

Quantitative trait locus analysis for flowering-related traits using two F₂ populations derived from crosses between Japanese common wheat cultivars and synthetic hexaploids

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Flowering time is an important trait for Japanese wheat breeding. *Aegilops tauschii*, the D-genome donor of hexaploid wheat, is a useful resource to enlarge the D-genome diversity of common wheat. Previously, we identified flowering-related QTLs in F₂ populations of synthetic hexaploid wheat lines between the tetraploid wheat cultivar Langdon and *Ae. tauschii* accessions. Here, to evaluate the usefulness of the early-flowering alleles from *Ae. tauschii* for Japanese wheat breeding, QTL analyses were conducted in two F₂ populations derived from crosses between Japanese wheat cultivars and early-flowering lines of synthetic hexaploid wheat. Only two chromosomal regions controlling flowering-related traits were identified, on chromosomes 2DS and 5AL in the mapping populations, and no previously identified QTLs were found in the synthetic hexaploid lines. The strong effect of the 2DS QTL, putatively corresponding to *Ppd-D1*, was considered to hide any significant expression of other QTLs with small effects on flowering-related traits. When F₂ individuals carrying *Ae. tauschii*-homozygous alleles around the 2DS QTL region were selected, the *Ae. tauschii*-derived alleles of the previously identified flowering QTLs partly showed an early-flowering phenotype compared with the Japanese wheat-derived alleles. Thus, some early-flowering alleles from *Ae. tauschii* may be useful for production of early-flowering Japanese wheat cultivars.

Key words: *Aegilops tauschii* Coss., allopolyploidization, flowering time, *Triticum aestivum* L.

INTRODUCTION

Flowering time (FLT) determination is one of the most important events for higher plant adaptation to regional growth habitats. Common wheat cultivars show wide variation in heading time (HT) and FLT, and control of HT and FLT has been a target for wheat breeding (Snape et al., 2001). Early-maturing wheat cultivars are required, particularly in Japan, because the rainy season overlaps with the season of wheat maturation. High moisture at maturation time (MAT) results in degradation of wheat grain quality through preharvest sprouting, *Fusarium* damage, and so on.

Wheat HT and FLT are controlled by three major genetic components, vernalization requirement, photoper-

iodic sensitivity and narrow-sense earliness (Murai et al., 2005). A few major genes control the vernalization requirement and photoperiodic sensitivity, whereas some minor genes have been identified as quantitative trait loci (QTLs) controlling narrow-sense earliness (Cockram et al., 2007). The vernalization requirement and photoperiodic sensitivity are mainly determined by the *Vrn-1* loci on the long arms of homoeologous group 5 chromosomes and the *Ppd-1* homoeologous loci on the short arms of group 2 chromosomes, respectively (Murai et al., 2005). Most Japanese wheat cultivars, except those in the Hokkaido area, carry the *Ppd-D1a* allele on 2DS, which contributes to their early-flowering phenotype (Seki et al., 2011; Nguyen et al., 2013a). The *Ppd-D1a* mutant allele is photoperiod-insensitive because of a 2-kb deletion upstream of the coding region in a pseudo-response regulator gene (Beales et al., 2007; Nishida et al., 2013). Most spring-type landraces of southwest Japan carry a domi-

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nant *Vrn-D1* allele on 5DL (Iwaki et al., 2001), and the spring habit caused by this allele shows slightly shorter narrow-sense earliness (Kato et al., 2001). Structural mutations at the *Vrn-1* loci, such as insertion/deletion events in the promoter region and large deletions in the first intron, generate dominant alleles in spring-type cultivars of einkorn, durum and common wheat (Yan et al., 2004; Fu et al., 2005). Moreover, *Vrn-D4* is known as another vernalization gene located in the centromeric region of chromosome 5D in common wheat, and wheat varieties with the dominant allele of *Vrn-D4* show the spring growth habit (Yoshida et al., 2010).

