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Natural variation in photoperiodic flowering pathway and identification of photoperiod-insensitive accessions in wild wheat, *Aegilops tauschii*

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Abstract

The D-genome progenitor of hexaploid wheat, *Aegilops tauschii* Coss., has a wide natural species range in central Eurasia and possesses wide natural variation in heading and flowering time. Here, we report identification of two *Ae. tauschii* accessions insensitive to short day length. Similarly to a loss or reduced degree of vernalization requirement, the photoperiod-insensitive mutations were found only in the early flowering sublineage (TauL1b) of *Ae. tauschii*. Quantitative trait locus (QTL) analyses using two F₂ mapping populations showed that a QTL for heading time on the long arm of chromosome 5D was related to the early heading phenotype of the photoperiod-insensitive accessions under short-day conditions. In the photoperiod-insensitive accession, expression patterns of two flowering-related genes were altered under short-day conditions compared with the patterns in photoperiod-sensitive accessions. This study indicates that analysis of natural variations in the *Ae. tauschii* population is useful to find novel genetic loci controlling agronomically important traits.

Keywords: Flowering time, Natural variation, Photoperiod sensitivity, Quantitative trait locus, Wheat

Introduction

Timing of flowering is a critical trait for environmental adaptation in higher plants, and thus adjusting flowering time to the growth environment is an important target in crop breeding. In wheat, three major genetic components, the vernalization requirement, photoperiod sensitivity and narrow-sense earliness, largely control heading and flowering time (Murai et al. 2005). As major genes for the vernalization requirement, the *Vrn-1* homoeologous loci are located on the long arms of homoeologous group 5 chromosomes (Snape et al. 1996). Similarly, genotypes of the three homoeologous *Ppd-1* loci strongly affect photoperiod sensitivity, and the *Ppd-1* loci are located on the short arms of homoeologous group 2 chromosomes (Law et al. 1978; Scarth and Law 1983). Unlike vernalization requirement and photoperiod sensitivity, narrow-sense earliness is regulated by multiple quantitative trait loci (QTLs) with small effects (Cockram et al. 2007). Wheat is a long-day plant, and thus long-day conditions exceeding the critical day length shorten heading and flowering time (Jackson 2009). Mutated alleles of some wheat photoperiod sensitivity-related genes such as *Ppd-1* and *PHYTOCLOCK 1* result in photoperiod insensitivity and an early flowering phenotype (Beales et al. 2007; Mizuno et al. 2016).

Aegilops tauschii Coss., a wild diploid wheat relative, is well known as the D genome donor of common wheat, and habitats of *Ae. tauschii* are widely distributed from northern Syria and Turkey to western China in central Eurasia (Van Slageren 1994). The D genome of *Ae. tauschii* was brought into the allohexaploid genome of common wheat through interspecific crossing to tetraploid wheat and subsequent amphidiploidization about 8,000 years ago (Matsuoka 2011). This evolutionary process can be artificially reproduced as the generation of synthetic allohexaploids which are obtained through chromosome doubling in triploid hybrids from interspecific crosses between cultivated tetraploid wheat and *Ae. tauschii* (Matsuoka and Nasuda 2004; Kajimura et al. 2011; Matsuoka et al. 2013). Therefore, *Ae. tauschii* is regarded as a directly available resource for common wheat breeding (Mujeeb-Kazi et al. 1996; Jones et al. 2013; Nguyen et al. 2015).

Recent studies with genome-wide marker genotyping showed that the *Ae. tauschii* populations could be genealogically divided into two major lineages, TauL1 and TauL2, and a minor lineage, TauL3 (Mizuno et al. 2010; Wang et al. 2013; Matsuoka et al. 2013, 2015). The TauL1 accessions were originally collected from western habitats, the Transcaucasian and northern Iran regions, to eastern habitats such as Pakistan and Afghanistan (Mizuno et al. 2010; Matsuoka et al. 2015), and in addition two sublineages have genetically diverged in both TauL1 and TauL2 (Matsuoka et al. 2013). The diversity center of *Ae. tauschii* is considered to be present in the western habitats, and the species habitats could have expanded from the western ones to the eastern ones (Matsuoka et al. 2008). The eastward dispersal could be explained by expansion of the TauL1 habitat range, which appears to be brought especially by one of the sublineages, Tau1b (TauL1b). The TauL1b accessions provide peculiar characteristics such as early heading time, high seed production ability, and salt stress tolerance during seedling growth (Matsuoka et al. 2015; Saisho et al. 2016). Since the early flowering accessions were found mainly in the eastern habitats, the early heading phenotype was presumed as one of the adaptive characters to the eastern habitats (Matsuoka et al. 2008, 2015). Therefore, genetic bases of the early heading phenotype should be studied to elucidate the adaptive mechanism. Genetic variations related to the early heading phenotype have been partly studied in *Ae. tauschii* (Xiang et al. 2008; Takumi et al. 2011; Huang et al. 2012; Kippes et al. 2016). Vernalization-insensitive accessions were formerly found in the *Ae. tauschii* germplasm collected from Afghanistan and Pakistan (Tsunewaki 1966; Golovnina et al. 2010; Takumi et al. 2011), and the lack of vernalization requirement was mainly due to structural mutations either in *Vrn-D^{tau1}* or *Vrn-D^{tau2}* (Takumi et al. 2011; Kippes et al. 2016). Haplotype variation at *Ppd-D^{tau1}* is considered to partly underlie the wide heading time variation in *Ae. tauschii* (Xiang et al. 2008; Huang et al. 2012). Synthetic hexaploid lines sharing identical A and B genomes derived from a tetraploid wheat cultivar Langdon and containing diverse D genomes originating from the *Ae. tauschii* pollen parents show wide variation in flowering-related traits, and the large variation in heading time observed in *Ae. tauschii* is also present in hexaploid synthetics (Kajimura et al. 2011). Several QTLs for

flowering-related traits were detected on various D-genome chromosomes of synthetic hexaploids, and the QTL on chromosome arm 2DS appears to be an allelic variant of *Ppd-D^{tau}1* (Nguyen et al. 2013). However, natural variation in photoperiodic responses has not been evaluated in *Ae. tauschii*, and information on causative genes for the early heading phenotype is quite limited in the eastern habitat accessions of *Ae. tauschii*.

The aims of the present study were (1) to clarify the genetic variation in photoperiodic response related to the control of heading time and (2) to elucidate the genetic loci controlling the photoperiod insensitivity in the wild D-genome progenitor of common wheat. We therefore screened photoperiod-insensitive mutant accessions and conducted QTL analysis for heading time under artificially controlled short-day conditions and field conditions using two mapping populations in *Ae. tauschii*.

Materials and Methods

Plant materials and mapping populations

Twenty-two accessions of *Ae. tauschii* from various habitats of the species' entire natural distribution range were used to evaluate their photoperiod sensitivities in this study (Table 1). Two sets of the F₂ populations were used to conduct linkage map construction and QTL analyses. Four *Ae. tauschii* accessions were selected as parental lines for mapping populations; AE1038 and KU-2042 for photoperiod-insensitive lines and KU-2069 and KU-2111 for photoperiod-sensitive lines. Two F₂ mapping populations were produced from the cross-combinations KU-2069/AE1038 and KU-2111/KU-2042. For each mapping population, all F₂ individuals were obtained from single F₁ plants. Two *Ae. tauschii* accessions, KU-2042 for a photoperiod-insensitive line and PI486267 for a photoperiod-sensitive line, were used for gene expression analysis.

