RESEARCH ARTICLE

# Molecular survey of *Tamyb10-1* genes and their association with grain colour and germinability in Chinese wheat and *Aegilops tauschii*

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## Abstract

To investigate allelic variation of *Myb10-1* genes in Chinese wheat and to examine its association with germination level in wheat, a total of 582 Chinese bread wheat cultivars and 110 *Aegilops tauschii* accessions were used to identify allelic variations of three *Myb10-1* genes. Identification results indicated that there is a novel *Tamyb10-B1* allele, designated *Tamyb10-B1c*, in the five Chinese landraces. The *Tamyb10-B1c* possibly has a large deletion including *Tamyb10-B1* gene. There are three novel *Tamyb10-D1* alleles (*Aetmyb10-D1c*, *Aetmyb10-D1d* and *Aetmyb10-D1e*) that were discovered in *Aegilops tauschii*. Of them, *Aetmyb10-D1c* allele possessed a 104-bp deletion and this resulted in a frame shift in the open reading frame of the *Aetmyb10-D1* gene. AETMYB10-D1d and AETMYB10-D1e proteins possessed three and two different amino acids when compared with TAMYB10-D1b protein, respectively. Association of *Tamyb10-1* allelic variation with grain germination level indicated that all five allelic combinations with red grains showed a significantly higher GP (germination percentage) and GI (germination index) values than those of white-grained *Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a* genotype after storing it for one year. Moreover, the *Tamyb10-A1b/Tamyb10-B1c/Tamyb10-D1b* genotype possesses the significantly highest GP and GI among the six different *Tamyb10-1* combinations. This study could provide useful information for wheat breeding programme in terms of grain colour and germination level.

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## Introduction

In wheat, grain with low level dormancy may directly germinate in spikes of plants before harvesting under wet-weather conditions. Therefore, grain dormancy is deterrent to pre-harvest sprouting (PHS) and the level of grain dormancy can be used for the assessment of PHS tolerance (Rehman-Arif *et al*. 2012; Lohwasser *et al.* 2013). Grain colour of wheat, influence the flour brightness by contaminating the red pigment in the milling process (Warner *et al.* 2000; Groos *et al.* 2002; Himi *et al.* 2002). Red grain colour of wheat has been associated with the development of grain dormancy (Himi *et al.* 2002), thereby to PHS tolerance. Compared to red-grained wheat, white-grained wheat is possibly susceptible to PHS in rain-affected area worldwide (Kottearachchi *et al.* 2008). Himi *et al.* (2002) found that at harvest the red-grained near-isogenic lines showed higher levels of grain dormancy than the white-grained near-isogenic lines. The association between dormancy and red pigmentation of the grain is likely due to a pleiotropic effect of the gene(s) controlling grain colour (Flintham 2000; Warner *et al.* 2000; Himi *et al.* 2002). The pigment contributing to wheat grain colour is synthesized through the pathway of flavonoid synthesis (Grotewold *et al.* 1994). The grain colour of wheat has been proven to be mainly controlled by the *R-1* (red) genes that is located on the chromosome 3 (3AL, 3BL and 3DL) (Li *et al*. 2010). A single *R-1* gene was able to upregulate four genes in the flavonoid biosynthesis pathway, suggesting that the *R-1* gene is a transcription factor (Himi and Noda 2005). Several studies have indicated that *R-1* genes could enhance grain dormancy to certain extent (Flintham 2000; Himi *et al*. 2002, 2005; Warner *et al*. 2000) by helping to accumulate germination inhibitors.

The red pigment of the grain coat in wheat is composed of catechin and proanthocyanidin synthesized through the flavonoid pathway (Winkel-Shirley 2001; Lepiniec *et al*. 2006). Himi *et al.* (2005) isolated three myb-type transcription

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factors that were transcriptional activators of the flavonoid biosynthesis genes and that differ in expression and structure in parallel with allelic differences at the *R-1* loci. These genes encode R2R3-type MYB domain proteins that act as a key determinant for PA accumulation in developing seed (Nesi *et al*. 2001). Subsequently, *Tamyb10-1* genes were cloned from bread wheat and cultivars with wild-type *Tamyb10-1* gene showed white grains (Himi *et al.* 2011). However, alternation of at least one of three *Tamyb10-1* genes would result in red-grained wheat cultivars.

