Theor Appl Genet (2014) 127:855–866
DOI 10.1007/s00122-014-2262-6

ORIGINAL PAPER

Cloning of seed dormancy genes (*TaSdr*) associated with tolerance to pre-harvest sprouting in common wheat and development of a functional marker

Yingjun Zhang · Xilei Miao · Xianchun Xia · Zhonghu He

Received: 4 August 2013 / Accepted: 3 January 2014 / Published online: 23 January 2014

Abstract

Key message **After cloning and mapping of wheat *TaSdr* genes, both the functional markers for *TaSdr-B1* and *TaVp-1B* were validated, and the distribution of allelic variations at *TaSdr-B1* locus in the wheat cultivars from 19 countries was characterized.**

Abstract Seed dormancy is a major factor associated with pre-harvest sprouting (PHS) in common wheat (*Triticum aestivum* L.). Wheat *TaSdr* genes, orthologs of *OsSdr4* conferring seed dormancy in rice, were cloned by a comparative genomics approach. They were located on homoeologous group 2 chromosomes, and designated as *TaSdr-A1*, *TaSdr-B1* and *TaSdr-D1*, respectively. Sequence analysis of *TaSdr-B1* revealed a SNP at the position -11 upstream of the initiation codon, with bases A and G in cultivars with low and high germination indices (GI), respectively. A cleaved amplified polymorphism sequence marker Sdr2B was developed based on the SNP, and subsequently functional analysis of *TaSdr-B1* was conducted by association and linkage mapping. A QTL for GI co-segregating with Sdr2B explained 6.4, 7.8 and 8.7 % of the phenotypic variances in a RIL population derived from Yangxiaomai/Zhongyou 9507 grown in Shijiazhuang, Beijing and the averaged data from those environments, respectively. Two sets of Chinese wheat cultivars were used for association mapping, and results indicated that *TaSdr-B1* was significantly associated with GI. Analysis of the allelic distribution at the *TaSdr-B1* locus showed that the frequencies of *TaSdr-B1a* associated with a lower GI were high in cultivars from Japan, Australia, Argentina, and the Middle and Lower Yangtze Valley Winter Wheat Region and Southwest Winter Wheat Region in China. This study provides not only a reliable functional marker for molecular-assisted selection of PHS in wheat breeding programs, but also gives novel information for a comprehensive understanding of seed dormancy.

Communicated by M. E. Sorrells.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-014-2262-6) contains supplementary material, which is available to authorized users.

Y. Zhang · X. Miao · X. Xia · Z. He (✉)
Institute of Crop Science, National Wheat Improvement Center, Chinese Academy of Agricultural Sciences (CAAS), 12 Zhongguancun South Street, Beijing 100081, China
e-mail: zhhecaas@163.com

Y. Zhang
Institute of Cereal and Oil Crops, Hebei Academy of Agricultural and Forestry Sciences, 162 Hengshan Street, Shijiazhuang 050035, China

Z. He
International Maize and Wheat Improvement Center (CIMMYT) China Office, c/o CAAS, 12 Zhongguancun South Street, Beijing 100081, China

Introduction

Seed dormancy allows germination to occur in more favorable seasons, and is therefore regarded as an important adaptive trait that enables the survival of plants in hostile environments. In cereal crops, dormancy is a desired trait because it prevents the early germination of grains (Rikiishi and Maekawa 2010; Lan et al. 2012). It is believed that pre-harvest sprouting (PHS) resistance of wheat is predominantly due to dormancy (Bailey et al. 1999; Flintham 2000; Li et al. 2004; Gubler et al. 2005; Tan et al. 2006). Early breakage of seed dormancy has been considered as the major component of PHS. The lack of harvest dormancy

in many wheat cultivars led to a serious problem, resulting in large economic losses caused by the adverse effects of pre-harvest sprouted wheat grains on end-product quality (Groos et al. 2002; Gubler et al. 2005). Improvement of tolerance to PHS is a major breeding objective in several regions in China as well as in many countries such as Japan, Australia, Canada, and the USA. Therefore, an understanding of genetic control of seed dormancy and development of functional markers are very important for marker-assisted breeding targeting for PHS tolerance in wheat.

Seed dormancy can be divided into coat-imposed and embryo-imposed dormancy. The seed coat imposes dormancy and acts as a physical barrier to imbibition and radicle growth. Red grain color controlled by *R* genes in wheat is associated with coat-imposed dormancy (Flintham 2000; Warner et al. 2000; Himi et al. 2002). *R* genes are located on the long arms of chromosomes 3A, 3B and 3D, approximately 30 cM proximal to the *Vp-1* loci that also affect dormancy (Groos et al. 2002; Himi and Noda 2005; Yang et al. 2007).

Embryonic dormancy is controlled more finely by the seed developmental program. Abscisic acid (ABA) plays a fundamental role in regulating embryonic dormancy. Many of the genes involved in seed dormancy are known to be involved in ABA synthesis and ABA signal transduction (Gubler et al. 2005). In Arabidopsis, *AtABI3* is an orthologous gene of *ZmVp-1* in maize and *OsVp-1* in rice (Koornneef et al. 1989; McCarty et al. 1991; Hattori et al. 1994). The abundance of the *AtABI3* is regulated by *AtFUS3*, *AtLEC1* and *AtAIP2* (Parcy et al. 1997; Kurup et al. 2000). The *AtLEC2*, *AtDOG1*, *AtHUB1* and *KYP/SUVH4* genes are also important for controlling seed dormancy in Arabidopsis (Stone et al. 2001; Bentsink et al. 2006; Liu et al. 2007b; Zheng et al. 2012). In wheat, the *TaVp-1* genes involved in ABA signal transduction are also important seed dormancy-related transcription factors (Nakamura and Toyama 2001; McKibbin et al. 2002). Three alleles of *TaVp-1B* were identified, designated as *TaVp-1Ba*, *TaVp-1Bb* and *TaVp-1Bc*. Based on the allelic variation, an STS marker Vp1B3 was developed. It was significantly associated with PHS tolerance (Yang et al. 2007). Later, *TaVp-1Bd* was reported in a collection of EU winter wheat cultivars and two further alleles, *TaVp-1Be* and *TaVp-1Bf*, were found in Chinese wheat cultivars (Xia et al. 2008; Chang et al. 2010a, b). Chang et al. (2011) identified six alleles of *TaVp-1A* located on chromosome 3A, designated as *TaVp-1Aa* to *TaVp-1Af*. Two orthologous genes *TaDOG1L1* and *HvDOG1L1* of *AtDOG1* were cloned. Although the sequence similarities of *TaDOG1L1* and *HvDOG1L1* with *AtDOG1* were low and the tissue-specific expression pattern of *AtDOG1* was not conserved in either gene, transformation in Arabidopsis clearly indicated that *TaDOG1L1* and *HvDOG1L1* promoted seed dormancy. Overexpression of *TaDOG1L1* and *HvDOG1L1* reduced seed germination percentage by 20 and 30 %, respectively, (Ashikawa et al. 2010).

In addition to the above genes, many QTL associated with PHS and seed dormancy have been reported in wheat; they are distributed over all 21 chromosomes (Mohan et al. 2009; Munkvold et al. 2009; Kulwal et al. 2012; Rehman Arif et al. 2012). Most of them were located on homoeologous group 3 and 4 chromosomes (Mares et al. 2005; Chen et al. 2008; Rasul et al. 2009; Liu and Bai 2010). A QTL linked with marker *Xwmc349* on chromosome 4B was significantly associated with seed germination index (GI) (Rasul et al. 2009). Chen et al. (2008) reported a major QTL controlling seed dormancy on chromosome 4A, and it was also confirmed in other studies (Zhang et al. 2008; Hickey et al. 2009). Osa et al. (2003) found a major QTL on chromosome 3AS associated with seed dormancy, and later, another QTL on 3AS was finely mapped (Liu and Bai 2010).

