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RESEARCH ARTICLE

Haplotype variations of gene *Ppd-D1* in *Aegilops tauschii* and their implications on wheat origin

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Abstract The *Ppd-D1* controlling photoperiod response is an important gene for wheat adaptation since it affects heading time. In the present study, three haplotypes, i.e. haplotype I without deletion, haplotype II with a 24 bp deletion, and haplotype III with two deletions of 24 and 15 bp, were identified in the upstream of the coding region in 80 *Ae. tauschii* accessions. The haplotype distribution was related to subspecies taxon. All typical ssp. *tauschii* accessions had haplotype I, whereas all ssp. *strangulata* had haplotype III. The three haplotypes were observed in *Ae. tauschii* with morphologically intermediate forms between the two typical subspecies. Present results supported that ssp. *strangulata* or intermediate form was the D-genome donor of common wheat since only haplotype III were found in wheat. Moreover, a 16 bp deletion in exon 8 of gene *Ppd-D1* exists in common wheat. However, none of *Ae. tauschii* accessions analyzed had the 16 bp deletion.

Keywords *Aegilops tauschii* · Common wheat · D-genome donor · *Ppd-D1*

Lin Huang and Qing Wang have contributed equally to this research.

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Introduction

Common wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) has a wide distribution in a diverse range of environments from Norway and Russia at 65°N to Argentina at 45°S (Dubcovsky and Dvorak 2007). The successful worldwide cultivation of common wheat is greatly influenced by heading time (Snape et al. 2001). The gene *Ppd-D1* on chromosomes 2D for photoperiod response has a strong action on heading time (Welsh et al. 1973; Law et al. 1978). The dominant allele *Ppd-D1a* confers photoperiod insensitivity, whereas the recessive allele *Ppd-D1b* confers photoperiod sensitivity. *Ppd-D1* gene has been considered to be a member of the pseudo-response regulator (*PRR*) family (Laurie 1997; Börner et al. 1998; Turner et al. 2005; Beales et al. 2007). A 2,089 bp deletion upstream of the coding region was only found in photoperiod insensitive wheat with gene *Ppd-D1a*, which leads to misexpression of the *Ppd-D1* gene and caused early flowering in both short- and long-day

conditions (Beales et al. 2007). Moreover, gene *Ppd-D1* in all analyzed common wheat varieties had a 16 bp deletion in exon 8 (Beales et al. 2007; Guo et al. 2010).

Common wheat was originated by hybridization of *T. turgidum* L. (2n = 4x = 28, AABB) with wild grass *Aegilops tauschii* Coss. (2n = 2x = 14, DD). The addition of the genome DD contributed by the *Ae. tauschii* is postulated to increase the adaptation of common wheat to an even world range of environments (Gororo et al. 2001). *Ae. tauschii* has a wide geographic distribution spreading westwards to Turkey and eastwards to Afghanistan and China (Kihara et al. 1965; Yen et al. 1983; Jaaska 1981; Van Slageren 1994) and shows a lot of variations on heading time (Xiang et al. 2009; Matsuoka et al. 2008). Based on the spike morphology, *Ae. tauschii* is classified into two subspecies, i.e. ssp. *tauschii* with elongated cylindrical spikes and *strangulata* (Eig) Tzvel. with markedly moniliform spikes (Hammer 1980). However, morphologically intermediate forms between the two typical subspecies had been also observed (Kihara and Tanaka 1958; Kim et al. 1992; Aghaei et al. 2008; Matsuoka et al. 2009).

In the present study, we analyzed the upstream region of the coding region and exon 8 of gene *Ppd-D1* in 80 *Ae. tauschii* accessions. The 2,089 bp deletion in upstream region and the 16 bp deletion in exon 8 existed in common wheat (Beales et al. 2007; Guo et al. 2010) were not observed in analyzed *Ae. tauschii*. Three haplotypes were found in the upstream of the coding region and their haplotype distribution was related to subspecies taxon and heading time.

Materials and methods

Plant materials

A total of 80 *Ae. tauschii* accessions were used in this study. Based on the description by Aghaei et al. (2008) and Matsuoka et al. (2009), 11, 51, and 18 accessions belonged to ssp. *stragulata*, ssp. *tauschii*, and intermediate form, respectively (Table 1).

