GENETIC LOCI FOR VERNALIZATION REQUIREMENT DURATION IN WINTER WHEAT

By

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CHAPTER I

ABSTRACT

Wheat is traditionally categorized into two classes, winter wheat and spring wheat. Winter wheat requires a period of low temperature to accelerate the transition from vegetative to reproductive development, a phenomenon known as vernalization, whereas spring wheat has no vernalization requirement for flowering. The three vernalization genes, *VRN1*, *VRN2* and *VRN3*, have been cloned, based on variation in vernalization requirement between spring and winter types of wheat and barley. Winter wheat cultivars have various durations of vernalization requirements that can be manipulated to regulate the developmental process for different purposes in wheat production, but little is known about how this important trait is genetically controlled. In this study, two diploid winter wheat accessions, PI362553 and G3116 were found to require 2 weeks and 5 weeks of low temperature, respectively, to attain their vernalization saturation points. An F_2 population was developed from a cross between these two accessions, and the population was treated with 2 weeks of low temperature in order to observe the most sufficient segregation in vernalization requirement duration. A total of 114 markers including 104 SSR markers transferred from hexaploid wheat and 10 PCR markers developed for 6 known flowering time genes and 4 RFLP loci were mapped in 94 lines of this population. A major QTL was located on the long arm of chromosome $7A^m$ in a 21.8 cM region between two SSR markers *Xwmc96* and *Xbarc172*, explaining 22.8% of phenotypic

variation. This QTL, designated *QVrd.osu.7A*^{m}, was not associated with orthologues of either*TmVRN3* or *TmVRT2*, two known flowering time genes that were mapped on the same chromosome. Therefore, *QVrd.osu.7A*^{m} locus should contain new gene(s) responsible for the vernalization requirement duration in diploid winter wheat. *TmVRN2* but not other five flowering time genes (*TmVRN1, TmVRN3, TmPPD1, TmGI,* and *TmVRT2*) had a significant effect (P<0.05) on heading date in the vernalized population by statistical analysis, although a minor QTL associated with the *TmVRN2* locus on chromosome 5A^{m} did not reach the LOD value of 2.5 for a common threshold. Based on these findings, we conclude that vernalization requirement duration in the genetic background of the diploid wheat applied in this study is regulated by two QTLs, explaining part of the total phenotypic variation.

CHAPTER II

INTRODUCTION

Winter wheat for dual purposes is a major management system in the southern Great Plains

Wheat is one of the grain crops extensively grown worldwide, due to its adaptation to a wide range of environmental conditions. This adaptation is partly conferred by its genetic diversity in growth habit, which classified wheat into winter and spring types. Winter wheat requires a period of exposure to low temperature to accelerate the transition from vegetative to reproductive development for flowering, a phenomenon known as vernalization. For spring wheat, however, no vernalization is required to induce flowering (Dubcovsky et al. 1998; Law 1966; Law and Wolfe 1966; Pugsley 1971, 1972).

Winter wheat is a major type in the world; for example, it is planted on approximately 43.5 million acres each year in the United States. This amount represents 75% of the total wheat grown in the United States and the grains from this area of wheat account for approximately 11% of the world supply and nearly 35% of world exports. In order to maintain the United States' role as a major grower and exporter of wheat to meet increasing population, it is important to understand the genetic basis and molecular mechanisms of winter wheat growth and development.

Oklahoma is one of the biggest wheat states in the USA, and wheat grains are one of the most important economic sources in this state. Almost all of the wheat in Oklahoma is of winter type. Breeding winter wheat in the southern Great Plains is the

foundation of many agricultural enterprises. Particularly important is that the Oklahoma wheat crop is managed as grain only, grazing only, or a dual purpose (grain plus grazing) crop (Carver et al. 2001). Almost two-third of the wheat planted in the fall was intended for dual-purpose based on the survey data for the crop years of 1996 and 2000 (Edwards et al. 2007). The dual purpose wheat is usually planted in early September. Then, the cattle will be released into the wheat field for grazing from late November until development of the first hollow stem, usually occurring in late February or early March. At this stage, the cattle will be removed from the field, and the wheat will continue to produce a grain crop for harvest in June (Edwards et al. 2007). The dual-purpose wheat provides high quality forage when other forage resources are low in quantity and quality in the winter season. Therefore, the use of wheat pasture for feed constitutes an essential role in the U.S. beef industry (Khalil et al. 2002). An overarching goal of the dual purpose wheat improvement program is to produce more forage biomass by extending the vegetative phase but with minimal delay in flowering time.

Genetic factors for the transition timing from vegetative to reproductive development of winter wheat

The life cycle of wheat from sowing to maturity is traditionally divided into several critical physiological and morphological stages, including seedling emergence, tillering, stem elongation or jointing, heading, flowering, and maturity (Gonzalez et al. 2002; Hay and Ellis 1998). When sown in fall, winter wheat should complete the transition from vegetative to reproductive development with the induction by low temperature or relatively short days during the winter season, so that the plants start to grow reproductive organs when favorable temperature and light conditions are met in the next

spring season. A later developmental transition time is desirable when wheat is used as a winter forage resource in the dual-purpose system (Redmon et al. 1996). Responses of plants to vernalization and photoperiod are believed to be the two most important mechanisms determining the developmental transition time in winter wheat.

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Winter wheat varieties are reported to have various vernalization requirement durations to attain a vernalization saturation point, from which further exposure to low temperature will not result in any more acceleration of flowering time or reduction of final leaf number (Berry et al. 1980; Wang et al. 1995a; Wang et al. 1995b). Based on the length of exposure to low temperature at which the plants attain a vernalization saturation point, winter wheat cultivars are generally classified into three types: a weak winter type that is stimulated to flower by brief exposure to low temperature, a semi-winter type that requires 2 to 4 weeks of cold exposure for flowering, and a strong winter type requiring 4 to 6 weeks of cold exposure (Baloch et al. 2003; Berry et al. 1980; Crofts 1989). It has been reported that as many as 12 weeks at low temperature was required for winter wheat cultivar Yeoman to attain a vernalization saturation point (Berry et al. 1980). The varied durations of vernalization in winter wheat cultivars depend on the regions the cultivars are grown. More vernalization requirement is typical for cultivars from zones with longer winters (Fayt et al. 2007).

Based on response to photoperiod, wheat cultivars are classified into sensitive and insensitive types. Wheat is usually classified as a long-day (LD, >14 h light) plant, because it typically flowers earlier when exposed to longer days and flowers later when exposed to short days (Laurie et al. 1994; Snape et al. 2001). However, it was reported that short day (SD, <10 h light) can also accelerate the developmental transition of some

winter cultivars sensitive to photoperiod, thus resulting in earlier flowering without vernalization (Evans 1987). Effects of SD on accelerating flowering could be detected only when the plants were treated for a period of SD and then were moved to LD as they were treated with low temperature for vernalization effects and finally treated with moderately high temperature (Dubcovsky et al. 2006).

In addition to vernalization and photoperiod, a group of genetic factors called *earliness per se* (*eps*) genes provide an additional mechanism to regulate flowering time in wheat (Snape et al. 2001), which enables winter wheat to eventually flower without vernalization or SD. Other adaptive mechanisms include regulation of flowering by plant age, hormones, and nitrogen fertilizers (McMaster 2005)

Genes responsible for developmental process of winter wheat

The three vernalization genes, *TmVRN1* (Yan et al. 2003), *TmVRN2* (Yan et al. 2004b) and *VRN3* (Yan et al. 2006), have been cloned from wheat and barley using a positional cloning strategy. All of them, however, were cloned based on the variation in vernalization requirement between spring and winter types, which differ in a qualitative trait, either vernalization-responsive or non-responsive. Yet, varying vernalization requirement durations (VRD) among winter wheat cultivars almost always yield quantitative responses (Wang et al. 1995). It could be reasonable to speculate that genetic mechanisms which account for the qualitative difference between these two types of wheat cannot be used to explain the variation in the developmental transition among winter wheat cultivars that require 2 weeks up to 12 weeks of low temperatures to attain a vernalization saturation point. However, little is known about how various vernalization

requirements are genetically controlled in different genotypes of winter wheats. Moreover, no specific genetic locus responsible for various vernalization requirement durations in winter wheat has been located, although it is reported that this trait is controlled by multiple loci, according to analysis of substitution lines (Košner and Pánková 1998) and isogenic lines (Fayt et al. 2007).

Genes which confer photoperiod response in wheat have not been cloned, but the orthologous photoperiod gene *PPD-H1* was cloned in barley (*Hordeum vulgare* L.). This will provides valuable sequence information to map genes in the photoperiod pathway in wheat (Beales et al. 2007; Turner et al. 2005). So far, no genes in the *earliness per se* pathway have been cloned from wheat.

Diploid wheat as a model for gene discovery in common wheat

Common wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is one of the cultivated wheat species that is most extensively grown worldwide and serves as food, feed, and industrial ends (Allan 1980). Within the Poaceae, the grass genera *Aegilops* and *Triticum* constitute an interesting model to explain the origin and evolution of common wheat genomes. From a hybridization event and allopolyploidization, diploid species produce several tetraploid and hexaploid wheat species (Chantret et al. 2005) . The three homoeologous genomes of common wheat arose from natural hybridization involving the A-genome donor (*T. urartu*, 2n = 2X = 14, AA), a currently unknown B-genome donor (probably in the section *Sitopsis* including *Ae. Speltoides*, 2n = 2X = 14, SS or BB), and the D-genome donor (*Ae. tauschii*, 2n = 2X = 14, DD). However, complex genome features consisting of three homoeologous genomes A, B and D of

common wheat have limited direct analysis on the gene network of flowering in this species. Instead, diploid progenitor species of common wheat have been used for developing genetic linkage maps based on the presence of high-levels of polymorphisms and ease of working with a single genome (Boyko et al. 1999; Dubcovsky et al. 1996).

