J Appl Genet 49(4), 2008, pp. 333–341

Original article

Mapping QTLs for pre-harvest sprouting tolerance on chromosome 2D in a synthetic hexaploid wheat×common wheat cross

Ren Xiao-bo[1], Lan Xiu-jin[1, 2], Liu Deng-cai[1, 2], Wang Jia-li[1], Zheng You-liang[1]

[1] Triticeae Research Institute, Sichuan Agricultural University, Sichuan, China
[2] Key Laboratory of Crop Genetic Resources and Improvement, Ministry of Education, Sichuan Agricultural University, Sichuan, China

Abstract. Based on segregation distortion of simple sequence repeat (SSR) molecular markers, we detected a significant quantitative trait loci (QTL) for pre-harvest sprouting (PHS) tolerance on the short arm of chromosome 2D (2DS) in the extremely susceptible population of F_2 progeny generated from the cross of PHS tolerant synthetic hexaploid wheat cultivar 'RSP' and PHS susceptible bread wheat cultivar '88-1643'. To identify the QTL of PHS tolerance, we constructed two SSR-based genetic maps of 2DS in 2004 and 2005. One putative QTL associated with PHS tolerance, designated *Qphs.sau-2D*, was identified within the marker intervals *Xgwm261-Xgwm484* in 2004 and in the next year, nearly in the same position, between markers *wmc112* and *Xgwm484*. Confidence intervals based on the LOD-drop-off method ranged from 9 cM to 15.4 cM and almost completely overlapped with marker interval *Xgwm261-Xgwm484*. Flanking markers near this QTL could be assigned to the C-2DS1-0.33 chromosome bin, suggesting that the gene(s) controlling PHS tolerance is located in that chromosome region. The phenotypic variation explained by this QTL was about 25.73–27.50%. Genotyping of 48 F_6 PHS tolerant plants derived from the cross between PHS tolerant wheat cultivar 'RSP' and PHS susceptible bread wheat cultivar 'MY11' showed that the allele of *Qphs.sau-2D* found in the 'RSP' genome may prove useful for the improvement of PHS tolerance.

Keywords: Pre-harvest sprouting, QTL mapping, segregation distortion, simple sequence repeat, synthetic hexaploid wheat, *Triticum aestivum.*

Introduction

Pre-harvest sprouting (PHS) defined as the germination of grains in the ear before harvesting is one of the most important factors negatively affecting grain weight and end use quality of kernels. Improving tolerance to PHS is difficult based on the phenotypic choice since PHS is strongly affected by environmental factors. Overall resistance to sprouting damage results from the combined effect of multiple characters influencing spike water uptake and drying, grain dormancy and the rate of degradation of grain components during germination.

In the past, several studies on PHS/dormancy in common wheat have been conducted, treating them as qualitative traits controlled by digenic recessive, trigenic recessive or as a quantitative mode resistance (Olsson et al. 1975; Baker et al. 1981). At present PHS/dormancy are perceived as quantitative traits and interval mapping is commonly performed using the MAPMAKER/QTL and QTL Cartographer. Mapping data are often generated by SSR or RFLP markers for mapping populations of various genetic stocks, including common bread wheat, synthetic hexaploid wheat and spelt (Anderson et al. 1993; Zanetti et al. 2000; Flintham et al. 2002; Osa et al. 2003; Kuwal et al. 2004; Mori et al. 2005; Torada et al. 2005).

Received: March 16, 2008. Accepted: June 27, 2008.
Correspondence: L. Xiu-jin, Triticeae Research Institute, Sichuan Agricultural University, Dujiangyan 611830, Sichuan, China; e-mail: lanxiujin@yahoo.com.cn

Up to now, QTLs for PHS/dormancy in wheat have been shown to be present on 20 chromosomes, the only exception being chromosome 1D. Most consistently QTLs for PHS/dormancy are shown on wheat chromosomes 3A, 3B, 3D, 4A (Groos et al. 2002; Osa et al. 2003; Kuwal et al. 2004; Mori et al. 2005).

Our previous study indicated that the wild species of *Aegilops tauschii* Cosson, the D-genome donor of bread wheat, is characterized by a large variation in PHS tolerance. One accession of *Ae. tauschii*, native to Henan, China, showing good tolerance to PHS has been isolated as a result of several years of observations (Liu et al. 1998). That individual has been used for synthesizing an artificial amphiploid wheat 'RSP'. The synthetic hexaploid wheat 'RSP' shows high tolerance to PHS controlled by a major gene (Lan et al. 1997), localized on chromosome 2D, which has been confirmed through monosomic analysis (Lan et al. 2002). The present study has been undertaken to map the locus for PHS tolerance on wheat chromosome 2D to a specific chromosome region.