Growth conditions and phenotypic evaluation

Two-week-old seedlings of the 22 *Ae. tauschii* accessions were grown at 22°C under 12 h day

length ($182 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and transferred to conditions of 4°C and 12 h day length. After a 40- or 50-day vernalization, the seedlings were grown at 22°C under short day conditions (12 h light/12 h darkness) and under long day conditions (16 h light/8 h darkness) to examine photoperiod sensitivity. Heading time was recorded as days after vernalization using three individual plants of each accession.

The two F₂ populations were grown under short-day conditions and under field conditions for QTL analyses. After a 30-day vernalization under short-day conditions, seedlings of the two F₂ populations (83 from KU-2069/AE1038 and 74 from KU-2111/KU-2042) were grown at 22°C under short-day and long-day conditions. Heading time of the F₂ individuals was recorded as days after vernalization.

For field assay, seeds of the two F₂ populations were sown in November 2010 and grown in the 2010-2011 season, with 102 from the KU-2069/AE1038 and 98 from the KU-2111/KU-2042 population. All two F₂ populations as well as two plants of each parent were grown individually in pots arranged randomly in a field of Kobe University (34°43'N, 135°13'E). Heading time of the F₂ individuals was recorded as days after sowing.

Genotyping with molecular markers

To perform genotyping of the F₂ individuals, PCR-based simple sequence repeat (SSR) markers were used. Total DNA was extracted from leaves of the parental *Ae. tauschii* accessions and F₂ individuals. Information on primer sequences of the SSR markers on the D genome and the respective annealing temperatures was obtained from the National BioResource Project (NBRP) KOMUGI web site (<http://www.shigen.nig.ac.jp/wheat/komugi/strains/aboutNbrpMarker.jsp>) and the GrainGenes web site (<http://wheat.pw.usda.gov/GG2/maps.shtml>). The PCR-based genotyping was performed according to our previous report (Nguyen et al. 2013).

To supplement the regions with scarce SSR markers, four gene-specific markers were used. Polymorphism at the *Ppd-D^{ta}1* locus on chromosome 2D was detected using allele-specific primers according to Beales et al. (2007). Insertion and deletion (indel)

polymorphisms at *Vrn-D^{tau}1* and *VERNALIZATION INSENSITIVE (VIN3)-like1 (AetVIL1)* were used as markers on chromosome 5D (Takumi et al. 2011; Koyama et al. 2012). A cleaved amplified polymorphic sequence (CAPS) marker for the phytochrome A (*PhyA*) locus on chromosome 4D was designed; the *PhyA*-specific primer set was 5'-AGTGTTTGCCGCTTCTCATT-3' and 5'-GTATGATGAAGTGCTACTAAATACGC-3'. The PCR conditions for the *PhyA* marker were 1 cycle of 94°C for 1 min and 40 cycles of 94°C for 20 s, 54°C for 30 s, and 68°C for 20 s; the amplified products were digested with the restriction enzyme *HhaI* for recognition of a single nucleotide polymorphism in *PhyA*.

PCR products and their digests were resolved in 2% agarose or 13% nondenaturing polyacrylamide gels according to our previous report (Nguyen et al. 2013).

Construction of linkage maps and QTL analysis

Genetic mapping was performed using MAPMAKER/EXP version 3.0b software (Lander et al. 1987), and QTL analyses were carried out by composite interval mapping with Windows QTL Cartographer version 2.5 software using the forward and backward method (Wang et al. 2011). The parameters for the map construction and QTL analysis were set according to our previous report (Nguyen et al. 2013). A LOD score threshold for each trait was determined by computing a 1000 permutation test. The percentage of phenotypic variation explained by a QTL for a trait and any additive effects were also estimated using the Windows QTL Cartographer software.

Gene expression analysis of wheat flowering-related genes

Seeds were germinated for 48 h at 22°C in darkness. Four or five synchronously germinating seeds were transferred to pots containing soil and incubated at 22°C under either short day conditions (12 h light/12 h darkness) or long day conditions (16 h light/8 h darkness). Total RNA was extracted from leaves using Sepasol-RNA I solution (Nacalai Tesque, Kyoto, Japan), and transcript accumulation patterns of four genes, including *Ppd-D^{tau}1*, two wheat *CONSTANS*-like genes, *WCO1* and *AetHd1* (Mizuno et al. 2016), and the wheat *GIGANTEA*

clock gene (*AetGI*; Mizuno et al. 2016), were detected by RT-PCR. First-strand cDNA was synthesized from DNase I-treated mRNA samples with oligo-dT primers using the high fidelity ReverTra Ace reverse transcriptase (Toyobo, Osaka, Japan). RT-PCR was conducted with the following gene-specific primer sets: 5'-AAATGGTGCCGCCGACTTCTTG-3' and 5'-TTGATTGGAGACCGCGCACGTA-3' for *Ppd-D^{tau1}*, 5'-GCAGTGCATGAACACACACGA-3' and 5'-AGGGCTGCAGTTGAGAACGTC-3' for *WCO1*, 5'-CATTCTGCTAACCGTGTGGC-3' and 5'-ATTGTTCTTCTGGTCTTGGGTCA-3' for *AetHd1*, and 5'-GAAGGTCAGAAGATGTGGAGAGTCAAC-3' and 5'-GGCAGCGGATGGTAGGTGATAG-3' for *AetGI*. The ubiquitin gene (*Ubi*) was used as an internal control (Kobayashi et al. 2005). The PCR conditions were 1 cycle of 94°C for 1 min and 30 cycles of 94°C for 30 s, 50°C (*WCO1*, *AetHd1* and *AetGI*) or 62°C (*Ppd-D^{tau1}*) for 30 s, and 72°C for 1 min; the PCR products were separated by electrophoresis through a 1.5% agarose gel and stained with ethidium bromide.

Results

Natural variation in photoperiodic response and vernalization requirement

Twenty-two accessions of *Ae. tauschii* that widely covered the species' distribution range were used to examine photoperiod sensitivity in regulation of heading time. In preliminary common garden experiments, the TauL1b accessions showed an earlier heading phenotype than the other lineages (Table 1; Matsuoka et al. 2008, 2015). Under long-day conditions after the 40-day vernalization, all accessions reached the heading and flowering stages, and wide variation in heading time was observed. However, heading was not observed in twelve accessions until 120 days of short-day conditions after the 40-day vernalization treatment. In these twelve accessions, the 40-day vernalization was insufficient for phase transition from vegetative to reproductive growth. At least four of the twelve accessions (KU-2834, IG126489, AT80 and KU-2078) could grow to the heading stage under short-day conditions

after the 50-day vernalization. Under long-day conditions, the 50-day vernalization shortened heading time in a few accessions, for example, KU-2132, KU-2042, AT80 and KU-2088, compared with the 40-day vernalization. Thus, the vernalization requirement varied greatly in the 22 *Ae. tauschii* accessions.