To enrich D-genome variation in common wheat, synthetic hexaploid wheat lines produced through hybridization of tetraploid wheat and *Aegilops tauschii* are useful resources (Mujeeb-Kazi et al., 1996; Trethowan and Mujeeb-Kazi, 2008; Jones et al., 2013). Several agronomically important traits such as disease resistance have been transferred from *Ae. tauschii* to common wheat through synthetic hexaploids (Kerber, 1987; Ma et al., 1995; Mujeeb-Kazi et al., 1996). Our empirical studies using a series of wheat synthetics have also shown enriched diversity of the D genome for some morphological and physiological characters (Takumi et al., 2009; Kajimura et al., 2011; Iehisa and Takumi, 2012; Okamoto et al., 2012). The wide variation in HT observed among *Ae. tauschii* accessions is retained in the synthetic hexaploids (Kajimura et al., 2011). Early heading and flowering in synthetic hexaploids are presumably associated with shortened narrow-sense earliness due to effects of the added D-genome (Fujiwara et al., 2010). Recently, we selected three early-flowering lines from the series of wheat synthetics and determined QTLs for flowering-related traits in four mapping populations of the synthetic wheat lines with distinct D-genome origins (Nguyen et al., 2013b). These QTLs significantly contribute to the variation in flowering-related traits among the synthetic wheat lines, implying that D-genome QTLs might be at least partly effective in the hexaploid wheat background. However, their utility for determination of flowering-related traits remains unclear in progeny crossed with Japanese common wheat cultivars. The *Ae. tauschii* populations used as the direct D-genome donors for common wheat are considered to be distributed in a narrow range relative to the entire species range (Feldman, 2001), suggesting that the huge genetic diversity of *Ae. tauschii* is not necessarily represented in common wheat and that the early-flowering lines of wheat synthetics should include early-flowering D-genome alleles that have not been introduced into the D-genome of Japanese common wheat cultivars (Matsuoka et al., 2013; Wang et al., 2013). Here, we conducted QTL analyses for flowering-related traits using two F_2 populations derived from crosses between Japanese common wheat cultivars and two selected lines of the early-flowering wheat synthetics

to examine whether the D-genome QTLs from *Ae. tauschii* are responsible for flowering-related trait variations in the cross progeny. Based on the results, we discuss the usefulness of the synthetic wheat lines for wheat breeding.

MATERIALS AND METHODS

Plant materials Two cultivars of common wheat (*Triticum aestivum* L.) from Japan, Kitanokaori and Norin 61, and two synthetic hexaploid wheat lines were used in this study. Kitanokaori is a winter-type elite cultivar of bread wheat grown on Hokkaido, and Norin 61 is a standard cultivar grown in the central to southwestern parts of Honshu. Synthetic wheat lines showing an early-flowering phenotype were produced from two cross combinations between a tetraploid wheat variety, *Triticum turgidum* ssp. *durum* (Desf.) Husn. cv. Langdon (Ldn), and two *Aegilops tauschii* Coss. accessions, KU-2097 and PI476874 (Kajimura et al., 2011; Nguyen et al., 2013b). These two synthetic wheat lines, Ldn/KU-2097 and Ldn/PI476874, were selected from the 82 wheat synthetic lines derived from crosses between Ldn and various *Ae. tauschii* accessions (Fujiwara et al., 2010; Kajimura et al., 2011) as showing early flowering. Ldn contains a dominant allele of *Vrn-A1* for the spring habit and no *Ppd-1* dominant allele for photoperiodic insensitivity (Fu et al., 2005; Fujiwara et al., 2010). Two F_2 populations were derived from crosses between Kitanokaori and Ldn/KU-2097 and between Norin 61 and Ldn/PI476874. F_2 seeds in the Kitanokaori/Ldn/KU-2097 population ($N = 132$) and the Norin 61/Ldn/PI476874 population ($N = 108$) were sown in November and respectively grown in the 2012–2013 and 2010–2011 seasons. From each of the 10 F_2 plants of Kitanokaori/Ldn/KU-2097, 16 selfed seeds (F_3 generation) were sown in November 2013 and grown in the 2013–2014 season. All F_2 and F_3 individuals as well as four plants of each parent were grown individually in pots arranged randomly in a glasshouse of Kobe University as previously reported (Kajimura et al., 2011).

Phenotype measurement and statistical analyses

Four flowering-related traits were measured in the field. HT and FLT were recorded as days after sowing (Kajimura et al., 2011). MAT was measured as the number of days that had passed before the peduncle turned yellow (Kajimura et al., 2011). The grain-filling period (GFP) was the number of days from flowering to maturation. HT, FLT and MAT were measured for the three earliest tillers of each plant, and mean values were calculated using the data for each F_2 and F_3 plant. The data were statistically analyzed using JMP software ver. 5.1.2 (SAS Institute, Cary, NC, USA). Pearson's correlation coefficients were estimated among the traits measured in each mapping population.

Detection of polymorphisms and genotyping with markers To amplify PCR fragments of simple sequence repeat (SSR) markers, total DNA was extracted from the parents and F₂ individuals using standard procedures. SSR genotyping was performed according to our previous report (Nguyen et al., 2013b). Information on SSR markers and their respective annealing temperatures was obtained from the NBRP KOMUGI website (<http://www.shigen.nig.ac.jp/wheat/komugi/strains/aboutNbrpMarker.jsp>) and the GrainGenes website (<http://wheat.pw.usda.gov/GG2/maps.shtml>). Polymorphisms at the *Ppd-D1* and *Vrn-A1* loci were detected using allele-specific primers (Fu et al., 2005; Beales et al., 2007; Nguyen et al., 2013a, 2013b). The PCR products were separated by electrophoresis through a 1.2% agarose gel and stained with ethidium bromide.