Although many works have been done, knowledge of the molecular mechanisms underlying seed dormancy is far from complete, and novel genes controlling seed dormancy to improve PHS tolerance in wheat are urgently required. However, wheat is a hexaploid plant, having three homoeologous genomes, A, B, and D, and it is very difficult to isolate genes by map-based cloning. Comparative genomics provide an excellent approach to identify and clone genes in common wheat based on information from rice or other grass-family crops (Sun et al. 2005; He et al. 2007, 2008, 2009; Yang et al. 2007; Ma et al. 2010), and functional markers were developed for wheat genes (Bagge et al. 2007; Liu et al. 2012). Recently, a rice gene *OsSdr4*, which contributed substantially to differences in seed dormancy between *japonica* and *indica* cultivars, was cloned (Sugimoto et al. 2010). The seeds of cultivars with *OsSdr4-n* had lower dormancy than those with *OsSdr4-k*. *OsSdr4* may act as an intermediate seed dormancy-specific regulator under the control of the regulator *OsVp-1*. *OsSdr4* expression was reduced in an *Osvp-1* mutant, and the expression levels of several germination-related genes such as *OsGA20ox-1* (Oikawa et al. 2004), *OsPIP* (Liu et al. 2007a) and *OsEXPB3* (Lee and Kende 2001) were significantly higher in the *Ossdr4* mutant than in the wild type (Sugimoto et al. 2010).

However, the functions of orthologous genes of *OsSdr4* on seed dormancy in wheat remain to be elucidated. The objectives of this study were to (1) isolate the orthologous genes *TaSdr* in common wheat, (2) develop a functional marker for use in marker-assisted selection for seed dormancy, and (3) examine the geographic distribution of allelic variants at the *TaSdr* locus in Chinese wheats and

those from 18 other countries. Cloning of *TaSdr* genes will provide an opportunity to characterize the regulatory mechanisms and related processes underlying seed dormancy in wheat.

Materials and methods

Plant materials

Four wheat cultivars with high germination index (GI) and four with low GI were chosen for cloning *TaSdr* genes. They were Zhongyou 9507 (GI 70.8), Jing 411 (GI 81.5), Han 6172 (GI 67.0), Heng 7228 (GI 68.4), Yangxiaomai (GI 7.5), Daqingmang (GI 3.5), Xiaobaiyuhua (GI 4.0) and Fengchan 3 (GI 3.9). A total of 201 recombinant inbred lines (RIL) derived from Yangxiaomai/Zhongyou 9507 (Table S1) were used to map *TaSdr* genes. Yangxiaomai, a Chinese landrace, had a low GI (7.5), whereas Zhongyou 9507 had a high GI (70.8). A set of Chinese Spring (CS) nulli-tetrasomic lines was used to determine the chromosomal locations of the *TaSdr* genes. Two sets of Chinese wheat cultivars (Tables S2 and S3) were used for validation of the Sdr2B marker and association analysis of the *TaSdr-B1* gene. Set I included 117 cultivars and Set II 84 cultivars. The two sets of wheat cultivars were previous or current cultivars from different major wheat production regions of China, and all had white grain color. Four hundred and forty-four cultivars from eight Chinese Wheat Regions (Table S4), covering almost all wheat areas of China, and 617 cultivars and advanced lines from 18 other countries (Table S5) were used to investigate allelic variation at *TaSdr-B1* locus.

Field trials and seed dormancy assay

Set I was planted at Beijing and Zhengzhou, Henan province, during the 2000–2001 and 2001–2002 cropping seasons. Set II was planted at Anyang, Henan province, in the 2005–2006 and 2006–2007 cropping seasons. The Yangxiaomai/Zhongyou 9507 RIL population was grown in randomized complete blocks with three replicates at Beijing and Shijiazhuang, Hebei province, in the 2011–2012 cropping season.

Seed dormancy measurement was based on GI values (Walker-Simmons 1988). Spikes were harvested at the dough-yellow ripping stage, naturally air dried for 2 days, and hand threshed to avoid damage to the embryos. The seeds were sterilized with 5 % (V/V) NaClO for 15 min, and washed three times with sterile water. Fifty seeds were placed crease down on two layers of filter paper in each Petri dish; 10 ml of sterile water were added and held at 20–24 °C. Germinated seeds were counted daily and removed. Germination index was calculated according to GI value (Walker-Simmons 1988):

$$\mathrm{GI} = \frac{7 \times n_1 + 6 \times n_2 + 5 \times n_3 + 4 \times n_4 + \ldots + 1 \times n_7}{7 \times \text{total grains}} \times 100$$

where n_1, $n_2 \ldots n_7$ were the number of seeds germinated on the first, second, and the subsequent days until the seventh day, respectively. Three replications of each sample were performed.

Cloning and sequence analysis of *TaSdr* genes

Common wheat *TaSdr* genes were cloned by a comparative genomics approach. The DNA sequence of rice *OsSdr4* (GenBank accession AB506455) was used in a BLAST search against the wheat genome database (http://www.cerealsdb.uk.net). Primers were designed based on the sequence using the software Premier Primer V5.0 (http://www.premierbiosoft.com) and synthesized by Shanghai Sangon Biotech Co., Ltd. (http://www.sangon.com). All PCR primers used in this study were described in Table 1.

The primer pair Sdr-1 was used to amplify the full length *TaSdr* genes. The sequences amplified were divided into three groups corresponding to chromosomes of the

Table 1 Primer sets for cloning *TaSdr* genes and STS markers for testing seed dormancy developed in this study

Marker	Primer sequences (5′-3′)	Tm (°C)	Amplified target
Sdr-1	F: CACTGGACGCCACTGGAATC	56	Full length *TaSdr*
	R: CGTAGGCAATCACCAGTCATTATC		
Sdr-2	F: CGTCGGCAGACATCGACTCC	59	A genome-specific
	R: GAAGCTCACTAGCTCAGAACACGC		
Sdr-3	F: CGTCAGCAGACTTCGACTCGC	57	B genome-specific
	R: CAAGAAGCTCACTATCTCAGAACACAA		
Sdr-4	F: GCCGCCGACCGCGTGCTA	57	D genome-specific
	R: CCACTAAACATGCACCTTAACCTAGTA		
Sdr-5	F: CGCCTACGTGTCGGCCC	66	The PCR for CAPS
	R: TCCGTGACGACCGCCGGG		

A, B and D genomes. Based on the sequence divergences, genome-specific primers Sdr-2, Sdr-3 and Sdr-4 were designed to amplify the A, B and D genome sequences of *TaSdr* genes, respectively. Each primer pair was tested on the CS nulli-tetrasomic lines to determine the chromosomal locations of the amplified genes.

Genomic DNA was extracted from three kernels using a method modified from Lagudah et al. (1991). Because of GC richness (~72 %) in *TaSdr* gene sequences, GC Buffer I was used to enhance PCR efficiency. PCR was performed in volumes of 20 μl, including 10 μl 2 × GC buffer I, 100 μM of each dNTP, 2 pmol of each primer, 1 U of ExTaq and 80 ng of template DNA. All reagents were from Takara Biotechnology Co., Ltd. (http://www.takara.com.cn). Reaction conditions were 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, annealing at 56–59 °C for 1 min (depending on primers), 72 °C for 2 min, and a final extension of 72 °C for 5 min.

PCR products were separated by electrophoresis on 1 % agarose gels, and targeted bands were recovered and cloned into the pEASY-T1 simple vector (Beijing TransGen Biotech Co., Ltd. http://www.transgen.com.cn) and transformed to DH5α competent *E. coli* cells by the heat shock method. Positive clones were sequenced from both strands by Shanghai Sangon Biotech Co., Ltd. Sequence analysis and characterization were performed using the software DNAMAN (http://www.lynnon.com) and Vector NTI Advance 10 (http://www.invitrogen.com). PCR and DNA sequencing were repeated two times to ensure the accuracy of nucleotide sequences. *TaSdr* homologs were searched by BLAST, and displayed by an unrooted phylogenetic tree using MEGA 4 (http://www.megasoftware.net).

Development of a functional marker

Based on the two alleles of *TaSdr-B1* locus that were characterized, a cleaved amplified polymorphism sequence (CAPS) marker was designed to identify allelic variants of *TaSdr-B1* that were validated using Set I and Set II Chinese wheat cultivars and confirmed with the Yangxiaomai/Zhongyou 9507 RIL population. PCR was performed in a total volume of 50 μl, including 25 μl 2 × GC buffer I, 200 μM of each dNTP, 4 pmol of each primer, 1.5 U of ExTaq and 80 ng of template DNA. Reaction conditions were 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 66 °C for 45 s with a decrease of 0.3 °C per cycle, 72 °C for 1 min, with a final extension of 72 °C for 5 min. The PCR products were purified by Purification Kit (http://www.tiangen.com), then digested with *Pml*I (http://www.neb-china.com) according to the manufacturer's direction and digested fragments were separated on 2 % agarose gels.

