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Mapping QTLs for salt tolerance with additive, epistatic and QTL × treatment interaction effects at seedling stage in wheat

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With 1 figure and 4 tables

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Abstract

Quantitative trait loci (QTLs) for salt tolerance with additive, epistatic and QTL × treatment interaction effects at seedling stage in wheat were identified. A set of 131 recombinant inbred lines derived from cross Chuan 35050 × Shannong 483 were evaluated under salt stress and normal conditions. Wide variation was found for all studied traits. A total of 18 additive and 16 epistatic QTLs were detected, among which five and 11 were with significant QTL × treatment effects. Ten QTL clusters were identified, and each may represent a single gene or closely linked genes. The locus controlling shoot K^+/Na^+ concentration ratio and shoot Na^+ concentration on chromosome 5A may be identical to *Nax2*. The interval *Xgwm6-Xgwm538* on chromosome 4B for total dry weight was also identified in a previous study, both near the marker *Xgwm6*. The marker *Xgwm6* may be useful for marker-assisted selection. Six pairs of homoeologous QTLs were detected, showing synteny among the A, B and D genomes. These results facilitate understanding the mechanisms and the genetic basis of salt tolerance in wheat.

Key words: salt tolerance — quantitative trait locus (QTL) — seedling — wheat (*Triticum aestivum* L.)

Salinity is one of the major problems constraining plant productivity and food security worldwide (Pitman and Läuchli 2004). More than 6% of the world's land is affected by either salinity or sodicity (FAO 2004). Wheat (*Triticum aestivum* L., 2n = 6*x* = 42, AABBDD) is one of the most important food crops in the world. Understanding the genetic control of salt tolerance in wheat is the basis for breeding new cultivars with improved productivity in saline environments. At the genetic level, salt tolerance is a quantitative trait controlled by several genes and can be significantly modulated by environment factors. Quantitative trait locus (QTL) analysis provides an effective approach to dissect complicated quantitative traits into component loci to study their relative effects on a specific trait (Doerge 2002) and thereby providing breeders with targets for marker-assisted selection (MAS) (Quarrie et al. 2005). Many QTLs for salt tolerance have already been identified in wheat, barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.) and other plant species. More information about these QTLs has been described in recently published articles (Genc et al. 2010, Roy et al. 2011).

The K^+, Na^+ concentration and K^+/Na^+ ratio are critical measures of salt tolerance in plants (Tester and Davenport 2003, Munns and Tester 2008). Some genes with major effects on Na^+ and K^+ homoeostasis have already been identified in wheat. The *Kna1* on the distal region of chromosome 4DL contributes to a higher K^+/Na^+ ratio and salt tolerance in bread wheat (Dubcovsky et al. 1996). Two Na^+ exclusion genes, *Nax1* on chromosome 2AL (Lindsay et al. 2004) and *Nax2* on chromosome 5AL (James et al. 2006, Byrt et al. 2007), were identified by QTL analysis in durum wheat (*T. turgidum* ssp. *durum* Desf.) (James et al. 2006). The gene *Nax1* reduces the rate of transport of Na^+ from the roots to the shoots and enhances the retention of Na^+ in the leaf sheath, thus restricts further passage to the leaf blade, while *Nax2* also reduces the transport of Na^+ from the roots to the shoots, but instead has a higher rate of K^+ transport, results in an enhanced K^+/Na^+ ratio in the leaf. A gene, *TmHKT1;5-A*, that encodes a Na^+-selective transporter was cloned in the *Nax2* locus. The presence of *TmHKT1;5-A* reduces leaf Na^+ concentration significantly and increases grain yield by 25% compared with near-isogenic lines without the *Nax2* locus in durum wheat (Munns et al. 2012).

Epistatic and QTL × environment ($Q \times E$) interaction effects are important genetic components. Most quantitative traits are greatly affected by either one of them or both (Xu and Crouch 2008). Epistasis and $Q \times E$ analysis have been conducted in wheat for water-soluble carbohydrates (Yang et al. 2007), plant height (Zhang et al. 2008), heading date (Zhang et al. 2009) and salt tolerance (Xu et al. 2012). Early seedling growth was considered to be the most critical stage for wheat establishment, especially under stress (Blum 1996). QTL analyses for salt tolerance in wheat at seedling stage have been conducted in previous studies (Lindsay et al. 2004, Ma et al. 2007, Genc et al. 2010, Xu et al. 2012). However, these genes and/or QTLs are not sufficient for enhancing salt tolerance of wheat cultivars. More studies on QTL analysis, especially on epistasis and $Q \times E$ interaction, are still needed to provide enough information to facilitate understanding the genetic basis and the genetic improvement of salt tolerance in wheat. In this study, we compared the relationships between biomass traits, Na^+, K^+ concentrations, K^+/Na^+ ratio and salt tolerance in wheat; compared the differences in salt tolerance between the shoots and roots; and identified QTLs with additive, epistatic and QTL × treatment interaction effects for biomass and physiological traits associated with salt tolerance, to better understand the genetic basis of salt tolerance at seedling stage.

Materials and Methods

Plant materials: A population of 131 F_{16} recombinant inbred lines (RILs) derived from a cross 'Chuan 35050 × Shannong 483' of wheat was used in this study (Li et al. 2007). A preliminary experiment using the parents and 20 of the RILs showed nice transgressive segregation for the biomass traits and physiological traits evaluated, that is, the root, shoot and total dry weight, K^+, Na^+ concentration and K^+/Na^+ ratio. So, the population was considered to be proper for mapping salt tolerance QTLs. Chuan 35050 has been planted in the South-western Winter Wheat Region of China. Shannong 483 has been grown in the Huanghuai Winter Wheat Region. Shannong 483 was derived from 'Ai-Meng-Niu', one of the most famous germplasm and founder parent in Chinese wheat breeding programmes. 'Ai-Meng-Niu' has led to the development of 16 successful varieties, which had been planted to more than 30 million hectares since 1980.

Experimental design and trait measurements: The experiments were conducted in hydroponic culture under greenhouse condition at Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang, China, in November and December 2008. The 131 RILs and their parents were evaluated for salt tolerance at two salt concentrations (control and 150 mM NaCl, designated as the normal (N) treatment and the salt stress (S) treatment, respectively). Each treatment had three replicates.

