

Independent mis-splicing mutations in *TaPHS1* causing loss of preharvest sprouting (PHS) resistance during wheat domestication

Shubing Liu^{1,2}, Sunish K. Sehgal^{3,4}, Meng Lin², Jiarui Li⁴, Harold N. Trick⁴, Bikram S. Gill⁴ and Guihua Bai^{2,5}

¹State Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University, Tai'an 271018, China; ²Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA; ³Department of Plant Science, South Dakota State University, Brookings, SD 57006, USA; ⁴Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA; ⁵Hard Winter Wheat Genetic Research Unit, USDA-ARS, Manhattan, KS 66506, USA

Summary

Author for correspondence:

Guihua Bai

Tel: +1 785 532 1124

Email: guihua.bai@ars.usda.gov

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- Preharvest sprouting (PHS) is one of the major constraints of wheat production in areas where prolonged rainfall occurs during harvest. *TaPHS1* is a gene that regulates PHS resistance on chromosome 3A of wheat, and two causal mutations in the positions +646 and +666 of the *TaPHS1* coding region result in wheat PHS susceptibility. Three competitive allele-specific PCR (KASP) markers were developed based on the two mutations in the coding region and one in the promoter region and validated in 82 wheat cultivars with known genotypes. These markers can be used to transfer *TaPHS1* in breeding through marker-assisted selection.
- Screening of 327 accessions of wheat A genome progenitors using the three KASP markers identified different haplotypes in both diploid and tetraploid wheats. Only one *Triticum monococcum* accession, however, carries both causal mutations in the *TaPHS1* coding region and shows PHS susceptibility. Five of 249 common wheat landraces collected from the Fertile Crescent and surrounding areas carried the mutation (C) in the promoter (−222), and one landrace carries both the causal mutations in the *TaPHS1* coding region, indicating that the mis-splicing (+646) mutation occurred during common wheat domestication.
- PHS assay of wheat progenitor accessions demonstrated that the wild-types were highly PHS-resistant, whereas the domesticated type showed increased PHS susceptibility.
- The mis-splicing *TaPHS1* mutation for PHS susceptibility was involved in wheat domestication and might arise independently between *T. monococcum* and *Triticum aestivum*.

Introduction

Preharvest sprouting (PHS) in wheat, germination of kernels in a spike before harvest when prolonged rainfall occurs, causes significant losses in both yield and quality. The malady also affects worldwide production of other cereal crops, including corn, rice, barley, sorghum and millet (Imtiaz *et al.*, 2008). Many factors contribute to PHS resistance, such as seed dormancy (SD), germination inhibitory substances in chaff tissue, physical barriers to water penetration in the spike, and spike structure, etc., whereas SD is the major factor (Mares *et al.*, 2005; Baurle & Dean, 2006; Bentsink *et al.*, 2006; Sugimoto *et al.*, 2010). SD controls the timing of seed germination and provides an important strategy for seed survival in adverse environments by delaying seed germination and allowing time for seed dispersal (Fenner & Thompson, 2005).

Seed dormancy is one of the major factors involved in domestication syndrome. Originally, wild and weedy species developed SD mechanisms during evolution to survive under adverse

natural or in human-disturbed environments by selecting optimum times to germinate; therefore, most of their seeds have long dormancy. However, the domestication process has reduced the degrees of SD in many cereal crops through human selection for rapid and uniform germination and breeding activities to improve crop productivity (Harlan *et al.*, 1973). In rice, the majority of wild species possess strong SD (Vaughan, 1994), and wild and traditional rice also have higher degrees of SD than modern cultivars (Simpson, 1990; Veasey *et al.*, 2004; Wan *et al.*, 2006). Barley and oats are similar to rice (Adkins *et al.*, 1986; Gutterman *et al.*, 1996; Vanhala & Stam, 2006).

Common wheat is polyploid with three genomes (A, B, and D). *Triticum boeoticum* (A^bA^b), the A genome species, was one of the first domesticated species in the mountains of southeast Turkey and was further domesticated as a cultivated species, *Triticum monococcum* ($2n = 2x = 14$, A^mA^m), c. 13 000 yr ago (Nesbitt & Samuel, 1996). Wild tetraploid wheat *Triticum dicoccoides* arose c. 500 000 yr ago from natural hybridization between *Triticum urartu* ($2n = 2x = 14$, A^uA^u), the A genome donor, and

Aegilops speltoides ($2n=2x=14$), or a species related to it (Huang *et al.*, 2002), and was domesticated into the cultivated species *Triticum turgidum* ssp. *dicoccum* ($2n=4x=28$, AABB) c. 9000 yr ago (Nesbitt & Samuel, 1996). During the course of cultivation of domesticated tetraploid wheat *T. dicoccum* (AABB), it hybridized with diploid species *Aegilops tauschii* ($2n=2x=14$, DD) to form hexaploid wheat, *T. aestivum* ($2n=6x=42$, AABBDD) (McFaden & Sears, 1946; Dvorak, 1976; Dubcovsky & Dvorak, 2007; Matsuoka, 2011).

