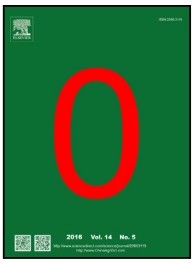
Journal of Integrative Agriculture 2016, 15(0): 60345-7

Available online at www.sciencedirect.com

ScienceDirect



RESEARCH ARTICLE

QTL mapping revealed *TaVp-1A* conferred pre-harvest sprouting resistance in wheat population Yanda 1817×Beinong 6

ZHOU Sheng-hui[1], FU Lin[1], WU Qiu-hong[2], CHEN Jiao-jiao[1], CHEN Yong-xing[1], XIE Jing-zhong[1], WANG Zhen-zhong[3], WANG Guo-xin[1], ZHANG De-yun[1], LIANG Yong[1], ZHANG Yan[1], YOU Ming-shan[1], LIANG Rong-qi[1], HAN Jun[4], LIU Zhi-yong[1, 2]

[1] *State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100193, P.R.China*
[2] *Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, P.R.China*
[3] *China Rural Technology Development Center, Beijing 100045, P.R.China*
[4] *College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, P.R.China*

Abstract

Pre-harvest sprouting (PHS) occurs frequently in most of the wheat cultivation area worldwide, which severely reduces yield and end-use quality, resulting in substantial economic loss. In this study, quantitative trait loci (QTL) for PHS resistance were mapped using an available high-density single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) genetic linkage map developed from a 269 recombinant inbred lines (RILs) population of Yanda 1817×Beinong 6. Using phenotypic data on two locations (Beijing and Shijiazhuang, China) in two years (2012 and 2013 harvesting seasons), five QTLs, designated as *QPhs.cau-3A.1*, *QPhs.cau-3A.2*, *QPhs.cau-5B*, *QPhs.cau-4A*, and *QPhs.cau-6A*, for PHS (GP) were detected by inclusive composite interval mapping (ICIM) (LOD≥2.5). Two major QTLs, *QPhs.cau-3A.2* and *QPhs.cau-5B,* were mapped on 3AL and 5BS chromosome arms, explaining 6.29–21.65% and 4.36–5.94% of the phenotypic variance, respectively. Precise mapping and comparative genomic analysis revealed that the *TaVp-1A* flanking region on 3AL is responsible for *QPhs.cau-3A.2*. SNP markers flanking *QPhs.cau-3A.2* genomic region were developed and could be used for introgression of PHS tolerance into high yielding wheat varieties through marker-assisted selection (MAS).

Keywords: wheat, pre-harvest sprouting, quantitative trait loci, SNP, *TaVp-1A*

1. Introduction

Pre-harvest sprouting (PHS) is a phenomenon that germination of grains occurred in the spikes before harvesting under humid and wet weather conditions. This situation in cereals negatively affects yield and end-use quality (Wahl and Orourke 1993) and remains a major concern in all wheat-growing regions of the world (Cowan 2002). Improving PHS resistance is one of the most important objectives in wheat breeding program (Zanetti *et al*. 2000; Li *et al*. 2004). However, breeding for PHS resistance is difficult for its quantitatively inheritance affected by genetic and environmental factors. Dormancy is one of the most dramatic factors related to PHS resistance (Flintham *et al*. 2002; Kulwal *et al*. 2004). Higher grain dormancy usually related to PHS resistance. However, contribution of grain dormancy to total PHS resistance in the field remains un-

Received 1 February, 2016 Accepted 21 March, 2016
ZHOU Sheng-hui, E-mail: zhoushenghui826@gmail.com
Correspondence LIU Zhi-yong, Tel: +86-10-64806422, E-mail: zyliu@genetics.ac.cn

doi: 10.1016/S2095-3119(16)61361-8

known. In addition, it has been found that PHS has a close relationship with wheat seed coat color. Red wheat tends to be more resistant to PHS than white wheat (Flintham 2000; Groos *et al*. 2002). Moreover, spike morphology (King and Richards 1984), physical barriers to water (Gale 1989) and environmental factors such as moisture and temperature (Ceccato *et al*. 2011) also have been considered to contribute to overall PHS resistance.

A number of QTL or genes related to PHS, dormancy and grain color have been identified and used for marker-assisted selection (MAS) of PHS resistance wheat varieties. Many PHS resistant QTLs have been identified in wheat (McIntosh *et al*. 2013). Several QTLs related to PHS were found on homoeologous group 3 chromosomes (Mori *et al*. 2005; Liu *et al*. 2008; Kumar *et al*. 2009; Mohan *et al*. 2009; Nakamura *et al*. 2011). Among them, major QTLs *QPhs.ocs-3A.1* (Mori *et al*. 2005) and *Qphs.pseru-3AS* (Liu *et al*. 2008) for PHS resistance were mapped on chromosome 3AS, respectively. Using reverse genetics approach, Nakamura *et al*. (2011) found *TaMFT*, an ortholog of *MOTHER OF FT AND TFL1* (*MFT*) in the *Arabidopsis*, was responsible for *QPhs.ocs-3A.1*. Subsequently, Liu *et al*. (2013) also isolated *Qphs.pseru-3AS* (*TaPHS1*) using comparative genetics mapping and map-based cloning approaches, and found *TaPHS1* is same as *TaMFT*. QTL for grain color were found to be co-located with QTL for PHS. Fofana *et al*. (2009) and Groos *et al*. (2002) identified 7 and 5 QTLs for seed coat color that were co-incident with PHS traits on chromosomes 3A, 3B and 3D, respectively.

Genome sequence information for wheat (International Wheat Genome Sequencing Consortium), *Brachypodium* (IBI 2010), rice (IRGSP 2005), and sorghum (Paterson *et al*. 2009) facilitated comparative genetic mapping genes/QTLs responsible for important quantitative traits in wheat. The *Brachypodium*, rice and sorghum genes found in syntenic conserved regions might be beneficial to comparative mapping and cloning genes from wheat genome (Wu *et al*. 2013; Wang *et al*. 2014).

Viviparous-1 (*VP-1*) encodes a dormancy related transcription factor in maize (McCarty *et al*. 1991) and plays important roles in seed maturation processes in wheat (Nakamura and Toyama 2001). Three homologs of *Vp-1*, *TaVp-1A*, *TaVp-1B*, and *TaVp-1D* were identified in wheat chromosomes 3AL, 3BL and 3DL, respectively. Genetic mapping revealed that the allelic variations of *TaVp-1B* were associated with PHS tolerance (Chang *et al*. 2010). Allelic variation analysis suggested the *TaVp-1A* locus is also related to PHS tolerance (Chang *et al*. 2011; Yang *et al*. 2014). However, no allelic variation of *TaVp-1D* was found in wheat (Yang *et al*. 2014).

In the present study, we report the QTL mapping of PHS tolerance using an integrated high-density single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) genetic linkage map and a RIL population derived from the cross between landrace Yanda 1817 (white-grained; PHS susceptible) and semi-dwarf high-yielding line Beinong 6 (red-grained; PHS tolerant). Precise mapping and comparative genomics analysis indicated that *TaVp-1A* was a candidate for the major QTL *QPhs.cau-3A.2*.

