

Detection of quantitative trait loci (QTLs) for seedling traits and drought tolerance in wheat using three related recombinant inbred line (RIL) populations

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Abstract In order to detect quantitative trait loci (QTLs) for drought tolerance in wheat during seed germination conditional and unconditional QTL analyses of eight seedling traits were conducted under two water regimes using three related F₉ recombinant inbred line populations with a common female parent. A total of 87 QTLs for the eight seedlings traits and 34 specific QTLs related to drought tolerance were detected. Seventy-one of these QTLs were major QTLs with contributions to phenotypic variance of >10 %. Of the 34 QTLs related to drought tolerance only eight were also detected by unconditional analysis of seedling traits under osmotic stress conditions indicating that most of the QTLs related to drought tolerance could not be detected by unconditional QTL analysis. Therefore, conditional QTL analysis of stress-tolerance traits such as drought tolerance was feasible and effective. Of 11

important QTL clusters located on chromosomes 1BL, 1D, 2A, 2B, 2D, 4A, 6B, and 7B, nine were detected in multiple populations and eight were detected by both unconditional and conditional analyses.

Keywords *Triticum aestivum* · Mapping population · Unconditional and conditional QTL analysis

Abbreviations

CL	Coleoptile length
PH	Plant height
RL	Longest root length
RN	Root number
SDW	Seedling dry weight
SLDW	Stem-and-leaf dry weight
RDW	Root dry weight
RSDWR	Root-to-shoot dry weight ratio
WL	Recombinant inbred line population derived from Weimai 8 × Luohan 2
WY	Recombinant inbred line population derived from Weimai 8 × Yannong 19
WJ	Recombinant inbred line population derived from Weimai 8 × Yannong 19

Introduction

Drought imposes constraints on wheat productivity. Improvement of drought tolerance during germination and early development of wheat can overcome the influence of soil water deficit, ensure seedling number,

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and provide a solid base for obtaining high and stable yields. Drought tolerance is commonly measured by using high molecular mass polyethylene glycol (PEG) to mimic osmotic stress (Blum et al. 1980; Almansouri et al. 2001; Dhanda et al. 2004; Mujtaba et al. 2005), as this approach avoids much of the environmental noise associated with field experiments and induces a plant response similar to that induced by drought.

Drought tolerance is a complex quantitative trait controlled by minor genes. Quantitative trait loci (QTL) mapping has become an effective tool for genetic analysis of quantitative factors such as agronomic traits (Perretant et al. 2000; Sourdille et al. 2000; Börner et al. 2002), disease tolerance (Anderson et al. 2001; Simón et al. 2004; Faris and Friesen 2005; Schmolke et al. 2005), and abiotic stress tolerance (Galiba et al. 2005; Bálint et al. 2007). Several studies have documented QTL analysis of seedling drought tolerance of wheat (Spielmeyer 2005; Zhou et al. 2005; Rebetzke et al. 2007). However, most of them used a single mapping population and used only unconditional QTL analysis.

Conditional genetic analysis can be used to exclude contributions of causal traits to variation of the resultant trait (Zhu 1995). The remaining variation is defined as conditional variation, and indicates the extra effects of genes that are independent of the causal trait. QTL analysis based on conditional variation is defined as conditional QTL analysis, whereas that based on total variation is called unconditional or conventional QTL analysis. A comparison between unconditional and conditional QTL could provide an outline of the genetic relationships between the given causal trait and the resultant trait at the individual QTL level. This method has been widely used to identify QTL expressed at certain developmental stages of plants (Zhu 1995; Atchley and Zhu 1997; Yan et al. 1998; Cao et al. 2001) or to analyze contributions of component traits to a complex trait (Guo et al. 2005; Liu et al. 2008; Cui et al. 2011). This method was also recently proposed for analyzing the influence of different agrotechnologies or agronomic practices on crop growth and development with an aim of discovering QTLs expressed specifically in stress environments. Jiang et al. (2008) used the method to determine genes expressed specifically in low-nitrogen stress environments. However, determination of QTLs expressed specifically under osmotic stress by conditional analysis has not been reported in wheat.

Using three related recombinant inbred line (RIL) populations, we carried out both unconditional and

Fig. 1 Locations of QTLs for wheat seedling traits and drought tolerance in three mapping populations. The positions of the marker loci and the QTL are listed to the left of individual chromosomes; marker loci are listed to the right. DArT markers are prefixed “wpt,” and the remainders are PCR-based markers. Intervals for QTL are LOD >2.0 with LOD peak values more than 2.5. Black, green, and red colors of the QTL symbols indicate QTL detected in the WL, WY, and WJ, respectively. (Color figure online)

conditional QTL analyses of eight seedling traits under both normal water and osmotic stress conditions during germination and early development of wheat. Our study aimed to (1) identify QTLs with significant contributions to drought tolerance during germination, (2) improve our understanding of the genetic basis of drought tolerance, and (3) provide useful information for molecular marker-assisted selection (MAS) in breeding for increased drought tolerance.

Materials and methods

Experimental materials

Three F₉ RIL populations derived from crosses between four common Chinese wheat varieties, namely, Weimai 8 × Luohan 2 (WL), Weimai 8 × Yannong 19 (WY) and Weimai 8 × Jimai 20 comprising 179, 175 and 172 lines, respectively, were used. Weimai 8 is a drought susceptible variety, released by the Weifang Municipal Academy of Agricultural Sciences, Shandong, in 2003; Luohan 2 is a drought-tolerant variety, released by the Crop Research Institute, Luoyang Municipal Academy of Agricultural Sciences, Henan, in 2001; Yannong 19 is a water efficient variety, released by the Yantai Municipal Academy of Agricultural Sciences, Shandong, in 2001. Jimai 20, also drought susceptible, was released by the Crop Research Institute, Shandong Academy of Agricultural Sciences in 2003. All of the materials were created and conserved by Taian Sub-center of the National Wheat Improvement Center.

An integrated genetic map

An integrated genetic map constructed by our laboratory in 2011 using the WL, WY and WJ populations will be published elsewhere. The map consists of 1,127 loci distributed across all 21 wheat chromosomes and covers 2,976.75 cM with an average distance of 2.64 cM between adjacent loci. The map includes 576 DArT marker loci, 496 of which are common to at least two maps (Fig. 1).