Different degrees of SD have been identified among modern hexaploid wheat cultivars. Seeds of most cultivars are dormant for only a few days after harvest, whereas seeds of some cultivars can be dormant for months (Greer & Hutchinson, 1945; Wverson & Hart, 1961; Simpson, 1990; Munkvold *et al.*, 2009). In cultivated tetraploid wheat, only a limited number of accessions have been evaluated for SD (Clarke *et al.*, 1994; Tavakkol-Afshari & Hucl, 2002), and a low to moderate degree of SD was identified in *Triticum durum*, *T. turgidum*, *Triticum turanicum*, *Triticum carthlicum*, and *Triticum polonicum* (Tavakkol-Afshari & Hucl, 2002). A high degree of SD was reported in Tibetan semiwild wheat accessions (AABBDD) (Lan *et al.*, 2005), but SD variation among domesticated diploid wheat (*T. monococcum*) and wild wheat species, *T. boeoticum*, *T. urartu* and *T. dicoccoides*, has not been characterized. Therefore, further characterization of SD of wheat relatives and its progenitor species may shed light on the evolution of wheat SD genes and facilitate effective manipulation of the genes to improve wheat production in adverse environments.

An appropriate degree of wheat SD is desirable to avoid seed germination before or during harvest in summer, but SD needs to break down after a period of storage so seeds can germinate uniformly after sowing in the fall. Thus, proper SD can be considered as an adaptive trait that optimizes germination timing, one of key demands in wheat breeding program (Bewley, 1997). In regions where PHS is a critical problem, a prolonged wet period occurs during harvest seasons, and growing cultivars with appropriate SD is important to improve wheat production. Therefore, identifying dormancy genes to develop cultivars with an appropriate degree of dormancy is vital to combating PHS in wheat. To date, many quantitative trait loci (QTLs) for SD or PHS resistance have been mapped on almost all wheat chromosomes (Groos *et al.*, 2002; Mori *et al.*, 2005; Ogbonnaya *et al.*, 2008; Munkvold *et al.*, 2009). More recently, a *MOTHER OF FT AND TFL1* gene was reported to be involved in seed germination in wheat. A single nucleotide polymorphism (SNP) in the promoter region (-222) was reported to be associated with SD (Nakamura *et al.*, 2011). In another study, a previously mapped QTL on 3AS for PHS resistance was cloned from a US white wheat cv Rio Blanco and designated as *TaPHS1* (Liu *et al.*, 2013). Two mutations at the positions +646 and +666 of *TaPHS1* caused a truncated transcript that resulted in the loss of SD and PHS resistance in susceptible wheat. This study was designed to: develop user-friendly diagnostic SNPs that can be used in breeding for PHS resistance in wheat; study allelic variation among diploid, tetraploid and hexaploid domesticated and wild wheat germplasm to determine

the impact of domestication on the sequence evolution of *TaPHS1*; and elucidate the relationship between the *TaPHS1* sequence variation and PHS resistance in wheat.

Materials and Methods

Plant materials

A total of 327 accessions of wild and domesticated wheat progenitors, including 39 *T. boeoticum* (A^bA^b), 57 *T. monococcum* ($A^m A^m$), 23 *T. urartu* ($A^u A^u$) and 208 tetraploid (AABB) accessions, and 249 wheat landraces originated from Iran, a part of the Fertile Crescent, were kindly provided by the USDA National Small Grain Collection (Aberdeen, ID, USA), and the Wheat Genetics Resource Center at Kansas State University (Manhattan, KS, USA). The 208 tetraploid (AABB) accessions include 59 *T. dicoccoides*, the only wild-type of tetraploid wheat, and 149 domesticated accessions that include 93 *T. durum* collected from different countries and different durum growing states of the US, five *T. turgidum*, five *T. carthlicum*, 38 *T. dicoccum*, three *T. polonicum*, four *T. tranicum* and one *T. paleocolchicum* accessions. The accession number and geographical origin of these accessions are listed in Supporting Information Table S1. A total of 82 bread wheat cultivars sequenced for *TaPHS1* (Liu *et al.*, 2013) were used to validate KASP SNP assays.