Recently, diploid progenitor species *T. monococcum* (2n=2x=12, A^mA^m) has been successfully used as a model to clone wheat genes using positional cloning strategy, e.g., *TmVRN1* (Yan et al. 2003), *TmVRN2* (Yan et al. 2004b), *Lr10* (Feuillet et al. 2003), *Q* locus (Faris et al. 2003). The sequence information of cloned genes is then used to characterize their orthologues in hexaploid wheat; for example, orthologues of *TmVRN1* have been validated in polyploidy wheat and other grass species (Danyluk et al. 2003; Dubcovsky et al. 2006; Murai et al. 2003; Trevaskis et al. 2003; Trevaskis et al. 2006; vonZitzewitz et al. 2005; Yan et al. 2006; Yan et al. 2004; Yan et al. 2005).

In this study we used the diploid *T. monococcum* wheat as a model species to map genetic loci responsible for the vernalization requirement duration in winter wheat.

Construction of genetic linkage map using molecular markers

The genetic linkage map could be useful for gene tagging, marker assisted selection and map based cloning of genes (Singh et al. 2007). In many studies, genetic maps have been constructed using different molecular markers such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism(AFLP), single nucleotide polymorphism (SNP), and simple sequence repeat (SSR). Because of the presence of large variability, co-dominance, PCR-based techniques and ease of handling or manipulation, SSR markers are very valuable in many

plant species. The available series of SSR markers have been developed and mapped in common wheat, as well as in diploid *T. urartu* and *Ae. tauschii* (Singh et al. 2007). However, no SSR markers specifically for diploid *T. monococcum* have been developed, and it was reported that a great number of SSR markers available in hexaploid wheat have high percentage of transferability (ability to amplify) into *T. monococcum* (Kuraparthy et al. 2007). A genome wide of 132 SSR markers were integrated with RFLP and EST markers to construct linkage maps in a RIL population generated from *T. monococcum* and its diploid relative genome (Singh et al.. 2007). The strategy integrating SSR markers and gene markers will facilitate genetic mapping of agriculturally important traits specifically characterized in diploid wheat species. For example, SSR markers were first used to successfully map the *tin3* gene, a tiller mutant producing only one main culm, on the long arm of chromosome $3A^m$ in an F_2 population of *T. monococcum* (Kuraparthy et al. 2007), which has facilitated construction of the *tin3* physical map and its further cloning (Kuraparthy et al. 2008).

CHAPTER III

OBJECTIVE

In this study we developed an F_2 population from a cross between two diploid winter wheat accessions that have different vernalization requirement durations. The objective of this study was to identify key genetic loci responsible for vernalization requirement duration in winter wheat. Mapping information from diploid species will be used to make a cross-map in common wheat, which should facilitate our long-term goal to eventually clone genes responsible for vernalization requirement duration in winter wheat.

CHAPTER IV

MATERIALS AND METHODS

Plant materials

Vernalization tests on two winter accessions, the wild type G3116 and the cultivated type PI 362553 (referred to as Tm53 hereafter) of diploid wheat *T. monococcum*, were carried out in the cold room at 4°C under long photoperiod (16-h light). The Tm53 and G3116 plants at 5th leaf stage were moved to the cold room after they were grown in the greenhouse at 20-25°C and 16 hours of light (long day photoperiod). Some Tm53 and G3116 plants were grown in the greenhouse without vernalization as controls. The vernalized plants in the cold room were moved back to the greenhouse every week for 6 weeks. These partially and fully vernalized plants were scored for heading date. It was found that accession G3116 required only 2 weeks of low temperature to attain a vernalization saturation point, whereas accession Tm53 needed as many as 5 weeks of low temperature to complete its vernalization process. These two accessions of diploid wheat were crossed, and the F_1 hybrid was self-pollinated to develop an F_2 population that was expected to segregate in vernalization requirement duration. The 94 plants of the F_2 population were vernalized for 2 weeks, so that the maximum segregation in the vernalization requirement duration could be detected. The F_2 population was grown in the same greenhouse until the 6th or 7th leaf stage, so that each F_2 plant could be split into two parts. One was transferred into the cold room at 4°C with

long days for two weeks to vernalize and the other one was kept in the greenhouse as a control. These two sets of split plants should have identical genetic backgrounds; therefore the effects of 2-weeks of vernalization on each F_2 line can be calculated by comparison with its second-half grown in the greenhouse.

Analysis on molecular markers

DNA from parents (G3116 and Tm53) and the population was extracted following a simple DNA extraction method (Yan et al. 2004a). Five hundred and thirty two SSR markers mapped in common wheat were used for screening the parental lines for polymorphism, including: 120 BARC, 37 CFA, 41 CFD, 8 GDM, 122 WMS (GWM), 186 WMC, 4 DUPW, 7 KSM, 2 CNL, and 5 STM. These SSR markers mapped in genome A of hexaploid wheat were preferentially selected, but SSR markers mapped in genomes B and D of hexaploid wheat were also tested in order to increase marker density in the genetic map. The PCRs were performed in 25-µl reaction in a PTC-100 or in a PTC-200 thermal cycler. The reaction mixture contained ~100ng of template DNA, 200 µM of each dNTPs, 1 X PCR buffer, 1.5 mM $MgCl_2$, 10 µM forward and reverse primers, and 2.5 units of Taq polymerase. Thermal cycling conditions included: 95°C for 5 min followed by 40 cycles of 94°C for 30", 55°C for 30", 72°C for 30", and followed by an extension step of 10 min at 72°C. In addition to SSR markers, PCR markers were developed to map six flowering time genes and four RFLPs that have been located in wheat or barley. The primers for these known genes and RFLP markers were designed based on genomic DNA or EST sequences of these genes available in GenBank databases, *VRN1* (=*AP1*) (Yan et al. 2003), *VRN2* (=*ZCCT1*) (Yan et al. 2004b), *VRN3*

(=*FT*) (Yan et al. 2006), *PPD1* (Beales et al. 2007), *GI* (*Gigantea*) (Park et al. 1999), and *VRT2* (=*TaVRT2*) (Kane et al. 2005) (Table 1). According to the above PCR protocol, the extension time was modified to amplify the predicted size of PCR products, usually 1 min for 1-kb. PCR products from both the parental lines were purified with PERFORMA® DTR Gel Filtration Cartridges for sequencing that were carried out in the Core Facility, Oklahoma State University. The sequences from each pair was aligned using BLAST from GenBank. Polymorphisms caused by deletions with more than 3-bp were used directly to map in the F_2 population using 9% acrylamide-bisacrylamide gels. For those markers that showed a single nucleotide polymorphism (SNP), however, an appropriate restriction enzyme was used to digest the PCR products to create polymorphic bands that could be distinguished between two parental lines on 1-2% agarose gel for mapping. The restriction enzymes used for digestion of different gene PCR products are included in Table 1.

Construction of linkage maps and discovery of QTLs

Genetic linkage maps were first constructed using MapMaker 3.0 (Whitehead Institute for Biomedical Research, Cambridge, MA) (Lander et al. 1987). WinQTLCart 2.5 (North Carolina State University, Raleigh) was then used to search for QTLs using interval mapping (IM) function. A QTL was declared when logarithm of the odds (LOD) value was higher than the threshold of 2.5 calculated at 300 and significance level at 0.05. Centimorgan values were calculated based on the Kosambi mapping function. The PROC GLM function of SAS Version 9 (SAS Institute, Cary, NC) was also used to test the effects of a single marker and to test interactions between the genes and SSR markers.

The linkage maps were compared with those published in *T. monococcum* and common wheat.

CHAPTER V

RESULTS

Variation in vernalization requirement duration between two parental lines

Neither G3116 nor Tm53 flowered when grown in the greenhouse with continuous 22°C and 16 hours of photoperiod after 200 days from sowing (Fig.1), confirming that they are a winter type as identified using *TmVRN1* and *TmVRN2* genes (Yan et al. 2003; Yan et al. 2004b). Vernalization could accelerate flowering, but different vernalization requirement durations were detected between two parental lines. For the wild type G3116, 1 week of low temperature was not sufficient to promote heading, but only 2 weeks of low temperature could have accelerated heading by approximately three months (Fig. 1). The further extended low temperature treatment (*i.e.*, more than 2 weeks) resulted in a gradual delay of heading when compared with the maximum effect detected with 2 weeks treatment, although significant vernalization effects could be detected when compared with control plants continuously grown in the greenhouse (Fig.1). The observations showed that G3116 required 2 weeks of low temperature to attain a vernalization saturation point. For the cultivated type Tm53, the maximum vernalization effect (143 days) was detected when the plant was treated with low temperature for up to 5 weeks, and the delayed effect of overexposure to low temperature on heading was 17 days when the low temperature treatment was extended to 6 weeks (Fig. 1).

When treated with the same period of low temperature for 2 weeks, G3116 and Tm53 showed the most significant difference of 95 days in heading date, but this difference was narrowed down to 13 days when treated with low temperatures for 5 weeks. The observation suggested that when the population plants generated from these two parental lines were treated with 2 weeks of low temperature, variation in vernalization requirement duration could be most efficiently segregated.

Statistical distribution of heading date in vernalized and unvernalized populations

Heading date of plants of the F_2 population generated from a cross between Tm53 x G3116 ranged from 150-270 days after sowing when they were grown in the greenhouse. Two weeks of vernalization narrowed the heading duration to 171-230 days after sowing (Fig. 2). The average heading date of the vernalized plants was 14 days earlier than that of the unvernalized plants. The heading date of the population plants with and without vernalization treatment showed a quantitative trait distribution (Fig. 2), indicating that segregation of heading date was controlled by multiple genes. The combinations of these genes resulted in earlier or later heading date than both of the two parental lines, suggesting that segregation of the heading date in the winter x winter population was controlled not only by vernalization genes but also other flowering time genes that were not promoted or even repressed by low temperature.

Sixty-five lines in the cold-treated population flowered earlier than the corresponding lines in the control population, indicating that these lines had a vernalization effect. The remaining 26 lines in the cold-treated population flowered at the same time or even later compared with their controls, suggesting that the heading date of

these lines were controlled by other flowering genes such as *earliness per se* genes.