As a secondary origin centre of bread wheat, China holds a highly diverse stock of wheat germplasm (Chen *et al*. 2006). With regard to grain colour, red-grained wheat cultivars occupied predominantly higher percentage than that of white-grained wheat cultivars, whereas white-grained wheat cultivars are more preferable in Chinese wheat market. The purpose of this study was to investigate the distribution of *Tamyb10-1* genes in Chinese winter wheat cultivars, and to uncover the novel *Tamyb10-1* alleles in *Ae. tauschii*, and to search for the relatively superior *Tamyb10-1* genotypes on germination level.

## Materials and methods

### *Plant material*

The 582 bread wheat cultivars (table 1 in electronic supplementry material at http://www.ias.ac.in/jgenet), including landraces, historical cultivars and currently popular cultivars, were planted at the Zhengzhou Scientific Research and Education Center of Henan Agricultural University according to local management practices in 2011–2012 cropping seasons. All cultivars grew well with the aid of supporting nets. The genotypes were collected from 10 provinces of China, including Henan, Hebei, Shandong, Shaanxi, Anhui, Jiangsu, Beijing, Heilongjiang, Jilin and Liaoning. Of these, 372 cultivars played an important role in wheat breading programme of the Yellow and Huai valley of China. The remaining 210 cultivars were unique landrace since they were collected from various parts of China in the early 1950s and remained as unselected populations for wheat quality since an intensive selection for quality improvement did not start until the late 1990 s (Chen *et al*. 2006).

The 110 *Ae. tauschii* accessions from Xinjiang, Henan, Shaanxi etc., were kindly provided by Prof. Jia Jizeng from Chinese Academy of Agricultural Sciences and Prof. Li Shuoping from Henan University.

### *Investigation of grain germination percentage and germination index*

Of the Chinese cultivars surveyed, 194 were planted in 2011–2012 and 2012–2013 cropping seasons for investigation of grain germination percentage and germination index due to their diverse *Tamyb10-1* genotypes.

All 194 cultivars and 110 *Ae. Tauschi* accessions were stored for one year before they were used to identify their germination levels. One-hundred intact grains from each wheat cultivar were randomly selected to test their germination levels. Germination was defined as pericarp rupture over the embryo. After storing for one year the germination percentage (GP) and germination index (GI) (under the condition of RH 35% and temperatures 25°C) were calculated according to the following formulae (Walker-Simmons 1988; Himi *et al.* 2002):

$$\mathrm{GI} = (7 \times n_1 + 6 \times n_2 + 5 \times n_3 + 4 \times n_4 + 3 \times n_5 + 2 \times n_6 + 1 \times n_7)/(7 \times 100).$$

$$\mathrm{GP} = M/100.$$

Where $n_1$, $n_2$, ..., $n_7$ are the number of grains that germinated on the first, second and subsequent days until the seventh day, respectively. $M$ indicates the total grains that germinated during seven days.

### *Identification of Myb10-1 alleles in bread wheat and cloning of Myb10-D1 in Ae. tauschii*

Genomic DNA from three kernels of each bread cultivar was extracted from pulverized kernels following the method of Chen *et al.* (2011). Allele-specific primers in table 1 were used to identify *Tamyb10-1* alleles (Himi *et al.* 2011). Further, two markers T3B-2 and T3B-3 (table 1) were developed based on sequences of *Tamyb10-B1* gene of Chinese Spring (AB191459.1) and *Tamyb10-B1* gene for myb-related protein of Norin 61 (AB599722.1) (Himi *et al.* 2011), to more precisely identify *Tamyb10-B1a* and *Tamyb10-B1b* alleles.

Genomic DNA of *Ae. tauschii* surveyed was extracted from two-month seedlings following the method of Xia *et al.* (2005). Genome-specific primers T3D-2 (table 1) of *Tamyb10-D1* were designed to clone *Tamyb10-D1* gene in *Ae. Tauschii* according to published sequences of AB191460 against AB191458, AB599721, AB191459 and AB599722 from NCBI. Then two codominant molecular markers (T3D-3 and T3D-4 in table 1) were developed to identify polymorphism of *Tamyb10-1* gene in *Ae. tauschii*.