2. Materials and methods

2.1. Plant materials and field experiments

A mapping population consisting of 269 RILs derived from a cross Yanda 1817×Beinong 6, and a high-density genetic linkage map with a total length of 3 213.2 cM and an averaged distance of 1.26 cM between markers (Wu *et al*. 2015) were used for this study. The parents and their RILs were grown in two-row plots 2 m in length with 25 cm between rows. A randomized complete block (RCB) design with three replicates were used at Beijing (Beijing, 116.10°E, 40.08°N) and Shijiazhuang (Hebei, 114.36°E, 37.38°N) in 2012 and 2013 at three environments for PHS evaluations, *viz*., Beijing 2012, Beijing 2013 and Shijiazhuang 2013.

2.2. Evaluation of PHS

Ten intact spikes from each of the RILs were harvested at the dough-yellow ripping stage, surface-sterilised with 5% (v/v) NaClO for 10 min, and then immersed in distilled water for 8 h after naturally air dried for 5 d. The 10 wet spikes were placed upright in a chamber at room temperature (26±0.5)°C. After 5 d, the numbers of germinated and non-germinated kernels in each spike were counted. Percentage of germinated kernels (GP) was used as phenotypic data for PHS QTL analysis.

2.3. Statistical analysis and QTL analysis

Mean PHS was calculated for each RIL. Heritability on an entry mean basis was calculated using the mixed model analysis (Holland *et al*. 2003). QTL analysis was carried out by inclusive composite interval mapping using the program IciMapping 3.2/4.0 based on stepwise regression of simultaneous consideration of all marker information (http://www.isbreeding.net/). The 'Deletion' command was used to accommodate the missing phenotypes and the step size chosen was 1.0 cM. A threshold was considered significant when the LOD score of QTL detection was greater than 2.5.

2.4. DNA isolation

From each lines (Yanda 1817, Beinong 6 and 269 RILs), 2–3

leaves were harvested, lyophilized, ground, and stored at –20°C in individually labeled vials. Genomic DNA isolation was performed using the cetyltrimethyl ammonium bromide (CTAB) method (Allen *et al*. 2006). DNA was quantified using 1% agarose gel electrophoresis with λ DNA as the standard. All molecular markers and PCR amplification of *TaVp-1A* systems were assayed using this genomic DNA source.

2.5. SSR and STS markers development and precise mapping QTL on 3AL

To precise mapping the QTL on 3AL, the SNPs linked to the QTL were converted to SSR and sequence-tagged site (STS) markers and re-mapped in the RIL population. The SNPs and its flanking sequences were used to search the IWGSC Chinese Spring survey sequences (http://www.wheatgenome.org/) to find homologous contigs for polymorphic marker development. These contigs were firstly used to screen SSR motifs using BatchPrimer3 (You *et al*. 2008). If no SSR polymorphisms were detected between the parental lines, the Chinese Spring orthologous contigs were used to design STS primer pairs to identify length polymorphisms using DNAMAN software with the following parameters: amplification product size of 200–800 bp with the optimum 500 bp, primer length of 18–22 bp, Tm of 55–65°C, GC content of 40–60%. The PCR amplification was carried out with an ABI9700 in 10 μL reaction mixtures containing 10 mmol L^{-1} Tris-Hcl, pH 7.5, 50 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTP, 25 ng of each primer, 0.75 U of *Taq* polymerase, and 50 ng of genomic DNA as the template. After an initial denaturing step for 5 min at 94°C, 35 cycles were performed for 45 s at 94°C, 55–60°C (depending on the specific primers) for 45 s, and 72°C for 70 s, with a final extension at 72°C for 10 min. PCR products were separated in 8% non-denaturing polyacrylamide gels, visualized by silver staining and photographed. To integrate polymorphic markers with previous genetic linkage map, Mapmaker/Exp ver. 3.0 (Incoln and Ander 1993) with a minimum LOD of 3.0 and maximum recombination fraction of 0.372 was used.

2.6. Comparative genomic analysis

The polymorphic SNP markers or corresponding contigs sequences flanking *QPhs.cau-3A.2* were used to perform a BIASTN (evalue 1e-10) search against the genome sequence databases of *Brachypodium* (http://mips.helmholtz-muenchen.de/plant/Brachypodium/), rice (http://rice.plantbiology.msu.edu/) and sorghum (http://mips.helmholtz-muenchen.de/plant/sorghum/) to identify orthologous gene pairs.

2.7. Allelic variation of *TaVp-1A* between Yanda 1817 and Beinong 6

Four pairs of primers were used to amplify the *TaVp-1A* gene (Sun *et al*. 2012). The PCR products were sequenced from both strands with three repetitions by TSINGKE Biological Technology Co. Ltd. (China, http://www.tsingke.net/). Sequence analysis and characterization were performed using software DNAMAN. Moreover, the *TaVp-1A* allele of Soleil (AJ400712, McKibbin *et al*. 2002) deposited in GenBank was used as a reference sequence.

3. Results

3.1. Percentage of germinated kernels (GP) in parents and RILs

The PHS (GP) was phenotyped in three environments at Beijing and Shijiazhuang, China, in 2012 and 2013. Significant differences of GP were found between the parental line Yanda 1817 (25.21 to 64.65%) and Beinong 6 (7.16 to 43.43%). Continuous variations and transgressive segregation were observed for GP frequency distributions in the RIL population (Table 1, Fig. 1). The heritability frequency for GP was 55.78% across the three environments, indicating that the genetic influence on PHS is moderate (Table 1).

3.2. QTL mapping for GP

Five QTLs, *QPhs.cau-3A.1*, *QPhs.cau-3A.2*, *QPhs.cau-5B*, *QPhs.cau-4A*, and *QPhs.cau-6A* (Table 2), for PHS (GP) were identified in the Yanda 1817×Beinong 6 RILs populations using an integrated high-density SNP and SSR genetic linkage map (Wu *et al*. 2015). A major QTL *QPhs.cau-3A.2*, mapped on 3AL and flanked by molecular markers *wsnp_Ex_c4923_8767234* and *wsnp_BQ160970A_Ta_2_7*, was consistently detected in all the three environments, and explained 6.29–21.65% of the phenotypic variances. The

Table 1 Phenotypic performance and distribution parameters of germinated kernels (GP) trait

Environment	Parents		RIL population				h^2 (%)
	Yanda 1817 (%)	Beinong 6 (%)	Mean (%)	SD	Max. (%)	Min.	
Beijing 2012	25.21	8.56	19.21	20.11	85.00	0	55.78
Beijing 2013	44.85	7.16	14.16	15.42	73.42	0	
Shijiazhuang 2013	64.65	43.43	36.18	24.77	88.54	0	