Molecular marker analysis and sequencing

DNA was extracted from leaves using 2×CTAB method according to Zhang et al. (2004). Primers Ppd-D1_F (acgcctcccactacactg) and Ppd-D1_R1 (gttggttcaaacagagagc) is used to amplify a part of sequence within the 2,089 bp region upstream of the coding sequence of *Ppd-D1* gene (Beales et al. 2007). Primers Ppd-D1 exon 8_F1 (gatgaacatgaaacggg) and Ppd-D1 exon 8_R1 (gtctaaatagtaggtactagg) was used to survey the 16 bp deletion in exon 8 (Beales et al. 2007). The PCR was performed in a Gene Amp PCR system 9700 (ABI) in 25 µl reaction volumes containing 1× buffer, 80 ng of template DNA, 250 nmol of each primer, 1.5 mM of $MgCl_2$, 200 µM of each dNTP and 1 U of *Taq* DNA polymerase. The cycling program consisted of 95°C for 5 min, followed by 38 cycles of 94°C for 40 s, 54°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were separated on 6% denaturing polyacrylamide gels and visualized by silver staining (Chen et al. 2008). PCR products were further used in cloning and sequencing as described by Chen et al. (2008).

Evaluation on heading date of *Ae. tauschii*

A total of 50 *Ae. tauschii* accessions (Table 1, indicated by *) were used for analysis on heading date. They were sown on 30 October in 2010 in the field of Triticeae Research Institute, located in Wenjiang of Chengdu City, Sichuan Province, China. *Ae. tauschii* was grown as 30 cm between rows with a length of 2 m. The date at which 50% of the heads had fully emerged from the flag leaf sheath (heading date) was recorded. The correlations between *Ppd-D1* haplotypes and heading date were done by using the SPSS 15.0 software (SPSS Inc; Chicago, Illinois, US).

Results and discussion

Haplotypes in the 2,089 bp region upstream of the coding sequence

All the 80 *Ae. tauschii* accessions successfully amplified PCR products with primers Ppd-D1_F and Ppd-D1_R1. This indicated that they did not contain the 2,089 bp deletion in the upstream of the coding sequence of *Ppd-D1* gene. Three haplotypes were observed among the 80 accessions based on the electrophoresis patterns of PCR products (Fig. 1, Table 1). Haplotype I, II, and III included 55 (51

Table 1 Haplotype distributions among 80 *Aegilops tauschii* accessions

Haplotypes	Accessions (origin)	Note
Type I	AS60* (Iran), AS61*, AS62*, AS64*, AS65* (Former Soviet Union), AS67* (Iran), AS68*, AS69*, AS71* (Xinjiang, China), AS72* (Xinjiang, China), AS74* (Shannxi, China), AS75* (Shannxi, China), AS76* (Shannxi, China), AS77* (Henan, China), AS78* (Henan, China), AS79* (Henan, China), AS80* (Henan, China), AS81* (Henan, China), AS82* (Henan, China), AS84, AS85*, AS86*, AS90*, AS91*, AS92*, AS93, AS94*, AS95*, AS96, AS2389* (KU2079, Astara, Iran), AS2390, AS2392* (TQ-2), AS2395* (TQ-12-2), AS2406* (TQ-56), AS2410, AS2564* (TQ-16), CIae5 (Afghanistan), PI476874 (Afghanistan), PI486271 (Van, Turkey), PI486274 (Kars, Turkey), PI486275 (Kars, Turkey), PI486276 (Kars, Turkey), PI486277 (Kars, Turkey), PI499262 (Xinjiang, China), PI511362 (Baluchistan, Pakistan), PI511366 (Zabul, Afghanistan), PI511367 (Kabul, Afghanistan), PI511370 (Mazandaran, Iran), PI511375, PI560536 (Van, Turkey), PI560538 (Bitlis, Turkey), PI560754 (Hakkari, Turkey), PI574469 (India), PI603220 (Western Asia), PI603222 (Former Soviet Union)	Among the 55 accessions, PI511370, PI560536, PI574469 and PI603222 belong to intermediate form, the other 51 belong to typical ssp. *tauschii*
Type II	AS63*, AS66* (Former Soviet Union), AS87*, AS88*, AS89*, AS2399* (TQ-22-2), AS2394* (TQ-22-2), AS2402* (TQ-27), PI486265 (Hakkari, Turkey), PI486266 (Hakkari, Turkey)	All the 10 accessions belong to intermediate form
Type III	AS2386* (KU2074, Behshahr, Iran), AS2387* (KU2075, Behshahr, Iran), AS2388* (KU2076, Gorgan, Iran), AS2396* (TQ-13), AS2397* (TQ-17-1), AS2398* (TQ-18-12), AS2403* (TQ-28), AS2404* (TQ-29), AS2405* (TQ-38), AS2407* (TQ-81), AS2409* (TQ-90), PI560755 (Hakkari, Turkey), PI574464 (Azerbaijian), PI574465 (Azerbaijian), PI574466 (Georgia)	Among the 15 accessions, PI560755, PI574464, PI574465 and PI574466 belong to intermediate form, the other 11 belong to typical ssp. *strangulata*