PCR amplification was performed in a thermal cycler and PCR reactions were conducted in a 25 $\mu$L volume containing 100 ng of genomic DNA, 10 pmol of each primer, 250 $\mu$mol of each dNTP, 1× reaction buffer (50 mmol of KCl, 10 mmol of Tris-HCl, 1.5 mmol of $MgCl_2$, pH 8.3), and 2.0 units of *Taq* DNA polymerase (Promega, Madison, USA). PCR amplification conditions used were: one cycle of 94°C for 5 min; followed by 35 cycles of 94°C for 30 s, 52–64°C (detailed annealing temperature of each primer set is presented in table 1) for 30 s and 72°C for1.5 min; and a final 10-min elongation at 72°C. PCR products were analysed and separated on 1.5–2.5% (w/v) agarose gels, stained with ethidium bromide, and visualized with UV light.

### *DNA sequencing*

PCR products were purified using Quick DNA Extraction kit (Takara, Japan) and ligated into pGEM-T Easy vector and transformed into competent cells of the *Escherichia coli*

**Table 1.** PCR primers used for identification of *Tmyb10-1* alleles in bread wheat and *Ae. tauschii*.

| Name | Allele | Forward primer | Reverse primer | Annealing Temp. (°C) | Fragment size (bp) | Reference |
|---|---|---|---|---|---|---|
| T3A-1 | *Tamyb10-A1b* | CTATGTGGATGGCCTTGGAT | CTACCAGCTCGTTTGGGAAG | 64 | 665 | Himi *et al.* (2011) |
| T3A-2 | *Tamyb10-A1a* (CS type) | TTTCAATCGAGTGGGCATAA | CCTGACGATGAGCTCCTCTT | 61 | 536 | Himi *et al.* (2011) |
| T3A-3 | *Tamyb10-A1a* (NO17 type) / *Tamyb10-A1b* or *Tamyb10-A1a* (CS type) | TCCCTACATGGGAGACAGAGA | TGTTATCACATGCTGATCCTGA | 61 | 2750/565 | Himi *et al.* (2011) |
| T3B-1 | *Tamyb10-B1b/Tamyb10-A1a* | AGCAAGAGGAACCTGCAGTC | GATGCCCTCCAGATCAAGGT | 61 | 282/263 | Himi *et al.* (2011) |
| T3B-2 | *Tamyb10-B1b/Tamyb10-A1a* | AGGAACCTGCAGTCTCACGG | CTCGTGAACCCCCTCTGCT | 61 | 183/164 | Present study |
| T3B-3 | – | CAGACACATGCACACACCG | TCCTCCAGATCATTGCCCA | 56 | 1913 | Present study |
| T3D-1 | *Tamyb10-D1b* | TAGGCCAACACCTTCTAAACG | AGGCACACCAGCTTATTTGG | 64 | 1353 | Himi *et al.* (2011) |
| T3D-2 | – | CAGAGGGAATGGGGAGGAA | TGACAGTTCCCACGGCTAG | 60 | 1991 | Present study |
| T3D-3 | *Tamyb10-D1d* or *Tamyb10-D1e*/ *Tamyb10-D1c* | ACCACGCTGAGCAAGAGGA | TAGCAAAGCCACGCCAACT | 58 | 470/376 | Present study |
| T3D-4 | *Tamyb10-D1d*/*Tamyb10-D1c* or *Tamyb10-D1e* | CACATCTATGTTGTTTTCCTTT | TAATACTCCTCCACGACCA | 52 | 189/175 | Present study |

DH-5$\alpha$ strain. Plasmids with targeted fragments detected by Colony PCR were extracted by Plasmid Rapid Isolation kit (BioDevtech Company, Beijing, China). Five clones for each new allele were sequenced from both strands by SinoGeno-Max (Shanghai, China). Multiple alignments of sequences and translations of nucleotide into amino acid sequence were performed by software DNAMAN ver. 6.0. Graphic data of sequencing results were analysed by Chromas ver 1.4.5 and FinchTV 1.4.0.