Two phenotypic markers, *Iw2* and *B1*, were used for genotyping the Kitanokaori/Ldn/KU-2097 population. *Iw2* for non-glaucousness and *B1* for awn suppression are well-known markers on 2DS and 5AL, respectively (Tsunewaki and Koba, 1979; Nishijima et al., 2014).

For map construction of chromosome 2D in the Norin 61/Ldn/PI476874 population, we used genotyping data from our previous report (Iehisa et al., 2014). In these genotyping data were included two EST-derived sequence-tagged site markers, TE6 and WE6, that were developed with the *Iw2*-linked markers (Liu et al., 2007; Nishijima et al., 2014). Some single nucleotide polymorphism (SNP) markers, which were converted to cleaved amplified polymorphic sequence (CAPS) or high resolution melting (HRM) markers, were also included to enrich the number of markers. The SNP information was originally obtained from our D-genome SNP database based on RNA-seq data for *Ae. tauschii* (Iehisa et al., 2012, 2014). PCR and analysis of the CAPS and HRM markers were performed according to our previous studies (Iehisa et al., 2012; Matsuda et al., 2012).

Map construction and QTL analysis Polymorphic markers of the parents were genotyped and used for map construction. Genetic mapping was performed using MAPMAKER/EXP software ver. 3.0b (Lander et al., 1987). The threshold value for log-likelihood (LOD) scores was set at 2.5, and the genetic distances were calculated using the Kosambi mapping function (Kosambi, 1944). Chromosomal assignment of SSR markers was generally based on reported reference maps (Somers et al., 2004; Torada et al., 2006; Kobayashi et al., 2010).

QTL analyses were carried out by composite interval mapping using Windows QTL Cartographer software ver. 2.5 (Wang et al., 2011) with the forward and backward method. A LOD score threshold for each trait was determined by computing a 1,000-permutation test. The percentage of phenotypic variation explained by a QTL for a trait and any additive effects were also estimated using this software.

RESULTS

Flowering-related traits in two F₂ populations and their parental accessions The mean values of the parental lines involved in each mapping population differed for flowering-related traits (Table 1). HT, FLT and MAT were significantly shorter in Ldn/KU-2097 than in Kitanokaori. Norin 61 showed significantly earlier attributes for HT, FLT and MAT than Ldn/PI476874, whereas GFP values were significantly shorter in Ldn/PI476874 than in Norin 61. Little variation in the four traits was observed among individual plants of each parental line, but all four traits varied widely in the F₂ populations. Much earlier- and later-heading F₂ individuals were present than in the parental lines, especially in the Kitanokaori/Ldn/KU-2097 population, which suggested transgressive segregation.

Significant ($P < 0.001$) positive correlations were

Table 1. Parental and F₂ population means for four flowering-related traits measured in two F₂ mapping populations

	Heading time	Flowering time	Maturation time	Grain-filling period
Kitanokaori/Ldn/KU-2097 in 2012–2013 season				
Kitanokaori	152.0 ± 1.00*	161.7 ± 0.58*	189.0 ± 1.00*	27.3 ± 0.58
Ldn/KU-2097	148.7 ± 1.53	158.3 ± 1.15	186.7 ± 0.583	28.3 ± 0.58
F ₂ plants	149.4 ± 8.43	157.9 ± 7.85	187.7 ± 5.84	29.8 ± 3.49
Range in F ₂ plants	127.0 – 168.0	139.3 – 176.0	174.5 – 202.5	24.0 – 42.0
Norin 61/Ldn/PI476874 in 2010–2011 season				
Norin 61	115.3 ± 1.28***	124.4 ± 1.17***	163.4 ± 0.88***	39.8 ± 0.87***
Ldn/PI476874	134.9 ± 1.93	144.2 ± 1.20	177.5 ± 1.11	32.1 ± 0.55
F ₂ plants	123.8 ± 8.81	133.2 ± 7.26	170.7 ± 5.65	37.4 ± 3.08
Range in F ₂ plants	110.0 – 146.3	121.6 – 153.3	159.0 – 187.6	22.0 – 44.6

Data are represented as mean (days) ± standard deviation.

Student's *t*-test was used to determine the statistical significance (* $P < 0.05$, *** $P < 0.001$) of parental differences.

observed among HT, FLT and MAT in the two F_2 populations (Table 2). However, GFP was negatively correlated with HT, FLT and MAT in the two F_2 populations. The negative correlation between GFP and the other three traits indicated that the earlier-flowering F_2 individuals required a longer period for grain maturation, and that GFP of the late-flowering F_2 individuals tended to be shorter.