Asterisk used in the heading time analysis. AS is the code of Triticeae Research Institute of Sichuan Agriculture University. The known origins of *Ae. tauschii* accessions are indicated in brackets. Chinese *Ae. tauschii* accessions were collected by Prof. Chi Yen of Triticeae Research Institute of Sichuan Agricultural University in 1980's. The lines with code PI or CIae were kindly provided by USDA-ARS, USA; KU, collected from Kyoto University, Japan; TQ, collected from Dr. Yigal Avivi, Weizmann Institute of Science, Israel

typical ssp. *tauschii* and four intermediate form), 10 (intermediate form), and 15 accessions (11 ssp. *strangulata* and four intermediate form), respectively. All the typical ssp. *tauschii* accessions belonged haplotype I, while all the typical ssp. *strangulata* accessions belonged haplotype III. In intermediate form *Ae. tauschii*, three haplotypes I–III were observed.

The PCR products from 12 accessions, including six ssp. *tauschii* (AS77, AS80, AS81, AS82, AS95, and AS2395) with haplotype I, two intermediate form (AS89 and AS2394) with haplotype II, and four ssp. *strangulata* (AS2386, AS2387, AS2388, and AS2403) with haplotype III were further sequenced

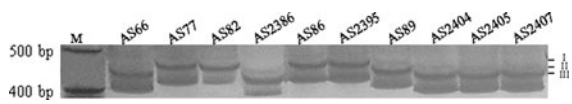

Fig. 1 PCR products amplified with *Ppd-D1* primer in 6% denaturing polyacrlamide gels. M, marker; haplotype I: AS77, AS82, AS86, and AS2395; haplotype II: AS66 and AS89; haplotype III: AS2386, AS2404, AS2405 and AS2407

(GeneBank accessions JN196544 to JN196555). Haplotypes I, II, and III had the sizes of 453, 429, 414 bp, respectively. Compared to haplotype I, haplotype II had a 24 bp deletion, while haplotype III had two deletions of 24 and 15 bp. Common wheat Chinese Spring (CS) belonged to haplotype III

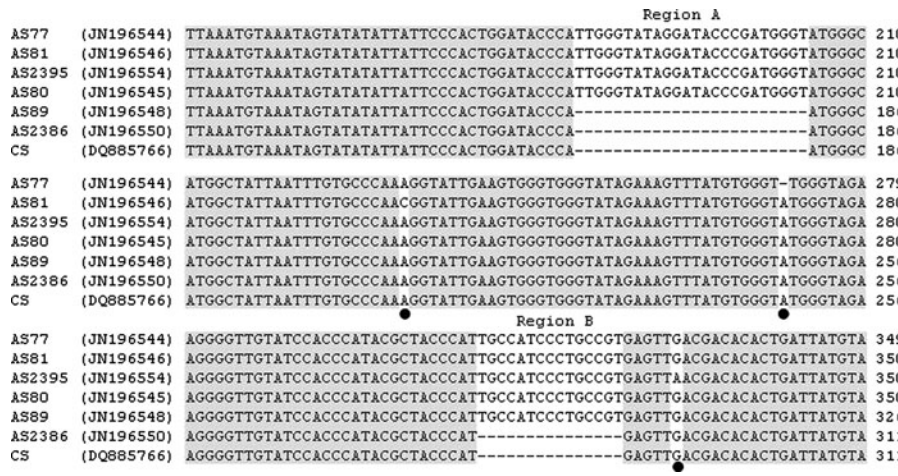

Fig. 2 Comparison of DNA sequence upstream of the coding region of gene *Ppd-D1* among three haplotypes. CS, common wheat Chinese Spring. Region A, the 24 bp deletion; Region B, the 15 bp deletion; Base pair substitution is marked with *black circle*

(Fig. 2). No SNP was found in haplotypes II and III. However, three SNPs were found in the haplotype I (Fig. 2).