It is noteworthy that when two parental lines were treated with two weeks of vernalization at the same time with the population, the heading date of the parental lines showed a difference in heading date by only 32 days, much less than what was observed in the previous study on the parental lines only. The decreased difference in heading date between the two parental lines was probably because the two experiments were done in different seasons although they were carried out in the same greenhouse and treated with cold in the same growth chamber.

Polymorphisms of SSR markers transferred from hexaploid wheat to diploid wheat *T. monococcum*

A total of 532 SSR markers developed in hexaploid wheat were used to screen for polymorphism between G3116 and Tm53. Out of these SSR markers, 292 markers showed amplification in one or both of the two parents (Table 2; Table 3), but the remaining 240 SSR markers did not show amplification in either one of the parents. These results indicated that approximately 55% of the SSR markers in hexaploid wheat can be transferred to construct genetic maps in diploid wheat.

Among the SSR markers in hexaploid wheat, they are specific to genome A, B or D in this species. When transferred to diploid wheat *T. monococcum*, these markers showed different transferring rates. Of 285 genome A-specific markers, 122 (43.6%) did not show amplification in either of the parental lines, and 163 markers (57.2%) markers were transferable in *T. monococcum* (Table 3). The genome B-specific markers showed a 37.5% rate for transferring, and genome D-specific markers showed a 50.8% rate for transferring.

Out of 292 transferable markers, 211 had polymorphic DNA band patterns between G3116 and Tm53 (Table 4) and 104 markers were mapped in the population. The overall rate of polymorphic markers exhibited between these two parental lines was 72.3%, which was much higher than that in hexaploid wheat. An average of SSR marker polymorphism is approximately 35% in hexaploid wheat (Chen et al., unpublished data). The higher rate of polymorphism in SSR markers in diploid wheat is probably due to highly diverse genetic backgrounds between the wild type G3116 and the cultivated type Tm53.

Based on the comparison of markers on chromosomal locations with consensus genetic maps in wheat (http://wheat.pw.usda.gov) (Somers et al. 2004), 71 of 104 polymorphic markers (68%) were mapped in orthologous chromosomes to homoeologous chromosomes in hexaploid wheat, and the 33 remaining polymorphic markers were located on different chromosomes. Compared with SSR markers mapped in *T. monococcum* by Singh et al. (2007), 33 of 104 polymorphic markers were mapped in similar locations, two polymorphic markers were located on different chromosomes, and the 69 remaining markers were for the first time mapped in *T. monococcum*.

When the SSR markers were utilized in diploid wheat, they showed different patterns of DNA bands. Fig.3 showed three examples of polymorphic bands between G3116 and Tm53 that were used to map our population. These SSR markers facilitated construction of a genetic map for each chromosome in diploid wheat.

Development and mapping of PCR markers for the known flowering time genes and RFLP loci

Many RFLP markers have been mapped in hexaploid wheat and diploid wheat in

the early stage of studies on genetic mapping. The RFLP markers were developed from a single or low copy of genomic DNA or EST clone; therefore, they can be used to anchor a certain chromosome. Some SSR markers are not mapped on a certain chromosomal location, due to their multiple copies, but their locations can be determined when they are mapped in the same group as a RFLP or gene marker. The sequence for a RFLP marker was recovered from GrainGene databases and then used to search in GenBank EST databases. Multiple wheat EST sequences, probably from genome A, B or D in hexaploid wheat, were aligned up and conserved sequences were used to design primers to amplify orthologous genes from two parental lines. Sequence polymorphisms were used to develop PCR markers. Four RFLP markers were transferred into PCR markers that were mapped in expected chromosomal sites (Table 1). In a similar way, six PCR markers were developed to map orthologues of the known flowering time genes in diploid wheat, including three genes *VRN1*, *VRN2*, and *VRN3* that were reported to function in the vernalization pathway (Yan et al. 2003; Yan et al. 2004b; Yan et al. 2006); two genes *PPD1* and *GI* that were reported to act in the photoperiod pathway (Beales et al. 2007; Park et al. 1999), and the gene *VRT2* that was reported to interact with *VRN-A1* and regulated by both low temperature and photoperiod (Kane et al. 2005). The sequence of each RFLP and flowering time gene marker is provided in the Appendix.

All the PCR markers for 6 known flowering time genes were analyzed by digestion of PCR products with restriction enzymes, except for *TmPPD1* that can be distinguished by polymorphism between the two parental lines with a 3-bp section present in G3116 but absent in Tm53 (Fig.4). Expected sizes of the PCR products and digested products with appropriate restriction enzymes were listed in the Appendix. The mapped bands were shown in Fig.4.

A genetic map for diploid winter x winter wheat population

A total of 116 markers including 106 polymorphic SSR markers, 6 flowering time genes and 4 RFLP markers, were used to construct a genetic map for the winter x winter wheat population. One hundred and fourteen markers were linked into 7 groups, which matched up with seven chromosomes in diploid wheat (Fig.5), based on the comparison of markers on chromosomal locations with consensus genetic maps in wheat (http://wheat.pw.usda.gov). Two additional unlinked SSR markers were *Xwms471* and *Xwmc382* that were supposed to locate on the telomere region of the short arm of chromosome $7A^m$ and distal region of the short arm of chromosome $2A^m$ respectively (data not shown).

A total length of 1,155.2 cM was determined in the genetic map consisting of 7 chromosomes, with 16 gaps that had more than 20-cM genetic distance, 2 on chromosome $1A^m$, 3 on chromosome $2A^m$, 2 on chromosome $3A^m$, 4 on chromosome $4A^m$, 2 on chromosome $5A^m$ and 3 on chromosome $7A^m$, respectively. The largest linkage group was mapped to chromosome $2A^m$ (224.5 cM), and the smallest one was for chromosome $6A^m$ (88.9 cM). The average genetic distance between two markers was 11.1 cM, which was expected to cover most of the QTLs responsible for the heading dates of the segregates in the populations.

Chromosome $1A^m$: Twelve SSR markers covering 146.0 cM were mapped on chromosome 1 A^m (Fig.5) A gap spanning 64.8 cM was between *Xbarc28* and *Xcfa2153*, and another gap spanning 38.9 cM was between *Xwmc95* and *Xcfd59*. Seven markers *Xbarc287*, *Xbarc28*, *Xcfa2153*, *Xwmc818*, *Xwmc95*, *Xcfd59*, and *Xwms135* were mapped in chromosome 1A of hexaploid wheat (Somers et al. 2004) and the

consensus map of wheat (http://wheat.pw.usda.gov) and an additional marker *Xcfd65* that was mapped in chromosome $1A^m$ (Singh et al. 2007) helped to identify the location of this linkage group in chromosome $1A^m$ in our population. Three markers, *Xwmc728* (2A, 1B, and 5B), *Xwmc243* (6A, 2B, 2D, and 7D), and *Xcfa2099* (2A and 7D) that were reported to reside at different chromosomal locations from previous studies, but all of them were for the first time, located on chromosome $1A^m$ in our population.

Chromosome $2A^m$: Twelve SSR markers covering 224.5 cM were mapped on chromosome $2A^m$ in our population, with three large gaps (27.5 cM, 72.3 cM and 47.7 cM) (Fig. 5). Three markers, *Xwmc83*, *Xbarc116*, and *Xbarc122*, were mapped on this chromosome but they were reported to be located on chromosome 7A, 5B, and 5A in previous studies, respectively (Somers et al. 2004) (http://wheat.pw.usda.gov). Another nine SSR markers have been reported on chromosome 2A of hexaploid wheat (Somer et al. 2004). Markers *Xwms71*, *Xwms515*, *Xwmc407*, *Xwms382*, and *Xbarc122* were also mapped in chromosome $2A^m$ (Singh et al. 2007). In addition, a photoperiod gene, *TmPPD1* that was previously reported to reside on the short arm of group 2 in hexaploid wheat and barley anchored this linkage group on chromosome $2A^m$.

Chromosome $3A^m$: Twenty SSR markers, 1 RFLP marker and 1 flowering time gene marker spanned 167.3 cM in genetic distance for chromosome $3A^m$, forming a high density genetic map for this chromosome (Fig. 5). There were two gaps spanning more than 20-cM in this chromosome; one was located between *Xwms369* and *TmGI* and the other was located between *Xstm731tc* and *Xwmc169*. There was a large gap in the region close to *Xwms369* on the short arm of group 3 in almost all previous studies in hexaploid wheat (Somer et al. 2004; Torada et al. 2006), probably because it is a low recombination

region. Five SSR markers, *Xcfd30* (1A, 4A, 7D), *Xwmc475* (5A, 7B), *Xcfa2226* (1A), *Xwmc723* (7B) and *Xdupw227* (3B), that were previously reported on different chromosomal locations were located on chromosome $3A^m$ in our population. Markers *Xcfd79*, *Xbarc19*, *Xwmc269*, *Xbarc67*, and *Xcfa2134* were also mapped in diploid *T. monococcum* (Singh et al. 2007). The RFLP marker *Xcdo189* that was reported to locate on the telomere region of the long arm of chromosome $3A^m$ (Dubcovsky et al. 1996) anchored the chromosomal location of this linkage group.

Chromosome $4A^m$: Fourteen SSR markers covering 169.4 cM were mapped on chromosome $4A^m$, leaving four gaps with more than 20-cM to close up (Fig. 5). Four markers *Xwmc651*, *Xwmc118*, *Xwms5* and *Xbarc1005* were previously mapped on chromosome 3A, 5B, 3A and 7A, respectively, but they were all mapped on chromosome $4A^m$ in this study. The chromosomal location of this linkage group was not validated by any RFLP markers or gene markers, but by the remaining 10 SSR markers that were mapped in the same chromosome in previous studies (Singh et al. 2007; Somers et al. 2004).