Construction of a linkage map and QTL analysis for flowering-related traits In the Kitanokaori//Ldn/KU-2097 population, 914 primer sets were tested and 194 (21.2%) were found to be polymorphic. Of these, 155 markers including *Iw2*, *B1* and *Vrn-A1* formed 22 linkage groups. The total map length was 1929.0 cM with an average spacing of 12.4 cM between markers. In the Norin 61//Ldn/PI476874 population, 270 (28.2%) of the 958 tested primer sets were polymorphic between the parental lines. In total, 241 loci including *Ppd-D1* were available for construction of a genetic map with 26 linkage groups. The total map length was 3117.0 cM with an average distance of 12.9 cM between markers.

In total, seven QTLs for flowering-related traits were detected on chromosomes 2D and 5A on the Kitanokaori//Ldn/KU-2097 map (Table 3). Of these, four QTLs for HT, FLT, MAT and GFP, with LOD scores of 12.20, 14.20, 9.56 and 4.24, respectively, were found in the same chromosomal region of 2DS (Fig. 1A). These 2DS QTLs explained 16.69 to 46.56% of the phenotypic variation for flowering-related traits. The remaining three QTLs for HT, FLT and MAT, with LOD scores of 9.00, 9.00 and 6.55, respectively, were assigned to the *Vrn-A1* region of 5AL. The 5A QTLs contributed 19.25, 20.44 and 18.65% of the HT, FLT and MAT variation in the Kitanokaori//Ldn/KU-2097 population.

Four QTLs for flowering-related traits were found on the Norin 61//Ldn/PI476874 map (Table 3). These QTLs for the four flowering-related traits were found in the *Ppd-D1* region of 2DS (Fig. 1B). The HT, FLT, MAT and GFP QTLs, with LOD scores of 40.03, 36.25, 24.65 and 12.31, respectively, explained 39.58 to 73.55% of the flowering-related trait variation in the Norin 61//Ldn/PI476874 population.

For all 2DS QTLs identified for HT, FLT and MAT,

Table 2. Correlation coefficient (r) matrices for four traits measured in two F_2 populations

	Heading time	Flowering time	Maturation time
Kitanokaori//Ldn/KU-2097 in 2012–2013 season			
Flowering time	0.9840***		
Maturation time	0.8797***	0.9079***	
Grain-filling period	–0.7301***	–0.7189***	–0.3613***
Norin 61//Ldn/PI476874 in 2010–2011 season			
Flowering time	0.9887***		
Maturation time	0.9023***	0.9108***	
Grain-filling period	–0.6356***	–0.6417***	–0.3282***

Levels of significance are indicated by asterisks; *** $P < 0.001$.

Table 3. Summary of QTLs for flowering-related traits identified in the two F_2 mapping populations

Trait	Chromosome	Map location	LOD score	LOD threshold	Contribution (%)	Additive effect
Kitanokaori//Ldn/KU-2097 in 2012–2013 season						
HT	2D	<i>Xwmc503–Xhbg256</i>	12.20	3.7	39.45	–7.79
FLT	2D	<i>Xwmc503–Xhbg256</i>	14.20	3.6	46.56	–7.18
MAT	2D	<i>Xwmc503–Xhbg256</i>	9.56	4.1	40.03	–4.72
GFP	2D	<i>Xcfd53–Xgwm484</i>	4.24	3.8	16.69	1.97
HT	5A	<i>Xbarc151–Xcfa2163</i>	9.00	3.7	19.25	4.91
FLT	5A	<i>Xbarc151–Xcfa2163</i>	9.00	3.6	20.44	4.11
MAT	5A	<i>Xbarc151–Xcfa2163</i>	6.55	4.1	18.65	2.37
Norin 61//Ldn/PI476874 in 2010–2011 season						
HT	2D	<i>Xwmc112–HRM13</i>	40.03	7.1	73.55	–8.85
FLT	2D	<i>Xwmc112–HRM13</i>	36.25	5.8	58.95	–7.85
MAT	2D	<i>Xwmc112–HRM13</i>	24.65	4.1	56.72	–5.23
GFP	2D	<i>Xwmc112–HRM13</i>	12.31	3.8	39.58	2.45