The seeds of each line were surface sterilized in 10% H_2O_2 for 5 min, rinsed with deionized water and then germinated in Petri dishes containing distilled water for 10 days. The 12 most uniform seedlings of each line were selected and after removing their residual endosperm, they were transplanted into plastic tanks and attached to the cover of the tanks using soft sponge rubber, with two seedlings per genotype and replicate. The tanks were opaque, and each tank contained 62 l deionized water. Two days after transplanting, half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) was introduced and increased to full strength after another 3 days. Twenty-five milli-molar NaCl solution was added to the solution twice daily during 3 days to reach a final concentration of 150 mM for the S treatment, while no NaCl was added for the N treatment. The solution was continuously aerated by an air compressor and renewed every 7 days; the pH was maintained at 6.0–6.2, and the air temperature ranged from 15 to 30°C. Tanks were placed randomly and rearranged every week.

After 3 weeks in 150 mM NaCl, chlorophyll content (CHL) was estimated using leaf chlorophyll meter (SPAD-502 meter; Minolta, Osaka, Japan) for both the two treatments. Mean leaf chlorophyll content for each genotype was derived from three readings taken at the base, middle and tip of the youngest fully expanded leaf for every seedling. Salt injury index (SII) was recorded using a scale of 1 for green leaves to 5 for leaf death (Liu et al. 2001). After 4 weeks at the 150 mM NaCl treatment, the shoots and the roots of each genotype for both the two treatments were separately harvested and rinsed with distilled water. Maximum root length (RL) and shoot height (SH) were recorded. Then, the roots and shoots were oven-dried at 80°C for 48 h, and root dry weight (RDW) and shoot dry weight (SDW) were determined. Total dry weight (TDW) was calculated as *RDW+SDW*. Harvested roots and shoots were digested in a '5 ml HNO_3 + 0.5 ml H_2SO_4 + 0.5 ml 60% TCA' solution at 90°C for 5 min, and the Na^+ and K^+ concentrations were determined with atomic absorption flame emission spectrophotometer (AA-6501F; SHIMADZU, Tokyo, Japan). The root K^+ (RKC), root Na^+ (RNC), shoot K^+ (SKC), shoot Na^+ (SNC) concentrations were determined, and root K^+/Na^+ concentration ratio (RKN) and shoot K^+/Na^+ concentration ratio (SKN) were calculated.

Data analysis and QTL mapping: Analysis of variance (ANOVA) of the data was performed using SPSS 16.0 software (SPSS Inc, Chicago, IL, USA). Trait measurements were averaged over three replications prior to QTL analysis. The linkage map of the 'Chuan 35050× Shannong 483' population was used in the QTL analysis. The map included 719 markers distributed on 21 wheat chromosomes, comprising 361 diversity arrays technology (DArT) markers, 170 simple sequence repeats (SSRs), 100 expressed sequence tag-SSRs (EST-SSRs) and 88 other molecular and biochemical loci. This map covered 4008.4 cM with average marker distance of 7.15 cM (Li et al. 2007, Wang et al. 2011). Mixed linear composite interval mapping was performed in the software QTLNetwork 2.1 to map QTLs with additive, epistatic as well as QTL by treatment interaction effects (Yang et al. 2008). Composite interval analysis was undertaken using forward–backward stepwise, multiple linear regression with 1-cM walking speed, 2D genome scan, a probability into and out of the model of 0.05 and window size set at 10 cM. Significant thresholds for QTL detection were calculated with 1000 permutations and a genome-wide error rate of 0.10 (suggestive) and 0.05 (significant).

Results

Phenotypic variation and correlations among traits

In the N treatment, the two parents showed little difference for RL, SDW, CHL, SNC, SKC, RKC and SKN, while Chuan 35050 was significantly superior to Shannong 483 for the other traits except for RNC. In the S treatment, Chuan 35050 produced significantly higher values for all the traits except for SII, SH, CHL, SNC, SKC, RNC and SKN (Table 1).

The phenotypic values for the traits exhibited wide ranges among the 131 RILs, with the coefficient of variation (CV) higher than 10% for all traits except for SH, CHL, SKC and RNC (Table 1). The RILs' mean values were higher in the normal (N) treatment than that in the salt stress (S) treatment for all traits except CHL, SNC and RNC (Table 1). The frequency distributions of all the traits showed continuous variation and transgressive segregation in both directions (Table 1), indicating that these traits may be polygenically inherited.

In the S treatment, SNC was significantly and positively correlated with SII ($r = 0.39$, $P \leq 0.01$) but negatively correlated with biomass traits ($-0.52 \leq r \geq -0.50$, $P \leq 0.01$); whereas SKC and SKN were negatively correlated with SII but positively correlated with biomass traits (Table 2). This indicated that SNC is a major factor constraining biomass production under salt stress, while increased SKC and SKN facilitate salt tolerance in wheat. SNC and SKC were positively correlated in the N treatment ($r = 0.33$, $P \leq 0.01$), but negatively correlated under salt stress ($r = -0.31$, $P \leq 0.01$), which suggested that the accumulation of K^+ in shoot suppresses the transportation of Na^+ from root to shoot, indicating a competitive relationship between SNC and SKC. In the S treatment, SII was significantly correlated with SNC, SKC and SKN of the shoots, but had no significant correlation with the corresponding traits of roots, such as RNC, RKC and RKN. This indicated that different mechanisms control the response to salt stress in the shoots and roots.

QTL analysis

A total of 18 additive and 16 epistatic QTLs for 12 traits were detected on 17 chromosomes (Tables 3 and 4, Fig. 1). The 18 additive QTLs explained 3.06–12.98% of the phenotypic variation (Table 3); 13 of the QTLs presented main additive effects, whereas five presented interactions with the environment. The epistatic QTLs explained 1.22%–17.20% of the phenotypic variation (Table 4); among them, 11 were with significant epistasis × treatment interaction effects.