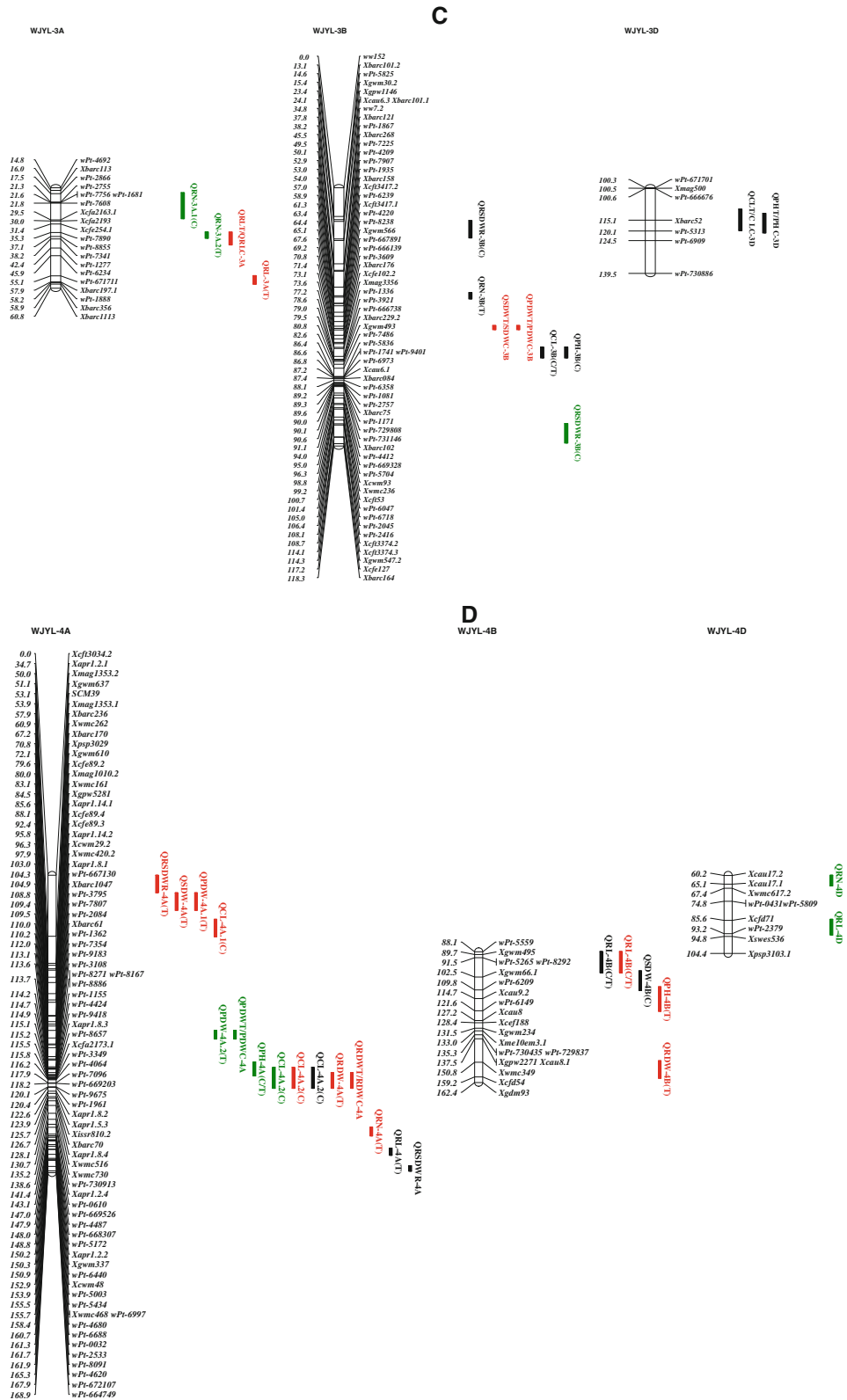


Fig. 1 continued

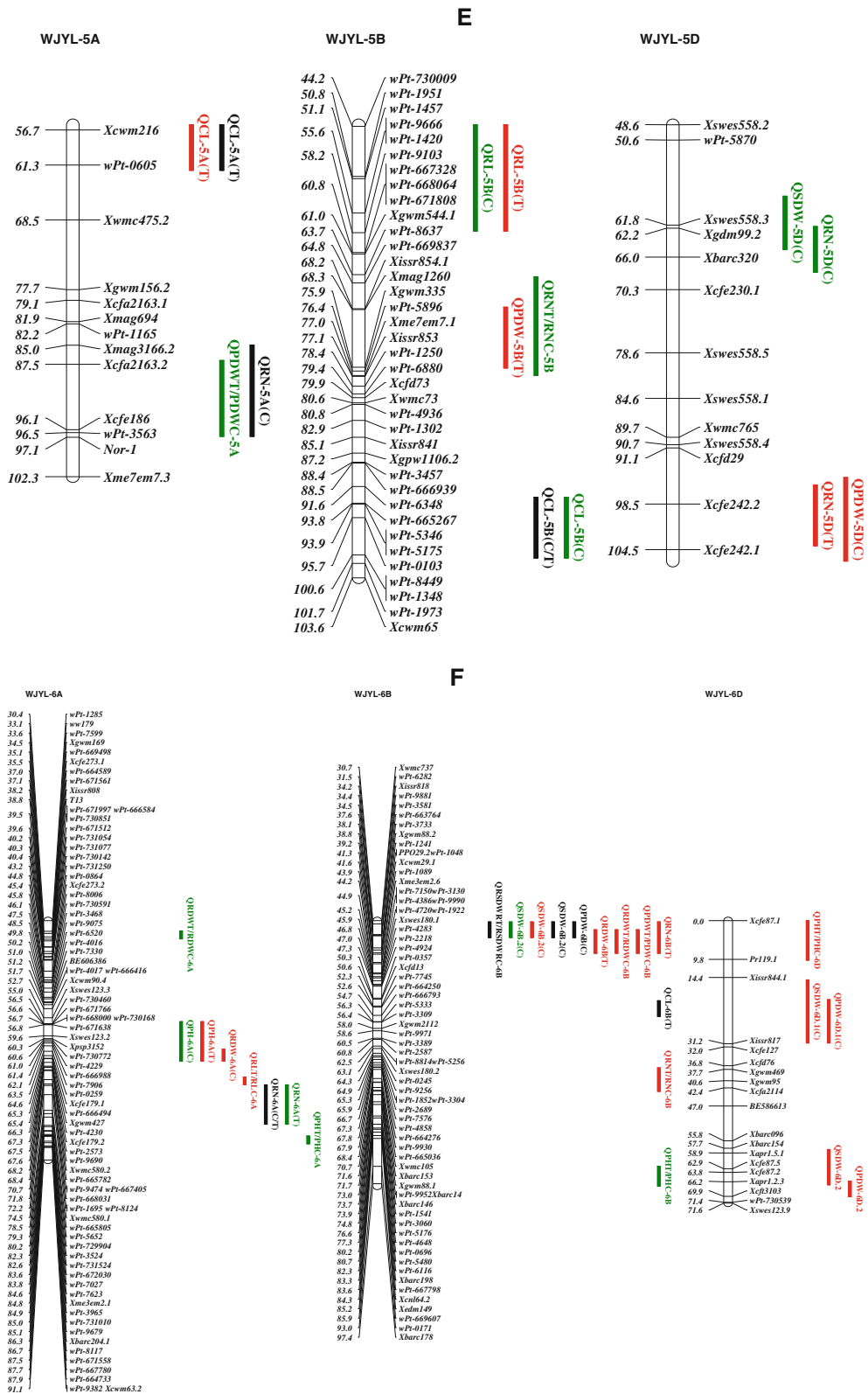


Fig. 1 continued

$$\Psi = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$$

where Ψ is the osmotic potential in bars, C is the concentration of PEG-6000 in g/kg H₂O and T is the temperature in °C.

All the beakers were placed in a plastic box, covered with a thin plastic film, and cultured at 25 °C in the dark for three days. On the fourth day, 5 ml of water were added to each beaker with concurrent illumination. On the eighth day, five uniform seedlings were selected from each beaker to measure coleoptile length (CL), plant height (PH), the longest root length (RL), and root number (RN). The seedlings were placed in an oven for 20 min at 100 °C, and then dried to constant weight at 80 °C. The following parameters were then measured: seedling dry weight (SDW), stem and leaf dry weight (SLDW) and root dry weight (RDW). The root-to-shoot dry weight ratio (RSDWR) was calculated. All weights and lengths reported herein were in grams and centimeters, respectively.

QTL analysis

Statistical analysis of the phenotypic data from the three RIL populations was carried out using the software SPSS13.0 (SPSS, Chicago, IL, USA). The estimated broad-sense heritability of the corresponding trait was calculated using the formula $h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_e^2)$, where σ_G^2 is the genetic variance and σ_e^2 is the experimental error. QTL screening was conducted using inclusive composite-interval mapping by Ici-Mapping 3.0 (Li et al. 2007; <http://www.isbreeding.net/>). The threshold logarithm of odds ratio (LOD) scores were calculated using 1,000 permutations, and the QTLs with LOD values <2.5 were ignored to ensure the authenticity and reliability of the QTLs reported herein. Conditional QTL screening was conducted based on conditional phenotypic values of the eight traits obtained by software QGASStation 1.0 (Zhu 1995). To obtain the conventional phenotypic value of each trait data were assembled according to the format of QTL data. The first two columns in order were environment and genotype and the following columns were trait means detected under osmotic stress and normal water conditions. QTL data menus of QGASStation 1.0 were selected as follows: ‘Have environment effect (No)’; ‘Have Block Effect (No)’.

The selected method of analysis included ‘Ge Var’ and ‘Conditional Final’. The assignment of a QTL name was based on the following rules: italicized uppercase ‘Q’ denoting ‘QTL’, followed by trait abbreviation, chromosome designation and QTL sequence number for multiple QTL on the same chromosome.

Results

Analysis of phenotypic data

The trait values for the three RIL populations and their parents under the two water regimes are shown in Table 1. ANOVA indicated significant differences ($P < 0.05$) between Weimai 8 and Luohan 2 in PH, RL, RN, RDW, SLDW, and PDW under both water regimes, and RSDWR under osmotic stress. Weimai 8 and Yannong 19 differed significantly in CL, RL, RN, PDW, and SLDW under both water regimes and RSDWR under normal water conditions. In regard to Weimai 8 and Jimai 20, there were significant differences in CL, RL, and RN under both water regimes, PH and PDW under normal water conditions, and SLDW under osmotic stress conditions. The variances for treatment effects of PH, RL, RN, PDW, SLDW, RDW, and RSDWR were significant in the three populations, whereas CL was significant only in the WY population. Estimated broad-sense heritabilities of the eight traits ranged from 15.4 to 89.6 %.