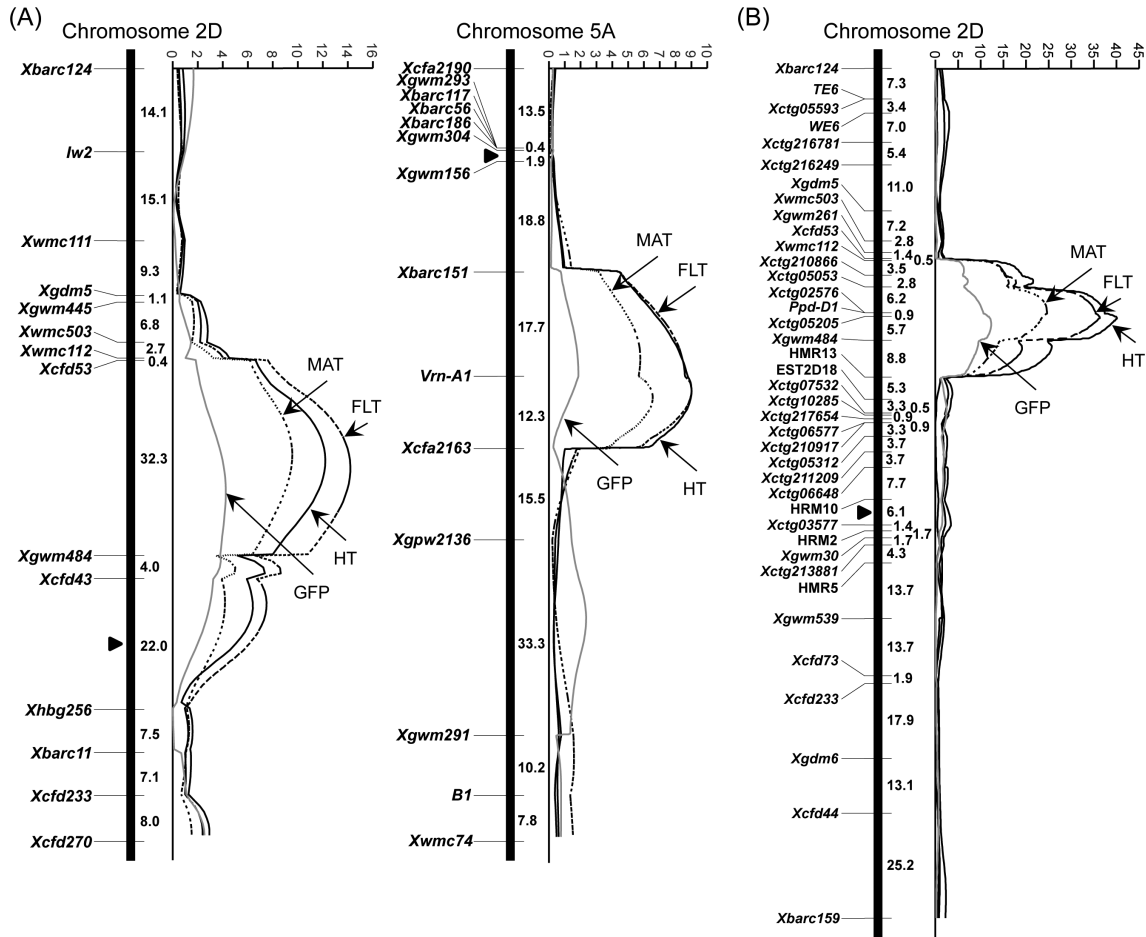


Fig. 1. Linkage maps for chromosomes 2D and 5A and QTL likelihood curves of LOD scores for four flowering-related traits. Genetic distances (in centimorgans) are given to the right of each chromosome. Black arrowheads indicate the putative positions of centromeres. (A) Kitanokaori/Ldn/KU-2097 mapping population. (B) Norin 61/Ldn/PI476874 mapping population.

alleles from the Japanese wheat cultivars showed negative values when assessed for additive effects (Table 3), indicating that the alleles for these QTLs that are derived from the Japanese wheat cultivars contribute to early phenotypes for HT, FLT and MAT. For the 5A QTLs for HT, FLT and MAT, alleles from the Japanese wheat cultivar showed positive values for additive effects, indicating that the alleles for these QTLs that are from the synthetic wheat line contribute to early phenotypes. The GFP QTL on chromosome 2DS showed a positive value for additive effect. Thus, the GFP allele for prolonged filling period was derived from the Japanese wheat cultivars for the 2DS QTL.

Effects of known QTLs on flowering-related traits Our previous QTL analyses using the F_2 mapping populations of synthetic wheat lines identified QTLs for flowering-related traits on chromosomes 7D in the Ldn/PI476874/Ldn/KU-2069 population and 1D, 5D and 6D in the Ldn/KU-2097/Ldn/IG126387 population (Nguyen et al., 2013b). However, we failed to identify the 1D, 5D,

6D or 7D QTLs in the Kitanokaori/Ldn/KU-2097 or Norin 61/Ldn/PI476874 populations. Therefore, data for each flowering-related trait were grouped based on the genotype around the reported QTL regions of each F_2 individual to study the effects of the QTLs identified previously. No significant differences were observed among genotypes around the 1D, 5D and 6D QTLs for HT in the Kitanokaori/Ldn/KU-2097 population (Fig. 2A). A similar result was obtained for the 7D QTL in the Norin 61/Ldn/PI476874 population (data not shown).