Two additive QTLs and one epistatic QTL were detected for RL. The additive effect of *QRl-2A* came from Chuan 35050-derived allele, while *QRl-6D* was contributed by Shannong 483-derived allele. The QTL *QRl-6D* was colocated with the other two QTLs for biomass production, *QSh-6D* and *QTdw-6D*, which were also contributed by Shannong 483-derived alleles. The epistatic

Table 1: Phenotypic performance for traits related to seedling growth of recombinant inbred lines (RILs) and their parents determined in normal (N) and salt stress (S) treatments

Traits	Treatments	Parents		RILs				
		Chuan 35050	Shannong 483	Mean	Min.	Max.	SD	CV (%)
Salt injury index, SII	S	3.58 ± 0.41	3.33 ± 0.48	3.64	2.67	5.00	0.44	11.97
Maximal root length, RL (cm)	N	32.2 ± 1.0	29.9 ± 0.7	33.3	22.1	48.9	6.7	20.26
	S	21.2 ± 0.6	18.9 ± 0.6*	20.9	14.4	28.4	3.4	16.30
Shoot height, SH (cm)	N	41.5 ± 2.8	37.0 ± 1.9*	39.0	32.1	47.2	3.1	7.97
	S	29.2 ± 1.3	29.0 ± 1.1	29.7	22.8	36.8	2.9	9.74
Root dry weight, RDW (g per plant)	N	0.049 ± 0.004	0.040 ± 0.004*	0.046	0.024	0.073	0.009	18.97
	S	0.041 ± 0.004	0.031 ± 0.003*	0.035	0.016	0.056	0.008	24.12
Shoot dry weight, SDW (g per plant)	N	0.225 ± 0.015	0.207 ± 0.011	0.213	0.121	0.339	0.041	19.29
	S	0.140 ± 0.008	0.120 ± 0.005*	0.110	0.057	0.165	0.022	20.32
Total dry weight, TDW (g per plant)	N	0.274 ± 0.021	0.247 ± 0.014*	0.259	0.150	0.402	0.048	18.65
	S	0.182 ± 0.013	0.151 ± 0.007*	0.145	0.073	0.215	0.030	20.69
Chlorophyll content (SPAD value), CHL	N	33.8 ± 0.9	34.2 ± 0.4	32.1	28.2	36.9	1.6	5.13
	S	38.7 ± 0.7	38.9 ± 1.0	36.2	31.1	42.5	2.5	7.00
Shoot Na^+ concentration, SNC (mmol/g DW)	N	0.21 ± 0.01	0.21 ± 0.01	0.23	0.11	0.42	0.06	26.60
	S	1.48 ± 0.10	1.43 ± 0.09	1.46	0.91	2.35	0.28	19.32
Shoot K^+ concentration, SKC (mmol/g DW)	N	1.76 ± 0.03	1.87 ± 0.02	1.84	1.57	2.34	0.12	6.56
	S	1.06 ± 0.02	1.08 ± 0.02	1.15	0.92	1.40	0.10	8.65
Root Na^+ concentration, RNC (mmol/g DW)	N	0.09 ± 0.00	0.12 ± 0.01*	0.10	0.08	0.13	0.01	7.69
	S	1.42 ± 0.10	1.40 ± 0.09	1.38	0.91	1.81	0.15	10.63
Root K^+ concentration, RKC (mmol/g DW)	N	1.17 ± 0.02	1.21 ± 0.01	1.27	0.92	1.61	0.15	11.76
	S	0.65 ± 0.01	0.56 ± 0.01*	0.59	0.43	0.76	0.07	11.67
Shoot K^+/Na^+ concentration ratio, SKN	N	8.34 ± 0.39	8.91 ± 0.56	8.57	3.85	15.99	2.30	26.89
	S	0.72 ± 0.03	0.76 ± 0.02	0.82	0.45	1.41	0.19	23.42
Root K^+/Na^+ concentration ratio, RKN	N	12.33 ± 0.77	10.50 ± 0.47*	12.71	9.98	16.18	1.30	10.23
	S	0.46 ± 0.05	0.40 ± 0.04*	0.43	0.29	0.58	0.06	15.00

*The parents were significantly different at the 0.05 probability level.

Table 2: Correlation coefficients among traits related to seedling growth in N (below diagonal) and S (above diagonal) treatments

	RL	SH	RDW	SDW	TDW	CHL	SNC	SKC	RNC	RKC	SKN	RKN
SII	−0.29**	−0.42**	−0.50**	−0.57**	−0.56**	−0.37**	0.39**	−0.44**	0.05	−0.13	−0.47**	−0.09
RL		0.36**	0.51**	0.45**	0.48**	0.12	−0.10	0.29**	0.13	−0.04	0.18*	−0.10
SH	0.03		0.70**	0.79**	0.79**	0.40**	−0.50**	0.33**	0.13	−0.04	0.48**	−0.11
RDW	0.28**	0.48**		0.87**	0.93**	0.42**	−0.50**	0.22**	0.31**	0.17*	0.46**	−0.07
SDW	0.05	0.66**	0.79**		0.99**	0.63**	−0.51**	0.25**	0.17*	0.10	0.47**	−0.05
TDW	0.09	0.64**	0.86**	0.99**		0.59**	−0.52**	0.25**	0.22*	0.12	0.48**	−0.06
CHL	−0.14	0.25**	0.12	0.41**	0.37**		−0.33**	−0.01	0.04	0.06	0.25**	−0.01
SNC	−0.05	0.03	−0.16	−0.10	−0.11	−0.13		−0.31**	0.03	−0.30**	−0.90**	−0.22*
SKC	−0.04	0.06	−0.05	−0.01	−0.02	−0.06	0.33**		−0.13	−0.06	0.62**	0.01
RNC	0.08	0.01	−0.02	−0.13	−0.11	−0.01	0.25**	0.24**		0.01	−0.07	−0.59**
RKC	−0.05	−0.13	−0.03	−0.15	−0.13	−0.10	0.32**	0.48**	0.52**		0.20*	0.73**
SKN	0.07	−0.02	0.20*	0.10	0.12	0.11	−0.91**	−0.15	−0.21*	−0.26**		0.14
RKN	−0.15	−0.17*	−0.06	−0.11	−0.10	−0.10	0.16	0.36**	−0.13	0.77**	−0.14	

*Correlation is significant at $P \leq 0.05$.
**Correlation is significant at $P \leq 0.01$.

QTL *QRl-7A.1/QRl-7A.2* had significant epistasis × treatment interaction effect. The parental type $Q_1Q_1Q_2Q_2$ increased RL by 2.20 cm in the N treatment, but only by 0.48 cm in the S treatment, with the recombination type $q_1q_1Q_2Q_2$ had the reverse effect of $Q_1Q_1Q_2Q_2$.

Two additive QTLs were identified for SH. The QTL *QSh-6A* was colocated with *QSdw-6A.2*, while *QSh-6D* was clustered with *QRl-6D* and *QTdw-6D*, as indicated above. The five loci were all contributed by Shannong 483-derived alleles.

Two and four additive QTLs were detected for SDW and TDW, respectively. The QTLs *QSdw-5A*, *QTdw-4B* and *QTdw-5A* were contributed by Chuan 35050-derived alleles, while *QSdw-6A.2*, *QTdw-1D* and *QTdw-6D* conferred by Shannong 483-derived alleles. The QTLs *QSdw-5A* and *QTdw-5A* were colocated.