#### *Statistical analysis*

SPSS ver. 19.0 software and least significant range (LSR) multiple comparisons were used to calculate averages of the surveyed characteristics and compare the significant difference of GP and GI values of different *Tamyb10-1* genotypes.

## Results

#### *Distribution of Tamyb10-1 gene in Chinese bread wheat cultivars*

A series of molecular markers were used to identify molecular characterization of *Tamyb10-1* genes in Chinese bread wheat cultivars. Three primer sets T3A-1, T3A-2 and T3A-3 (table 1) were used to identify allelic variations of *Tamyb10-A1* gene. Results indicated that 313 and 269 out of 582 Chinese wheat cultivars possessed *Tamyb10-A1a* (Chinese Spring type) (accounting for 53.8%) and *Tamyb10-A1b* (accounting for 46.2%), respectively. However, no No17-type *Tamyb10-A1a* (a 2.2-kb insertion in its second intron) was found in Chinese wheat cultivars surveyed.

The primer set T3B-1 was first used to identify allelic variations of *Tamyb10-B1* gene. However, to more precisely identify *Tamyb10-B1a* and *Tamyb10-B1b* alleles on agarose gel, we developed a new codominant marker T3B-2 (figure 1) giving a 164-bp fragment for *Tamyb10-B1a* allele and a 183-bp fragment for *Tamyb10-B1b* allele due to only 19-bp difference between *Tamyb10-B1a* and *Tamyb10-B1b* alleles. Sequencing results confirmed the reliability of new marker T3B-2. Identification results indicated that 564 and 13 out of 582 Chinese wheat cultivars possessed *Tamyb10-B1a* (accounting for 96.9%) and *Tamyb10-B1b* alleles (accounting for 2.2%), respectively. However, there were still five remaining cultivars that did not belong to *Tamyb10-B1a* and *Tamyb10-B1b* alleles. Further identification indicated that these five cultivars possessed a large deletion in *Tamby10-B1* locus. This allele was designated *Tamyb10-B1c* allele according to the nomenclature of Himi *et al.* (2011).

The primer sets T3D-1, T3D-2 and T3D-3 (table 1) were used to identify allelic variations of *Tamyb10-D1* gene. Identification results indicated that 417 and 165 out of 582 Chinese wheat cultivars possessed *Tamyb10-D1a* (accounting for 71.6%) and *Tamyb10-D1b* alleles (accounting for 28.4%), respectively.