To mask the large effects of 2DS QTLs identified here on flowering-related traits, F_2 individuals carrying the Ldn/KU-2097-homozygous (photoperiod-sensitive) and Ldn/PI476874-homozygous (photoperiod-sensitive) alleles around the 2DS QTL region were selected and the genotypic effects of the 1D, 5D, 6D and 7D QTLs for HT were compared based on the genotype around the reported QTL regions of each selected F_2 individual. Around the 6D QTL, the selected F_2 individuals with heterozygous and Ldn/KU-2097-homozygous alleles showed significantly ($P < 0.05$) earlier HT than those with Kitanokaori-homozygous

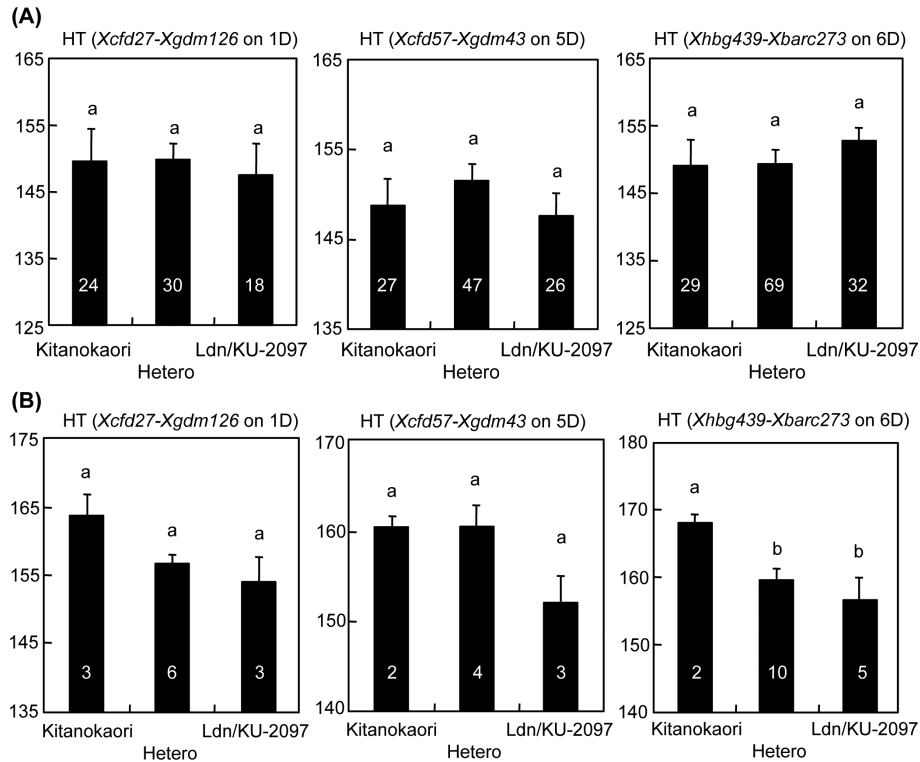


Fig. 2. Genotypic effects at each previously detected QTL on the observed variation in flowering-related traits in the Kitanokaori/Ldn/KU-2097 mapping population. Markers used to deduce the genotype at a QTL are listed above each graph, and the number of the F_2 individuals is indicated within each bar. Means (days) $\pm$ standard error with the same letter are not significantly different ($P > 0.05$, Tukey-Kramer HSD test). (A) F_2 individuals selected independently of the genotype around the *Ppd-D1* region. (B) F_2 individuals with the Ldn/KU-2097-homozygous allele around the *Ppd-D1* region.

alleles (Fig. 2B). The F_2 individuals carrying Ldn/KU-2097-homozygous alleles around the 1D and 5D QTLs for HT appeared to display earlier HT attributes than those with the Kitanokaori-homozygous allele, although these differences were not significant (Fig. 2B). In the Norin 61/Ldn/PI476874 population, no significant differences in flowering-related traits were observed among genotypes around the 7D QTL.

We then chose 10 F_2 individuals with Ldn/KU-2097-homozygous alleles around the 2DS QTL region from the Kitanokaori/Ldn/KU-2097 population, and the effects of the 1D, 5D and 6D QTLs for HT on flowering-related traits were compared in their selfed progeny (F_3 generation). The mean values of each $F_{2.3}$ plant varied for the four flowering-related traits (Table 4). $F_{2.3}$ plants with Ldn/KU-2097-homozygous alleles around the 5D QTL for HT tended to display earlier attributes for HT, FLT and MAT than other $F_{2.3}$ progeny or Kitanokaori.

DISCUSSION

Allohexaploidization between the tetraploid wheat (AABB) and *Ae. tauschii* (DD) genomes affects gene expression profiles in synthetic hexaploid wheat