Materials and methods

Plant materials

Two mapping populations from a cross between a synthetic hexaploid wheat cultivar 'RSP', PHS tolerant, and bread wheat cultivar '88-1643', PHS susceptible, were used to analyze the QTL for PHS tolerance. The PHS tolerant parent of the mapping population, synthetic hexaploid wheat cultivar 'RSP' was obtained by crossing one *Ae. tauschii* accession with high PHS tolerance and a tetraploid wheat (*Triticum turgidum*) landrace Ailanmai, which shows susceptibility to sprouting. This synthetic hexaploid wheat cultivar 'RSP' was characterized by germination percentages ranging from 0 to 1.2% in different years. The PHS susceptible parent of the mapping population, a bread wheat cultivar '88-1643' was obtained from the Sichuan Agricultural University and was characterized by germination percentages ranging from 41 to 58% in different years. In 2004, the mapping population consisted of 91 F_2 plants. Twenty F_2 plants, which showed germination rates higher than the PHS susceptible parent '88-1643' (58.0%) were used as the group of segregants with extreme values of sprouting susceptibility. The mapping population developed in 2005 consisted of 140 F_2 plants. Among the 140 plants, 20 showed germination rates higher than the PHS susceptible parent '88-1643' (41.0%) and were used as the extremely sprouting susceptible segregants.

A bread wheat cultivar 'MY11' also has lower tolerance to PHS than the synthetic hexaploid wheat cultivar 'RSP', with its germination percentages ranging from 14 to 28% in different years (Lan et al. 2002). It was used for hybridization with the 'RSP' cultivar. Validation of the QTL for tolerance to PHS was conducted on 48 extremely PHS tolerant plants of the F_6 generation (germination percentage only up to 1.75%) from a cross between 'RSP' and 'MY11' cultivars. Selection of F_6 extremely tolerant progenies of the 'RSP' ×'MY11' hybrid was performed starting from the F_2 generation, from which random spikes were evaluated for PHS tolerance, and spikes with an extremely high level of tolerance to PHS were selected for further work. Threshed seeds from these spikes were mixed and were advanced to F_3 and further to F_6 plants with similar selection for PHS tolerance after each propagation.

The parents, the bread wheat cultivar '88-1643', 'MY11' and the synthetic hexaploid wheat cultivar 'RSP' as well as their F_2 and F_6 progenies were grown in 2 m rows with a 10 cm distance between plants and 30 cm between rows. The synthetic hexaploid RSP was grown as a 10-row plot and other parents were grown as 4–6 row plots. The F_2 populations and the F_6 population were planted as a 40-row plot. The plant materials were planted in a field of Dujiangyan City, Sichuan, China. There was little rain and the mean day/night temperature was 28/18°C during the month of May (the month of harvest) for Dujiangyan.

Evaluation of PHS

Three intact uninfected spikes from each of the F_2 and F_6 plants were randomly selected at wax maturity when all traces of green had disappeared from the spikes. Twenty intact spikes for each parent were selected in the same way. Spikes were uniformly surrounded with filter paper soaked in distilled water and were incubated in an upright position in incubators at 25°C for 7 days, when the spikes were threshed and PHS response was assessed. A grain was considered germinated if the pericarp over the embryo ruptured (Lan et al. 1997).

DNA isolation from plant material and PCR amplification of SSR bands

DNA was isolated from leaves of field-grown plants using a modified CTAB method (Saghai-Maroof et al. 1984).

Simple sequence repeat (SSR) markers for chromosome 2D (Röder et al. 1998; Pestsova et al. 2000; Somers et al. 2004) were used in this study (*gwm, gdm* and *wmc*). A polymerase chain reaction (PCR) was performed in a 25-μL volume containing approximately 50–100 ng of genomic DNA, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1 unit *Taq* polymerase, 300 nM of each primer (TakaRa Biotechnology Co., Ltd). The thermocycling program consisted of an initial denaturation at 94°C for 3 min, followed by 40 cycles of 1 min at 94 °C, 1 min at 50–60 °C, 2 min at 72°C and a final step of 10 min at 72°C. PCR products were separated on a 3.0% agarose gel and visualized by staining with ethidium bromide.