Phenotypic variation among the eight traits in the RIL lines was obvious, and strong transgressive segregation occurred for each trait in both water regimes in all three populations, indicating that alleles with positive effects differed between the respective parents. All traits showed continuous variation in each population, indicating they were typical quantitative traits controlled by a few minor genes and that the data were suitable for QTL analysis (Table 1).

Evaluations of phenotypic correlations of the eight traits under the two water regimes in all three populations are shown in Table 2. All traits showed significant positive correlations under both water regimes with correlation coefficients ranging from 0.24 to 0.91, and showing consistency between the populations. CL showed the highest correlation coefficients, ranging from 0.83 to 0.91 in the three populations. The correlation coefficients for RL, RDW, and RSDWR

Table 1 Statistics for seedling traits of RILs and parents under normal water and osmotic stress treatments

Trait (t ² %) ^a	Treatment ^b	Parent	WL ^c										WJ									
			Yannong 19					Jimai 20					WY					WJ				
			Weimai 8	Luohan 2	Yannong 19	Jimai 20	Mean ^e	Std ^d	Skewness	Kurtosis	Mean	Std	Skewness	Kurtosis	Mean	Std	Skewness	Kurtosis				
CL (cm)	C	3.15	2.82	2.74	2.27	2.81 aA	0.40	-0.06	-0.51	2.64 aA	0.43	0.27	0.00	2.81 aA	0.33	0.24	0.56					
(81.7/89.6/ 79.2)	T	3.46	3.46	3.21	2.24	2.84 aA	0.43	0.03	-0.41	2.56 bA	0.41	0.64	0.32	2.86 aA	0.30	0.01	0.21					
PH (cm)	C	11.27	12.58	10.89	9.19	12.64 aA	1.21	-0.15	0.54	10.16 aA	1.40	-0.12	0.25	12.99 aA	1.01	-0.31	1.15					
(42.8/39.2/ 38.9)	T	8.90	10.33	8.58	8.74	11.23 bB	1.33	-0.36	0.24	8.35 bB	1.30	-0.16	0.22	11.72 bB	1.10	0.03	-0.15					
RL (cm)	C	10.57	9.19	8.64	8.16	9.49 aA	1.80	-0.05	-0.12	7.50 aA	1.98	0.01	-0.40	10.13 aA	1.60	0.05	0.21					
(31.0/34.7/ 19.0)	T	8.02	9.35	6.16	6.13	7.44 bB	1.20	0.13	-0.22	5.82 bB	1.14	0.32	-0.30	7.17 bB	0.98	0.04	-0.30					
RN	C	3.00	4.20	4.60	4.9	5.35 aA	0.46	-0.08	0.45	4.70 aA	0.53	-0.21	-0.07	5.08 aA	0.29	0.03	0.62					
(64.8/68.4/ 38.2)	T	3.60	5.80	5.40	4.8	5.57 bB	0.56	0.00	0.39	4.84 bB	0.49	-0.38	0.44	5.26 bB	0.34	0.52	0.36					
PDW (mg)	C	18.54	24.36	18.89	15.9	21.18 aA	2.63	0.26	-0.16	14.65 aA	2.79	-0.23	0.23	20.64 aA	2.22	-0.03	0.44					
(46.3/40.1/ 32.1)	T	14.24	20.06	16.94	12.6	18.29 bB	2.83	-0.20	0.17	12.34 bB	2.45	0.14	0.27	18.30 bB	2.11	-0.17	0.20					
SLDW (mg)	C	11.83	15.23	12.85	11.3	14.07 aA	1.76	0.30	0.42	9.75 aA	2.11	0.11	0.71	13.60 aA	1.56	0.19	0.10					
(47.9/44.9/ 46.0)	T	9.41	12.23	11.30	8.2	12.34 bB	1.98	-0.16	0.14	8.36 bB	1.65	0.13	0.19	12.61 bB	1.58	-0.16	0.01					
RDW (mg)	C	6.71	9.13	6.04	6.6	7.12 aA	1.28	-0.01	-0.29	4.84 aA	1.14	0.06	0.31	7.04 aA	1.16	0.27	0.16					
(50.1/41.5/ 15.4)	T	4.83	7.83	5.64	4.4	5.94 bB	1.15	-0.10	-0.36	3.97 bB	1.07	0.69	1.48	5.69 bB	0.88	0.25	0.80					
RSDWR (%)	C	56.72	59.87	47.00	53.66	50.84 aA	8.46	0.17	-0.10	50.92 aA	12.53	0.55	0.79	52.13 aA	8.83	0.54	0.26					
(54.6/45.6/ 19.1)	T	51.33	63.93	49.91	47.71	48.53 bB	8.05	0.36	0.32	47.85 bB	10.10	0.55	1.27	45.50 bB	6.98	0.41	0.23					

^a Arabic numerals in parentheses are estimated broad-sense heritabilities of the corresponding traits for WL, WY, and WJ in turn

^b C, T normal and osmotic stress conditions, respectively

^c WL, WY, and WJ, crosses Weimai 8 × Luohan 2, Weimai 8 × Yannong 19, and Weimai 8 × Jimai 20, respectively

^d Std Standard deviation

^e Small letters indicate significant differences at $P = 0.05$, large letters indicate significant differences at $P = 0.01$

Table 2 Phenotypic correlations of wheat seedling traits under normal water and osmotic stress conditions in three populations

Population ^a	CL	PH	RL	RN	PDW	SLDW	RDW	RSDWR
WL	0.906**	0.660**	0.590**	0.704**	0.687**	0.649**	0.696**	0.608**
WY	0.897**	0.769**	0.586**	0.686**	0.604**	0.626**	0.557**	0.468**
WJ	0.831**	0.633**	0.325**	0.466**	0.508**	0.541**	0.287**	0.239**

^a WL, WY and WJ represent populations derived from Weimai 8 × Luohan 2, Weimai 8 × Yannong 19, and Weimai 8 × Jimai 20, respectively

** Significant at $P = 0.01$

were the smallest, indicating a larger influence of osmotic stress on root growth of wheat seedlings. The significant correlations of all the traits under the two water treatments made it possible and meaningful to conduct conditional QTL analysis and determine the specific expression of QTL for the corresponding traits in wheat seedlings under osmotic stress.

Unconditional QTL mapping

Up to 87 QTLs distributed across all 21 wheat chromosomes were identified by unconditional QTL analysis for the eight traits, individually explaining 2.1–26.4 % of the phenotypic variation (Table 3; Fig. 1). Among them, 18 QTLs were detected under both water regimes, whereas 38 and 31, respectively, were detected only under normal water and osmotic stress conditions. 19, 25, and 31 QTLs, respectively, were detected in the WL, WY and WJ populations, with 10, 20, and 20 QTL increasing the corresponding traits originating from the common parent Weimai 8 in WL, WY, and WJ, respectively.

Of the 18 QTLs detected under both water regimes, 11 were major QTLs individually accounting for >10 % of the phenotypic variation. These QTLs were *QCL-3B*, *QPH-4A*, *QRL-2A*, *QRN-4B*, *QRN-2B.1*, *QRN-2B.2*, *QRN-7B.1*, *QSLDW-1D*, *QRDW-2B*, *QPDW-2A.2*, and *QPDW-2B* (Table 3). The stability of these QTLs across both water environments implied expression of drought tolerance and indicated that they were potentially important for breeding for increased drought tolerance.

Twelve QTLs were detected in at least two populations, accounting for 13.8 % of unconditional QTLs. Of them, *QPH-1BL*, *QRL-2A*, *QRL-4B*, *QSLDW-2B.2*, and *QSLDW-6B.2*, were major QTLs individually accounting for >10 % of the phenotypic variation (Table 3). Notably, *QCL-4A.2* and *QSLDW-6B* were jointly detected in all three populations. The

coincidence of these QTLs in chromosomal regions across different mapping populations not only implied the reliability of the QTLs reported herein, but also indicated a potentially high efficiency of the corresponding flanking markers in MAS.

Conditional QTL mapping

When the eight trait values detected under osmotic stress were conditioned on those found under normal water conditions, conditional analysis detected a total of 34 conditional QTLs, individually explaining 4.9–28.6 % of the phenotypic variation (Table 4). These QTLs were located on all 21 chromosomes except 1A, 1B, 4B, 4D, and 5D, 19 of them were major QTLs individually accounting for >10 % of the phenotypic variation.