Heading time under short-day conditions was delayed in many of the 22 accessions compared with long-day conditions, whereas no difference in heading time between the short- and long-day conditions was observed in two accessions, AE1038 and KU-2042 (Table 1). The ratio of heading time under short-day conditions to long-day conditions varied in the *Ae. tauschii* accessions, indicating existence of wide natural variation in photoperiod sensitivity for phase transition from vegetative to reproductive growth. The two accessions KU-2042 and AE1038 showed ratios of 1.00 and 1.17 in heading time after the 50-day vernalization, respectively, indicating that these accessions are insensitive to day length for heading. After the 40-day vernalization, the ratio of heading time was smaller in these two accessions than in many others, indicating low photoperiod sensitivity of these accessions.

QTL analysis for heading time under short-day conditions

Two F₂ populations were produced through crosses between photoperiod-insensitive accessions and late-heading accessions; one was derived from a cross between KU-2069 and AE1038 and the other from a cross between KU-2111 and KU-2042. The F₂ individuals were grown under short-day conditions after a 30-day vernalization. The two late-heading accessions KU-2069 and KU-2111 did not transition from vegetative to reproductive growth phase, and some non-heading F₂ individuals segregated in the F₂ populations (Fig. 1A, 1B).

In the KU-2069/AE1038 population, 127 of more than 400 SSR and CAPS markers were polymorphic between the parental accessions, and 65 markers formed eight linkage groups. The total map length was 936.1 cM with an average spacing of 14.4 cM between markers (Supplementary material 1, Fig. S1). In the KU-2111/KU-2042 population, 59 of 99 markers polymorphic between the parental accessions mapped to nine linkage groups, and the total map length was 828.5 cM with an average spacing of 14.0 cM between markers

(Supplementary material 1, Fig. S2).

QTLs for heading time under short-day conditions were detected using the two linkage maps. A QTL with a LOD score of 12.3 was assigned only to the long arm of chromosome 5D in the KU-2069/AE1038 population, while a QTL with a LOD score of 10.1 was found on chromosome 5D in the KU-2111/KU-2042 population (Table 2). The chromosomal position of one 5D QTL detected in the KU-2069/AE1038 population corresponded to that in the KU-2111/KU-2042 population. The commonly detected QTLs mapped proximal to the *Vrn-D1* locus (Fig. 2A), and explained more than 50% of the heading time variation in the two mapping populations. For the two commonly detected QTLs on 5DL, alleles from the late flowering accessions showed additive effects (Table 2), indicating that they contribute to the early heading phenotype in the photoperiod-insensitive accessions.

We chose 18 F₂ individuals carrying KU-2069 homozygous alleles and 16 individuals with AE1038 homozygous or heterozygous alleles based on the genotype around the 5D QTL region (*Xcfd7* – *Vrn-D1*) from the KU-2069/AE1038 population. The genotypic effect of the 5D QTL for heading time was compared under short-day conditions, and the F₂ individuals selected with heterozygous and AE1038 homozygous alleles showed significantly earlier heading time than those with KU-2069 homozygous alleles (Fig. 3A). Similarly, we compared the effects of the 5D QTL region (*Xgdm43* – *Xcfd3*) on heading time in the KU-2111/KU-2042 population. The F₂ individuals carrying heterozygous and KU-2042 homozygous alleles showed significantly earlier heading time than those with KU-2111 homozygous alleles under short-day conditions (Fig. 3B).

QTL analysis for heading time under field conditions

The F₂ individuals in the KU-2069/AE1038 and KU-2111/KU-2042 populations were grown under field conditions. Heading time of the F₂ individuals varied from 146 to 174 days after sowing, and the frequency distributions of heading time were distinct in field and short-day conditions (Fig. 1C, 1D).

In the KU-2069/AE1038 population grown in the field, 60 markers formed nine linkage

groups, and the total map length was 739.5 cM with an average spacing of 12.3 cM between markers (Supplementary material 1, Fig. S3). Nine linkage groups were formed with 53 markers in the KU-2111/KU-2042 population, and the total map length was 797.1 cM with an average spacing of 15.0 cM between markers (Supplementary material 1, Fig. S4).

Three QTLs for heading time in field conditions were found in the two linkage maps. Two QTLs with LOD scores of 7.9 and 11.3 were respectively assigned to chromosomes 4D and 7D in the KU-2069/AE1038 population, whereas only one QTL with a LOD score of 4.1 was detected on the short arm of chromosome 7D in the KU-2111/KU-2042 population (Table 2). The 7D QTLs coincided in chromosomal position, and mapped between *Xbarc92* and *Xctg05313* (Fig. 2B). The commonly detected QTLs on 7D explained 37.2% and 19.5% of the heading time variation in the KU-2069/AE1038 and KU-2111/KU-2042 populations, respectively. For the three QTLs on 4D and 7D, alleles from the late flowering accessions showed additive effects (Table 2), indicating that the alleles for early heading time were derived from the photoperiod-insensitive accessions.

The genotypic effects of the 7D QTLs for heading time were examined under field conditions using F₂ individuals selected from the two populations. In the KU-2069/AE1038 population, F₂ individuals carrying AE1038 homozygous alleles in the 7D QTL region (*Xbarc92* – *Xcfd68*) showed significantly earlier heading time than those with KU-2069 homozygous alleles (Fig. 3C). Heading time of the heterozygous F₂ individuals was intermediate between the homozygous individuals. Similarly, the F₂ individuals with KU-2042-homozygous alleles in the 7D QTL region (*Xbarc92* – *Xwmc405*) showed significantly earlier heading time than those with heterozygous or KU-2111 homozygous alleles under field conditions (Fig. 3D).

Expression patterns of some flowering-related genes

Many flowering-related genes are regulated by the circadian clock, and their expression patterns are affected by day length (Bendix et al. 2015). To examine the expression patterns of flowering-related genes in the photoperiod-insensitive accession of *Ae. tauschii*, we compared

expression of four genes (*Ppd-D1*, *WCO*, *AetHd1* and *AetGI*) functioning in photoperiodic responses in wheat (Mizuno et al. 2016). In the photoperiod-sensitive accession PI486267, expression patterns of the four genes within a day differed in short-day conditions and long-day conditions (Fig. 4). Gene expression patterns of *WCO* and *AetHd1* were clearly altered under short-day conditions in the photoperiod-insensitive accession KU-2042 compared to PI486267. However, no clear changes in *Ppd-D1* or *AetGI* expression patterns were observed between the two accessions.