QTL mapping

The linkage map was generated using MAPMARKER/EXP version 3.0b (Lander et al. 1987) with Kosambi's mapping function (Kosambi, 1944). The commands "group" and minimum LOD scores ≥ 3.0 were used to develop the linkage map. QTL analysis by Composite Interval Mapping (CIM) was performed using Windows QTL Cartographer version 2.5 (Wang et al. 2007). Another software package, QTLMapper version 2.0 (Wang et al. 1999), which detected QTLs by Mixed-model-based Composite Interval Mapping (MCIM), was used simultaneously. An LOD score of 3.0 was used to declare the presence of a putative QTL. Confidence intervals (CI) were obtained by marking positions ±1 LOD from the peak. The QTL for PHS tolerance detected in the present study was designated according to the standard nomenclature for QTLs as recommended for wheat.

Results

Evaluation of PHS

A large difference in germination rate between parental cultivars, 'RSP' and '88-1643', was detected in 2004 and 2005 (Figure 1). The tolerant parental cultivar, 'RSP', germinated slightly in 2004 (1.2% germination rate) and did not germinate under experimental conditions in 2005, while the susceptible cultivar, '88-1643', germinated strongly in 2004 at 58.0% and in 2005 at 41.0%. Germination rates at day 7 of the germination test among F_2 populations ranged from 0 to 97.3% in 2004 and 0 to 90.3% in 2005. Germination rates for F_2 populations showed a skewed distribution, biased to the most tolerant class (Figure 1).

Segregation distortion of molecular markers analysis in the extreme population

Twenty plants of F_2 progeny of the 'RSP'× '88-1643' population with germination rates higher than that of cultivar '88-1643', the PHS susceptible parent, represented the extremely susceptible groups in 2004 and 2005. These extreme groups were analyzed by using 12 SSR primer sets (Table 1). In 2004 there were significant segregation distortions at a probability level of 0.05 from

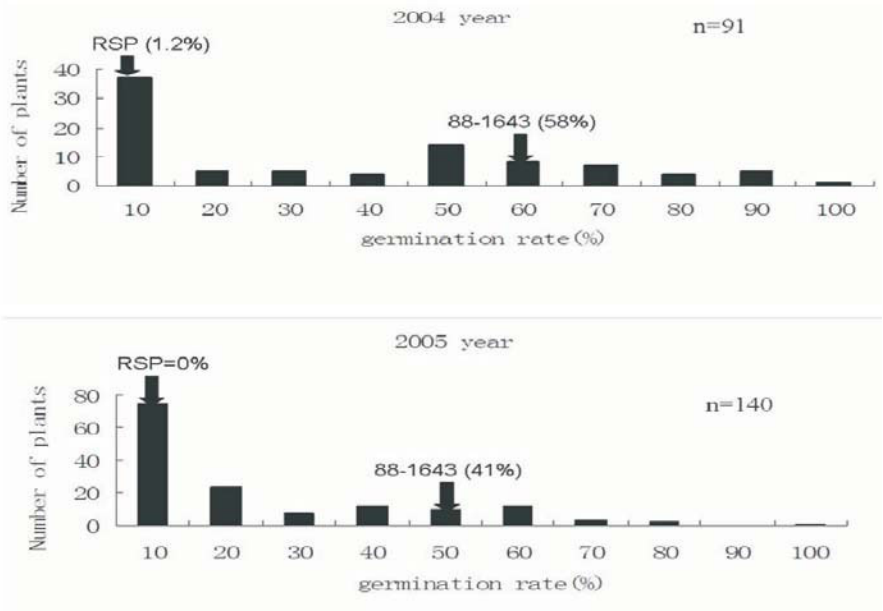

Figure 1. Frequency distributions for germination percentages during 7 days in populations consisting of F_2 plants from a cross between a synthetic hexaploid PHS tolerant wheat cultivar 'RSP' and a PHS susceptible bread wheat cultivar '88-1643'. Germination rates at day 7 of germination test ranged from 0 to 97.3% among 91 F_2 plants in 2004 and 0 to 90.3% among 140 F_2 plants in 2005.

the expected ratios 1:2:1 or 3:1 for markers *Xgdm35* and *Xgwm484*, and at a probability level of 0.01 for markers *Xgdm5* and *Xgwm26*. In 2005 significant segregation distortions were detected for *Xgwm455*, *wmc503*, *Xgwm261*, *wmc112* and *Xgwm484* markers at the 0.01 probability level (Table 2). All of markers showing segregation distortion were located on the short arm of chromosome 2D (2DS) (Röder et al. 1998; Pestsova et al. 2000; Somers et al. 2004; http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker). Molecular markers on 2DS exhibited distorted segregation within the selected extremely susceptible groups, suggesting that there was a significant QTL for PHS tolerance on 2DS.

