



Quantitative trait locus analysis for flowering time in hexaploid wheat

Nguyen, Tuan Anh

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Doctoral Dissertation

**Quantitative trait locus analysis
for flowering time in hexaploid wheat**

**6倍性コムギの開花期を
制御する量的形質遺伝子座の研究**

Nguyen Tuan Anh

July, 2013

Laboratory of Plant Genetics, Department of Agrobioscience
Graduate School of Agricultural Science
Kobe University

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Chapter I

General Introduction

Flowering time, one of the fundamental events in the life cycle of many higher plants, is precisely controlled by environmental conditions and developmental regulation. Extensive studies on the complexity of floral transition in higher plants have revealed an intricate network of signaling pathways that transduce and integrate developmental and environmental signals to promote or inhibit the transition to flowering. In *Arabidopsis thaliana*, an excellent higher plant in which to approach this complexity, genetics and molecular interaction analyses under various environmental conditions indicated many of the genes involved to be assigned to distinct regulatory pathways which are generally referred to by the environmental and endogenous cues that they respond to, i.e. the vernalization, photoperiod, gibberellin and autonomous pathways (reviewed by Bäurle1 and Dean 2009). These convergences of pathways on a common a set of ‘floral integrator’ genes that integrate the outputs of the various pathways and, under favorable conditions, directly activate floral meristem identity genes, which specify the formation of floral meristems that will develop into flowers (**Fig.1-1**).

Like model plant *A. thaliana*, heading date/flowering time for life cycle of wheat is one of the most important traits in wheat breeding because it affects the adaptability of the crop to various environmental conditions, so adjusting flowering time to the growth environment is also a significant objective in crop breeding. Now it is well known that the wheat flowering time is controlled by three major genetic elements, vernalization requirement, photoperiodic sensitivity and narrow earliness. The first two genes are vernalization (*Vrn-1* genes) and photoperiodic response (*Ppd-1* genes) act in response to the environmental stimuli cold and day length; while the latter narrow earliness *per se* (*Eps* genes) act independently of the environment, determining the number of vegetative

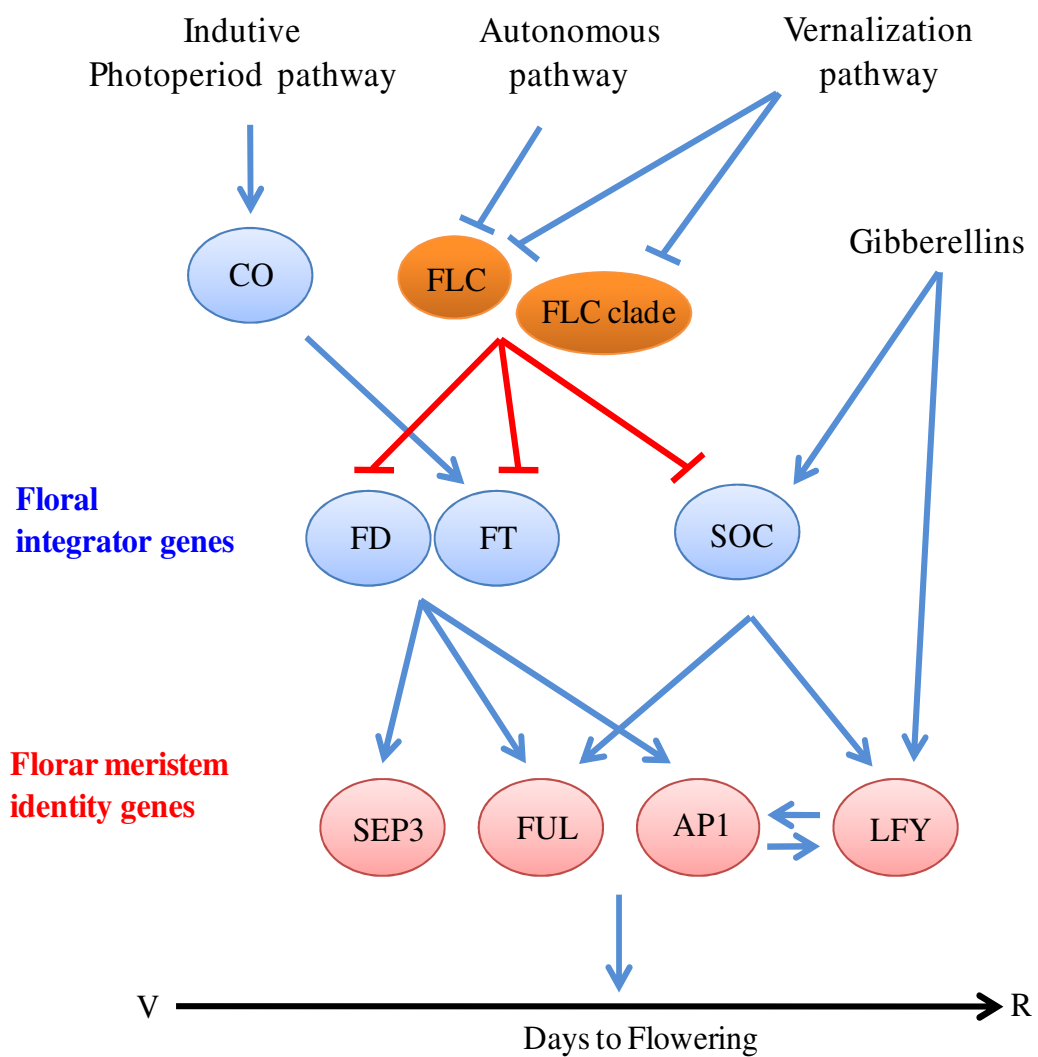


Fig. 1-1. Outline of flowering pathways in *Arabidopsis thaliana*

and floral primordia being initiated. The major genes controlling sensitive to vernalization response, the *Vrn* gene, determine the control of the spring wheat/winter wheat difference. This requirement is determined by four genes, namely, *Vrn-A1* (formerly *Vrn1*) on chromosome 5A, *Vrn-B1* (formerly *Vrn2*) on chromosome 5B, *Vrn-D1* (formerly *Vrn3*) on chromosome 5D, and *Vrn-B4* on chromosome 7B (Law et al. 1976; Snape et al. 1996; Iwaki et al. 2001). The *Vrn1* encodes a MADS-box transcription factor with high similarity to *Arabidopsis* floral meristem-identity genes *APETALA1* (*API*) and *FRUITFULL* (*FUL*) (Murai et al. 2003; Fu et al. 2005) and is required for flowering (Shitsukawa et al. 2007). The *Vrn2* encodes a CCT-domain protein that has no clear homolog in *Arabidopsis* (Yan et al. 2004). *Vrn2* acts as a repressor of flowering by blocking the expression of *Vrn3*, which encodes a RAF kinase inhibitor like protein with homolog to *FLOWERING LOCUS T* (*FT*) (Yan et al. 2006). The genetic control of photoperiod (long-day) response is mainly determined by a homoeologous series of genes *Ppd-A1*, *Ppd-B1*, and *Ppd-D1* (formerly *Ppd3*, *Ppd2*, and *Ppd1*) located on short arms of chromosomes 2A, 2B, and 2D, respectively (Welsh et al. 1973; Law et al. 1978; Scarth and Law 1983). Under long day conditions, the photoperiod pathway functions of wheat plant similarly to that in *Arabidopsis*, *CONSTANS* (*CO*) like gene, *Ppd1* activates *Vrn3* (Turner et al. 2005). The *Ppd1* has a stronger action on heading time than the other two. It seems to encode a pseudo-responsive regulator (PRR) protein related with plant circadian clock and have stronger action on heading time than other two (Beales et al. 2007). Narrow-sense earliness is the earliness of fully vernalized plants grown under long days. Nevertheless, the earliness *per se* genes are known to be located on several chromosomes including group 2 homoeologous chromosomes (Scarth and Law 1983). It has been reported that some chromosomes include quantitative trait loci (QTLs) controlling narrow-sense earliness (Hoogendoorn 1985; Law 1987; Miura and Worland

1994, Hanocq et al. 2004; Lin et al. 2008). The three sets of genes influencing flowering time act together to determine the exact time of flowering and hence the suitability of a genotype for flowering under particular environmental conditions (**Fig.1-2**) (Worland 1996; Worland et al. 1998). The harvesting earliness consists of two wheat developmental processes; one is flowering time control described above and another is grain filling period that has a little information (**Fig.1-3**) and only the *Gpc-B1* locus on chromosome 6B has been shown to affect this trait. The *Gpc-B1* gene has pleiotropic effects on multiple traits such as the grain protein content, grain micro-nutrient concentration, flag leaf senescence and the grain-filling period (Uauy et al. 2006).

Common wheat, hexaploid wheat, is one of the most important cereal crops grown in many regions of the world for human consumption, providing over 20% of total food calories and protein in human nutrition, and supplying a staple food in more than 40 countries for over 35% of the world's population. It is the most widely distributed from the southern regions of South America and Australia to the northern latitudes of Canada and China, and can grow over a wide range of elevations, climatic conditions and soil fertility (Bushuk 1998). The successful worldwide of wheat crop growing in a diverse range of environments, all over the world, is because of, in part, breeding and selection for appropriate heading time and its cultivars show varied heading time in order to adapt to different regional environments (Snape et al. 2001). Due to the importance of common wheat for human consumption, especially human being is faced with warming of the earth, growing population, and natural disasters, so physiological and molecular mechanisms controlling changes in the developmental phase of wheat plant including flowering time that have been studied extensively during last four decades. A main objective of wheat breeders is to match genotypes to environments to reach the maximum yield stability across location and years. This effort allows a better targeting of germplasm to specific environments, reduces the risk of crop failure, increases the

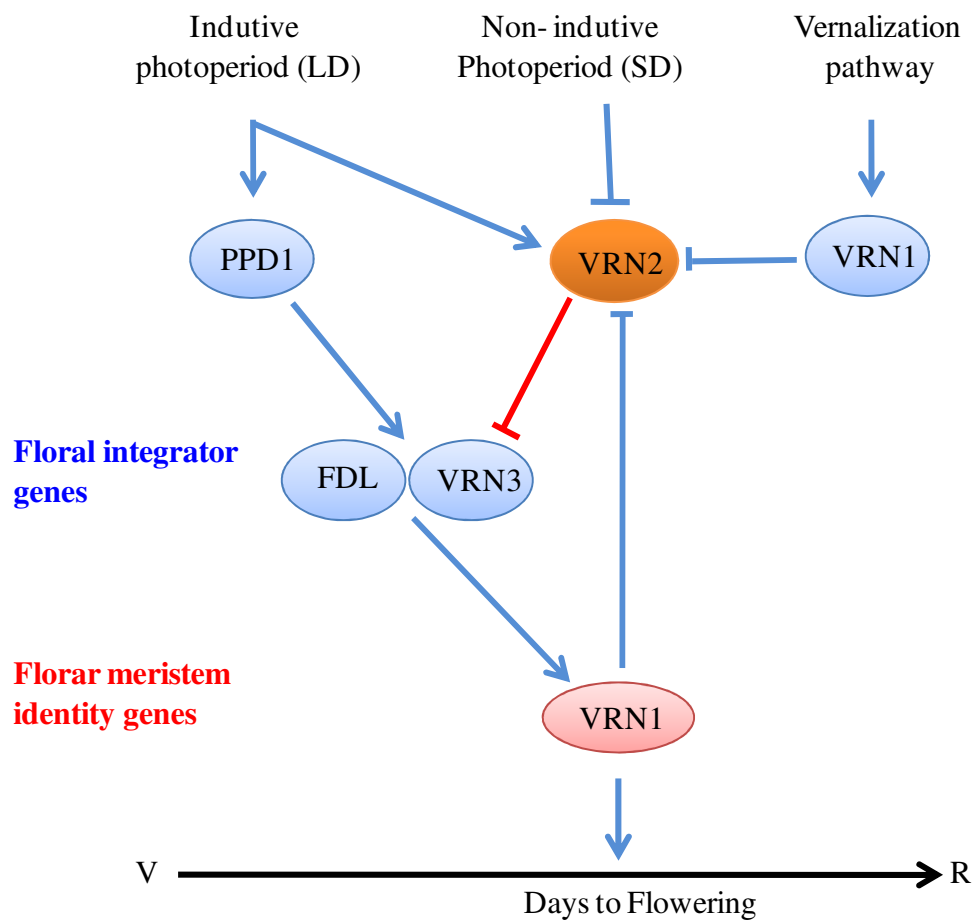


Fig. 1-2. Outline of flowering pathways in wheat

number of agricultural products over an area limitation, and insures diverse environments by optimization of heading time.

In Japan, more early harvesting cultivars are strongly required for wheat breeding, because wheat harvesting season is usually June which is a rainy season. Moisture condition in the harvesting season reduces quality of the wheat grain and induces both pre-harvest sprouting and *Fusarium* diseases. So, if the period of the flowering time or grain filling period will enable to be controlled, early-maturing varieties with genetically shorter were selected for short growing seasons, i.e. the periods until grain harvesting will be earlier and escape the damages caused by rain or hot temperature. While late-maturing varieties were selected where long growing seasons prevail (Kato and Yokoyama 1992; Law and Worland 1997). However, there is little information about genetics of harvesting earliness and narrow earliness especially in landraces and wild wheat relatives. Understanding the genetic control of the harvesting times of the life cycle will enable plant breeders to fine-tune growth and development to the demands of a particular environment.

Landraces (the local varieties), which have appeared through a combination of natural selection and chosen by farmers (Belay et al. 1995), often have a broader genetic base and therefore can provide characteristics critical value for breeding (Keller et al. 1991; Tesemma et al. 1998). They had have an abundant genetic variation in many agricultural traits including flowering time (Tesemma et al. 1998; Kato and Yokoyama 1992; Iwaki et al. 2001; Terasawa et al. 2009), and their lines supplied useful traits for modern wheat breeding (Feldman and Sears 1981). In previous studies, we found that many wheat landraces in Nepal, Bhutan and Tibet show wide variation in flowering traits (Kato and Yokoyama 1992; Takumi 2009). A common garden experiment revealed the presence of early-flowering landraces in Nepal, which have flowering time as short as that of the Japanese early-flowering cultivar Shiroganekomugi (Takumi 2009). Heading

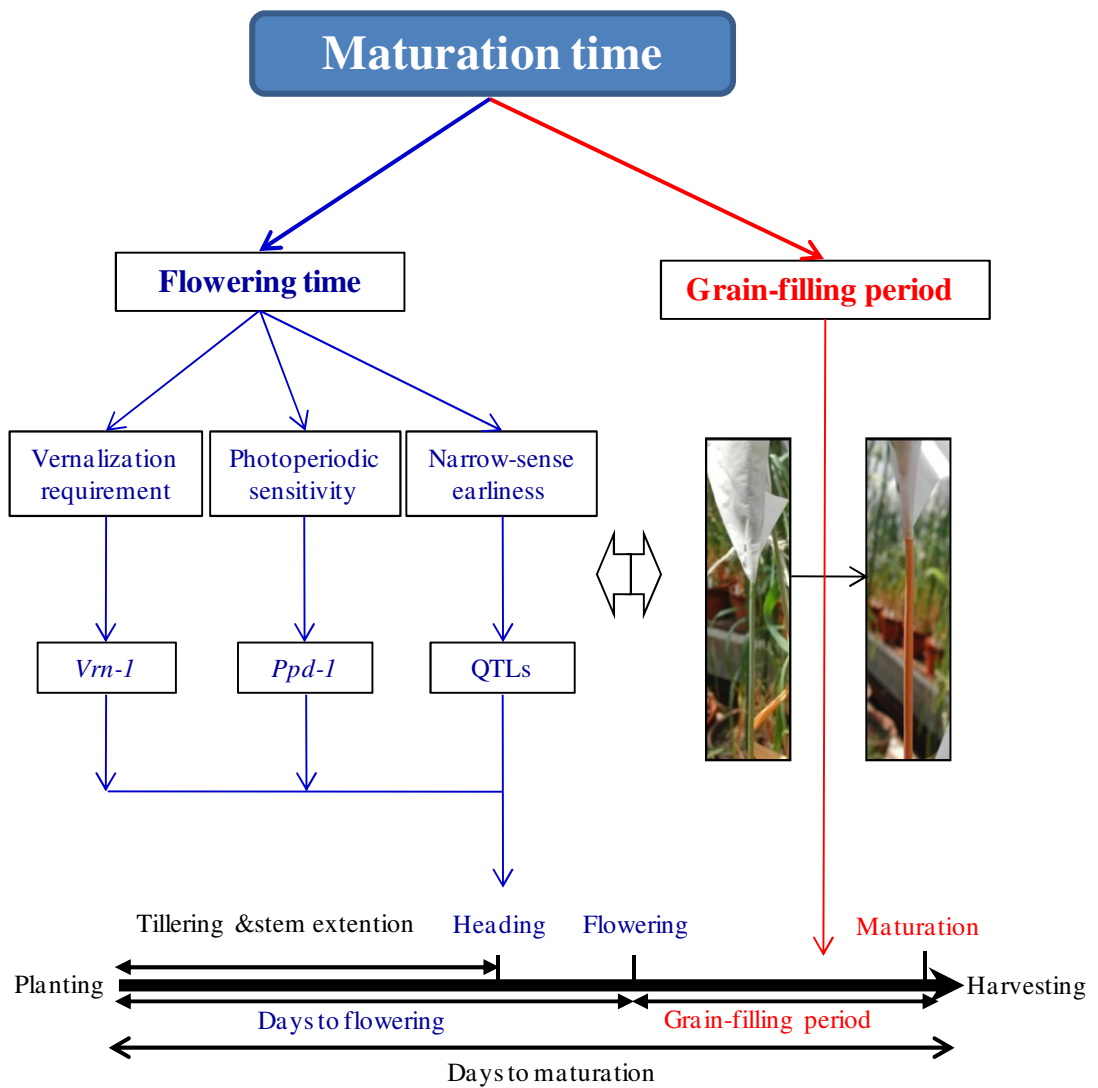


Fig. 1-3. Progression through growth phases during post-embryonic development in wheat plant.

time is highly correlated with photoperiodic response and narrow-sense earliness in the Nepalese and Bhutanese landraces, and in particular, narrow-sense earliness shows significant association with altitude of landrace collection sites in Nepal (Kato and Yokoyama 1992). However, information about the genetic basis of flowering traits in the Nepalese and Bhutanese wheat landraces has under controlled. Those studies indicated that early flowering landraces from Nepal might be useful for Japanese wheat breeding. One of goals of the present research is an identification of novel early flowering-related alleles from the Nepal landraces.

Wild wheat relatives are important genetic resources for wheat breeding. Especially, a D-genome donor of common wheat, *Aegilops tauschii* Coss. is significant because genetic variation of D genome is relatively smaller than those of A and B genomes in hexaploid wheat. Common wheat is an allohexaploid species that originated from natural hybridization between tetraploid wheat (*Triticum turgidum* L.), including emmer and durum wheats, and *Ae. tauschii* (Kihara 1944; McFadden and Sears 1944). Thus, allohexaploid wheat share identical A and B genomes derived from Langdon and contain diverse D genomes originating from the *Ae. tauschii* pollen parents. The *Ae. tauschii* accessions are widely distributed in Eurasia and shows abundant genetic variation (Dvorak et al. 1998; Matsuoka et al. 2008; Mizuno et al. 2010). The birthplace of common wheat is considered to lie within the area comprising Transcaucasia and the southern coastal region of the Caspian Sea (Feldman 2001). Therefore, the *Ae. tauschii* populations involved in the origin of common wheat are limited to a narrow distribution range relative to the entire species range, suggesting that this species holds vast genetic diversity that is not represented in common wheat (Feldman 2001). Natural variation in the *Ae. tauschii* population provides potential for improving modern varieties of common wheat. In fact, hexaploid synthetic wheat derived from crosses between tetraploid wheat and *Ae. tauschii* has the potential to provide new genetic variation for abiotic stress

tolerance (Trethowan and Mujeeb-Kazi 2008). Takumi et al. (2009) and Kurahashi et al. (2009) have developed hexaploid wheat synthetic lines by generating from interspecific crosses between a tetraploid wheat *Triticum durum* cv. Langdon and various *Ae. tauschii* accessions (**Fig. 1-4**). These lines are useful to study the D-genome effects on various agronomical traits including flowering and harvesting time. A preliminary study with these lines showed that they exhibited wide variation in the flowering-related traits, including grain filling period, and that the large variation in heading time observed in *Ae. tauschii* is also present in the hexaploid synthetic wheat (Kajimura et al. 2011) and that the D genome at least partly affects the flowering of hexaploid wheat (Nguyen et al. 2013). Nguyen et al. (2013) showed that synthetic wheat lines can be useful for the identification of new agriculturally important loci which were detected on synthetic wheat lines can be transferred to, and used for the modification of flowering and grain maturation. But the genetic basis of variation in the flowering-related traits present in the D genome remains unknown. The goal of the present research is identification of D-genome loci for controlling the flowering and harvesting time through the hexaploid synthetic lines.

Together with wild wheat *Ae. tauschii*, tetraploid wheat such as emmer and durum (*Triticum turgidum* L.) have played a critical role for wheat breeding, their A and B-genomes have been used as genetic sources for creating hexaploid synthetic wheat. However, morphological and molecular diversities of tetraploid wheat genetics have not been fully clarified. Recently, many leaf rust resistance genes, strip rust, stem rust and powdery mildew have been transferred from the relatives of wheat, such as durum wheat (Knot 1989; Lutz et al. 1995). Our previous study has shown that tetraploid wheat genome mainly contribute to the grain shape in synthesized hexaploid wheat. (Okamoto et al. 2012). It was regarded as a mostly genomic control the morphological characteristics including spike shape, grain shape, and thickness of empty glumes in allopolyploid wheat

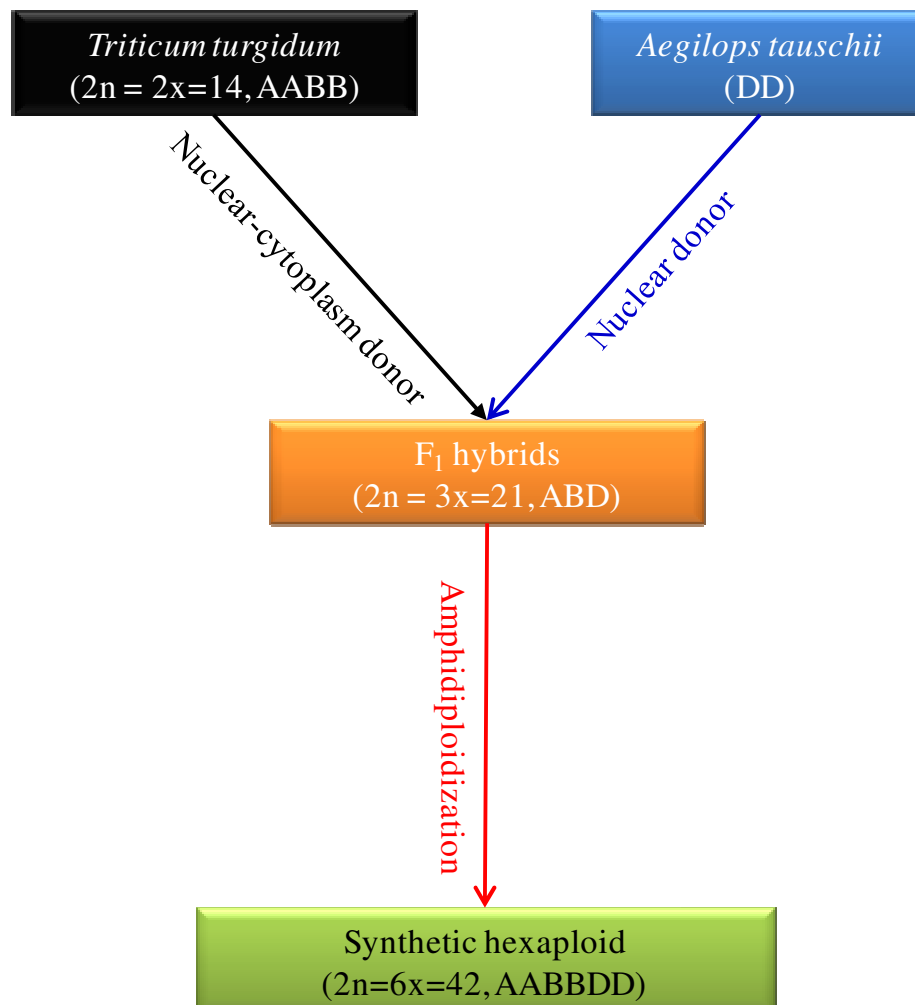


Fig. 1-4. Synthetic hexaploid wheat line derived from interspecific hybridization between tetraploid wheat contributed the AB genomes and *Aegilops tauschii* Coss *Aegilops tauschii* contributed the D genome

(Peng et al. 2003; Zhang et al. 2011). Because the important genetic backgrounds of tetraploid wheats that have a lot of useful agronomical trait were used to wheat breeding. Together with detection of valuable QTLs from D-genome, QTLs from A, B-genome for controlling the flowering and harvesting time were also commended by using crosses between Japanese wheat line and hexaploid synthetic wheat line, which derived from tetraploid wheat Langdon (*Triticum turgidum* L.) and diploid wheat *Ae. tauschii*.

In sum, the landraces and hexaploid synthetic wheat lines, as important gene pools for wheat breeding, showed wide variations in flowering time and another duration for seed maturation. In recent years, genetic analyses of heading and other traits have been conducted mainly by using F₂ population and/ or aneuploid or chromosome substitution lines. Significant progress has been made in the use of molecular approaches for plant breeding. So flowering of their line related crossing F₂ mapping populations have been used to construct the linkage maps for identification of quantitative traits loci (QTLs). Determining the location of genes and QTLs controlling complex characters such as heading time can be possible by using marker mediated techniques. A, B and D-genome-specific, polymorphic SSR markers should be screened. Their markers have been greatly exploited in wheat and assigned to wheat chromosomes (Röder et al. 1998; Somers et al. 2004; Song et al. 2005; Torada et al. 2006). However, the number of SSR markers reported is not sufficient to identify closely linked markers useful in physical map. For high-density-map, additional molecular markers such as SNP-based markers were derived from leaf transcripts of *Ae. tauschii* accession (Iehisa et al. 2012). The additional markers were selected from a high confidence SNP dataset constructed by comparing the next generation sequencing of leaf transcripts between two genetically distinct accessions of *Ae. tauschii* in previous study (Iehisa et al. 2012). Then, QTL mapping will be done using the F₂ population, and gene expression studies will be

conducted to characterize the molecular nature and roles of the identified QTLs for wheat flowering processes. Through these studies, novel A, B and D-genome loci for controlling the flowering time should be identified, and their usefulness of the identified QTLs should be examined.

2. Objectives of the doctoral dissertation

Chapter II. We conducted QTL analyses for flowering-related traits using four F₂ populations between early and late flowering synthetic wheat lines to identify genetic loci responsible for variation observed in the hexaploid background. Based on the results, we discuss the usefulness of the synthetic wheat lines for wheat breeding.

Chapter III. Identify causal loci for early flowering in the Nepalese landrace and Shiroganekomugi. Based on the results, genetic differences in FLT between the Nepalese and Japanese landraces and their usefulness for wheat breeding were discussed.