Detection of *TaVp-1* gene in set I and set II wheat cultivars

The primer sequences of Vp1B3 marker for *TaVp-1* gene were from Yang et al. (2007). PCR was performed in a total volume of 20 μl, including 10 μl 2 × GC buffer I, 100 μM of each dNTP, 2 pmol of each primer, 1.0 U of rTaq polymerase and 80 ng of template DNA. The reaction conditions were 94 °C for 5 min, followed by 36 cycles of 94 °C for 1 min, 61 °C for 1 min and 72 °C for 1 min, with a final extension of 72 °C for 10 min. PCR products were separated by electrophoresis in 1.5 % agarose gels stained with ethidium bromide and visualized using UV light. The genotypes *TaVp-1Ba*, *TaVp-1Bb* and *TaVp-1Bc* yielded 652, 845 and 569 bp bands, respectively.

Statistical analysis

The analysis of variance (ANOVA) and correlation coefficients among environments were validated with the Statistical Analysis System (SAS Institute, V8). ANOVA was performed using PROC GLM and the correlation coefficients among environments were estimated by PROC CORR. The differences in GI among genotypes with different PCR profiles were tested using Duncan's multiple comparison. The software IciMapping V3.2 (Li et al. 2007, 2008) was employed for linkage map construction and QTL analysis, and the genetic distance (centiMorgans cM) was generated with Kosambi mapping function (Kosambi 1944). The LOD (logarithm of odds) threshold was set at 3.0 to claim linkage between markers with an algorithm SERiation and criterion SARF (sum of adjacent recombination frequencies). QTL detection was conducted using inclusive composite interval mapping (ICIM, Li et al. 2007, 2008), and a LOD threshold, calculated from 2,000 permutations at a probability of 0.01, was used for declaring definitive QTL. The phenotypic variance explained (PVE) by individual QTL was estimated through stepwise regression at a probability of 0.001 (Li et al. 2007).

Results

Cloning and characterization of *TaSdr* genes

The BLAST search against the wheat genome database identified a genomic sequence, contig 360323, exhibiting 77.6 % of sequence identity with *OsSdr4* (GenBank accession AB506455). The primer set Sdr-1 (Table 1), designed according to the putative sequence, yielded a single ~1.5 Kb fragment. Sequence analysis demonstrated three similar but different sequences of PCR products, and they were divided into three groups corresponding to chromosomes of the A, B and D genomes. Based on the sequence

divergences, genome-specific primer pairs Sdr-2, Sdr-3 and Sdr-4 (Table 1) were designed to amplify the corresponding genes in the A, B and D genomes, respectively. Each primer pair was tested on the CS nulli-tetrasomic lines to determine the chromosomal locations of amplified PCR fragments. For example, amplifications were observed in all nulli-tetrasomic lines except N2A-T2D, indicating that *TaSdr* amplified by the primer pair Sdr-2 was located on wheat chromosome 2A; this gene was designated as *TaSdr-A1*. Likewise, *TaSdr-B1* and *TaSdr-D1* amplified by primer sets Sdr-3 and Sdr-4, respectively, were located on chromosomes 2B and 2D (Fig. S1).

TaSdr-A1 contains a 993 bp open reading frame (ORF), a 254 bp 5′ untranslated region (UTR), and a 266 bp 3′UTR, with 1,513 bp in total. *TaSdr-B1* has a 981 bp ORF, a 255 bp 5′UTR, and a 267 bp 3′UTR, and is 1,503 bp in total. *TaSdr-D1* contains a 987 bp ORF, a 254 bp 5′UTR, and a 266 bp 3′UTR, 1,507 bp in total (Fig. S2). The ORF of *TaSdr* genes was slightly shorter than that of *OsSdr4* which is 1,029 bp. The sequence identity of *TaSdr-A1*, *TaSdr-B1* and *TaSdr-D1* was ~96 %, and the similarity of each sequence to *OsSdr4* was ~75 %. The deduced polypeptides of *TaSdr* shared ~68 % sequence identity with *OsSdr4*. Like *OsSdr4* there was no intron in the *TaSdr* genes. The GC content of *TaSdr* was ~72.0 %, thus showing GC-rich characteristics. Based on the DNA sequences and predicated proteins of *TaSdr*, we concluded that *TaSdr* were orthologs of *OsSdr4*.

Homologous genes of *TaSdr*

Fifteen *TaSdr* homologs besides *OsSdr* were found by BLAST, including three genes from *Brachypodium distachyon* (XM_003559980, XM_003559981, XM_003576883), two from maize (NM_001151595, NM_001152143), one from sorghum (XM_002460891), seven from Arabidopsis (NM_001123893, NM_123444, NM_124417, NM_123445, NM_114709, NM_125730, BT_012643) and one from grape (XM_002283317). Phylogenetic analysis based on these homologous sequences placed them in two groups. Group 1 contained the homologous genes from wheat, rice, *Brachypodium distachyon*, maize and sorghum. The genes from wheat, *B. distachyon* and rice showed high similarity, but the wheat genes were more similar to *B. distachyon* than to rice. Maize *ZmSdr* genes showed close relationship with sorghum. Most of the Arabidopsis homologs were in group 2 (Fig. 1).

Allelic variants for *TaSdr* genes

Sequence analysis showed a SNP at the position 643 in *TaSdr-A1*, with G in genotypes with lower GI values and A in those with higher GI; the two alleles were designated as *TaSdr-A1a* (GenBank accession KF021988) and *TaSdr-A1b* (GenBank accession KF021989), respectively. The G/A mutation resulted in amino acid change from Valine to Isoleucine. DNA sequencing of the *TaSdr-B1* locus revealed no diversity in the coding sequence (CDS), but a SNP was found at position −11 in the promoter region, with A in genotypes with lower GI values and G in those with higher GI (Fig. 2); the two alleles were designated as *TaSdr-B1a* (GenBank accession KF021990) and *TaSdr-B1b* (GenBank accession KF021991), respectively. No diversity was found at *TaSdr-D1* locus (GenBank accession KF021992).

Development of a CAPS marker for *TaSdr-B1*

The SNP at the position -11 of *TaSdr-B1* produced a restriction enzyme *Pml*I recognition site (CACGTG) in low GI genotypes, but not in high GI genotypes. Based on this SNP, a cleaved amplified polymorphism sequence (CAPS) marker, designated as Sdr2B, was developed to discriminate the two alleles. The chromosome 2B-specific primer set Sdr-5 was used firstly to amplify an 826 bp fragment of *TaSdr-B1* from all cultivars. Because of a second *Pml*I recognition site in the amplified region (Fig. 2) three fragments of 60, 116 and 650 bp were generated in genotype *TaSdr-B1a*, whereas two fragments of 60 and 766 bp were produced in *TaSdr-B1b*. The specific bands of 650 and 766 bp can be used to distinguish between *TaSdr-B1a* and *TaSdr-B1b*.

Linkage and association mapping of *TaSdr-B1*

The phenotypic data in the Yangxiaomai/Zhongyou 9507 RIL population showed continuous distributions of GI indicating a typical quantitative inheritance (Fig. 3). Linkage analysis showed that Sdr2B was closely linked (3.2 cM) to *Xwmc477*, an SSR marker located the near centromere of chromosome 2B (Fig. 4). The mapped location was consistent with the results obtained from CS nulli-tetrasomic lines. Based on 2,000 permutations at a probability of 0.01, the LOD scores to declare a significant QTL for GI were 2.13, 2.18 and 2.06, respectively, in the environments Beijing, Shijiazhuang and the averaged data from both environments, thus a LOD score of 2.2 was set as the threshold for declaring significant QTL. A QTL (*TaSdr-B1*) for GI co-segregating with Sdr2B, with LOD scores 3.5, 2.9 and 4.0, explained 7.8, 6.4 and 8.7 % of the phenotypic variances in the Yangxiaomai/Zhongyou 9507 RIL population in the environments Beijing, Shijiazhuang and the averaged data from both environments, respectively, (Fig. 4). Two sets of Chinese wheat cultivars used for association mapping of *TaSdr-B1* (Table S2 and S3) were genotyped by Sdr2B (Fig. S3). Cultivars with the genotype *TaSdr-B1b* showed significantly higher GI than *TaSdr-B1a* genotypes