Discussion

Wide variation in heading time has been naturally observed among *Ae. tauschii* accessions (Matsuoka et al. 2009; Huang et al. 2011). Here, we showed the presence of natural variation in two major components controlling heading time, vernalization requirement and photoperiodic sensitivity in *Ae. tauschii* (Table 1). Our previous study reported on *Ae. tauschii* accessions with full loss of the vernalization requirement due to a large deletion in the first intron of the *Vrn-D^{tau}1* locus (Takumi et al. 2011). Structural mutation of either *ZCCT1* or *ZCCT2* at the *Vrn-D^{tau}2* locus also led to loss of vernalization requirement (Kippes et al. 2016). In the present study, great line differences were observed in the degree of vernalization requirement of the 22 *Ae. tauschii* accessions. Thus, a 40-day vernalization was enough for heading in some accessions, whereas even a 50-day vernalization did not cause a change in the vegetative growth phase to the reproductive phase in several accessions. In barley, similar quantitative variation in the grade for vernalization requirement was reported (Saisho et al. 2011). Spring growth habits are frequently found in western barley accessions, while Far Eastern accessions show extreme winter growth habits (Saisho et al. 2011). TauL1, distributed from the western to the eastern habitats of *Ae. tauschii* (Mizuno et al. 2010; Matsuoka et al. 2015), showed a lower degree of vernalization requirement than TauL2, which is distributed only in the western habitats, and many TauL1 accessions could transition to the reproductive stage after a 40-day vernalization (Table 1). Therefore, a contrasting distribution of grade in vernalization

requirement was observed between barley and *Ae. tauschii*.

Two photoperiod-insensitive accessions, AE1038 and KU-2042, were screened in the present study, and the insensitivity was observed after a 50-day vernalization but not after a 40-day vernalization (Table 1). Some early heading accessions including AE1038 required around 20 days after the 40-day and 50-day vernalization under long-day conditions, but in most, short-day conditions delayed heading time. The delayed heading time was shorter after the 50-day vernalization than after the 40-day vernalization under short-day conditions. Thus, longer vernalization treatment accelerated the phase change to reproductive stage under short-day conditions. Therefore, the photoperiod sensitivity could interact with the vernalization requirement in photoperiod-sensitive accessions.

A sublineage of TauL1, TauL1b, is associated with the eastward expansion of *Ae. tauschii* and shows significantly earlier heading and flowering time than another TauL1 sublineage or TauL2 (Matsuoka et al. 2015). The photoperiod-insensitive mutation of AE1038 and KU-2042 presumably occurred in the TauL1b sublineage. Loss of vernalization requirement due to the deleterious mutation in *Vrn-D^{tau}1* was also found only in the TauL1b sublineage (Takumi et al. 2011). These observations suggest that these two types of mutations were naturally selected and survived under growth environments to which early flowering plants were well adapted. In Norwegian *Arabidopsis* populations, vernalization requirement and photoperiod sensitivity are associated with altitude, and local climatic factors create the clinal variations in these flowering time-controlling components (Lewandowska-Sabat et al. 2012, 2017). Further studies using many more TauL1b accessions will be required to clarify the association of variation in vernalization response and photoperiod sensitivity with local adaptation in the eastern habitats of *Ae. tauschii*.

Our QTL analyses for heading time under short-day conditions using the two F₂ mapping populations indicated that the causal genes for photoperiod insensitivity in AE1038 and KU-2042 are on the long arm of chromosome 5D (Table 2). In common wheat, a photoperiod-insensitive locus *Ppd-D1* is well known as an early flowering gene on 2DS (Beales et al. 2007). At the *Ppd-D^{tau}1* locus, two haplotypes were recognized to contribute

partly to the heading time variation in *Ae. tauschii* (Xiang et al. 2008; Huang et al. 2012). However, the heading time difference between the two *Ppd-D^{tau}1* haplotypes was quite small and perhaps photoperiodic response-independent. The map positions of the causal genes were corresponded on 5DL in the two populations and proximal to *Vrn-D^{tau}1* (Fig. 2A). Wheat and barley phytochrome C (*PHYC*) genes are tightly linked to *Vrn-1* on the homoeologous group 5 chromosomes, and loss-of-function mutations in *PHYC* result in late flowering phenotypes under long-day conditions (Chen et al. 2014). Therefore, the causal gene for photoperiod insensitivity to short-day conditions might be different from *Vrn-1* and *PHYC*. The photoperiod-insensitive mutation in AE1038 and KU-2042, acting as a dominant allele, accelerated heading time under short-day conditions (Fig. 3), and the photoperiod-insensitive mutation affected the expression patterns of *WCO* and *Aethd1* (Fig. 4). These results suggest that the 5D QTL could be a novel locus involved in control of wheat flowering time through the photoperiod sensitivity-related pathway. Dominant photoperiod-insensitive alleles of *Ppd-1* alter expression profiles of flowering-related genes (Jackson 2009), but further studies are required to clarify the relationship between *Ppd-1* and the 5D QTL in regulation of flowering time.

The photoperiod-insensitive gene on 5DL was not detected as a QTL for heading time under field conditions in the two mapping populations (Fig. 2B). The early heading effect of the photoperiod-insensitive allele appears to be dependent on the growth conditions. The effect of photoperiod insensitivity in AE1038 and KU-2042 on control of heading time should be examined in more detail in further studies. Presumably, the photoperiod-insensitive allele at the 5DL QTL would affect the heading time under comparatively shorter day-length conditions after short vernalization periods, although the assumption should be validated in future experiments. Instead of the 5D QTL, QTLs for heading time were found on the short arm of chromosome 7D. The 7D QTL for heading time could be associated with intraspecific differentiation of early flowering TauL1b accessions in *Ae. tauschii*. At a similar position to the 7D QTL, a flowering time QTL was reported in a mapping population of synthetic wheat hexaploids (Nguyen et al. 2013). These observations suggest that the recessive allele of the 7D

QTL found in AE1038 and KU-2042 could function at least partly in the hexaploid background of synthetic wheat.

The present study is a good example of finding genetic loci controlling agronomically important traits through analysis of natural variation in the *Ae. tauschii* population. In the present study, the minimum numbers of F₂ individuals were used for the QTL mapping experiments. Toward future breeding use of the QTLs detected in the present study, their fine-map construction should be conducted using mapping populations of larger size. Recent advances such as whole-genome genotyping methods enable efficient detection of QTLs in *Ae. tauschii* via genome-wide association studies (Liu et al. 2015; Arora et al. 2017). Therefore, comprehensive research on natural variation in various traits of *Ae. tauschii* should give quite useful information for wheat breeding.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1. Natural variation in photoperiod sensitivity in the 22 accessions of *Ae. tauschii*