Chapter IV. QTL analysis for early flowering related traits using F₂ population derived from synthetic wheat line and Japanese elite variety and to compare the location of genes for early flowering from different genetic backgrounds of hexaploid wheat

Chapter 2

Differential contribution of two *Ppd-1* homoeoalleles to early-flowering phenotype in Nepal and Japanese varieties of common wheat

1. Abstract

Wheat landraces carry abundant genetic variation in heading and flowering times of leading varieties. Here, we studied flowering-related traits of two Nepalese varieties, KU-4770 and KU-180, and a Japanese wheat cultivar, Shiroganekomugi (SGK). These three wheat varieties showed similar flowering time in a common garden experiment. In total, five significant quantitative trait loci (QTLs) for three examined traits, the heading, flowering and maturation times, were detected using an F₂ population of SGK/KU-4770. The QTLs were found at the *Ppd-1* loci on chromosomes 2B and 2D, and the 2B QTL was also confirmed in another F₂ population of SGK/KU-180. The *Ppd-D1* allele from SGK and the *Ppd-B1* alleles from the two Nepalese varieties might be causal for early-flowering phenotype. The SGK allele of *Ppd-D1* contained a 2-kb deletion in the 5' upstream region, indicating a photoperiod-insensitive *Ppd-D1a* allele. Real-time PCR analysis estimating the *Ppd-B1* copy number revealed that the two Nepalese varieties included two intact copies of *Ppd-B1*, putatively resulting in photoperiod insensitivity and an early-flowering phenotype. The two photoperiod-insensitive homoeoalleles of *Ppd-1* could independently contribute to segregation of early-flowering individuals in the two F₂ populations. Therefore, wheat landraces are genetic resources for discovery of alleles useful for improving wheat heading or flowering times.

2. Introduction

Common wheat cultivars show widely varying heading time (HT) in order to adapt to different regional environments (Snape et al. 2001). Heading/flowering time (HT/FLT)

is one of the important traits in wheat breeding. Wheat HT/FLT is controlled by three major genetic components, vernalization requirement, photoperiodic sensitivity and narrow-sense earliness (earliness *per se*) (reviewed in Murai et al. 2005). A few major genes control the vernalization requirement and determine spring and winter habits of each wheat cultivar. The photoperiodic sensitivity is mainly determined by the homoeologous loci *Ppd-A1*, *Ppd-B1*, and *Ppd-D1*, which are located on the short arms of chromosomes 2A, 2B, and 2D, respectively (Welsh et al. 1973; Law et al. 1978; Scarth and Law 1983). Narrow-sense earliness is the earliness of fully vernalized plants grown under long days, and no major genes have been detected for this character (Murai et al. 2005). Nevertheless, the earliness *per se* genes are known to be located on several chromosomes including group 2 homoeologous chromosomes (Scarth and Law 1983). It has been reported that some chromosomes include quantitative trait loci (QTLs) controlling narrow-sense earliness (Hoogendoorn 1985; Law 1987; Miura and Worland 1994; Hanocq et al. 2004; Lin et al. 2008).

Most spring type landraces of southwest Japan carry a dominant *Vrn-D1* allele (Gotoh 1979; Iwaki et al. 2000; Seki et al. 2011). The spring habit caused by the *Vrn-D1* allele shows slightly short narrow-sense earliness (Kato et al. 2001). It is significant that photoperiodic sensitivity is an adaptive trait in common wheat cultivars (Worland et al. 1998). A *Ppd-D1a* mutant allele is photoperiod insensitive because of a 2-kb deletion upstream of the coding region in a pseudo-response regulator gene on wheat chromosome 2D (Beales et al. 2007; Nishida et al. 2013). The photoperiodic-insensitive allele has been widely distributed for breeding of early-flowering cultivars, and was originally derived from the Japanese wheat variety Akakomugi (Worland et al. 1998; Borojevic and Borojevic 2005). Recently, haplotype analysis of the *Ppd-D1* gene implied that the *Ppd-D1a* allele might have originated from a photoperiod-sensitive *Ppd-D1b* allele in Asia (Guo et al. 2010). Most Japanese wheat cultivars, except those in the

Hokkaido area, carry the *Ppd-D1a* allele, which contributes to their early-flowering phenotype, helping to avoid damage from preharvest rain (Seki et al. 2011). Moreover, structural variations have been found at the *Ppd-B1* locus (Beales et al. 2007; Takenaka and Kawahara 2012; Nishida et al. 2013). It was recently reported that the copy number of *Ppd-B1* is associated with FLT variation in common wheat (Díaz et al. 2012), and that structural variation exists between the 5' upstream region of the photoperiod-insensitive *Ppd-B1a* allele and the photoperiod-sensitive *Ppd-B1b* allele (Nishida et al. 2013).

Wheat landraces provide abundant genetic variation in many agricultural traits including FLT (Kato and Yokoyama 1992; Iwaki et al. 2001; Terasawa et al. 2009), and some landraces supply useful traits for modern wheat breeding (Feldman and Sears 1981). In previous studies, we found that many wheat landraces in Nepal, Bhutan and Tibet show wide variation in flowering traits (Kato and Yokoyama 1992; Takumi 2009). A common garden experiment revealed the presence of early-flowering landraces in Nepal, which have FLT as short as that of the Japanese early-flowering cultivar Shiroganekomugi (SGK) (Takumi 2009). HT is highly correlated with photoperiodic response and narrow-sense earliness in the Nepalese and Bhutanese landraces, and in particular, narrow-sense earliness shows significant association with altitude of landrace collection sites in Nepal (Kato and Yokoyama 1992). However, there is little information about the genetic basis of flowering traits in the Nepalese and Bhutanese wheat landraces.

Early-maturing cultivars of common wheat are required in Japan, because the rainy season overlaps with that of wheat maturation. High moisture at maturation time (MAT) results in preharvest sprouting and *Fusarium* damage and reduction of wheat grain quality. Earliness of HT/FLT in the previously identified Nepalese landraces might be caused by different genetic loci than the early-flowering phenotype of SGK.

3.Objective

Our objective was to identify causal loci for early flowering in the Nepalese landrace and SGK. Based on the results, genetic differences in FLT between the Nepalese and Japanese landraces and their usefulness for wheat breeding were discussed.

4. Materials and methods

4.1. Plant materials

Three accessions of common wheat, including the Japanese cultivar Shiroganekomugi (SGK) and two Nepalese landraces, KU-4770 and KU-180, were used in this study. These three accessions exhibited spring growth habit, and one-week treatment of vernalization (4°C) was required for heading. The two Nepalese landraces were identified in a screen of 41 accessions from Nepal, Bhutan and Tibet as early-flowering varieties (Takumi 2009). Two F₂ populations were obtained from F₁ plants crossed between SGK and the two Nepalese landraces. Seeds of the first F₂ population ($N = 171$), SGK/KU-4770, were sown on 12th November 2008, and plants were grown in the 2008–2009 season in a field at Kobe University (34°43' N, 135°13' F). In the second F₂ population, SGK/KU-180, F₂ seeds ($N = 100$) were sown on 25th November 2009, and plants were grown in the 2009–2010 season. In addition, two common wheat cultivars, Chinese Spring (CS) and Cheyenne (Cnn), were used for estimation of *Ppd-B1* copy number.

4.2. Phenotype measurement and statistical analyses

Four flowering-related traits were measured at the field. Heading time (HT) and flowering time (FLT) were recorded as days after sowing. Maturity time (MAT) was measured as the number of days that had passed before the peduncle turned yellow, according to our previous report (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 F₂ 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.

4.3. Detection of polymorphisms and genotyping with molecular markers

To amplify PCR fragments of simple sequence repeat (SSR) markers, total DNA was extracted from the parents and F₂ individuals using standard procedures. For SSR genotyping, 40 cycles of PCR were performed using 2x Quick Taq HS DyeMix (TOYOBO, Osaka, Japan) with the following conditions: 10 s at 94°C, 30 s at the annealing temperature, and 30 s at 68°C. The last step was incubation for 1 min at 68°C. Information on the SSR markers and their 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 products were separated in 2% agarose or 13% nondenaturing polyacrylamide gels and visualized under UV light after staining with ethidium bromide. For polyacrylamide gel electrophoresis, the high efficiency genome scanning system (Nippon Eido, Tokyo, Japan) of Horii et al. (2003) was used.

Polymorphism at the *Ppd-D1* locus was detected using allele-specific primers according to Beales et al. (2007). A common forward primer, Ppd-D1_F, 5'-ACGCCTCCCACTACTACTG-3', and two reverse primers, Ppd-D1_R1, 5'-GTTGGTTCAAACAGAGAGC-3', and Ppd-D1_R2, 5'-CACTGGTGGTAGCTGAGATT-3', were used for this PCR-based analysis. PCR products amplified with Ppd-D1_F and Ppd-D1_R2 showed a 2,089-bp deletion in the 5' upstream region of *Ppd-D1*, indicative of the photoperiod-insensitive *Ppd-D1a* allele (Beales et al. 2007). *Ppd-B1* alleles were determined using the following allele-specific

primers for amplification by PCR: Ppd-B1_2ndcopy_F1, 5'-TAACTGCTCGTCACAAGTGC-3', and Ppd-B1_2ndcopy_R1, 5'-CCGGAACCTGAGGATCATC-3' (Beales et al. 2007). PCR products amplified with Ppd-B1_2ndcopy_F1 and Ppd-B1_2ndcopy_R1 gave a 425 bp fragment derived from the partly deleted second *Ppd-B1* copy in CS (Beales et al. 2007). The PCR products were separated by electrophoresis through a 1.2% agarose gel and stained with ethidium bromide.

4.4. Map construction and QTL analysis

The polymorphic SSRs, *Ppd-B1*, and *Ppd-D1* 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 3.0, 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 (Röder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; 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) using 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.

4.5. Copy number estimation of *Ppd-B1*

Real-time PCR analysis was carried out for estimation of copy number of the *Ppd-B1* genes using a LightCycler 480 Real-Time PCR System II (Roche Diagnostics, Basel, Switzerland). The wheat *CONSTANS2* gene, *TaCO2*, was used as an internal control. Gene-specific primer sets were based on a previous report (Díaz et al. 2012): 5'-GCGTAAGTTACTATCTCTCATGGTGTATC-3' and

5'-TTTGTTTTAGTACCCAGTACCATAACCAG-3' for *Ppd-B1*, and 5'-TGCTAACCGTGTGGCATCAC-3' and 5'-GGTACATAGTGCTGCTGCATCTG'-3 for *TaCO2*. The rate of amplification was monitored using THUNDERBIRD SYBR qPCR mix (Toyobo, Osaka, Japan) according to the manufacturer's protocol. The relative copy number of *Ppd-B1* was calculated as $2^{-\Delta\Delta C_t}$, where ΔC_t is the difference in number of PCR cycles required to reach the log phase of amplification of *Ppd-B1* relative to *TaCO2*; representative values were expressed relative to the *Ppd-B1* copy number in the *Cnn* genome.

4.6. Assay for flowering-related traits under short-day conditions

For estimation of photoperiod requirement, five imbibed seeds of each wheat cultivar were planted in a pot with soil and incubated for two weeks at 24°C with a 12 h light/12 h dark photoperiod at a light intensity of 55–65 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by cool white fluorescent lamps. The seedlings were treated at 4°C for one week for vernalization, and then transferred to 24°C with a 12 h photoperiod. The four flowering-related traits, HT, FLT, MAT and GFP, were recorded for each cultivar.

5. Results

5.1. Flowering-related traits in two F₂ populations and their parental accessions

The mean values of two Nepalese varieties, KU-4770 and KU-180, were compared with SGK. HT of the Nepalese varieties was 4 to 8 days later than SGK, whereas there were no significant differences for FLT, MAT and GFP (**Table 2-1**). Little variation in the four traits was observed among individual plants of each parental variety. However, all four traits varied widely in the F₂ populations (**Fig. 2-1a**). The SGK/KU-180 population showed larger variance in GFP than the SGK/KU-4770 population compared with the variance in the other three traits. Much earlier and later heading/flowering F₂ plants were present than in the parental lines, which indicated transgressive segregation (**Fig. 2-1b**).

Table 2-1. Parental and F₂ population means for four flowering-related traits measured in each of the two F₂ mapping populations

	Heading time	Flowering time	Maturation time	Grain-filling period
SGK/KU-4770 in 2008-2009 season				
SGK	130.3 ± 1.18*	148.0 ± 0.47	179.8 ± 0.88	31.8 ± 0.57
KU-4770	138.9 ± 0.83	149.9 ± 0.50	180.1 ± 0.91	30.1 ± 0.57
F ₁ plants	138.1 ± 0.87	150.0 ± 0.59	180.0 ± 1.10	30.0 ± 0.71
Mean in F ₂ plants	136.0	149.9	180.6	30.7
Range in F ₂ plants	124.0-153.0	143.0-161.0	168.3-193.6	19.6-36.6
Variance in F ₂ plants	43.96	11.51	16.41	4.99
SGK/KU-180 in 2009-2010 season				
SGK	132.6 ± 0.89	147.0 ± 1.00	181.6 ± 1.14	34.6 ± 1.89
KU-180	136.0 ± 1.22	147.8 ± 0.45	179.8 ± 0.83	32.0 ± 0.70
Mean in F ₂ plants	133.1	145.8	183.2	37.4
Range in F ₂ plants	123.3-157.0	137.6-163.0	174.6-196.5	27.33-49.7
Variance in F ₂ plants	33.66	25.33	21.83	22.30

*Means (days) with standard deviations.

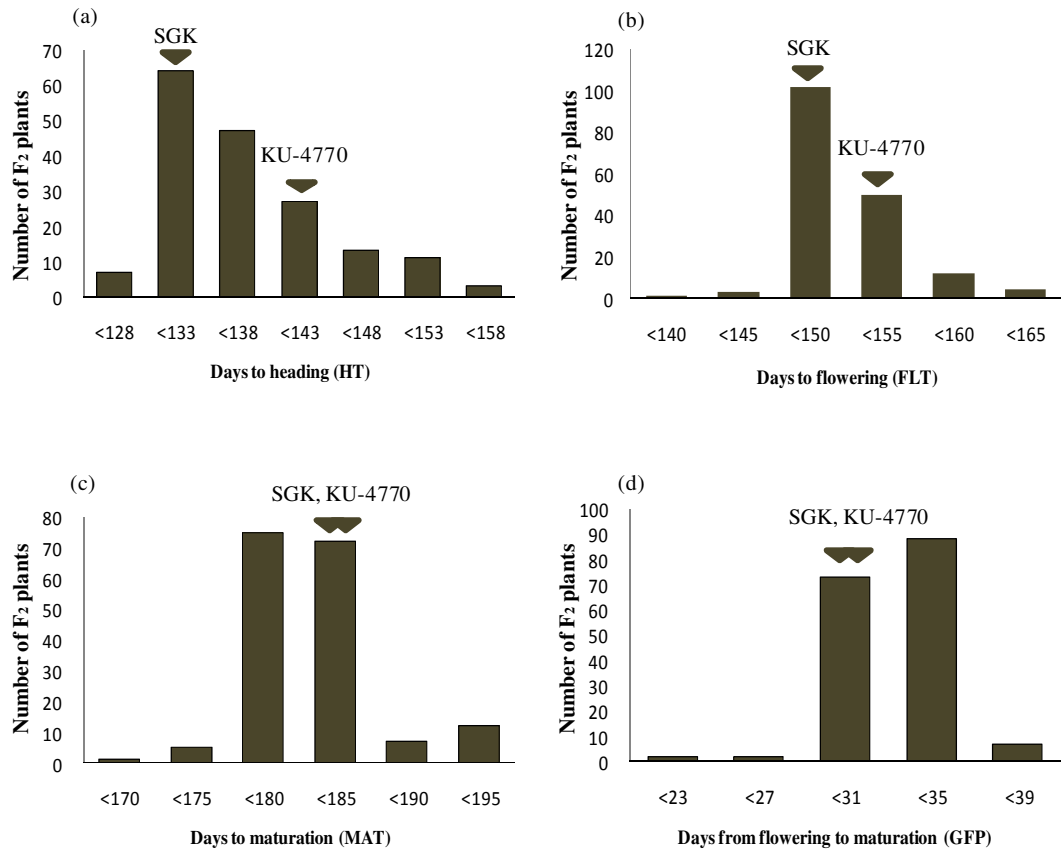


Fig. 2-1a. Frequency distribution of flowering-related traits in the F₂ population: (a) Days to heading; (b) Days to flowering; (c) Days to maturation; (d) Days from flowering to maturation. Arrows indicate the data of their parental accessions Shiroganekomugi//KU-4770.

These observations implied that genes controlling the early heading/flowering of the Nepalese varieties may be distinct from those of SGK.

Significant ($P < 0.001$) positive correlations were observed among HT, FLT and MAT in the two F₂ populations (**Table 2-2**). MAT correlated significantly ($P < 0.001$) with GFP, while HT and FLT did not show any positive correlation with GFP in the two F₂ populations. In the SGK/KU-180 population, GFP was negatively correlated with HT and FLT. The negative correlation between GFP and HT/FLT indicated that the earlier flowering F₂ individuals required a longer period for grain maturation, and that GFP in the late flowering F₂ individuals tended to be shorter.

5.2. Construction of a linkage map and QTL analysis for flowering-related traits in the SGK/KU-4770 population

In the SGK/KU-4770 population, 889 primer sets were tested, including 887 SSR primer sets and *Ppd-D1* and *Ppd-B1* allele-specific primers, and 166 (16.7%) were found to be polymorphic between the parental accessions. Of these, 145 SSR markers, *Ppd-D1* and *Ppd-B1* formed 33 linkage groups. The total map length was 1,649 cM with an average spacing of 11.4 cM between markers.

QTLs for the three flowering-related traits other than GFP were detected using the SGK/KU-4770 genetic map. In total, 5 QTLs, located on chromosomes 2B and 2D, showed significant LOD scores ($P < 0.05$) (**Fig. 2-2**). Two, one and two QTLs were respectively detected for HT, FLT and MAT (**Table 2-3**).

For HT, two QTLs were detected on the short arms of chromosomes 2B and 2D with LOD scores of 5.26 and 18.84, respectively (**Table 2-3**). The two QTLs, located at the *Ppd-B1* and *Ppd-D1* chromosomal regions, respectively explained 8.8% and 58.0% of the variation in HT in the SGK/KU-4770 population. The additive effect of the HT QTLs indicated that the KU-4770 allele at the 2B QTL promoted heading earlier than the SGK allele, while the SGK allele at the 2D QTL has an earlier heading effect than the

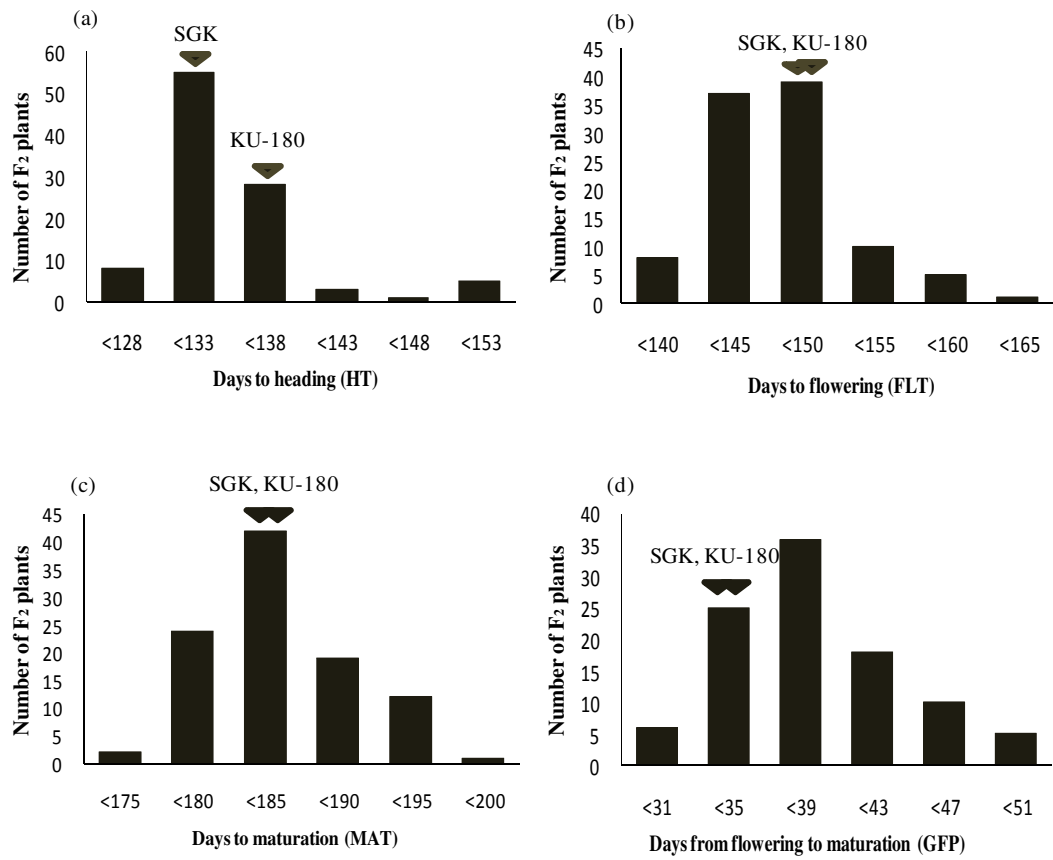


Fig. 2-1b. Frequency distribution of flowering-related traits in the F₂ population: (a) Days to heading; (b) Days to flowering; (c) Days to maturation; (d) Days from flowering to maturation. Arrows indicate the data of their parental accessions Shiroganekomugi//KU-180 .

KU-4770 allele.

For FLT, one QTL with a LOD score of 15.39 was found on chromosome 2D. The 2D QTL for FLT was located at the *Ppd-D1* chromosomal position similar to that of HT, and contributed 50.7% of the FLT variation. The additive effect of the FLT QTL on chromosome 2D showed the same direction as that of the 2D QTL for HT.

For MAT, two QTLs with LOD scores of 6.36 and 11.66 were respectively found in the *Ppd-B1* and *Ppd-D1* chromosomal regions. The 2B and 2D QTLs explained 9.1% and 37.2%, respectively, of the variation in MAT. The SGK allele at the MAT QTL on 2D had an earlier effect on MAT than the KU-4770 allele, whereas the KU-4770 allele at the MAT QTL on 2B contributed to early maturation.

To study the effects of the identified QTLs, data on each of the flowering-related traits, HT, FLT and MAT, were grouped based on the genotypes at the QTL regions of each F₂ individual. For all three traits, there were significant ($P < 0.05$) differences among genotypes at the QTLs (**Fig. 2-3**). The F₂ individuals homozygous for the KU-4770 allele at *Ppd-B1* showed significantly earlier HT, FLT and MAT than those with the allele from SGK. Conversely, the F₂ individuals with the SGK allele at *Ppd-D1* exhibited earlier attributes than those with the allele from KU-4770.

5.3. Confirmation of effects of the identified QTLs in the SGK/KU-180 population

To confirm the expression of the 2B QTL identified in the SGK/KU-4770 population, another F₂ population of SGK/KU-180 was examined. A linkage map for the short arm of chromosome 2B was constructed for the SGK/KU-180 population. No polymorphism was detected using the *Ppd-B1_2ndcopy_F1* and *Ppd-B1_2ndcopy_R1* primers for *Ppd-B1*, but in total, 9 SSR markers could be mapped in 2BS. QTL analyses for the four flowering-related traits were conducted using the linkage map, and three QTLs for HT, FLT and MAT with respective LOD scores of 3.44, 3.46 and 3.76 were found on chromosome 2B in the SGK/KU-180 population (**Fig. 2-2**). The 2B QTLs were located

Table 2-2. Correlation coefficient (*r*) matrices for four traits measured in two F₂ populations

	Heading time	Flowering time	Maturation time
SGK/KU-4770 in 2008-2009 season			
Flowering time	0.9414***		
Maturation time	0.7991***	0.8278***	
Grain-filling period	0.0129	-0.0112	0.5375***
SGK/KU-180 in 2009-2010 season			
Flowering time	0.9704***		
Maturation time	0.5181***	0.5441***	
Grain-filling period	-0.5439***	-0.5496***	0.3979***

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

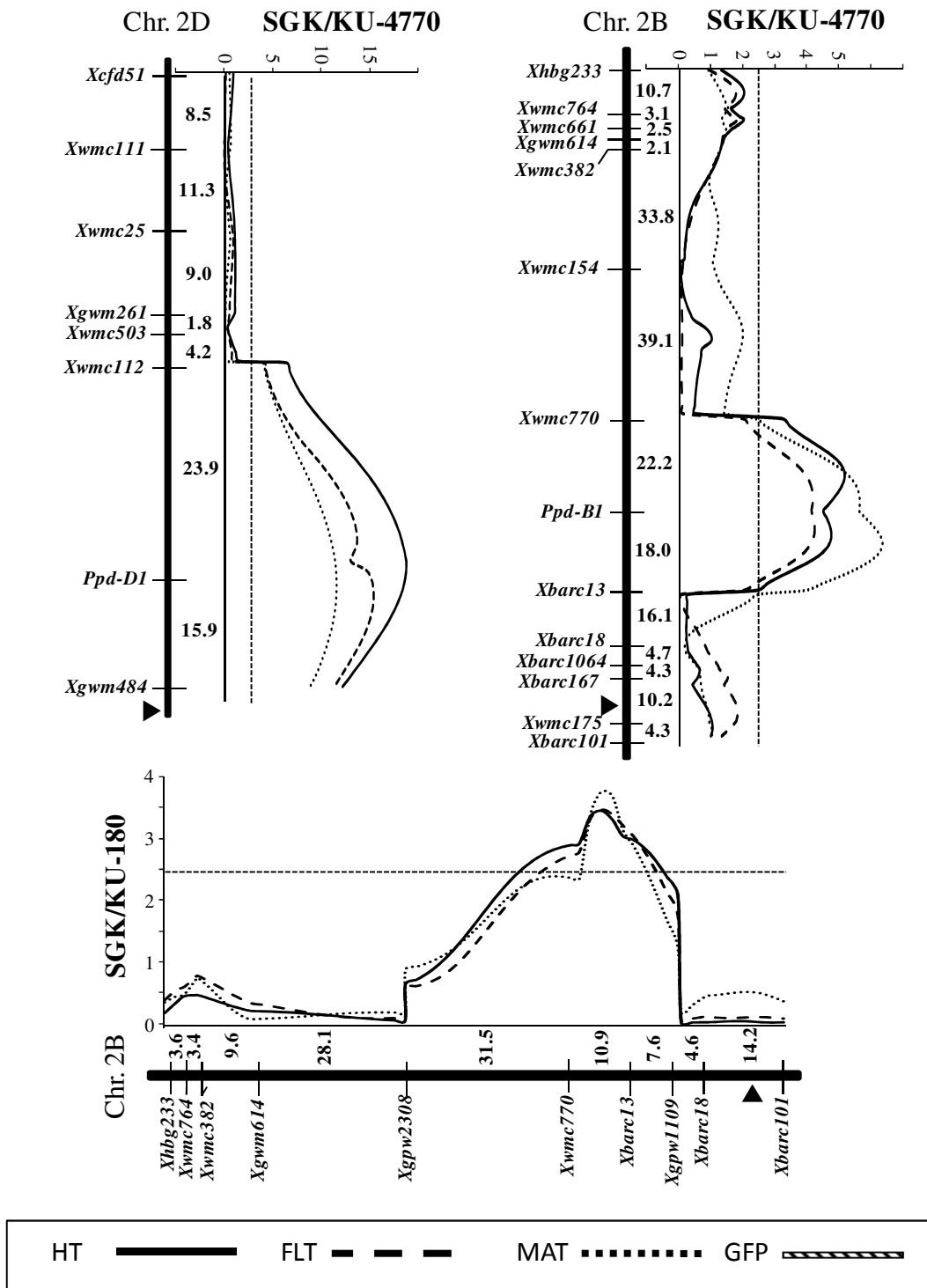


Figure 2-2. Linkage maps and positions of QTLs identified on the short arms of chromosomes 2B and 2D for three flowering-related traits. QTLs with LOD scores above the threshold are indicated, and genetic distances (in centiMorgans) are given to the right of each chromosome. Black arrowheads indicate the putative positions of centromeres.

between *Xwmc770* and *Xbarc13*, and the chromosomal positions corresponded in the two F₂ populations. These QTLs contributed 18–30% of the variation in HT, FLT and MAT in the SGK/KU-180 population (**Table 2-3**). In addition, additive effects indicated that the KU-180 alleles at the 2B QTLs show earlier attributes than the SGK alleles.