Chromosome $5A^m$: Eighteen SSR markers and 2 PCR markers developed from two vernalization genes *TmVRN1* and *TmVRN2* were mapped on chromosome $5A^m$ covering 160.2 cM in genetic distance and forming a high density genetic map for this chromosome (Fig. 5). There were two gaps spanning more than 20-cM; one was located between *Xcfa2234* and *Xwms156* and the other one was located between *Xwms156* and *Xwms639*. Two markers, *Xwms328* and *Xcfa2234*, that were previously mapped on chromosomes 2A and 3A respectively (Singh et al. 2007; Somers et al. 2004) were placed in this linkage group.

Chromosome 6A^m: Six SSR markers and 2 PCR markers transferred from two RFLP markers, *Xpsr113* and *Xmwg798*, were mapped in the same group covering 88.9 cM on chromosome 6A^m (Fig. 5). Two SSR markers, *Xcfd25* (4A, 2D, 5D, and 7D) and *Xwmc773* (5B and 6D) that were previously mapped on different chromosomal locations (Somers et al. 2004) were for the first time mapped on chromosome 6A^m. Chromosome 6A^m has a relatively short genetic length (Singh et al. 2007).

Chromosome 7A^m: Twenty two SSR markers, 1 PCR marker developed from RFLP marker *Xcdo673*, and 2 PCR markers developed from flowering time genes *TmVRN3* and *TmVRT2*, were mapped on chromosome 7A^m covering 198.9 cM including 183.9 cM for the first group and 15.0 cM for the second group. These two groups have a large gap between them but they were placed on the same chromosome, because both two markers, *Xgwm344* and *Xcfa2040* in the second group (Fig. 5), were mapped on chromosome 7A in hexaploid wheat (Somer et al. 2004). Seven SSR markers that were previously reported on different chromosomes, *Xwmc177* (2A), *Xwmc26*4 (2A, 3A, and 5D), *Xgdm88* (4A and 7D), *Xgdm145* (4A and 7D), *Xbarc231* (2A and 7B), *Xbarc172* (5B and 7D), *Xwmc698* (4A and 7D) were also mapped on this chromosome.

QTLs for vernalization requirement duration

The heading date of two sets of split population plants with and without vernalization treatment were analyzed with the IM program of WinQTL. As a result, a major QTL on chromosome 7A^m was found affecting heading date of the vernalized population. This QTL affecting *v*ernalization *r*equirement *d*uration on chromosome 7A^m, designated *QVrd.osu-7A^m*, was flanked by two SSR markers *Xwmc96* and

Xbarc172, spanning 21.8 cM on the long arm of this chromosome (Fig.5). Fortunately, RFLP marker *Xcdo673* on chromosome 7Am was mapped to be centered on the peak of this QTL. The LOD of *QVrd.osu-7A*^{m} was 3.68, explaining 22.8% phenotypic variation in heading date. This QTL was not found when the heading date of the unvernalized population was analyzed, suggesting that *QVrd.osu-7A*^{m} was caused by vernalization for 2 weeks. It has been clearly demonstrated that *QVrd.osu-7A*^{m} has no association with either of the flowering time genes, *TmVRN3* or *TmVRT2*, that were located on this chromosome (Fig.5), suggesting that *QVrd.ous-7A*^{m} that was found in this study is a new locus related to responses of winter wheat to vernalization.

TmVRN2 locus on chromosome 5A^m was also found associated with a minor QTL (designated *QVrd.osu-5A*^{m}) in the vernalized population (data not shown). This QTL was not present in the unvernalized population, suggesting that it was regulated by vernalization. The LOD value of *QVrd.osu-5A*^{m} did not reach 2.5, a common threshold that is used to declare the presence of a QTL. Statistical analysis, however, showed that *TmVRN2* had a significant effect on heading date in the vernalized population ($Pr<0.05$). The remaining five flowering time genes (*TmVRN1*, *TmVRT2*, *TmVRN3*, *TmPPD1*, and *TmGI*) were not detected genetic effects in either vernalized population or unvernalized population.

Homozygous G3116 allele, homozygous Tm53 allele and their heterozygous allele at *QVrd.osu-7A*^{m} had an average heading date of 195.2 days, 204.1 days, and 197.6 days in the vernalized population respectively. It was estimated that the G3116 allele of the gene responsible for *QVrd.osu-7A*^{m} is dominant for early heading, as the mid-parent value (X=199.7 days) was significantly higher than the heterozygous allele. Homozygous G3116 allele, homozygous Tm53 allele and their heterozygous allele at *QVrd.osu-5A*^{m}

had an average heading date of 195.6 days, 202.1 days, and 200.2 days in the vernalized population respectively. It was estimated that the Tm53 allele of the gene responsible for *QVrd.osu-5A*^{m} is dominant for late heading, as the mid-parent value (X=198.9 days) was significantly lower than the heterozygous allele. No significant interaction was detected between *QVrd.osu-7A*^{m} and at *QVrd.osu-5A*^{m} (*Pr*=0.4239), indicating that the genes residing within these two QTLs play an independent role in controlling vernalization requirement duration in winter wheat.

CHAPTER VI

DISCUSSIONS

Variation in vernalization requirement duration in winter wheat

The difference in vernalization requirement duration among winter wheat is mainly caused by both amplitude and duration of low temperatures in varying geographical areas. Vernalization usually has significant effects at temperatures between 2-10°C. Its rate will decline, however, at temperatures above 11°C and it will be apparently ineffective above 18 °C (Brooking 1996). If the fully vernalized plants continue to stay at the low temperature, the developmental process of these over-vernalized plants will be delayed due to a longer phyllochron and a slower growth rate (McMaster 2005). In almost all previous studies, vernalization tests were conducted with 6 weeks of low temperature under long day conditions, under which the vernalization requirement has been fully met and the acceleration of flowering can be detected. The results on vernalization effects, however, could be incorrectly interpreted in a certain genotype, because of the paradoxes between the effects of low temperature on the acceleration of the developmental transition and the effects of low temperature on the delay of growth rate after vernalization. In order to solve this experimental confusion, it is critical that the fully vernalized plants need to be transferred into favorable temperature and light conditions for further growth upon reaching vernalization saturation point.

We used two diploid winter wheat accessions that have different vernalization requirement durations to develop an F_2 population, and segregation for the trait was obtained. Although we carried out experiments in the same growth chamber and greenhouse with well controlled conditions, the difference in heading date between the two parental lines showed variation between experimental seasons. A difference between parents of as many as three months was detected when the experiment was performed in winter season, but a difference of only 32 days when the experiment was performed in summer season. Irrespective of the difference detected with different seasons, the wild type G3116 always had less vernalization requirement than Tm53. When vernalized for two weeks, the segregates generated from a cross between these two parental lines varied in vernalization requirement duration, which enabled us to determine genetic loci responsible for this trait. Previous studies showed that this trait is present in extensive cultivars in hexaploid wheat (Baloch et al. 2003; Wang et al. 1995) or between substitution lines and isogenic lines (Fayt et al. 2007). A precise molecular characterization of the genes responsible for the quantitative vernalization requirement would provide innovative and critical information for the genetic basis of this important trait.

A major locus for vernalization requirement duration is located on an unreported genomic region involved in developmental process

We mapped 114 molecular markers in the F_2 segregating population and expected to locate genome-wide genetic loci associated with vernalization requirement duration. A major QTL responsible for vernalization is located on the long arm of chromosome $7A^m$, *QVrd.osu-7A*^{m}, and it has no association with two known flowering time genes *TmVRN3*

(=*TmFT*) and *TmVRT2* that were mapped in the same chromosome; therefore, *QVrd.osu-7A*^{m} is a new genetic locus responsible for the vernalization requirement duration in winter wheat. It was the first time to find that a genetic locus on the long arm of chromosome 7A^{m} affects flowering time in wheat or other temperate cereals. This gene may play a coordinating role in the vernalization process, since it displayed the most significant genetic effect in the presence of genetic effect from *TmVRN2*, a major flowering repressor identified so far in wheat. A tight association of this major QTL with a PCR marker for RFLP marker *Xcdo673* would greatly facilitate construction a fine map and cloning this first gene for vernalization requirement in winter wheat.

In addition to a major locus that was located on chromosome 7A^{m}, *TmVRN2* was found to have association with segregation in heading date in the vernalized population. *TmVRN2* was cloned based on variation between spring wheat and winter wheat (Yan et al. 2004). *TmVRN2* is a transcription factor containing a conserved CCT domain present in *CONSTANS (CO), CO-like* and *TOC* genes in plants (Putterill et al. 1995). A dominant *Vrn2* is responsible for winter growth habit, whereas a recessive *vrn2* allele was caused by a point mutation at the conserved CCT domain of the TmVRN2 protein or complete deletion of this gene in the diploid wheat (Yan et al. 2004b). The two parental lines tested in this study both have no point mutation or deletion but possess a dominant allele at *Tm VRN2*, resulting in winter growth habit (Yan et al. 2004b). Genetic effect of *TmVRN2* detected in this study has pointed to the possibility that *TmVRN2* could be regulated by different mechanisms. Alternatively, a new gene tightly linked to *TmVRN2* may be involved in the regulation of reproductive development in winter wheat.

A genetic model for vernalization requirement duration

Vernalization requirement duration in the genetic background of the diploid wheat applied in this study is regulated by two QTLs, explaining part of the total phenotypic variation. Our observation is consistent with the previous reports that vernalization requirement duration is controlled by QTLs including genes on chromosome group 5, according to analysis of substitution lines (Košner and Pánková 1998) and isogenic line (Fayt et al. 2007). However, the conclusion that vernalization requirement duration is controlled by multiple genes might not apply to other experiments when different genetic backgrounds of winter wheat are investigated. An experiment on hexaploid wheat Jagger x 2174 population in our laboratory indicated that the vernalization requirement duration in mainly determined by a locus on chromosome 5A, which explained most of the total phenotypic variation and is different from either of the two QTLs found in diploid wheat.