(Pumphrey et al., 2009; Chagué et al., 2010). This alteration of gene expression is considered to be due to epistatic interaction between the AB and D genomes and genetic and epigenetic modification of the chromatin structure (Khasdan et al., 2010; Mestiri et al., 2010; Feldman et al., 2012). For control of flowering-related traits, the *Ppd-1* and *Vrn-1* genotypes of the A and B genomes largely affect the expression of flowering-related traits in synthetic hexaploid wheat (Gororo et al., 2001). On the other hand, when Ldn was used as the single AB donor parent for production of synthetic wheat, D-genome variation for some morphological and physiological traits including flowering-related traits in *Ae. tauschii* was at least partly expressed in the hexaploid background (Kajimura et al., 2011; Iehisa and Takumi, 2012; Okamoto et al., 2012). In the present study, QTL analyses revealed only two chromosomal regions controlling flowering-related traits in each mapping population, and failed to find any QTL previously identified using mapping populations of the synthetic hexaploid wheat lines. The chromosomal location of the QTLs identified on 2DS corresponds to that of *Ppd-D1* in both the Kitanokaori/Ldn/KU-2097 and Norin 61/Ldn/PI476874 populations (Fig. 1). At the centromeric region of barley chromosome 2H, *EPS2*, a

Table 4. Parental lines and F_{2:3} means for four flowering-related traits in *ppd-D1*-homozygous individuals of the Kitanokaori//Ldn/KU-2097 population

Accession	1D QTL genotype	5D QTL genotype	6D QTL genotype	Heading time	Flowering time	Maturation time	Grain-filling period
Kitanokaori	Kitanokaori	Kitanokaori	Kitanokaori	162.2 ± 0.38 ^{ab}	167.4 ± 0.35 ^{abc}	198 ± 0 ^{abc}	30.6 ± 0.35 ^{ab}
Ldn/KU-2097	KU-2097	KU-2097	KU-2097	147.5 ± 1.41 ^c	157.8 ± 0.35 ^d	188 ± 0 ^d	30.3 ± 0.35 ^{ab}
F ₂ -#5	KU-2097	KU-2097	KU-2097	150.8 ± 8.42 ^c	158.8 ± 6.96 ^d	189.9 ± 6.20 ^d	31.1 ± 4.42 ^a
F ₂ -#17	KU-2097	heterozygous	KU-2097	168.7 ± 1.92 ^a	173.2 ± 2.07 ^a	201.0 ± 1.39 ^a	27.8 ± 1.14 ^b
F ₂ -#30	heterozygous	heterozygous	KU-2097	165.2 ± 2.33 ^a	170.1 ± 2.08 ^{ab}	199.9 ± 2.03 ^{ab}	29.7 ± 1.43 ^{ab}
F ₂ -#46	Kitanokaori	heterozygous	heterozygous	164.9 ± 3.24 ^a	171.3 ± 3.12 ^a	198.8 ± 2.77 ^{ab}	27.5 ± 1.78 ^b
F ₂ -#47	heterozygous	Kitanokaori	KU-2097	165.4 ± 1.81 ^a	170.8 ± 1.52 ^{ab}	200.5 ± 1.86 ^a	29.7 ± 1.51 ^{ab}
F ₂ -#80	Kitanokaori	heterozygous	Kitanokaori	166.8 ± 2.31 ^a	172.0 ± 1.69 ^a	200.0 ± 1.30 ^{ab}	28.0 ± 0.94 ^b
F ₂ -#92	KU-2097	KU-2097	heterozygous	159.8 ± 2.62 ^b	166.4 ± 2.80 ^c	195.2 ± 1.77 ^c	28.8 ± 1.95 ^{ab}
F ₂ -#95	Kitanokaori	KU-2097	heterozygous	160.4 ± 3.45 ^b	167.6 ± 3.24 ^{bc}	197.0 ± 2.20 ^{bc}	29.4 ± 1.93 ^{ab}
F ₂ -#104	heterozygous	Kitanokaori	heterozygous	166.7 ± 2.33 ^a	172.0 ± 2.17 ^a	200.5 ± 1.00 ^a	28.5 ± 1.55 ^b
F ₂ -#125	heterozygous	heterozygous	KU-2097	165.6 ± 3.40 ^a	169.8 ± 2.44 ^{abc}	198.7 ± 2.70 ^{ab}	28.9 ± 1.53 ^{ab}

Data are represented as mean (days) ± standard deviation.

Means with the same letter were not significantly different ($P > 0.05$, Tukey-Kramer HSD test).