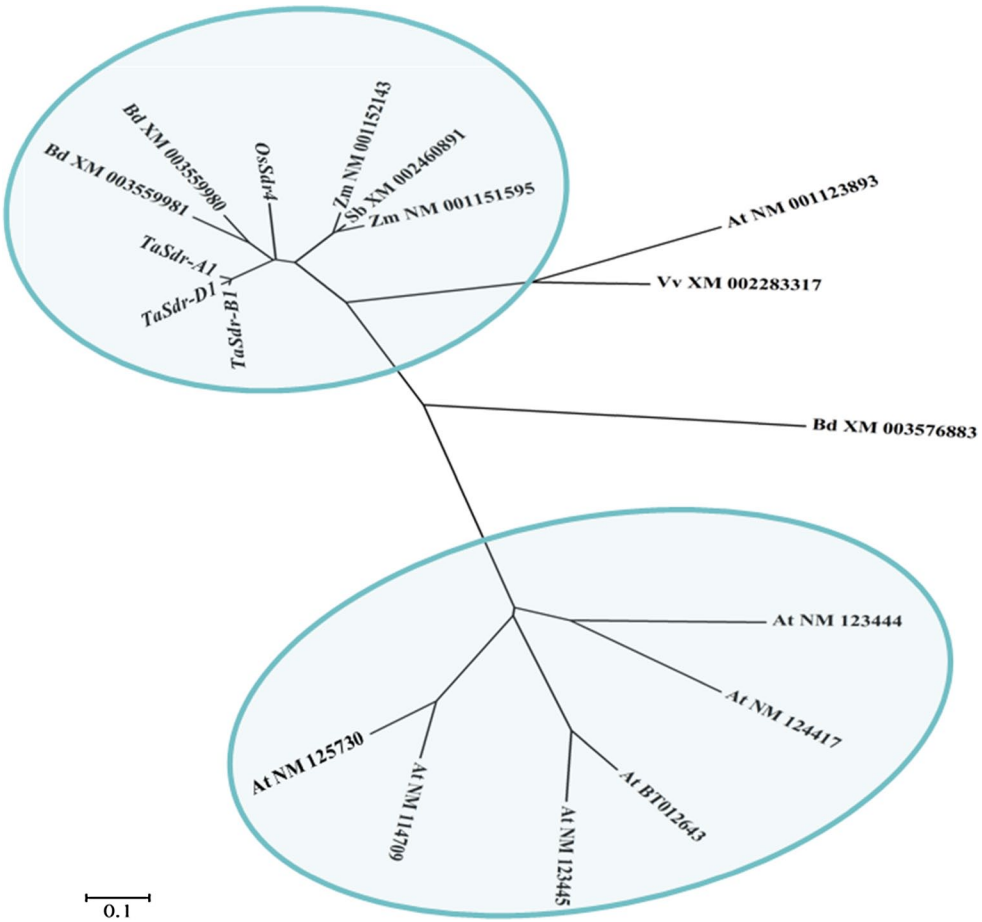

Fig. 1 Phylogenetic tree of *TaSdr* homologs. Fifteen *TaSdr* homologs were obtained by BLAST. Phylogenetic analysis based on sequence classified these homologous genes into two groups. Wheat, *Brachypodium distachyon* and rice genes had highly similar sequences

(Table 2) on average, confirming the association of *TaSdr-B1* with GI values.

Combined phenotypic effects of *TaSdr-B1* and *TaVp-1B*

TaVp-1B is also involved in the regulation of seed dormancy. PHS-tolerant cultivars have alleles *TaVp-1Bb* or *TaVp-1Bc*, and susceptible cultivars frequently have *TaVp-1Ba* (Yang et al. 2007). The *TaVp-1Ba* and *TaVp-1Bc* are common in wheat cultivars and *TaVp-1Bb* is a rare allele. When markers for the *TaSdr-B1* and *TaVp-1B* genes were used to test Set I and Set II cultivars significant associations were identified between genotypes and phenotypes (Table 2). In set I cultivars, the genotype *TaSdr-B1b/TaVp-1Ba* exhibited the highest GI (54.8), whereas *TaSdr-B1a/TaVp-1Bc* had the lowest GI (15.6). Among 39 cultivars with genotype *TaSdr-B1b/TaVp-1Ba*, 20 had GI high than 50, and 11 had GI values of 30–50. Whereas among 18 cultivars with genotype *TaSdr-B1a/TaVp-1Bc*, 16 had GI lower than 31, the proportion of tolerant cultivars was 88.9 %. Similar results were found in Set II cultivars. Genotype *TaSdr-B1b/TaVp-1Ba* also exhibited the highest mean GI (45.8), and *TaSdr-B1a/TaVp-1Bc* showed a lower average GI value (31.9). However, the genotype *TaSdr-B1b/TaVp-1Bb* exhibited the lowest GI value (15.8) in Set II. Therefore, the combination of two STS markers, Sdr2B and Vp1B3, could improve the accuracy of marker-assisted breeding for improving PHS tolerance.

Geographic distribution of *TaSdr-B1* alleles

Four hundred and forty-four cultivars from eight Chinese Wheat Regions (Table S4) and 617 lines from 18 other countries (Table S5) were used for testing the geographic distribution of *TaSdr-B1* alleles. Among the Chinese cultivars, 166 (37.4 %) carried *TaSdr-B1a*. The proportions of *TaSdr-B1a* were higher in the Middle and Lower Yangtze Valley Winter Wheat Region (III) (48.9 %) and Southwest Winter Wheat Region (IV) (41.9 %) (Table S6; Fig. 5). The Xinjiang Winter–Spring Wheat Region (X), where annual rainfall was only 0–200 mm, had the lowest frequency of *TaSdr-B1a* (14.8 %). The frequencies of the *TaSdr-B1a* allele in Japanese, Australian and Argentine cultivars were 62.5, 37.1 and 36.4 %, respectively, (Table S7; Fig. 6). Canadian (33.3 %) and Chilean (20.5 %) cultivars had

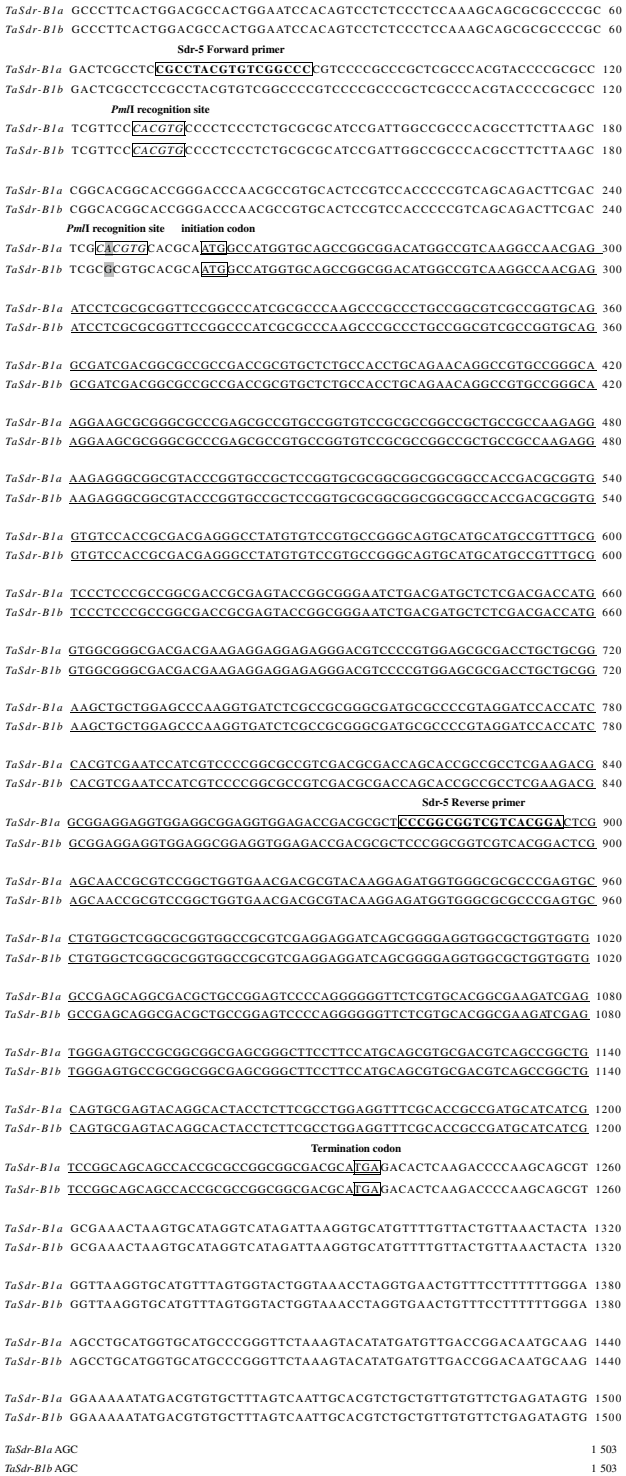

Fig. 2 Alignment of alleles *TaSdr-B1a* and *TaSdr-B1b* located on wheat chromosome 2B. The open reading frame (ORF) is *underlined*; the initiation and termination codons are *boxed*; the SNP is *shadowed*; the forward and reverse primers of Sdr-5 are *boxed* and *bolded*; the *Pml*I recognition site is *boxed* and *italicized*

somewhat lower frequencies of *TaSdr-B1a*. No *TaSdr-B1a* genotype was found among cultivars from Germany, Romania, Russia, Ukraine and Serbia.