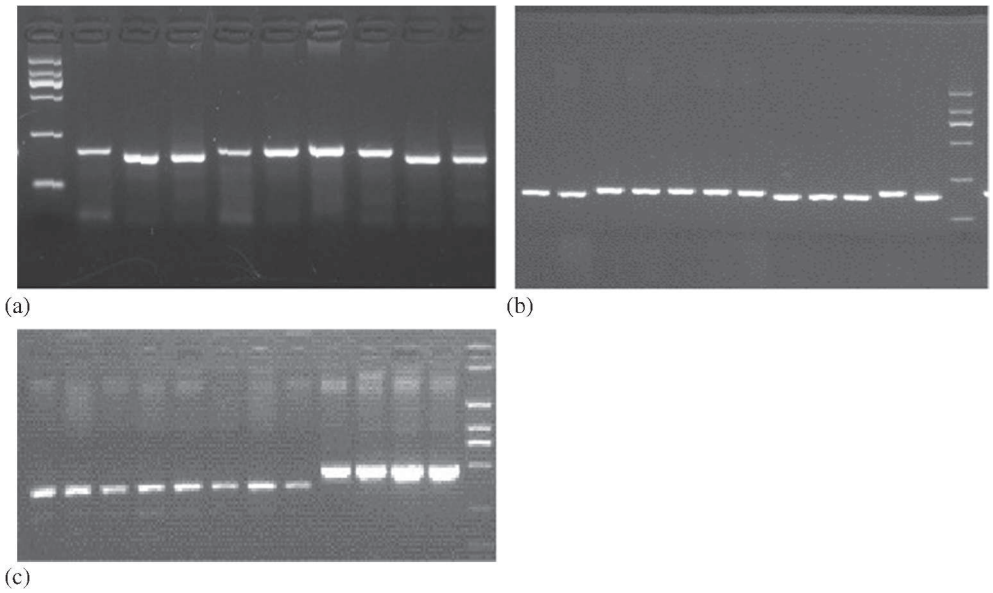


**Figure 1.** Identification of *Tamyb10-B1* and *Tamyb10-D1* alleles by molecular markers R3B-2 (a: 183 bp and 164 bp), T3D-4 (b: 189 bp and 175 bp) and T3D-3 (c: 470 bp and 376 bp). DNA ladder: 2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp and 100 bp.

***Polymorphism of Myb10-1 gene in accessions of Ae. tauschii surveyed***

Due to no amplification obtained from bread wheat cultivars with *Tamyb-D1a* allele by 10 primer sets designed at different positions in *Tamyb-D1* locus (data not provided), the collected 110 accessions of *Ae. Tauschii* were used to clone *Myb10-1* gene with primer set T3D-2 (table 1). Sequencing results indicated that three new types of *Myb10-1* genes were found in these accessions. We found that the first type possessed a 4-bp deletion in first intron, a 14-bp deletion in second intron and a 104-bp deletion in third exon of *Myb10-1* gene (figure 1 in electronic supplementary material) when compared with *Tamyb10-D1b* allele (NCBI no: AB191460), and this new type was designated *Aetmyb10-D1c* allele (NCBI no: KP279635) according to the nomenclature of (Himi *et al*. (2011). The second new type we found only possessed the same 4-bp deletion to *Aetmyb10-D1c* allele in first intron and this new type was designated *Aetmyb10-D1d* allele (NCBI no: KP279636). The third type we found possessed the same 4-bp and14-bp deletions to *Aetmyb10-D1c* allele and was designated *Aetmyb10-D1e* allele (NCBI no: KP279637). Additionally, these three new *Aetmyb10-D1* alleles possessed several SNPs differences from *Tamyb10-D1b* allele. The full alignments of four *Myb10-D1* alleles are listed in figure 1 in electronic supplementary material, at the DNA level. Moreover, full alignments of deduced amino acid sequences of four *Myb10-D1* alleles (figure 2 in electronic supplementary material) showed that the *Aetmyb10-D1c* allele encoded a premature TAMYB10-D1c protein due to the 104-bp deletion. This mutation resulted in a frame shift in the open reading frame (ORF) of the *Myb10-D1* gene and, subsequently, a stop codon at position 189 in the deduced amino acid sequence of MYB10-D1 protein *Aetmyb10-D1d* and *Aetmyb10-D1e* alleles possessed three and two different amino acids when compared to *Tamyb10-D1b* allele, respectively.

Based on molecular characterization of *Myb10-D1* gene in *Ae. tauschii*, two codominant markers (T3D-3 and T3D-4 in table 1) were developed to identify *Aetmyb10-D1c*, *Aetmyb10-D1d* and *Aetmyb10-D1e* alleles. Identification results by the codominant marker T3D-3 indicated that 40 out of 110 *Ae. tauschii* accessions surveyed possessed *Aetmyb10-D1c* allele. Further, identification results by the codominant marker T3D-4 indicated that 61 and 9 out of the remaining 70 *Ae. Tauschii* accessions possessed *Aetmyb10-D1d* and *Aetmyb10-D1e* alleles.

**Figure 2.** Comparison of grain colour of bread wheat cultivars with different allelic variation combination of *Tamyb10-1* gene. From left to right: Baikebai (*Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a*), Yanjingbaimangbai (*Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a*), Zaosui 30 (*Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a*), Luohan 2 (*Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a*), Hongmai (*Tamyb10-A1a/Tamyb10-B1b/Tamyb10-D1a*), Hongmanghong (*Tamyb10-A1b/Tamyb10-B1c/Tamyb10-D1b*), Honglumai (*Tamyb10-A1b/Tamyb10-B1b/Tamyb10-D1a*), Hongquanmang (*Tamyb10-A1b/Tamyb10-B1a/Tamyb10-D1b*), Luochengxiaomai (*Tamyb10-A1b/Tamyb10-B1b/Tamyb10-D1b*).