PHS resistance evaluation in wheat progenitors and cultivars

The wheat species accessions were evaluated for PHS resistance in the glasshouses at Kansas State University. Experiments were conducted twice in the glasshouse in the fall of 2012 and 2013 with two replications per experiment. Three to four spikes per accession were harvested from each replication at physiological maturity as characterized by loss of green color on the spike. Harvested spikes were air-dried for 5 d in the glasshouse at $25 \pm 5^\circ\text{C}$, and stored in a freezer at -20°C to maintain SD. After all accessions were harvested, the spikes from the freezer were dried in the glasshouse for another 2 d before sprouting test, as described previously (Liu *et al.*, 2008). Sprouting assays were conducted in a moist chamber for 7 d at $23 \pm 2^\circ\text{C}$ with 100% humidity. Terracolor (Southern Agricultural Insecticide Inc., Rubonia, FL, USA) solution (0.025%) was sprayed on the wheat spikes using a hand mister to control fungal contamination when needed. For accessions with brittle rachis, a transparent plastic bag was placed over the spikes after flowering and sealed at the bottom to prevent dropping of shattered spikelets. The harvested spikes were dried in an envelope for drying, stored at -20°C , and then placed in a Petri dish in a moist chamber for sprouting evaluation following the protocol described earlier. Twelve wheat cultivars (Chinese Spring, Rio Blanco, NW97S186, NW97S078, Zenkujji, Trego, CO03W239, CO04W210, Karl92, AUS1408, Tutoumai A and Jagalene) with known PHS resistance were used as controls. PHS resistance of an additional 82 wheat cultivars was reported previously (Liu *et al.*, 2013).

DNA extraction, sequencing and data analysis

Procedures for wheat tissue collection and DNA extraction were described previously (Liu *et al.*, 2008). An 850 bp fragment of *TaPHS1*, including exons 1, 2 and 3 and introns 1, 2 and part of 3, was amplified and sequenced in 32 accessions, including four of *T. boeoticum*, two of *T. urartu*, four of *T. monococcum*, two of *T. dicoccoides*, one of *T. dicoccum*, two of *T. carthlicum*, two of *T. durum*, one of *T. turanicum*, two of *T. polonicum*, one of *T. paleocolchicum* and 11 of *T. aestivum*, using the primer pair 5'GTGAGAGACACGCAAGAACG3' and 5'TTTGTACAGGTCGTCTGGTGG3'. The PCR products were sequenced using big dye-terminator chemistry after they were cleaned up by adding 2 U shrimp alkaline phosphatase and 0.4 U exonuclease I, and incubated at 37°C for 1 h and 75°C for 15 min to eliminate enzyme activity. DNA sequence data were checked for sequencing errors using 'Sequencer' software (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were aligned using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). All statistical analyses were done using SAS for Windows version 9.0 (SAS, Cary, NC, USA).

Conversion of SNP markers to KASP assay

To develop SNP markers that can effectively differentiate three contrasting alleles of *TaPHS1* in the promoter (−222) and in the coding region (+646 and +666), the SNPs were converted to KASP assays following instruction from the manufacturer (http://www.kbioscience.co.uk/reagents/KASP_manual.pdf). The primer sequences for the *SNP.646* KASP assay included GGTGGAACAGATGCAACTAAAGG as the *SNP.646*FAM forward primer, GGTGGAACAGATGCAACTAAAGA as the *SNP.646*HEX forward primer, and GTGAGT GTTATATGAAACTAATGATCCATT as the *SNP.646* reverse primer; the primer sequences for the *SNP.666* KASP assay include GTGAGTGTATATGAAACTAATGATCCATTT as the *SNP.666*FAM forward primer, GTGAGTGTATATGAAACTAATGATCCATTA as the *SNP.666*HEX forward primer, and ACCGGGTGGAACAGATGCAACTAAA as the *SNP.666* reverse primer; the primer sequences for the *SNP.-222* KASP assay include CCATGCACGCATCAGCGATCGAT as the *SNP.-222*FAM forward primer, CCATGCACGCATCAGCGATCGAC as the *SNP.-222*HEX forward primer, and CCAACGACGACCTGTACAAA as the *SNP.-222* reverse primer. The KASP assays were validated in a collection of 82 wheat cultivars, and then used to analyze the 327 wheat progenitor accessions of different wheat species and the 249 Iranian common wheat landraces.

A 6 µl KASP assay PCR mix includes 3 µl of 2× reaction mix (LGC Genomics, Beverly, MA, USA), 0.106 µl of primer assay mix and 3 µl genomic DNA at a concentration of *c.* 15 ng µl^{−1}. The initial PCR mix and post-PCR fluorescent endpoint readings were carried out using an ABI 7900HT Real-Time PCR System (Life Technology, Grand Island, NY, USA). Thermal cycling conditions for PCR were determined according

to the manufacturer's instructions (http://www.kbioscience.co.uk/reagents/KASP_manual.pdf).