A total of five epistatic QTLs were identified for biomass production, with one, two and two for RDW, SDW and TDW, respectively. Among them, *QRdw-1D/QRdw-5A* and *QTdw-1D/QTdw-5A* were located together, with the recombination type $q_1q_1Q_2Q_2$ had the positive effects. The QTLs *QSdw-1B.1/QSdw-1B.2* and *QTdw-1B.1/QTdw-1B.2* were colocated and had significant epistasis × treatment interaction effects, with the recombination type $q_1q_1Q_2Q_2$ increasing SDW and TDW in the N treatment, but had little effects in the S treatment. The QTL *QSdw-6A.1/QSdw-7A* increased SDW in the parental type $Q_1Q_1Q_2Q_2$.

For CHL, only one epistasis (*QChl-4A/QChl-6B*) was detected. The CHL value was increased by 1.24 in the recombination type $q_1q_1Q_2Q_2$. The locus *QChl-6B* was colocated with *QSnc-6B.2*.

Table 3: QTLs with additive effects (*a*) and additive × treatment interaction effects (*at*) detected at seedling stage in the N (t_1) and S (t_2) treatments

Traits	QTLs	Marker intervals[1]	Site[2] (cM)	a[3]	h^2 (*a*)	at_1	at_2	h^2(*at*)
RL	*QRl-2A*	*Xwmc522a-Xwmc522b*	0	1.04***	6.22			
	QRl-6D	*wPt667006-wPt667726*	0	−1.91***	12.80			
SH	*QSh-6A*	*wPt7127-wPt730631*	0	−1.15***	12.98			
	QSh-6D	*Xbarc21a-wPt667006*	5	−0.84***	5.92			
SDW	*QSdw-5A*	*Xtrap3-Xissr22c*	18	9.80***	4.59			
	QSdw-6A.2	*wPt7127-wPt730631*	1	−6.30***	4.24			
TDW	*QTdw-1D*	*wPt665480-wPt666067*	0	−6.90***	6.20			
	QTdw-4B	*Xgwm6-Xwmc413*	17	12.00***	7.62			
	QTdw-5A	*Xtrap3-Xissr22c*	19	9.00***	3.51			
	QTdw-6D	*wPt667726-wPt668152*	0	−9.10***	9.31			
RKC	*QRkc-2B*	*wPt4559-wPt3378*	0	0.024***	3.80	0.027**	−0.026**	5.50
RNC	*QRnc-3A*	*wPt666438-wPt4692*	4	0.035***	10.97	−0.034***	0.034***	9.12
RKN	*QRkn-2B.2*	*Xwmc445d-wPt4559*	7	0.193***	5.77	0.170*	−0.172*	4.79
SKC	*QSkc-2B*	*wPt3378-wPt0047*	8	0.042***	9.30			
SKN	*QSkn-1B*	*Xswes1079b-Xswes1079a*	0	0.256**	3.86			
	QSkn-1D	*Xwmc336b-Xwmc432b*	0	0.279**	3.06	0.290*	−0.288*	3.65
	QSkn-4A	*Xswes620-Xswes1060b*	3	−0.310**	5.34			
	QSkn-5A	*Xswes921a-Xswes921b*	1	0.352***	7.30	0.317**	−0.320**	5.34

RL, root length; SH, shoot height; SDW, shoot dry weight; TDW, total dry weight; RKC, root K^+ concentration; RNC, root Na^+ concentration; RKN, root K^+/Na^+ concentration ratio; SKC, Shoot K^+ concentration; SKN, shoot K^+/Na^+ concentration ratio.
*, ** and ***Significant at 0.05, 0.01 and 0.001 probability levels, respectively.
[1]Marker interval means the interval of the *F*-value peak for QTLs.
[2]Site means the distance of *F*-value peak for QTL after the first marker in the marker interval.
[3]Positive and negative effects indicated the increased effect contributed by Chuan 35050 and Shannong 483, respectively.

Table 4: QTLs with epistatic effects (*aa*) and epistatic × treatment interaction effects (*aat*) detected at seedling stage in the N (t_1) and S (t_2) treatments

Traits	QTLi	Marker intervals[1]	Site[2] (cM)	QTLj	Marker intervals	Site (cM)	aa [3]	h^2(*aa*)	aat_1	aat_2	h^2(*aat*)
RL	*QRl-7A.1*	*Xubc859a-Xswes624e*	13	*QRl-7A.2*	*Xgwm471-Xwmc497a*	2	1.33***	4.65	0.87*	−0.85*	2.36
RDW	*QRdw-1D*	*wPt665480-wPt666067*	3	*QRdw-5A*	*Xissr22c-Xgwm666a*	0	−2.00***	4.30			
SDW	*QSdw-1B.1*	*Xswes579-Xwmc128*	5	*QSdw-1B.2*	*Xubc856a-Xubc853b*	0	−10.50***	11.94	−7.90**	8.00**	6.49
	QSdw-6A.1	*wPt7475-wPt9075*	6	*QSdw-7A*	*wPt6668-Xgwm635*	0	8.70***	9.54			
TDW	*QTdw-1D*	*wPt665480-wPt666067*	0	*QTdw-5A*	*Xtrap3-Xissr22c*	19	−6.50**	1.61			
	QTdw-1B.1	*Xwmc128-Xswes189*	0	*QTdw-1B.2*	*Xubc856a-Xubc853b*	0	−8.70***	3.49	−8.80**	9.00**	3.61
CHL	*QChl-4A*	*Xwmc313-wPt0032*	6	*QChl-6B*	*Xswes1106a-wPt8015*	4	−1.24***	17.20			
RNC	*QRnc-4A*	*Xswes620-Xswes1060b*	2	*QRnc-5B*	*Xbarc140-wPt0935*	0	−0.037***	8.54	0.034***	−0.032***	6.18
RKN	*QRkn-1A*	*Xswes131a-wPt2847*	0	*QRkn-2A*	*Xubc873b-Xwmc179a*	19	0.267***	9.42	0.261***	−0.263***	7.01
	QRkn-2B.1	*wPt0100-wPt6627*	1	*QRkn-6A*	*wPt730711-wPt730456*	0	−0.211***	2.23	−0.188**	0.196**	3.92
SNC	*QSnc-2B*	*wPt6158-wPt6223*	0	*QSnc-6B.3*	*wPt7576-wPt8412*	2	−0.053***	8.90	0.042**	−0.041**	6.02
	QSnc-3B	*wPt4412-wPt2416*	3	*QSnc-5D*	*Xswes558a-Xswes555a*	0	−0.048***	6.76	0.045***	−0.045***	7.26
	QSnc-4B	*Xswes30-Xbarc1096*	10	*QSnc-7B*	*wPt7887-Xgwm577*	17	−0.048***	3.29	0.040*	−0.040*	2.26
	QSnc-5A	*Xswes921a-Xswes921b*	0	*QSnc-6A*	*wPt665782-wPt666208*	5	−0.032**	5.10	0.033*	−0.034*	4.46
	QSnc-6B.1	*wPt0171-wPt5885*	4	*QSnc-6B.2*	*Xswes1106a-wPt8015*	0	−0.054***	6.91	0.050***	−0.049***	5.71
SKN	*QSkn-1D*	*Xwmc336b-Xwmc432b*	0	*QSkn-4A*	*Xswes620-Xswes1060b*	3	−0.273*	1.22			

