qRDW.CK-1B</i>	1B	<u><i>XDUPw7.2-Xbarc119.2</i></u>	3.4	7.9	1.2
	CK	<i>qRDW.CK-4B</i>	4B	<u><i>Xgwm192.1-Xgwm368</i></u>	4.5	11.6	-1.4
	ST	<i>qRDW.ST-4A</i>	4A	<u><i>Xbarc170-Xbarc1136.2</i></u>	3.3	19.0	0.5
MRL	CK	<i>qMRL.CK-2B</i>	2B	<u><i>Xbarc1138.2-Xcfd238</i></u>	5.1	15.2	2.1
	ST	<i>qMRL.ST-4D</i>	4D	<u><i>Xbarc98-Xgwm55.2</i></u>	3.0	8.3	0.4
	ST	<i>qMRL.ST-6B</i>	6B	<u><i>X239630-Xcfd13</i></u>	4.5	16.0	-0.6
SDW	CK	<i>qSDW.CK-3D</i>	3D	<u><i>Xbarc226-Xgwm645</i></u>	3.2	13.7	7.3
	CK	<i>qSDW.CK-4B</i>	4B	<u><i>Xbarc90-Xbarc20</i></u>	4.2	9.8	-6.2
	CK	<i>qSDW.CK-6D</i>	6D	<u><i>Xgwm55.5-Xgwm133.4</i></u>	3.7	9.4	-6.1
	CK	<i>qSDW.CK-7B</i>	7B	<u><i>Xbarc1116-Xbarc258</i></u>	3.4	12.1	-6.9
	ST	<i>qSDW.ST-7A</i>	7A	<u><i>Xbarc1136.4-Xgdm14.3</i></u>	3.6	12.4	-1.7
TDW	CK	<i>qTDW.CK-3D</i>	3D	<u><i>Xbarc226-Xgwm645</i></u>	3.7	15.3	8.8
	CK	<i>qTDW.CK-4B</i>	4B	<u><i>XDUPw270-Xgwm192.1</i></u>	3.1	9.3	-7.0
	CK	<i>qTDW.CK-6D</i>	6D	<u><i>Xgwm55.5-Xgwm133.4</i></u>	4.1	10.2	-7.2
	CK	<i>qTDW.CK-7B</i>	7B	<u><i>Xbarc1116-Xbarc258</i></u>	3.3	9.5	-7.0
	ST	<i>qTDW.ST-3A</i>	3A	<u><i>Xgwm156.2-Xbarc324</i></u>	3.51	12.3	-2.1
	ST	<i>qTDW.ST-7A</i>	7A	<u><i>Xbarc1136.4-Xgdm14.3</i></u>	3.8	12.0	-2.0
TDWR	ST/CK	<i>qTDWR-1A</i>	1A	<u><i>Xgwm558.2-XGluA1</i></u>	3.6	13.7	0.044
	ST/CK	<i>qTDWR-3A</i>	3A	<u><i>Xgwm156.2-Xbarc324</i></u>	4.3	15.0	-0.038

^aChr means chromosome name. ^bMarkers underlined were the nearest marker to the QTL. ^cLOD means Logarithm of odds. ^dAdditive effects, a positive sign means that positive allele comes from the parent Xiaoyan 54, while a negative sign means positive allele comes from the parent Jing 411.

and Jing411, but Jing 411 had higher SDW and TDW than Xiaoyan 54 under under 150mM salt stress condition. The RDW, MRL, SDW and TDW of the RIL population were significantly decreased under salt stress condition comparing to those of under normal condition (Table 1, 2), which is in consistence with previous study (WU et al., 2007; REN et al., 2012a; TOUNSI et al., 2016).

In total, we detected 11 and six QTLs under normal and salt stress conditions respectively and two QTLs for TDWR (Table 2). These 19 QTLs explained phenotypic variations varying from 7.9% to 19.0% (Table 2). The locus for MRL on chromosome 2B, which tightly linked with SSR marker *Xbarc1138*, explained 15.2% MRL phenotypic variation (Figure 1 and 2, Table 2). In fact, this locus has been reported controlling multi-root morphologic parameters and a number of QTLs for yield component (explained 19.1% and 17.3% of phenotypic variations in grain weight per ear and grain number per ear, respectively) (REN et al., 2012b; HAI et al., 2008). However, we did not detect any QTLs for root traits in this chromosomal region under ST condition, indicated that the expression of this locus is inhibited and very sensitive to salt stress. Actually this QTL has been

proved to be involved in brassinosteroids (BRs) signaling pathway (HE et al., 2014) and BRs is also known to confer salt stress tolerance (KRISHNA et al., 2003; ZHU et al., 2016). The *qTDWR-3A* located on chromosome 3A between SSR marker *Xgwm156.2* and *Xbarc324*, and explained 15.0% TDWR phenotypic variation. We found that some chromosomal regions governed more than one trait under salt stress condition. For example, *qTDW.ST-3A* and *qTDWR-3A* were mapped in the same marker interval (*Xgwm156.2-Xbarc324*) on chromosome 3A. The marker interval *Xbarc1136.4-Xgdm14.3* on chromosome 7A also located two QTLs (*qSDW.ST-7A* and *qTDW.ST-7A*). These salt-tolerance related loci may be pleiotropic.

We found that some QTLs detected in this study were tightly linked or coincided with previously reported salt tolerant QTLs in wheat. *qTDWR-1A* was located on chromosome 1A and tightly linked with SSR marker *Xgwm558*. Actually, this chromosomal region has been reported harboring two QTLs for salt tolerance, named *QTdw-1A* and *QSkn-1A* (controlling Shoot K⁺/Na⁺ concentration ratio), which also linked with SSR marker *Xgwm558* on chromosome 1A. *qTDWR-3A* and *qTDW.ST-3A*

were located between SSR marker *Xgwm156.2* and *Xbarc324* on chromosome 3A and coincided with previously reported *QTDW-3A* and *QSkn-3A* (XU et al., 2012). Genc had proved that QTLs for Na⁺ exclusion was associated with an increase (10%) in seedling biomass. Of the five QTLs identified for Na⁺ exclusion in the literature, two were co-located with seedling biomass (GENC et al., 2010). SHAHZAD also verified that the biomass of wheat seedlings could be used as selection criteria in salt tolerance (SHAHZAD et al., 2012). Taken together, the clustering of *qTDWR-3A* and *qTDW.ST-3A*, and coinciding with previously reported QTL for K⁺/Na⁺ concentration ratio indicated that this chromosomal region may harbor crucial salt-tolerance genes. The QTLs detected repeatedly in different trials described above may facilitate MAS of wheat salt tolerance.

CONCLUSION

We identified a total of 19QTLs for wheat seedling traits, of which 11 were detected under normal condition and six under salt stress condition. The other two QTLs controlled TDWR. Some salt-tolerance related loci may be pleiotropic. Chromosome 1A, 3A and 7A may harbor crucial salt-tolerance related loci and the linked marker could be utilized in wheat breeding for improving salt tolerance.

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