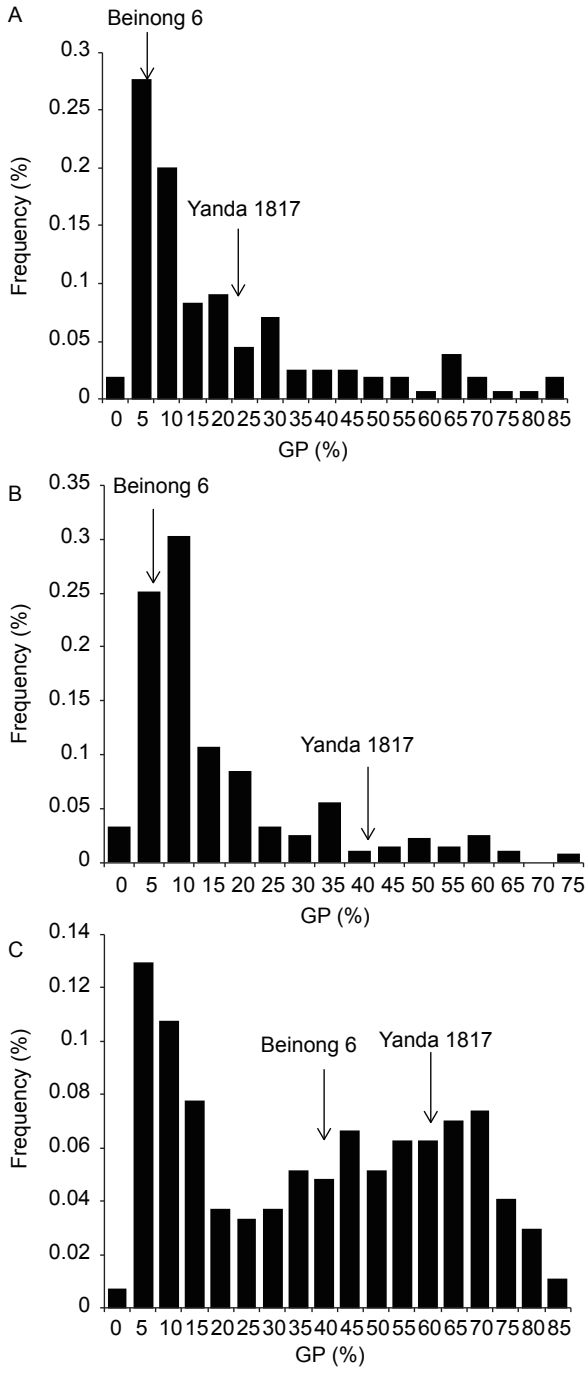


Fig. 1 Frequency distributions for germinated kernels (GP) of recombinant inbred lines (RILs) population derived from Yanda 1817×Beinong 6 in three environments. A, Beijing 2012. B, Beijing 2013. C, Shijiazhuang 2013.

positive additive effects of *QPhs.cau-3A.2* suggested the greater GP was contributed by the lower PHS resistance parental Yanda 1817 allele. Another QTL *QPhs.cau-5B* was mapped between *wsnp_Ex_c936_1797024* and *wsnp_Ex_c214_421541*, and accounted for 4.36 and 5.94% of the phenotypic variance in two environments. The high GP was also contributed by the allele derived from Yanda 1817. Three minor QTLs, *QPhs.cau-3A.1*, *QPhs.cau-6A* and *QPhs.cau-4A* were detected in only one environment. Under the condition of a single environment, the total phenotypic variations explained by all detected QTLs ranged from 18.52 to 33.61%.

3.3. Precise mapping of *QPhs.cau-3A.2* in 3AL

In order to determine the accurate location of *QPhs.cau-3A.2,* SNP markers flanking the *QPhs.cau-3A.2* locus on chromosome 3AL were converted into 15 STS markers (WGGC, Table 3) that can be easily used in MAS to improve PHS resistance. These STS markers were used to construct an integrated genetic linkage map of the *QPhs.cau-3A.2* (Fig. 2). The *QPhs.cau-3A.2* was mapped between *WGGC3260* and *Xcfd152* and explained 6.16–12.03% of the phenotypic variance in the three environments with positive additive effects of 7.34, 4.60 and 6.27, respectively (Table 4), suggesting the genetic interval is most like responsible for *QPhs.cau-3A.2*.

3.4. Comparative genomics analyses and candidate gene for *QPhs.cau-3A.2* in 3AL

The corresponding sequences of two STS markers *WGGC3241* and *WGGC3293* (Table 3) were used as queries to search orthologous regions from genome sequences of *Brachypodium*, rice and sorghum. Both *WGGC3241* and *WGGC3293* revealed orthologs on *Brachypodium* chromosome 2L (*BRADI2G56910* and *BRADI2G59480*) rice chromosome 1L (*Os01g0881500* and *Os01g0927600*) and sorghum chromosome 3L (*Sb03g041760* and *Sb03044630*), respectively (Fig. 2). Three genomic regions span 2.0, 2.4, and 2.2 Mb in *Brachypodium*, rice and sorghum, respectively. The orthologous genomic regions of *QPhs.cau-3A.2* around markers *WGGC3267* and *WWGC3264* in wheat were also identified in *Brachypodium*, rice, and sorghum. The genomics region *BRADI2G58277–BRADI2G58460* in *Brachypodium* showed high collinearity and share gene orders with rice and sorghum. Marker *WGGC3267* was developed from Chinese Spring contig2541758 and homologous to genomic sequence between genes *BRADI2G58360* and *BRADI2G58370* in *Brachypodium* (Fig. 2). Markers *WGGC3260* and *WGGC3264* were developed from Chinese Spring contig4251860 and are corresponding with *BRADI2G58360*, *Os01g0911100* and *Sb03g043450*, suggesting possible gene duplication in wheat. Marker *WGGC3249* was developed from SNP marker *wsnp_Ex_rep_c67588_66227926* and is corresponding with *BRADI2G58370*, *Os01g0911200* and *Sb03g043460*. Gene annotation revealed that *BRADI2G58390*, *Os01g0911700* and *Sb03g043480* are homologs of maize *Viviparous1* (*Vp-1*)

Table 2 Quantitative trait loci (QTL) for pre-harvest sprouting (PHS) detected in the Yanda 1817×Beinong 6 recombinant inbred lines (RILs) population

QTL	Environment	Left marker	Right marker	Position	LOD[1)]	PVE(%)[2)]	Add[3)]
QPhs.cau-3A.1	Beijing 2012	*wsnp_JG_c2509_1153697*	*Xgwm30*	46	4.64	11.96	–7.31
QPhs.cau-3A.2	Beijing 2012	*wsnp_Ex_c4923_8767234*	*wsnp_Ra_c19079_28210937*	107	8.06	21.65	9.82
	Beijing 2013	*wsnp_Ex_rep_c67588_66227926*	*wsnp_BQ160970A_Ta_2_7*	125	5.24	7.85	4.40
	Shijiazhuang 2013	*wsnp_Ex_c5623_9891427*	*wsnp_Ex_rep_c67588_66227926*	124	4.16	6.29	6.33
QPhs.cau-4A	Shijiazhuang 2013	*wsnp_Ex_c12812_20324273*	*wsnp_Ex_c12812_20324622*	90	4.31	6.29	6.33
QPhs.cau-5B	Beijing 2013	*wsnp_Ex_c936_1797024*	*wsnp_Ku_c6125_10773757*	49	3.25	4.36	4.90
	Shijiazhuang 2013	*wsnp_CAP8_c1210_739365*	*wsnp_Ex_c214_421541*	50	4.02	5.94	6.14
QPhs.cau-6A	Beijing 2013	*wsnp_Ku_rep_c68790_67934066*	*wsnp_Ex_c31149_39976103*	1	3.96	5.73	3.75

[1)] LOD score from the location with the underlined *P*-value.
[2)] PVE (%), phenotypic variance estimated from marker regression against phenotype.
[3)] Add., additive effect. Positive values indicate a positive effect of Yanda 1817 alleles, whereas negative values indicate the contribution of the Beinong 6 allele.
The same as below.