There are two alternative mechanisms which can explain quantitative vernalization results in winter type of *Arabidopsis* accessions. One is that vernalization results in a quantitative reduction in *FLOWERING LOCUS C* (*FLC*) mRNA levels that negatively correlates with the flowering time (Michaels and Amasino 1999; Sheldon et al. 1999; Sheldon et al. 2000). The other is that the multiple signals from a flowering network, mainly including vernalization and photoperiod pathways, are integrated to determine the transcript level of *FLC*. Those vernalization genes include *FRIGIDA* (*FRI*) that up-regulates *FLC*, and *VERNALIZATION-INSENSITIVE 3* (*VIN3*) that down-regulates *FLC* (Michaels et al. 2001; Reeves and Coupland 2001; Simpson and Dean 2002; Sung and Amasino 2004; Amasino 2004). Those photoperiod genes include *GIGANTEA* (*GI*), *CONSTANS* (*CO*), and *FLOWERING LOCUS T* (*FT*), which form

flowering signals in the *GI-CO-FT* streamline to repress *FLC* to induce the meristem identity gene *AP1* to promote flowering (Kardailsky et al. 1999; Kobayashi et al. 1999; Samach et al. 2000; Suarez-Lopez et al. 2001; Corbesier et al. 2007; Tamaki et al. 2007).

In Arabidopsis, a central role of *FLC* was used to explain these mechanisms for the quantitative vernalization requirements, but no *FLC* homologue has been found in grass species. The wheat *VRN2* plays a similar function to the Arabidopsis *FLC* in repressing flowering, but the wheat *VRN2* gene has no homologues in an entire genome of sequences in *Arabidopsis*, further suggesting that these two species have evolved different vernalization mechanisms (Yan et al. 2004b).

We suggest that regardless of whether the one-gene model or multiple-genes model, the vernalization variation duration in a species even a variety in the same species depends on the genetic backgrounds of the materials studied. When two parental lines with diverse genetic backgrounds are used to generate a population, the vernalization requirement duration may be mapped by a major gene or quantitative trait loci (QTLs), and one QTL may appear in one population but not in another one. All of these genes and QTLs identified under various genetic backgrounds can be used to make specific genetic combinations for winter wheat to adapt to diverse environments and changing climate shift.

CHAPTER VII

CONCLUSIONS

- Simple sequence repeat (SSR) markers that were extensively developed in hexaploid wheat can be transferred to map specific traits in the diploid wheat *T. monococcum*.

- Vernalization requirement duration in the genetic background of the diploid wheat applied in this study is regulated by two QTLs. The segregation of the vernalization requirement duration in winter wheat was greatly benefited from a specific vernalization condition that was provided for one line but not the other line to attain a vernalization saturation point.

- A major QTL affecting vernalization requirement duration is located on the long arm of chromosome 7A, *QVrd.osu-7A*^{m}, and it has no association with two known flowering time genes *TmVRN3* (=*TmFT*) and *TmVRT2* that were mapped in the same chromosome; therefore, *QVrd.osu-7A*^{m} is a new genetic locus responsible for the vernalization requirement duration in winter wheat.

- The known vernalization genes *TmVRN2* but not *TmVRN1* and *TmVRN3* might be involved in regulation of vernalization requirement based on their association with minor QTLs found in this study.

- All of these genes and QTLs can be used to make specific genetic combinations for winter wheat to adapt to diverse environments and changing climate shift.

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Table 1. PCR markers transferred from RFLP markers and known flowering time genes

Marker name	Primers	Enzymes	Deletion
MWG798-F[a]	5'-CAATTATCATCTGTGTAAGCC-3'	*Ban*I	
MWG798-R[b]	5'-CATTCGACGAGAGTGTTCTTC-3'		
CDO673-F	5'-CAGCGAAAGGGTTTTCAGAG-3'		4-bp
CDO673-R	5'-CCCATGATATCAACCAGCAA-3'		
CDO189-F	5'-CTGGATCTTCTTGAAACCATTG-3'	*Rsa*I	
CDO189-R	5'-CTGTCGAAACTGAACTTTTCG-3'		
PSR113-F	5'-GCCTACAAGGTGTACGACGTG-3'	*Nde*I	
PSR113-R	5'-TGTAATTTACATCTGACACAACC-3'		
TmVRN1-F	5'-TCTCATGGGAGAGGATCTTGA-3'	*Bam*HI	
TmVRN1-R	5'-GGGAGCATCCCTCATCATGAA-3'		
TmVRN2-F	5'-CATCCATGCTATTATCATGG-3'	*Bgl*II	
TmVRN2-R	5'-TGGATCAGGAGAGATGACCC-3'		
TmVRN3-F	5'-AGAGACCCGCTGGTGGTT-3'	*Apa*LI	
TmVRN3-R	5'-CACGAGCACGAAGCGATG-3'		
TmPPD1-F	5'-GTCTTTGGCCATGGATCATT-3'		3-bp
TmPPD1-R	5'-GACCGACTCCGCACTTCTAC-3'		
TmVRT2-F	5'-CATGAGGTGCCAACTGCTAG-3'	*Dde*I	
TmVRT2-R	5'-GCGAAACTCACGCAAGTCTCA-3'		
TmGI-F	5'-TTTGGCGAACTTCCTTACCATG-3'	*Mse*I	
TmGI-R	5'-TCCCACGTGAGAAATGAACA-3'		

[a] F in each marker name indicates forward primer

[b] R in each marker name indicates reverse primer

Table 2. Transferability (ability to amplify) of different types of SSR markers from hexaploid wheat to diploid wheat

Marker type	Markers in 6X wheat tested	Amplification in one or both the parents	Transferability to 2X wheat (%)
BARC	120	79	65.8
CFA	37	27	73.0
CFD	41	25	61.0
CNL	2	2	100.0
DUPW	4	3	75.0
GDM	8	5	62.5
GWM(WMS)	122	56	45.9
KSM	7	5	71.4
WMC	186	85	45.7
STM	5	5	100.0
Total	532	292	54.9

Table 3. Transferability of genome-specific SSR markers from hexaploid wheat to diploid wheat

Genome	Markers in 6X wheat tested	Amplification in one or both the parents	Transferability to 2X wheat (%)
A	285	163	57.2
B	56	21	37.5
D	63	32	50.8
A and B	50	27	54.0
A and D	41	28	68.3
A, B and D	37	21	56.8
Total	532	292	54.9

Table 4. The level of polymorphism of different SSR markers from hexaploid wheat to diploid wheat

Marker type	Amplification in one or both the parents	Polymorphic markers	Parental polymorphism (%)
BARC	79	58	73.4
CFA	27	24	88.8
CFD	25	13	52.0
CNL	2	0	0.0
DUPW	3	1	33.3
GDM	5	5	100.0
GWM(WMS)	56	45	80.4
KSM	5	4	80.0
WMC	85	56	65.9
STM	5	5	100.0
Total	292	211	72.3

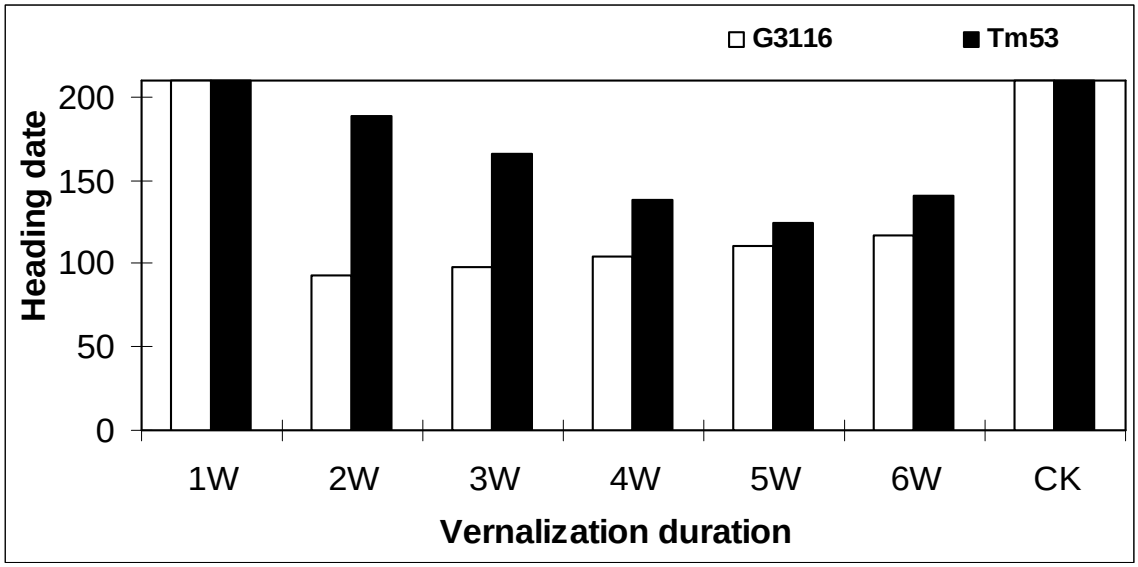

Fig.1. Vernalization tests with different periods of low temperatures in the parental lines. The two parental lines, Tm53 and G3116, were treated with different periods of low temperature at 4-6°C with 16 hours of lights then moved back to a greenhouse where control plants were grown under 22-25°C and 16 hours of light. 1W=1 week, 2W=2 weeks, 3W=3 weeks, 4W=4 weeks, 5W=5 weeks, 6W=6 weeks, and CK= check.