Eight QTLs on chromosomes 2A, 2B, 3D, 6B, 7A, and 7D were detected in the WL population; all increasing the corresponding traits originating from Luohan 2. Only *QRLT/QRLC-2B* had been detected by unconditional analysis showing reduced additive effects compared to the unconditional QTL.

Nine QTLs located on chromosomes 2B, 2D, 4A, 5A, 5B, 6A, 6B, and 7B were detected in the WY population; four alleles increasing the corresponding traits originating from Yannong 19, and five from Weimai 8. Only *QPDWT/PDWC* was detected by unconditional analysis of PDW, again showing decreased additive effects compared to the unconditional QTL analysis.

Seventeen QTLs located on chromosomes 1D, 2B, 2D, 3A, 3B, 4A, 6A, 6B, and 6D were detected in the WJ population; eight alleles increased the corresponding traits originating from Weimai 8, and nine originating from Jimai 20. Five QTLs were also detected by the unconditional analysis; of those, *QRSDWRT/RSDWRC-1D.1* and *QRSDWRT/RSDWRC-1D.2* showed decreased

Table 3 Summary of unconditional QTL for wheat seedling traits under two water regimes

Trait	QTL	Treat ^a	Population	Chr ^b	Left marker ^c	Right marker	LOD	PVE (%) ^d	Add ^e	
CL	<i>QCL-1A</i>	C	WL	1A	<i>wPt-731282</i>	<i>Xwmc120</i>	2.69	8.1	-0.11	
	<i>QCL-3B</i>	C/T	WL	3B	<i>Xmag3356</i>	<i>wPt-1336</i>	6.59/4.10	17.5/9.9	0.19/0.15	
	<i>QCL-4A.1</i>	C	WJ	4A	<i>Xcft3034.2</i>	<i>Xapr1.2.1</i>	5.81	15.6	-0.16	
	<i>QCL-4A.2</i>	C	WL/WY/WJ	4A	<i>wPt-2084</i>	<i>wPt-1362</i>	2.78/3.69/3.71	6.7/8.3/8.5	-0.11/-0.12/-0.13	
	<i>QCL-5A</i>	T/T	WJ/WL	5A	<i>Xcwm216</i>	<i>wPt-0605</i>	3.50/3.56	8.9/11.2	0.08/0.14	
	<i>QCL-5B</i>	(C/T)/C	WL/WY	5B	<i>wPt-5175</i>	<i>wPt-8449</i>	(3.03/4.33)/3.21	(6.5/9.2)/10.0	(-0.16/-0.13)/0.14	
	<i>QCL-6B</i>	T	WY	6B	<i>wPt-664250</i>	<i>wPt-666793</i>	5.35	18.7	0.20	
	<i>QCL-7A</i>	C	WL	7A	<i>wPt-6668</i>	<i>Xmag2931.3</i>	2.81	9.7	-0.15	
	<i>QCL-7B.1</i>	C	WY	7B	<i>Xwmc517.2</i>	<i>wPt-3833</i>	2.72	10.1	0.15	
	<i>QCL-7B.2</i>	C	WY	7B	<i>wPt-7108</i>	<i>wPt-9925</i>	2.61	13.6	0.18	
	<i>QCL-7B.3</i>	T	WY	7B	<i>ww121</i>	<i>wPt-1553</i>	3.80	10.2	-0.13	
	PH	<i>QPH-1BL</i>	C/C	WY/WJ	1BL	<i>wPt-0260</i>	<i>wPt-2230</i>	6.29/3.65	13.5/15.2	-0.51/0.39
		<i>QPH-2A.1</i>	C	WJ	2A	<i>Xgwm382.2</i>	<i>wPt-665330</i>	5.54	20.6	0.61
<i>QPH-2A.2</i>		C	WY	2A	<i>Xcfe175.2</i>	<i>wPt-3565</i>	3.87	9.2	0.47	
<i>QPH-2B</i>		C	WJ	2B	<i>wPt-9736</i>	<i>wPt-8004</i>	7.50	21.4	-0.67	
<i>QPH-3B</i>		C	WL	3B	<i>Xmag3356</i>	<i>wPt-1336</i>	3.18	9.3	0.42	
<i>QPH-4A</i>		C/T	WY	4A	<i>Xbarc1047</i>	<i>wPt-7354</i>	5.61/5.01	12.7/11.3	-0.49/-0.46	
<i>QPH-4B</i>		T	WJ	4B	<i>wPt-6209</i>	<i>wPt-6149</i>	3.85	10.2	-0.38	
<i>QPH-6A</i>		C/T	WY/WJ	6A	<i>wPt-0259</i>	<i>wPt-4230</i>	3.89/2.56	6.1/4.1	-0.35/0.21	
<i>QPH-7B</i>		C/T	WY	7B	<i>wPt-7934</i>	<i>wPt-9467</i>	4.78/3.73	8.9/8.1	0.41/0.37	
RL		<i>QRL-1A</i>	C	WY	1A	<i>wPt-4029</i>	<i>wPt-668160</i>	5.70	14.5	-0.83
	<i>QRL-1BL</i>	C/T	WY/WL	1BL	<i>wPt-6975</i>	<i>Glu-b1</i>	2.88/3.72	5.9/10.0	-0.48/-0.38	
	<i>QRL-2A</i>	C/T	WY/WJ	2A	<i>Xcfe175.2</i>	<i>wPt-3653</i>	5.33/3.27	15.2/7.6	0.88/-0.29	
	<i>QRL-2B</i>	T	WL	2B	<i>wPt-3109</i>	<i>wPt-8460</i>	8.11	15.8	-0.50	
	<i>QRL-3A</i>	T	WJ	3A	<i>wPt-671711</i>	<i>wPt-1888</i>	7.04	19.7	0.46	
	<i>QRL-4A</i>	T	WL	4A	<i>wPt-6997</i>	<i>wPt-4680</i>	3.28	11.0	-0.40	
	<i>QRL-4B</i>	C/T	WL/WJ	4B	<i>Xgwm495</i>	<i>wPt-5265</i>	3.27/3.55	10.7/11.3	-0.62/0.67	
	<i>QRL-4D</i>	T	WY	4D	<i>Xcfd71</i>	<i>wPt-2379</i>	2.78	6.5	-0.29	
	<i>QRL-5B</i>	C/T	WY/WJ	5B	<i>wPt-730009</i>	<i>wPt-9103</i>	2.53/4.62	6.0/12.5	0.57/0.63	
	<i>QRL-7B</i>	C	WJ	7B	<i>wPt-6936</i>	<i>Xgwm344</i>	3.33	12.9	0.61	
	RN	<i>QRN-1D</i>	C	WY	1D	<i>wPt-7697</i>	<i>wPt-4988</i>	4.01	6.8	0.14
<i>QRN-2A</i>		C	WJ	2A	<i>Xgwm558</i>	<i>Xgwm372</i>	3.69	10.1	0.09	
<i>QRN-2B.1</i>		C/T	WL	2B	<i>wPt-669273</i>	<i>wPt-9736</i>	7.79/6.64	15.5/11.3	0.20/0.21	
<i>QRN-2B.2</i>		C/T	WY	2B	<i>wPt-3132</i>	<i>wPt-2106</i>	2.60/8.47	7.6/18.5	-0.05/-0.26	
<i>QRN-2D.1</i>		C	WY	2D	<i>Xcwm83</i>	<i>wPt-4144</i>	3.40	5.9	-0.14	
<i>QRN-2D.2</i>		C	WY	2D	<i>Xcfd168.1</i>	<i>Xcfd168.2</i>	3.39	6.3	-0.13	
<i>QRN-3A.1</i>		C	WY	3A	<i>wPt-2866</i>	<i>Xcfa2163.1</i>	2.59	8.5	-0.15	
<i>QRN-3A.2</i>		T	WY	3A	<i>wPt-7890</i>	<i>wPt-8855</i>	4.76	15.8	-0.20	
<i>QRN-3B</i>		T	WL	3B	<i>wPt-7225</i>	<i>wPt-4209</i>	3.59	6.6	0.15	
<i>QRN-4A</i>		T	WJ	4A	<i>wPt-0610</i>	<i>wPt-669526</i>	3.62	8.4	0.10	
<i>QRN-4D</i>		C	WY	4D	<i>Xcau17.2</i>	<i>Xcau17.1</i>	5.08	8.9	-0.18	
<i>QRN-5A</i>		C	WL	5A	<i>Xmag3166.2</i>	<i>wPt-3563</i>	2.70	4.5	-0.10	
<i>QRN-5D</i>		C	WY	5D	<i>Xgdm99.2</i>	<i>Xbarc320</i>	6.02	10.8	0.17	
<i>QRN-5D</i>		T	WJ	5D	<i>Xswes558.4</i>	<i>Xcfe242.1</i>	2.68	9.9	-0.11	
<i>QRN-6A</i>		(C/T)/T	WL/WY	6A	<i>wPt-8124</i>	<i>wPt-3524</i>	(4.14/5.65)/3.20	(7.6/8.8)/12.8	(0.13/0.17)/-0.21	
<i>QRN-6B</i>	T	WJ	6B	<i>wPt-663764</i>	<i>wPt-3733</i>	3.62	9.1	-0.10		
<i>QRN-7B.1</i>	C/T	WL	7B	<i>Xmag1036</i>	<i>wPt-9467</i>	5.28/9.2	14.4/18.8	-0.18/-0.27		
<i>QRN-7B.2</i>	T	WL	7B	<i>wPt-6936</i>	<i>wPt-666615</i>	5.25	9.2	-0.17		