Table 3 Polymorphic simple sequence repeat (SSR) and sequence-tagged site (STS) markers for precise mapping QTL on 3AL

Polymorphic markers	Forward primer	Reverse primer	SNP name	Chinese Spring contig (IWGSC)
WGGC3243	CAAAGAGCGAGGCATTTC	TGGGTGGTGGCTTATGTC	*wsnp_Ex_c5623_9891427*	contig4227543
WGGC3244	TGGGAGTGAAACAGGAAAC	GGCTTTGACATTGGACATC	*wsnp_Ex_c5623_9891427*	contig4227543
WGGC3241	ACCTGGCTGATTATTATCCG	TGTTTCCTGTTTCACTCCC	*wsnp_Ex_c5623_9891427*	contig4227543
WGGC3267	ACGCTTAGTTCACTGTTTCG	GTTTGGTTCGGGAAGAGA	*wsnp_Ex_c14400_22381548*	contig2541758
WGGC3260	GCTCAGGGATGATAAGTCG	TTACCGAAGAAGACAGCAAC	*wsnp_Ex_c14202_22145136*	contig4251860
WGGC3249	TCATCTCCACATCTGACCA	TTCTTTCTCAAATGGCACAG	*wsnp_Ex_rep_c67588_66227926*	contig2463602
WGGC3264	GTGGTAGATGTCATTTGGTTG	TCCTCCTCCTTTGTATTCG	*wsnp_Ex_c14202_22145136*	contig4251860
WGGC3291	CAGAGATGACCTTATCCAACC	AATGACCAGCGGCTTTAC	*wsnp_Ex_c14400_22381548*	contig2541758
WGGC3293	TCACTAATGGAAGTCAAGGG	GTCATCCCAACCTCGTAAA	*wsnp_CAP11_rep_c4226_1995152*	contig4027470
WGGC1674	AAGTTGAGATGGGAACGC	AAACACGATAAGAGCCTCG	*wsnp_Ex_c12341_19693090*	contig1871758
WGGC864	ATACATAGCAGTGCGGGA	TGGATGCGATTTGTGAAC	*wsnp_BQ160970A_Ta_2_7*	contig4444496
WGGC861	GTGTTGTTGGTCCCGTC	CCATTTGAACCTGAAGGG	*wsnp_BG262734A_Ta_2_3*	contig4414650
WGGC1688	TGATGGGTAAGACGCTTTC	CTGTTAGGCAGTGTTCCAAG	*wsnp_BG262734A_Ta_2_3*	contig4414650
WGGC862	TGTTCATTCACACGCCA	TTGTTGACACTGTTCCTTGA	*wsnp_BG262734A_Ta_2_3*	contig4414650
WGGC856	GCTCCTCTGTTCTTGACCTG	GTAATCTGTTGTCCCGTTTG	*wsnp_Ex_c15475_23757972*	contig4374263

and *Arabidopsis* ABSCISIC ACID INSENSITIVE (ABI) which are related to seed dormancy and PHS resistance (Table 5), suggesting their ortholog in the corresponding wheat genomic region could be served as candidate of *QPhs.cau-3A.2* (Fig. 2).

3.5. Allelic variation of *TaVp-1A* between Yanda 1817 and Beinong 6 and polymorphism marker development

To characterize the sequence variations of *TaVp-1A* between the parental line Yanda 1817 and Beinong 6, four primer pairs (Sun *et al*. 2012) were used to amplify the whole length genomic sequences of *TaVp-1A*. Sequence polymorphisms between Yanda 1817 and Beinong 6 were found only in the 3rd and 4th introns amplified by primer set *Vp-1AF2/R2*. Compared with the reference allele *Vp-1Aa* in Soleil (AJ400712, McKibbin *et al*. 2002), Yanda 1817 and Beinong 6 had 21 and 9 TTC repeats in the third intron, respectively (Table 6, Fig. 3). Meanwhile, Beinong 6 had a C deletion at position 2 718 bp, a C insertion at position 2 858 bp, and a A/T SNP at positions 2 862 bp in the 3rd intron, and one SNPs (T/C at positions 3 004) in the 4th intron (Table 6, Fig. 3). Based on the sequence polymorphism, a *TaVp-1A* specific marker *WGGC180* was developed and co-segregated with markers *WGGC3260* and *WGGC3249* in the genetic linkage map (Fig. 2).

4. Discussion

PHS is a complex quantitative trait influenced by many genetic loci and environmental factors. Numbers of methods are available for assessment of PHS resistance (Hagemann and Ciha 1984; Depauw and Mccaig 1991; Xiao *et al*. 2012). In the present study, we used intact spikes and GP to evaluate the PHS resistance for its stable values in different