Polymorphisms between SGK and KU-180 were found at the *Ppd-D1* locus using allele-specific primers for *Ppd-D1*. To study the effects of the 2B QTLs and *Ppd-D1*, data on each flowering-related trait were grouped based on the genotypes at the QTL regions of each F₂ individual in the SGK/KU-180 population. For HT, FLT and MAT, there were significant ($P < 0.05$) differences among genotypes at the 2B QTLs and *Ppd-D1* (**Fig. 2-4**). The F₂ individuals homozygous for the KU-180 allele at the 2B QTL showed significantly earlier HT, FLT and MAT than those with the allele from SGK. Conversely, the F₂ individuals with the SGK allele at *Ppd-D1* exhibited earlier attributes than those with the allele from KU-180. The opposite effects of SGK and KU-180 alleles corresponded to those found in the SGK/KU-4770 population.

5.4. Comparison of effects of the 2B and 2D QTLs on flowering-related traits

To compare effects of the 2B and 2D QTLs on flowering-related traits, the data were grouped into four categories based on the genotypes at the QTL regions of each F₂ individual in the two populations (**Fig. 2-5**). No significant difference in the three flowering-related traits was observed among the following three groups in the two populations: F₂ individuals with only the Nepalese variety's allele at the 2B QTL, F₂ individuals with only the SGK allele at the 2D QTL, and F₂ individuals with both the Nepalese variety's allele at the 2B QTL and the SGK allele at the 2D QTL. These three groups showed comparatively earlier HT, FLT and MAT than the other group with neither the Nepalese variety's allele at the 2B QTL nor the SGK allele at the 2D QTL.

Table 2-3. A summary of QTLs for flowering-related traits that were identified in the two F₂ mapping populations

Traits	Chromosome	Map location	LOD score	LOD threshold	Contribution (%)	Additive effect
SGK/KU-4770						
HT	2B	<i>Ppd-B1-Xbarc13</i>	5.26	4.9	8.84	-2.63
HT	2D	<i>Ppd-D1-Xgwm484</i>	18.84	4.9	57.98	6.34
FLT	2D	<i>Ppd-D1-Xgwm484</i>	15.39	4.5	50.73	4.01
MAT	2B	<i>Ppd-B1-Xbarc13</i>	6.36	6.2	9.09	-1.62
MAT	2D	<i>Ppd-D1-Xgwm484</i>	11.66	6.2	37.17	3.91
SGK/KU-180						
HT	2B	<i>Xwmc770-Xbarc13</i>	3.44	2.4	18.51	-3.43
FLT	2B	<i>Xwmc770-Xgwm484</i>	3.46	2.3	20.85	-3.45
MAT	2B	<i>Xwmc770-Xbarc13</i>	3.76	2.4	30.41	-3.66

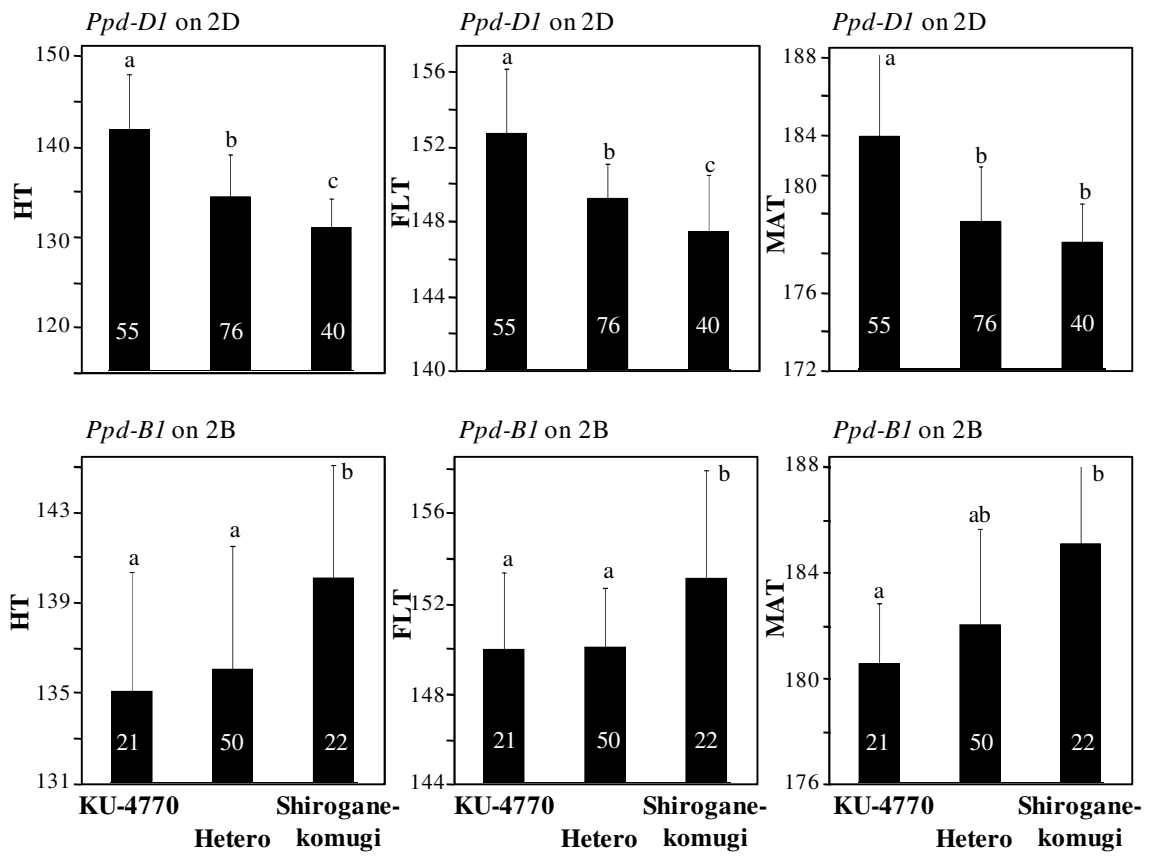


Figure 2-3. The genotype effects at each QTL on the observed variation in the flowering-related traits in the SGK/KU-4770 population. Markers that were used to deduce the genotype at a QTL are listed above each graph. The number of F₂ individuals with each genotype was indicated in the closed bars. Means \pm standard deviation with the same letter were not significantly different ($P > 0.05$) (Tukey-Kramer HSD test).

5.5. Copy number estimation of *Ppd-B1*

Although no polymorphism between SGK and KU-180 was observed using the *Ppd-B1* allele-specific primer set, significant QTLs for flowering-related traits were detected in the *Ppd-B1* region. It was recently reported that alleles with an increased copy number of *Ppd-B1* confer an early-flowering, photoperiod-insensitive phenotype (Díaz et al. 2012). Therefore, we estimated the *Ppd-B1* copy number in SGK and the two Nepalese varieties. As reported in Díaz et al. (2012), the copy number of *Ppd-B1* in CS was calculated as being four times higher than in Cnn (**Fig. 2-6**). Real-time PCR analysis showed that SGK contains one copy of *Ppd-B1*, and KU-4770 and KU-180 respectively contain three and two *Ppd-B1* copies per haploid genome. Because the 425-bp PCR fragment from the partly deleted *Ppd-B1* copy was observed in KU-4770, KU-4770 was presumed to contain a truncated copy of *Ppd-B1* which was previously reported (Beales et al. 2007; Díaz et al. 2012).

To examine the photoperiod insensitivity in the two Nepalese varieties, flowering-related traits were measured under short-day conditions. Both KU-4770 and KU-180 transited as well as SGK to reproductive phase under short-day conditions (**Table 2-4**), indicating that the two Nepalese varieties are photoperiod insensitive for heading and flowering. In addition, HT, FLT and MAT of KU-4770 and KU-180 were significantly earlier than the corresponding values for SGK under short-day conditions.

6. Discussion

SGK is an early-flowering Japanese cultivar of common wheat. A common garden experiment revealed that two varieties selected from Nepalese landraces showed an early flowering phenotype similar to SGK. We then performed QTL analysis to elucidate the genetic basis underlying the early FLT in these wheat varieties. Two chromosomal regions contributed large parts of the variation in HT, FLT and MAT in the two F₂

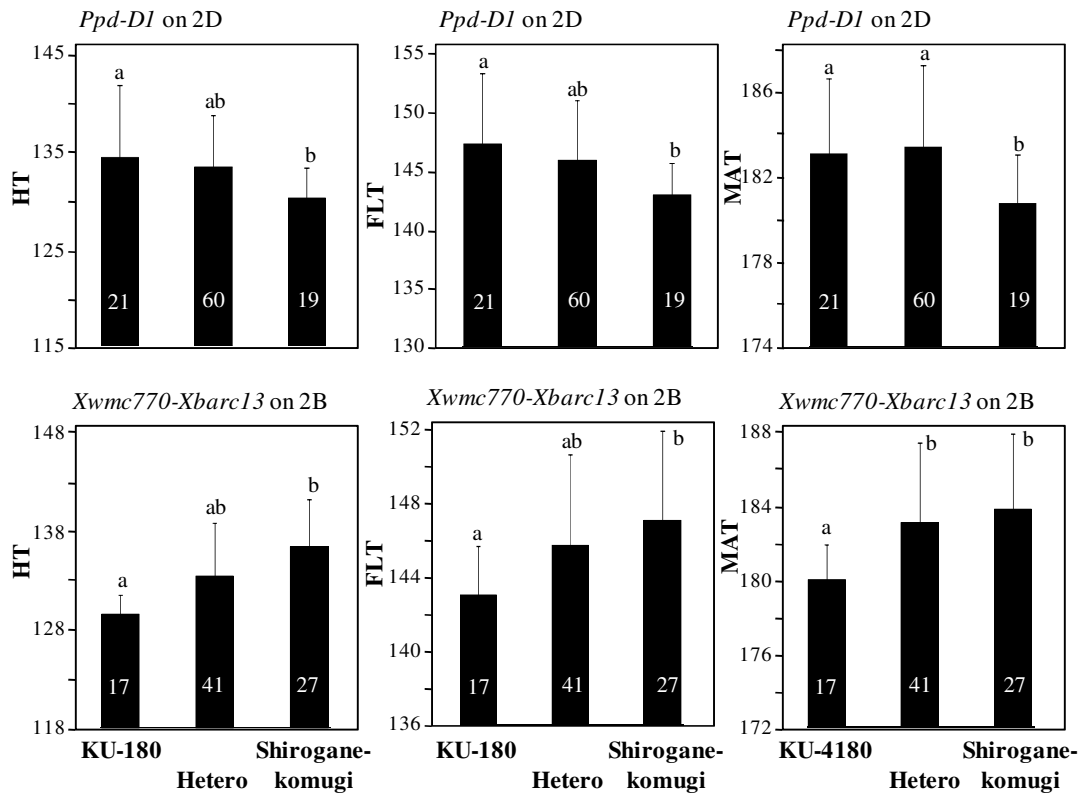


Figure 2-4. The genotype effects at each QTL on the observed variation in the flowering-related traits in the SGK/KU-180 population. Markers that were used to deduce the genotype at a QTL are listed above each graph. The number of F₂ individuals with each genotype was indicated in the closed bars. Means \pm standard deviation with the same letter were not significantly different ($P > 0.05$) (Tukey-Kramer HSD test).

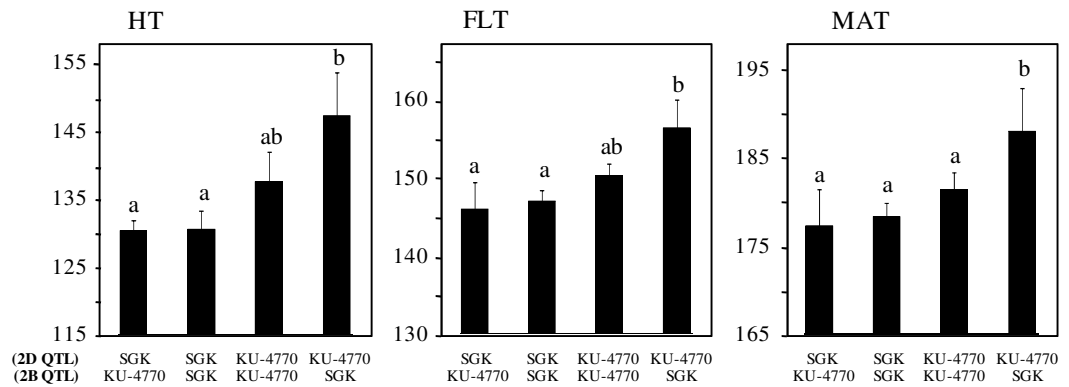
populations, and the two major QTL regions seem to be *Ppd-B1* and *Ppd-D1* (**Fig. 2-2, Table 2-3**). The effects of each allele of the *Ppd-D1* and *Ppd-B1* on HT, FLT and MAT (**Figs. 2-3, 2-4**) strongly suggested that the *Ppd-D1* allele from SGK and the *Ppd-B1* alleles from the two Nepalese varieties caused the early-flowering phenotype in the two F₂ populations. The SGK allele of *Ppd-D1* contained a 2,089-bp deletion in the 5' upstream region, indicating a photoperiod-insensitive *Ppd-D1a* allele (Beales et al. 2007). Most of the Japanese early-flowering cultivars, including SGK, carry the *Ppd-D1a* allele (Seki et al. 2011), an observation supported by the present study. On the other hand, introduction of the photoperiod-insensitive allele of *Ppd-B1* into wheat cultivars has been extremely limited in Japan except in the Hokkaido region (Seki et al. 2011). The 2B QTL position identified in the present study corresponds to the *Ppd-B1* locus, and the early attribute alleles at the 2B QTLs were derived from the two Nepalese varieties. Because the two Nepalese varieties lost photoperiod sensitivity and showed accelerated HT, FLT and MAT under short-day conditions (**Table 2-4**), it could be assumed that the two Nepalese varieties carry the photoperiod-insensitive allele of *Ppd-B1*. The photoperiod-insensitive alleles of *Ppd-B1* may be the main cause of earliness of HT, FLT and MAT in the two Nepalese varieties. The two photoperiod-insensitive homoeoalleles of *Ppd-1* could independently contribute to segregation of early-flowering individuals in the two F₂ populations.

No significant differences in the three flowering-related traits were observed among the F₂ individuals with either or both of the photoperiod-insensitive homoeoalleles (**Fig. 2-5**), although the LOD scores, contribution to variation and the additive effects of *Ppd-D1* were apparently larger than those of *Ppd-B1* in the SGK/KU-4770 and SGK/KU-180 populations (**Table 2-3**). These results did not necessarily correspond with previous reports (Worland et al. 1998, Snape et al. 2001), in which it was demonstrated that plants carrying the *Ppd-D1a* allele are able to undergo earlier flowering than those

with the insensitive allele of *Ppd-B1*, *Ppd-B1a*, from CS. The *Ppd-B1a/Ppd-D1a* genotype previously exhibited earlier HT than *Ppd-B1b/Ppd-D1a*, suggesting a significant expression of the *Ppd-B1a* effect in the *Ppd-D1a* genetic background (Seki et al. 2011). On the other hand, it was recently reported that the effect of the photoperiod-insensitive *Ppd-B1a* allele (named *Ppd-B1a.1*) of the cultivar ‘Winter-Abukumawase’ on HT is stronger than that of the CS *Ppd-B1a* allele (*Ppd-B1a.2*) (Nishida et al. 2013). A comparative study of the *Ppd-1* homoeoalleles using doubled haploid lines of common wheat also showed that HT of the genotypes with two or three insensitive homoeoalleles was significantly earlier than that of the single insensitive allele-containing genotypes and that the *Ppd-1* insensitive homoeoalleles exhibit significant interaction (Nishida et al. 2013). The significant interaction between *Ppd-B1* and *Ppd-D1* was also reported using near-isogenic lines for these photoperiod-insensitive alleles (Tanio and Kato 2007). In the present study, significant differences of HT and MAT between the SGK and KU-4770 alleles of the 2B QTL were observed in either of the two mapping populations under the KU-4770 allele of the 2D QTL, whereas no difference of each flowering-related trait was found in both populations under the SGK allele of the 2D QTL (**Fig. 2-5**). This observation suggested the presence of interaction between *Ppd-B1* and *Ppd-D1*. It remains unclear whether the function of the photoperiod-insensitive alleles of the Nepalese varieties is identical to that of *Ppd-B1a*. Production of near-isogenic lines of the photoperiod-insensitive alleles may be necessary to clarify the allele identities.

The two Nepalese varieties exhibited earlier HT, FLT and MAT than SGK under short-day conditions, suggesting that early-flowering genes other than *Ppd-B1* exist in the two Nepalese varieties. In the SGK/KU-4770 population, the high LOD score thresholds based on a permutation test might hide minor QTLs for the flowering-related traits. QTL analyses using recombinant inbred lines have been effective in identifying

(A) SGK/KU-4770



(B) SGK/KU-180

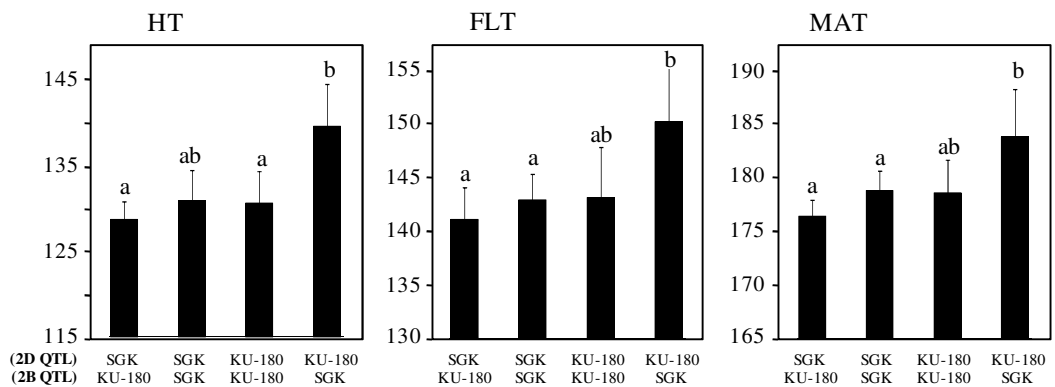


Figure 2-5. Effects of the two identified QTLs on the flowering-related traits in the two F_2 populations, SGK/KU-4770 (A) and SGK/KU-180 (B). The genotype at each QTL was deduced by linked markers. Means \pm standard deviation with the same letter were not significantly different ($P > 0.05$) (Tukey-Kramer HSD test).

minor QTLs for HD and FLT in common wheat, many of which were associated with narrow-sense earliness (Hanocq et al. 2004; Lin et al. 2008). Novel QTLs for flowering-related traits should be found using recombinant inbred lines of SGK/KU-4770 under controlled growth conditions. In addition, we failed any QTL for GFP in the present study although wide variations in GFP were observed in the F₂ populations (**Table 2-1**), which implied that GFP might be easily affected by environmental conditions.

In addition to the structural variations in the 5' and 3' untranslated regions of *Ppd-B1* (Nishida et al. 2013), copy number variation has been found in *Ppd-B1*, and the increased copy number alters *Ppd-B1* gene expression and shortens FLT (Díaz et al. 2012). At the *Ppd-B1* locus of CS, three intact and one truncated copies of *Ppd-B1* exist (Díaz et al. 2012). The *Ppd-B1a* allele-specific PCR primers we used in the present study recognize the truncated copy of *Ppd-B1* (Beales et al. 2007). Our genotyping study, therefore, indicated that KU-4770 includes the *Ppd-B1* truncated copy, whereas no truncated copy was found in KU-180. Real-time PCR analysis to estimate the *Ppd-B1* copy number revealed that KU-4770 and KU-180 respectively contain three and two copies of *Ppd-B1* (**Fig. 2-5**), suggesting that the copy number difference between the two Nepalese varieties is due to the presence or absence of the truncated copy, and that the two Nepalese varieties include two intact *Ppd-B1* copies. Since no significant difference in HT, FLT and MAT was observed between the two Nepalese varieties under short-day conditions, the truncated copy of *Ppd-B1* apparently has no influence on these flowering-related traits or photoperiod sensitivity. Therefore, the number of intact copies of *Ppd-B1* is important for photoperiod sensitivity in wheat. Some wheat cultivars have been estimated to have two or three copies of *Ppd-B1* (Díaz et al. 2012). We also found copy number variation in *Ppd-B1* of the Nepalese wheat landraces and discovered varieties containing two and three copies of this allele. The intact copy numbers of

Table 2-4. Parental means for four flowering-related traits of the three wheat cultivars measured under short-day (12 h light/12 h dark) conditions

Accessions	Heading time	Flowering time	Maturation time	Grain-filling period
KU-4770	46.3 ^a ± 2.5*	48.5 ^a ± 2.1	66.5 ^a ± 3.1	18.0 ^a ± 2.2
KU-180	48.3 ^a ± 1.7	51.0 ^a ± 2.2	69.5 ^a ± 2.4	18.5 ^a ± 2.0
SGK	63.5 ^b ± 3.1	65.3 ^b ± 2.6	87.5 ^b ± 3.1	22.3 ^a ± 2.6

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

Ppd-B1 in the two Nepalese varieties were different from that of CS, meaning that the effect of the photoperiod-insensitive allele in *Ppd-B1* of the Nepalese varieties on flowering-related traits could be inconsistent with that of CS. The increased copy number of *Ppd-B1* pulls up basal accumulation levels of *Ppd-B1* transcripts, putatively resulting in alteration of photoperiod sensitivity (Díaz et al. 2012). To characterize precisely the photoperiod-insensitive alleles of the Nepalese varieties, near-isogenic lines for the insensitive alleles should be produced for future study, and *Ppd-B1* gene expression in the near-isogenic lines should be analyzed. To confirm the polymorphisms in the *Ppd-B1* locus reported in Nishida et al. (2013), the nucleotide sequences should be determined in the two Nepalese varieties in future study.

Wide genetic variation in flowering-related traits have been found in landraces of common wheat (Kato and Yokoyama 1992; Iwaki et al. 2001). Nepalese landraces contain both late- and early-heading varieties, and their HT is correlated with both photoperiod sensitivity and narrow-sense earliness (Kato and Yokoyama 1992). Our study has suggested that the phenotypes of the two early-flowering varieties of Nepalese wheat are at least partly due to the photoperiod-insensitive allele *Ppd-B1*, although we failed to identify any significant QTL for narrow-sense earliness. Therefore, wheat landraces are genetic resources for discovery of alleles useful for improvement of HT/FLT of elite cultivars.

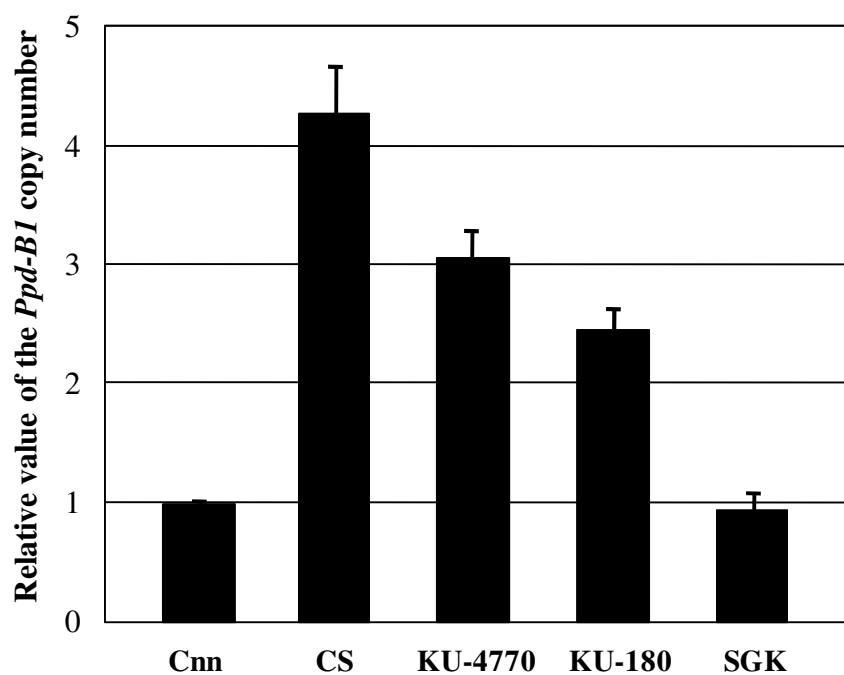


Figure 2-6. Copy number variation of *Ppd-B1* in five wheat varieties. Relative mean values of PCR amplification levels of the *Ppd-B1* copy are represented with standard deviation.

Chapter III

Identification of quantitative trait loci for flowering-related traits in the D genome of synthetic hexaploid wheat lines

1. Abstract

The gene pool of *Aegilops tauschii*, the D-genome donor of common wheat (*Triticum aestivum* L.), can be easily accessed in wheat breeding, but remains largely unexplored. In our previous studies, many synthetic hexaploid wheat lines were produced through interspecific crosses between the tetraploid wheat cultivar Langdon (Ldn) and various *Ae. tauschii* accessions. The synthetic hexaploid wheat lines showed wide variation in many characteristics. To elucidate the genetic basis of variation in flowering-related traits, we analyzed quantitative trait loci (QTL) affecting time to heading, flowering and maturity, and the grain-filling period using four different F₂ populations of synthetic hexaploid wheat lines. In total, 10 QTLs located on six D-genome chromosomes (all except 4D) were detected for the analyzed traits. The QTL on 1DL controlling heading time appeared to correspond to a flowering time QTL, previously considered to be an ortholog of *Eps-A^m1* which is related to the narrow-sense earliness in einkorn wheat. The 5D QTL for heading time might be a novel locus associated with wheat flowering, while the 2DS QTL appears to be an allelic variant of the photoperiod response locus *Ppd-D1*. Some of the identified QTLs seemed to be novel loci regulating wheat flowering and maturation, including a QTL controlling the grain filling period on chromosome 3D. The exercise demonstrates that synthetic wheat lines can be useful for the identification of new, agriculturally important loci that can be transferred to, and used for the modification of flowering and grain maturation in hexaploid wheat.

2. Introduction

Flowering time (FLT) is a critical adaptation trait in higher plants, so adjusting FLT to the growth environment is also a significant objective in crop breeding. The worldwide cultivation of common wheat in diverse environments was in part made possible by the optimization of heading time (Snape et al. 2001). Late-maturing varieties were selected where long growing seasons prevail, while early-maturing varieties were selected for short growing seasons (Kato and Yokoyama 1992; Law and Worland 1997).