Genetic map of 2DS and QTL analysis

To identify QTLs for PHS tolerance, six SSR markers (*Xgdm35*, *Xgdm5*, *Xgwm261*, *Xgwm30*, *Xgwm102*, *Xgwm484*) in 2004 and seven SSR markers (*Xgwm30*, *wmc503*, *wmc112*, *Xgwm48*, *Xgwm102*, *Xgwm455, Xgwm261*) in 2005 were employed to construct an SSR-based genetic map of 2DS. The CIM conducted for both mapping populations suggested the presence of a significant QTL on 2DS. The QTL designated *Qphs.sau-2D* was identified within the marker intervals *Xgwm261-Xgwm484* in 2004 and *wmc112-Xgwm484* in 2005, respectively. The LOD scores were 5.21 in 2004 and 11.66 in 2005 (Table 3 and Figure 2), which were above the LOD score for declaring the presence of a putative QTL. Negative values of the additive effect of the QTL in the two years showed that the allele of the synthetic hexaploid wheat 'RSP' contributed to a reduction of the germination rate. The data were also subjected to QTL analysis using QTLMapper version 2.0 (based on mixed linear models). One putative QTL associated with PHS tolerance was also identified in both years (2004 and 2005) in that region using QTL Cartographer (Table 3 and Figure 2).

The phenotypic variation explained by the detected QTL is about 25% (25.73% in 2004 as calculated using the CIM algorithm and 27.50% in 2005 using MCIM). Confidence intervals based on the LOD-drop-off method ranged from 9 (2005 CIM) to 15.4 cM (2004 MCIM), which were almost completely within marker intervals *Xgwm261-Xgwm484* (Figure 2).

Validation of QTL for breeding aimed at the improvement of PHS tolerance

The two markers *Xgwm261* and *Xgwm484*, which flanked the QTL on 2DS, were used for its validation on 48 PHS tolerant plants representing F_6 progeny of the cross between the PHS tolerant cultivar 'RSP' and the susceptible bread wheat cultivar 'MY11'. There were 42 homo- and 6 heterozygotes detected with the *Xgwm261* marker and 45 homozygotes and 3 heterozygotes detected with the *Xgwm484* marker. The *Xgwm261* marker amplified the DNA product specific for the tolerant parent, the 'RSP' cultivar, in 40 (83.3%) and *Xgwm484* in 34 PHS tolerant plants (70.8%) of F_6 progeny from the cross 'RSP' x 'MY11' (Table 4 and Figure 3). Both *Xgwm261* and *Xgwm484* amplified the product specific for the 'RSP' PHS resistant, parental cultivar in 31 F_6 PHS tolerant plants (64.4%). On the other hand, none of the

Table 1. A list of molecular markers used for mapping QTLs for pre-harvest sprouting tolerance on chromosome 2D in a synthetic hexaploid wheat × common wheat cross. Information about probe name, locus name, locus chromosome and breakpoint interval on chromosome 2D – Röder et al. 1998; Pestsova et al. 2000; Somers et al. 2004; http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker.

Probe name	Locus name	Locus chromosome	Breakpoint interval on chromosome 2D
GDM35	Xgdm35	2D	2DS5-0.47-1.00
WMS455	Xgwm455	2D, 6B	2DS5-0.47-1.00
GDM5	Xgdm5	2D, 2A	2DS
WMC503	Xwmc503	2D	2DS
WMS261	Xgwm261	2D	C-2DS1-0.33
WMC112	Xwmc112	2D	2DS
WMS484	Xgwm484	2D	C-2DS1-0.33
WMS102	Xgwm102	2D	2DS1-0.33-0.47
WMS30	Xgwm30	2D, 2A, 3A, 4A	C-2DL3-0.49
WMS157	Xgwm157	2D	2DL3-0.49-0.76
WMS539	Xgwm539	2D	C-2DL3-0.49
WMS349	Xgwm349	2D	C-2DL3-0.49