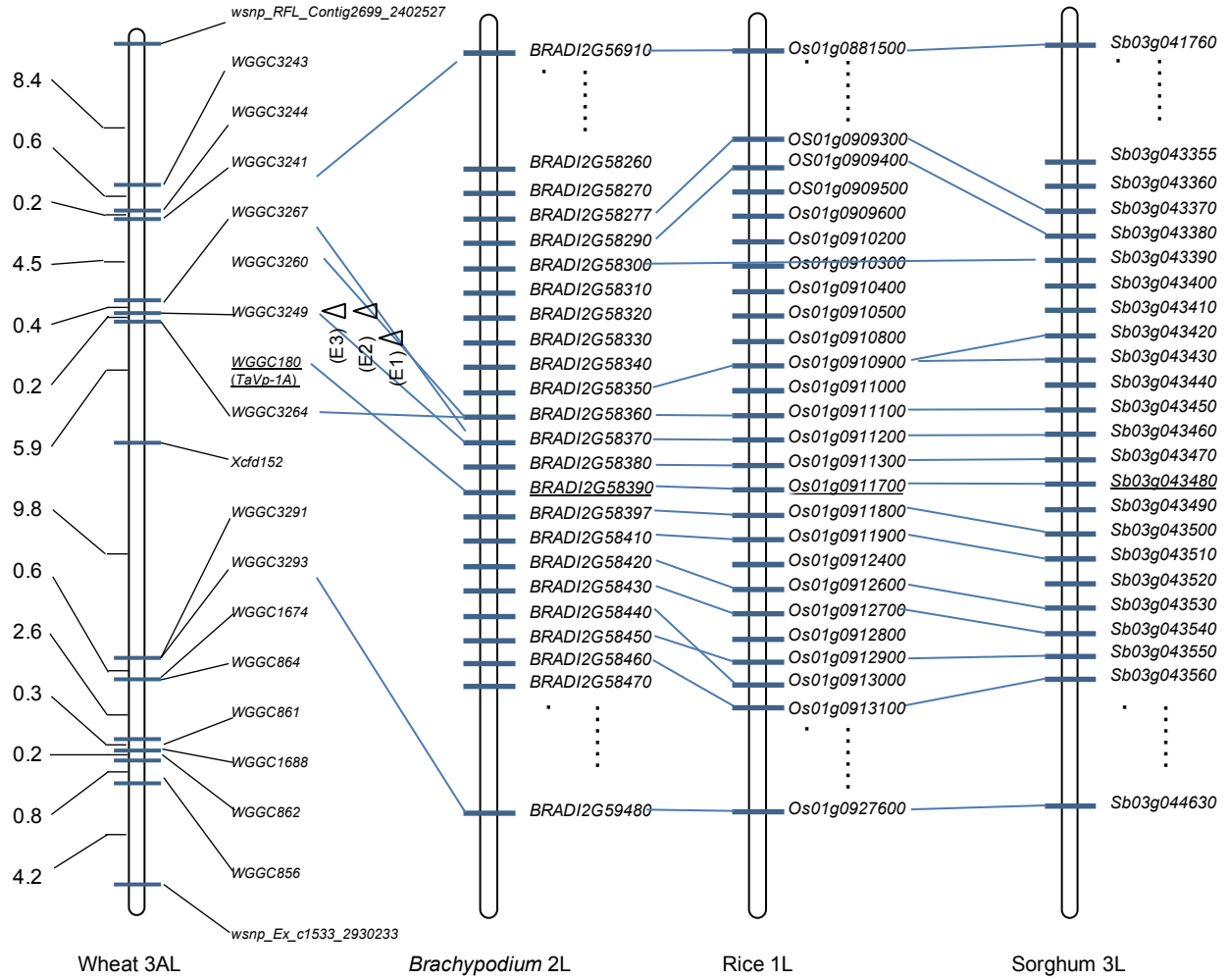


Fig. 2 Comparative genetic mapping of *QPhs.cau-3A.2*. E1, Beijing 2012; E2, Beijing 2013; E3, Shijiazhuang 2013. *TaVp-1A* and their orthologs are shown in blod underlined.

Table 4 Precise QTL mapping of *QPhs.cau-3A.2*

QTL	Environment	Left marker	Right marker	Position	LOD	PVE (%)	Add.
QPhs.cau-3A.2	Beijing 2012	*WGGC3264*	*Xcfd152*	128	4.43	12.03	7.34
	Beijing 2013	*WGGC3260*	*WGGC3264*	123	5.95	8.59	6.33
	Shijiazhuang 2013	*WGGC3260*	*WGGC3264*	123	4.25	6.16	6.27

environments. Significant variations in PHS resistance were observed for the parental lines Yanda 1817 and Beinong 6, as well as the RILs in two locations at different years (Table 1, Fig. 1), indicating significant environmental effects on PHS. However, the moderate heritability frequency of GP among the three environments revealed the contributions of genetic factors to PHS. The continuous distribution and transgressive segregation of GP in the RIL population (Table 1, Fig. 1) demonstrated high PHS resistance could be achieved by pyramiding multiple PHS resistance genes or QTL in wheat breeding program.

QPhs.cau-3A.1, *QPhs.cau-6A* and *QPhs.cau-4A* were identified only in one environment. *QPhs.cau-3A.1* was mapped on the terminal region of the short arm of chromosome 3A and explained up to 11.96% of the phenotypic variation at Beijing in 2012. Based on its mapping position, *QPhs.cau-3A.1* is likely to be the same locus as *Qphs.pseru-3AS* (Liu *et al*. 2008; Liu and Bai 2010), *QPhs.ocs-3A.1* (Mori *et al*. 2005) and *TaMFT* (Nakamura *et al*. 2011).

Two major QTLs for PHS, *QPhs.cau-3A.2* and *QPhs.cau-5B*, were consistently detected in at least two environmental conditions in this study. The QTL *QPhs.cau-5B*, flanked by SNP markers *wsnp_Ex_c936_1797024* and *wsnp_Ex_c214_421541* on 5BS (Table 2), was detected

Table 5 Gene annotation of the collinear genomic regions in *Brachypodium*, rice and sorghum[1)]

Brachypodium	Rice	Sorghum	Predicted function
BRADI2G58260			Histone deacetylase 2C
BRADI2G58270			Dicer-like 3
		Sb03g043355	Ribonuclease III family protein
		Sb03g043360	Hypothetical protein
BRADI2G58277	*OS01g0909300*	*Sb03g043370*	Hypothetical protein
BRADI2G58290	*OS01g0909400*	*Sb03g043380*	Hypothetical protein
BRADI2G58300		*Sb03g043390*	Hypothetical protein
	OS01g0909500		Hypothetical protein
	OS01g0909600		Hypothetical protein
	OS01g0910200		Hypothetical protein
	OS01g0910300		Hypothetical protein
	OS01g0910400		Hypothetical protein
	OS01g0910500		Hypothetical protein
	OS01g0910800		Hypothetical protein
BRADI2G58310			Hypothetical protein
BRADI2G58320			BTB-POZ and MATH domain 6
		Sb03g043400	Hypothetical protein
		Sb03g043410	Hypothetical protein
BRADI2G58330			Ankyrin repeat family protein
BRADI2G58340			Hypothetical protein
BRADI2G58350	*OS01g0910900*	*Sb03g043420*	Hypothetical protein
	OS01g0911000		Sas10/Utp3/C1D family
		Sb03g043440	Leucine zipper factor-related
BRADI2G58360	*OS01g0911100*	*Sb03g043450*	P-loop containing nucleoside triphosphate hydrolases superfamily protein
BRADI2G58370	*OS01g0911200*	*Sb03g043460*	RPN2 family protein
BRADI2G58380	*OS01g0911300*	*Sb03g043470*	Transporter associated with antigen processing protein 1
BRADI2G58390	***OS01g0911700***	***Sb03g043480***	***VP1*; ABA INSENSITIVE 3**
		Sb03g043490	THAXTOMIN A RESISTANT 1
BRADI2G58397	*OS01g0911800*	*Sb03g043500*	BLISTER
BRADI2G58410	*OS01g0911900*	*Sb03g043510*	Hypothetical protein
		Sb03g043520	AGAMOUS-LIKE 103; transcription factor
	OS01g0912400		Hypothetical protein
BRADI2G58420	*OS01g0912600*	*Sb03g043530*	snRNA activating complex family protein
BRADI2G58430	*OS01g0912700*	*Sb03g043540*	Hypothetical protein
	OS01g0912800		Hypothetical protein
BRADI2G58440	*OS01g0913000*		Thioredoxin F-type, chloroplastic
BRADI2G58450	*OS01g0912900*	*Sb03g043550*	PPR-like superfamily protein
BRADI2G58460	*OS01g0913100*	*Sb03g043560*	Hypothetical protein
BRADI2G58470			Nitrate transporter 1.7

[1)] Genes related to seed dormancy and PHS resistance are shown in blod underlined.

in two locations at 2013. QTL for PHS resistance on chromosome 5BS have been previously reported. Using DArT markers and a DH population between PHS resistance cultivar Cayuga and susceptible cultivar Caledonia, two weak QTL *QPhs.cnl-5B.1* and *QPhs.cnl-5B.2* were detected when using the overall mean but not detected in any one location (Munkvold *et al*. 2009). However, the relationship between *QPhs.cau-5B* and *QPhs.cnl-5B.2* could not be established because of the different type of markers used and the coverage of genetic linkage maps in the two studies.