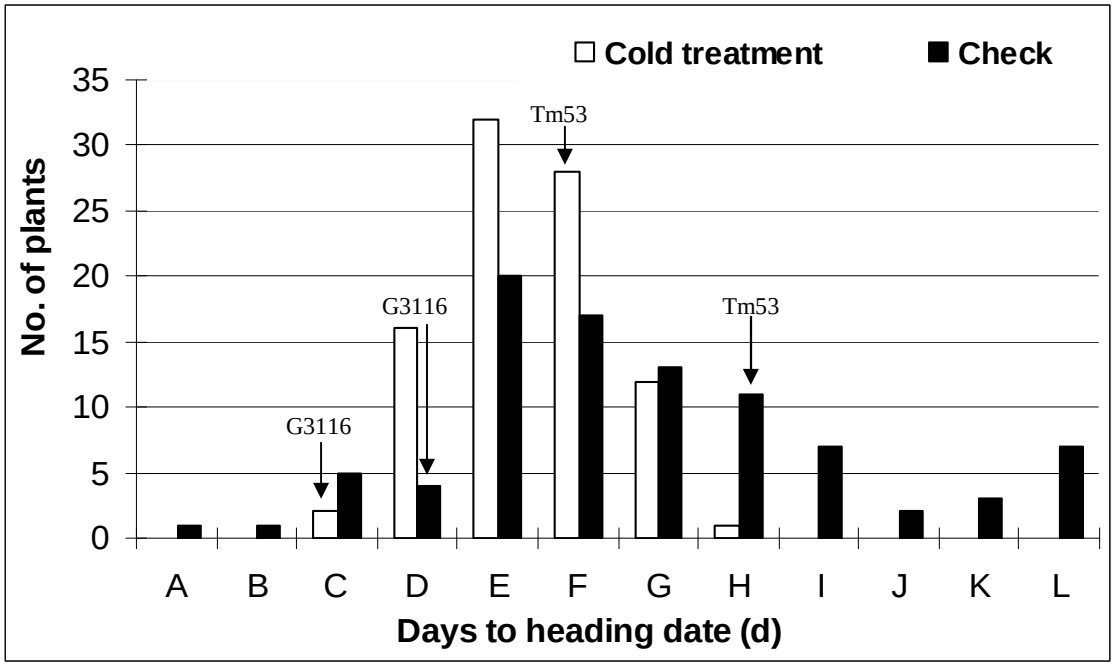

Fig.2. Frequency distribution for days to heading of plants in vernalized and unvernalized F_2 populations. Scale for days to heading time: A=151-160, B=161-170, C=171-180, D=181-190, E=191-200, F=201-210, G=211-220, H=221-230, I=231-240, J=241-250, K=251-260, L=261-270.

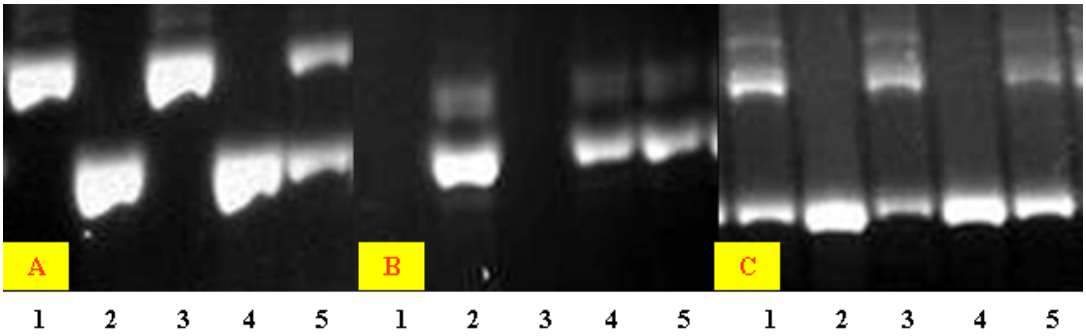

Fig.3. SSR marker band patterns. A) *Xwmc169*, a dominant marker with the upper band for one parental line but the lower band for the other parental line; B) *Xwmc188*, a co-dominant marker present in one parental line but not in the other; C) *Xwmc705*, a co-dominant marker with the upper band but with the lower band that is common in all plants. 1) G3116; 2) Tm53; 3-5) three F_2 plants from Tm53 x G3116 population.

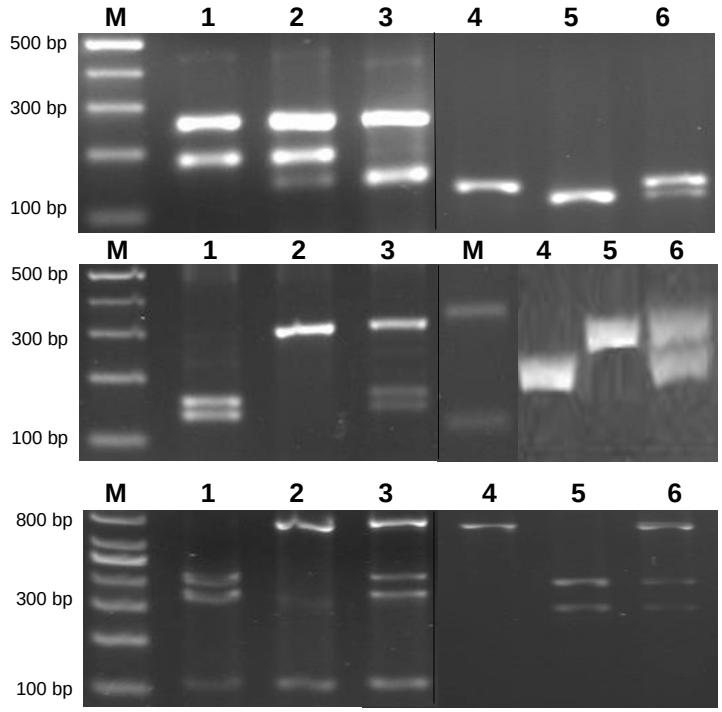

Fig.4. PCR markers for six known flowering time genes. Genotypes of the plants were scored as A for the PI362553 allele, B for the G3116 allele, and H for the heterozygous allele. Primer sequences and enzymes used to digest PCR products are listed in Table 1. (Top) Lanes 1-3 indicate the *TmGI* gene marker for the A allele (~254-bp and ~186-bp), the H allele, and the B allele{~252-bp, ~143-bp and ~43bp (out of the gel)}. Lanes 4-6 indicate the *TmVRT2* gene marker for the A allele (not digested), the B allele {~142-bp and ~22-bp (out of the gel)}, and the H allele. (Center) Lanes 1-3 indicate the *TmVRN3* gene marker for the A allele (~546-bp, ~157-bp and ~132-bp), the B allele (~545-bp and ~289-bp), and the H allele. Lanes 4-6 indicate the *TmPPD1* gene marker for the A allele (3-bp deletion), the B allele (3-bp insertion), and the H allele. 100-bp and 200-bp of marker size are also indicated. (Bottom) Lanes 1-3 indicate the *TmVRN2* gene marker the A allele (~411-bp, ~333-bp and ~308-bp), the B allele (not digested), and the H allele. Lanes 4-6 indicate the *TmVRN1* gene marker for the A allele (not digested), the B allele (~510-bp and ~284-bp), and the H allele.

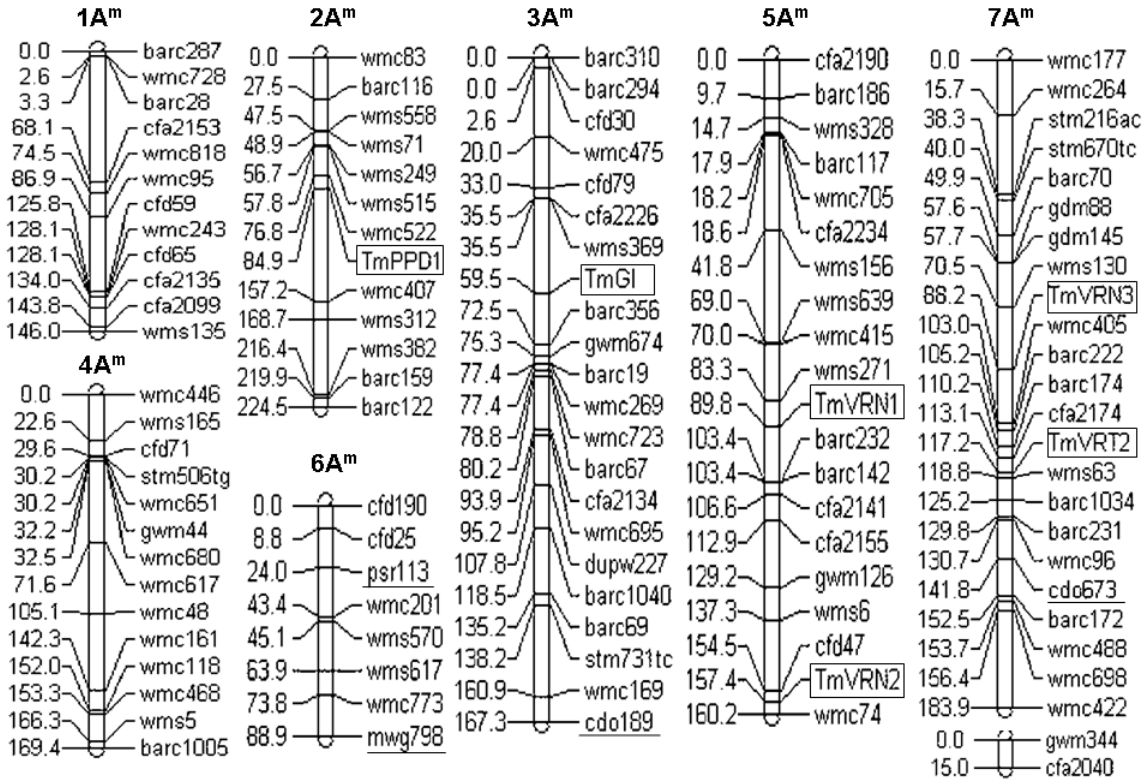

Fig.5. Linkage groups of 114 markers and their chromosomal locations. Six markers for flowering time genes are squared and 4 markers for RFLP loci are underlined. Numbers on the left side of each chromosome are genetic distance scaled in centi-Morgan (cM).