barley homolog of *Antirrhinum CENTRORADIALIS* and *Arabidopsis TERMINAL FLOWER1*, is located as a flowering time regulator (Comadran et al., 2012). The 2DS QTL position was much more closely linked to the *Ppd-D1* locus than was the *EPS2* centromeric region. The *Ppd-D1* dominant allele is frequently found in Japanese wheat cultivars, and contributes to their early flowering phenotype (Seki et al., 2011; Nguyen et al., 2013a). In the Kitanokaori//Ldn/KU-2097 and Norin 61//Ldn/PI476874 populations, the 2DS QTLs showed high LOD scores and explained large amounts of the phenotypic variation for flowering-related traits (Table 3). The strong effects of the 2DS QTL could hide significant expression of other QTLs with weak effects on flowering-related traits. In fact, when F₂ individuals with the Ldn/KU-2097-homozygous allele around the *Ppd-D1* region were selected, the 6D QTL for HT on the D genome, identified previously in the synthetic wheat population (Nguyen et al., 2013b), significantly affected HT in the F₂ progeny of Kitanokaori//Ldn/KU-2097 (Fig. 2). In addition, the Ldn/KU-2097-homozygous allele around the 5D QTL tended to cause earlier HT, FLT and MAT than the Kitanokaori-homozygous allele under *ppd-D1* recessive conditions (Table 4). On the other hand, we failed to detect statistically significant effects of the 1D and 5D QTLs on HT in the Kitanokaori//Ldn/KU-2097 population and of the 7D QTLs on the flowering-related traits in the Norin 61//Ldn/PI476874 population even when F₂ individuals with the Ldn/KU-2097-homozygous allele and the Ldn/PI476874-homozygous allele around the *Ppd-D1* region were selected. Because the number of available F₂ and F_{2:3} plants was limited due to the restricted size of

the F₂ populations, precise effects of the three D-genome QTLs on HT should be evaluated in future studies using near-isogenic lines with the genetic background of Japanese wheat cultivars.

The negative correlations between MAT and GFP were significant in both the Kitanokaori//Ldn/KU-2097 and Norin 61//Ldn/PI476874 populations (Table 2). A similar negative correlation between MAT and GFP was previously reported in synthetic hexaploid wheat lines (Kajimura et al., 2011), whereas positive correlations between MAT and GFP were detected in four F₂ mapping populations of synthetic hexaploids and two F₂ mapping populations of common wheat (Nguyen et al., 2013a, 2013b). In the Kitanokaori//Ldn/KU-2097 and Norin 61//Ldn/PI476874 populations, the strong effect of the *Ppd-D1* region on GFP might have been responsible for the negative correlations. As previously reported, GFP can be influenced by temperature in wheat (Wiegand and Cuellar, 1981; Knott and Gebeyehou, 1987). The grain-filling process seemed to progress more quickly under higher temperatures in the late-flowering F₂ individuals with the *ppd-D1* allele than in the early-flowering individuals with the *Ppd-D1* allele in both populations in the present study.

The 5AL QTLs detected in the Kitanokaori//Ldn/KU-2097 population appeared to correspond to *Vrn-A1* (Fig. 1A). Ldn contains a spring allele with a large deletion in the first intron of *Vrn-A1* (Fu et al., 2005), whereas Kitanokaori, a winter-type cultivar, putatively has a recessive allele with no deletions in the promoter or first intron regions of *Vrn-A1*. Thus, the *Vrn-A1* dominant allele from Ldn could contribute to earliness for HT and

FLT in the mapping population. This suggestion supports previous data showing slightly shorter narrow-sense earliness for the spring allele of *Vrn-D1* (Kato et al., 2001). In addition, a phytochrome gene, *PHYC*, acts as one of the major flowering accelerators under long-day conditions in tetraploid wheat, and the *PHYC* loci are linked to the *Vrn-1* loci in wheat and barley (Chen et al., 2014). An allelic difference of *PHYC* might contribute to the HT and FLT variations in the mapping population, although little information about natural variation in *PHYC* has been reported in common wheat. The relationship between the 5A QTL and *PHYC* in the Kitanokaori//Ldn/KU-2097 population should be clarified in further studies. On the other hand, although the vernalization requirement of Norin 61 should be affected by the spring allele of *Vrn-D1* (Yan et al., 2004), no *Vrn-1* locus was found for flowering-related traits in the Norin 61//Ldn/PI476874 population. These observations suggest that detection of the *Vrn-1* loci as QTLs for flowering-related traits depends on the environment during the growing season, or that effects of the spring allele of *Vrn-1* on earliness are small after over-winter growth of wheat plants. Dominant alleles of the *Ppd-1* loci more markedly affect earliness of HT, FLT and MAT than do the *Vrn-1* loci in the wheat mapping populations.

The narrow-sense earliness genes are thought to be involved in the fine-tuning of wheat flowering (Griffiths et al., 2009; Zikhali et al., 2014), and allelic divergence in a narrow-sense earliness gene results in a one- to five-day difference in flowering time in wheat (Valarik et al., 2006; Zikhali et al., 2014). The KU-2097-derived alleles of the 5D and 6D QTLs for HT showed a significantly early-flowering phenotype under *ppd-D1* recessive conditions (Fig. 2, Table 4), suggesting that the earliness alleles may be useful for fine-tuning of wheat flowering. For efficient marker-assisted selection, molecular markers linked to the two KU-2097-derived alleles of the 5D and 6D QTLs for HT should be developed. Although epistatic interaction between the AB and D genomes makes it difficult to detect the effects of useful alleles from *Ae. tauschii* on flowering-related traits in hexaploid wheat, at least some of the early-flowering alleles from *Ae. tauschii* should be made available for production of early-flowering Japanese wheat cultivars.

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