Results

KASP assays for *TaPHS1* and their variations in wheat cultivars

The SNPs (−222, +646 and +666) of *TaPHS1* were successfully converted into KASP assays and validated in a panel of 82 wheat cultivars that have sequence data at the SNPs (Liu *et al.*, 2013). The KASP assays generated haplotypes that completely matched with the sequence data from all 82 accessions. At the positions +646 and +666, haplotype G-A in 66 cultivars was associated with long dormancy or PHS resistance, and A-T in 16 cultivars was associated with short dormancy or PHS susceptibility across the 82 accessions (Fig. 1). For the SNP (−222) in the promoter region, C presents in 20 cultivars and T in 62 cultivars, however, the association between the PHS resistance and the SNP was not significant. Thus, the KASP assays for the SNPs in the coding region can effectively distinguish resistance and susceptibility alleles of *TaPHS1* in the panel and can be used for diagnosis of *TaPHS1* in marker-assisted breeding.

Sequence variations in *TaPHS1* in the wheat progenitors

The three KASP assays were used to determine genotypes of 327 wheat progenitor accessions. All the accessions carried the T allele at −222, indicating that no mutation occurs at this locus for all progenitor accessions tested. Sequence variations, however, were observed in the two SNPs at +646 and +666 (Tables 1, S1).

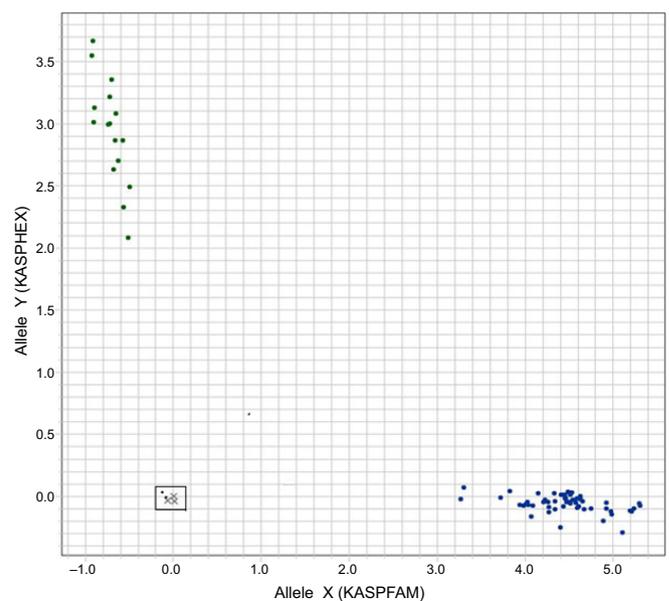


Fig. 1 KASP assay of single nucleotide polymorphism (SNP) *TaPHS1.646* in 82 wheat accessions. Allele X (KASPFAM, blue) shows the G nucleotide, and allele Y (KASPHEX, green) shows the A nucleotide. The black dots and × in the rectangular box are water and blank controls, respectively.

Among these progenitor accessions, only PI 168804, a domesticated diploid wheat *T. monococcum* (A^mA^m), had the mis-splicing mutation (A) at +646 and the stop codon mutation (T) at +666 of *TaPHS1* that together determine a PHS-susceptible wheat genotype. All other accessions have the wild-type G at +646, although mutation (T) was found at +666 in some diploid and tetraploid wheat accessions. All 93 *T. durum* accessions showed the PHS-resistant haplotype G-A (Table S1). Two haplotypes identified in *T. monococcum* were G-T (39 accessions) and the wild-type G-A (18 accessions). In *T. boeoticum* (A^bA^b), the G-T haplotype is predominant with wild-type haplotype G-A in only one accession; by contrast, *T. urartu* (A^uA^u) and tetraploid accessions had mainly the G-A haplotype (wild-type), with only one accession each having G-T haplotype in *T. urartu*, *T. dicoccum* and *T. turanicum* (Table S1).

Sequence variations of *TaPHS1* in common wheat landraces

A total of 249 wheat landraces from Iran, a part of the Fertile Crescent, were evaluated for the three SNPs in *TaPHS1*. Among 175 accessions that generated scorable data for SNP at -222, only five landraces carry the mutant allele C, whereas 170 carry the wild-type allele T. The C/T allele ratio is 5/170, which is lower than that in cultivated cultivars (20/82). For the SNP at +646, the mutant allele A appeared only in one landrace (PI 623421) and all other 243 landraces carried the wild-type allele G, while for the SNP at +666, 22 landraces carried the mutant allele T and 225 carried the wild-type allele A (Tables 1, S1). One landrace carried double mutations at the two SNPs with the A-T haplotype, and the ratio of A-T haplotype to other haplotypes is much lower in landraces (1/249) than in the cultivars tested (16/82). That mis-splicing mutation (+646) was only detected in *T. monococcum*, common wheat landraces and cultivars (Tables 1, S1) indicates that this mutation for increased PHS susceptibility evolved independently in *T. monococcum* and *T. aestivum* during wheat domestication (Fig. 2).