Accession number	Country	Lineage*	Heading time after 40-d vernalization			Heading time after 50-d vernalization			Heading time in field**
			LD (d)	SD (d)	SD/LD	LD (d)	SD (d)	SD/LD	
IG126293	Armenia	TauL1a	52	> 120	> 2.31	n.d.	n.d.	n.d.	164
KU-2132	Turkey	TauL1a	85	> 120	> 1.41	41	> 120	> 2.93	166
KU-2834	Georgia	TauL1a	43	> 120	> 2.79	47	88	1.87	165
IG47259	Syria	TauL1x	20	84	4.20	20	49	2.45	150
AE1038	Tajikistan	TauL1b	18	48	2.67	16	16	1.00	144
KU-2042	Afghanistan	TauL1b	14	34	2.43	6	7	1.17	149
KU-2058	Afghanistan	TauL1b	19	66	3.47	17	50	2.94	152
PI476874	Afghanistan	TauL1b	19	64	3.37	20	48	2.40	149
KU-2001	Pakistan	TauL1b	21	70	3.33	18	50	2.78	154
IG126489	Turkmenistan	TauL1b	26	> 120	> 4.62	24	86	4.58	152
AT80	China	TauL1b	28	> 120	> 4.29	18	88	4.89	n.d.
PI508262	China	TauL1b	79	> 120	> 1.52	48	> 120	> 2.50	181
PI508264	China	TauL1b	28	83	2.96	19	60	3.16	n.d.
KU-20-8	Iran	TauL2a	65	> 120	> 1.85	45	> 120	> 2.67	160
KU-2111	Iran	TauL2a	72	> 120	> 1.67	61	> 120	> 1.97	170
KU-2126	Iran	TauL2a	39	84	2.15	n.d.	n.d.	n.d.	154
PI486267	Turkey	TauL2a	25	120	4.80	43	86	2.00	162
IG120866	Dagestan	TauL2x	38	> 120	> 3.16	48	> 120	> 2.50	166
KU-2078	Iran	TauL2x	29	> 120	> 4.14	24	99	4.13	158
KU-20-10	Iran	TauL2b	52	> 120	> 2.31	n.d.	n.d.	n.d.	164
KU-2069	Iran	TauL2b	69	> 120	> 1.74	72	> 120	> 1.67	161
KU-2088	Iran	TauL2b	35	81	2.31	24	81	3.38	161

n.d., not determined; LD, long-day conditions; SD, short-day conditions

*Matsuoka et al. (2015). TauL1x and TauL2x indicate admixtures possessing <85% membership.

**Matsuoka et al. (2008)

Table 2. Summary of QTLs for heading time in the two F₂ populations under the short-day and field conditions

Population	Chr.	Marker interval	Length ^a	LOD score	R ² (%)	Additive effect
Short-day conditions						
KU-2069/AE1038	5D	<i>Xcfd7 – Vrn-D1</i>	37.8	12.32	52.9	46.24
KU-2111/KU-2042	5D	<i>Xgwm583 – Xcfd003</i>	29.1	10.16	51.1	14.43
Field conditions						
KU-2069/AE1038	4D	<i>Xgdm61 – Xcfd39</i>	21.7	7.86	18.0	4.72
KU-2069/AE1038	7D	<i>Xbarc092 – Xcfd68</i>	33.2	11.32	37.2	4.49
KU-2111/KU-2042	7D	<i>Xbarc092 – Xctg05313</i>	24.2	4.08	19.5	2.43

^aLength is the distance (cM) of the interval in which the QTL is located.

Figure legends

Figure 1. Frequency distributions of heading time in the two F₂ populations. Arrows indicate the data for the parental *Ae. tauschii* accessions. (A) KU-2069/AE1038 F₂ populations grown under the phytotron short-day conditions (12 h day length) after a 30-day vernalization. (B) KU-2111/KU-2042 F₂ populations under short-day conditions after a 30-day vernalization. (C) KU-2069/AE1038 F₂ populations grown in field conditions. (D) KU-2111/KU-2042 F₂ populations grown in field conditions.

Figure 2. Linkage map and QTL-likelihood curves of LOD scores showing the locations of QTLs for heading time on chromosomes 5D and 7D. Genetic distances are shown in centimorgans on the left of each chromosome. The 3.0 LOD score threshold is indicated by a black line. The putative position of each centromere is represented by a double-headed arrow. (A) KU-2069/AE1038 F₂ population grown under short-day conditions. (B) KU-2111/KU-2042 F₂ population under short-day conditions. (C) KU-2069/AE1038 F₂ population grown under field conditions. (D) KU-2111/KU-2042 F₂ population under field conditions.

Figure 3. Comparison of heading time among putative genotypes at the 5D and 7D QTLs. Statistical significance of the QTL effect on the examined traits was estimated by analysis of variance. Means with the same letters were not significantly different ($P > 0.05$) (Tukey-Kramer's HSD test). (A) KU-2069/AE1038 F₂ population genotype between *Xcfd7* and *Vrn-D1* on chromosome 5D under short-day conditions. (B) KU-2111/KU-2042 F₂ population genotype between *Xgdm43* and *Xcfd003* on chromosome 5D under short-day conditions. (C) KU-2069/AE1038 F₂ population genotype between *Xbarc92* and *Xcfd68* on chromosome 7D under field conditions. (D) KU-2111/KU-2042 F₂ population genotype between *Xbarc92* and *Xwmc405* on chromosome 7D under field conditions. SD, short-day.

Figure 4. Expression analyses of four flowering-related genes in two *Ae. tauschii* accessions under short-day (12 h day length) and long-day (16 h day length) conditions. RT-PCR was conducted to compare the transcript accumulation profiles within 1 d between photoperiod-sensitive PI486267 and photoperiod-insensitive KU-2042. The ubiquitin gene (*Ubi*) was used as a control.