Table 2. Segregation distortion of molecular markers analysis in extremely susceptible population of F_2 progeny generated from the cross of PHS tolerant synthetic hexaploid wheat cultivar 'RSP' and PHS susceptible bread wheat cultivar '88-1643'

2004					Marker	2005				
AA[b]	aa[b]	Aa[b]	a_[b]	χ^2		χ^2	AA[b]	aa[b]	Aa[b]	a_[b]
1	9	10		6.40*	*Xgdm35*					
					Xgwm455	13.90**	1	12	7	
0	10	10		10.00**	*Xgdm5*					
					Xwmc503	13.90**	1	12	7	
0	10	10		10.00**	*Xgwm261*	13.90**	1	12	7	
					Xwmc112	17.60**	1	13	6	
0	9	11		8.30*	*Xgwm484*	17.60**	1	13	6	
2			18	2.40	*Xgwm102*	2.40	2			18
3			17	1.06	*Xgwm30*	0.2667	4			16
					Xgwm157	0	5	5	10	
4	5	11		0.3	*Xgwm539*	0	5	5	10	
					Xgwm349	0	5			15

* and **, significant at the 0.05 and 0.01 level, respectively; Chi-square (χ^2) values in 2004 and 2005 are shown on the left and right of the marker column, respectively. Goodness of fit (χ^2) of ratios of different markers in F_2 population to that expected for a single gene (1:2:1 or 3:1); [b] Represents marker genotypes, AA for 'RSP', aa for '88-1643', Aa for heterozygosity, and a_ for '88-1643' or heterozygosity

PHS tolerant plants gave both *Xgwm261* and *Xgwm484* amplification products specific for the susceptible parent 'MY11' (Table 4 and Figure 3). It suggests that the RSP allele of *Qphs-sau.2D* may increase the PHS tolerance in different genetic backgrounds.

Discussion

The present study indicates that the molecular markers putatively linked to the PHS locus may be identified by distorted segregation within the selected extreme subpopulations. The segregation distortion and QTL analysis gave similar results. Genotype evaluation based on sprouting phenotype was valid in the population extremely susceptible to PHS, not containing a significant tolerance gene for PHS.

In the present study, the significant QTL for PHS tolerance located on 2DS was detected in the marker interval *Xgwm261-Xgwm484.* The *Xgwm261* and *Xgwm484* markers were located on the chromosome region between the centromere and breakpoint of 2DS1-0.33 (data on website: .gov/ggpages/SSRclub/GeneticPhysical). Flanking markers near the QTL could be assigned to the C-2DS1-0.33 chromosome bin, suggesting that the gene(s) controlling PHS tolerance is located in this chromosome region (Figure 4). The QTL for PHS tolerance on 2DS was detected in the International Triticeae Mapping Initiative population (ITMI*pop*), which was a hybrid between a synthetic wheat cultivar 'W7984' and a Mexican wheat variety 'Opata85' (Kuwal et al. 2004). The QTL identified in the present study seems to be consistent with the previous location of the PHS tolerance locus, because both QTLs are linked with common SSR markers,

Table 3. A comparison of mapping results obtained using Composite Interval Mapping (CIM) and Mixed-model-based Composite Interval Mapping (MCIM) in 2004 and 2005. (LOD log-likelihood, CI confidence interval, Add additive effect, R^2 phenotypic variation explained by individual QTLs)

Year	Method	Marker interval	Position	LOD value	CI (cM)*	Add	R%
2004	CIM	Xgwm261-Xgwm484	14.7cM	5.21	14.3–27.7	–21.63	25.73
	MCIM	Xgwm261-Xgwm484	19.7cM	6.64	11.3–26.7	–22.08	26.95
2005	CIM	wmc112-Xgwm484	21.2cM	11.66	16.2–25.2	–17.84	26.67
	MCIM	wmc112-Xgwm484	18.2cM	10.28	11.9–24.2	–17.46	27.50