Maturation time (MAT), determined by the sum of the FLT and the grain-filling period (GFP), is one of the most important traits in wheat breeding. Due to the agronomic importance of common wheat, physiological and molecular mechanisms controlling changes in the developmental phase have been studied extensively. In wheat, FLT is controlled by three major genetically controlled components, namely the vernalization requirement, photoperiod sensitivity and narrow-sense earliness (Murai et al. 2005). Major genes for the vernalization requirement are the *Vrn-1* homoeologous loci, *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, located on the long arms of chromosomes 5A, 5B and 5D, respectively (Snape et al. 1996). Photoperiod sensitivity is primarily determined by the three homoeologous loci, *Ppd-A1*, *Ppd-B1*, and *Ppd-D1*, which are located on the short arms of chromosomes 2A, 2B, and 2D, respectively (Law et al. 1978; Scarth and Law 1983). The narrow-sense earliness is controlled by several quantitative trait loci (QTL) (Cockram et al. 2007). On the other hand, there is little information about GFP, and only the *Gpc-B1* locus on chromosome 6B has been shown to affect this trait. The *Gpc-B1* gene has pleiotropic effects on multiple traits such as the grain protein content, grain micronutrient concentration, flag leaf senescence and the grain-filling period (Uauy et al. 2006).

Common wheat is an allohexaploid species derived from interspecific hybridization between tetraploid wheat and *Aegilops tauschii* Coss. *Aegilops tauschii* contributed the D genome. Natural habitats of *Ae. tauschii* are widely distributed, from northern Syria and

Turkey to western China in Eurasia. The birthplace of common wheat is considered to lie within the area comprising Transcaucasia and the southern coastal region of the Caspian Sea (Tsunewaki 1966; Dvorak et al. 1998). The narrowness of the presumed region of origin of common wheat relative to the entire range of *Ae. tauschii* suggests that this species has large genetic diversity that is not represented in common wheat (Feldman 2001; Mizuno et al. 2010a, 2010b). Natural variation in the *Ae. tauschii* populations offers potential for improving modern varieties of common wheat. Natural variation in FLT shows significant longitudinal and latitudinal clines in *Ae. tauschii* (Matsuoka et al. 2008; Takumi et al. 2009a). Several agronomically important traits such as pest and disease resistance have been transferred from *Ae. tauschii* to common wheat (Kerber 1987; Ma et al. 1995; Mujeeb-Kazi et al. 1996).

The tetraploid wheat cultivar Langdon (Ldn) has been used as the A and B genome parent for the production of hexaploid wheat synthetics (Matsuoka and Nasuda 2004). Numerous synthetic hexaploid wheat lines have been produced through crosses of Ldn with 69 *Ae. tauschii* accessions (Takumi et al. 2009b; Kajimura et al. 2011) followed by chromosome doubling of the interspecific ABD hybrids. Thus, the synthetic hexaploids share identical A and B genomes derived from Ldn and contain diverse D genomes originating from the *Ae. tauschii* pollen parents. A preliminary study with these synthetic wheat lines showed that they exhibited wide variation in the flowering-related traits, including GFP, and that the large variation in heading time (HT) observed in *Ae. tauschii* is also present in the hexaploid synthetics (Kajimura et al. 2011), but the genetic basis of variation in the flowering-related traits present in the D genome remains unknown.

3. Objectives

We conducted QTL analyses for flowering-related traits using four F₂ populations between early and late flowering synthetic wheat lines to identify genetic loci

responsible for variation observed in the hexaploid background. Based on the results, we discuss the usefulness of the synthetic wheat lines for wheat breeding.

4. Materials and Methods

4.1. Plant materials

In our previous study, 82 wheat synthetic lines were independently produced through endoreduplication in interspecific hybrids obtained by crossing Ldn with 69 different *Ae. tauschii* accessions (Kajimura et al. 2011). For this study, three early flowering and three late flowering lines were selected as parental lines for mapping populations. The three early flowering lines were derived from crosses between Ldn and *Ae. tauschii* accessions PI476874, KU-2097 and KU-2159; the three late flowering lines were from crosses of Ldn with *Ae. tauschii* accessions, IG126387, KU-2090 and KU-2069. Four F₂ mapping populations were produced from the following cross-combinations, Ldn/PI476874//Ldn/KU-2090, Ldn/KU-2097//Ldn/IG126387, Ldn/PI476874//Ldn/KU-2069, and Ldn/IG126387//Ldn/KU-2159. Seeds of the first two F₂ populations were sown in November 2010, with the numbers of individuals in each being 99 and 96. The third population (Ldn/PI476874//Ldn/KU-2069) contained 106 F₂ individuals, and was grown in the 2009-10 season. The last population, Ldn/KU-2159//Ldn/IG126387, with 100 individuals was grown in the 2011-12 season. For each mapping population, all F₂ individuals were obtained from single F₁ plants. All four F₂ populations as well as four plants of each parent were grown individually in pots arranged randomly in a glasshouse of Kobe University (34°43'N, 135°13'E) as previously reported (Kajimura et al. 2011). The wheat synthetics under greenhouse conditions started heading about 20 days earlier than in the experimental field of Kobe University, and the flowering-related traits represented good correlations between both growth conditions (Kajimura et al. 2011).

4.2. Phenotype measurement and statistical analyses

Four flowering-related traits were measured. Heading time (HT) and flowering time (FLT) were recorded as days after sowing. Maturity time (MAT) was measured as the number of days that had passed until the peduncle turned yellow. The grain filling period (GFP) was the number of days from flowering to maturation. HT, FLT and MAT were measured for the five earliest tillers of each plant, and mean values were calculated using the data for each F₂ 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.

4.3. Genotyping with molecular markers

To amplify PCR fragments of simple sequence repeat (SSR) markers, total DNA was extracted from the parents and F₂ individuals using standard procedures. For SSR genotyping, 40 cycles of PCR were performed using 2x Quick Taq HS DyeMix (TOYOBO, Osaka, Japan) at the following conditions: 10 s at 94°C, 30 s at the annealing temperature, and 30 s at 68°C. The last step was incubation for 1 min at 68°C. Information on the SSR markers and their 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 products were separated in 2% agarose or 13% nondenaturing polyacrylamide gels and visualized under UV light after staining with ethidium bromide. For polyacrylamide gel electrophoresis, the high efficiency genome scanning system (Nippon Eido, Tokyo, Japan) of Hori et al. (2003) was used.

To supplement the regions with scarce SSR markers in the Ldn/KU-2097//Ldn/IG126387 population, eight single nucleotide polymorphisms (SNPs) and one SSR polymorphism derived from leaf transcripts of *Ae. tauschii* (Iehisa

et al. 2012) were used. The additional markers were selected from a high confidence SNP dataset constructed by comparing the next generation sequencing of leaf transcripts between two genetically distinct accessions of *Ae. tauschii* in our previous study (Iehisa et al. 2012). **Table 3-1** summarizes the relevant information on the gene-specific primer sequences, the chromosomes involved, marker types and the restriction enzymes used. The PCR conditions for cleaved amplified polymorphic sequence (CAPS) markers were 1 cycle of 94°C for 2 min and 40 cycles of 94°C for 30 s, 60°C for 30 s, and 68°C for 30 s. After amplification, PCR products were digested with a restriction enzyme, and the digested fragments were separated by electrophoresis on 2% agarose gels and stained with ethidium bromide.

Polymorphism at the *Ppd-D1* locus on chromosome 2D was detected using allele-specific primers according to Beales et al. (2007). Insertion and deletion (indel) polymorphism at *WDREB2* was used as a marker on chromosome 1D (Egawa et al. 2006; Koyama et al. 2012). The PCR conditions for the gene-specific markers were 1 cycle of 94°C for 2 min and 40 cycles of 94°C for 30 s, 55°C for 30 s, and 68°C for 30 s (**Table 3-1**). The amplified PCR products were separated by electrophoresis on 2% agarose gels and stained with ethidium bromide.

4.4. Construction of linkage maps and QTL analysis

Genetic mapping was performed using MAPMAKER/EXP version 3.0b (Lander et al. 1987). The threshold for log-likelihood scores was set at 3.0, and genetic distances were calculated with the Kosambi function (Kosambi 1944). Chromosomal assignment of SSR markers was generally based on reference maps (Somers et al. 2004; Torada et al. 2006; Kobayashi et al. 2010).

QTL analyses were carried out by the composite interval mapping with Windows QTL Cartographer ver. 2.5 software (Wang et al. 2011) using the forward and backward method. A log-likelihood (LOD) score threshold for each trait was determined by

Table 3-1. List of DNA markers developed in this study to aid the construction of a linkage map in the Ldn/KU-2097//Ldn/IG126387 population

Marker locus	Chromosome	Primer sequence	Type	Restriction enzyme
<i>WDREB2</i>	1D	GGACACTCACGGCAAGAAAC GTCGCTGGGGGCTGAGTC	Indel	-
<i>Xctg07195</i>	1D	CGTTCGGTAAGAAGCAGCTC TCGGTTGGCAACATGATCT	CAPS	<i>HaeIII</i>
<i>Ppd_D1</i>	2D	ACGCCTCCCACTACTACTG GTTGGTTCAAACAGAGAGC	Indel	-
<i>Xctg05205</i>	2D	TACGCTCCTCTGGTTTCCTC GAAGAGTTGCCAAAGCAAGG	CAPS	<i>MspI</i>
<i>Xctg03017</i>	6D	TCCAATAAAGGCAACGGATA GGAGGCAATCAAGCATGTG	SSR	-
<i>Xctg05512</i>	6D	TCGTCGCTGGTGAAGATGTA ATGACGACGACGGAGAAGAT	CAPS	<i>MboI</i>
<i>Xctg02103</i>	6D	GGTGTATTTTCGGCACGACTT TTGCCACCATCCATTACAAA	CAPS	<i>MspI</i>
<i>Xctg05183</i>	6D	TCGCTTGATAGTGCATGTGA ACTAGCTGCACCCTTTGCAT	CAPS	<i>HindIII</i>
<i>Xctg03037</i>	7D	CAAGTGGTGGGAGTCAGGAT GCTCCAAAACAACCTTCTCG	CAPS	<i>SphI</i>
<i>Xctg03322</i>	7D	CCAGGCACTGTTCGCTTACT TGCTCGTGATTGGTTCTGAG	CAPS	<i>HhaI</i>
<i>Xctg06255</i>	7D	AAACGTTGTTTGGCTCAAGG CTCCCTGTGCGCTCTTATC	CAPS	<i>MboI</i>

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 this software.

5. Results

5.1. Phenotype evaluation in the F₂ populations

The mean values of the parental lines involved in each mapping population differed significantly for three of the flowering-related traits (HT, FLT and MAT); there was no significant difference for GFP (**Table 3-2**). Little variation in the four traits was observed among individual plants of each parental line (data not shown). However, all four traits varied widely in the F₂ populations. Much earlier and later heading F₂ plants were present, which suggested transgressive segregation, compared with their parental lines (**Fig. 3-1a, 1b, 1c, 1d**). The dates for the four flowering-related traits of most F₂ plants ranged within the dates of their parental lines. These results indicated the involvement of multiple loci.

Significant ($P < 0.001$) positive correlations were observed among HT, FLT and MAT in all four F₂ populations (**Table 3-3**). HT and FLT showed no correlation with GFP, while MAT correlated significantly ($P < 0.001$) to GFP in the four F₂ populations. Only in the Ldn/KU-2097//Ldn/IG126387 population, was GFP negatively correlated with FLT. The negative correlation between GFP and FLT suggested that the earlier flowering wheat synthetic (Ldn/KU-2097) required a longer period for grain maturation, and that GFP in the late flowering line (Ldn/IG126387) tended to be shorter.

5.2. Construction of linkage maps in the four F₂ populations

In the Ldn/PI476874//Ldn/KU-2090 population, 472 SSR primer sets targeting the D genome were tested and 89 (18.9%) were found polymorphic. Of these, 83 markers formed 6 linkage groups that did not involve chromosome 4D. The total map length was 910.6 cM with the average spacing of 11.0 cM between markers.

Table 3-2. Parental and F₂ population means for four flowering-related traits measured in each of the four F₂ mapping populations

	Heading time	Flowering time	Maturation time	Grain-filling period
Ldn/PI476874//Ldn/KU-2090 in 2010 – 2011 season				
Ldn/PI476874	143.5 ± 1.98*	152.4 ± 2.01	182.5 ± 1.38	30.1 ± 1.28
Ldn/KU-2090	152.2 ± 1.08	164.9 ± 0.79	191.1 ± 1.11	26.2 ± 1.98
F ₂ population	149.3 ± 14.81	159.2 ± 19.62	191.2 ± 25.86	32.0 ± 9.76
Ldn/KU-2097//Ldn/IG126387 in 2010 - 2011 season				
Ldn/KU-2097	139.9 ± 1.28	147.6 ± 0.88	182.7 ± 1.78	35.1 ± 1.99
Ldn/IG126387	150.1 ± 1.17	158.1 ± 1.33	189.1 ± 1.46	31.0 ± 1.23
F ₂ population	147.5 ± 12.51	156.7 ± 13.52	189.2 ± 14.81	32.5 ± 6.58
Ldn/PI476874//Ldn/KU-2069 in 2009 - 2010 season				
Ldn/PI476874	138.5 ± 1.38	144.4 ± 1.98	174.6 ± 1.58	30.2 ± 1.34
Ldn/KU-2069	147.2 ± 0.78	151.8 ± 1.25	183.3 ± 2.00	30.5 ± 0.98
F ₂ population	139.2 ± 14.34	147.9 ± 11.15	178.9 ± 15.90	31.9 ± 5.24
Ldn/KU-2159//Ldn/IG126387 in 2011 – 2012 season				
Ldn/KU-2159	145.7 ± 0.58	155.3 ± 1.15	184.0 ± 2.00	28.7 ± 1.15
Ldn/IG126387	153.7 ± 0.58	159.3 ± 0.58	188.3 ± 1.53	29.0 ± 1.00
F ₂ population	153.5 ± 2.94	159.7 ± 2.17	188.5 ± 3.32	28.7 ± 2.32

*Means (days) with standard deviations.

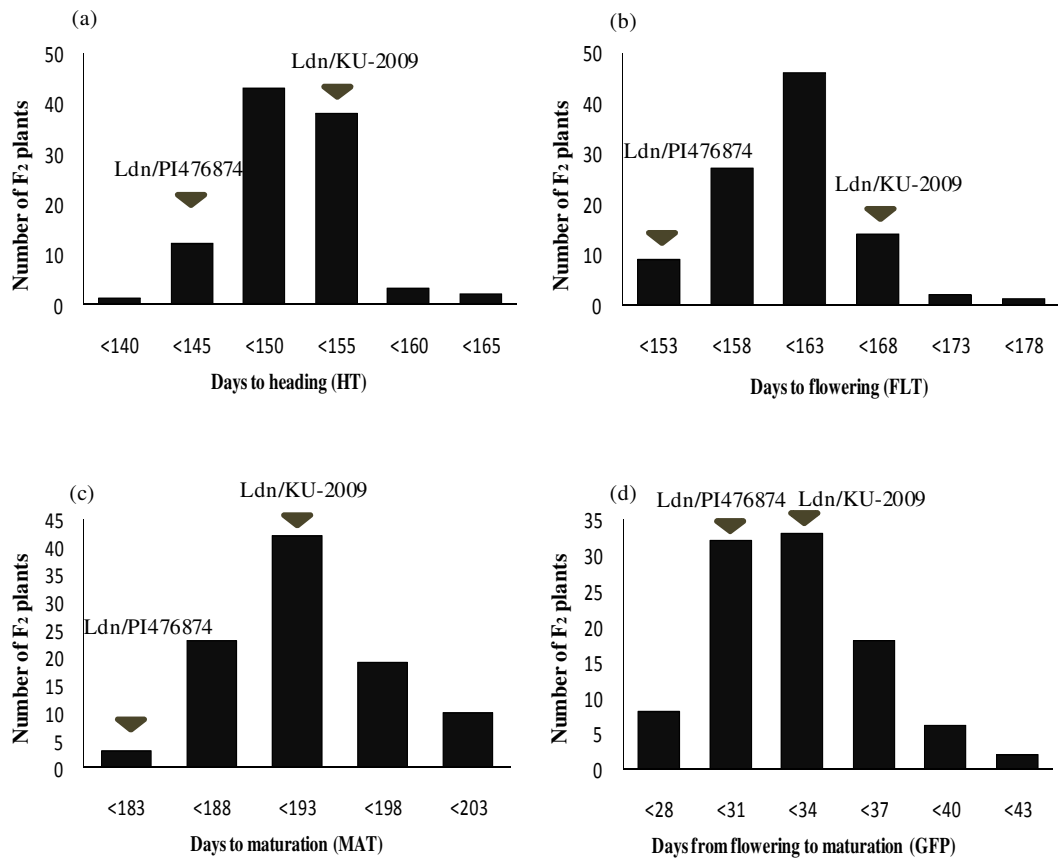


Fig. 3-1a. Frequency distribution of flowering-related traits in the F₂ population: (a) Days to heading; (b) Days to flowering; (c) Days to maturation; (d) Days from flowering to maturation. Arrows indicate the data of their parental accessions Ldn/PI476874//Ldn/KU-2090.

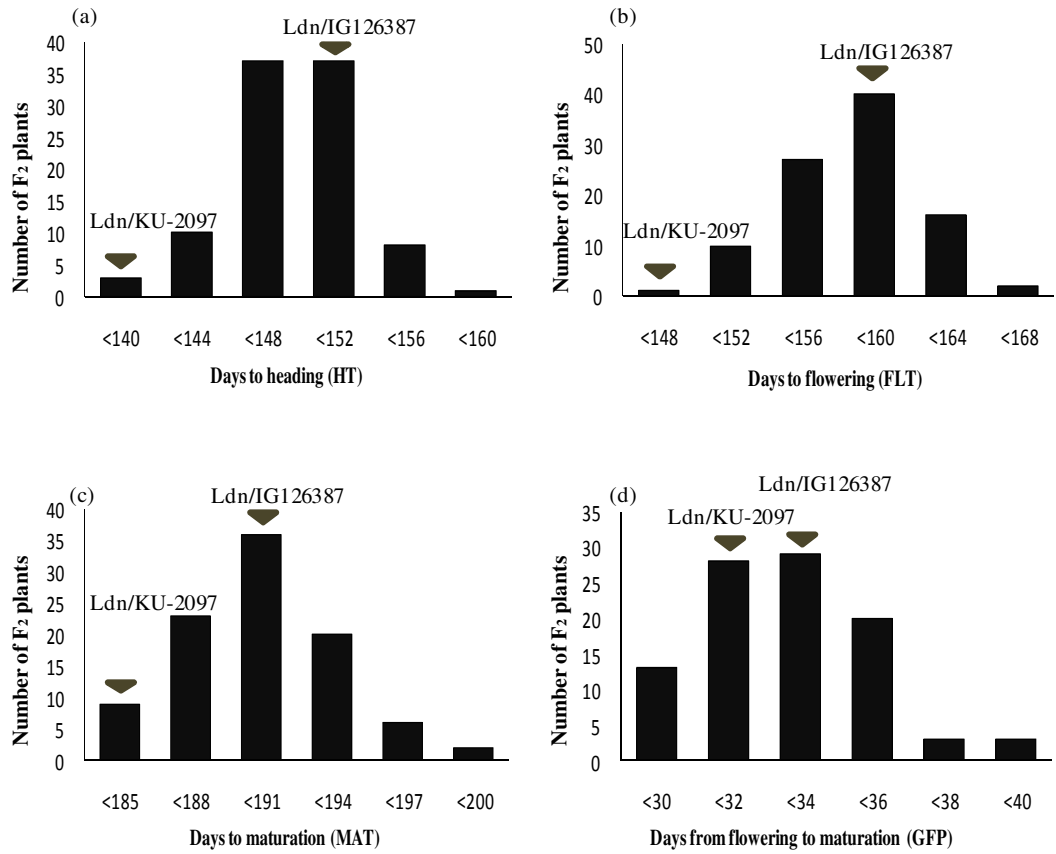


Fig. 3-1b. Frequency distribution of flowering-related traits in the F₂ population: (a) Days to heading; (b) Days to flowering; (c) Days to maturation; (d) Days from flowering to maturation. Arrows indicate the data of their parental accessions Ldn/KU-2097//Ldn/IG126387 .

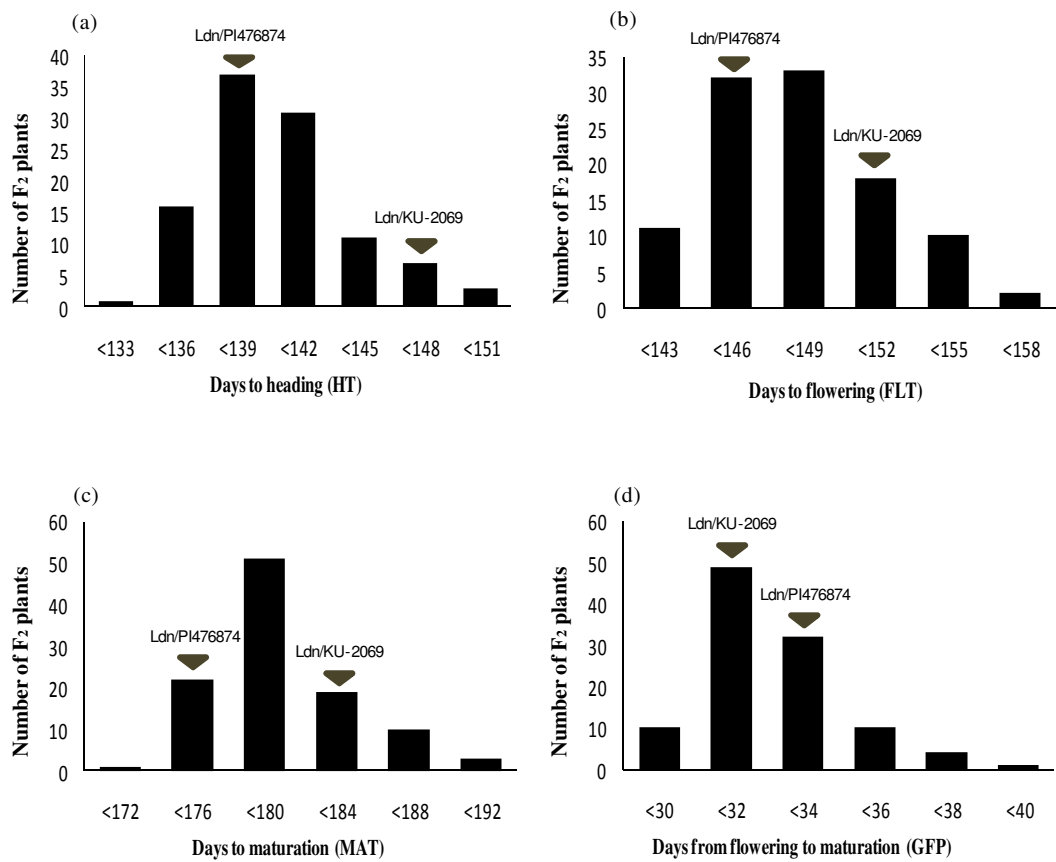


Fig. 3-1c. Frequency distribution of flowering-related traits in the F₂ population: (a) Days to heading; (b) Days to flowering; (c) Days to maturation; (d) Days from flowering to maturation. Arrows indicate the data of their parental accessions Ldn/PI476874//Ldn/KU-2069.

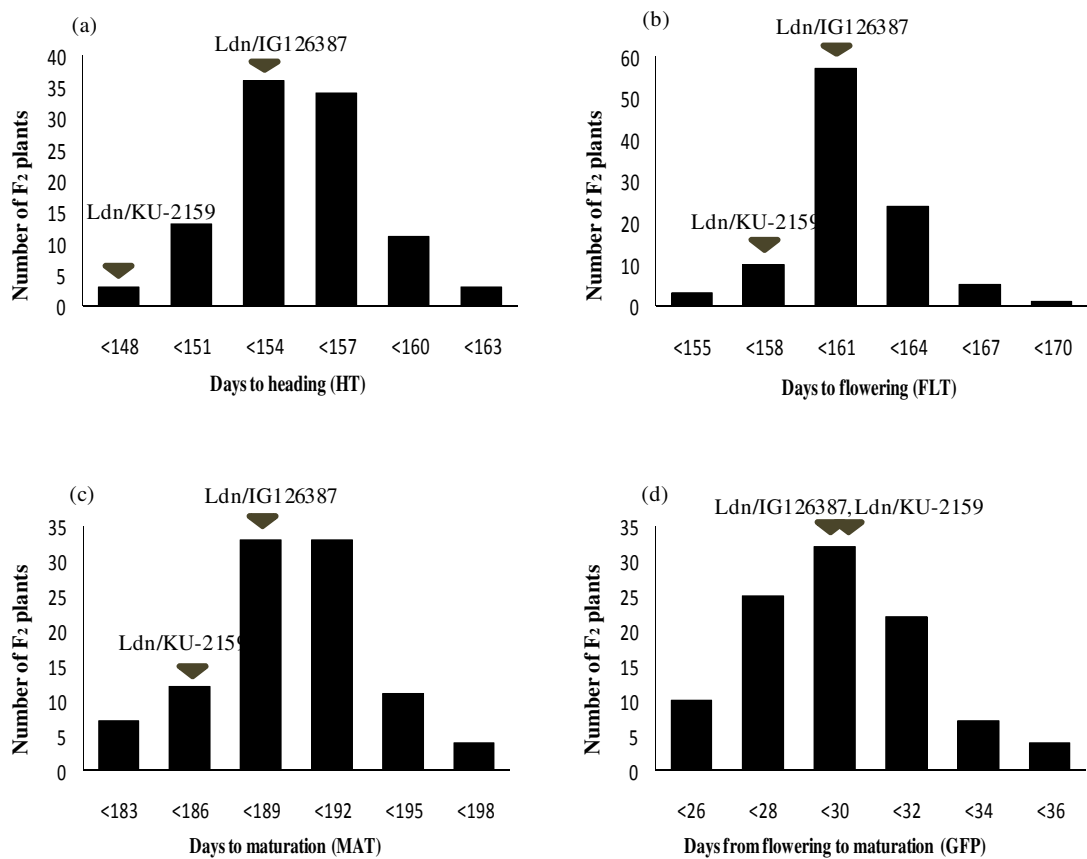


Fig. 3-1d. Frequency distribution of flowering-related traits in the F₂ population: (a) Days to heading; (b) Days to flowering; (c) Days to maturation; (d) Days from flowering to maturation. Arrows indicate the data of their parental accessions Ldn/KU-2159//Ldn/IG126387 .

In the population Ldn/KU-2097//Ldn/IG126387, 98 (19.6%) out of 474 SSR, 25 CAPS and 2 indel markers were polymorphic between the parental synthetic lines. In total, 83 loci were available for construction of a genetic map with 7 linkage groups. The total map length was 824.4 cM with the average spacing of 9.9 cM between markers.

In the population Ldn/PI476874//Ldn/KU-2069, 97 (20.6%) out of 470 SSR markers were polymorphic between the two parental synthetic lines. A total of 59 SSR markers were assigned to 9 linkage groups. The total map length was 1073 cM with the average distance of 18.1 cM between SSR loci.

In the last population Ldn/KU-2159//Ldn/IG126387, 132 (30.3%) out of 434 SSR and 2 indel markers were polymorphic; 105 markers were assigned to 7 linkage groups, with a total map length of 520.1 cM and the average interval of 4.9 cM between markers.

5.3. QTL analysis for flowering-related traits in the four F₂ populations

QTL for all studied flowering-related traits were detected using the four genetic maps. In total, 10 QTL, located on each D-genome chromosome except for 4D, showed significant LOD scores ($P < 0.05$) (**Fig. 3-2**). Five, two, two and one QTLs were detected for HT, FLT, MAT and GFP, respectively (**Table 3-4**).