Ae. tauschii ssp. *strangulata* has been recognized as the D-genome donor of common wheat (Nishikawa 1974; Jaaska 1981; Nakai 1979; Lagudah et al. 1991; Dvorak et al. 1998). However, the contribution of subspecies *tauschii* to wheat D-genome is also suggested (see review by Kilian et al. 2011). Previous studies found that common wheats had either haplotype III for photoperiod sensitive wheat with allele *Ppd-D1b* or a 2,089 bp deletion in region upstream of the coding sequence for photoperiod insensitive wheat with allele *Ppd-D1a* (Beales et al. 2007; Yang et al. 2009; Guo et al. 2010). All the analyzed *Ae. tauschii* accessions in this study and previous study by Guo et al. (2010) did not found the 2,089 bp deletion. These results suggested that common wheat with *Ppd-D1b* was originated by hybridization of *T. turgidum* with *Ae. tauschii* with haplotype III. The 2,089 bp deletion after the origin of common wheat might result in the appearance of allele *Ppd-D1a*. This is agreed with the suggestion that *Ppd-D1b* is come from the mutation of *Ppd-D1a* (Thomas and Vince-Prue 1997). Haplotype III existed in all analyzed ssp. *strangulata* and in some intermediate forms. Present results supported that ssp. *strangulata* or intermediate form was the D-genome donor of common wheat.

In the present study, all the typical ssp. *tauschii* had haplotype I, while all the ssp. *strangulata* had haplotype III. This reflected that the haplotype distribution were related to subspecies taxon. However, both haplotypes I and III existed in the intermediate forms. This may be caused by the natural hybridization and gene flow between ssp. *strangulata* and ssp. *tauschii* (Kihara et al. 1965; Lubbers et al. 1991; Dvorak et al. 1998; Lelley et al. 2000; Pestsova et al. 2000; Mizuno et al. 2010). On the other hand, haplotype II was only observed in intermediate form in the present study. This haplotype was not observed in a previous study involved 30 *Ae. tauschii* accessions and 25 synthetic hexaploid wheats (Guo et al. 2010). To elucidate the origin of haplotypes II, more *Ae. tauschii* accessions need to be analyzed.

Exon 8 in *Ae. tauschii*

The *Ppd-D1* of all analyzed common wheat varieties had a 16 bp deletion in exon 8 (Beales et al. 2007; Guo et al. 2010). Primers Ppd-D1 exon 8_ F1 and Ppd-D1 exon 8_ R1 was used to survey the deletion in *Ae. tauschii* accessions. All the 80 *Ae. tauschii* accessions amplified products with same size. The PCR products from nine accessions, including five ssp. *tauschii* (AS72, AS79, AS80, AS94, and AS2389), one intermediate form (AS2402), and three ssp. *strangulata* (AS2386, AS2388, and AS2409) were further sequenced (GeneBank accessions JN196556 to JN196564). They had a size of 336 bp with four SNPs. Compared to that of common wheat, *Ae. tauschii* accessions did not have the 16 bp deletion (Fig. 3).

Since only a limited *Ae. tauschii* samples were used in this study, present results can not allow us to

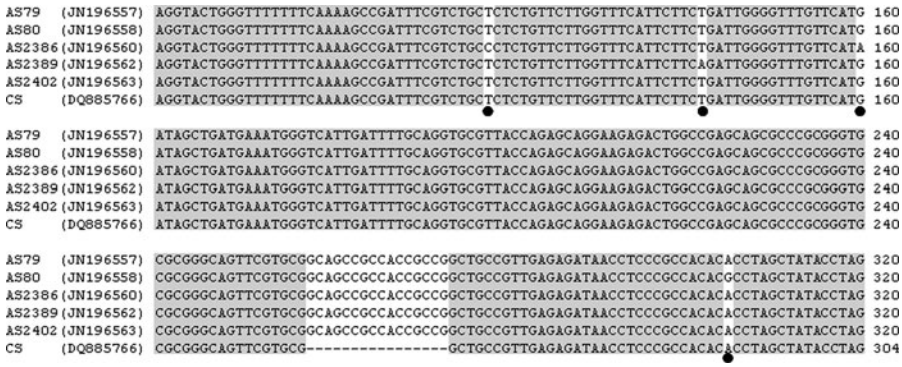

Fig. 3 Sequence comparison of exon 8 in *Ppd-D1* between *Ae. tauschii* and wheat Chinese Spring (CS). Base pair substitution is marked with *black circle*

determine when the 16 bp deletion in common wheat originated. There were several possibilities for its origin. This deletion may occur after the origin of common wheat or it has existed in some *Ae. tauschii* accessions that excluded in this study. An alternative possibility was that the deletion appeared in the allohexaploidization process of common wheat between *T. turgidum* and *Ae. tauschii*.