Table 3 continued

Trait	QTL	Treat ^a	Population	Chr ^b	Left marker ^c	Right marker	LOD	PVE (%) ^d	Add ^e
SLDW	<i>QSLDW-1D</i>	C/T	WJ	1D	<i>wPt-7946</i>	<i>Xwmc429.3</i>	2.92/6.18	10.4/21.7	-0.5/-0.8
	<i>QSLDW-2A</i>	T	WJ	2A	<i>wPt-3565</i>	<i>wPt-4197</i>	4.69	10.8	-0.6
	<i>QSLDW-2B.1</i>	T	WJ	2B	<i>Xwmc617.1</i>	<i>wPt-2314</i>	3.43	11.0	-0.6
	<i>QSLDW-2B.2</i>	C/C	WL/WY	2B	<i>wPt-3132</i>	<i>wPt-4301</i>	2.71/3.13	12.4/20.4	-0.7/1.2
	<i>QSLDW-4A</i>	T	WJ	4A	<i>Xcft3034.2</i>	<i>Xapr1.2.1</i>	2.74	20.1	0.9
	<i>QSLDW-4B</i>	C	WL	4B	<i>wPt-8292</i>	<i>wPt-6209</i>	2.75	12.5	-0.6
	<i>QSLDW-5D</i>	C	WY	5D	<i>wPt-5870</i>	<i>Xgdm99.2</i>	4.49	12.2	0.8
	<i>QSLDW-6B</i>	C/C/C	WY/WJ/WL	6B	<i>wPt-6282</i>	<i>wPt-9881</i>	5.16/3.49/3.21	15.0/15.8/14.2	0.7/0.7/0.7
	<i>QSLDW-6D.1</i>	C	WJ	6D	<i>Xissr844.1</i>	<i>Xissr817</i>	4.36	17.2	0.7
	<i>QSLDW-6D.2</i>	C	WJ	6D	<i>Xapr1.5.1</i>	<i>Xapr1.2.3</i>	2.85	6.4	-0.6
RDW	<i>QRDW-1A</i>	C/T	WL	1A	<i>wPt-668160</i>	<i>wPt-664778</i>	3.07/3.66	7.8/10.7	-0.4/-0.4
	<i>QRDW-1BL</i>	C	WY	1BL	<i>wPt-8267</i>	<i>wPt-7094</i>	2.74	7.4	-0.3
	<i>QRDW-1D</i>	T	WY	1D	<i>Xcfd48.2</i>	<i>Xcfe78.2</i>	3.88	12.1	-0.4
	<i>QRDW-2B</i>	C/T	WL	2B	<i>wPt-3132</i>	<i>wPt-4301</i>	7.51/5.93	26.3/17.9	-0.7/-0.5
	<i>QRDW-4A</i>	T	WJ	4A	<i>wPt-4064</i>	<i>wPt-9675</i>	3.28	8.6	0.3
	<i>QRDW-4B</i>	T	WJ	4B	<i>Xwmc349</i>	<i>Xcfd54</i>	2.50	8.4	0.3
	<i>QRDW-6A</i>	C	WJ	6A	<i>wPt-0259</i>	<i>wPt-4230</i>	2.71	10.2	0.4
	<i>QRDW-6B</i>	T	WJ	6B	<i>wPt-3581</i>	<i>wPt-663764</i>	3.37	11.6	-0.3
PDW	<i>QPDW-1D</i>	T	WJ	1D	<i>wPt-7946</i>	<i>Xwmc429.3</i>	3.82	14.4	-0.8
	<i>QPDW-2A.1</i>	T	WJ	2A	<i>wPt-3565</i>	<i>wPt-4197</i>	4.18	10.2	-0.7
	<i>QPDW-2A.2</i>	C/T	WY	2A	<i>Xwmc177</i>	<i>Xcwm109.7</i>	4.57/3.83	12.5/9.6	1.1/0.9
	<i>QPDW-2B</i>	C/T	WL	2B	<i>wPt-3132</i>	<i>wPt-4301</i>	6.51/3.79	26.4/14.8	-1.4/-1.2
	<i>QPDW-4A.1</i>	T	WJ	4A	<i>Xcft3034.2</i>	<i>Xapr1.2.1</i>	2.86	26.2	1.4
	<i>QPDW-4A.2</i>	T	WY	4A	<i>Xcfe89.4</i>	<i>Xcfe89.3</i>	3.40	9.0	0.8
	<i>QPDW-5B</i>	T	WJ	5B	<i>Xissr854.1</i>	<i>Xgwm335</i>	2.68	8.7	0.7
	<i>QPDW-5D</i>	C	WJ	5D	<i>Xswes558.4</i>	<i>Xcfe242.1</i>	3.03	7.8	-0.6
	<i>QPDW-6B</i>	C	WL	6B	<i>wPt-6282</i>	<i>wPt-9881</i>	2.73	13.7	1
	<i>QPDW-6D.1</i>	C	WJ	6D	<i>Xissr844.1</i>	<i>Xissr817</i>	4.58	11.0	0.8
<i>QPDW-6D.2</i>	C	WJ	6D	<i>Xapr1.2.3</i>	<i>Xcft3103</i>	2.56	9.9	-1	
RSDWR	<i>QRSRWR-1BL</i>	C	WL	1BL	<i>wPt-8627</i>	<i>wPt-665375</i>	5.27	13.1	3.05
	<i>QRSRWR-1D</i>	T	WY	1D	<i>Xcfd48.2</i>	<i>Xcfe78.2</i>	3.30	8.8	-3.12
	<i>QRSRWR-1D</i>	T	WJ	1D	<i>wPt-671990</i>	<i>wPt-4196</i>	5.56	13.5	2.75
	<i>QRSRWR-1D</i>	T	WJ	1D	<i>wPt-671990</i>	<i>wPt-4196</i>	3.55	8.5	-2.21
	<i>QRSRWR-2D</i>	C	WJ	2D	<i>wPt-731134</i>	<i>wPt-1554</i>	2.81	7.3	-2.78
	<i>QRSRWR-3B</i>	C	WL	3B	<i>Xgwm30.2</i>	<i>Xgpw1146</i>	3.94	9.4	-2.62
	<i>QRSRWR-3B</i>	C	WY	3B	<i>wPt-2416</i>	<i>Xcfe127</i>	4.73	16.3	-6.27
	<i>QRSRWR-4A</i>	T	WL	4A	<i>wPt-4620</i>	<i>wPt-672107</i>	2.84	6.4	-2.11
	<i>QRSRWR-4A</i>	T	WJ	4A	<i>Xcft3034.2</i>	<i>Xapr1.2.1</i>	3.41	22.2	-4.62
	<i>QRSRWR-7B</i>	T	WY	7B	<i>wPt-2994</i>	<i>wPt-669158</i>	2.52	12.2	-3.68

^a Letters indicate different treatments; C normal water conditions, T osmotic stress

^b Chromosome on which the QTL was detected

^c Flanking marker

^d Percentage phenotypic variance explained by additive effects

^e Estimate of the additive effect of the QTL. Positive values indicate that Weimai 8 alleles increase the trait value; negative values indicate that Weimai 8 alleles reduce the trait value

additive effects, whereas *QSDWT/SDWC-1D* and *QRDWT/RDWC-2B* showed increased effects, and *QRDWT/RDWC-6B* showed additive effects similar to that of the unconditional QTL.