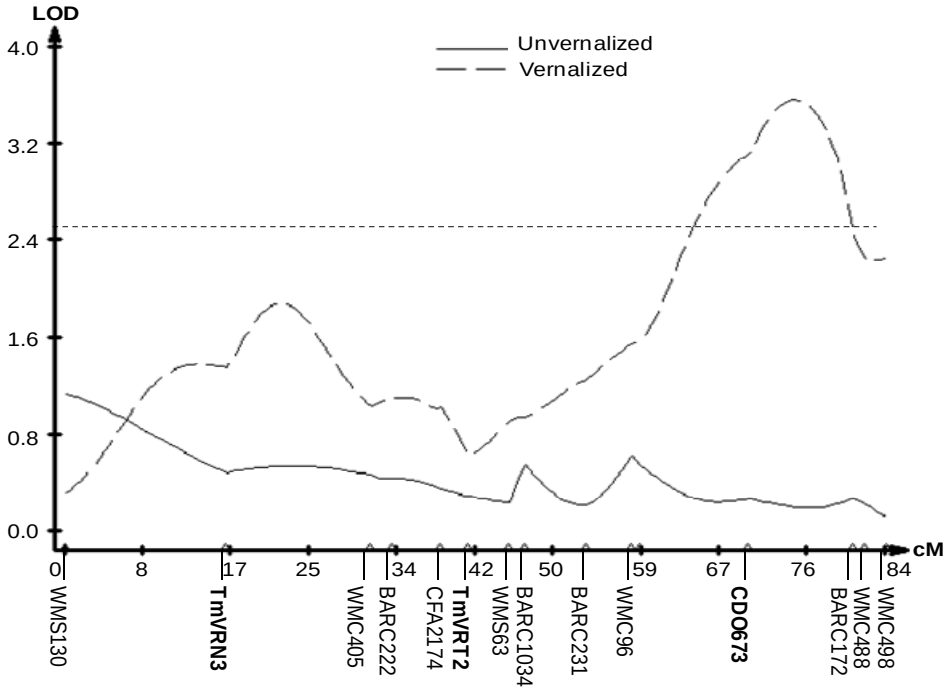

Fig.6. Location of a major QTL, *QVrd.osu-7A^m* on chromosome 7A^m.

APPENDIX

Sequences of PCR markers developed from RFLPs and known flowering time genes

RFLP marker: MWG798

Tm53

ACCGTACCCCTCATGCGCGTTTGCTCTGGTGCCTCTATTTTCTCTCTTTCCTCGACAATGTCCTTCAATTCATCGTAGG
CGGTCGTGCAAAGGTTTCTTCTGGTTCCTGCCATTAGCATTTCAGGATTCAAAAACAGTAGATACATCATGTAGTTGGA
CATTTCTCTGCACTGGATGGCCCTCTTGTGATGACCGGATCTTTTGCACCAAGCAGGGCATGCATAAGCATGAGGACAT
GAATCTTGCGTGCACAGCAGTCCGTGGTGAGAACGAGACACAACCGTGTCGTAGTAGCAGAAATCCGTGGCGAGATGCC
AAAGAAGAACACTCTCGTCGAATGAGCCGCGAGGAAATAAGGCACACACAGATGATAAGTGGACCCGCCTTGTGTGTTT
GCATAACCTCTTATGCACCAGATGACCACGTCGCATATATGTATTCC

G3116

TTACTTCTCTCTCCTTCGCGCTGGTGCCTCTATTTTCTCTCTTTCCTCGACAATGTCCTTCAATTCATCGTAGGCGGTC
GTGCAAAGGTTTCTTCTGGTGCCTGCCATTAGCATTTCAGGATTCAAAAACAGTAGATACATCATGTAGTTGGACATTT
CTCTGCACTGGATGGCCCTCTTGTGATGACCGGATCTTTTGCACCAAGCAGGGCATGCATAAGCATGAGGACATGAATC
TTGCGTGCACAGCAGTCCGTGGTGAGAACGAGACACAACCGTGTCGTAGTAGCAGAAATCCGTGGCGAGATGCCAAAGA
AGAACACTCTCTCGAATGAGAG

Left primer = 5’-CAATTATCATCTGTGTAAGCC-3’
Right primer = 5’-CATTCGACGAGAGTGTTCTTC-3’
Restriction enzymes: *Ban*I (5’-G/GPyPuCC-3’)
Expected product size: Tm53 (484-bp), G3116 (118-bp + 262-bp)

RFLP marker: CDO673

Tm53

ACTTTCGTCGAGGCATCCGGTCGCTCGGATCCCGGCCGTGCAGTTGAGGGCCCCTCAGTCCCTTGCTGAGGGAGTCCAC
CGCGAATGTGCCGCGGATCGTCGGGTCGGCCATGTCTTTCAGCATGTCCGGGTCTTCGGCCAGGGCCTCGCTCAGCTAC
ACGGATTGACACGGGATGCATATTCAGAATCAAAATGTAATAATTTGTTCAGAAGGACCAAGCTCTAAGCTTTTTGGTG
CTAGGAGTAGTATCTTTCAGTGTGATGTGAACATGAAGCTTGAGAATTGAGAGTCACCTGAGCTCTCAGGGATTCCAGC
TCGCTTTTCGACGCTGCTGGTTTGCCTGTGATCACCTCGAGAAGGATTTGGCCGAACTGGTATATGTCATCCCTGTCCC
CTTGTTCTGCAGGTTGAGCGCTGCAAGCAGACAGAATCATAAACGTTTTAGGTTGCAAGTTGAGTGGCCTAATCAAATC
CACATTCAGTCTTAAAAGGTTAGGTTCGTATCTCTTGAGTTCCAACAACTAATCATTAGGTTCATGTCTGTTCTCTGTT
CAGATAACTAGTAGCTCTGCCCTAGTTCCAGTTTATTAAGTGCGGATGTCACTTTCTTTCTGCAAGAACACTGTACCTG
CCATGGTCGTTTTCCCCGTCGACAGCGAAAGGGTTTTCAGAGAATAGCTGCAAGATAAGAACGGTTACAACATATCTAT
CCGCTAGGCAAGCGAGGGACTTCACCCATAATCTATCTAAATAAATAAAGAAACTTGACAATATTCTGAAGGTACCTTG
CCATTCTTGCTGGTTGATATCATGGGGAGACTGAAGTCACTAATTTTCGACGTGAGGGTCTTGTCCAGCAGATATTTTC
AATGTTGAGATCATTCTGTACAATTTCTGGGGCAGTCACATTGTGCAAGACTGGATCCCTCTCGCGATACCAATGCAGA
AAAACCCGC

G3116

AAGCTTTTTTATCGATTATATTCTATGATGCCGGCTACACGGGGAGCGGAGATGAGGGGACCTAGTCACCTTGCTGAGG
GAGTCCACCGCGAATGTGCCGCGGATCGTCGGGTCGGCCATGTCTTTCAGCATGTCCGGGTCTTCGGCCAGGGCCTCGC
TCAGCTACACGGATTGACACGGGATGCATATTCAGAATCAAAATGTAATAATTTGTTCAGAAGGACCAAGCTCTAAGCT
TTTTGGTGCTAGGAGTAGTATCTTTCAGTGTGATGTGAACATGAAGCTTGAGAATTGAGAGTCACCTGAGCTCTCAGGG
ATTCCAGCTCGCTTTTCGACGCTGCTGGTTTGCCTGTGATCACCTCGAGAAGGATTTGGCCGAACTGGTATATGTCATC
CCTGTCCCCTTGTTCTGCAGGTTGAGCGCTGCAAGCAGACAGAATCATAAACGTTTTAGGTTGCAAGTTGAGTGGCCTA
ATCAAATCCACATTCAGTCTTAAAAGGTTAGGTTCGTATCTCTTGAGTTCCAACAACTAATCATTAGGTTCATGTCTGT
TCTCTGTTCAGATAACTAGTAGCTCTGCCCTAGTTCCAGTTTATTAAGTGCGGATGTCACTTTCTTTCTGCAAGAACAC
TGTACCTGCCATGGTCGTTTTCCCCGTCGACAGCGAAAGGGTTTTCAGAGAATAGCTGCAAGATAAGAACGGTTACAAC
ATATCTATCCGCTAGGCAAGCGAGGGACTTCACCAATAATCTAAATAAATAAAGAAACTTGACAATATTCTGAAGGTAC
CTTGCCATTCTTGCTGGTTGATATCATGGGGAGACTGAAGTCACTAATTTTCGACGTGAGGGTCTTGTCCAGCAGATAT
TTTCAATGTTGAGATCATTCTGTACAATTTCTGGGGCAGTCACATTGTGCAAGACTGGATCCCTCTCGCGATACCAATG
GCAGCAGAAACCCGA

Left primer = 5’-CAGCGAAAGGGTTTTCAGAG-3’
Right primer = 5’-CCCATGATATCAACCAGCAA-3’
Polymorphism = 4-bp deletion in G3116
Expected product size = Tm53 (162-bp); G3116 (158-bp)

RFLP marker: CDO189

Tm53

GCCGGCAGTTAGTTAGTACCAAGGAGCAACTAGCATGAATGTTCTAAGCTTGTATTCATGCATAGTTTCTCCCCAGGCT
TTGAAGGAAGATTCAGTTTTCGTGGTCCCATCCAAAAGGTCAACTTTGGACAGGTGCAAGCTCCCGGTTGGGATCCGCC
TGTTCGTATCAGCTGGTCACACGGAATCAGACATCTCCAAAGTGTGTTCATCCTTGAAGAAGGTTTCTGCGTCGGTCCT
TTCAAACCACAATTGATTCCACGTTCCTTGAAAGACGAGAAGAGACGTTGGTCGTTTTCGACGCCCTGTGTACGGTAGC
CTGATTGTGGTTGGTATATGTACATAACAATTATGTACATGTGGACTATGTTGCTGCTTAAACTTCAGGTTGAGCAGGG
TTTTAGGTGTCGAAAAGTTCAGTTC

Following sequence is noise

GACAGAAAAACCCTGCTCAACCTGAAGTTTAAGCAGCAACATAGTCCACATGTACATAATTGTTATGTACATATACCAA
CCACAATCAGGCTACCGTACACAGGGCGTCGAAAACGACCAACGTCTCTTCTCGTCTTTCAAGGAACGTGGAATCAATT
GTGGTTTGAAAGGACCGACGCAGAAACCTTCTTCAAGGATGAACACACTTTGGAGATGTCTGATTCCGTGTGACCAGCT
GATACGAACAGGCGGATCCCAACCGGGAGCTTGCACCTGTCCAATGTTGACCTTTTGGATGGAACCACGAAAACTGAAT
CTTCCTTCAGAGCCTGGTGGAGAAACTATGCATGAATACAAAGCTTATGAACATTCATGCTAGTTGCATCTCAAAGTCT
AACTTACCTGCAAGGGCAAGGATATACAAAACTATC