Correlation between *Ppd-D1* haplotype and heading date

There was a significant correlation ($R^2 = 0.2524$, $P < 0.01$) between heading date and haplotype in analyzed 50 *Ae. tauschii* accessions. Haplotype I showed significantly earlier heading with average days of 177.06 ± 0.93 than haplotype II with 181.88 ± 1.63 at $P = 0.05$ or haplotype III with 183.45 ± 1.19 at $P = 0.01$ (Fig. 4). The analyzed 31 accessions with haplotype I belonged to ssp. *tauschii*, 11 with haplotype III belonged to ssp. *strangulata*, and the other 8 with haplotype II belonged to intermediate form. This is agreed with previous results that ssp. *tauschii* showed earlier heading than ssp. *strangulata* (Matsuoka et al. 2008; Xiang et al. 2009).

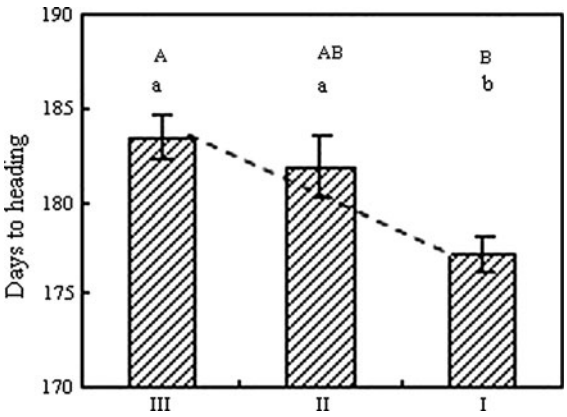

Fig. 4 Days to heading of three *Ppd-D1* haplotypes. *Capital letters* represent significance at $P = 0.01$ and *small letters* represent significance at $P = 0.05$

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References

Aghaei MJ, Mozafari J, Taleei AR, Naghavi MR, Omidi M (2008) Distribution and diversity of *Aegilops tauschii* in Iran. Genet Resour Crop Evol 55:341–349

Beales J, Turner A, Griffiths S, Snape JW, Laurie DA (2007) A *pseudo-response regulator* is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). Theor Appl Genet 115:721–733

Börner A, Korzun V, Worland AJ (1998) Comparative genetic mapping of loci affecting plant height and development in cereals. Euphytica 100:245–248

Chen QJ, Zhang LQ, Yuan ZW, Yan ZH, Zheng YL, Sun GL, Liu DC (2008) Empirical verification of heterogeneous DNA fragments generated from wheat genome-specific SSR primers. Can J Plant Sci 88:1065–1071

Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. Science 316:1862–1866

Dvorak J, Luo MC, Yang ZL, Zhang HB (1998) The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor Appl Genet 97:657–670

Gororo NN, Flood RG, Eastwood RF, Eagles HA (2001) Photoperiod and vernalization responses in *Triticum turgidum* × *T. tauschii* synthetic hexaploid wheats. Ann Bot (London) 88:947–952

Guo ZA, Song YX, Zhou RH, Ren ZL, Jia JZ (2010) Discovery, evaluation and distribution of haplotypes of the wheat *Ppd-D1* gene. New Phytol 185:841–851

Hammer K (1980) Vorarbeiten zur monographischen Darstellung von Wildpflanzensortimenten: *Aegilops* L. Kulturpflanze 28:33–180

Jaaska V (1981) Aspartate aminotransferase and alcohol dehydrogenase isoenzymes: intraspecific differentiation in *Aegilops tauschii* and the origin of the D genome polyploids in the wheat group. Plant Syst Evol 137:259–273

Kihara H, Tanaka M (1958) Morphological and physiological variation among *Aegilops squarrosa* strains collected in Pakistan, Afghanistan and Iran. Preslia 30:241–251

Kihara H, Yamashita K, Tanaka M (1965) Morphological, physiological, genetical and cytological studies in *Aegilops* and *Triticum* collected from Pakistan, Afghanistan and Iran. In: Yamashita K (ed) Results of the Kyoto University Scientific Expedition to the Karakoram and Hindukush. Kyoto University, Kyoto, pp 1–118