RL, root length; RDW, root dry weight; SDW, shoot dry weight; TDW, total dry weight; CHL, chlorophyll content (SPAD value); RNC, root Na^+ concentration; RKN, root K^+/Na^+ concentration ratio; SNC, shoot Na^+ concentration; SKN, shoot K^+/Na^+ concentration ratio.
*, **, ***, [1] and [2] can refer to Table 3.
[3]Positive and negative effects indicated the increased effect contributed by the parental and the recombination types, respectively.

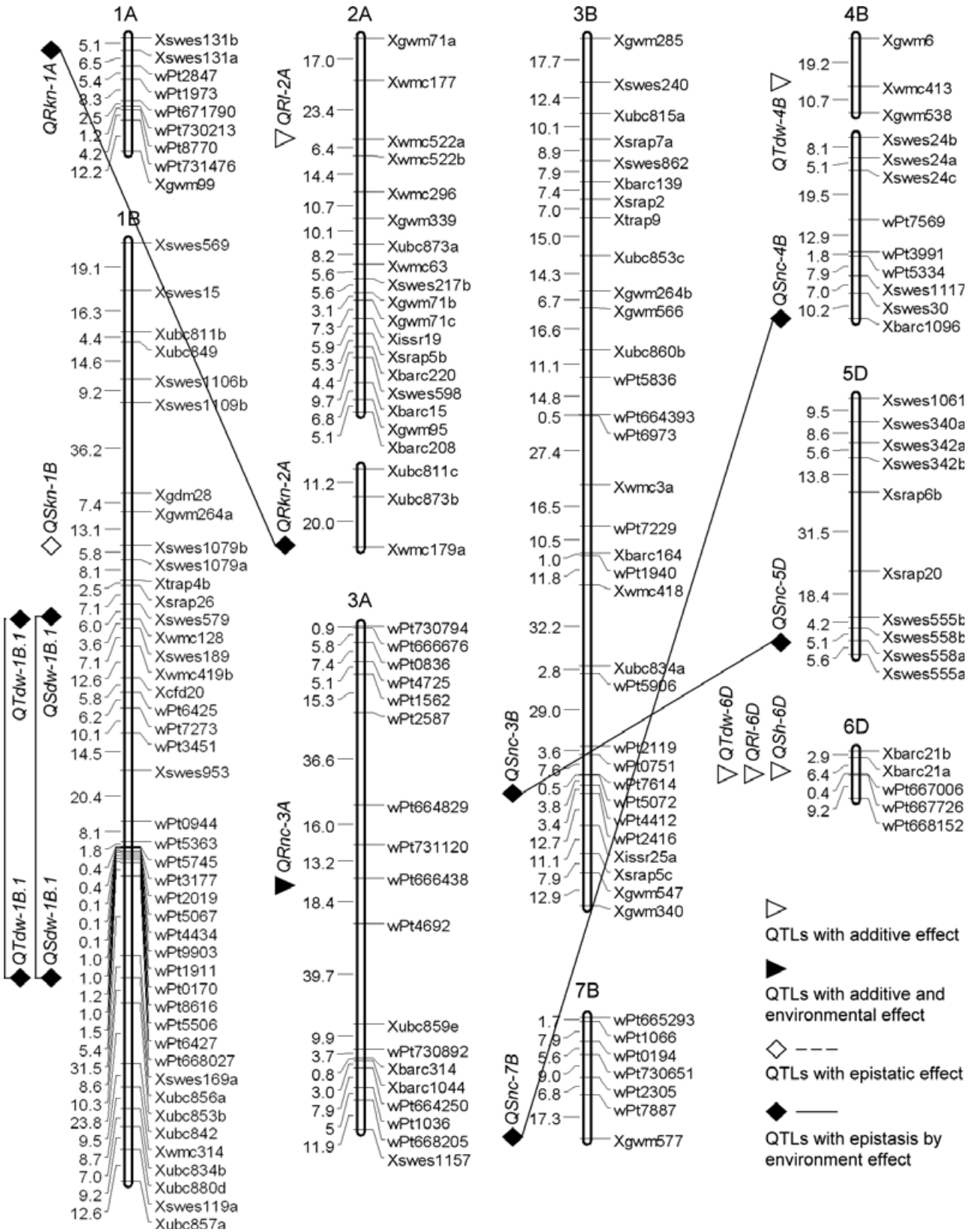

Fig. 1: : Locations of QTLs for traits related to seedling growth in N and S treatments based on recombinant inbred lines derived from Chuan 35050× Shannong 483. QTLs are indicated on the left side of each chromosome; markers are shown on the right. The lines indicated QTLs with digenic epistatic effects

One additive QTL was identified for each of RKC, RNC, RKN and SKC. The three QTLs *QRkc-2B*, *QRkn-2B.2* and *QSkc-2B* were located in the nearby chromosome interval. Chuan 35050-derived alleles increased RKC and RKN in the N treatment and increased SKC in both the N and S treatments. The QTL *QRnc-3A* was also contributed by Chuan 35050-derived allele, but only effective in the S treatment.