After precise mapping and comparative genomic analysis, *QPhs.cau-3A.2* was located in the *TaVp-1A* flanking region in 3AL, indicating the effect associated with *QPhs.cau-3A.2* is probably contributed by *TaVp-1A*. A previous study reported a minor QTL *QPhs.caas-3AL* mapped on 3AL in about 15 cM proximal to the *TaVp-1A* (Miao *et al*. 2013). Another weaker QTL *Qphs.ocs-3A.2* for PHS was founded on the pericentromeric region of 3AL flanked by SSR markers *Xcdo345* and *Xbcd141*, and not associated with *TaVp-1A* (Osa *et al*. 2003). A major QTL, *Qphs.ccsu-3A.1*, was simultaneously detected in an interval between markers *Xwmc153* and *Xgwm155* at the end of 3AL in two studies (Kulwal *et al*. 2005; Mohan *et al*. 2009). And a QTL *Qphs.usask-3A* for seed dormancy linked with markers *Xgwm340* and *XwPt1596* was positioned approximately 30 cM distal to the *TaVp-1A* locus on 3AL (Singh *et al*. 2010). Therefore, the *QPhs.cau-3A.2* identified in the current study demonstrated the effects of *TaVp-1A* locus to PHS resistance in the first

time through QTL mapping. The STS markers associated with *QPhs.cau-3A.2* provided an effective tool for selection of PHS resistance in the breeding program through MAS.

Since no assembled reference genome information is available for wheat genomics research, comparative genomics approach using the conserved gene order among grasses provides an important tool for mapping and cloning genes from the complex wheat genome. Many wheat genes have been narrowed to sub-centimorgan intervals by applying comparative genomic analyses (Wu *et al*. 2013; Ouyang *et al*. 2014). Comparative genomics analyses revealed the QTL *QPhs.cau-3A.2* interval on chromosome 3AL is orthologous to the highly collinearity genomic regions of *Brachypodium* 2L, rice 1L and sorghum 3L (Fig. 2). Only *BRADI2G58390*/*Os01g0911700*/*Sb03g043480*, orthologs of transcription factor *Viviparous1* (*Vp-1*) involved in abscisic acid (ABA) hormone signaling pathway (McCarty *et al*. 1991; Gale *et al*. 2002; Li *et al*. 2004), were identified in the corresponding genomic regions of *Brachypodium*, rice and sorghum and related to PHS in wheat and other cereals.

The *TaVp-1A* is the *Vp-1* homologue on chromosome 3A and was found to have an effect on seed dormancy/PHS. The maize *Vp-1* homolog enhanced seed dormancy of hexaploid wheat with significant reduction of alpha-amylase activity (Huang *et al*. 2012). Alternative splicing was found for *TaVp-1A* transcripts, while no *Vp-1A* expression differences were observed between the PHS-susceptible and -resistant cultivars (Yang *et al*. 2007). Association analyses indicated that allelic variations of *TaVp-1A* were related with seed dormancy and PHS of common wheat (Chang *et al*. 2011; Sun *et al*. 2012; Yang *et al*. 2014). Rich sequence variations were found in the 3rd, 4th and 5th introns and the 6th exon of the *TaVp-1A* gene. The TTC repeats are the most abundant sequence variations in the third intron and might be an important repeat sequence regulating *TaVp-1A* expression (Sun *et al*. 2012). Compared with Yanda 1817, a 12 TTC repeats deletion is identified in the 3rd intron of *TaVp-1A* in Beinong 6 (Table 6, Fig. 3). Furthermore, SNPs were also reported in the 3rd and 4th introns, and a 134-bp deletion in the 5th intron and a 160-bp insertion in the 6th exon were detected in International Maize and Wheat Improvement Center (CIMMYT) and Chinese wheat germplasm (Chang *et al*. 2011; Sun *et al*. 2012; Yang *et al*. 2014). We also found single nucleotide insertion and deletions, as well as SNPs in the 3rd intron and SNPs in the 4th intron between Yanda 1817 and Beinong 6 (Table 6, Fig. 3). From all the available data, it is very hard to establish a relationship between the PHS resistance and the TTC repeats number, InDels and SNPs in the 3rd, 4th and 5th introns and the 6th exon of the *TaVp-1A* locus. Only some of the *TaVp-1A* haplotypes expressed PHS resistance. The *TaVp-1A* allele of Yanda 1817 was same as *TaVp-1Ae* identified by Sun *et al*. (2012) and Chang *et al*. (2011) exhibiting higher average germination index with less dormancy. The *TaVp-1A* allele of Beinong 6 was same as the most common allele *TaVp-1Ain* in wheat germplasm reported by Yang *et al*. (2014). However, the *TaVp-1Ain* allele was susceptible to PHS with higher average germination index values (Yang *et al*. 2014), which is inconsistent with our results of Beinong 6. It seems the expression level of *TaVp-1A* shows close consistency with the PHS resistance (Yang *et al*. 2014). The PHS resis-

Table 6 Allelic variation of *TaVp-1A* in Yanda 1817 and Beinong 6

Cultivar	3rd intron (bp)				4th intron (bp)	Reference
	2719	2720–2794	2858	2862	3004	
Soleil (AJ400712)	C	25 TTC repeats	–	A	T	McKibbin *et al.* (2002)
Yanda 1817	C	21 TTC repeats	–	A	T	*TaVp-1Ae* reported by Chang *et al.* (2011) and Sun *et al.* (2012)
Beinong 6	–	9 TTC repeats	C	T	C	*TaVp-1Ain* reported by Yang *et al.* (2014)

```
TaVp-1A      TTCCATCTTCTTCTTCC.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.CTTCTTCTTCTTCTTCTTCTTCTTCCAAAGTGTATCATATGTTGCTAGTTAGC 2822
Beinong 6    TTCCATCTTCTTCTTC.....TTCTTCTTCTTCTTCTTCTTCTTCTTCCAAAGTGTATCATATGTTGCTAGTTAGC 443
Yanda 1817   TTCCATCTTCTTCTTCC.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.C.....TTCTTCTTCTTCTTCTTCTTCTTCTTCCAAAGTGTATCATATGTTGCTAGTTAGC 480

TaVp-1A      TAGAGCTGTCAAAGTAAGGAGAAAAAAACTAGCTAG_TTGATTCTGTATGATAACAAATTGCTGGTACAATGATCGATCTTGATTTTCATGCATGCAGATTTTGGCCCAACAACAAGAG 2941
Beinong 6    TAGAGCTGTCAAAGTAAGGAGAAAAAAACTAGCTAGCTTG_ATTCTGTATGATAACAAATTGCTGGTACAATGATCGATCTTGATTTTCATGCATGCAGATTTTGGCCCAACAACAAGAG 551
Yanda 1817   TAGAGCTGTCAAAGTAAGGAGAAAAAAACTAGCTAG_TTGATTCTGTATGATAACAAATTGCTGGTACAATGATCGATCTTGATTTTCATGCATGCAGATTTTGGCCCAACAACAAGAG 587

TaVp-1A      CAGAATGTATCTTCTAGAGAACACTGGTGAGAGAAGAGAACCAAACCTGCCTGATACAATTT_GAAACCAAATATATAGCTAGCTTCCCTTGTGTTCACAAAACGTTTCTTGTGCTTGAA 3061
Beinong 6    CAGAATGTATCTTCTAGAGAACACTGGTGAGAGAAGAGAACCAAACCTGCCTGATACAATTT1GAAACCAAATATATAGCTAGCTTCCCTTGTGTTCACAAAACGTTTCTTGTGCTTGAA 671
Yanda 1817   CAGAATGTATCTTCTAGAGAACACTGGTGAGAGAAGAGAACCAAACCTGCCTGATACAATTT_GAAACCAAATATATAGCTAGCTTCCCTTGTGTTCACAAAACGTTTCTTGTGCTTGAA 707
```