Fig. 1

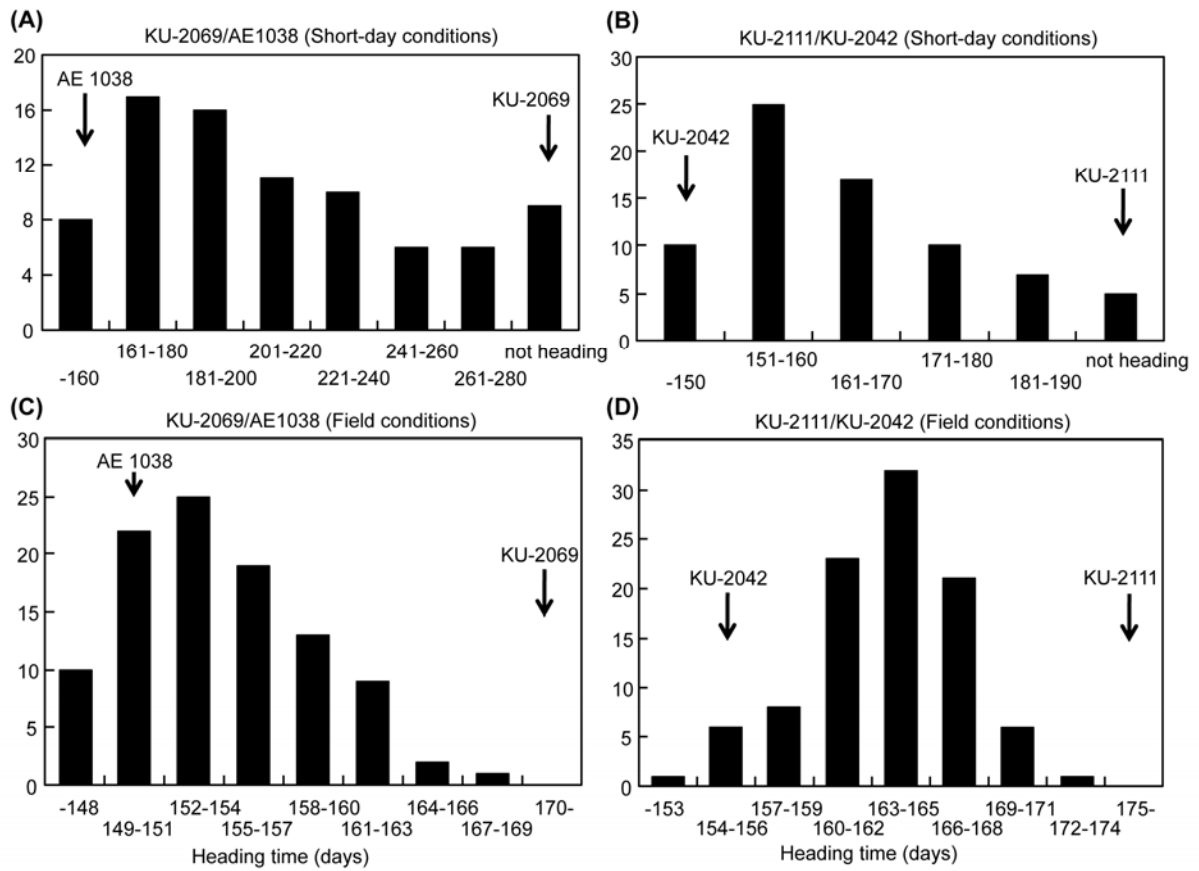


Fig. 2

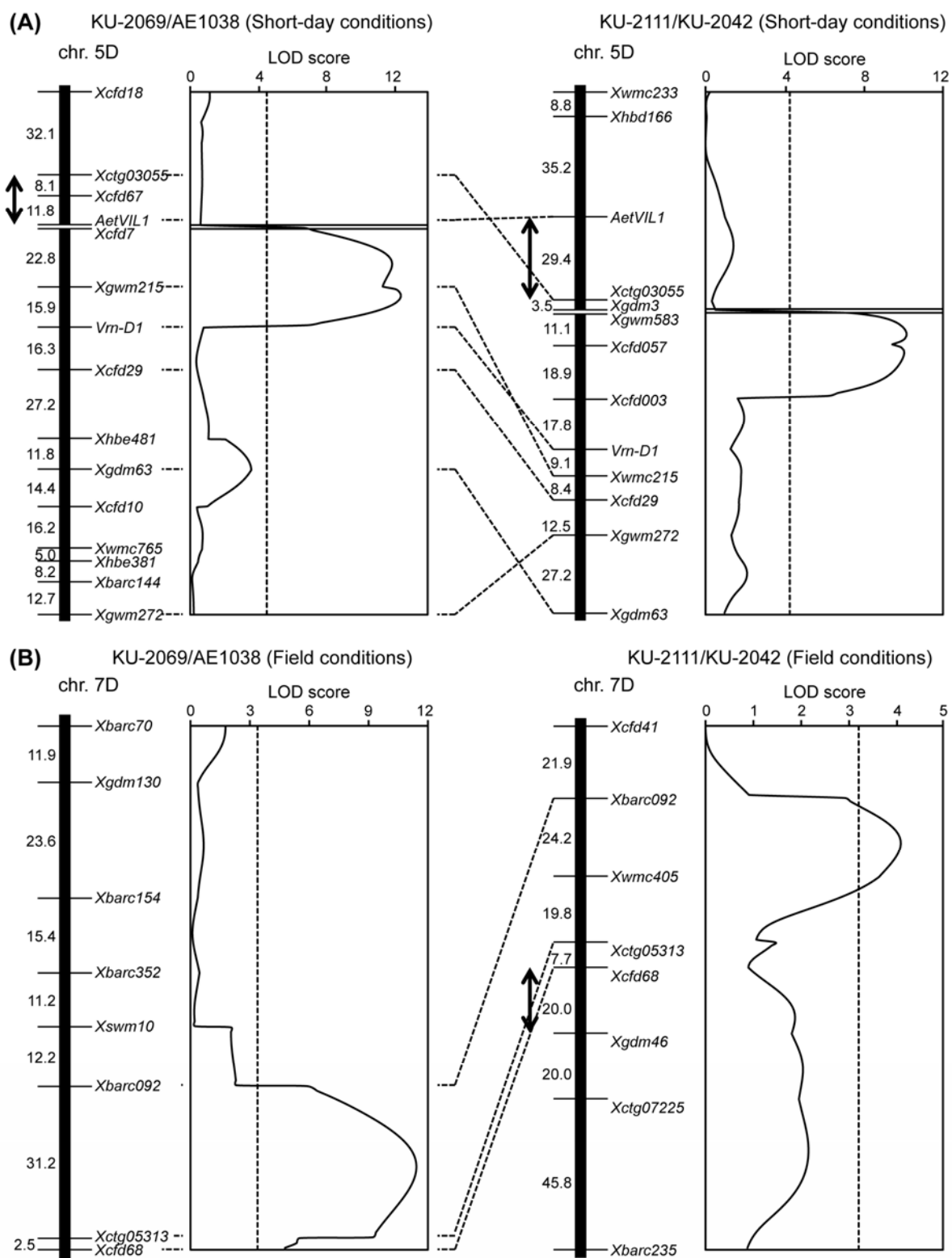


Fig. 3

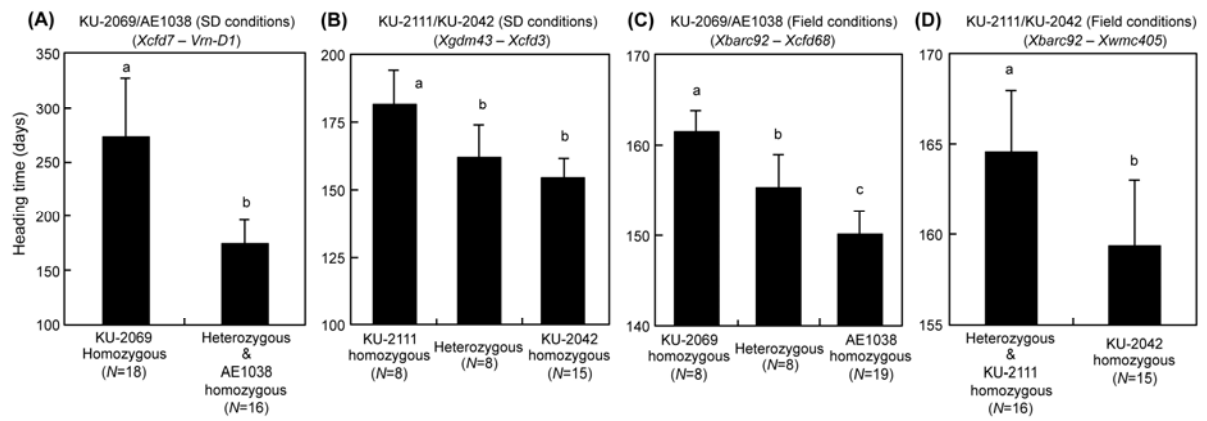
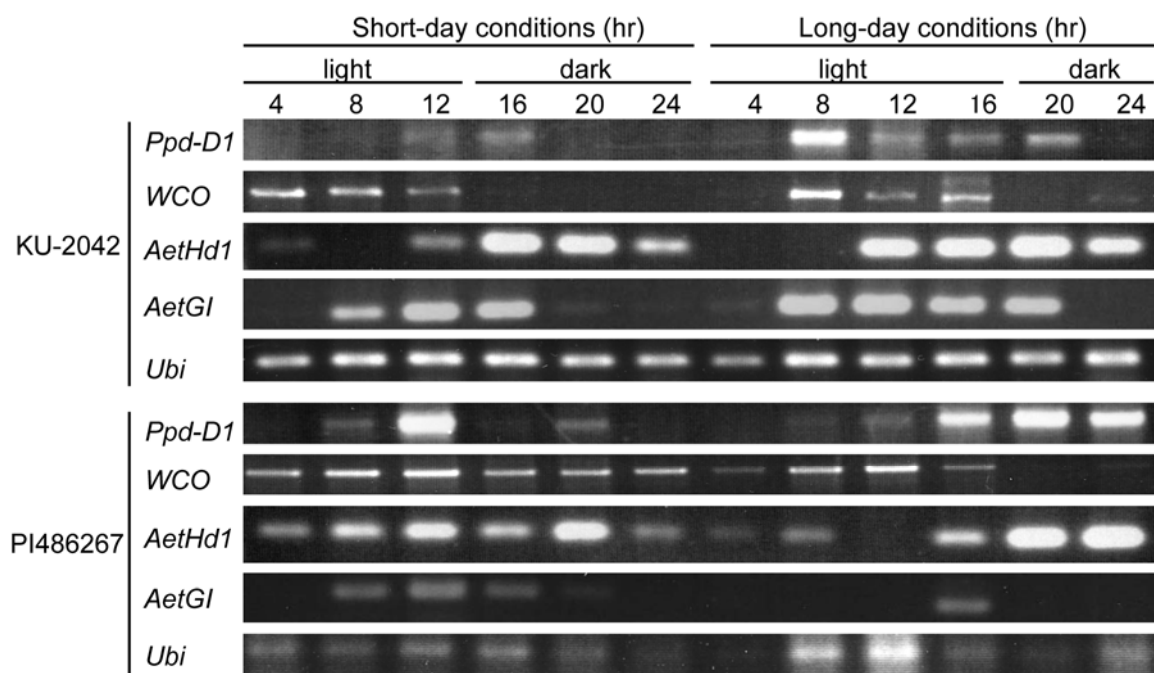


Fig. 4



Supplementary material 1, Fig. S1. Linkage map of the KU-2069/AE1038 mapping population for analysis under short-day conditions. Genetic distances (cM) and markers are shown on the left and right, respectively.

Supplementary material 1, Fig. S2. Linkage map of the KU-2111/KU-2042 mapping population for analysis under short-day conditions. Genetic distances (cM) and markers are shown on the left and right, respectively.

Supplementary material 1, Fig. S3. Linkage map of the KU-2069/AE1038 mapping population for analysis in field conditions. Genetic distances (cM) and markers are shown on the left and right, respectively.

Supplementary material 1, Fig. S4. Linkage map of the KU-2111/KU-2042 mapping population for analysis in field conditions. Genetic distances (cM) and markers are shown on the left and right, respectively.