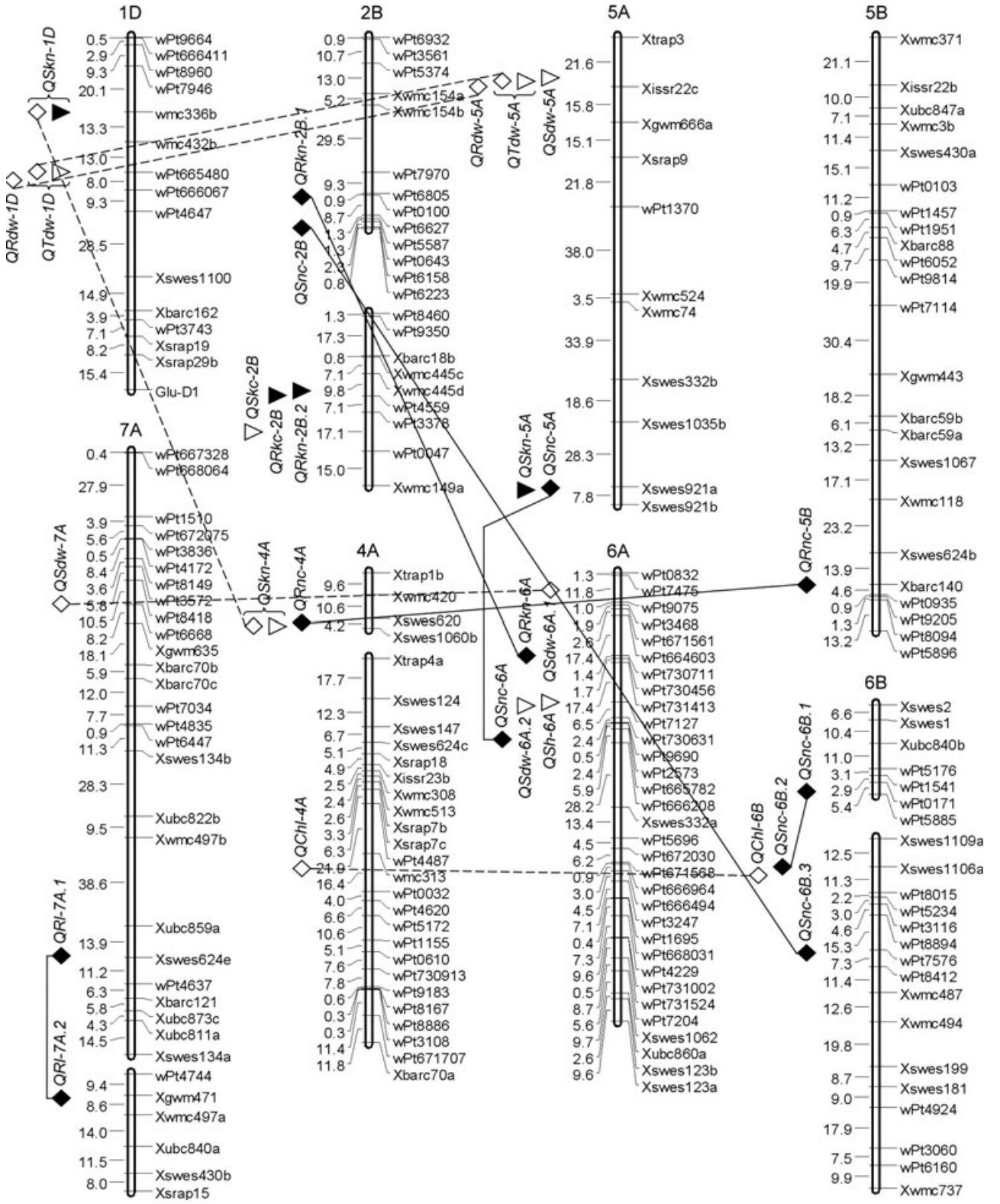

Fig. 1: (Continued)

Four additive QTLs were detected for SKN. Three of them, *QSkn-1B*, *QSkn-1D* and *QSkn-5A* were contributed by Chuan 35050-derived alleles. *QSkn-1D* and *QSkn-5A* were with significant additive × treatment interaction effects, with Chuan 35050-derived alleles increasing SKN in the N treatment. The locus *QSkn-4A* was conferred by Shannong 483-derived allele.

Nine epistatic QTLs were identified for traits related to K^+ and Na^+ concentrations, among which eight had significant epistasis × treatment interaction effects. The QTL *QSkn-1D/QSkn-4A* was the only one without epistasis × treatment interaction effect, with the recombination type $Q_1Q_1q_2q_2$ increasing SKN in both the N and S treatments and $q_1q_1Q_2Q_2$ had the reverse effect. For *QRkn-*

1A/QRkn-2A, the parental type $Q_1Q_1Q_2Q_2$ had the positive effect in the N treatment, while $q_1q_1Q_2Q_2$ was negative. The recombination type $q_1q_1Q_2Q_2$ of *QRnc-4A/QRnc-5B*, *QSnc-2B/QSnc-6B.3*, *QSnc-3B/QSnc-5D*, *QSnc-4B/QSnc-7B*, *QSnc-5A/QSnc-6A* and *QSnc-6B.1/QSnc-6B.2* resulted in increased values for the corresponding traits in the S treatment and of *QRkn-2B.1/QRkn-6A* for RKN in the N treatment, while the parental type $Q_1Q_1Q_2Q_2$ had the negative effect.

Discussion

Na^+, K^+, K^+/Na^+ ratio and salt tolerance

The ability to exclude Na^+ from shoot is a critical factor for salinity tolerance in plants (Cattivelli et al. 2002, Munns and James 2003, Tester and Davenport 2003), and the modification of specific Na^+ transport processes can improve salinity tolerance (Pardo 2010). An appropriate intracellular K^+/Na^+ balance is critical for salt tolerance. Actually, the K^+/Na^+ ratio rather than Na^+ concentration and any other agronomical or physiological traits has always been regarded as the most important determinant of salt tolerance (Chhipa and Lal 1995, Shavrukov et al. 2009, Pardo 2010). In the present study, SNC was positively correlated with SII ($r = 0.39$, $P \leq 0.01$), but negatively correlated with biomass traits, while SKC and SKN were negatively correlated with SII, but positively correlated with biomass traits (Table 2), which was the same as our previous study using another RIL population (Xu et al. 2012). This result indicated that the exclusion of Na^+ from the shoots, and thereby, reduced Na^+ concentrations and an increased K^+/Na^+ ratio were important factors for improving salt tolerance in wheat.

Na^+ and K^+ are physicochemically similar and may interact in plant's homoeostatic mechanisms. The element Na^+ strongly inhibits K^+ uptake by cells (Qi and Spalding 2004, Rodriguez-Navarro and Rubio 2006, Pardo 2010) and can compete with K^+ for binding sites in the enzymes essential for cellular functions and may result in cytotoxicity (Serrano 1996). Therefore, an abundance of K^+ can protect plants against Na^+ toxicity (Qi and Spalding 2004). In our study, SKC had a significantly positive relationship with SNC ($r = 0.33$, $P \leq 0.01$) in the N treatment, but a negative relationship ($r = -0.31$, $P \leq 0.01$) in the S treatment (Table 2), indicated that the uptake of K^+ may restrain the uptake of Na^+ and there is a competition relationship. The result is corresponding to our previous study in wheat (Xu et al. 2012) and a study in rice (Lin et al. 2004).