QTL clusters

Eleven QTL clusters related to more than three traits were observed on chromosomes 1BL, 1D, 2A, 2B, 2D,

Table 4 Summary of conditional QTL for wheat seedling traits

Trait ^a	QTL	Population	Chr ^b	Position	Left marker	Right marker	LOD	PVE (%)	Add ^c
RSDWRTIRSDWRC	<i>QRSDWRTIRSDWRC-1D.1</i>	WJ	1D	45.79	<i>wPt-671990</i>	<i>wPt-4196</i>	3.84	9.0	21.9 (–)
RSDWRTIRSDWRC	<i>QRSDWRTIRSDWRC-1D.2</i>	WJ	1D	52.79	<i>wPt-671990</i>	<i>wPt-4196</i>	3.14	7.2	–19.7 (+)
SLDWTISLDWC	<i>QSDWTISLDWC-1D</i>	WJ	1D	57.79	<i>wPt-7946</i>	<i>Xwmc429.3</i>	5.17	18.5	–0.6 (–)
RDWTIRDWC	<i>QRDWTIRDWC-2A</i>	WL	2A	62.00	<i>wPt-666857</i>	<i>Xcfa2263.2</i>	2.58	9.3	–0.3
RNTIRNC	<i>QRNTIQRNC-2A</i>	WJ	2A	69.00	<i>Xdupw210</i>	<i>Xgwm558</i>	2.51	7.6	–0.08
RSDWRTIRSDWRC	<i>QRSDWRTIRSDWRC-2B</i>	WJ	2B	12.65	<i>wPt-2314</i>	<i>wPt-665550</i>	4.86	17.3	47.8
PHTIPHC	<i>QPHTIPHC-2B</i>	WJ	2B	21.65	<i>Xbarc98</i>	<i>Xcfd188</i>	2.65	12.7	–0.54
RLTIRLC	<i>QRLTIQRLC-2B</i>	WL	2B	38.00	<i>wPt-3109</i>	<i>wPt-8460</i>	3.51	8.4	–0.29 (–)
RNTIRNC	<i>QRNTIQRNC-2B</i>	WJ	2B	42.65	<i>wPt-6144</i>	<i>Xedm97.1</i>	3.93	10.4	–0.1
RDWTIRDWC	<i>QRDWTIRDWC-2B</i>	WY	2B	63.61	<i>wPt-3132</i>	<i>wPt-2106</i>	2.52	28.6	1.5 (–)
RDWTIRDWC	<i>QRDWTIRDWC-2D</i>	WY	2D	93.00	<i>wPt-2781</i>	<i>wPt-666887</i>	3.31	8.8	–0.4
PDWTIPDWC	<i>QPDWTIPDWC-2D</i>	WJ	2D	98.34	<i>wPt-8713</i>	<i>Xmag3596</i>	4.19	10.6	0.6
RLTIRLC	<i>QRLTIQRLC-3A</i>	WJ	3A	38.34	<i>wPt-7341</i>	<i>wPt-6234</i>	3.95	9.1	0.29
PDWTIPDWC	<i>QPDWTIPDWC-3B</i>	WJ	3B	64.23	<i>wPt-4220</i>	<i>wPt-8238</i>	2.62	7.3	0.4
SLDWTISLDWC	<i>QSDWTISLDWC-3B</i>	WJ	3B	64.23	<i>wPt-4220</i>	<i>wPt-8238</i>	3.2	9.2	0.5
CLTICLC	<i>QCLTIQCLC-3D</i>	WL	3D	114.95	<i>wPt-666676</i>	<i>wPt-5313</i>	4.75	18.4	–0.08
PHTIPHC	<i>QPHTIPHC-3D</i>	WL	3D	119.95	<i>wPt-666676</i>	<i>wPt-5313</i>	3.48	9.1	–0.37
PDWTIPDWC	<i>QPDWTIPDWC-4A</i>	WY	4A	92.17	<i>Xcfe89.4</i>	<i>Xcfe89.3</i>	2.52	7.5	0.6 (–)
RDWTIRDWC	<i>QRDWTIRDWC-4A</i>	WJ	4A	119.00	<i>wPt-4064</i>	<i>wPt-9675</i>	4.65	12.1	0.3 (=)
PDWTIPDWC	<i>QPDWTIPDWC-5A</i>	WY	5A	90.48	<i>Xcfa2163.2</i>	<i>wPt-3563</i>	2.88	21.5	1.3
RNTIRNC	<i>QRNTIQRNC-5B</i>	WY	5B	72.95	<i>wPt-669837</i>	<i>wPt-5896</i>	2.98	18.5	–0.17
RDWTIRDWC	<i>QRDWTIRDWC-6A</i>	WY	6A	34.44	<i>wPt-7599</i>	<i>Xgwm169</i>	2.55	7.2	–0.4
RLTIRLC	<i>QRLTIQRLC-6A</i>	WJ	6A	71.00	<i>wPt-667405</i>	<i>wPt-668031</i>	4.19	14.8	–0.42
PHTIPHC	<i>QPHTIPHC-6A</i>	WY	6A	86.44	<i>Xbarc204.1</i>	<i>wPt-8117</i>	3.82	13.9	–0.34
RSDWRTIRSDWRC	<i>QRSDWRTIRSDWRC-6B</i>	WL	6B	32.00	<i>wPt-6282</i>	<i>wPt-9881</i>	3.16	11.9	23.4
PDWTIPDWC	<i>QPDWTIPDWC-6B</i>	WJ	6B	37.00	<i>wPt-3581</i>	<i>wPt-663764</i>	3.57	11.5	–0.3
RDWTIRDWC	<i>QRDWTIRDWC-6B</i>	WJ	6B	37.00	<i>wPt-3581</i>	<i>wPt-663764</i>	3.22	11.0	–0.6 (=)
RNTIRNC	<i>QRNTIQRNC-6B</i>	WJ	6B	69.00	<i>wPt-665036</i>	<i>wPt-1541</i>	4.27	14.4	–0.11
PHTIPHC	<i>QPHTIPHC-6B</i>	WY	6B	97.00	<i>wPt-0171</i>	<i>Xbarc178</i>	2.51	7.1	0.22
PHTIPHC	<i>QPHTIPHC-6D</i>	WJ	6D	2.00	<i>Xcfe87.1</i>	<i>Pr119.1</i>	2.61	23.3	–0.87
RLTIRLC	<i>QRLTIQRLC-7A</i>	WL	7A	73.00	<i>Xbarc49</i>	<i>Yp-7A</i>	3.46	10.3	–0.31
RNTIRNC	<i>QRNTIQRNC-7A</i>	WL	7A	98.00	<i>wPt-1928</i>	<i>wPt-6495</i>	2.64	8.6	–0.12
RDWTIRDWC	<i>QRDWTIRDWC-7B</i>	WY	7B	58.27	<i>wPt-669158</i>	<i>wPt-665293</i>	2.74	15.0	1.4
CLTICLC	<i>QCLTIQCLC-7D</i>	WL	7D	60.99	<i>wPt-0934</i>	<i>wPt-1405</i>	2.56	4.9	–0.04

^a The trait value surveyed under osmotic stress was subjected to conditional QTL analysis on that detected under normal water conditions

^b Chromosome on which the QTL was detected

^c (–), (+), and (=) indicate that the QTL was also detected by unconditional analysis, and showed a reduced, increased, and similar additive effect, respectively, compared with that of the unconditional QTL analysis

4A, 6B, and 7B, explaining 7.2–28.6 % of the phenotypic variation in single traits, involving 44 QTLs. Among them, eight clusters involved QTLs for both seedling traits and drought tolerance, and three involved QTLs only for seedling traits (Table 5; Fig. 1). Four QTL clusters were detected in three populations, five in two populations, and two in one population.