Discussion

Genes involved in seed dormancy

Seed dormancy in wheat is controlled by many genes. Red grain color controlled by *R* genes is known to be associated with the seed dormancy (Flintham 2000; Warner et al. 2000; Himi et al. 2002). The *R* genes affect water imbibition, and the red grain coat mostly acts as a physical barrier to radicle growth. However, most of the genes associated with seed dormancy are involved in abscisic acid (ABA) pathways. In Arabidopsis, *AtABI3*, *AtFUS3*, *AtLEC1*, *AtAIP2*, *AtHUB1* and *KYP/SUVH4* are involved in ABA synthesis and ABA signal transduction (Parcy et al. 1997; Stone et al. 2001; Kurup et al. 2000; Liu et al. 2007b; Zheng et al. 2012). A *CYP707A* family that catalyzes 8′-hydroxylation of ABA to PA was identified in Arabidopsis (Kushiro et al. 2004; Saito et al. 2004). The *CYP707A2* sequence was used to identify barley homologs by BLAST search, and barley *HvCYP707A1* was cloned by a comparative genomics approach. Increased expression of *HvCYP707A1* led to a rapid ABA decrease and high germination percentage (Chono et al. 2006). *TaCYP707A1* was highly similar to *HvCYP707A1*, at both the nucleotide and protein levels. Chromosome localization indicated the *TaCYP707A1* gene resided on chromosome 6BL in wheat (Zhang et al. 2009). The *Viviparous-1* developmental gene in maize encodes a transcriptional activator (McCarty et al. 1991; Hattori et al. 1992); homologs of the maize *ZmVp1* gene were isolated from rice and wheat (Hattori et al. 1994; Bailey et al. 1999). New alleles of *TaVp-1* were reported and STS markers for PHS tolerance were developed in wheat (Yang et al. 2007; Xia et al. 2008; Chang et al. 2010a, b, 2011). The *TaDOG1L1* and *HvDOG1L1* genes had similar function to *AtDOG1* (Bentsink et al. 2006; Ashikawa et al. 2010). *OsSdr4*, an intermediate regulator of dormancy between *OsVp-1* and *OsDOG1*-like genes in the seed maturation program, was cloned (Sugimoto et al. 2010). Recently, a detailed functional analysis showed that *AtMFT* is a negative regulator of ABA sensitivity during seed germination in Arabidopsis. The *AtMFT* expression is directly regulated by *AtABI3*, *AtABI5* and DELLA proteins (Xi et al. 2010). In wheat, *TaMFT* expression suppressed germination, opposite to the action of *AtMFT*. *TaMFT* was also the causal gene for the wheat seed dormancy QTL *QPhs.ocs-3A.1* (Nakamura et al. 2011). Liu et al. (2013) cloned and characterized a gene on the chromosome 3AS for PHS resistance in white wheat using map-based cloning. This gene, designated *TaPHS1*, is a homolog of *TaMFT*. RNA interference-mediated knockdown of the gene confirmed that *TaPHS1* positively regulates PHS resistance. In wheat, genes associated with seed dormancy are still relatively few in number, and functional markers that could be used for

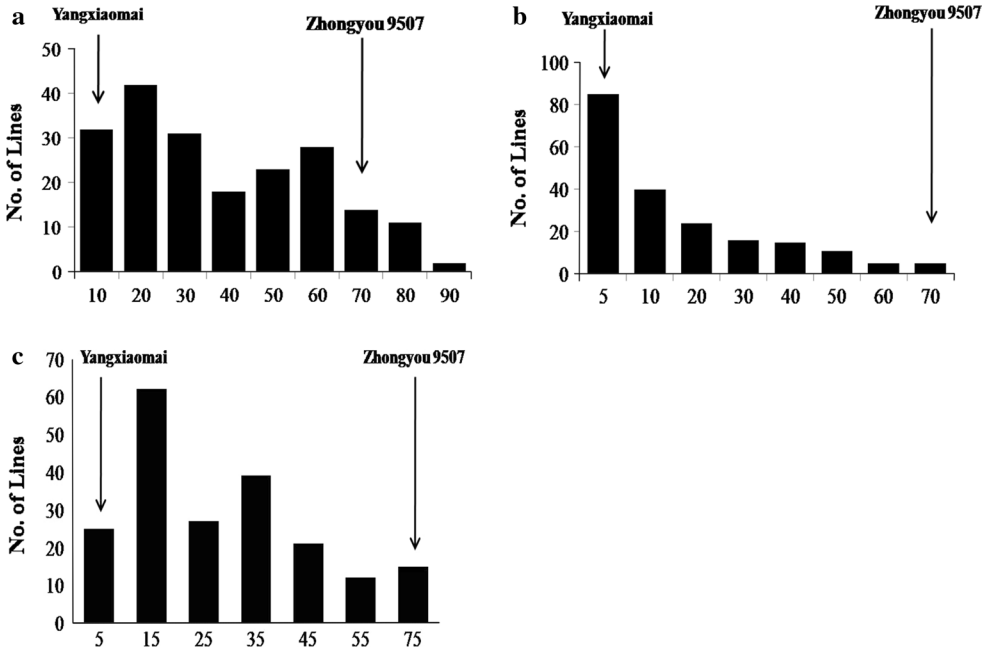

Fig. 3 Frequency distributions of germination index (GI) in Yangxiaomai/Zhoungyou 9507 RIL population in the environments (**a**, **b**) as well as the averaged data (**c**). *a*, Beijing; *b*, Shijiazhuang

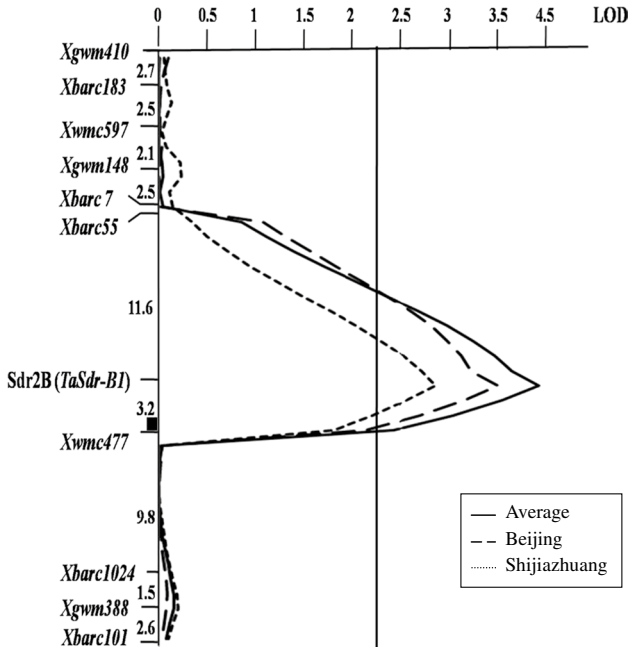

Fig. 4 Linkage map of functional marker Sdr2B and SSR markers on wheat chromosome 2B and mapping of *TaSdr-B1* using Yangxiaomai/Zhongyou 9507 RILs. Sdr2B was 3.2 cM from SSR marker *Xwmc477* located near the centromere. A QTL for GI co-segregating with Sdr2B explained 6.4, 7.8 and 8.7 % of the phenotypic variance in environments Shijiazhuang, Beijing and the averaged data from both environments, respectively. The *solid black box* indicated the centromere

screening low GI cultivars are also limited. It is therefore necessary to clone novel genes and develop reliable functional markers for seed dormancy.