For HT, two 1D QTLs with LOD scores of 3.54 and 4.68, respectively, were found at the same chromosomal region in two mapping populations, i.e. Ldn/KU-2097//Ldn/IG126387 and Ldn/KU-2159//Ldn/IG126387. The 1D QTL, named as *QHt.kpg-1D.1*, contributed 15-21% of the variation in HT in each population (**Table 3-4**). A QTL for HT with the LOD score of 5.58 was detected on chromosome 5D, and named *QHt.kpg-5D.1*. It explained 17% of the HT variation in the Ldn/KU-2097//Ldn/IG126387 population. QTLs for HT with LOD scores of 5.02 and 4.08, respectively, were found on chromosomes 2D and 6D, and contributed 17.1% (*QHt.kpg-2D.1*) and 16.8% (*QHt.kpg-6D.1*) of the HT variation in the Ldn/KU-2097//Ldn/IG126387 population.

For FLT, a QTL with the LOD score of 4.30 was found on chromosome 1D in the Ldn/KU-2159//Ldn/IG126387 mapping population. This 1D QTL, named *QFlt.kpg-1D.1*, explained 18% of the FLT variation. A QTL for FLT (LOD score = 5.47), named *QFlt.kpg-7D.1*, was also found on chromosome 7D, and contributed 24% of the FLT variation in the Ldn/PI476874//Ldn/KU-2069 population.

For MAT, two QTLs with LOD scores of 3.28 and 8.33 were found on chromosomes 2D and 7D, respectively. The 7D QTL, named *QMat.kpg-7D.1*, was detected in the Ldn/PI476874//Ldn/KU-2069 population, and the 2D QTL, named *QMat.kpg-2D.1*, was detected in the Ldn/KU-2097//Ldn/IG126387 population. *QMat.kpg-7D.1* and *QMat.kpg-2D.1* contributed 45 and 14% of the MAT variation, respectively. On the other hand, only one QTL for GFP was found. It was located on the long arm of chromosome 3D, and was present in the Ldn/PI476874//Ldn/KU-2090 mapping population. It was named *QGfp.kpg-3D.1*; its LOD score was 3.59, and it explained 15% of the GFP variation.

In all QTLs identified for HT, FLT and MAT, alleles from the early flowering parent showed negative values when assessed for additive effects (**Table 3-4**), indicating that the alleles derived from the early synthetic lines produced early phenotypes for HT, FLT and MAT. *QGfp.kpg-3D.1* showed a positive additive effect, indicating that the GFP allele for prolonged filling period was derived from the early flowering parent Ldn/PI476874.

5.4. The effects of the identified QTLs on flowering-related traits

To study the effects of the identified QTL, data of each flowering-related trait were grouped based on the genotypes at the QTL regions of each F₂ individual. For all four traits there were significant ($P < 0.05$) differences among genotypes at the QTLs (**Fig. 3-3**). The F₂ individuals carrying alleles from the early flowering parent at most of the QTLs showed significantly earlier attributes than those with the alleles from the late

Table 3-3. Correlation coefficient (r) matrices for four traits measured in four F_2 mapping populations

	Heading time	Flowering time	Maturation time
Ldn/PI476874//Ldn/KU-2090			
Flowering time	0.9504***		
Maturation time	0.7039***	0.7974***	
Grain-filling period	-0.1956	-0.1141	0.5008***
Ldn/KU-2097//Ldn/IG126387			
Flowering time	0.9412***		
Maturation time	0.6963***	0.8278***	
Grain-filling period	-0.3009	-0.2838*	0.4065***
Ldn/PI476874//Ldn/KU-2069			
Flowering time	0.9126***		
Maturation time	0.7528***	0.8565***	
Grain-filling period	-0.0218	0.0374	0.5477***
Ldn/KU-2159//Ldn/IG126387			
Flowering time	0.9314***		
Maturation time	0.6159***	0.7172***	
Grain-filling period	0.0084	0.0889	0.7578***

Levels of significance are indicated by asterisks, * $P < 0.05$, *** $P < 0.001$.

flowering parent. At *QGfp.kpg-3D.1*, the F₂ individuals with the late flowering parent allele exhibited shorter GFP than those with the early flowering parent allele. No significant differences were observed between the late flowering parent alleles and the heterologous alleles at the 1D and 2D QTLs, indicating that early flowering genotypes are determined by homozygous recessive alleles at the 1D and 2D QTL.

6. Discussion

Synthetic hexaploid wheat constitutes an effective genetic bridge for transferring agronomically important genes from *Ae. tauschii* to common wheat. Our previous studies have shown that the wide variation in HT observed among *Ae. tauschii* accessions was retained in hexaploid synthetic wheat lines derived from these *Ae. tauschii* accessions to Langdon durum wheat (Takumi et al. 2009b; Kajimura et al. 2011). When all vernalization and photoperiod requirements are satisfied, the effects of the parental *Ae. tauschii* genes on HT and FLT of the synthetic hexaploid wheat lines become evident (Fujiwara et al. 2010). Therefore, early flowering accessions of *Ae. tauschii* must contain substantial genetic variation that can be used for the development of early flowering cultivars of common wheat. In this study, in four mapping populations ten QTLs affecting flowering-related traits were identified (**Fig. 3-2**). These QTLs significantly contributed to the variation of flowering time among the synthetic wheat lines. For most QTLs, the early flowering synthetic parents contained early-type alleles. Only *QGfp.kpg-3D.1* was originated from the late flowering parental line.

QHt.kpg-1D.1 found in the Ldn/KU-2097//Ldn/IG126387 population was located on the long arm of chromosome 1D, at the same position as the HT and FLT QTLs identified in the Ldn/KU-2159//Ldn/IG126387 population (**Fig. 3-2**). *QHt.kpg-1D.1* seems to correspond to the FLT QTL, *QFlt.nau-1D*, detected among recombinant inbred lines (RILs) derived from a hybrid of two spring cultivars (Lin et al. 2008).

Table 3-4. A summary of QTLs for flowering-related traits that were identified in four F₂ mapping populations

Traits	Locus	Map location	LOD score	LOD threshold	Contribution (%)	Additive effect
Ldn/PI476874//Ldn/KU-2090						
GFP	<i>QGfp.kpg-3D.1</i>	<i>Xhbg270-Xbarc71</i>	3.59	3.1	15.14	1.75
Ldn/KU-2097//Ldn/IG126387						
HT	<i>QHt.kpg-1D.1</i>	<i>Xctg07195-Xcfd282</i>	3.54	3.2	15.83	-1.87
HT	<i>QHt.kpg-2D.1</i>	<i>Xctg05205-Ppd-D1</i>	5.02	3.2	17.13	-1.97
MAT	<i>QMat.kpg-2D.1</i>	<i>Xctg05205-Ppd-D1</i>	3.28	3.2	14.86	-1.65
HT	<i>QHt.kpg-5D.1</i>	<i>Xgwm583-Xcfd57</i>	5.58	3.2	17.90	-2.49
HT	<i>QHt.kpg-6D.1</i>	<i>Xcfd33-Xctg05512</i>	4.08	3.2	16.81	-0.41
Ldn/PI476874//Ldn/KU-2069						
FLT	<i>QFlt.kpg-7D.1</i>	<i>Xwmc121-Xgwm295</i>	5.47	3.9	24.66	-1.29
MAT	<i>QMat.kpg-7D.1</i>	<i>Xwmc121-Xgwm295</i>	8.33	5.4	45.08	-3.35
Ldn/KU-2159//Ldn/IG126387						
HT	<i>QHt.kpg-1D.1</i>	<i>Xgdm126-Xcfd282</i>	4.68	3.1	21.47	-1.59
FLT	<i>QFlt.kpg-1D.1</i>	<i>Xgdm126-Xcfd282</i>	4.30	3.1	18.10	-1.04

QFlt.nau-1D is considered to be homoeologous to another FLT QTL, *QFlt.nau-1B*, and they seem to be orthologs of *Eps-A^{m1}*, a narrow-sense earliness QTL in einkorn wheat (Bullrich et al. 2002; Lin et al. 2008). *Eps-A^{m1}* was originally identified in an F₂ population and RILs from a cross between cultivated and wild einkorn wheats. The wild wheat derived allele of *Eps-A^{m1}* produces an early flowering effect (Bullrich et al. 2002). The three homoeologous QTL controlling wheat HT and FLT are in the distal regions of chromosomes 1AL, 1BL and 1DL. In the present study, *QHt.kpg-1D.1* was identified in F₂ populations from crosses between early and late flowering wheat synthetics, indicating that the early flowering allele was derived from the wild wheat progenitor, the same as the early flowering allele of *Eps-A^{m1}*. *QHt.kpg-1D.1* is located near *Xctg07195* in the Ldn/KU-2097//Ldn/IG126387 mapping population (**Fig. 3-2**). Using the GenomeZipper approach (Mayer et al. 2011), the *ctg07195* CAPS marker was selected as one linked to an ortholog of barley *EARLY MATURITY 8 (EAM8)*, which encodes an ortholog of the *Arabidopsis EARLY FLOWERING3* gene (Faure et al. 2012). The GenomeZipper approach also revealed that the nucleotide sequence of *Xcdo393*, an RFLP marker linked to *Eps-A^{m1}* (Bullrich et al. 2002; Faricelli et al. 2010), is located near the *EAM8* region. The barley *eam8* mutant shows an early flowering phenotype, and the recessive allele seems to allow adaptation to high-latitude habitats (Faure et al. 2012). The recessive homozygote of *QHt.kpg-1D.1* and *QFlt.kpg-1D.1* also exhibited an early flowering phenotype (**Fig. 3-3**). Thus, a wheat ortholog of *EAM8* is a candidate gene for *QHt.kpg-1D.1* and *QFlt.kpg-1D.1*, a hypothesis that should be studied further.

There is little information regarding flowering-related loci in the chromosomal region of *QHt.kpg-5D.1* identified in the Ldn/KU-2097//Ldn/IG126387 population. One vernalization requirement gene, *Vrn-D4*, is located proximally on chromosome 5DL of common wheat (Yoshida et al. 2010), and the einkorn wheat *VERNALIZATION-INSENSITIVE3*-like (*VIL*) gene *TmVIL1* is closely linked to *Vrn-D4*

(Fu et al. 2007). However, *Vrn-D4* mapped to the centromeric region of 5D (Yoshida et al. 2010), and the *Ae. tauschii* ortholog of *TmVIL1*, *AetVIL1*, is also located near the 5D centromeric region (Koyama et al. 2012). Therefore, the chromosomal position of *QHt.kpg-5D.1* does not correspond to *Vrn-D4* (**Fig. 3-2**). *QHt.kpg-5D.1* seems to be a novel locus associated with wheat flowering.

Several studies have reported identifying FLT QTL that appears to be identical to *Ppd-D1* in common wheat (Hanocq et al. 2004; Xu et al. 2005; Lin et al. 2008; Wang et al. 2009). *QHt.kpg-2D.1* and *QMat.kpg-2D.1* in the present study were located in the same region of chromosome 2DS: region of the QTL included *Ppd-D1* (**Fig. 3-2**). The gene-specific primer set we used for *Ppd-D1* was designed for amplification of a 414 bp product from the intact *Ppd-D1* sequence (Beales et al. 2007). No amplified product appears in the *Ppd-D1a* allele that carries a deletion in the photoperiod-insensitive cultivars (Beales et al. 2007). Of the two parental synthetics used here, the early flowering Ldn/KU-2097 generated a 414 bp PCR product, whereas the late flowering Ldn/IG126387 generated a product about 40 bp longer (data not shown). In *Ae. tauschii*, three haplotypes of *Ppd-D1* are present, two of which are specific to the *Ae. tauschii* population and are missing in hexaploid wheat (Guo et al. 2010). The *Ppd-D1* sequences of the *Ae. tauschii*-specific haplotypes contain either two insertions of 24 and 15 bp or one insertion of 15 bp in the 5'-upstream region (Guo et al. 2010; Huang et al. 2012). Therefore, IG126387 may belong to the *Ae. tauschii*-specific haplotype group. The haplotype variation at *Ppd-D1* is considered to partly underlie the wide HT variation in *Ae. tauschii* (Matsuoka et al. 2008; Xiang et al. 2008; Huang et al. 2012). *QHt.kpg-2D.1* and *QMat.kpg-2D.1* identified here appear to be an allelic difference at the *Ppd-D1* locus, which was transmitted from the parental *Ae. tauschii* accessions to the synthetic hexaploid lines. Early and late flowering lines of wheat synthetics (Ldn/KU-2097 and Ldn/IG126387, respectively) failed to transition from vegetative to

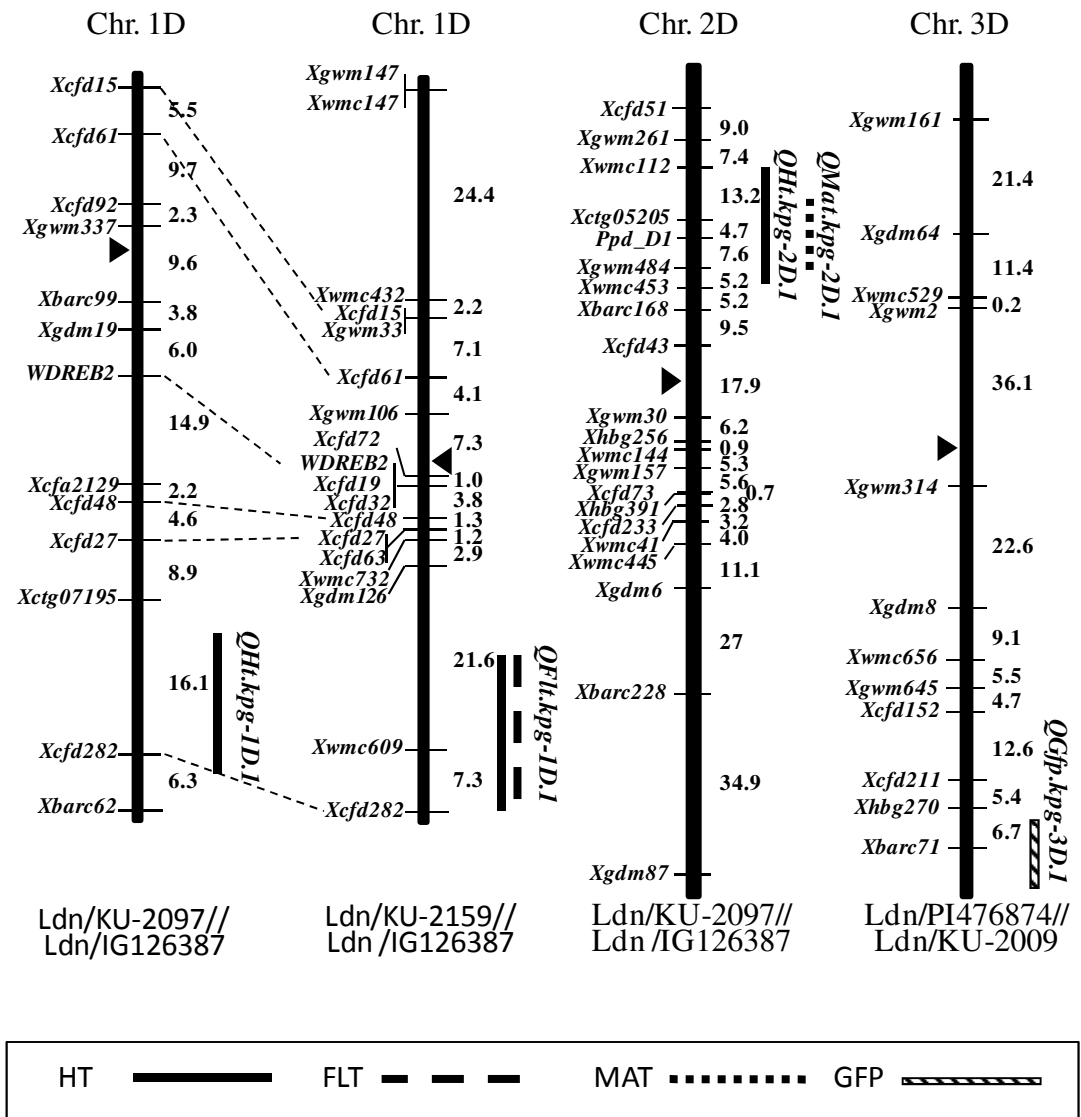


Figure 3-2. Linkage maps and positions of identified QTLs for four flowering-related traits in four mapping populations of synthetic hexaploid wheat. Only those chromosomes with QTL are shown. QTLs with LOD scores above the threshold are indicated, and genetic distances (in centiMorgans) are given to the right of each chromosome. Black arrowheads indicate the putative positions of centromeres.

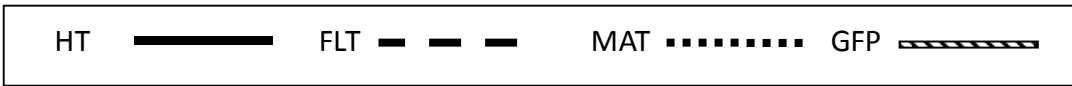
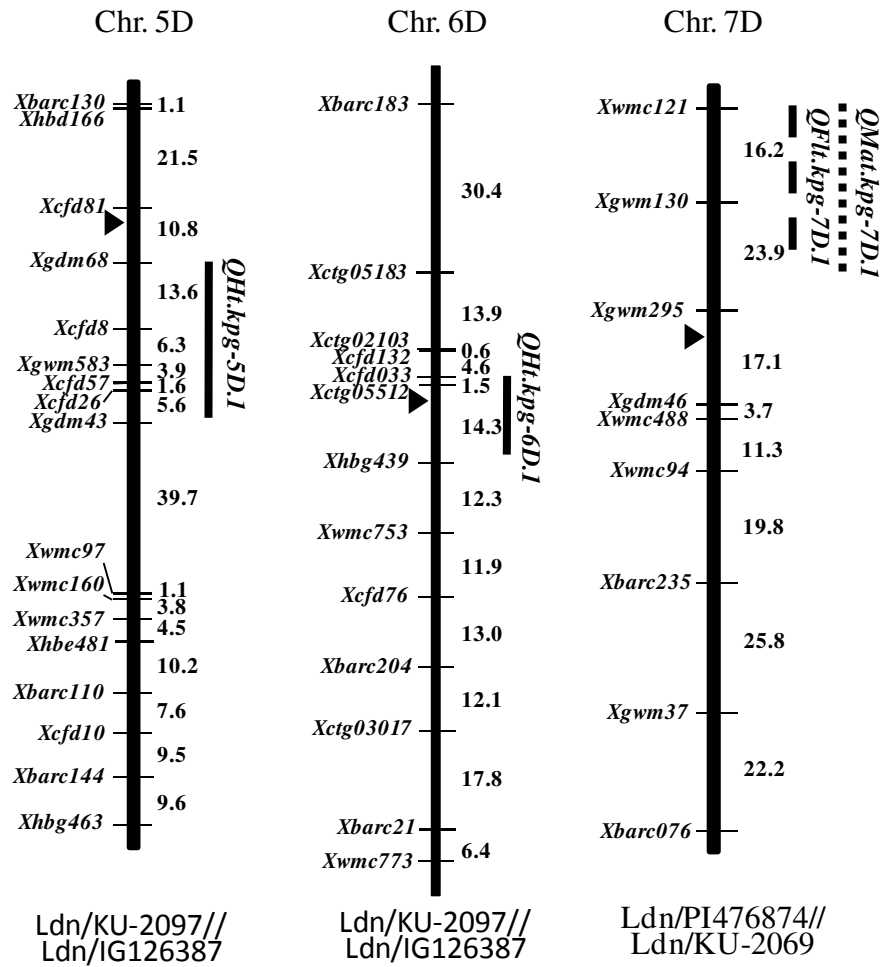


Figure 3-2 (continued). Linkage maps and positions of identified QTLs for four flowering-related traits in four mapping populations of synthetic hexaploid wheat. Only those chromosomes with QTL are shown. QTLs with LOD scores above the threshold are indicated, and genetic distances (in centiMorgans) are given to the right of each chromosome. Black arrowheads indicate the putative positions of centromeres.

reproductive growth phase under short days (data not shown), indicating that both synthetic lines retained their photoperiod sensitivity. These observations suggest that, if *QHt.kpg-2D.1* and *QMat.kpg-2D.1* correspond to *Ppd-D1*, the two indels of 24 and 15 bp in the 5'-upstream region of *Ppd-D1* could affect HT and FLT without any influence on the photoperiodic sensitivity. An analysis of the expression of genes downstream from *Ppd-D1* should clarify the effects of the indel mutation on wheat flowering.

QGfp.kpg-3D.1 was located in the distal part of chromosome 3DL, and no other QTL for HT, FLT and MAT were present in this region (**Fig. 3-2**). Highly positive correlations were observed among HT, FLT and MAT, while GFP was uncorrelated to HT and FLT in the four mapping populations (**Table 3-3**). These results indicated that GFP is regulated separately from HT and FLT. As shown in our previous study (Kajimura et al. 2011), two major genetic pathways independently determine wheat MAT; one that controls HT and FLT, and the other regulates GFP. However, little is known about the molecular control of GFP in wheat. Up to now, *Gpc-B1* on chromosome 6B has been the only genetic locus shown to affect GFP in tetraploid wheat (Uauy et al. 2006). *QGfp.kpg-3D.1* seems to be a novel locus for regulating GFP. GFP is a trait that is strongly affected by plant growth conditions, and shorter GFP was observed under higher temperature conditions during grain maturation (Kajimura et al. 2011). Genetic modification of GFP may in the future become more important as a means to optimize grain quality for different environments. Therefore, it is important to elucidate the molecular nature of *QGfp.kpg-3D.1* and to identify additional QTLs.

Synthetic wheat lines are useful resources for the identification of agronomically important loci that function in hexaploid wheat. This and previous studies (Kerber 1987; Ma et al. 1995; Mujeeb-Kazi et al. 1996) have shown that numerous alleles useful for wheat breeding can be identified in natural populations of wild progenitors of common wheat. Interactions among the A, B and D genomes affect gene expression profiles in

hexaploid wheat. The A and B genomes of Ldn appeared to have a strong effect on the observed phenotypes of the synthetic hexaploid wheat lines used in the present study. Two major flowering characters, photoperiod sensitivity and the vernalization requirement, in the synthetic lines were dependent on the characteristics of Ldn (data not shown). Therefore, 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 as has been reported previously (Gororo et al. 2001). On the other hand, chromosome 2D of *Ae. tauschii* significantly affected HT in the hexaploid wheat background (Xiang et al. 2008). The epistatic interactions among genes of the A, B and D genomes of hexaploid wheat and its effects on flowering should be carefully analyzed in future studies.

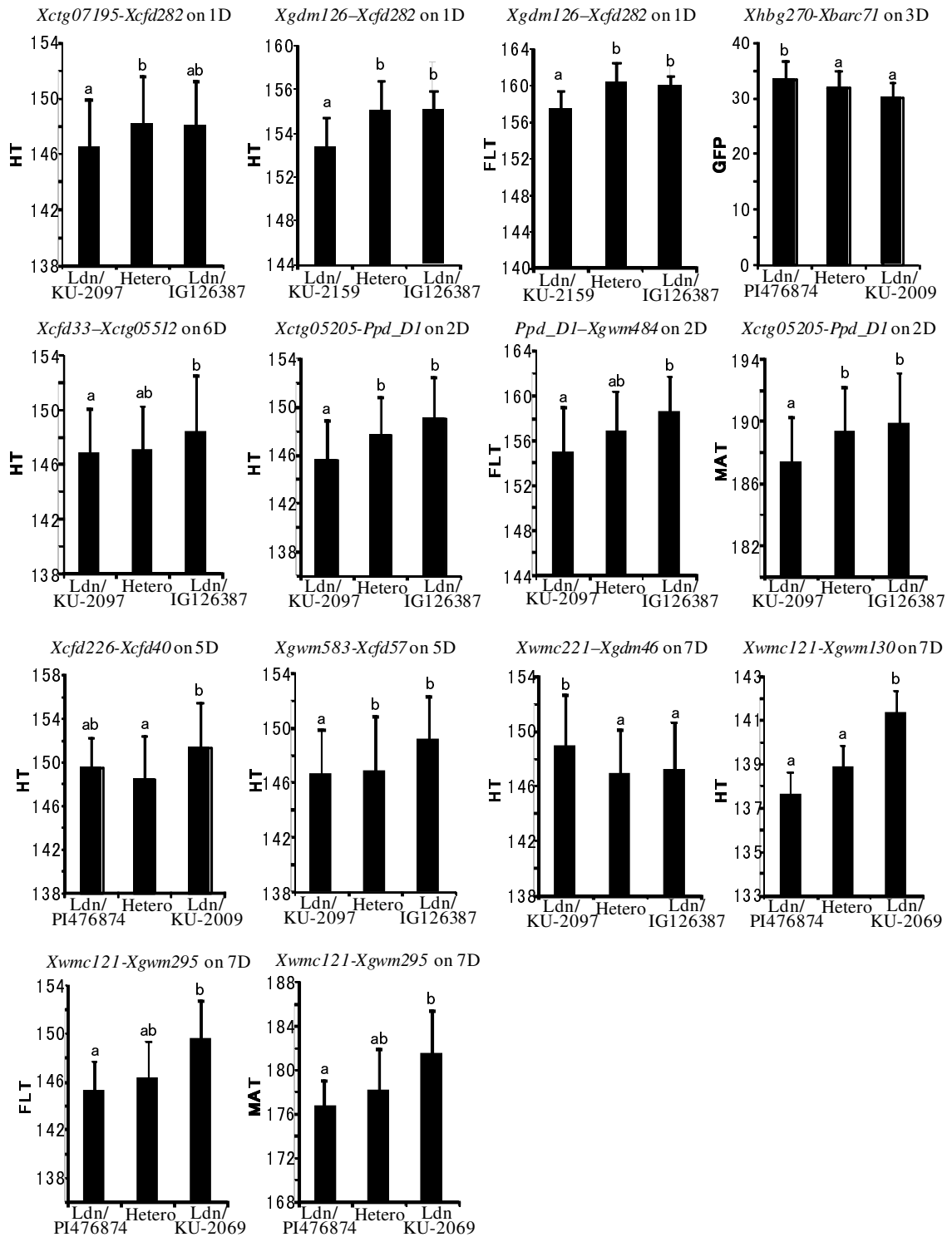


Figure 3-3. The genotype effects at each QTL on the observed variation in the flowering-related traits. Markers that were used to deduce the genotype at a QTL are listed above each graph. Means \pm standard deviation with the same letter were not significantly different ($P > 0.05$) (Tukey-Kramer HSD test).

Chapter IV

Indetification and mapping of QTL for early flowering from early synthetic wheat and Japanese elite varieties.

1. Summary

Our previous study showed that synthetic wheat line, which derived from crosses between tetraploid wheat and *Ae. tauschii*, has the potential to provide new genetic variation in heading and flowering times of leading varieties. In this study, we investigated flowering traits under glasshouse conditions by comparing synthetic wheat lines and Japanese elite wheat cultivars. Two F₂ mapping populations were crossed respectively between two Japanese wheat varieties Norin 61 and Kitanokaori with two synthetic wheat lines Ldn/PI476874 and Ldn/KU-2097. In total, twelve significant quantitative trait loci (QTLs) for four flowering related traits were detected, of which 3 were for HT, 3 for FLT, 3 for MAT and 3 for GFP. The *Ppd-D1* locus from the two Japanese elite varieties was causal for early-flowering phenotype and might strongly affect to expression of other QTLs, which were found in synthetic wheat line from previous study. Further, two QTL regions were separately detected on chromosome 5A and alleles from the synthetic wheat lines were associated with early days to flowering in the two F₂ populations. The 5AL QTL region controlling flowering time found in the Kitanokaori// Ldn/KU-2097 population appeared correspond to locus of *Vrn-A1*, which is related to the vernalization requirement in tetraploid and hexploidy wheat. Another 5AS QTL seems to be novel loci regulating days to maturation. The present study indicated that favorable QTL alleles could be transferred from wild wheat relatives into an elite wheat variety for improvement of quantitative trait loci like flowering time.