**Table 2.** Comparison of grain colour and germination level of Chinese wheat cultivars with different *Tmyb10-1* allelic combinations.

| Genotype | Sample no. | Grain colour | Germination percentage (%) | | | | | | | GP (%) | GI (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | | |
| *Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a* | 50 | White | 49.5 | 17.7 | 7.2 | 3.2 | 1.3 | 1.0 | 0.7 | 80.6d | 72.5d |
| *Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1b* | 21 | Red | 52.1 | 17.2 | 6.8 | 3.5 | 1.7 | 1.5 | 0.9 | 83.8c | 75.0cd |
| *Tamyb10-A1b/Tamyb10-B1a/Tamyb10-D1a* | 14 | Red | 55.8 | 17.2 | 6.5 | 2.6 | 0.6 | 0.6 | 0.6 | 84.0c | 77.2bc |
| *Tamyb10-A1b/Tamyb10-B1a/Tamyb10-D1b* | 94 | Red | 58.5 | 15.0 | 7.5 | 3.2 | 1.6 | 0.7 | 0.6 | 87.1b | 79.5b |
| *Tamyb10-A1b/Tamyb10-B1b/Tamyb10-D1b* | 10 | Red | 66.9 | 11.7 | 5.4 | 1.9 | 0.8 | 0.4 | 0.5 | 87.6b | 82.4a |
| *Tamyb10-A1b/Tamyb10-B1c/Tamyb10-D1b* | 5 | Red | 56.5 | 24.5 | 5.8 | 3.6 | 1.7 | 0.6 | 0.4 | 93.1a | 84.7a |
| Total | 194 | – | 55.7 | 16.1 | 7.1 | 3.2 | 1.4 | 0.8 | 0.6 | 85.0 | 77.3 |

Different letters indicated a significant difference at the 5% level. GP, germination percentage; GI, germination index.

***Characterization of the allelic combination of Tamyb10-1 genes and their associations with grain colour and grain germination level***

Characterization of the allelic combination of *Tamyb10-1* genes revealed that 281 out of 582 Chinese winter wheat cultivars surveyed had *Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a* (accounting for 48.3%), 134 cultivars had *Tamyb10-A1b/Tamyb10-B1a/Tamyb10-D1a* (accounting for 23.0%), 118 cultivars had *Tamyb10-A1b/Tamyb10-B1a/Tamyb10-D1b* (accounting for 20.3%), 31 cultivars had *Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1b* (accounting for 5.3%), 11 cultivars had *Tamyb10-A1b/Tamyb10-B1b/Tamyb10-D1b* (accounting for 1.9%), five cultivars had *Tamyb10-A1b/Tamyb10-B1c/Tamyb10-D1b* (accounting for 0.86%), one cultivar (Hongmai) had *Tamyb10-A1a/Tamyb10-B1b/Tamyb10-D1a* (accounting for 0.17%) and one cultivar (Honglumai) had *Tamyb10-A1b/Tamyb10-B1b/Tamyb10-D1a* (accounting for 0.17%). In the above eight allelic combinations, all 281 cultivars with *Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a* showed white colour grains and the remaining 301 cultivars showed red colour grains (figure 2).

Associations of six *Tamyb10-1* allelic combinations with GP and GI of wheat grains were analysed in 194 cultivars. Results indicate that all five allelic combinations with red-colour grains showed the significantly higher GP and GI values than that of *Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a* genotype with white-colour grains (table 2) ($P < 0.05$). Of five allelic combinations with red-colour grains, the *Tamyb10-A1b/Tamyb10-B1c/Tamyb10-D1b* genotype possesses the significantly highest GP and GI amongst the six different *Tamyb10-1* genotypes ($P < 0.05$). Further, both *Tamyb10-A1b/Tamyb10-B1a/Tamyb10-D1b* and *Tamyb10-A1b/Tamyb10-B1a/Tamyb10-D1b* genotypes possessed significantly higher GP and GI values than those of *Tamyb10-A1b/Tamyb10-B1a/Tamyb10-D1a* and *Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1b* genotypes ($P < 0.05$). Interestingly, on the first day, *Tamyb10-A1b/Tamyb10-B1b/Tamyb10-D1b* genotype possessed the significantly highest GP among the six *Tamyb10-1* allelic combinations (figure 3), whereas *Tamyb10-A1a/Tamyb10-B1a/Tamyb10-D1a* genotype with white-colour grains possessed the significantly lowest GP (table 2; figure 2). Comparison of cumulative GPs of Chinese landrace wheat cultivars with different *Tamyb10-1* allelic combinations from first day to seventh day is illustrated in figure 3.