Fig. 3 Sequence variations between Yanda 1817, Beinong 6 and *TaVp-1A*. The bold underlined letters show mutant positions.

tance landrace Liangzhongbaimai had greater abundance of *TaVp-1A* transcript and the PHS susceptible cultivar Zhongyou 9507 has the least abundance of *TaVp-1A* transcript. Transcription analyses of the *TaVp-1A* locus could be used to characterize the mechanism of PHS resistance in Beinong 6 in the future.

5. Conclusion

Two major QTLs for PHS, *QPhs.cau-3A.2* and *QPhs.cau-5B*, have been identified using the high-density genetic linkage map developed from the RILs of Yanda 1817×Beinong 6. Precise mapping and comparative genomic analysis revealed that the *TaVp-1A* flanking region on 3AL is responsible for *QPhs.cau-3A.2*.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (31271710, 31301312).

References

Allen G, Flores-Vergara M, Krasynanski S, Kumar S, Thompson W. 2006. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. *Nature Protocols*, **1**, 2320–2325.

Ceccato D V, Bertero H D, Batlla D. 2011. Environmental control of dormancy in quinoa (*Chenopodium quinoa*) seeds: Two potential genetic resources for pre-harvest sprouting tolerance. *Seed Science Research*, **21**, 133–141.

Chang C, Zhang H P, Feng J M, Yin B, Si H Q, Ma C X. 2010. Identifying alleles of *Viviparous-1B* associated with pre-harvest sprouting in micro-core collections of Chinese wheat germplasm. *Molecular Breeding*, **25**, 481–490.

Chang C, Zhang H P, Zhao Q X, Feng J M, Si H Q, Lu J, Ma C X. 2011. Rich allelic variations of *Viviparous-1A* and their associations with seed dormancy/pre-harvest sprouting of common wheat. *Euphytica*, **179**, 343–353.

Cowan A K. 2002. Pre-harvest sprouting: Safari in southern Africa. *Euphytica*, **126**, 1–2.

Depauw R M, Mccaig T N. 1991. Components of Variation, Heritabilities and correlations for indexes of sprouting tolerance and seed dormancy in *Triticum* spp. *Euphytica*, **52**, 221–229.

Flintham J, Adlam R, Bassoi M, Holdsworth M, Gale M. 2002. Mapping genes for resistance to sprouting damage in wheat. *Euphytica*, **126**, 39–45.

Flintham J E. 2000. Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. *Seed Science Research*, **10**, 43–50.

Fofana B, Humphreys D G, Rasul G, Cloutier S, Brule-Babel A, Woods S, Lukow O M, Somers D J. 2009. Mapping quantitative trait loci controlling pre-harvest sprouting resistance in a red×white seeded spring wheat cross. *Euphytica*, **165**, 509–521.

Gale M D. 1989. The genetics of preharvest sprouting in cereals, particularly in wheat. In: Derera N F, ed., *Preharvest Field Sprouting in Cereals*. CRC Press, USA. pp. 85–110.

Gale M D, Flintham, J E, Devos, K M. 2002. Cereal comparative genetics and preharvest sprouting. *Euphytica*, **126**, 21–25.

Groos C, Gay G, Perretant M R, Gervais L, Bernard M, Dedryver F, Charmet D. 2002. Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a whitexred grain bread-wheat cross. *Theoretical and Applied Genetics*, **104**, 39–47.

Hagemann M G, Ciha A J. 1984. Evaluation of methods used in testing winter-wheat susceptibility to preharvest sprouting. *Crop Science*, **24**, 249–254.

Holland J B, Nyquist W E, Cervantes-Martinez C T. 2003. Estimating and interpreting heritability for plant breeding: An update. *Plant Breeding Reviews*, **22**, 9–112.

Huang T, Qu B, Li H P, Zuo D Y, Zhao Z X, Liao Y C. 2012. A maize viviparous 1 gene increases seed dormancy and preharvest sprouting tolerance in transgenic wheat. *Journal of Cereal Science*, **55**,166–173.

IBI (The International Brachypodium Initiative). 2010. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature, 463:763–768.

Incoln L, Ander E. 1993. *Constructing Genetic Linkage Maps with MAPMAKER/EXP Version 3.0: A Tutorial and Reference Manual*. Lander ES, Cambridge. pp. 1–9.

IRGSP (International Rice Genome Sequencing Project). 2005. The mapbased sequence of the rice genome. *Nature*, **436**, 793–800.

King R W, Richards R A. 1984. Water-uptake in relation to pre-harvest sprouting damage in wheat-ear characteristics. *Australian Journal of Agricultural Research*, **35**, 327–336.

Kulwal P L, Kumar N, Gaur A, Khurana P, Khurana J P, Tyagi A K, Balyan H S, Gupta P K. 2005. Mapping of a major QTL for pre-harvest sprouting tolerance on chromosome 3A in bread wheat. *Theoretical and Applied Genetics*, **111**, 1052–1059.

Kulwal P L, Singh R, Balyan H S, Gupta P K. 2004. Genetic basis of pre-harvest sprouting tolerance using single-locus and two-locus QTL analyses in bread wheat. *Functional & Integrative Genomics*, **4**, 94–101.

Kumar A, Kumar J, Singh R, Garg T, Chhuneja P, Balyan H S, Gupta P K. 2009. QTL analysis for grain colour and pre-harvest sprouting in bread wheat. *Plant Science*, **177**, 114–122.

Li C D, Ni P X, Francki M, Hunter A, Zhang Y, Schibeci D, Li H, Tarr A, Wang J, Cakir M, Yu J, Bellgard M, Lance R, Appels R. 2004. Genes controlling seed dormancy and pre-harvest sprouting in a rice-wheat-barley comparison. *Functional & Integrative Genomics*, **4**, 84–93.

Liu S B, Bai G H. 2010. Dissection and fine mapping of a major QTL for preharvest sprouting resistance in white wheat Rio Blanco. *Theoretical and Applied Genetics*, **121**, 1395–1404.

Liu S B, Cai S B, Graybosch R, Chen C X, Bai G H. 2008. Quantitative trait loci for resistance to pre-harvest sprouting

in US hard white winter wheat Rio Blanco. *Theoretical and Applied Genetics*, **117**, 691–699.