2. Introduction

Common wheat (*Triticum aestivum* L.), hexaploid wheat, is one of the most important crops in the world and it provides over 20% of calories and protein in human nutrition, and is the staple food in more than 40 countries for over 35% of the world's population (Bushuk 1998). However, increases in wheat productivity and quality have not kept pace with consumer demand and increases in population. Both are, in part, strongly influenced by environmental cues and pathogens. For example, raining or high temperature at harvesting time can create many hazards, such as fungal diseases and lower quality and quantity. So limiting the impact of environment cues and pathogens to the development of crops as well as expanding growing in a diverse range of environments are the major purpose of the wheat breeders.

The breeding and selection for appropriate heading time is one of the critical success factors that help, in part, to expand the cultivated area of hexaploid wheat in diverse range of environments. Because different wheat cultivars show varied heading time in order to adapt to different regional environments (Snape et al. 2001). Maturation time is one of the most important traits for improvement of modern wheat varieties. It is determined by the sum of flowering time and the grain filling period. Flowering time/heading time is the most well-known that it is controlled by three major genetic elements, vernalization requirement, photoperiodic sensitivity and narrow earliness. The vernalization requirement and photoperiodic sensitivity are respectively regulated by major quantitative trait loci (QTLs), *Vrn-1* and *Ppd-1*. The narrow earliness is polygenic. The *Vrn-1* encodes a transcription factor homologous to *Arabidopsis APETALA1* (Murai et al. 2003; Fu et al. 2005). This requirement is determined by four genes, namely, *Vrn-A1* (formerly *Vrn1*) on chromosome 5A, *Vrn-B1* (formerly *Vrn2*) on chromosome 5B, *Vrn-D1* (formerly *Vrn3*) on chromosome 5D, and *Vrn-B4* on chromosome 7B (Law et al. 1976; Snape et al. 1996; Iwaki et al. 2001). The *Ppd-1* seems to encode a

pseudo-responsive regulator (PRR) protein related with plant circadian clock (Beales et al. 2007). The photoperiodic sensitivity is mainly determined by the homoeologous loci *Ppd-A1*, *Ppd-B1*, and *Ppd-D1*, which are located on the short arms of chromosomes 2A, 2B, and 2D, respectively. (Welsh et al. 1973; Law et al. 1978; Scarth and Law 1983). However, there is little information about genetics of the grain filling period. Uauy et al. (2006a) identified a NAC transcription factor gene as a major QTL controlling grain protein content. They found that the QTL, *Gpc-1*, showed pleiotropic effects including senescence of flag leaf, peduncle and seed and water content of seed (Uauy et al. 2006b).

In Japan, more early harvesting cultivars, except in the Hokkaido area, are strongly required on the wheat breeding spot, because wheat harvesting season is usually June which is a rainy season. Moisture condition in the harvesting time reduces quality of the wheat grain and induces both pre-harvest sprouting and Fusarium head blight. The harvesting earliness consists of two wheat developmental processes; one is heading time control and another is grain filling period. So, if varieties with their processes will enable to be genetically shorter, the periods until grain harvesting will be earlier and escape the damages caused by rain or high temperature, which is a major environmental factor affecting crop production. Whereas, late time varieties were selected where long growing seasons prevail (Kato and Yokoyama 1992; Law and Worland 1997).

On the other hand, the restriction of genetic background among wheat cultivars related to flowering time that might cause the limitation of expanded cultivated areas, for instance, winter wheat Kitanokaori cultivar which grows in according with the condition in Hokkaido area (Seki et al. 2011). The range of flowering responses on cultivated wheat could be limited by genetic backgrounds, because they are often genetically similar, with a rather narrow genetic base. Thus, understanding the genetic control and indentifying new genetic loci responsible of the harvesting times of wheat relatives will offers potential for crop improvement because they introduce new sources of genetic

diversity (Edwards et al. 2009).

Common wheat is an allohexaploid species carrying the genomes AABBDD ($2n = 6x = 42$) that originated from natural hybridization between tetraploid wheat (*Triticum turgidum* L.), including emmer and durum wheats, and diploid *Aegilops tauschii* ($2n = 14$, DD) (Kihara 1944; McFadden and Sears 1944). Allohexaploid wheat plant can be artificially produced through hybridization of tetraploid wheat Langdon and the *Ae. tauschii*. Consequently, they share identical A and B genomes derived from tetraploid wheat Langdon and contain diverse D genomes originating from the *Ae. tauschii* pollen parents. The relatives of wheat plant both are important genetic resources for wheat breeding (Knot 1989; Lutz et al. 1995).

The wild wheat *Ae. tauschii* is widely distributed in Eurasia and shows abundant genetic variation (Dvorak et al. 1998; Matsuoka et al. 2008; Mizuno et al. 2010). The birthplace of common wheat is considered to lie within the area comprising Transcaucasia and the southern coastal region of the Caspian Sea (Feldman 2001). Therefore, the *Ae. tauschii* populations involved in the origin of common wheat are limited to a narrow distribution range relative to the entire species range, suggesting that this species holds vast genetic diversity that is not represented in common wheat (Feldman 2001). Results from previous studies shown that hexaploid synthetic wheat derived from crosses between tetraploid wheat and *Ae. tauschii* has the potential to provide new genetic variation for abiotic stress tolerance as well as several agronomically important traits such as pest and disease resistance (Kerber 1987; Ma et al. 1995; Mujeeb-Kazi et al. 1996; Trethowan and Mujeeb-Kazi 2008). In our previous studies have shown that the wide variation in flowering time observed among *Ae. tauschii* accessions is retained in the hexaploid synthetic wheat lines Kajimura et al. (2011), and that the D genome at least partly affects the flowering of hexaploid wheat (Nguyen et al. 2013). Nguyen et al. (2013) showed that synthetic wheat lines can be

useful for the identification of new agriculturally important loci that were detected on synthetic wheat lines can be transferred to and used for the modification of flowering and grain maturation.

The tetraploid emmer wheat is available as the A-and B-genome parent for efficient production of hexaploid synthetic wheat (Matsuoka and Nasuda 2004). The gene pool of wild emmer, being larger and richer than that of the cultivated wheat, contains many agronomically valuable alleles that are easily exploitable for wheat improvement (Feldman and Millet 1995). A lot of studies showed that wild wheat harbours rich genetic resources for wheat improvement such as yield components genes, leaf rust resistance genes and drought stress genes has been reported in both durum and bread wheat (Sinha et al. 1986; Saleem et al. 2003; Maccaferri et al. 2008). In our previous studies showed that the A and B genomes of tetraploid wheat Langdon appeared to have a partly effect on flowering traits (Nguyen et al. 2013) as well as grain shape (Okamoto et al. 2012). 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 as has been reported previously (Gororo et al. 2001). However, the tetraploid emmer wheat are genetically and morphologically diverse and their evolution under domestication has not been fully elucidated (Matsuoka 2011).

In this study, the present research objectives are to clarify the presence of the genes controlling the flowering time and to compare the length of the seed maturation process among hexaploid wheat cultivars. Naturally occurring genetic variation is useful to identify novel alleles available to study the genetic mechanisms of flowering-related pathways (Koornneef et al. 2004).

3. Objectives

The empirical study indicated that early flowering relatives of wheat might be useful

for Japanese wheat breeding. One of goals of the present research is identification of novel early flowering-related alleles using F₂ population derived from known synthetic wheat line and Japanese elite variety and to compare the location of genes for early flowering from different genetic backgrounds of elite wheat and their relatives.

4. Materials and Methods

4.1. Plant materials

Two F₂ populations from crosses between Japanese wheat cultivars and synthetic wheat lines were used in the present study. The first F₂ population was derived from crosses between Japanese wheat cultivar Norin 61 and synthetic hexaploid wheat, Ldn/PI476874. Seeds of the first F₂ population were sown in November 2009-2010 season, with the numbers of individuals in being 108. The second F₂ population of 132 individual lines derived from the cross between Japan winter wheat Kitanokaori and Ldn/KU-2097. The F₂ population of Kitanokaori//Ldn/KU-2097 was grown in the 2012–2013 season. Those synthetic wheat parents, the PI476878 and Ldn/KU-2097, were obtained through interspecific crosses between a tetraploid wheat accession *Triticum durum* cv. Langdon and two *Ae. tauschii* accessions PI476878 and KU-2097, respectively (Takumi et al.2009). The synthetics lines Ldn/PI476874 and Ldn/KU-2097 both were used as the early flowering parent lines in the F₂ mapping populations produced from crosses-combination between synthetic hexaploid wheat lines (Nguyen et al. 2013). Some flowering related QTLs were detected in their F₂ populations and the Ldn/PI476874 and Ldn/KU-2097 alleles at these loci were found to cause early day to flowering time related traits (Nguyen et al. 2013). For each mapping population, F₂ individuals were obtained from a single F₁ plant crossed between wheat cultivars and synthetic wheat lines. All two F₂ populations were grown individually in pots arranged randomly in a glasshouse of Kobe University (34°43'N, 135°13'F), phenotypic was

recorded from each of F₂ individual plants of each populations.

4.2. Phenotype measurement and statistical analyses

On two F₂ populations, four flowering-related traits were measured using the three earliest tillers of each plant, and the trait averages and standard deviations were calculated using the data for each F₂ plant. Heading time (HT) and flowering time (FLT) were recorded as days after sowing. Maturity time (MAT) was measured as the number of days that had passed before the peduncle turned yellow, according to our previous report (Kajimura et al. 2011). The grain filling period (GFP) was the number of days from flowering to maturation. 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.

4.3. Detection of polymorphisms and genotyping with molecular markers

To amplify PCR fragments of simple sequence repeat (SSR) markers, total DNA was extracted from the parents and F₂ individuals using standard procedures. For SSR genotyping, 40 cycles of PCR were performed using 2x Quick Taq HS DyeMix (TOYOBO, Osaka, Japan) with the following conditions: 10 s at 94°C, 30 s at the annealing temperature, and 30 s at 68°C. The last step was incubation for 1 min at 68°C. Information on the SSR markers and their 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 products were separated in 2% agarose or 13% nondenaturing polyacrylamide gels and visualized under UV light after staining with ethidium bromide. For polyacrylamide gel electrophoresis, the high efficiency genome scanning system (Nippon Eido, Tokyo, Japan) of Horii et al. (2003) was used. Genetic mapping was performed using MAPMAKER/EXP version 3.0b software (Lander et al. 1987).

To provide for a number of regions with less SSR markers in the Norin 61//Ldn/PI476874 mapping population, single nucleotide polymorphisms (SNPs) derived from leaf transcripts of *Ae. tauschii* (Iehisa et al. 2012) were used. The additional markers were selected from a high confidence SNP dataset which was constructed by comparison of the next generation sequencing of leaf transcripts between two genetically distinct accessions of *Ae. tauschii* in our previous study (Iehisa et al. 2012). The PCR conditions for cleaved amplified polymorphic sequence (CAPS) markers were 1 cycle of 94°C for 2 min and 40 cycles of 94°C for 30 s, 60°C for 30 s, and 68°C for 30 s. After amplification, PCR products were digested with a restriction enzyme, and the digested fragments were separated by electrophoresis in a 2% agarose gel and stained with ethidium bromide.

Polymorphism at the *Ppd-D1* locus was detected using two allele-specific primers according to Beales et al. (2007). A common forward primer, Ppd-D1_F, 5'-ACGCCTCCCACTACTACTG-3', and two reverse primers, Ppd-D1_R1, 5'-GTTGGTTCAAACAGAGAGC-3', and Ppd-D1_R2, 5'-CACTGGTGGTAGCTGAGATT-3', were used for this PCR-based analysis. PCR products amplified with Ppd-D1_F and Ppd-D1_R2 showed a 2,089-bp deletion in the 5' upstream region of *Ppd-D1*, indicative of the photoperiod-insensitive *Ppd-D1a* allele (Beales et al. 2007). The another primer set is Ppd-D1exon8_F1 5'-GATGAACATGAAACGGG-3' and Ppd-D1exon8_R1 5'-GTCTAAATAGTAGGTACTAGG-3', which generated a 320 bp amplicon from genotypes with the deletion and 336 bp from genotypes with the intact gene (Beales et al. 2007). The PCR products were separated by electrophoresis through a 1.2% agarose gel and stained with ethidium bromide.

4.4. Map construction and QTL analysis

The polymorphic SSRs, SNPs and *Ppd-D1* 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 3.0, 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 (Röder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; 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) using 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.

5. Results

5.1. Flowering-related traits in two F₂ populations and their parental accessions

The mean values among the parental lines Norin 61 and Ldn/PI476874 were more largely differed for flowering related traits than that of Kiatnokaori and Ldn/KU-2097 (**Table 4-1**). **Fig. 4-1a** and **Fig. 4-1b** show the frequency distributions for four flowering related traits in the two F₂ populations. Much earlier and later heading F₂ plants occurred, which suggested transgressive segregation, compared with their parental lines. The dates for the four flowering-related traits of most F₂ plants ranged within the dates of their parental lines, suggesting segregation of a number of genetic factors effect on the studied traits.

Correlation analysis revealed significant positive or negative correlation between the traits examined in this study (**Table 4-2**). Significant ($P < 0.001$) positive correlations were observed among HT, FLT and MAT, and GFP was negatively correlated with HT, FLT and MAT in the two F₂ populations. The negative correlation between GFP and HT/FLT indicated that the earlier flowering F₂ individuals required a longer period for

Table 4-1. Parental and F₂ population means for four flowering-related traits measured in each of the two F₂ mapping populations

	Heading time	Flowering time	Maturation time	Grain-filling period
Norin 61// Ldn/PI476874				
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	147.5 ± 1.11	32.1 ± 0.55
Mean in F ₂ plants	122.1	131.7	169.7	37.7
Range in F ₂ plants	110.0-146.3	121.6-153.3	159.0-187.6	22.0-44.6
Kitanokaori// Ldn/KU-2097				
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 ± 0.59
Mean in F ₂ plants	149.5	157.9	187.7	29.8
Range in F ₂ plants	127.0-168.0	139.3-176.0	174.5-202.5	24.0-42.0

*Means (days) with standard deviations.

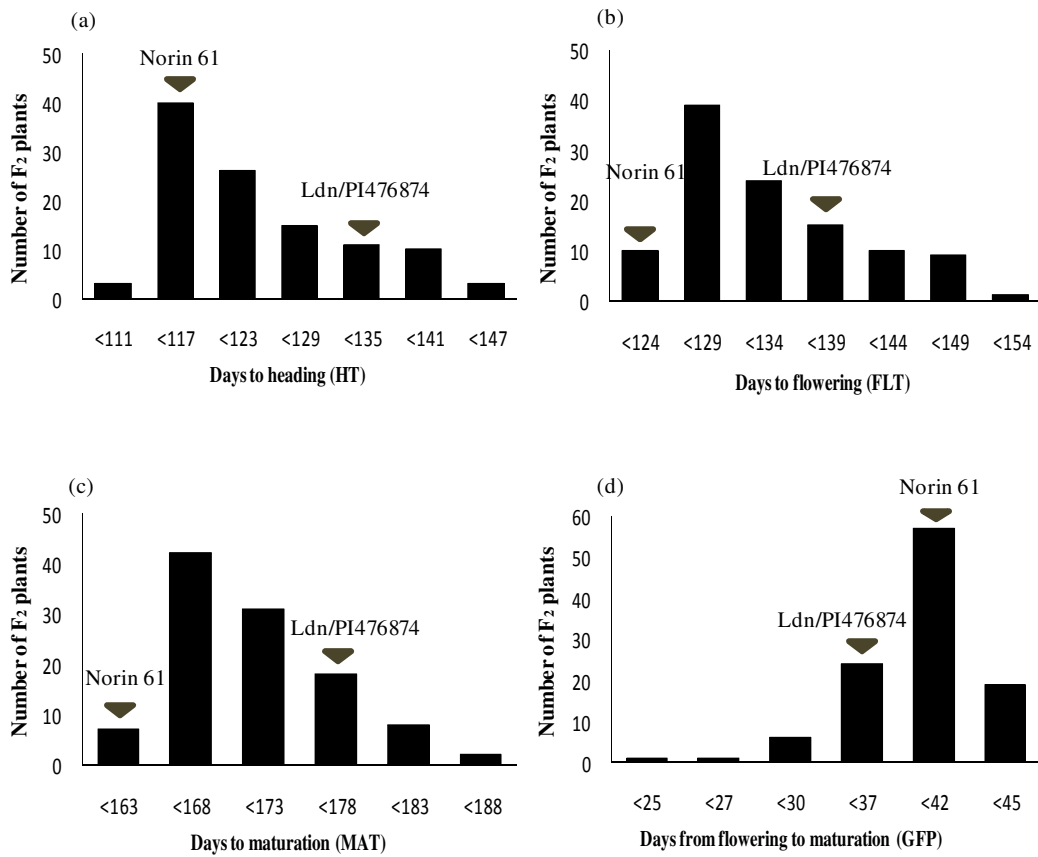


Fig. 4-1a. Frequency distribution of flowering-related traits in the F₂ population: (a) Days to heading; (b) Days to flowering; (c) Days to maturation; (d) Days from flowering to maturation. Arrows indicate the data of their parental accessions Norin 61// Ldn/PI476874.

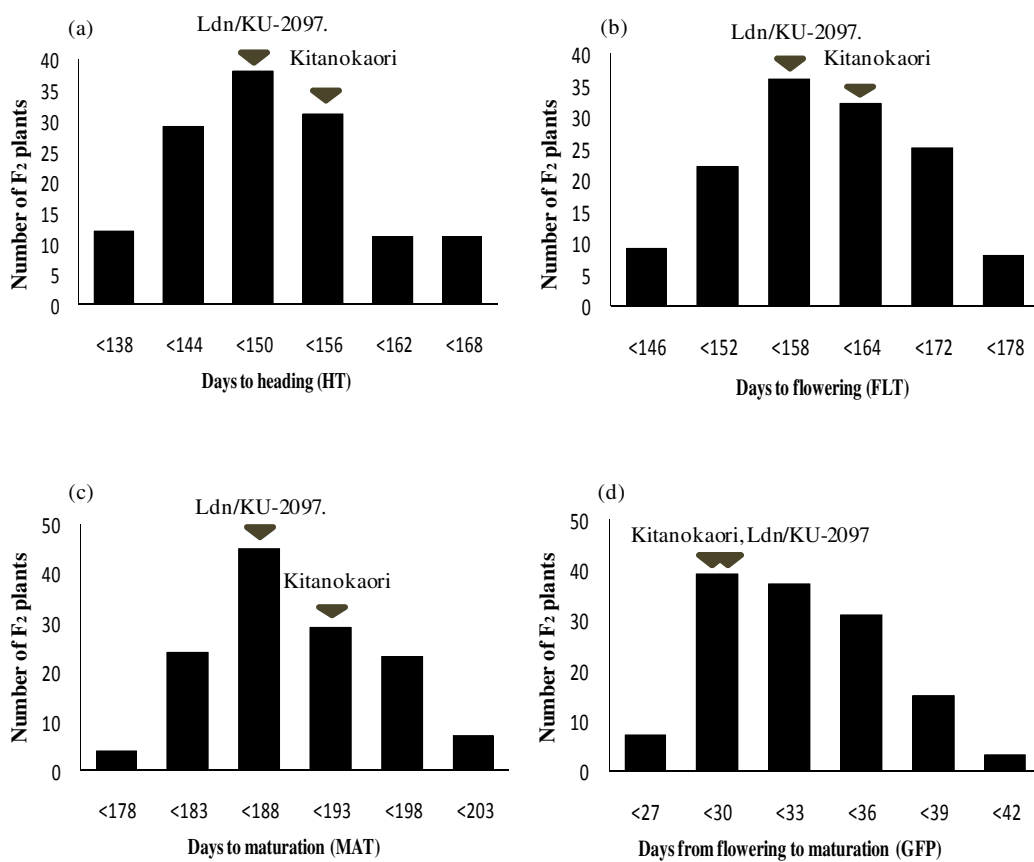


Fig. 4-1b. Frequency distribution of flowering-related traits in the F₂ population: (a) Days to heading; (b) Days to flowering; (c) Days to maturation; (d) Days from flowering to maturation. Arrows indicate the data of their parental accessions Kitanokaori/Ldn/KU-2097.

grain maturation, and that GFP in the late flowering F₂ individuals tended to be shorter. The synthetic hexploid wheat lines both provide the genetic loci for early flowering time (Nguyen et al. 2013). Therefore, if some molecular markers linked to the early flowering and maturing QTLs will identified, the synthetic line alleles from the D genome as well as A, B genomes will be respectfully available for wheat breeding in Japan.

5.2. Construction of a linkage map in the two F₂ populations.

In the Norin 61//Ldn/PI476874 population: To construct the map, 958 primer sets were initially screened for polymorphism and 270 polymorphic markers (28.2 %) were mapped using 108 plants. Genetic linkage map constructed with 241 marker loci, including *Ppd-D1* allele-specific and CAPS markers, were assigned to 26 linkage groups. The total map length was 3117 cM with the average spacing of 12.9 cM between markers. Chromosomes 1A, 2A, 3A, 7A and 3D each were represented by two linkage groups. The total number of markers per chromosome ranged from 5 (6A) to 39 (2D).

In the Kitanokaori//Ldn/KU-2097 population: The survey of the 912 molecular markers identified a total of 192 markers that were polymorphic between the parents. These polymorphic markers were mapped by using 132 F₂ individual plants. Mapmaker analysis at LOD 2.5 resolved the markers into 22 linkage groups with 154 marker loci, chromosomes 2A, 3A and 7D each separated into two linkage groups. The linkage map spanned 1853.6 cM in length with an average interval of 12.0 cM between adjacent markers. The total number of markers per chromosome ranged from 6 (3A) to 16 (5D).

5.3. QTL analysis for flowering-related traits in the two F₂ populations.

In the Norin61//Ldn/PI476874 populatioon. Five QTLs, located on chromosomes 5A and 2D, showed significant LOD scores ($P < 0.05$) (**Fig. 4-2a**). One, one, two and one QTLs were respectively detected for HT, FLT, MAT and GFP (**Table 4-3**).

For HT, one QTL was detected between interval markers *Ppd-D1-Xctg4491* on the short arms of chromosomes 2D with LOD scores of 40.0. The QTL, located at 2D QTL

explained 33.3 of the variation in HT in the Norin61//Ldn/PI476874 population. The additive effect of the HT QTL indicated that the Norin61 allele at the 2D QTL promoted heading earlier than the synthetic allele.

For FLT, one QTL with a LOD score of 36.2 was found on chromosome 2D. The 2D QTL for FLT was located at a chromosomal position similar to that of HT, and contributed 21.4 % of the FLT variation. The additive effect of the FLT QTL on chromosome 2D was similar to that of the 2D QTL for HT.

For MAT, two QTLs with LOD scores of 16.3 and 4.2 were respectively found in the chromosomal 2D and 5A. The 2D and 5A QTLs explained 16.4 % and 28.9 %, respectively of the variation in MAT. The Norin61 allele at the MAT QTL on 2D had an earlier effect on MAT than the synthetic wheat allele, whereas the synthetic allele at the MAT QTL on 5A contributed to early day to maturation.

For GFP, a QTL with the LOD score of 7.2 was found on chromosome 2D in the same region with HT, FLT and MAT QTLs of Norin61// Ldn/PI476874 mapping population. This 2D QTL for GFP explained 15.8 % of the GFP variation. The synthetic allele at this locus was found to cause earlier days to grain filling period.

In the Kitanokaori// Ldn/KU-2097 population. Four and three QTLs, located respectively on chromosomes 2D and 5A, showed significant LOD scores ($P < 0.05$) (**Fig. 4-2b**). On the chromosome 2D, QTLs for HT, FLT, MAT and GFP were co-located and located the interval of SSR markers *Xwmc112* – *Xgwm484*. In the chromosome 5A, QTLs controlling HT, FLT, and GFP trait were co-located on the middle of long arm of chromosome 5A (**Table 4-3**).

Among the detected QTLs on chromosome 2D on , the three QTLs of HT, FLT and MAT was found with LOD score of 9.94, 8.86 and 6.89, respectively, explained 22.6 %, 12.2 % and 13.9 % of the total phenotypic variation, respectively. They shared the same flanked SSR markers *Xgwm484* -*Xwmc112* at 32.1 cM interval. The additive effect in

Table 4-2. Correlation coefficient (*r*) matrices for four traits measured in two F₂ populations

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

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

HT, FLT and MAT QTLs indicated that the Kitanokaori allele at these loci promote to heading earlier than the synthetic wheat allele. For GFP, one QTL with a LOD score 4.24 was found located at the same place with the other traits such as HT, FLT and MAT, explaining 10.51 % of the variation. Early grain filling period was conferred by the synthetic wheat allele on chromosome 2D.

On chromosome 5A, Three QTLs for HT, FLT and GFP each were detected at the same position on the middle long arm of chromosome 5A. The 5D QTLs explained 11-17% of the variations. The two QTLs for HT and FLT detected on chromosome 5A QTL region, the synthetic wheat Ldn/KU-2097 allele showed earlier day to heading and flowering time. The opposite effects of Kitanokaori alleles corresponded to shorter GFP trait found in the Kitanokaori//Ldn/KU-2097 population.

5.4. Confirmation of effects of the identified QTLs in the D genome of synthetic hexaploid wheat population.

To know effects of the identified QTLs for flowering-related traits that were identified from previous study on synthetic wheat (Nguyen et al. 2013) related to two populations being studied. By comparative linkage map, SSR makers located in each region were used. Data on each flowering-related trait were grouped based on the genotypes at their QTL regions of each F₂ individual in the two F₂ populations.

In the population between Norin61 and Ldn/PI476874, for FLT and MAT traits, there were no significant ($P < 0.05$) differences among genotypes at their 7D QTL (**Fig. 4-3**). Similar result, in the Kitanokaori//Ldn/KU-2097 population, no significant difference in the HT traits was observed among genotypes at their QTLs on chromosome 1D, 5D and 6D. However, the group F₂ individuals carrying the synthetic wheat alleles were slightly earlier flowering time than that of the elite wheat alleles in both population, except at the 6D QTL from the Kitanokaori//Ldn/KU-2097 (**Fig. 4-3**).