*Confidence intervals were obtained by marking positions ± 1 LOD from the peak

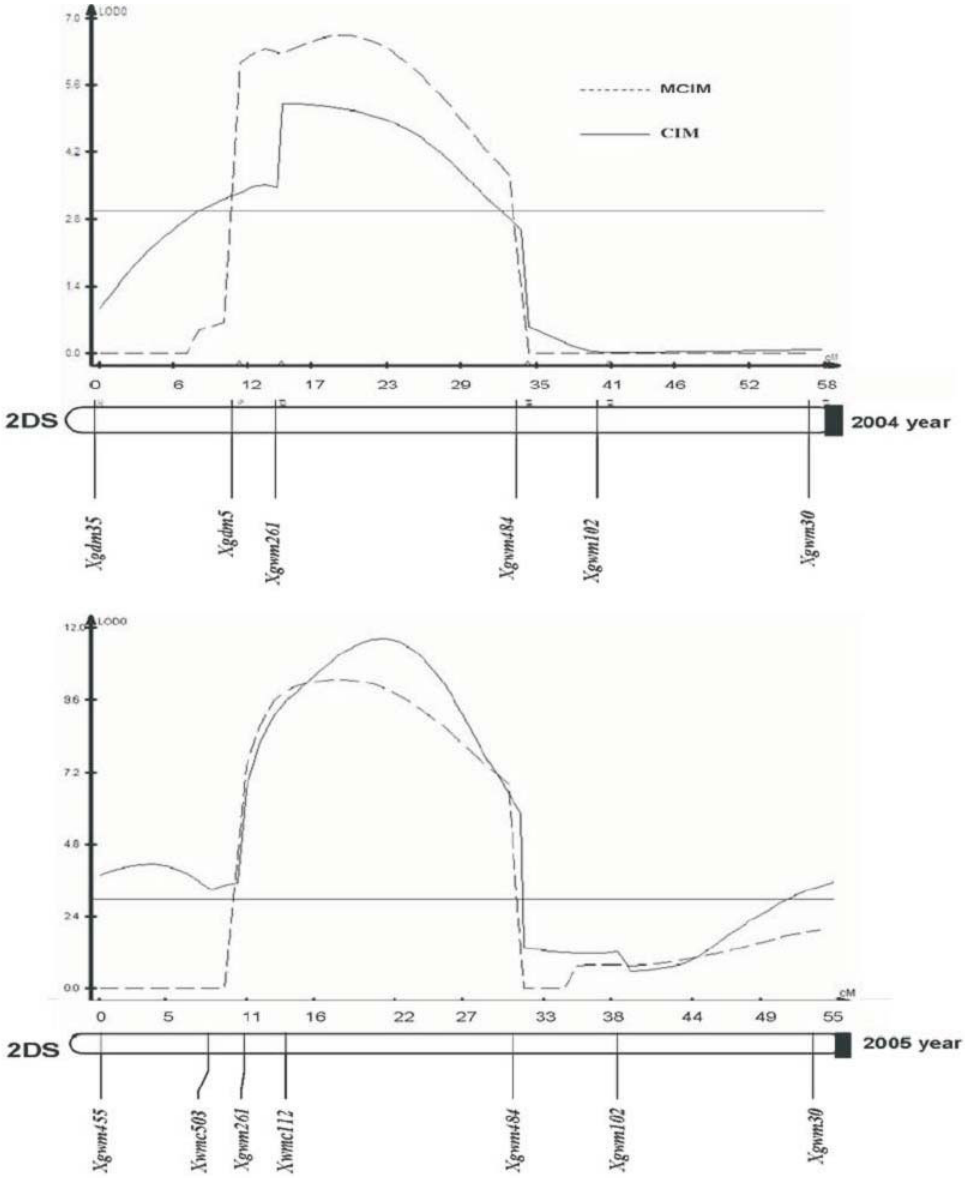

Figure 2. Genetic maps of chromosome 2DS generated on the basis of data from the years 2004 and 2005, and QTL-Likelihood curves of LOD scores showing locations of QTL for PHS tolerance for 2 years.