Putative mechanism of *TaSdr* genes for seed dormancy

TaSdr-B1 is involved in the pathway of seed dormancy regulation, but mutation sites differ between *TaSdr-B1* and *OsSdr4*. The coding region of *OsSdr4* contains clustered sequence differences corresponding to one 3-bp InDel and three amino acid substitutions, along with four SNPs, and three of them resulting in amino acid changes. The clustered sequence changes are resulted from an 18-bp direct repeat in *OsSdr4-n*, which could have been created by double-strand cleavage and repair in the corresponding region of *OsSdr4-k* (Sugimoto et al. 2010). However, there was only a SNP in the promoter region of *TaSdr-B1*.

In rice, *OsSdr4* expression is positively regulated by *OsVp-1*, which in turn positively regulates *OsDOG1-like* genes, suggesting that *OsSdr4* acts as an intermediate regulator of dormancy in the seed maturation program (Sugimoto et al. 2010). In wheat, an association of *TaVp-1* and *TaDOG1L1* genes with seed dormancy was identified previously (Yang et al. 2007; Ashikawa et al. 2010; Chang et al. 2010a, b, 2011). In the present study, a relationship of *TaSdr-B1* with seed dormancy was also elucidated. It is very likely that the *TaVp-1*, *TaSdr-B1* and *TaDOG1L1* genes have the similar functions to orthologous genes in rice.

Relationship between *TaSdr-B1* and QTL for PHS tolerance on chromosome 2B

Chromosomes 2B is of particular importance for PHS tolerance as many major QTL for PHS were found on this chromosome in both white and red wheats (Kulwal et al.

Table 2 Association between *TaSdr-B1* and *TaSdr-B1/TaVp-1B* genotypes and germination index (GI) values in Chinese wheat cultivars

Experiment	Genotype	Number of accessions	Mean GI[a]	Standard deviation	Range
Set I	*TaSdr-B1a*	37	24.8a	18.9	0.0–64.0
	TaSdr-B1b	80	38.3b	31.2	1.0–100
	TaSdr-B1b/TaVp-1Ba	39	54.8a	32.2	3.5–100.0
	TaSdr-B1a/TaVp-1Bb	1	47.5ab	–	–
	TaSdr-B1b/TaVp-1Bb	6	36.4ab	15.3	8.0–50.0
	TaSdr-B1a/TaVp-1Ba	18	32.8ab	18.3	0.0–64.0
	TaSdr-B1b/TaVp-1Bc	35	20.3ab	20.6	1.0–87.0
	TaSdr-B1a/TaVp-1Bc	18	15.6b	28.6	0.0–52.0
Set II	*TaSdr-B1a*	30	28.2a	15.8	3.9–66.7
	TaSdr-B1b	54	39.4b	18.3	6.7–70.8
	TaSdr-B1b/TaVp-1Ba	23	45.8a	17.2	6.7–70.8
	TaSdr-B1b/TaVp-1Bc	26	38.2ab	17.0	7.0–68.4
	TaSdr-B1a/TaVp-1Bc	14	31.9b	16.1	3.9–66.7
	TaSdr-B1a/TaVp-1Ba	16	25.0bc	13.5	4.0–47.3
	TaSdr-B1b/TaVp-1Bb	5	15.8c	8.0	6.9–24.0
	TaSdr-B1a/TaVp-1Bb	0	–	–	–

[a] Numbers followed by different letters are significantly different at $P = 0.05$

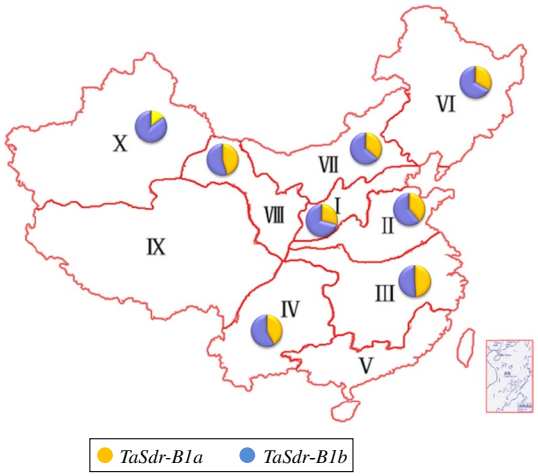

Fig. 5 Geographic distribution of *TaSdr-B1* alleles in Chinese Wheat Regions. The frequencies of *TaSdr-B1a* were highest in the Middle and Lower Yangtze River Valley Winter Wheat Region (III) and Southwest Winter Wheat Region (IV), at 48.9 and 41.9 %, respectively. The frequencies of *TaSdr-B1a* were also high in the Northern Winter Wheat Region (I), Yellow and Huai Valleys Winter Region (II), Northeastern Spring Wheat Region (VI), Northern Spring Wheat Region (VII) and Northwestern Spring Wheat Region (VIII). The Xinjiang Winter–Spring Wheat Region (X), where annual rainfall was only 0–200 mm, had the lowest frequency (14.8 %) of *TaSdr-B1a*

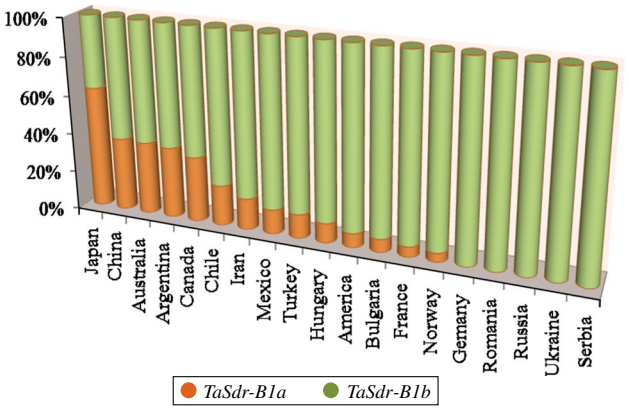

Fig. 6 Frequencies of *TaSdr-B1* alleles in cultivars from different countries. The proportion of *TaSdr-B1a* declined from Japan (62.5 %) to Serbia (0 %). Cultivars from Australia, Argentina, Canada and Chile also had high frequencies of *TaSdr-B1a*. The *TaSdr-B1a* allele was not present in cultivars from Germany, Romania, Russia, Ukraine and Serbia

2004, 2012; Liu et al. 2008; Mohan et al. 2009; Munkvold et al. 2009; Singh et al. 2010; Somyong 2010; Jaiswal et al. 2012; Lan et al. 2012; Rehman Arif et al. 2012; Lohwasser et al. 2013; Miao et al. 2013). In the present study, Sdr2B was spanned by *Xwmc477* and *Xbarc55* on chromosome 2B, with genetic distances of 3.2 and 11.6 cM, respectively. On the high-density microsatellite consensus map for bread wheat (Somers et al. 2004), the genetic distance of SSR marker *Xwmc474* to *Xwmc477* is 4.0 cM, thus Sdr2B and *Xwmc474* is likely to be in the same or close location. In previous studies, several QTL for PHS tolerance were reported in or nearby this region. For example, Munkvold et al. (2009) reported a major QTL *QPhs.cnl-2B.1* on the chromosome 2BS in the marker interval of *Xbarc55-Xwmc474*; it was detected in 16 environments with LOD scores of up to 27.1 and explained 24 % of the phenotypic variation using the mean PHS score. Another QTL, near *Xbarc328*, explain up to 5 % of the variation, was identified by Kulwal et al. (2012). Liu et al. (2008) detected two QTL on chromosome 2B using a population of 171 RILs. One QTL, *QPhs.pseru-2B.2*, flanked by

markers *Xbarc105* and *Xbarc334*, explained 4.5 % of the phenotypic variation for PHS resistance. *QPhs.pseru-2B.2* was 12–18 cM from marker *Xwmc474*. Somyong (2010) reported two QTL for PHS on chromosome 2BS; the first one was located between *Xwmc453c* and *Xbarc55*, and the second was in the interval between *Xwmc474* and *rCaPK*. Among these QTL, *TaSdr-B1* was likely to be in the same region as that reported by Munkvold et al. (2009) and Somyong (2010).

There is a large difference in GI between the population parents, Yangxiaomai (7.5) and Zhongyou 9507 (70.8), but a minor QTL effect near *TaSdr* was detected in the present study. The reason may be attributed to other genes that also contribute to seed dormancy in the Yangxiaomai/Zhongyou 9507 population, such as *R*, *TaVp-1*, *TaDOG1L1*, *TaCYP707A1*, and/or *TaMFT* (*TaPHS1*) genes.