No significant differences were observed on genotypes at the QTL regions of each F₂

Table 4-3. A summary of QTLs for flowering-related traits that were identified in the two F₂ mapping populations

Traits	Chromosome	Map location	LOD score	LOD threshold	Contribution (%)	Additive effect
Norin 61// Ldn/PI476874						
HT	2D	<i>Ppd_D1- Xctg4491</i>	40.03	7.1	33.23	-6.26
FLT	2D	<i>Ppd_D1- Xctg4491</i>	36.25	5.8	21.36	-5.08
MAT	2D	<i>Ppd_D1- Xctg4491</i>	16.29	4.1	16.41	-3.22
GFP	2D	<i>Ppd_D1- Xctg4491</i>	7.17	3.8	15.84	2.16
MAT	5A	<i>Xgwm293- Xbarc197</i>	4.21	4.1	8.91	2.23
Kitanokaori// Ldn/KU-2097						
HT	2D	<i>Xwmc112 – Xgwm484</i>	9.94	3.5	22.56	-5.60
FLT	2D	<i>Xwmc112 – Xgwm484</i>	8.86	3.6	12.21	-3.86
MAT	2D	<i>Xwmc112 – Xgwm484</i>	6.89	4	13.94	-3.10
GFP	2D	<i>Xwmc112 – Xgwm484</i>	4.24	3.8	10.51	1.71
HT	5A	<i>Xgpw2136-Xcfa2163</i>	5.62	3.5	13.54	4.36
FLT	5A	<i>Xgpw2136-Xcfa2163</i>	5.25	3.6	17.59	4.66
GFP	5A	<i>Xgpw2136-Xcfa2163</i>	5.35	4	11.50	-1.67

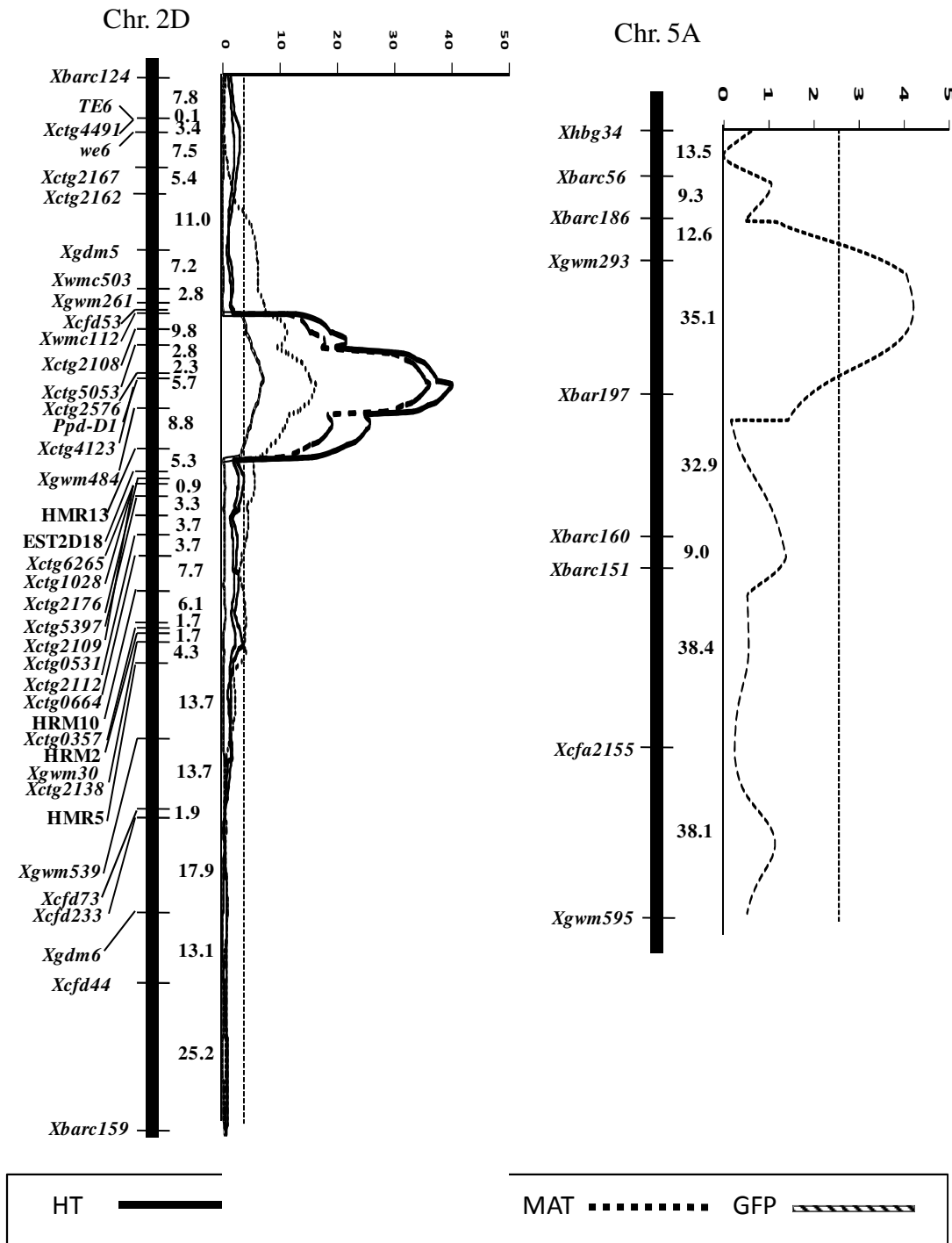


Figure 4-2a. I Positions of identified QTLs for four flowering-related traits in four mapping populations of synthetic hexaploid wheat. QTLs with LOD scores above the threshold are indicated, and genetic distances (in centiMorgans) are given to the right of each chromosome. Black arrowheads indicate the putative positions of centromeres. Norin 61//Ldn/PI476874.

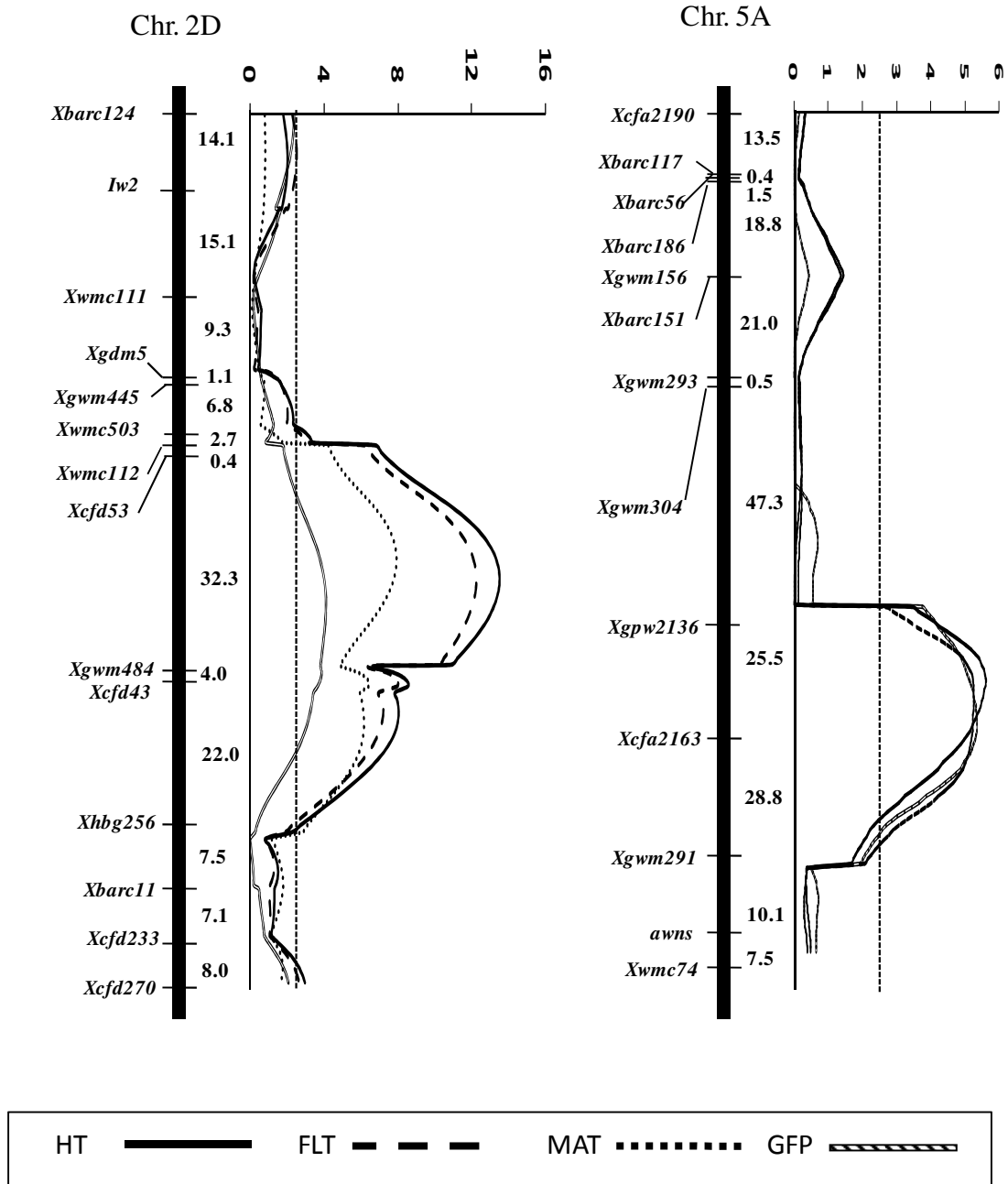


Figure 4-2b. Linkage maps and positions of identified QTLs for four flowering-related traits in four mapping populations of synthetic hexaploid wheat. QTLs with LOD scores above the threshold are indicated, and genetic distances (in centiMorgans) are given to the right of each chromosome. Black arrowheads indicate the putative positions of centromeres. Kitanokaori//Ldn/KU-2097.

individuals at the 7D QTL region from the Norin61//Ldn/PI476874 population and at 1D, 5D and 6D QTL regions from the Kitanokaori//Ldn/KU-2097 population. These results indicated that these QTLs related to flowering may be affected by the other genetic factors, which might causes expression inhibits the effects. Thus, the photoperiod responds *Ppd-D1* on chromosome 2D, which is known the major photoperiod response locus affect to flowering time of wheat, is used for determining the assumptions. Data from their QTL regions of each F₂ individual were grouped based on the genotypes of F₂ individual plants without elite wheat allele of the *Ppd-D1* in the two F₂ populations. In the Kitanokaori//Ldn/KU-2097 population, significant ($P < 0.05$) differences among groups of F₂ individuals at 1D, 5D and 6D QTLs were observed when the genotype of F₂ individuals at the *Ppd-D1* chromosomal region was homozygous for the synthetic wheat line KU-2097 allele, but not when that of F₂ individuals at the *Ppd-D1* locus was homozygous for Kitanokaori (**Fig.4-4**). In the Norin 61//Ldn/PI476874 population, no phenotypic ($P < 0.05$) difference caused by the genotype of 7D QTL controlling FLT and MAT was observed when the genotype F₂ individual at the *Ppd-D1* chromosomal region was either homozygous for synthetic wheat line (**Fig.4-4**).

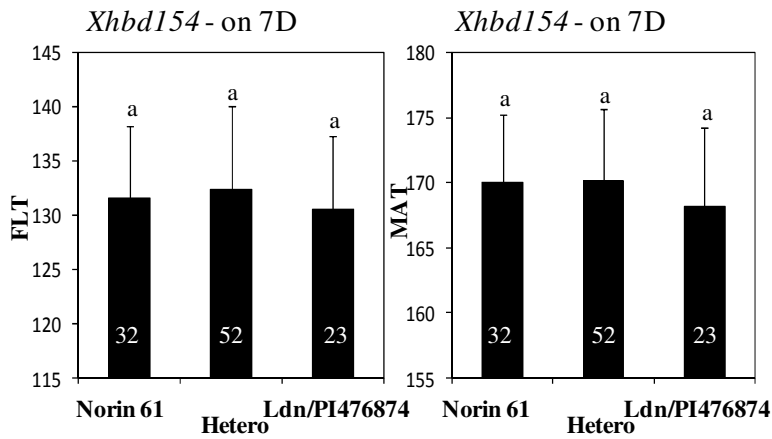
6. Discussion

Gene flow from wild to domesticated tetraploid wheat and from tetraploid wheat and *Ae. tauschii* to *T. aestivum* has been experimentally documented (Caldwell et al. 2004; Dvorak et al. 2006), but its impact on the evolution of the *T. aestivum* A, B, and D genomes is not clear. Recently, there have been many studies of important biological characteristics as genes for resistance to leaf rust, strip rust, stem rust and powdery mildew from relatives of wheat transferred to common wheat (Knot 1989; Lutz et al. 1995). Synthesis hexaploid wheat plays an important role in bridging to transfer agronomically important genes from tetraploid progenitor Langdon and the D-genome donor *Ae.tauschii*

to common wheat. Our studies have previously shown that the large variation in flowering traits among diploid wheat *Ae. tauschii* accessions has been observed and retained in the hexaploid synthetic wheat lines, which derived from *Ae. tauschii* accessions and Langdon durum wheat (Takumi et al. 2009b; Kajimura et al. 2011). Therefore, the *Ae. tauschii* accessions that must contain substantial genetic variation can be used for the development of early flowering cultivars of common wheat. In addition, Nguyen et al. (2013) found some QTLs from D-genome related early flowering traits on synthetic hexaploid wheat lines. Hence, these early flowering accessions of *Ae. tauschii* were selected for QTL analysis to identify beneficial loci from crosses between elite wheat cultivar and synthetic wheat line.

In present study, in the two F₂ mapping populations with twelve QTLs for flowering-related traits were identified (**Table 4-3; Fig. 4-2a, b**). Two chromosome regions on 2D contributed largely parts of the variation in HT, FLT and MAT. Although we failed to detect polymorphism between the Kitanokaori and Ldn/KU2097 by using two *Ppd-D1* primer specific sets, however, the 2D QTL region between flanking the SSR markers *Xwmc112-Xgwm484* identified in the present study appeared to be identical to *Ppd-D1* in common wheat (Hanocq et al. 2004; Xu et al. 2005; Lin et al. 2008; Wang et al. 2009). The *Ppd-D1* allele from two the elite wheat varieties caused the early-flowering phenotype in two F₂ populations of Norin61 and Kitanokaori. In the Norin61//Ldn/PI476874 population, Norin61 carrying allele of *Ppd-D1* contained a 2,089-bp deletion in the 5' upstream region, indicating a photoperiod-insensitive *Ppd-D1a* allele (Beales et al. 2007). Most of the Japanese early-flowering cultivars, including Norin61, carry the single allele for photoperiod insensitivity *Ppd-D1a* allele (Seki et al. 2011). Enhance, there is very little information about the Kitanokaori wheat *Ppd-D1* allele. Kitanokaori cultivar was produced by cross-breeding Japanese Horoshiri and Hungarian GK Szemes wheat varieties. Otherwise, in present study we fail to detect a

(A)



(B)

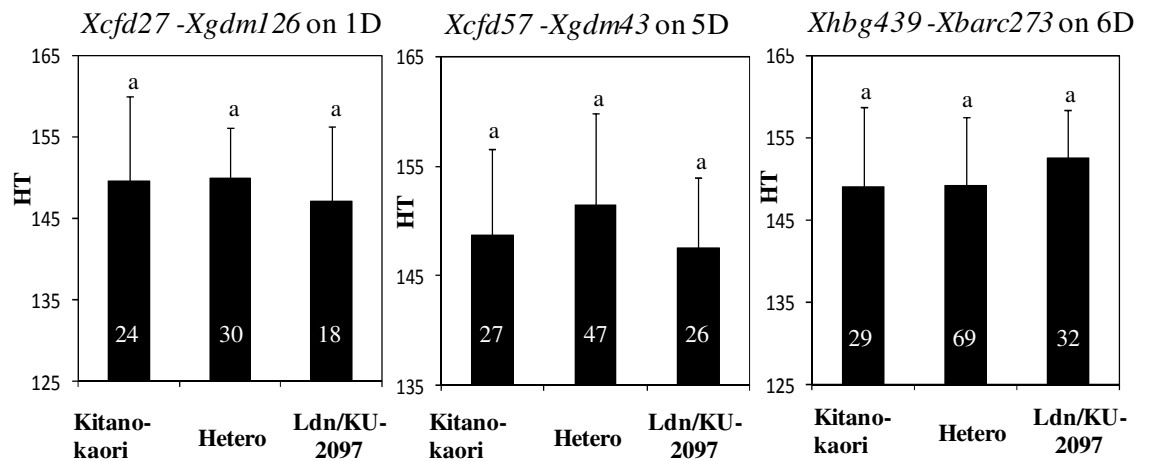


Figure 4-3. The genotype effects at each QTL on the observed variation in the flowering-related traits in the Norin 61/Ldn/Ldn/PI476874 (A) and Kitanokaori/Ldn/KU-2097 (B) populations. Markers that were used to deduce the genotype at a QTL are listed above each graph. The number of F_2 individuals with each genotype was indicated in the closed bars. Means \pm standard deviation with the same letter were not significantly different ($P > 0.05$) (Tukey-Kramer HSD test).

polymorphism between parental lines Kitanokaori and Ldn/KU-2097. Thus, further studies are necessary to conduct the effects of the Langdon, which might be causal no polymorphism in the F₂ population between Kitanokaori and Ldn/KU-2097. In addition, the photoperiod responds *Ppd-D1* allele of Kitanokaori cultivar might belong to different haplotypes of *Ppd-D1* allele from Norin 61. Recently the existence of at least six haplotypes of *Ppd-D1* has been reported (Guo et al. 2010), with apparent adaptive significance, suggesting many more opportunities for fine-tuning genotypes to environmental conditions.

The QTLs related to GFP trait were found in the chromosome 2D in the two F₂ populations. Negative correlations were observed among HT, FLT and MAT with GFP in the two F₂ mapping populations (**Table 4-2**). These results indicated that maturation time may be controlled by two independent genetic factors. One is heading time, one is duration for seed maturation. However, significant QTLs for GFP were co-located in the region of the other 2D QTLs of HT, FLT in the two F₂ mapping populations (**Fig. 4-2a,b**). Otherwise, the GFP trait is strongly affected by plant growth conditions. The plant with later HT, the shorter GFP was observed under higher temperature conditions during grain maturation (Kajimura et al. 2011). Moreover, QTLs affecting different traits fell near one another more frequently than would be expected by chance. This might suggested that either some QTL have pleiotropic effects of *Ppd-D1* locus or that different QTLs affecting these traits tend to be clustered together into closely linked groups in the two F₂ populations.

In the Kitanokaori//Ldn/KU-2097 population, QTLs for HT, FLT and GFP were co-located on the middle arm of chromosome 5A. In wheat, major genes for their vernalization requirement are the *Vrn-1* homoeologous loci, *Vrn-A1*, *Vrn-B1* and *Vrn-D1*. The *Vrn-1* and its allelic variants have been extensively studied. The dominant *Vrn-A1*

allele is associated with spring growth habit in durum wheat (*T. Turgidum* L. $2n = 4x = 28$, AABB), and hexaploid wheat (Yan et al. 2004a; Fu et al. 2005; Dubcovsky et al. 2006). The *Vrn-1* mapped to the middle long arms of chromosomes 5A, 5B and 5D, respectively (Snape et al. 1996). Using the linkage map (Somers et al. 2004), our region of 5D QTL seems to correspond to vernalization requirement *Vrn-A1*. The vernalization responds of *Vrn-A1* from Ldn contributed to segregation of early-flowering individuals in this F₂ population. In contrast, a QTL for MAT was found on the short arm of chromosome 5A in the Norin 61//Ldn/ PI476874 population. The QTL is, therefore, not correspond to *Vrn-1* locus. The synthetic wheat allele at this locus contributed to early MAT and it seems to be a novel locus associated with wheat flowering in synthetic wheat. In additional, molecular genetic data indicated that the A- and B-genome chromosomes between two species, the common wheat (*T. aestivum*) and tetraploid wheat (*T. turgidum*) are >99% identical (Huang at el. 2002). Thus, the region of 5A QTL in the Norin61//Ldn/PI476874 population should be required in further studies.

Two the synthetics wheat lines Ldn/PI476874 and Ldn/KU-2097 acted as early flowering parent lines in F₂ population by cross-combinations with other later flowering parent synthetic wheat lines in previous study. Several QTLs for early flowering-related traits were detected from their F₂ populations. The two synthetic wheat Ldn/PI476874 and Ldn/KU-2097 alleles at these loci were found to cause early day to flowering time (Nguyen et al. 2013). For the synthetics line Ldn/PI476874, two QTLs associated with early FLT and MAT traits were co-located on the short arm of chromosome 7D. In the other synthetic line Ldn/KU-2097, three QTLs associated with HT trait were separately detected on the chromosome 1D, 5D and 6D (Nguyen et al. 2013). Unfortunately, in the two current F₂ mapping populations, we did not find any QTLs effecting to flowering time from two synthetic wheat lines Ldn/PI476874 and Ldn/KU-2097 from previous study,

although the F₂ population from previous study and this study used the same synthetic wheat parents. It means that why was QTLs having a gene effect not detected in a current population. There could be several reasons that are mentioned as follows.

To further confirm the assumptions, differences in mean values for days to heading on three classes of F₂ individuals were grouped based on the genotypes at their QTL regions in the two F₂ populations. Because SSR markers were used in the F₂ previous map, no integrating SSR markers into the two F₂ current map. Thus, each genotype is represented by the two nearest SSR markers or a nearest SSR marker, which involved in regions of QTL from previous study. The data showed that the F₂ individuals carrying alleles from the synthetic wheat lines of their QTLs showed no significantly earlier attributes than those with the alleles from elite wheat in the two F₂ populations (**Fig. 4-3**). Therefore, synthetic wheat line Ldn/PI476874 and Norin 61 parents might carry the same genetic backgrounds at the 7D QTL regions as well as the same genetic background between Kitanokaori and synthetic wheat line KU-2097 at 1D, 5D and 6D QTL chromosome regions.

In addition, to study the effects of the *Ppd-D1* at 2D QTL, which might cause expression inhibits the gene effects. Data from 7D QTL regions from the Norin//Ldn/PI476874 and 1D, 5D and 6D QTL from the Kitanokaori//Ldn/KU2097 of each F₂ individual were grouped based on the genotypes of F₂ individual plants without elite wheat's *Ppd-D1* allele. **Fig. 4-4 (A)** shows no phenotypic difference caused by the genotype of F₂ individuals at 7D QTL. With the results discussed above, synthetic wheat line Ldn/PI476874 and Norin 61 parents might carry the same genetic backgrounds at their 7D QTL regions. The identification of QTLs linked with early flowering in synthetic wheat lines may be useful for in breeding for early flowering. Testing this hypothesis by crossing these QTLs into different genetic backgrounds and also combining QTLs for the same traits are needed

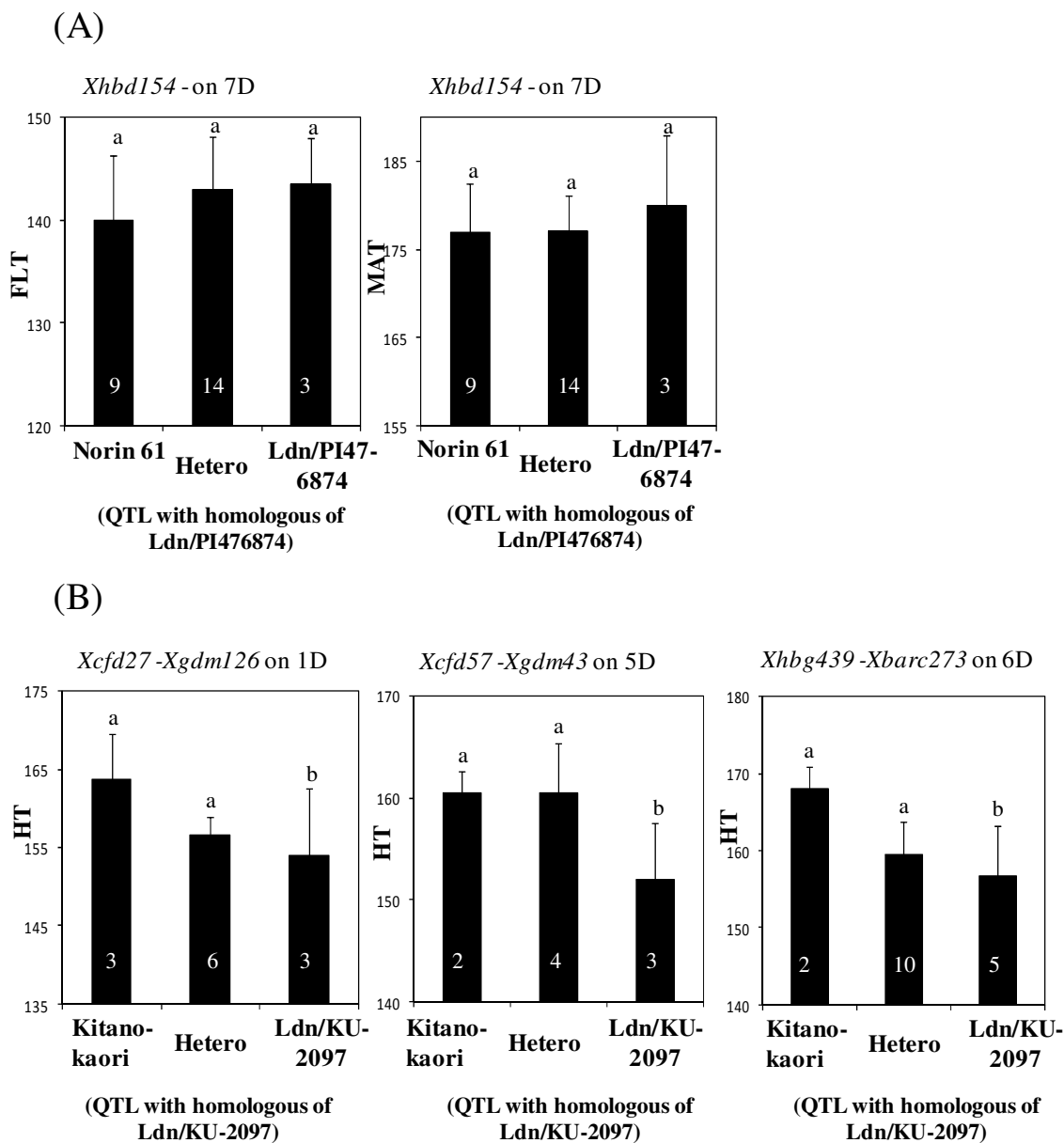


Figure 4-4. Effects of the identified 2D QTL on the flowering-related traits in the two F_2 populations, Norin 61//Ldn/Ldn/PI476874 (A) and Kitano-kaori//Ldn/KU-2097 (B) populations. The genotype at each QTL was deduced by linked markers. The number of F_2 individuals with each genotype was indicated in the closed bars. Means \pm standard deviation with the same letter were not significantly different ($P > 0.05$) (Tukey-Kramer HSD test).

In the Kitanokaori//Ldn/KU-2097 population, the group with F₂ individuals with the synthetic wheat line alleles showed also significantly earlier HT than those with the Kitanokaori variety alleles at 1D, 5D and 6D QTL regions (**Fig.4-4_B**). These results suggested that QTLs for HT might be strongly affected by the *Ppd-D1* locus, which cause predominant early flowering with high LOD score in both F₂ populations (**Table 4-3**). Therefore, putative QTLs for flowering-related traits should be found by using backcross population between elite wheat and synthetic wheat line under controlled growth conditions. The advanced backcross population is a way to identify beneficial QTL alleles from wild species when incorporated into an adapted genetic background (Tanksley and Nelson 1996).

The introgression of wild wheat and derived synthetic hexaploids into common cultivars will play a large role in the future. The incorporation of favorable alleles at QTL for flowering time into a wheat breeding program could result in better performing cultivars, which would be useful in many parts of the world suffering from particular environment. Experiments with wheat have led to the successful introduction of alleles from wild species into cultivated wheat. Some previous studies (Kerber 1987; Ma et al.1995; Mujeeb-Kazi et al.1996) have shown that numerous alleles useful for wheat breeding can be identified in natural populations of wild progenitors of common wheat. Our study has revealed that the putative QTLs regions, which found from previous study, were segregated in the F₂ of each population between elite wheat cultivars and synthetic wheat lines, although we failed to identify any significant QTL for flowering time in their regions. Together with the other QTLs found in synthetic wheat lines are, therefore, genetic resources for discovery of alleles useful for improvement of HT/FLT of elite cultivars.