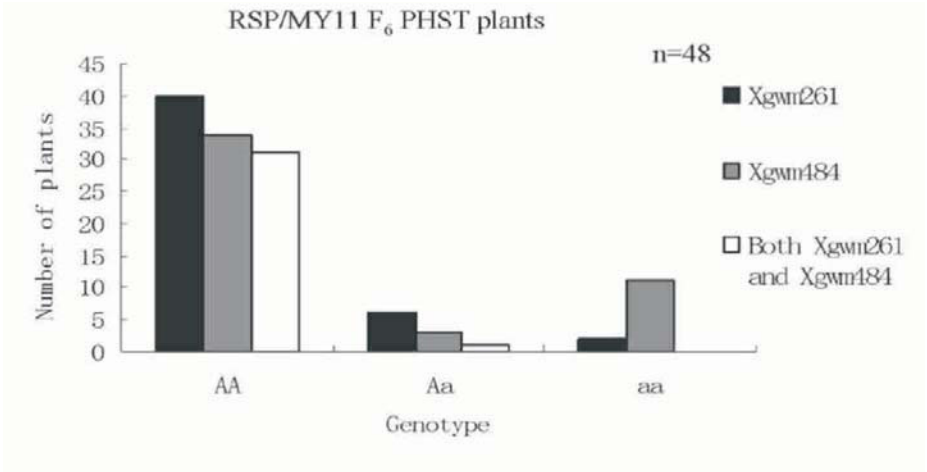

Figure 3. Frequency distributions of genotypes according to flanking markers, *Xgwm261* and *Xgwm484*, in the population consisting of 48 PHS tolerant plants representing F_6 progeny of the cross between a PHS tolerant cultivar 'RSP' and a susceptible bread wheat cultivar 'MY11'. Represents marker genotypes, AA for 'RSP', aa for 'MY11', and Aa for heterozygosity

Xgwm261 and *Xgwm484* (Figure 4). In other studies on common wheat, chromosome 2D was also related to dormancy or PHS tolerance. Anderson et al. (1993) identified one QTL for PHS tolerance that was linked to the RFLP marker *Xcdo64*, located on the chromosome region between the centromere and breakpoint of 2DS-3 (http://wheat.pw.usda.gov/cgi-bin/graingenes/reort.cgi?class=marker&name=*Xcdo64*). Noda et al. (2002) reported that 2DS appeared to carry major gene(s) for germination using ditelocentric lines of common wheat cv. Chinese Spring (CS). Miura et al. (2002) detected a smaller effect of 2D on dormancy in a PHS tolerant common wheat cultivar 'Zenkojikomugi' (Zen) using the backcross reciprocal monosomic method.

It is well-known that other chromosomes also carry major QTLs/genes for PHS/dormancy in common wheat. The QTLs for PHS/dormancy detected on the long arms of group 3 chromosomes have been reported to be linked with *taVP1* genes, that are orthologous to the maize *VP1* gene, which encoded a dormancy-related transcription and/or *R* genes for seed-coat color in several earlier stud-

Table 4. Validation of QTL for breeding aimed at improvement of PHS tolerance. The two markers *Xgwm261* and *Xgwm484*, which flanked *Qphs-sau.2D*, were used on 48 PHS tolerant plants representing F_6 progeny of the cross between PHS tolerant cultivar 'RSP' and susceptible bread wheat cultivar 'MY11'.

No.	*Xgwm261*	*Xgwm484*	No.	*Xgwm261*	*Xgwm484*	No.	*Xgwm261*	*Xgwm484*	No.	*Xgwm261*	*Xgwm484*
1	AA*	AA	13	AA	aa	25	AA	AA	37	AA	AA
2	AA	AA	14	AA	AA	26	AA	aa	38	aa	AA
3	AA	Aa*	15	AA	AA	27	AA	AA	39	AA	aa
4	AA	AA	16	aa	AA	28	AA	AA	40	AA	AA
5	AA	AA	17	AA	AA	29	AA	AA	41	AA	Aa
6	Aa	aa*	18	AA	aa	30	Aa	aa	42	AA	AA
7	AA	AA	19	AA	AA	31	AA	AA	43	AA	AA
8	AA	AA	20	AA	AA	32	AA	AA	44	AA	AA
9	AA	AA	21	AA	AA	33	AA	AA	45	AA	aa
10	AA	aa	22	AA	AA	34	AA	AA	46	Aa	AA
11	AA	aa	23	Aa	Aa	35	Aa	aa	47	AA	AA
12	AA	AA	24	Aa	aa	36	AA	AA	48	AA	AA

* Represents marker genotypes, AA for 'RSP', aa for 'MY11', and Aa for heterozygosity