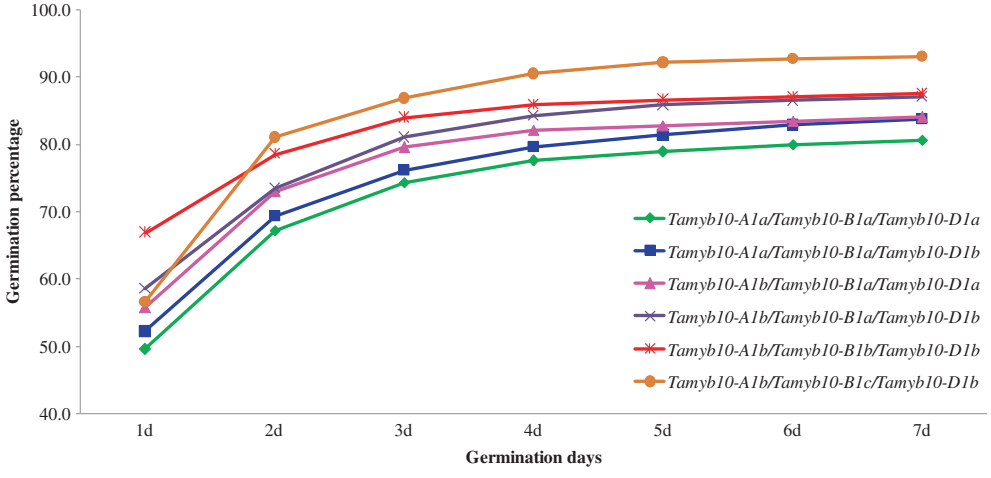


**Figure 3.** Comparison of cumulative GP of Chinese wheat landraces with different *Tamyb10-1* allelic combinations from 1st day to 7th day.

Associations of *Myb10-D1* alleles with GP and GI of wheat grains were also analysed in *Ae. tauschii*. Results indicated that accessions with *Aetmyb10-D1c* showed a significantly highest averages of GP and GI values among *Ae. tauschii* with three *Aetmyb10-D1* alleles ($P < 0.05$), whereas the averages of GP and GI values of *Ae. Tauschii* with *Aetmyb10-D1c* and *Aetmyb10-D1d* did not show significant difference.

## Discussion

Sprouting of grains in mature spikes before harvest is a major problem in wheat production worldwide. The function of the *R* gene in relation to grain dormancy has been speculated to accumulate germination inhibitors in the grain coat tissue (Miyamoto and Everson 1958). In this study, allelic variation of *Tamy10-1* genes mainly controlling grain colour is intimately associated with germinability in wheat. It may provide useful information for wheat breeding programme in terms of grain germinability in bread wheat.