Chapter V

Summary

Wheat is one of the first domesticated crops, and has contributed to human life as the basic staple food. Today, wheat is the most widely grown cereal in the commercial crops. The successful worldwide growing of common wheat in a diverse range of environments, is because of, at least in part, marked efforts of breeding for appropriate flowering time (FLT). Wheat cultivars show varied heading time (HD) and FLT in order to adapt to each regional environment (Snape et al. 2001). Wheat HT/FLT is controlled by three major genetic components, vernalization requirement, photoperiodic sensitivity and narrow-sense earliness (reviewed in Murai et al. 2005). The vernalization requirement and photoperiodic sensitivity are respectively regulated by major quantitative trait loci (QTLs) such as *Vrn-1* and *Ppd-1*. Nevertheless, the earliness *per se* genes are known to be located on various wheat chromosomes (Scarth and Law 1983; Kato and Yamagata 1988). However, increases in wheat yield and grain quality have not kept pace with consumer demand accompanied with extensive increase in human population. Both are strongly influenced by environmental conditions and pathogen attacks. Especially in Japan, rainy season is overlapped with wheat harvesting, resulting in pre-harvest sprouting, *Fusarium* damage, and reduction of grain quality. To restrict the impact of environmental conditions and pathogen attacks development of cultivars adaptive to a diverse range of environments is one of the major purposes of the wheat breeders.

Control of HT/FLT is a key trait in wheat breeding. Understanding the genetic control and indentifying new genetic loci responsible of the harvesting times of landraces and wild wheat relatives will offers potential for wheat improvement because they introduce new sources of genetic diversity (Matsuoka 2011).

Wheat landraces and wild wheat relatives provide abundant genetic variations in

many agricultural traits including FLT (Kato and Yokoyama 1992; Iwaki et al. 2001; Terasawa et al. 2009; Dvorak et al. 1998; Matsuoka et al. 2008), and supply useful traits for modern wheat breeding (Feldman and Sears 1981; Kerber 1987; Ma et al. 1995; Mujeeb-Kazi et al. 1996). Kato and Yokoyama (1992) and Takumi (2009) found that many wheat landraces in Nepal, Bhutan and Tibet show wide variations in flowering related traits. Natural variation in FLT shows significant longitudinal and latitudinal clines in *Ae. tauschii* (Matsuoka et al. 2008; Takumi et al. 2009a). The *Ppd-1* and *Vrn-1* genotypes of the A and B genomes in Ldn largely affect expression of flowering-related traits in synthetic hexaploid wheat (Fujiwara et al. 2010). However, hexaploid wheat synthetic constitutes an effective genetic bridge for transferring agronomically important genes from *Ae. tauschii* to common wheat (Takumi et al. 2009b; Kajimura et al. 2011).

To identify causal loci for the early flowering phenotype in the Nepalese landraces, first, two F₂ populations that were obtained from F₁ plants crossed between Shiroganekomugi (SGK), a Japanese early flowering cultivar and the two Nepalese landraces, KU-4770 and KU-180, were used for QTL analysis. The QTL analysis showed that the *Ppd-D1* allele from SGK and the *Ppd-B1* alleles from the two Nepalese varieties caused the early-flowering phenotype in the two F₂ populations. Real time PCR analysis estimating the *Ppd-B1* copy number revealed that the two Nepalese varieties included two intact *Ppd-B1* copies, putatively resulting in photoperiod insensitivity and an early flowering phenotype. The SGK *Ppd-D1* allele contained a 2-kb deletion in the 5'upstream region, indicating a photoperiod insensitive *Ppd-D1a* allele. Wheat landraces are genetic resources for discovery of alleles useful for improving wheat heading or flowering times.

Next, four F₂ mapping populations produced from the following cross-combinations between early and late flowering synthetic wheat lines (Takumi et al. 2009; Kajimira et al. 2011) were used for QTL analysis for flowering related traits. In total, 10 QTLs

located on six D-genome chromosomes (all except 4D) were detected for the analyzed traits. The QTL on 1DL controlling heading time appeared to correspond to a flowering time QTL, previously considered to be an ortholog of *Eps-Aml* which is related to the narrow-sense earliness in einkorn wheat. The 5D, 6D and 7D QTLs seem to be a novel loci associated with wheat flowering and maturation including a QTL controlling the grain filling period on chromosome 3D, while the 2DS QTL appears to be an allelic variant of the photoperiod response locus *Ppd-D1*.

Finally, two F₂ mapping populations, respectively crossed between two Japanese wheat varieties Norin 61 and Kitanokaori with two synthetic wheat lines Ldn/PI476874 and Ldn/KU-2097, were used for QTL analysis. In total, 12 QTLs for flowering related traits were detected, of which 3 were for HT, 3 for FLT, 3 for MAT and 3 for GFP. *Ppd-D1* on chromosome 2D from the two Japanese varieties was causal for early-flowering phenotype of HT, FLT and MAT and might affect to the expression of other QTLs controlling flowering. Further, two different QTL regions were found on chromosome 5A. The synthetic wheat line derived alleles on the 5A QTLs were associated with early flowering phenotype in the two F₂ populations. The 5AL QTL region for FLT in the Kitanokaori//Ldn/KU-2097 population appeared to be corresponding to the *Vrn-A1* locus, which is related to the vernalization requirement in tetraploid and hexaploid wheat (Chu et al. 2011). Another 5AS QTL in the F₂ population crosses between Norin 61 and Ldn/PI476874 seemed to be a novel locus regulating MAT. Combined with the previous research using the synthetic wheat population, the identified QTLs in the present study are available for improving modern wheat cultivars.

In the future, production of the advanced backcrossed lines having only desirable alleles under the genetic background of hexaploid wheat cultivars will allow to characterize more precisely the QTL alleles and to elucidate the molecular nature of QTLs responsible for agronomically important traits.

Literature Cited

- Beales, J., A. Turner, S. Griffiths, J. W. Snape and D. A. Laurie (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.
- Bäurle, I. and C. Dean (2006) The timing of developmental transitions in plants. *Cell.* 125: 655–664.
- Belay, G., T. Tesema, E. Bechere and T. Mitiku (1995) Natural and human selection for purple-grain tetraploid wheats in the Ethiopian highlands. *Genet. Resour. Crop. Evol.* 42: 387-391.
- Borojevic, K. and K. Borojevic (2005) Historic role of the wheat variety Akakomugi in southern and central European wheat breeding programs. *Breed. Sci.* 55: 253-256.
- Bullrich, L., M. L. Appendino and G. Tranquilli (2002) Mapping of a thermo-sensitive earliness gene on *Triticum monococcum* chromosome 1A^m. *Theor Appl Genet.* 105:585–593
- Bushuk, W. (1998b) Wheat breeding for end-product use. *Euphytica* 100: 137–45
- Chu, C. G., C. T. Tan, G. T. Yu, S. Zhong, S. S. Xu and L. Yan (2011) A Novel Retrotransposon Inserted in the Dominant *Vrn-B1* Allele Confers Spring Growth Habit in Tetraploid Wheat (*Triticum turgidum* L.). *G3*. 1: 637–645.
- Cockram, J., H. Jones, F. J. Leigh, D. O'Sullivan, W. Powell, D. A. Laurie and A. J. Greenland (2007) Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. *J Exp Bot.* 58:1231–1244
- Dubcovsky, J., A. Loukoianov, D. Fu, M. Valarik and A. Sanchez (2006) Effect of photoperiod on the regulation of wheat vernalization genes *VRN1* and *VRN2* *Plant Mol. Biol.* 60:469–480.
- Díaz, A., M. Zikhali, A. S. Turner, P. Isaac and D. A. Laurie (2012) Copy number variation affecting the *Photoperiod-B1* and *Vernalization-A1* genes is associated with altered flowering time in wheat (*Triticum aestivum*). *PLoS ONE* 7: e33234.
- Dvorak, J., L. C. Luo, Z. L. Yang and H. B. Zhang (1998) The structure of the *Aegilops tauschii* gene pool and the evolution of hexaploid wheat. *Theor Appl Genet.* 97:657–670
- Dvorak, J., D. Eduard, R. Alina, R. Karin, R. Deal and M. C. Luo (2006) Molecular

- Characterization of a Diagnostic DNA Marker for Domesticated Tetraploid Wheat Provides Evidence for Gene Flow from Wild Tetraploid Wheat to Hexaploid Wheat. *Mol. Biol. Evol.* 23:1386-1396
- Edwards, K.J., A.L.Reid, J.A.Coghill, S.T.Berry and G.L.A.Barker (2009) Multiplex single nucleotide polymorphism (SNP)-based genotyping in allohexaploid wheat using padlock probes. *Plant Bio. Jour.* 7:375-390.
- Egawa, C., F.Kobayashi, M.Ishibashi, T.Nakamura, C.Nakamura and S.Takumi (2006) Differential regulation of transcript accumulation and alternative splicing of a *DREB2* homolog under abiotic stress conditions in common wheat. *Genes Genet Syst.* 81:77-91
- Faricelli, M., Valárik and J.Dubcovsky (2010) Control of flowering time and spike development in cereals: the earliness *per se Eps-1* region in wheat, rice, and *Brachypodium*. *Funct Integr Genomics.* 10:293-306
- Faure, S., A.S.Turner, D.Gruszka, V.Christodoulou, S.J.Davis, M.Korff and D.A.Laurie (2012) Mutation at the circadian clock gene *EARLY MATURITY 8* adapts domesticated barley (*Hordeum vulgare*) to short growing seasons. *Proc Natl Acad Sci USA.* 109:8328-8333
- Feldman, M. and E.R.Sears (1981) The wild gene resources of wheat. *Sci. Am.* 244: 102-112.
- Gotoh, T. (1979) Genetic studies on growth habit of some important spring wheat cultivars in Japan, with special reference to the identification of the spring genes involved. *Jpn. J. Breed.* 29: 133-145.
- Feldman, M. (2001) Origin of cultivated wheat. In: Bonjean AP, Angus WJ (eds) *The World Wheat Book: A History of Wheat Breeding*, Lavoisier Publishing, rue Lavoisier, Paris, pp 3–53
- Feldman, M. and E.Millet (1995) Methodologies of identification, allocation and transfer of quantitative genes from wild emmer into cultivated wheat. *Proc VIII Int Wheat Genet Symp, Beijing* 1:19-27.
- Fu, D., P.Szücs, L.Yan, M.Helguera and J.S.Skinner (2005) Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. *Mol. Genet. Geno.* 273:54–65.
- Fu, D., M.Dunbar and J.Dubcovsky (2007) Wheat *VIN3*-like PHD finger genes are up-regulated by vernalization. *Mol. Genet. Geno.* 277:301-313

- Fujiwara, Y., S. Shimada, S. Takumi and K. Murai (2010) Differential effects of *Aegilops tauschii* genotypes on maturing-time in synthetic hexaploid wheats. *Breed Sci.* 60:286–292
- Gororo, N.N., R.G. Flood, R.F. Eastwood and H.A. Eagles (2001) Photoperiod and vernalization responses in *Triticum turgidum* x *T. tauschii* synthetic hexaploid wheats. *Ann Bot.* 88:947-952
- Guo, Z., Y. Song, R. Zhou, Z. Ren and J. Jia (2010) Discovery, evaluation and distribution of haplotypes of the wheat *Ppd-D1* gene. *New Phytol.* 185: 841-851.
- Gupta, P.K., H.S. Balyan, K.J. Edwards, P. Isaac, V. Kurzun, M. Röder and P. Leroy (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. *Theor. Appl. Genet.* 105: 413-422.
- Hanocq, E., M. Niarquin, E. Heumez, M. Rousset and J. LeGouis (2004) Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population. *Theor. Appl. Genet.* 110: 106-115.
- Hoogendoorn, J. (1985) A reciprocal F₁ monosomic analysis of the genetic control of time of ear emergence, number of leaves and number of spikelets in wheat (*Triticum aestivum* L.). *Euphytica* 34: 545-558.
- Hori, K., T. Kobayashi, A. Shimizu, K. Sato and S. Kawasaki (2003) Efficient construction of high-density linkage map and its application to QTL analysis in barley. *Theor. Appl. Genet.* 107: 806-813.
- Huang, S., A. Sirikhachornkit, X.J. Su, J. Faris and B. Gill (2002) Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum/Aegilops* complex and the evolutionary history of polyploid wheat. *Proc. Natl. Acad. Sci.* 99:8133–8138.
- Huang, L., Q. Wang, L.Q. Zhang, Z.W. Yuan, J.R. Wang, H.G. Zhang, Y.L. Zheng and D.C. Liu (2012) Haplotype variations of gene *Ppd-D1* in *Aegilops tauschii* and their implications on wheat origin. *Genet Resources Crop Evol.* 59:1027-1032
- Keller, L., J.E. Schmid and E.R. Keller (1991) Are cereal landraces a source for breeding? *Landwirtschaft Schweiz.* 4: 197–202.
- Kim, D.H., R.D. Mark, S. Sung and M.A. Richard (2009) Vernalization: Winter and the Timing of Flowering. *Plants Cell Dev. Biol.* 25:277–99
- Iehisa, J.C.M., A. Shimizu, K. Sato, S. Nasuda, S. Takumi (2012) Discovery of high-confidence SNPs from large-scale *de novo* analysis of leaf transcripts of

- Aegilops tauschii*, a wild wheat progenitor. DNA Res. 19: 487-497
- Iwaki,K., K.Nakagawa, H.Kuno and K.Kato (2000) Ecogeographical differentiation in east Asian wheat, revealed from the geographical variation of growth habit and *Vrn* genotype. Euphytica 111: 137-143.
- Iwaki,K., S.Haruna, T.Niwa and K.Kato (2001) Adaptation and ecological differentiation in wheat with special reference to geographical variation of growth habit and *Vrn* genotype. Plant Breed. 120: 107–114.
- Kajimura,T., K.Murai and S.Takumi (2011) Distinct genetic regulation of flowering time and grain-filling period based on empirical study of D genome diversity in synthetic hexaploid wheat lines. Breed. Sci. 61: 130-141.
- Katherine,S.C., J.Dvorak, S.L.Evans, A.Eduard, M.C.Luo, W.Petra and P.Wayne (2004) Sequence Polymorphism in Polyploid Wheat and Their D-Genome Diploid Ancestor. Genetics 167:941-947
- Kato,K. and H.Yokoyama (1992) Geographical variation in heading characters among wheat landraces, *Triticum aestivum* L., and its implication for their adaptability. Theor. Appl. Genet. 84: 259-265.
- Kato,H., S.Taketa, T.Ban, N.Iriki and K.Murai (2001) The influence of a spring habit gene, *Vrn-D1*, on heading time in wheat. Plant Breed. 120: 115-120.
- Kerber,E.R. (1987) Resistance to leaf rust in hexaploid wheat: *Lr32*, a third gene derived from *Triticum tauschii*. Crop Sci. 27:204–206
- Kobayashi,F., S.Takumi and H.Handa (2010) Identification of quantitative trait loci for ABA responsiveness at the seedling stage associated with ABA-regulated gene expression in common wheat. Theor. Appl. Genet. 121: 629-641.
- Koornneef, M., A.B.Carlos and D.Vreugdenhil (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. Annual Review of Plant Biology. 55:141-172
- Kosambi,D.D. (1944) The estimation of map distance from recombination values. Annals of Eugenics 12: 172-175.
- Koyama,K., H.Hatano, J.Nakamura and S.Takumi (2012) Characterization of three *VERNALIZATION INSENSITIVE3-like (VIL)* homologs in wild wheat, *Aegilops tauschii* Coss. Hereditas 149:62-71
- Knot,D.R. (1989) The wheat rusts: breeding for resistance. Theo. App. Gen.12
- Lander,E.S., P.Green, J.Abrahamson, A.Barlow, M.J.Daly, S.E.Lincoln and L.Newburg (1987) An interactive computer package for constructing primary genetic linkage

- maps of experimental and natural populations. *Genomics* 1: 174-181.
- Law,C.N., J.Sutka and A.J.Worland (1978) A genetic study of day length response in wheat. *Heredity* 41: 185-191.
- Law,C.N. (1987) The genetic control of day-length response in wheat. *In: Atherton,J.G. (ed.) Manipulation of flowering, Proc. 45th Nottingham Easter School in Agricultural Science, Butterworths, London, pp.225-240.*
- Law,C.N. and A.J.Worland (1997) Genetic analysis of some flowering time and adaptive traits in wheat. *New Phytol* 137:19-28
- Lin,F., S.L.Xue, D.G.Tian, C.J.Li, Y.Cao, Z.Z.Zhang, C.Q.Zhang and Z.Q.Ma (2008) Mapping chromosomal regions affecting flowering time in a spring wheat RIL population. *Euphytica* 164: 769-777.
- Lutz,J., S.L.K.Hsam, E.Limpert and F.J.Zeller (1995) Chromosomal location of powdery mildew resistance genes in *Triticum aestivum* L. (common wheat). 2. Genes *Pm2* and *Pm19* from *Aegilops squarrosa* L. *Heredity* 74:152–156
- Ma,H., P.Singh and A.Mujeeb-Kazi (1995) Resistance to stripe rust in *Triticum turgidum*, *T. tauschii* and their synthetic hexaploids. *Euphytica* 82:117–124.
- Maccaferri,M., P.Mantovani, R.Tuberosa, E.DeAmbrogio, S.Giuliani, A.Demontis, A.Massi and M.C.Sanguineti (2008) A major QTL for durable leaf rust resistance widely exploited in durum wheat breeding programs maps on the distal region of chromosome arm 7BL. *Theo. App. Gen.*117:1225-1240.
- Matsuoka,Y. and S.Nasuda (2004) Durum wheat as a candidate for the unknown female progenitor of bread wheat: an empirical study with a highly fertile F₁ hybrid with *Aegilops tauschii* Coss. *Theor Appl Genet.* 109:1710–1717
- Matsuoka,Y., S.Takumi and T.Kawahara (2008) Flowering time diversification and dispersal in entral Eurasian wild wheat *Aegilops tauschii* Coss.: genealogical and ecological framework. *PLoS ONE* 3:e3138
- Matsuoka,Y. (2011) Evolution of polyploid *Triticum* wheats under cultivation: the role of domestication, natural hybridization and allopolyploid speciation in their diversification. *Plant Cell Physiol* 52:750–764
- Mayer,K.F.X., M.Martis, P.E.Hedley, H.Simková, H.Liu, J.A.Morris, B.Steuernagel, S.Taudien, S.Roessner, H.Gundlach, M.Kubaláková, P.Suchánková, F.Murat, M.Felder, T.Nussbaumer, A.Graner, J.Salse, T.Endo, H.Sakai, T.Tanaka, T.Itoh, K.Sato, M.Platzer, T.Matsumoto, U.Scholz, J.Dolezel, R.Waugh and N.Stein (2011)

- Unlocking the barley genome by chromosomal and comparative genomics. *Plant Cell* 23:1249–1263
- Mizuno,N., M.Yamasaki, Y.Matsuoka, T.Kawahara and S.Takumi (2010a) 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
- Mizuno,N., N.Hosogi, P.Park and S.Takumi (2010b) Hypersensitive response-like reaction is associated with hybrid necrosis in interspecific crosses between tetraploid wheat and *Aegilops tauschii* Coss. *PLoS ONE* 5:e11326
- Miura,H. and A.J.Worland (1994) Genetic control of vernalization, day-length response, and earliness *per se* by homeologous group-3 chromosomes in wheat. *Plant Breed.* 113:160-169.
- Mujeeb-Kazi,A., V.Rosas and S.Roldan (1996) Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. non L.) in synthetic hexaploid wheats (*T. turgidum* L. s.lat. x *T. tauschii*; 2n=6x=42, AABBDD) and its potential utilization for wheat improvement. *Genet Resources Crop Evol.* 43:129–134
- Murai,K., C.Ikari and N.Shitsukawa (2005) Pathways that promote the floral transition in wheat. *In: Tsunewaki,K. (ed.) Frontiers of Wheat Bioscience, the 100th Memorial Issue of Wheat Information Service, Kihara Memorial Foundation for the Advancement of Life Sciences, Yokohama, Japan, pp.119-128.*
- Nguyen,T.A., J.C.M.Iehisa, T.Kajimura, K.Murai and S.Takumi (2013) Identification of quantitative trait loci for flowering-related traits in the D genome of synthetic hexaploid wheat lines. *Euphytica* (in press)
- Nishida,H., T.Yoshida, K.Kawakami, M.Fujita, B.Long, Y.Akashi, D.A.Laurie and K.Kato (2013) Structural variation in the 5' upstream region of photoperiod-insensitive alleles *Ppd-A1a* and *Ppd-B1a* identified in hexaploid wheat (*Triticum aestivum* L.), and their effect on heading time. *Mol. Breed.* 31: 27-37.
- Okamoto,Y., T.Kajimura, T.M.Ikeda and S.Takumi (2012) Evidence from principal component analysis for improvement of grain shape- and spikelet morphology-related traits after hexaploid wheat speciation. *Genes Genet. Syst.* 87: 299–310.

- Pestsova,E., M.W.Ganal and M.S.Röder (2000) Isolation and mapping of microsatellite markers specific for the *D*-genome of bread wheat. *Genome* 43: 689-697.
- Röder,M., V.Korzun, K.Vendehake, J.Plaschke, M.H.Tixier, P.Leroy and M.Ganal (1998) A microsatellite map of wheat. *Genetics* 149: 2007-2023.
- Saleem, M. (2003) Response of durum and bread wheat genotypes to drought stress: Biomass and yield components. *Asian journal of plant science* 2:290-293.
- Scarth,R. and C.N.Law (1983) The location of photoperiod gene *Ppd2*, and an additional genetic factor for ear emergence. *Heredity* 51: 607-619.
- Seki,M., M.Chono, H.Matsunaka, M.Fujita, S.Oda, K.Kubo, C.Kiribuchi-Otobe, H.Kojima, H.Nishida and K.Kato (2011) Distribution of photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a* and their effect on heading time in Japanese wheat cultivars. *Breed. Sci.* 61: 405-412.
- Shitsukawa,N., C.Tahira, K.I.Kassai, C.Hirabayashi, T.Shimizu, S.Takumi, K.Mochida, K.Kwaura, Y.Ogihara and K. Murai (2007) Genetic and epigenetic alteration among three homoeologous genes of a class E MADS box gene in hexaploid wheat. *Plant Cell* 19:1723-1737.
- Sinha,S.K., P.K.Aggarwal, G.S.Chaturvedi, A.K.Singh and K.Kailasnathan (1986) Performance of wheat and triticale cultivars in a variable soil-water environment. 1. Grain yield stability. *Field Crops Research* 13:289-299.
- Snape,J.W., S.A.Quarrie and D.A.Laurie (1996) Comparative mapping and its use for the genetic analysis of agronomic characters in wheat. *Euphytica* 89:27-31
- Snape,J.W., K.Butterworth, E.Whitechurch and A.J.Worland (2001) Waiting for fine times: genetics of flowering time in wheat. *Euphytica* 119: 185-190.
- Somers,D.J., P.Isaac and K.Edwards (2004) A high density microsatellite consensus map for wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109: 1105-1114.
- Song,Q.J., J.R.Shi, S.Singh, E.W.Fickus, J.M.Costa, J.Lewis, B.S.Gill, R.Ward and P.B.Cregan (2005) Development and mapping of microsatellite (SSR) markers in wheat. *Theor. Appl. Genet.* 110:550–560.
- Takenaka,S. and T.Kawahara (2012) Evolution and dispersal of emmer wheat (*Triticum* sp.) from novel haplotypes of *Ppd-1* (photoperiod response) genes and their surrounding DNA sequences. *Theor. Appl. Genet.* 125:999-1014.
- Takumi,S. (2009) Segregation analysis of heading time and its related traits in wheat F₂ populations between two Nepal landraces and a Japanese cultivar

- Shiroganekomugi. *Wheat Inf. Serv.* 107: 5-8.
- Takumi,S., E.Nishioka, H.Morihiro, T.Kawahara and Y.Matsuoka (2009a) Natural variation of morphological traits in wild wheat progenitor *Aegilops tauschii* Coss. *Breed Sci.* 59:579-588
- Takumi,S., Y.Naka, H.Morihiro and Y.Matsuoka (2009b) Expression of morphological and flowering time variation through allopolyploidization: an empirical study with 27 wheat synthetics and their parental *Aegilops tauschii* accessions. *Plant Breed.* 128:585-590
- Tanio,M. and K.Kato (2007) Development of near-isogenic lines for photoperiod-insensitive genes, *Ppd-B1* and *Ppd-D1*, carried by the Japanese wheat cultivars and their effects on apical development. *Breed. Sci.* 57: 65-72.
- Tanksley,S.D. and J.C.Nelson (1996) Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theo. App. Gen.* 92:191-203
- Terasawa,Y., T.Kawahara, T.Sasakuma and T.Sasanuma (2009) Evaluation of the genetic diversity of an Afghan wheat collection based on morphological variation, HMW glutenin subunit polymorphisms, and AFLP. *Breed. Sci.* 59: 361-371.
- Tesemma,T., S.Tsegaye, G.Belay, E.Bechere and D.Mitiku (1998) Stability of performance of tetraploid wheat landraces in the Ethiopian highland. *Euphytica* 102:301–308.
- Torada,A., M.Koike, K.Mochida and Y.Ogihara (2006) SSR-based linkage map with new markers using an intraspecific population of common wheat. *Theor. Appl. Genet.* 112: 1042-1051.
- Turner,A., J.Beales, S.Faure, R.P.Dunford and D.A.Laurie (2005) The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. *Science* 310:1031–34
- Tsunewaki,K. (1966) Comparative gene analysis of common wheat and its ancestral species. II. Waxiness, growth habit and awnedness. *J Jpn Bot.* 19:175–229
- Uauy,C., J.C.Brevis and J.Dubcovsky (2006) The high grain protein content gene *Gpc-B1* accelerates senescence and has pleiotropic effects on protein content in wheat. *J Exp Bot.*57:2785–2794
- Wang,S., B.Carver and L.Yan (2009) Genetic loci in the photoperiod pathway interactively modulate reproductive development of winter wheat. *Theor Appl Genet.*

- Wang,S., C.J.Basten and Z.B.Zeng (2011) Windows QTL cartographer 2.5. Department of Statics, North Carolina State University, Raleigh, NC. <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>
- Welsh,J.R., D.L.Keim, B.Pirasteh and R.D.Richards (1973) Genetic control of photoperiod response in wheat. *In: Sears,E.R. and L.E.S.Sears (eds.) Proc. 4th Int. Wheat Genet. Symp. University of Missouri, Columbia, USA, pp.879-884.*
- Worland,A.J., A.Börner, V.Korzun, W.M.Li, S.Petrovic and E.J.Sayers (1998) The influence of photoperiod genes to the adaptability of European winter wheats. *Euphytica* 100: 385-394.
- Xiang,Z.G, L.Q.Zhang, S.Z.Ning, Y.L.Zheng and D.C.Liu (2008) Evaluation of *Aegilops tauschii* for heading date and its gene location in a re-synthesized hexaploid wheat. *Agric Sci China.* 8:1-7
- Xu,X., G.Bai, B.F.Carver and G.E.Shaner (2005) A QTL for early heading in wheat cultivar Suwon 92. *Euphytica* 146:233-237
- Yoshida,T., H.Nishida, J.Zhu, R.Nitcher, A.Distelfeld, Y.Akashi, K.Kato and J.Dubcovsky (2010) *Vrn-D4* is a vernalization gene located on the centromeric region of chromosome 5D in hexaploid wheat. *Theor Appl Genet.* 120:543-552
- Yan,L., A.Loukoianov, G.Tranquilli, M.Helguera, T.Fahima and J.Dubcovsky (2003) Positional cloning of the wheat vernalization gene *VRN1*.*In: Proceedings of the National Academy of Sciences* 100:6263-6268.
- Yan,L., M.Helguera, K.Kato, S.Fukuyama, J.Sherman et al., (2004) A Allelic variation at the *VRN-1* promoter region in polyploid wheat.*Theor. Appl. Genet.* 109:1677–1686.

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