Additive, epistatic and QTL × treatment effects

In previous studies, many QTLs identified under salt or drought stress were also detected for the same traits under normal conditions (Landjeva et al. 2008, Peleg et al. 2009, Genc et al. 2010, Xu et al. 2012). In the present study, a total of 18 additive and 16 epistatic QTLs were detected, among which five and 11 were with significant $Q \times T$ interaction effects. Four of the 18 additive QTLs were involved in digenic effects. For biomass traits, only three of the six epistatic QTLs had significant $Q \times T$ interaction effects, while five of the eight additive QTLs and eight of the nine epistatic QTLs were involved in significant $Q \times T$ interactions. The results indicated that additive and epistatic effects were common, but the physiological traits rather than biomass traits are more likely to be involved in $Q \times T$ interactions at seedling stage of wheat. These results were corresponding to the little differences between the N and the S treatments for biomass traits and the great differences for traits related to Na^+, K^+, especially for Na^+ concentration (Table 1). The findings were similar to those of our previous study (Xu et al. 2012).

QTL clustering and QTL comparison

QTL clustering was common in previous studies (Groos et al. 2003, Quarrie et al. 2005, Marza et al. 2006, Li et al. 2007, Sun et al. 2009, Xu et al. 2012). In the present study, a total of 10 QTL clusters were detected on chromosomes 1B (two), 1D, 2B, 4A, 5A (two), 6A, 6B and 6D (Fig. 1). For each of the eight clusters expect that on chromosomes 4A and 6B, the correlations between the traits related were consistent with the additive effects of the corresponding QTLs. The clustering of the QTLs may actually be due to their strong correlations. Each of these clusters may represent a single gene or closely linked genes. The regions *Xswes579-Xswes189* and *Xubc856a-Xubc853b* on chromosome 1B had a major epistatic QTL for SDW and TDW. The interval *Xswes921a-Xswes921b* on chromosome 5A was detected to affect SNC and SKN. Previous studies have identified some genes with major effects on Na^+ and K^+ homoeostasis in wheat, that is, the *Kna1* locus on chromosome 4DL (Dubcovsky et al. 1996), *Nax1* on 2AL (Lindsay et al. 2004) and *Nax2* on 5AL (James et al. 2006, Byrt et al. 2007, Munns et al. 2012). The interval *Xswes921a-Xswes921b* on chromosome 5A may be identical to the *Nax2* locus on 5AL, which reduces leaf Na^+ concentration and increases durum wheat grain yield by 25% (Munns et al. 2012). The region *Xgwm6-Xgwm538* on chromosome 4B was a major locus for TDW. A QTL controlling seedling shoot biomass (SDW) and tiller number was located in a similar region, both near the marker *Xgwm6* (Genc et al. 2010). The marker *Xgwm6* may be useful in MAS breeding.

Homoeologous QTLs

As a result of the allopolyploid nature of the wheat genome, many traits are controlled by homoeologous genes. Homoeologous regions related to salt tolerance were detected for yield at the adult stage, SII, biomass traits and physiological traits at seedling stage (Quarrie et al. 2005, Ma et al. 2007, Xu et al. 2012). In the present study, we detected six pairs of QTLs with each pair for a same trait on seemingly homoeologous positions of four chromosome groups, that is, group 1 for SKN and TDW, group 2 for RKN, group 5 for SNC and group 6 for SNC and SH. The numerous homoeologous QTLs reflected synteny between the A, B and D genomes of wheat.

Differences between shoots and roots for salt tolerance

In the present study, SNC, SKC and SKN were all significantly correlated with SII and biomass traits; whereas for the root traits, there were only positive correlations between RNC and biomass traits, which were different from the negative relationships between SNC and biomass traits. Therefore, it is likely that shoots and roots had different responses to salt stress. For physiological traits like Na^+, K^+ and K^+/Na^+ ratio, no similar loci were detected between SNC and RNC, between SKC and RKC and between SKN and RKN. Only the interval *Xswes620-Xswes1060b* on chromosome 4A contained two QTLs for both shoot and root physiological traits, that is, *QSkn-4A* and *QRnc-4A*. As to biomass traits, two loci on chromosomes 5A (RDW and SDW) and 6D (RL and SH) were detected that controlled the corresponding trait for both roots and shoots (Fig. 1). This agreed with the results of our previous study, which detected colocating QTLs between shoots and roots for biomass traits, but none for physiological traits (Xu et al. 2012).

The QTLs detected in this study, especially those with epistatic effects and $Q \times T$ interactions, the QTL clusters and the major

QTLs, which have not been previously reported in wheat, provide a basis for further functional analysis of salt tolerance genes in wheat, and the identified molecular markers closely linked to QTLs for salt tolerance may facilitate wheat MAS breeding.

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References

Blum, A., 1996: Crop responses to drought and the interpretation of adaptation. Plant Growth Regul. **20**, 135—148.

Byrt, C. S., J. D. Platten, W. Spielmeyer, R. A. James, E. S. Lagudah, E. S. Dennis, M. Tester, and R. Munns, 2007: HKT1;5-like cation transporters linked to Na^+ exclusion loci in wheat, *Nax2* and *Kna1*. Plant Physiol. **143**, 1918—1928.

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

Chhipa, B. R., and P. Lal, 1995: Na/K ratios as the basis of salt tolerance in wheat. Aust. J. Agric. Res. **46**, 533—539.

Doerge, R. W., 2002: Mapping and analysis of quantitative trait loci in experimental populations. Nat. Rev. Genet. **3**, 43—52.

Dubcovsky, J., G. S. Maőa, E. Epstein, M. C. Luo, and J. Dvořák, 1996: Mapping of the K^+/Na^+ discrimination locus *Kna1* in wheat. Theor. Appl. Genet. **92**, 448—454.

FAO, 2004. Available at: http://www.fao.org/ (last accessed on 5 October 2004).

Genc, Y., K. Oldach, A. P. Verbyla, G. Lott, M. Hassan, M. Tester, H. Wallwork, and G. K. McDonald, 2010: Sodium exclusion QTL associated with improved seedling growth in bread wheat under salinity stress. Theor. Appl. Genet. **121**, 877—894.

Groos, C., N. Robert, E. Bervas, and G. Charmet, 2003: Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor. Appl. Genet. **106**, 1032—1040.

Hoagland, D., and D. Arnon, 1950: The water culture method for growing plants without soil. California Agricultural Experiment Station Circular, 347.