Table 1 Genotype at +646 and +666 of the wheat progenitors and landraces

Ploidy	Species	Genome	Accession no.	+646	+666	Accession no.	Domestication type
Diploid	<i>Triticum urartu</i>	A ^u A ^u	23	G	A	22	Wild
	<i>Triticum boeoticum</i>	A ^b A ^b	39	G	T	1	Wild
	<i>Triticum monococcum</i>	A ^m A ^m	57	G	A	38	Domesticated
Tetraploid	<i>Triticum dicoccoides</i>	AABB	59	G	A	18	Wild
	<i>Triticum turgidum</i>	AABB	149	G	A	38	Domesticated
				G	T	1	
Wheat landraces*	<i>Triticum aestivum</i>	AABBDD	249	G	A	59	Domesticated
				G	T	18	
				A	T	1	
Wheat cultivars	<i>T. aestivum</i>	AABBDD	82	G	A	225*	Domesticated
				A	T	16	

*The difference between total accession number and number of accessions with data is the number of missing data.

PHS resistance in hexaploid wheat cultivars and its progenitors

Preharvest sprouting resistance data were successfully collected from 82 hexaploid wheat cultivars and 197 of the 327 diploid and tetraploid progenitor accessions, including 28 of *T. boeoticum*, 17 of *T. urartu*, 30 of *T. monococcum*, seven of *T. dicoccoides*, and 115 of cultivated tetraploid (AABB). The wild accessions showed a significantly ($P < 0.01$) higher amount of PHS resistance than the domesticated accessions (Fig. 3). All *T. boeoticum* accessions showed no germinated kernels from all tested spikes. The same was true for most *T. urartu* and *T. dicoccoides* accessions; only one *T. urartu* accession showed some sprouted kernels in spikes. In the domesticated accessions, higher sprouting rates in spikes were observed in *T. monococcum*, *T. durum* and other tetraploid accessions, and *T. aestivum*. Among them, *T. aestivum* had the highest sprouting rates, *T. monococcum* had the lowest, and tetraploid wheat was intermediate (Fig. 3), indicating that SD decreased during wheat evolution and domestication.

Relationship between the causal mutations in *TaPHS1* and PHS resistance

In *T. monococcum*, only PI 168804 had the mis-splicing mutation G-to-A at +646 and the A-to-T mutation at +666 of *TaPHS1* and exhibited a relatively higher sprouting rate (43.9%) than all other *T. monococcum* accessions which had a low average sprouting rate of 8.2% (range 0–32%). This result indicates that concurrent occurrence of the mis-splicing and premature stop codon resulted in a significantly increased sprouting rate in PI 168804 ($P < 0.01$). However, some other *T. monococcum* accessions (PI 167627, PI 190939, PI 168805) also showed some sprouting, but did not share the *TaPHS1* haplotype with PI 168804. In addition, all the *T. monococcum* accessions had the same genotype T at -222. These results suggest that loci other than *TaPHS1* might affect SD in domesticated diploid wheat. Other mutations at -266 and +646 were not detected in the tested

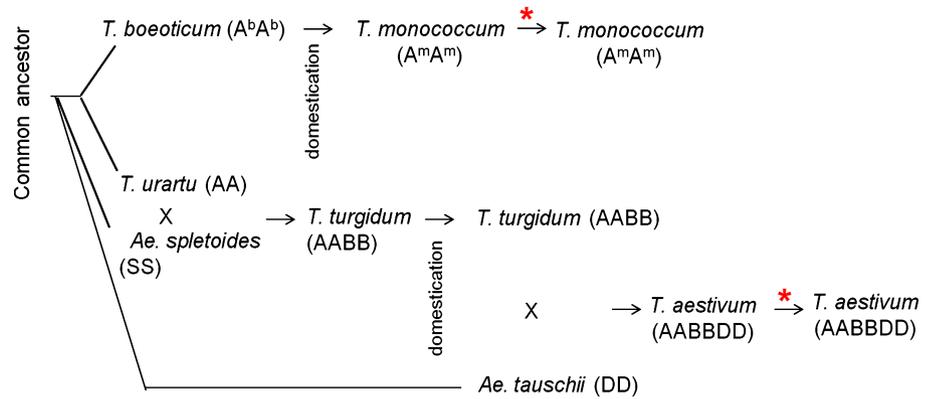


Fig. 2 Origin of the mis-splicing mutation of *TaPHS1* in wheat progenitors. The mis-splicing mutation occurred in *Triticum monococcum* and *Triticum aestivum* independently (marked by a red asterisk). Genera beginning with *T.* are *Triticum* and those with *Ae.* are *Aegilops*.