Fig. S1

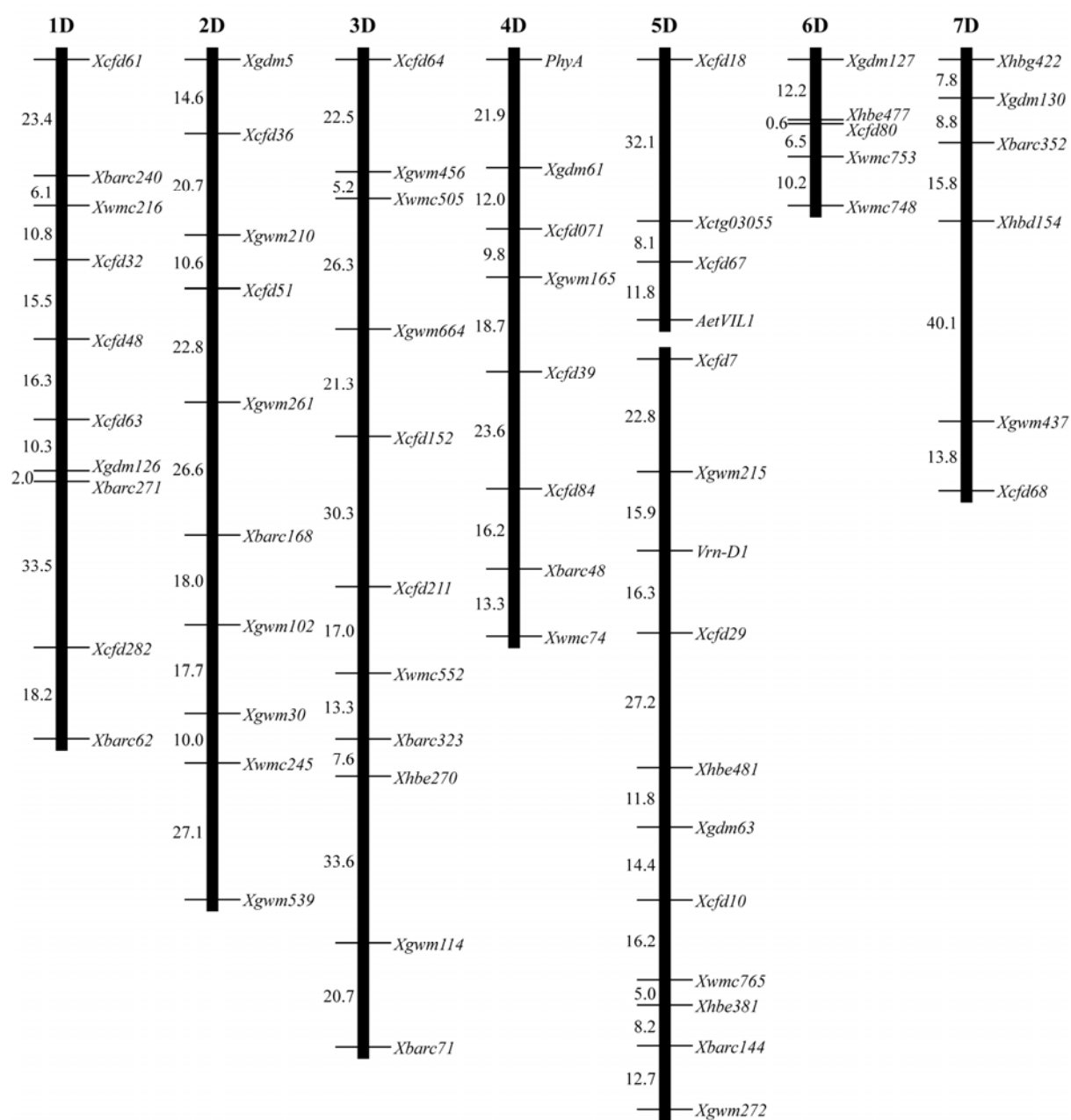


Fig. S2

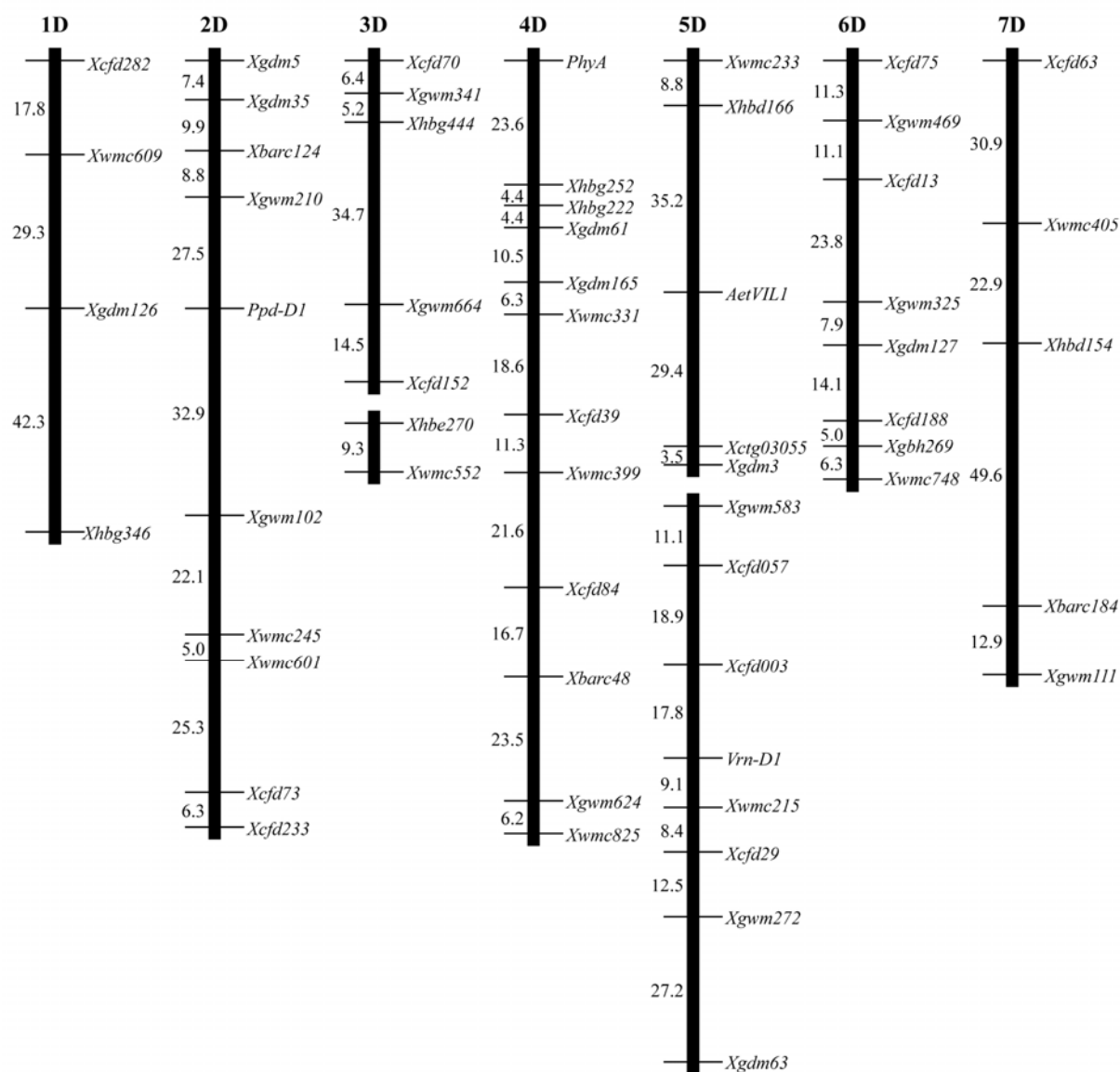


Fig. S3

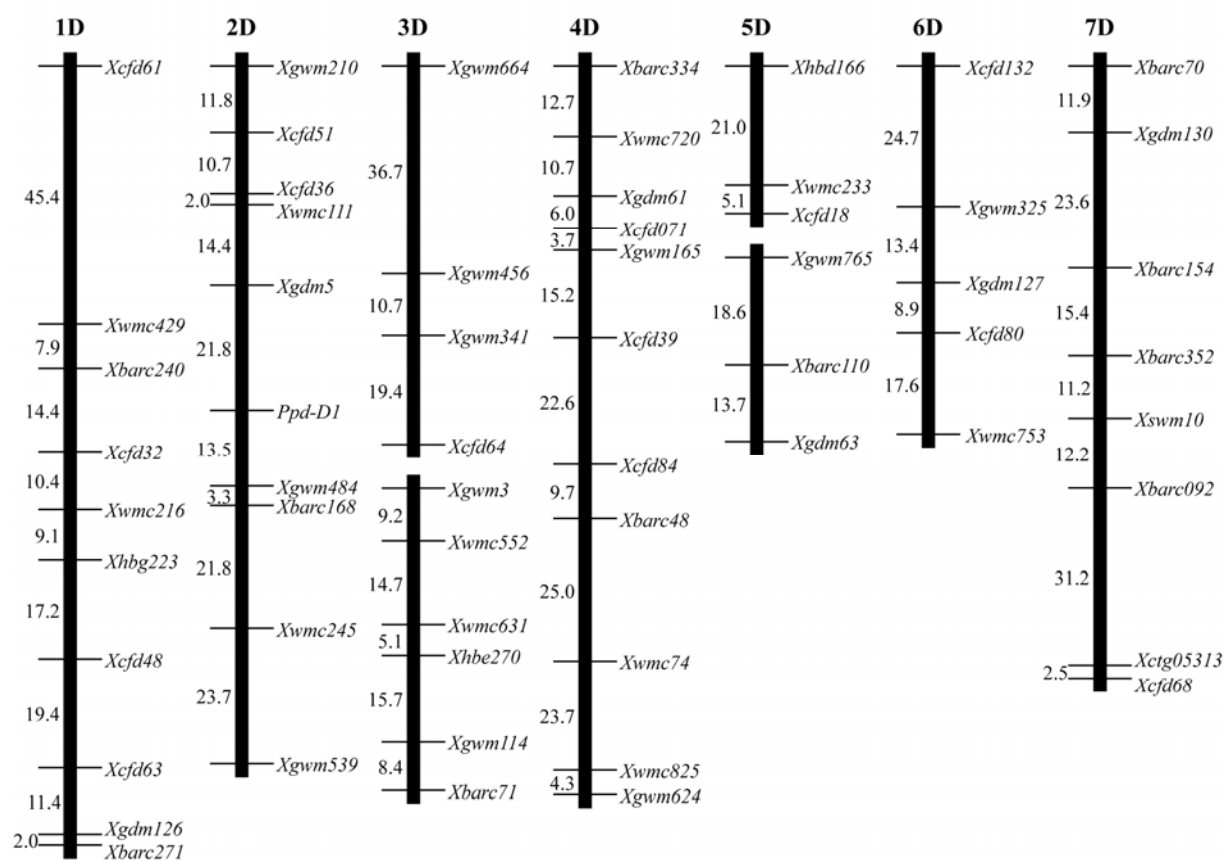


Fig. S4