James, R. A., R. J. Davenport, and R. Munns, 2006: Physiological characterization of two genes for Na^+ exclusion in durum wheat, *Nax1* and *Nax2*. Plant Physiol. **142**, 1537—1547.

Landjeva, S., K. Neumann, U. Lohwasser, and A. Borner, 2008: Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. Biol. Plant. **52**, 259—266.

Li, S. S., J. Z. Jia, X. Y. Wei, X. C. Zhang, L. Z. Li, H. M. Chen, Y. D. Fan, H. Y. Sun, X. H. Zhao, T. D. Lei, Y. F. Xu, F. S. Jiang, H. G. Wang, and L. H. Li, 2007: A intervarietal genetic map and QTL analysis for yield traits in wheat. Mol. Breed. **20**, 167—178.

Lin, H. X., M. Z. Zhu, M. Yano, J. P. Gao, Z. W. Liang, W. A. Su, X. H. Hu, Z. H. Ren, and D. Y. Chao, 2004: QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. Theor. Appl. Genet. **108**, 253—260.

Lindsay, M. P., E. S. Lagudah, R. A. Hare, and R. Munns, 2004: A locus for sodium exclusion (*Nax1*), a trait for salt tolerance, mapped in durum wheat. Funct. Plant Biol. **31**, 1105—1114.

Liu, X., J. Shi, X. Y. Zhang, Y. S. Ma, and J. Z. Jia, 2001: Screening salt tolerance germplasms and tagging the tolerance gene(s) using microsatellite (SSR) markers in wheat. Acta Bot. Sin. **43**, 948—954.

Ma, L. Q., E. F. Zhou, N. X. Huo, R. H. Zhou, G. Y. Wang, and J. Z. Jia, 2007: Genetic analysis of salt tolerance in a recombinant inbred population of wheat (*Triticum aestivum* L.). Euphytica **153**, 109—117.

Marza, F., G. H. Bai, B. F. Carver, and W. C. Zhou, 2006: Quantitative trait loci for yield and related traits in the wheat population Ning7840 × Clark. Theor. Appl. Genet. **112**, 688—698.

Munns, R., and R. A. James, 2003: Screening methods for salinity tolerance: a case study with tetraploid wheat. Plant Soil **253**, 201—218.

Munns, R., and M. Tester, 2008: Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. **59**, 651—681.

Munns, R., R. A. James, B. Xu, A. Athman, S. J. Conn, C. Jordans, C. S. Byrt, R. A. Hare, S. D. Tyerman, M. Tester, D. Plett, and M. Gilliham, 2012: Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. Nat. Biotechnol. **30**, 360—364.

Pardo, J. M., 2010: Biotechnology of water and salinity stress tolerance. Curr. Opin. Biotechnol. **21**, 185—196.

Peleg, Z., I. Cakmak, L. Ozturk, A. Yazici, Y. Jun, H. Budak, A. B. Korol, T. Fahima, and Y. Saranga, 2009: Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. Theor. Appl. Genet. **119**, 353—369.

Pitman, M. G., and A. Läuchli, 2004: Global impact of salinity and agricultural ecosystems, In: A. Läuchli, and U. Lüttge, (eds.) Salinity: Environment-Plants-Molecules, 3—20. Kluwer Academic, Dordrecht.

Qi, Z., and E. P. Spalding, 2004: Protection of plasma membrane K^+ transport by the salt overly sensitive1 Na^+-H^+ antiporter during salinity stress. Plant Physiol. **136**, 2548—2555.

Quarrie, S. A., A. Steed, C. Calestani, A. Semikhodskii, C. Lebreton, C. Chinoy, N. Steele, D. Pljevljakusic, E. Waterman, J. Weyen, J. Schondelmaier, D. Z. Habash, P. Farmer, L. Saker, D. T. Clarkson, A. Abugalieva, M. Yessimbekova, Y. Turuspekov, S. Abugalieva, R. Tuberosa, M. C. Sanguineti, P. A. Hollington, R. Aragues, A. Royo, and D. Dodig, 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.

Rodriguez-Navarro, A., and F. Rubio, 2006: High-affinity potassium and sodium transport systems in plants. J. Exp. Bot. **57**, 1149—1160.

Roy, S. J., E. J. Tucker, and M. Tester, 2011: Genetic analysis of abiotic stress tolerance in crops. Curr. Opin. Plant Biol. **14**, 232—239.

Serrano, R., 1996: Salt tolerance in plants and microorganisms: toxicity targets and defense responses. Int. Rev. Cytol. **165**, 1—52.

Shavrukov, Y., P. Langridge, and M. Tester, 2009: Salinity tolerance and sodium exclusion in genus *Triticum*. Breed. Sci. **59**, 671—678.

Sun, X. Y., K. Wu, Y. Zhao, F. M. Kong, G. Z. Han, H. M. Jiang, X. J. Huang, R. J. Li, H. G. Wang, and S. S. Li, 2009: QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. Euphytica **165**, 615—624.

Tester, M., and R. Davenport, 2003: Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. **91**, 503—527.

Wang, Y. Y., X. Y. Sun, Y. Zhao, F. M. Kong, Y. Guo, G. Z. Zhang, Y. Y. Pu, K. Wu, and S. S. Li, 2011: Enrichment of a common wheat genetic map and QTL mapping for fatty acid content in grain. Plant Sci. **181**, 65—75.

Xu, Y. B., and J. H. Crouch, 2008: Marker-assisted selection in plant breeding: from publications to practice. Crop Sci. **48**, 391—407.

Xu, Y. F., D. G. An, D. C. Liu, A. M. Zhang, H. X. Xu, and B. Li, 2012: Mapping QTLs with epistatic effects and QTL × treatment interactions for salt tolerance at seedling stage of wheat. Euphytica **186**, 233—245.

Yang, D. L., R. L. Jing, X. P. Chang, and W. Li, 2007: Identification of quantitative trait loci and environmental interactions for accumulation and remobilization of water-soluble carbohydrates in wheat (*Tiiticum aestivum* L.) stems. Genetics **176**, 571—584.

Yang, J., C. C. Hu, H. Hu, R. D. Yu, Z. Xia, X. Z. Ye, and J. Zhu, 2008: QTLNetwork: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics **24**, 721—723.

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

Zhang, K. P., J. C. Tian, L. Zhao, B. Liu, and G. F. Chen, 2009: Detection of quantitative trait loci for heading date based on the doubled haploid progeny of two elite Chinese wheat cultivars. Genetica **135**, 257—265.