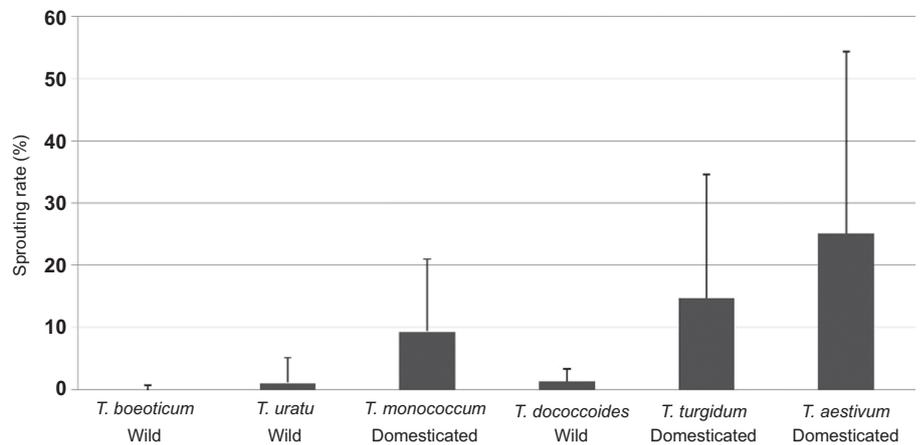


Fig. 3 Sprouting rate in wild and domesticated wheat (*Triticum*) accessions. Error bars, +SD.

wild and domesticated tetraploid accessions. In *T. aestivum*, 16 accessions carry the mutations at both SNPs in the coding region with significantly decreased SD ($P < 0.0001$) (Table 1; Fig. 3).

Sequence diversity of *TaPHS1* coding region in wheat and its progenitors

With further sequencing of the 850 bp fragment of *TaPHS1* harboring the two causal mutations in the coding region of *TaPHS1* in 32 selected accessions, 23 SNPs or InDels were detected. Cluster analysis of these accessions identified two clearly separated groups: group I includes four PHS-susceptible wheat cultivars (CO04W239, NW97S078, CO03W210 and NW97S186), four *T. monococcum* accessions, and four *T. boeoticum* accessions. Most of these accessions showed the A-T or G-T haplotype at +646 and +666 of *TaPHS1* (Fig. 4). The *T. monococcum* accession PI 168804 with the mis-splicing and premature stop codon mutations (A-T) was clustered in this group. Group II includes two *T. urartu*, all the tetraploid wheat accessions, and seven PHS-resistant wheat cultivars, including Chinese Spring, Zen, Rio Blanco, Karl 92, Trego, Jagalene and AUS1408, and all these accessions showed the G-A haplotype at +646 and +666 of *TaPHS1*.

Discussion

Many traits were selected during wheat cultivation to meet human needs, including increased seed size, shattering and

increased productivity, and decreased SD (Simpson, 1990; Meyer & Purugganan, 2013). Long SD was required for wild wheat to survive in harsh environments, but it is no longer essential for survival of domesticated wheat under human cultivation; indeed, short SD facilitates uniform and quick germination after planting to ensure the uniform and quick establishment of seedlings necessary for high productivity (Bewley, 1997; Veasey *et al.*, 2004). Wheat ancestors should have longer SD (higher PHS resistance) than most of their cultivated descendants (Simpson, 1990; Matsuoka, 2011; Meyer *et al.*, 2012), and dormancy duration of cultivated wheat needs to be diversified to meet the needs of producers in different environments. Results from the current study fully support this assumption and show that cultivated wheat had the most distinct SD for different cultivars ranging from long to short SD, with most accessions having shorter SD than their wild progenitors (Fig. 3).

Among wild wheat progenitors such as *T. boeoticum*, *T. urartu* and *T. dicoccoides*, most accessions are highly PHS-resistant with no or low sprouting, whereas some showed sprouting, but only a relatively low rate. Among the cultivated wheat species, *T. monococcum*, tetraploid and hexaploid wheat, a wider range of sprouting rates were observed, from a high rate of sprouting to low or no sprouting in a spike. The hexaploid wheat and *T. durum* accessions have the highest mean sprouting rates, indicating diversification of SD during cultivated species formation and domestication (Greer & Hutchinson, 1945; Clarke *et al.*, 1994; Tavakkol-Afshari & Hucl, 2002). Shuttle breeding and