*Ae. tauschii* (Coss.) is the D genome progenitor of bread wheat (*Triticum aesticum* L.). However, when compared with hexaploid wheat, *Ae. tauschii* possesses a large number of novel genes or more diverse alleles that were possibly lost during the formation of hexaploid wheat (Dvorak *et al.* 1998; Jia *et al.* 2013). The genus *Ae. tauschii* originated from the Middle East that was regarded as the centre of origin of *Ae. tauschii* (Van Slageren 1994) in the world. However, in China, *Ae. tauschii* accessions are mainly distributed in the Yili valley of Xinjiang and the middle and low Yellow River region (MLYRR) including Henan and Shaaxi (Wei *et al.* 2008). Moreover, *Ae. tauschii* of MLYRR possibly stemmed directly from Yili valley, whereas *Ae. tauschii* of Yili valley was possibly directly disseminated from the Middle East (Yen *et al.* 1983; Wei *et al.* 2008). In this study it seems to support this view due to discoveries of three novel *Myb10-1* alleles (*Aetmyb10-D1c*, *Aetmyb10-D1d* and *Aetmyb10-D1e*) in Yili valley and only one allele (*Aetmyb10-D1d*) in MLYRR. From this study, we concluded that *Myb10-1* genes are not very diverse in the Chinese wheat cultivars. Therefore, 110 *Ae. tauschii* accessions from Yili valley, MLYRR and other regions were selected to use for identification of *Myb10-1* allelic variations and three new types of *Aetmyb10-1* alleles were discovered in these accessions surveyed. The results could provide new germplasms for the Chinese wheat breeding programme in view of grain colour and germination level. However, it needs more work to do to further examine the influence of these three novel *Tamyb10-1* alleles on wheat grain colour and germination level before we use them for wheat breeding.

This results showed that both *Tamyb10-A1a* (CS type) and *Tamyb10-A1b* alleles were prevalent at the *Tamyb10-A1* locus, and *Tamyb10-B1a* and *Tamyb10-D1a* were predominant at the *Tamyb10-B1* and *Tamyb10-D1* loci in the Chinese winter wheat cultivars surveyed. However, *Aetmyb10-D1d* allele is the most prevalent in the collected 110 *Ae. tauschii* accessions. Interestingly, all the 40 *Ae. tauschii* accessions from Henan and Shanxi possessed *Aetmyb10-D1d* allele, whereas 37 out of 48 *Ae. tauschii* accessions from Xinjiang possessed *Aetmyb10-D1c* allele. Moreover, *Aetmyb10-D1c*, *Aetmyb10-D1d* and *Aetmyb10-D1e* alleles discovered in the above *Ae. tauschii* accessions were not found in the Chinese bread wheat cultivars surveyed.

The level of grain dormancy in white-grained wheat cultivars does not exceed that in red-grained wheat cultivars, suggesting that red-grained wheat cultivars carry genes for grain dormancy addition to the dormancy genes in white-grained wheat cultivars (Flintham 2000). In this study, wheat cultivars with *Tamyb10-1a* genes at the three loci would show white colour grains, whereas wheat cultivars with one or more mutations at the three *Tamyb10-1* loci would show red colour grains. In China, most of the farmers would like to store wheat seeds for future use. Although, it is a farmers' practice to store seeds for a year or more, the genotypic effects on germination should have been studied in periodic intervals to see is there is any pattern of change in germination pattern. This could help in deciphering the ageing effect of seed germinability. The ability of the stored seeds to germinate will decline with time. In this study, we found that cultivars with mutations in two of three *Tamyb10-1* loci have a significantly higher germination level than those with mutation in one of three *Tamyb10-1* loci, and cultivars with *Tamyb10-A1b/Tamyb10-B1c/Tamyb10-D1b* allele possessed the highest GP and GI values and cultivars with *Tamyb10-A1b/Tamyb10-B1b/Tamyb10-D1b* allele possessed the highest first-day GP value after storage of one year. The results may suggest that germination ability of cultivars with *Tamyb10-A1b/Tamyb10-B1c/Tamyb10-D1b* and *Tamyb10-A1b/Tamyb10-B1b/Tamyb10-D1b* alleles declines more slowly with time than those of cultivars with other *Tamyb10-1* alleles.

Molecular characterization of *Tamyb10-D1a* allele is still unknown eventhough *Tamyb10-D1b* allele has been characterized (Himi *et al.* 2011). In this study, we designed 10 primer sets surrounding *Tamyb10-D1* locus for the purpose of trying to examine the molecular characterization of *Tamyb10-D1a* allele. However, none of the PCR products could be successfully amplified with above primer sets. It suggests that *Tamyb10-D1a* genotype possibly has a large deletion including the whole *Tamyb10-D1* gene or a total different sequence from *Tamyb10-D1b* allele in the corresponding region of D genome of bread wheat.

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