Geographic distribution of *TaSdr-B1* alleles

In China, PHS is common in the Middle and Lower Yangtze Valley Winter Wheat Region (III), Southwest Winter Wheat Region (IV), Yellow and Huai Valleys Winter Region (II), Northeastern Spring Wheat Region (VI) and the Northern Winter Wheat Region (I) (Zhang et al. 1989). Wheat cultivars in other countries such as Japan, Australia, Canada, USA and Germany, are often damaged by PHS (Derera et al. 1977).

Various seed traits, including dormancy, have been selected through crop domestication. In general, wheat cultivars from areas with more serious PHS have a higher frequency of the PHS tolerance allele *TaSdr-B1a*, which can be attributed to a positive selection. The frequencies of *TaSdr-B1a* were highest in the Chinese wheat regions III and IV, consistent with the higher incidence of PHS in those regions. Similarly, higher frequencies of *TaSdr-B1a* allele were also found in Japanese, Australian and Argentina cultivars. In China, wheat cultivars from region X had the lowest frequency (14.8 %) of *TaSdr-B1a* probably due to the low annual rainfall (0–200 mm) and absence of a PHS problem in Xinjiang. No *TaSdr-B1a* was found in German, Romanian, Russian and Ukrainian wheat cultivars, but almost all of those tested in this study were red grained, and it is likely that *R* genes conferred seed dormancy in those cultivars. Combined selections using molecular markers of *R* genes and the PHS resistance alleles *TaSdr-B1a* and *TaVp-1Bc* alleles would certainly increase tolerance to PHS in red wheat cultivars.

Acknowledgments The authors are grateful to Prof. R. A. McIntosh, Plant Breeding Institute, University of Sydney, for his critical review of this manuscript. This work was supported by the Gene Transformation Projects (2011ZX08009-003, 2011ZX08002004-008), National 863 Project (2012AA101105), National Natural Science Foundation of China (31161140346), International Collaboration Project from the Ministry of Agriculture (2011-G3) and China Agriculture Research System (CARS-3-1-3).

Conflict of interest There are no conflicts of interest for this manuscript.

References

Ashikawa I, Abe F, Nakamura S (2010) Ectopic expression of wheat and barley *DOG1*-like genes promotes seed dormancy in *Arabidopsis*. Plant Sci 179:536–542

Bagge M, Xia XC, Lübberstedt T (2007) Functional markers in wheat. Curr Opin Plant Biol 10:211–216

Bailey PC, McKibbin RS, Lenton JR, Holdsworth MJ, Flintham JE, Gale MD (1999) Genetic map locations for orthologous *Vp1* genes in wheat and rice. Theor Appl Genet 98:281–284

Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. Proc Natl Acad Sci USA 103:17042–17047

Chang C, Feng JM, Si HQ, Yin B, Zhang HP, Ma CX (2010a) Validating a novel allele of *viviparous-1* (*Vp-1Bf*) associated with high seed dormancy of Chinese wheat landrace, Wanxianbaimaizi. Mol Breeding 25:517–525

Chang C, Zhang HP, Feng JM, Yin B, Si HQ, Ma CX (2010b) Identifying alleles of *Viviparous-1B* associated with pre-harvest sprouting in micro-core collections of Chinese wheat germplasm. Mol Breeding 25:481–490

Chang C, Zhang HP, Zhao QX, Feng JM, Si HQ, Lu J, Ma CX (2011) Rich allelic variations of *Viviparous-1A* and their associations with seed dormancy/pre-harvest sprouting of common wheat. Euphytica 179:343–353

Chen CX, Cai SB, Bai GH (2008) A major QTL controlling seed dormancy and pre-harvest sprouting resistance on chromosome 4A in a Chinese wheat landrace. Mol Breeding 21:351–358

Chono M, Honda I, Shinoda S, Kushiro T, Kamiya Y, Nambara E, Kawakami N, Kaneko S, Watanabe Y (2006) Field studies on the regulation of abscisic acid content and germinability during grain development of barley: molecular and chemical analysis of pre-harvest sprouting. J Exp Bot 57:2421–2434

Derera NF, Bhatt GM, McMaster GJ (1977) On the problem of pre-harvest sprouting of wheat. Euphytica 26:299–308

Flintham JE (2000) Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. Seed Sci Res 10:43–50

Groos C, Gay G, Perretant MR, Gervais L, Bernard M, Dedryver F, Charmet G (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white × red grain bread-wheat cross. Theor Appl Genet 104:39–47

Gubler F, Millar AA, Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. Curr Opin Plant Biol 8:183–187

Hattori T, Vasil V, Rosenkrans L, Hannah LC, McCarty DR, Vasil IK (1992) The *Viviparous-1* gene and abscisic acid activate the *C1* regulatory gene for anthocyanin biosynthesis during seed maturation in maize. Gene Dev 6:609–618

Hattori T, Terada T, Hamasuna ST (1994) Sequence and functional analyses of the rice gene homologous to the maize *Vp1*. Plant Mol Biol 24:805–810

He XY, He ZH, Zhang LP, Sun DJ, Morris CF, Fuerst EP, Xia XC (2007) Allelic variation of polyphenol oxidase (PPO) genes located on chromosomes 2A and 2D and development of functional markers for the PPO genes in common wheat. Theor Appl Genet 115:47–58

He XY, Zhang YL, He ZH, Wu YP, Xiao YG, Ma CX, Xia CX (2008) Characterization of phytoene synthase 1 gene (*Psy1*) located on common wheat chromosome 7A and development of a functional marker. Theor Appl Genet 116:213–221

He XY, He ZH, Ma W, Appels R, Xia XC (2009) Allelic variants of phytoene synthase 1 (*Psy1*) genes in Chinese and CIMMYT wheat cultivars and development of functional markers for flour colour. Mol Breeding 23:553–563

Hickey LT, Dieters MJ, DeLacy IH, Christopher MJ, Kravchuk OY, Banks PM (2009) Screening for grain dormancy in segregating generations of dormant × non-dormant crosses in white-grained wheat (*Triticum aestivum* L.). Euphytica 172:183–195

Himi E, Noda K (2005) Red grain colour gene (*R*) of wheat is a Myb-type transcription factor. Euphytica 143:239–242

Himi E, Mares DJ, Yanagisawa A, Noda K (2002) Effect of grain colour gene (*R*) on grain dormancy and sensitivity of the embryo to abscisic acid (ABA) in wheat. J Exp Bot 53:1569–1574

Jaiswal V, Mir RR, Mohan A, Balyan HS, Gupta PK (2012) Association mapping for pre-harvest sprouting tolerance in common wheat (*Triticum aestivum* L.). Euphytica 188:89–102

Koornneef M, Hanhart CJ, Hilhorst HWM, Karssen CM (1989) In vivo inhibition of seed development and reserve protein accumulation in recombinants of abscisic acid biosynthesis and responsiveness mutants in *Arabidopsis thaliana*. Plant Physiol 90:463–469

Kosambi DD (1944) The estimation of map distances from recombination values. Annals of Eugenics 12:172–175

Kulwal PL, Singh R, Balyan HS, Gupta PK (2004) Genetic basis of pre-harvest sprouting tolerance using single-locus and two-locus QTL analyses in bread wheat. Funct Integr Genomics 4:94–101

Kulwal P, Ishikawa G, Benscher D, Feng ZY, Yu LX, Jadhav A, Mehetre S, Sorrells ME (2012) Association mapping for pre-harvest sprouting resistance in white winter wheat. Theor Appl Genet 125:793–805

Kurup S, Jones HD, Holdsworth MJ (2000) Interactions of the developmental regulator ABI3 with proteins identified from developing *Arabidopsis* seeds. Plant J 21:143–155

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E (2004) The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8'-hydroxylases: key enzymes in ABA catabolism. EMBO J 23:1647–1656

Lagudah ES, Appels R, McNeil D (1991) The Nor-D3 locus of *Triticum tauschii*: natural variation and genetic linkage to markers in chromosome 5. Genome 34:387–395

Lan XJ, Wang JR, Wei YM, Chen GY, Jiang QT, Peng YY, Dai SF, Zheng YL (2012) Identification of seed dormancy on chromosome 2BS from wheat cv. Chinese Spring. Afr J Agric Res 7:6191–6196

Lee Y, Kende H (2001) Expression of beta-expansions is correlated with internodal elongation in deepwater rice. Plant Physiol 127:645–654

Li CD, Ni PX, 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. Funct Integr Genomics 4:84–93