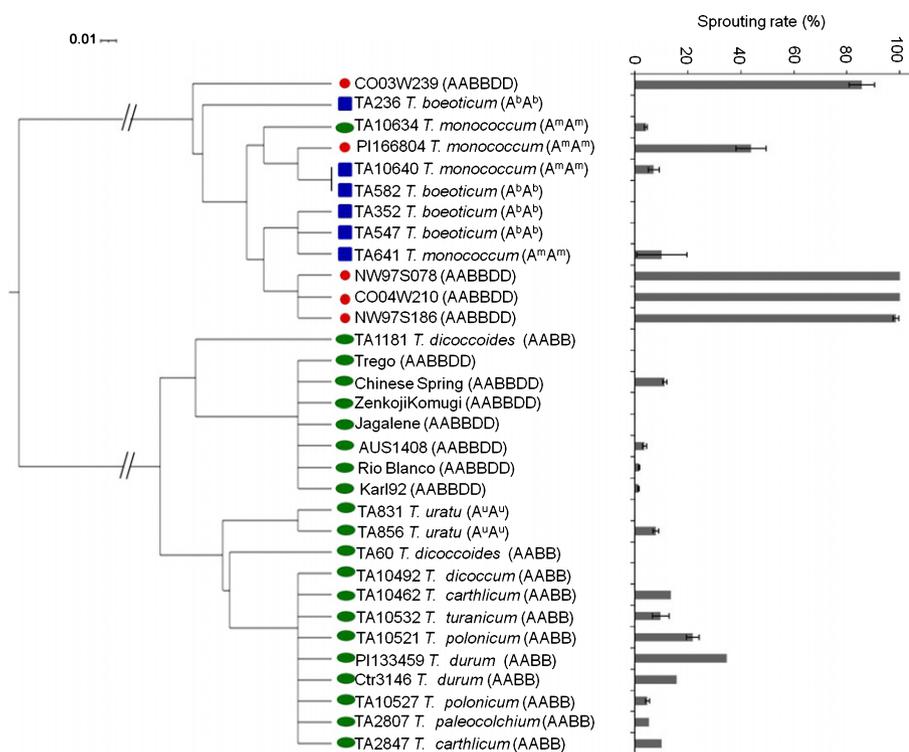


Fig. 4 Cluster analysis to show nucleotide variation at +646 and +666 and genetic relationship among wheat and its progenitor accessions based on sequence of *TaPHS1*. Red circle, A-T; blue square, G-T; green oval, G-A haplotype at +646 and +666 of *TaPHS1*. Sprouting rates (+ SD) of these accessions are shown in a bar graph on the right.

multiple generations yr^{-1} in modern wheat breeding can also contribute to the diversification of SD and the decrease of SD in cultivars, because these breeding approaches require short or no SD between generations, and only lines with short or no SD can be selected during the breeding process. Breeders might select for different genes or different alleles for one gene that reduce dormancy in different regions to meet their specific needs and preferences and to adapt wheat to adverse ecological growing environments (Bewley, 1997; Fenner & Thompson, 2005; Meyer & Purugganan, 2013).

Isolation of genes that underlie domestication and diversification traits provides an opportunity to examine how these genes are involved in the evolution of crop species. The A genome of all tetraploid and hexaploid wheat originated from the same wild ancestors, *T. urartu* (Matsuoka, 2011) (Fig. 2) and *TaPHS1*, involved in domestication was recently cloned from a hexaploid wheat for PHS resistance (Nakamura *et al.*, 2011; Liu *et al.*, 2013); therefore, investigation of the evolution of causative mutation of *TaPHS1* among these species and their ancestors can reveal the evolutionary process of genes involved in domestication syndrome (Sweeney *et al.*, 2007).

After screening the three candidate causative SNPs in *TaPHS1* across the wheat progenitor accessions, we found the variations for two SNPs at +646 and +666 of the gene coding region. The prevailing combinations of the two SNPs at +646 and +666 of *TaPHS1* are different among the three diploid progenitors (A genome), with G-A (wild type) in *T. urartu* and G-T in *T. boeoticum* and *T. monococcum*. Thus, the SNP (G) at +646 of *TaPHS1* remained the same in almost all accessions from the three species except in PI 168804, a *T. monococcum* accession; however, the SNP at +666 was changed from nucleotide A to

nucleotide T in many accessions from these species, and the change occurred early in the A genome ancestor, *T. urartu*, with one out of 23 samples of *T. urartu* accessions having T (Table 1). It is interesting to note that almost all *T. boeoticum* accessions carry T at +666, whereas more domesticated *T. monococcum* accessions carry A, which may be the consequence of human selection during domestication from *T. boeoticum* to *T. monococcum*, and nucleotide A might have some selection advantage under certain wheat-growing environments (Li & Gill, 2006; Jin *et al.*, 2008; Purugganan & Fuller, 2009). As in *T. urartu*, A to T mutation occurred rarely at +666 of *TaPHS1* in tetraploid wheat. The mutation was found in only one accession in each of two species after 208 accessions from eight species were screened (Tables 1, S1), which differs from *T. boeoticum* and *T. monococcum*. Among the 208 accessions screened, 93 were cultivated durum wheat and had the wild-type allele A. *T. urartu* is the A genome donor of all tetraploid species, including *T. durum* wheat (Dvorak, 1976; Dubcovsky & Dvorak, 2007; Matsuoka, 2011), and therefore *T. urartu* with the G-A haplotype (wild-type) was the most likely donor of the A genome of tetraploid wheat, which is supported by the evidence that most *T. urartu* accessions screened are wild-type (G-A haplotype), with the G-T haplotype being the only one exception. However, we cannot exclude the possibility that the G-T haplotype *T. urartu* was involved in the formation of tetraploid wheat, because only a limited number of *T. urartu* accessions were screened in this study, but data from *T. dicoccoides* provide some information to support this hypothesis, because 59 *T. dicoccoides* accessions screened were all G-A haplotype. In the 149 cultivated accessions screened, only two showed the G-T haplotype, suggesting that A to T

mutation occurred at position +666 of *TaPHS1* in cultivated tetraploid wheat during domestication.