Liu S B, Sehgal S K, Li J R, Lin M, Trick H N, Yu J M, Gill B S, Bai G H. 2013. Cloning and characterization of a critical regulator for preharvest dprouting in wheat. *Genetics*, **195**, 263.

McCarty D R, Hattori T, Carson C B, Vasil V, Lazar M, Vasil I K. 1991. The *Viviparous-1* developmental gene of maize encodes a novel transcriptional activator. *Cell*, **66**, 895–905.

McIntosh R A, Yamazaki Y, Dubcovsky J, rogers J, Morris C, Appelsr, Xia X C. 2013. Catalogue of gene symbols for wheat. In: *12 th International Wheat Genetics Symposium*, Yokohama, Japan. Springer, Germany.

McKibbin R S, Wilkinson M D, Bailey P C, Flintham J E, Andrew L M, Lazzeri P A, Gale M D, Lenton J R, Holdsworth M J. 2002. Transcripts of *Vp-1* homeologues are misspliced in modern wheat and ancestral species. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 10203–10208.

Miao X L, Zhang Y J, Xia X C, He Z H, Zhang Y, Yan J, Chen X M. 2013. Mapping quantitative trait loci for pre-harvest sprouting resistance in white-grained winter wheat line CA 0431. *Crop & Pasture Science*, **64**, 573–579.

Mohan A, Kulwal P, Singh R, Kumar V, Mir R R, Kumar J, Prasad M, Balyan H S, Gupta P K. 2009. Genome-wide QTL analysis for pre-harvest sprouting tolerance in bread wheat. *Euphytica*, **168**, 319–329.

Mori M, Uchino N, Chono M, Kato K, Miura H. 2005. Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. *Theoretical and Applied Genetics*, **110**, 1315–1323.

Munkvold J D, Tanaka J, Benscher D, Sorrells M E. 2009. Mapping quantitative trait loci for preharvest sprouting resistance in white wheat. *Theoretical and Applied Genetics*, **119**, 1223–1235.

Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T, Utsugi S, Ogawa T, Handa H, Ishida H, Mori M, Kawaura K, Ogihara Y, Miura H. 2011. A Wheat homolog of *MOTHER OF FT AND TFL1* Acts in the regulation of germination. *The Plant Cell*, **23**, 3215–3229.

Nakamura S, Toyama T. 2001. Isolation of a *VP1* homologue from wheat and analysis of its expression in embryos of dormant and non-dormant cultivars. *Journal of Experimental Botany*, **52**, 1952–1952.

Osa M, Kato K, Mori M, Shindo C, Torada A, Miura H. 2003. Mapping QTLs for seed dormancy and the *Vp1* homologue on chromosome 3A in wheat. *Theoretical and Applied Genetics*, **106**, 1491–1496.

Ouyang S H, Zhang D, Han J, Zhao X J, Cui Y, Song W, Huo N X, Liang Y, Xie J Z, Wang Z Z, Wu Q H, Chen Y X, Lu P, Zhang D Y, Wang L L, Sun H, Yang T M, Keeble-Gagnere G, Appels R, Dolezel J, *et al*. 2014. Fine physical and genetic mapping of powdery mildew resistance gene *MIIW172* originating from wild emmer (*Triticum dicoccoides*). *PLOS ONE*, **9**, e100160.

Paterson A H, Bowers J E, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H B, Wang X Y, Wicker T, Bharti A K, Chapman J, Feltus F A, Gowik U, Grigoriev I V, *et al*. 2009. The Sorghum bicolor genome and the diversification of grasses. *Nature*, **457**, 551–556.

Singh R, Matus-Cadiz M, Baga M, Hucl P, Chibbar R N. 2010. Identification of genomic regions associated with seed dormancy in white-grained wheat. *Euphytica*, **174**, 391–408.

Sun Y W, Jones H D, Yang Y, Dreisigacker S, Li S M, Chen X M, Shewry P R, Xia L Q. 2012. Haplotype analysis of *Viviparous-1* gene in CIMMYT elite bread wheat germplasm. *Euphytica*, **186**, 25–43.

Wahl T I, Orourke A D. 1993. The Economics of sprout damage in wheat. In: *Pre-Harvest Sprouting in Cereals 1992.* AACC International Press, USA. pp. 10–17.

Wang Z Z, Cui Y, Chen Y X, Zhang D Y, Liang Y, Zhang D, Wu Q H, Xie J Z, Ouyang S H, Li D L, Huang Y L, Lu P, Wang G X, Yu M H, Zhou S H, Sun Q X, Liu Z Y. 2014. Comparative genetic mapping and genomic region collinearity analysis of the powdery mildew resistance gene *Pm41*. *Theoretical and Applied Genetics*, **127**, 1741–1751.

Wu H B, Qin J X, Han J, Zhao X J, Ouyang S H, Liang Y, Zhang D, Wang Z Z, Wu Q H, Xie J Z, Cui Y, Peng H R, Sun Q X, Liu Z Y. 2013. Comparative high-resolution mapping of the wax inhibitors *Iw1* and *Iw2* in hexaploid wheat. *PLOS ONE*, **8**, e84691.

Wu Q H, Chen Y X, Zhou S H, Fu L, Chen J J, Xiao Y, Zhang D, Ouyang S H, Zhao X J, Cui Y, Zhang D Y, Liang Y, Wang Z Z, Xie J Z, Qin J X, Wang G X, Li D L, Huang Y L, Yu M H, Lu P, *et al*. 2015. High-density genetic linkage map construction and QTL mapping of grain shape and size in the wheat population Yanda1817×Beinong6. *PLOS ONE*, **10**, e0118144.

Xiao S H, Zhang H P, You G X, Zhang X Y, Yan C S, Chen X. 2012. Integration of marker-assisted selection for resistance to pre-harvest sprouting with selection for grain-filling rate in breeding of white-kernelled wheat for the Chinese environment. *Euphytica*, **188**, 85–88.

Yang Y, Ma Y Z, Xu Z S, Chen X M, He Z H, Yu Z, Wilkinson M, Jones H D, Shewry P R, Xia L Q. 2007. Isolation and characterization of *Viviparous-1* genes in wheat cultivars with distinct ABA sensitivity and pre-harvest sprouting tolerance. *Journal of Experimental Botany*, **58**, 2863–2871.

Yang Y, Zhang C L, Liu S X, Sun Y Q, Meng J Y, Xia L Q. 2014. Characterization of the rich haplotypes of *Viviparous-1A* in Chinese wheats and development of a novel sequence-tagged site marker for pre-harvest sprouting resistance. *Molecular Breeding*, **33**, 75–88.

You F M, Huo N, Gu Y Q, Luo M C, Ma Y, Hane D, Lazo G R, Dvorak J, Anderson O D. 2008. BatchPrimer3: A high throughput web application for PCR and sequencing primer design. *BMC Bioinformatics*, **9**, 253.

Zanetti S, Winzeler M, Keller M, Keller B, Messmer M. 2000. Genetic analysis of pre-harvest sprouting resistance in a wheat×spelt cross. *Crop Science*, **40**, 1406–1417.

(Managing editor WANG Ning)