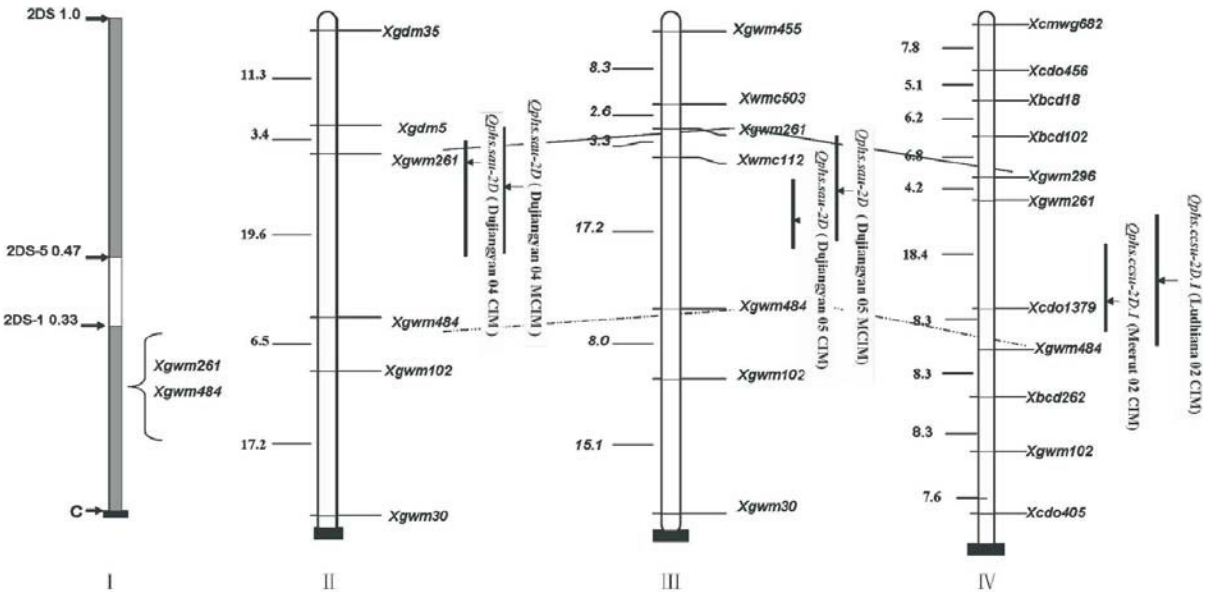

Figure 4. A comparison of genetic maps of the short arm of chromosome 2D generated in 2004 and 2005 (map II and map III), aligned with the 2DS framework map (map IV International Triticeae Mapping Initiative population (ITMI*pop*) (Kulwal et al. 2004)) with the physical map based on the deletion bin system (Sourdille et al. 2004; http://wheat.pw.usda. gov/ggpages/SSRclub/GeneticPhysical). Vertical bars and arrows indicated QTLs for pre-harvest sprouting tolerance (II–IV), vertical bars for confidence intervals and arrows for QTL peaks. Environment and year in which the QTL was detected as well as the method by which the QTL was detected are given in brackets. (Meerut and Ludhiana were Indian cities, where mapping populations were grown by Kulwal in 2002).

ies (Bailey et al. 1999; Flintham et al. 2000; Warner et al. 2000; Watanabe and Ikebata 2000; Groos et al. 2002; Kuwal et al. 2004). In other studies some important QTLs for PHS/dormancy were not related with the *taVP1* and/or *R* genes. A major QTL for PHS/dormancy was detected on the short arm of chromosome 3A, which suggested that it might be associated with the sensitivity of embryos to abscisic acid (ABA) (Osa et al. 2003; Mori et al. 2005). The long arm of chromosome 4A (4AL) carried at least two QTLs for PHS/dormancy. Most studies showed that a QTL for PHS/dormancy considered to be homologous to the barley gene *SD4* was located in the chromosome region between the breakpoints of 4AL-13 0.59 and 4AL-5 0.66 (Kato et al. 2001; Flintham et al. 2002; Mori et al. 2005; Torada et al. 2005). Some authors suggest that a QTL for PHS tolerance was located in the terminal region of 4AL (Anderson et al. 1993; Noda et al. 2002).

Conclusions

The present study is the first attempt indicating a significant QTL controlling PHS tolerance on the short arm of chromosome 2D. The QTL could be assigned to the C-2DS1-0.33 chromosome region. Flanking molecular markers may be used for marker-aided selection (MAS) for the improvement of PHS tolerance.

Acknowledgements. The authors are thankful to the National Natural Science Foundation of China (30471088, 30370883), the National High Technology Research and Development Program of China (863 program 2006AA10Z1F8), Changjiang Scholars and Innovative Research Team in University (PCSIRT), China for their financial supports.

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