Kilian B, Mammen K, Millet E, Sharma R, Graner A, Salamini F, Hammer K, Özkan H (2011) *Aegilops* L. In: Kole C (ed) Wild crop relatives: genomic and breeding resources cereals. Springer, Heidelberg, pp 1–76

Kim WK, Innes RL, Kerber ER (1992) Ribosomal DNA repeat unit polymorphism in six *Aegilops* species. Genome 35:510–514

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 36:387–395

Laurie DA (1997) Comparative genetics of flowering time. Plant Mol Biol 35:167–177

Law CN, Sutka J, Worland AJ (1978) A genetic study of day-length response in wheat. Heredity 41:185–191

Lelley T, Stachel M, Grausgruber H, Vollmann J (2000) Analysis of relationships between *Aegilops tauschii* and the D genome of wheat utilizing microsatellites. Genome 43:661–668

Lubbers EL, Gill KS, Cox TS, Gill BS (1991) Variation of molecular markers among geographically diverse accessions of *Triticum tauschii*. Genome 34:354–361

Matsuoka Y, Takumi S, Kawahara T (2008) Flowering time diversification and dispersal in central Eurasian wild wheat *Aegilops tauschii* Coss.: genealogical and ecological framework. PLoS ONE 3:e3138

Matsuoka Y, Nishioka E, Kawahara T, Takumi S (2009) Genealogical analysis of subspecies divergence and spikelet-shape diversification in central Eurasian wild wheat *Aegilops tauschii* Coss. Plant Syst Evol 279:233–244

Mizuno N, Yamasaki M, Matsuoka Y, Kawahara T, Takumi S (2010) Population structure of wild wheat D-genome progenitor *Aegilops tauschii* Coss.: implications for intraspecific lineage diversification and evolution of common wheat. Mol Ecol 19:999–1013

Nakai Y (1979) Isozyme variations in *Aegilops* and *Triticum*, IV. The origin of the common wheats revealed from the study on esterase isozymes in synthesized hexaploid wheats. Jpn J Genet 54:175–189

Nishikawa K (1974) Alpha-amylase isozymes and phylogeny of hexaploid wheat. In: Sears ER, Sears LMS (eds) Proceedings of 4th international wheat genetics symposium. University of Missouri, Columbia, USA, pp 851–855

Pestsova E, Korzun V, Goncharov NP, Hammer K, Ganal MW, Röder MS (2000) Microsatellite analysis of *Aegilops tauschii* germplasm. Theor Appl Genet 101:100–106

Snape JW, Butterworth K, Whitechurch E, Worland AJ (2001) Waiting for fine times: genetics of flowering time in wheat. Euphytica 119:185–190

Thomas B, Vince-Prue D (1997) Photoperiodism in plants, 2nd edn. Academic Press, San Diego

Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The *pseudo-response regulator Ppd-H1* provides adaptation to photoperiod in barley. Science 310:1031–1034

Van Slageren MW (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Wageningen Agricultural University, Wageningen

Welsh JR, Keim DL, Pirasteh B, Richards RD (1973) Genetic control of photoperiod response in wheat. In: Sears ER, Sears LMS (eds) Proceedings of the 4th international wheat genetics symposium. University of Missouri, Columbia, USA, pp 879–884

Xiang ZG, Zhang LQ, Ning SZ, Zheng YL, Liu DC (2009) Evaluation of *Aegilops tauschii* for heading date and its gene location in a re-synthesized hexaploid wheat. Agric Sci China 8:1–7

Yang FP, Zhang XK, Xia XC, Laurie DA, Yang WX, He ZH (2009) Distribution of the photoperiod insensitive *Ppd-D1a* allele in Chinese wheat cultivars. Euphytica 165:445–452

Yen C, Yang JL, Liu XD (1983) The distribution of *Aegilops tauschii* Cosson in China and with reference to the origin of the Chinese common wheat. In: Sakamoto S (ed) Proceedings of the 6th international wheat genetics symposium, Kyoto University, Japan, pp 55–58

Zhang LQ, Liu DC, Yan ZH, Lan XJ, Zheng YL, Zhou YH (2004) Rapid changes of microsatellite flanking sequence in the allopolyploidization of new synthesized hexaploid wheat. Sci China C Life Sci 47:553–561