Li HH, Ye GY, Wang JK (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361–374

Li HH, Ribaut JM, Li ZL, Wang JK (2008) Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. Theor Appl Genet 116:243–260

Liu SB, Bai GH (2010) Dissection and fine mapping of a major QTL for preharvest sprouting resistance in white wheat Rio Blanco. Theor Appl Genet 121:1395–1404

Liu HY, Yu X, Cui DY, Sun MH, Sun WN, Tang ZC, Kwak SS, Su WA (2007a) The role of water channel proteins and nitric oxide signaling in rice seed germination. Cell Res 17:638–649

Liu YX, Koornneef M, Soppe WJJ (2007b) The absence of histone H2B monoubiquitination in the *Arabidopsis hub1* (*rdo4*) mutant reveals a role for chromatin remodeling in seed dormancy. Plant Cell 19:433–444

Liu SB, Cai SB, Graybosch R, Chen CX, Bai GH (2008) Quantitative trait loci for resistance to pre-harvest sprouting in US hard white winter wheat Rio Blanco. Theor Appl Genet 117:691–699

Liu YN, He ZH, Appels R, Xia XC (2012) Functional markers in wheat: current status and future prospects. Theor Appl Genet 125:1–10

Liu SB, Sehgal SK, Li JR, Lin M, Trick HN, Yu JM, Gill BS, Bai GH (2013) Cloning and characterization of a critical regulator for pre-harvest sprouting in wheat. Genetics 195:263–273

Lohwasser U, Rehman Arif MA, Börner A (2013) Discovery of loci determining pre-harvest sprouting and dormancy in wheat and barley applying segregation and association mapping. Biol Plantarum 57:663–674

Ma DY, Yan J, He ZH, Wu L, Xia XC (2010) Characterization of a cell wall invertase gene *TaCwi-A1* on common wheat chromosome 2A and development of functional markers. Mol Breeding 29:43–52

Mares D, Mrva K, Cheong J, Williams K, Watson B, Storlie E, Sutherland M, Zou Y (2005) A QTL located on chromosome 4A associated with dormancy in white- and red-grained wheats of diverse origin. Theor Appl Genet 111:1357–1364

McCarty DR, Hattori T, Carson CB, Vasil V, Lazar M, Vasil IK (1991) The *Viviparous-1* developmental gene of maize encodes a novel transcriptional activator. Cell 66:895–905

McKibbin RS, Wilkinson MD, Bailey PC, Flintham JE, Andrew LM, Lazzeri PA, Gale MD, Lenton JR, Holdsworth MJ (2002) Transcripts of *Vp-1* homeologues are misspliced in modern wheat and ancestral species. Proc Natl Acad Sci USA 99:10203–10208

Miao XL, Zhang YJ, Xia XC, He ZH, Zhang Y, Yan J, Chen XM (2013) Mapping quantitative trait loci for pre-harvest sprouting resistance in white-grained winter wheat line CA 0431. Crop Pasture Sci 64:573–579

Mohan A, Kulwal P, Singh R, Kumar V, Mir RR, Kumar J, Prasad M, Balyan HS, Gupta PK (2009) Genome-wide QTL analysis for pre-harvest sprouting tolerance in bread wheat. Euphytica 168:319–329

Munkvold JD, Tanaka J, Benscher D, Sorrells ME (2009) Mapping quantitative trait loci for preharvest sprouting resistance in white wheat. Theor Appl Genet 119:1223–1235

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. J Exp Bot 52:875–876

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. Plant Cell 23:3215–3229

Oikawa T, Koshioka M, Kojima K, Yoshida H, Kawata M (2004) A role of *OsGA20ox1*, encoding an isoform of gibberellin 20-oxidase, for regulation of plant stature in rice. Plant Mol Biol 55:687–700

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. Theor Appl Genet 106:1491–1496

Parcy F, Valon C, Kohara A, Misera S, Giraudat J (1997) The *ABSCISIC ACID-INSENSITIVE3*, *FUSCA3*, and *LEAFY COTYLEDON1* loci act in concert to control multiple aspects of *Arabidopsis* seed development. Plant Cell 9:1265–1277

Rasul G, Humphreys DG, Brûlé-Babel A, McCartney CA, Knox RE, DePauw RM, Somers DJ (2009) Mapping QTLs for pre-harvest sprouting traits in the spring wheat cross 'RL4452/AC Domain'. Euphytica 168:363–378

Rehman Arif MA, Neumann K, Nagel M, Kobiljski B, Lohwasser U, Börner A (2012) An association mapping analysis of dormancy and pre-harvest sprouting in wheat. Euphytica 188:409–417

Rikiishi K, Maekawa M (2010) Characterization of a novel wheat (*Triticum aestivum* L.) mutant with reduced seed dormancy. J Cereal Sci 51:292–298

Saito S, Hirai N, Matsumoto C, Ohigashi H, Ohta D, Sakata K, Mizutani M (2004) Arabidopsis *CYP707As* encode (+)-Abscisic Acid 8′-Hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. Plant Physiol 134:1439–1449

Singh R, Matus-Cádiz M, Båga M, Hucl P, Chibbar RN (2010) Identification of genomic regions associated with seed dormancy in white-grained wheat. Euphytica 174:391–408

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Somyong S (2010) Comparative genetic analysis and fine mapping of a major preharvest sprouting QTL interval in white winter wheat. http://hdl.handle.net/1813/17215

Stone SL, Kwong LW, Yee KM, Pelletier J, Lepiniec L, Fischer RL, Goldberg RB, Harada JJ (2001) *LEAFY COTYLEDON2* encodes a B3 domain transcription factor that induces embryo development. Proc Natl Acad Sci USA 98:11806–11811

Sugimoto K, Takeuchi Y, Ebana K, Miyao A, Hirochika H, Hara N, Ishiyama K, Kobayashi M, Ban Y, Hattori T, Yano M (2010) Molecular cloning of *Sdr4*, a regulator involved in seed dormancy and domestication of rice. Proc Natl Acad Sci USA 107:5792–5797

Sun DJ, He ZH, Xia XC, Zhang LP, Morris CF, Appels R, Ma WJ, Wang H (2005) A novel STS marker for polyphenol oxidase activity in bread wheat. Mol Breeding 16:209–218

Tan MK, Sharp PJ, Lu MQ, Howes N (2006) Genetics of grain dormancy in a white wheat. Aust J Agr Res 57:1157–1165

Walker-Simmons M (1988) Enhancement of ABA responsiveness in wheat embryos by high temperature. Plant Cell Environ 11:769–775

Warner RL, Kudrna DA, Spaeth SC, Jones SS (2000) Dormancy in white-grain mutants of Chinese Spring wheat (*Triticum aestivum* L.). Seed Sci Res 10:51–60

Xi WY, Liu C, Hou XL, Yu H (2010) *MOTHER OF FT AND TFL1* regulates seed germination through a negative feedback loop modulating ABA signaling in *Arabidopsis*. Plant Cell 22:1733–1748

Xia LQ, Ganal MW, Shewry PR, He ZH, Yang Y, Röder MS (2008) Exploiting the diversity of *Viviparous-1* gene associated with preharvest sprouting tolerance in European wheat varieties. Euphytica 159:411–417

Yang Y, Zhao XL, Xia LQ, Chen XM, Xia XC, Yu Z, He ZH, Röder M (2007) Development and validation of a *Viviparous-1* STS marker for pre-harvest sprouting tolerance in Chinese wheats. Theor Appl Genet 115:971–980

Zhang HF, Zemetra RS, Liu CT (1989) Studies on preharvest sprouting resistance in winter wheat and its determination. Acta Agronomica Sinica 15:116–122

Zhang XQ, Li CD, Tay A, Lance R, Mares D, Cheong J, Cakir M, Ma JH, Appels R (2008) A new PCR-based marker on chromosome 4AL for resistance to pre-harvest sprouting in wheat (*Triticum aestivum* L.). Mol Breeding 22:227–236

Zhang CL, He XY, He ZH, Wang LH, Xia XC (2009) Cloning of *TaCYP707A1* gene that encodes ABA 8′-Hydroxylase in common wheat (*Triticum aestivum* L.). Agric Sci China 8:902–909

Zheng J, Chen F, Wang Z, Cao H, Li X, Deng X, Soppe WJJ, Li Y, Liu Y (2012) A novel role for histone methyltransferase *KYP/SUVH4* in the control of *Arabidopsis* primary seed dormancy. New Phytol 193:605–616