Domesticated tetraploid accessions had higher sprouting rates than the wild-type, *T. dicoccoides*. The mutation at +646 was not detected in the 208 tetraploid wheat accessions. *T. durum* is the second most cultivated wheat species. We screened a worldwide collection of *T. durum* in this study (Tables 1, S1) and did not find the mutated susceptible haplotype of *TaPHS1* (A-T type) in the coding region. Most *T. durum* accessions tested are dormant, but some showed 100% germination, as in some hexaploid wheat. The loss of SD in these accessions is unlikely to be the result of the causative mutation identified in Rio Blanco (Liu *et al.*, 2013), but it may be the result of different causal SNPs in *TaPHS1*, different genes, or different mechanisms controlling SD.

The mutation at +646 that caused the mis-splicing of *TaPHS1* presents only in domesticated species, diploid *T. monococcum* and hexaploid landraces and cultivars (Table 1); thus, this mutation occurred later than the mutation at +666. This mutation leads to phenotypic change (loss of SD) in both *T. monococcum* (PI 168804) and *T. aestivum* (Fig. 2). Because *T. monococcum* was not the ancestor of *T. aestivum* and tetraploid wheat does not have the functional mutation, the mis-splicing mutation could be an independent event between *T. monococcum* and hexaploid wheat. It might also be possible that the mutation in hexaploid wheat was from an ancient introgression of the *Taphs1* from *T. monococcum*, because spontaneous hybridization and introgression are observed between bread wheat and wild and weedy relatives (Hegde & Waines, 2004; Zaharieva & Monneveux, 2005). Some studies indicated that possible introgression happened between *T. monococcum* and *T. urartu* (Fricano *et al.*, 2014); however, chance of introgression between *T. monococcum* and hexaploid wheat is very low as a result of the hybridization barrier. Direct evidence to support introgression between *T. monococcum* and hexaploid wheat is not available.

For the SNP at position -222 of the promoter region, only nucleotide T was identified in diploid and tetraploid wheat progenitors, and therefore T is the wild-type for the SNP. Nucleotide C in the SNP is a mutant and presents only in some common wheat landraces and cultivars at a ratio of 5/170 (C/T) in landraces and 20/82 (C/T) in cultivars. This mutation appeared during common wheat domestication with an increased frequency in cultivars and was hypothesized to be associated with long dormancy or PHS resistance (Nakamura *et al.*, 2011). The phenotypic data from this study indicated that most of the wheat progenitor accessions were PHS-resistant and that domesticated wheat had higher sprouting rates than their wild-type, which contradicts to the hypothesis. Thus the SNP in the promoter region may not be a major causative SNP of *TaPHS1* for PHS resistance in most wheat.

In cluster analysis, four *T. monococcum* accessions, including the one susceptible accession with A-T (PI168804), and four *T. boeoticum* accessions were clustered together with four susceptible hexaploid wheat cultivars (Fig. 4), whereas all other accessions were grouped with PHS-resistant hexaploid wheat cultivars.

This result suggests that *TaPHS1* sequences might have co-evolved between the two species under similar selection pressure. Growing wheat in different environments that require different degrees of SD might be the driving force behind selection and diversification of these alleles (Shomura *et al.*, 2008; Meyer & Purugganan, 2013).

The KASP assay is a quick and cost-effective genotyping assay for single SNP analysis (Terracciano *et al.*, 2013; Semagn *et al.*, 2014) that has been applied successfully in polyploids such as wheat (Allen *et al.*, 2011) and cotton (Byers *et al.*, 2012); thus, it is a useful SNP genotyping platform for marker-assisted breeding. In this study, the two causal mutations that result in the non-functional transcript of *TaPHS1* were successfully converted into KASP markers, *SNP.646* and *SNP.666*. The genotypic data from the two KASP assays completely coincided with the data from sequencing results reported previously (Liu *et al.*, 2013), which validated the robustness of the KASP assays. Thus, the KASP markers are diagnostic indicators of the causal mutations responsible for the change in wheat SD as a result of *TaPHS1* and can be used effectively for marker-assisted selection for *TaPHS1* in wheat breeding.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 The accession number, geographical origin and genotype at –222, +646 and +666 of *TaPHS1* of wheat progenitors and landraces

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