The most important QTL clusters involving QTLs for drought tolerance were C9, C10, and C11 (Table 5) and were detected in all three populations. Cluster C9 on chromosome 4A in marker interval *wpt-1047–wpt-9675* involved QTLs for PH, CL, RDW and drought tolerance. Cluster C10 on chromosome 6B in marker interval *wpt-6282–wpt-3733* involved seven QTLs for SDW, PDW, RDW, RN, and drought

Table 5 QTL clusters detected by unconditional and conditional analyses of wheat seedling traits

Number	QTL ^a	Population ^b	Left marker	Right marker	PVE (%)
C1	<i>QPH-1BL, QRSDWR-1BL, QRL-1BL</i>	WL, WY, WJ	<i>wPt-0260</i>	<i>Glu-b1</i>	6.0–15.2
C2	<i>QRSDWR-1D, QRSDWRTIRSDWRC-1D.2, QSDW-1D, QPDW-1D, QSDWTISDWC-1D</i>	WJ	<i>wPt-671990</i>	<i>Xwmc429.3</i>	7.2–21.7
C3	<i>QPH-2A.2, QRL-2A, QSDW-2A, QPDW-2A.1</i>	WY, WJ	<i>Xcfe175.2</i>	<i>wPt-4197</i>	7.6–15.2
C4	<i>QRN-2B, QPHTPHC-2B, QPH-2B</i>	WL, WJ	<i>wPt-669273</i>	<i>wPt-8004</i>	11.3–21.4
C5	<i>QRL-2B, QRLTIQRLC-2B, QRNTIQRNC-2B</i>	WL, WJ	<i>wPt-3109</i>	<i>Xedm97.1</i>	8.4–15.8
C6	<i>QRDWTIRDWC-2B, QSDW-2B.2, QRDW-2B, QPDW-2B, QRN-2B</i>	WL, WY	<i>wPt-3132</i>	<i>wPt-2106</i>	12.4–28.6
C7	<i>QRDWTIRDWC-2D, QPDWTPDWC-2D, QRSDWR-2D</i>	WY, WJ	<i>wPt-2781</i>	<i>wPt-1554</i>	7.3–10.6
C8	<i>QRSDWR-4A, QSDW-4A, QPDW-4A.1</i>	WJ	<i>Xcft3034.2</i>	<i>Xapr1.2.1</i>	20.1–22.2
C9	<i>QPH-4A, QCL-4A.2, QRDW-4A, QRDWTIRDWC-4A</i>	WL, WY, WJ	<i>Xbarc1047</i>	<i>wPt-9675</i>	6.7–12.7
C10	<i>QRSDWRTIRSDWRC-6B, QSDW-6B, QPDW-6B, QRDW-6B, QRDWTIRDWC-6B, QPDWTPDWC-6B, QRN-6B</i>	WL, WY, WJ	<i>wPt-6282</i>	<i>wPt-3733</i>	9.1–15.8
C11	<i>QRDWTIRDWC-7B, QRL-7B, QRN-7B.2, QCL-7B.1</i>	WL, WY, WJ	<i>wPt-669158</i>	<i>wPt-3833</i>	9.2–15.0

^a QTL involved in the cluster

^b Populations in which the QTL cluster was detected

tolerance. Cluster C11 on chromosome 7B in marker interval *wpt-669158–wpt-3833* involved four QTLs for RL, CL, RN, and drought tolerance.

The other eight more important QTL clusters included C2 on chromosome 1D, C4, C5, and C6 on chromosome 2B, and C7 on chromosome 2D involving QTLs for both seedling traits and drought tolerance. Cluster C1 on 1BL, C3 on 2A, and C8 on 4A involved only QTLs for the seedling traits. The chromosomal regions where these QTL clusters were located might be important gene regions related to seedling traits and drought tolerance during seed germination.

Discussion

Comparison of QTL analyses using several populations and a single population

Beavis (1998) reported that expression of genetic effects of QTL was influenced by genetic background, environment, and interaction between them. Consistent with this, several QTLs that were detected under normal water conditions in the WY and WL populations were detected only under osmotic stress conditions in WJ. A possible reason for this is that the effects of these QTLs are small under normal water

conditions in WJ, whereas osmotic stress increases their effects making them detectable.

Analysis of phenotypic data indicated that the correlation coefficient of each seedling trait under the two water regimes in the three populations was sometimes different. The general trend was that the correlation coefficient of each trait in WL was the largest, and the correlation coefficient of each trait in the WY was the smallest. This means that the influence of osmotic stress on each seedling trait in the WL was small, whereas the influence of osmotic stress on each seedling trait in the WJ was large. This was also explained by the estimated broad-sense heritability of each investigated trait in the three populations. The number of QTLs detected in WL, WY, and WJ was 7, 9 and 22, respectively, under osmotic stress, and 8, 9 and 17, respectively, by the conditional analysis method. The number detected in WJ was more than that in WY and WL under osmotic stress conditions. It is worth considering if expression of some related genes might have been more easily induced by adverse conditions for WJ which is more sensitive to drought than WL.

With the ongoing rapid development in quantitative genetics, research on QTL effects in more than one genetic background, either different or related, is warranted (Kumar et al. 2007; Ma et al. 2007; Buckler et al. 2009; Cui et al. 2011). Although previous studies

on QTL effects were carried out using multiple populations, it was difficult to compare them across populations because of limited numbers of common markers. In this report, three related RIL populations with one common parent were used to construct an integrated genetic map with high density markers and subjected to unconditional and conditional QTL mapping for seedling traits during germination under two water regimes. QTL information that came from the three populations was displayed on a single genetic map (Fig. 1), making it easy to perform comparative analyses of QTLs from different populations, and thus to confirm important genetic regions controlling related traits. Some studies have shown that QTLs for closely related traits are often located in close proximity to each other (Fracheboud et al. 2002; Tuberosa et al. 2002; Liu et al. 2013). Our findings corroborate those results. We found 11 important QTL clusters related to seedling traits and drought tolerance during germination by comparing QTLs detected in WL, WY, and WJ. In addition, a total of 35 QTLs were detected in WL, 44 in WY, and 56 in WJ. Eighteen QTLs were detected in more than one population, enhancing the reliability of QTL identification.

Comparison of conditional and unconditional QTLs

QTL analysis of conventional drought tolerance is generally aimed at analyzing expression of phenotypic traits under osmotic stress (Zhou et al. 2005; Yue et al. 2006; Courtois et al. 2009). It is difficult to determine a direct relationship to drought tolerance because of the influence of trait expression, and the results are usually different with different traits. The phenotypic value of a trait is mainly influenced by genetic factors and the environment. Correlation analyses indicated that trait values of seedlings grown under osmotic stress were strongly influenced by those detected under normal water conditions. Therefore, we could use trait values of seedlings grown under normal water conditions as estimates of the genetic effects of the trait. Conditional QTL analysis involved taking the phenotypic value of the trait under normal water conditions as an estimation of the genetic effect of the trait under osmotic stress to calculate the effect value caused by drought. Then, QTL analysis was conducted using the effect value of each trait caused by drought, so expression of specific loci

under osmotic stress could be estimated by conditional QTL mapping. When conducting conditional QTL analysis of traits in wheat seedlings grown under osmotic stress on the corresponding traits under normal water conditions, four results are possible: (1) a QTL detected by the unconditional method is not identified, meaning that this QTL is not entirely related to drought tolerance; (2) a QTL detected by the unconditional method is identified with either a greatly reduced or a greatly enhanced effect, suggesting that this QTL is partially, but not completely, associated with drought tolerance; (3) a QTL detected by the unconditional method is identified with a similar or equal effect, indicating that this QTL is specifically associated with drought tolerance; and (4) an additional QTL is detected by conditional mapping, which means that expression of the QTL for the trait under osmotic stress is suppressed by traits expressed under normal water conditions and that these effects can only be identified by eliminating the influence of the traits expressed under normal water conditions. This suggests that the additional QTL has opposite additive effects on trait expression under the two water regimes.

Of the 49 unconditional QTLs for the eight seedling traits detected in the three populations, only eight related to drought tolerance with similar, reduced, and enhanced additive effects were detected by conditional analysis, and 41 were not detected. Thus most of the QTLs detected by unconditional analysis are not entirely concerned with drought tolerance. Furthermore, 26 new QTLs were detected by conditional analysis indicating that expression of these QTLs associated with drought tolerance was inhibited by expression of the trait itself. Our results showed that some unconditional QTLs for traits of wheat seedlings grown under osmotic stress contributed only to the development of those traits, and had no direct relation to drought tolerance. Most of the QTLs directly concerned with drought tolerance were detected only by conditional QTL analysis after excluding the influence of normal-trait representation. Therefore, carrying out conditional analysis for stress-tolerance traits such as drought tolerance is viable and effective.

Comparison of the present study to previous studies

Rebetzke et al. (2001, 2007) detected QTLs for CL on chromosomes 1A, 2B, 2D, 3B, 4A, 4B, 4D, 5A, 5D, 6B,

and 7A. Matsui et al. (1998) reported that final CL is controlled by genes on chromosomes 1A, 4A, 4D, 5A, and 5B. In the current work 11 QTLs for CL were detected on chromosomes 1A, 3B, 4A, 5A, 5B, 6B, 7A, and 7B. Spielmeier et al. (2007) detected a QTL controlling seedling height, coleoptile length, and leaf width on the short arm of chromosome 6A. We identified nine QTLs located on chromosome 1BL, 2A, 2B, 3B, 4A, 6A, 7B for height during germination. Interestingly, six of the nine QTLs were located on the same or closely linked intervals with QTLs detected by Cui et al. (2011) for mature PH using the WY and WJ populations (Table 6). This illustrated the close correlation between seedling height during germination and final PH at the molecular level and that it would be effective to select for PH at an early stage of growth.

Hamada et al. (2012) detected one QTL for seminal RL on chromosome 5A. Liu et al. (2013) reported QTLs for RL on chromosomes 1B, 2D, 3A, 5B, 5D and

7B, and for RN on chromosomes 2B, 3B, 3D, 5A and 7A under two different water regimes. Landjeva et al. (2008) detected a QTL for RL on chromosome 5B under normal water conditions and six QTLs on 1A, 6D and 7D under osmotic stress. Zhou et al. (2005) detected QTLs for RN on chromosomes 2B, 7A and 7B, three for RL on chromosomes 1D, 2B and 6B, two for RFW on chromosomes 5B and 7A, two for RDW on chromosomes 2A and 5B, and one for RSDWR on chromosome 5D using a doubled haploid population of 150 lines under two water regimes. Using a set of common wheat lines carrying known introgressed segments in the D genome, Landjeva et al. (2010) found QTL for germination *senso stricto* clustered in the region *Xgwm1291–Xgwm337* on chromosome 1DS and a region for seedling vigour linked to *Xgwm960* on chromosome 5DL. In similar regions of chromosomes 1DS and 5DL we detected QTL for SLDWR and drought tolerance, and for RN and SDW,

Table 6 Comparison of the QTL location between previous studies and this study

Chromosome	Marker	QTLs in this study	QTLs detected in previous studies	
		QTL name (treatment ^a)	Related traits (treatment ^a)	Reference
1A	<i>Glu-1A</i>	<i>QRL-1A</i> (C)	RL (T)	Landjeva et al. (2008)
1BL	<i>Wpt-9857</i>	<i>QPH-1BL</i> (C)	PH (normal water conditions in field trial)	Cui et al. (2011)
2A	<i>Xbarc212</i>	<i>QPH-2A</i> (C)	PH (normal water conditions in field trial)	Cui et al. (2011)
2B	<i>Wpt9736</i>	<i>QPH-2B</i> (C)	PH (normal water conditions in field trial)	Cui et al. (2011)
2B	<i>Xgwm374</i>	<i>QRL-2B</i> (T), <i>QRLTQRLC-2B</i> , <i>QRNTQRNC-2B</i>	Root surface areas (C/T), Project root area (T), Seminal root angles (T)	Liu et al. (2013)
2B	<i>Xgwm319</i>	<i>QRDWTIRDWC-2B</i> , <i>QSDW-2B</i> (C), <i>QRDW-2B</i> (C/T), <i>QPDW-2B</i> (C/T), <i>QRN-2B</i> (C/T)	Germination potentials (two different osmotic stress)	Hao et al. (2003)
3B	<i>Xbarc268</i>	<i>QRN-3B</i> (T)	RNs (T), RLs (T), Potential quantum efficiency of photosystems (T), Chlorophyll content (T)	Liu et al. (2013), Kumar et al. (2012)
3B	<i>Wpt-3921</i>	<i>QPH-3B</i> (C)	PH (normal water condition in field trial)	Cui et al. (2011)
4A	<i>Xbarc1047</i>	<i>QPH-4A</i> (C/T)	PH (normal water condition in field trial)	Cui et al. (2011)
4B	<i>Xgwm495</i>	<i>QRL-4B</i> (C/T)	RL, Project root area, root surface area (C)	Liu et al. (2013)
5A	<i>Xgwm156</i>	<i>QPDWTPDWC-5A</i>	Germination potential (two different osmotic stress)	Hao et al.(2003)
5A	<i>Xgwm156</i>	<i>QRN-5A</i> (C)	RN (C)	Liu et al. (2013)
5B	<i>Xgwm540</i>	<i>QRL-5B</i> (C/T)	RL (T)	Liu et al. (2013)
5D	<i>Xgdm99</i>	<i>QSDW-5D</i> (C), <i>QRN-5D</i> (C)	Shoot length (C), Root shoot length ratio (C)	Landjeva et al. (2010)
6A	<i>Wpt-0259</i>	<i>QPH-6A</i> (C/T)	PH (normal water condition in field trial)	Cui et al. (2011)

^a Letters indicate the different treatments in which the QTL was detected; C, T represent the normal water and osmotic stress conditions, respectively

respectively. We detected 10 QTLs for RL on chromosomes 1A, 1BL, 2A, 2B, 3A, 4A, 4B, 4D, 5B, and 7B, and 18 QTLs for RN on chromosomes 1D, 2A, 2B, 2D, 3A, 3B, 4A, 4D, 5A, 5D, 6A, 6B, and 7B. When we compared our results with those of previous studies, only three QTLs for RL and two QTLs for RN were within the same chromosome regions (Table 6). To our knowledge no QTL for SDW and SLDW under osmotic stress were previously reported.

Cattivelli et al. (2002) reported that there are genes related to drought tolerance on the first, second, fifth, sixth, and seventh homoeologous linkage groups of the *Triticeae*, and those on the sixth and seventh groups were the most important. Gorny (1999) found that chromosome 7D has positive effects on water use efficiency. They detected QTLs related to drought tolerance on different regions of group 2 chromosomes (both the short arm and the distal part of the long arm). Increased abscisic acid (ABA) concentration in tissues is thought to play a key role in drought response. A major QTL affecting drought-induced ABA accumulation was mapped on the long arm of chromosome 5A of wheat in the vicinity of the *Dhn1/Dhn2* locus (Quarrie et al. 1994). *Dreb* genes are involved in tolerances to stresses such as drought, salinity, low temperature and ABA. *Dreb-B1* was mapped between markers *Xmwig818* and *Xfbb117* on chromosome 3BL (Wei et al. 2009). The QTL related to drought tolerance detected on chromosome 3B in WJ was located in the same region as *Dreb-B1* with the common marker *Xgwm566* in our study. Landjeva et al. (2010) indicated that cultivars carrying only *Rht8* produced longer roots, coleoptiles and shoots, and had smaller root-to-shoot ratios in non-stress and osmotic stress conditions compared with those carrying *Rht-B1b/d* or *Rht-B1b/d + Rht8*. *Rht8* was shown to be closely linked to photoperiod sensitivity gene *Ppd-D1* on chromosome 2D (Korzun et al. 1998; Cane et al. 2013). In this study the QTL cluster C7 (Table 5) related to drought tolerance and RSDWR detected in the WY and WJ populations was in the region of *Rht8*. DELLA proteins are transcription factors that affect plant growth and stress tolerance. In common wheat DELLA proteins are encoded by the homoeoloci *Rht-A1*, *Rht-B1* and *Rht-D1* located in the group 4 chromosomes (Wilhelm et al. 2013). Liu et al. (2013) mapped QTL for root surface area in the approximate region of *Rht-B1* under different water regimes. In the present study the major QTL for RL detected on chromosome

4B in WL and WJ is located in the same region. We also detected a nearby QTL for seedling height under osmotic stress conditions in WJ. Hao et al. (2003) detected loci related to drought tolerance on chromosomes 1B, 2B, 5A, 6B, 7A, 7B by germination potential and germination rate of wheat seed under two osmotic stress regimes. Of those, a QTL on chromosome 2B accounted for 27.2 % of the phenotypic variation. In our study, QTL clusters related to PDW, SLDW, RDW, RN and drought tolerance were detected in the neighborhood of this locus. In addition, two other QTL clusters related to drought tolerance were detected on chromosome 2B, and additional QTL clusters related to drought tolerance were detected on chromosomes 1D, 2D, 6B, and 7B. However, it was difficult to judge whether the intervals between QTLs detected in this study and those reported in earlier studies are the same due to lack of common markers.

In summary, we detected 87 QTLs associated with 8 seedling traits and 34 specific QTLs related to drought tolerance of seedlings during germination by unconditional and conditional QTL analyses. Eleven important QTL clusters located on chromosomes 1BL, 1D, 2A, 2B, 2D, 4A, 6B, and 7B were detected. Identification of these QTLs and QTL clusters has an important significance for fine-mapping, map-based cloning, and molecular marker assisted selection for drought tolerance in wheat. Application of conditional analysis in conducting genetic research on drought tolerance has significant relevance for genetic study of stress tolerance traits in wheat.

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