G3116

GTTATCGCTCTCATATTTTTTCAAAGAGCATTCTCATGAATGTTGATAAGCTTGTATTCTGCATAGTTTCTCCACCAGG
CTTTGAAGGAAGATTCAGTTTTCGTGGTCCCATCCAAAAGGTCAACTTTGGACAGGTGCAAGCTCCCGGTTGGGATCCG
CCTGTTCGTATCAGCTGGTCACACGGAATCAGACATCTCCAAAGTGTGTTCATCCTTGAAGAAGGTTTCTGCGTCGGTC
CTTTCAAACCACAATTGATTCCACGTTCCTTGAAAGACGAGAAGAGACGTTGGTCGTTTTCGACGCCCTGTGTAAGGTA
GCCTGATTGTGGTTGGTATATGTACATAACAATTATGTACATGTGGACTATGTTGCTGCTTAAACTTCAGGTTCAGCAG
GGTTTTAGGTGTCGAAAAGTTCAGTTTCGACAG

Following sequence is noise

AAATACCCTGCTGAACCTGAAGTTTATGCAGCACCATAGTCCACATGTTCATAATTGTTATGTACATATACCAACCACA
ATCAGGCTACCGTACACAGGGCGTCGAAAACGACCAACGTCTCTTCTCGTCTTTCAAGGAACGTGGAACCAATTGTGGT
TTGAAAGGACCGACGCAGAAACCTTCTTCAAGGATGAACACACTTTGAGCTGTCTGATTCCGTGTGACCAGCTGTACGA
CAGGCGGATCCCAACCGGGAGCTTGCACCTGTCAAAGTTGACCTTTTGGATGGGACCACGAAAACTGAATCATCTTTCA
AGCCTGGTGGAGAAACTATGCATGAATACAAAGCTTATGAACCATTCATGCTAGTTGCATCTCAAGTCAAACTTACAGC
CCGGCATGGTTATC

Left primer: 5’-CTGGATCTTCTTGAAACCATTG-3’
Right primer: 5’-CTGTCGAAACTGAACTTTTCG -3’
Restriction enzymes: *Rsa*I (5’-GT/AC-3’)

RFLP marker: PSR113

Tm53

TGGAGGCAGTGAGGGGTCGGTGCGGCGCAGCGTCAAGTGGATCAGGTCAAGTGCTCCCGCGCCGTCTACGGCTGGTGAT
GAGCCGGCGATCCGTCCATCCATGGTTGCTCTGCCTCCGTCTGCTCCCGGTACGAGTTGTTGTGCAGCTGAGCTAGTAG
TACAGTAGTACGTATGAGCGCGCGCGTGTTGATGACTCTTCGGTTCTTTGCCCTTGTGCTTCCTCCTTTTTCTTTCATC
TTCTTGTAGTAGGATTTTTGACGGTATTTGATGTGGCATGTGTACACTAGTGTGAGCGGCGCTTCTGCTCGTGGTGTCC
TGCTTGTTGGCCACCGACAGAAGCTCCTCTGTGTTGTCCTTGTACATACGGATCCTTTCCTCCCCTTGTATCATCGTCT
ATCTGCTTATGTAATATAGACACTATGTAGAATAAGGTTGTGTCAGATGTAATTTACAACTCAGCAGATAGACGATGAT
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AGCAGAAGCGCCGCTCACACTAGTGTACACATGCCACATCAAATACCGTCAAAAATCCTACTACAAGAAGATGAAAGAA
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AGCTGCACAACAACTCGTACCGGGGAGCAGACGGAGGCAGAGCAACCATGGATGGACGGATCGCCGGCTCATCACCAGC
CGTAGACTGCGCGGAGCACTTGACCTTGATCCACTTGACGCTGCGCCCGCACCCGACCCTTCACCTTGCCCTCCCACGT
CGTACACCTTGTAGGCA

G3116

CCCGCTTCATTGATGGTGGGGTTGGTGCTGCGCAGCGTCAAGTGGATCAGGTCAAGTGCTCCCGCGCCGTCTACGGCTG
GTGATGAGCCGGCGATCCGTCCATCCATGGTTGCTCTGCCTCCGTCTGCTCCCGGTACGAGTTGTTGTGCAGCTGAGCT
AGTAGTACAGTAGTACGTATGAGCGCGCGCGTGTTGATGACTCTTCGGTTCTTTGCCCTTGTGCTTCCTCCTTTTTCTT
TCATCTTCTTGTAGTAGGATTTTTGACGGTATTTGATGTGGCATATGTACACTAGTGTGAGCGGCGCTTCTGCTCGTGG
TGTCCTGCTTGTTGGCCACCGACAGAAGCTCCTCTGTGTTGTCCTTGTACATACGGATCCTTTCCTCCCCTTGTATCAT
CGTCTATCTGCTTATGTAATATAGACACTATGTAGAATAAGGTTGTGTCAGATGTAATTACAACAAAGCAGATAGACGA
TGATACAAGGGGAGGAAAGGATCCGTATGTACAAGGACAACACAGAGGAGCTTCTGTCGGTGGCCAACAAGCAGGACAC
CACGAGCAGAAGCGCCGCTCACACTAGTGTACATGCCACATCAAATACCGTCAAAAATCCTACTACAAGAAGATGAA
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GCTCAGCTGCACAACAACTCGTACCGGGGAGCAGACGGAGGCAGAGCAACCATGGATGGACGGATCGCCGGCTCATCAC
CAGCCGTAGACGCGCGGAGCACTTGACCTTGATCCACTTGACGCTGCGCCGCACCGACCCCTTCACCTTGCCCTCCACG
TCGTACACCCTTGTAGGCA

Left primer: 5’-GCCTACAAGGTGTACGACGTG-3’
Right primer: 5’-TGTAATTTACATCTGACACAACC-3’
Restriction enzyme: *Nde*I (5’-CA/TATG-3’)
Expected product size: Tm53 (930-bp), G3116 (301-bp + 306-bp + 325-bp)

Flowering time gene marker: *TmVRN1*

Tm53

TACTTTGCTCTCGGATTGAGCAACTGGAGCAGCAGCTGGAAAGCTCACTGAAACATATCAGATCCAGGAAGGTACTGAT
TTAAATGATTTGATACAGCAGCACAATATATAAAAAAACAAGAAAAACACTTGCAGAGAAGTTCAGCAAAGTATATCTG
AAATCAGATTCTAGACTGAGATGTTCACAATATGTATATGCATTTTAGTCATATGCTCTTCATAGTTAAAAAATGACTA
ATTTTTTTCATTTTTTGTACTTGCAGAACCAACTTATGCACGAATCCATTTCTGAGCTGCAGAAGAAGGTAAGCTGTCA
ACCTTGCATACCTTATTCGGTATTCGAACTGGTCAACTTGTCATGAAGCCTTAGCTGGTTTCAAGATTTGTGACATTAT
AACATGTATGCAAGTAACTGGTCTACATGCACGTAACCTCATTACATCGTTCTTGCTGCAGGAGAGGTCACTGCAGGAG
GAGAATAAAGTTCTCCAGAAGGAAGTAAGCCCGTTATATCACCTTATGGTCCAACCGGTCTAAACTGTTCCGTATAGCA
AATTTTATTGACAGAGGTCCGTGTCCCTTCCCCACAGCTCGTGGAGAAGCAGAAGGCCCATGCGGCGCAGCAAGATCAA
ACTCAGCCTCAAACCAGCTCTTCTTCTTCTTCCTTCATGCTGAGGGATGCCTCCCTAGAACAGGATCAAAGAATAAAAA
AAACAACTGGTTTGAGGCTGAGTTTGATCTGCTGCAAGCAAGGGCCTTATGGTACACACGAGATGTAGGAAAGGACACG
AACTATTAAATAAAATTTTTTTACAGAACAATTTAGATCGTGGGACATATCGGGATATAACGGGCTTCCTTCGATTCTG
AAAGATAATTCCCACTGAAATACCTA

G3116

GGACTTTGAGGCGCGGCGTTACAGCAACTGGAGCAGCAGCTGGAAAGCTCACTGAAACATATCAGATCCAGGAAGGTAC
TGATTTAAATGATTTGATACAGCAGCACAATATATAAAAAAACAAGAAAAACACTTGCAGAGAAGTTCAGCAAAGTATA
TCTGAAATCAGATTCTAGACTGAGATGTTCACAATATGTATATGCATTTTAGTCATATGCTCTTCATAGTTAAAAAATG
ACTAATTTTTTTCATTTTTTGTACTTGCAGAACCAACTTATGCACGGATCCATTTCTGAGCTGCAGAAGAAGGTAAGCT
GTCAACCTTGCATACCTTATTCGGTATTCGAACTGGTCAACTTGTCATGAAGCCTTAGCTGGTTTCAAGATTTGTGACA
TTATAACATGTATGCAAGTAACTGGTCTACATGCACGTAACCTCATTACATCGTTCTTGCTGCAGGAGAGGTCACTGCA
GGAGGAGAATAAAGTTCTCCAGAAGGAAGTAAGCCCGTTATATCACCTTATGGTCCAACCGGTCTAAACTGTTCCGTAT
AGCAAATTTTATTGACAGAGGTCCGTGTCCCTTCCCCACAGCTCGTGGAGAAGCAGAAGGCCCATGCGGCGCAGCAAGA
TCAAACTCAGCCTCAAACCAGCTCTTCTTCTTCCTTCATGCTGAGGGATGCTCCCTAAACAGGATCTATGAAAAAA
GAAAAACAAGTGTGTTGCACCGCTCCGTTTCAGCCTGTCGTACTGATGCCCGTGCTGCCCC

Left primer: 5’-TCTCATGGGAGAGGATCTTGA-3’
Right primer: 5’-GGGAGCATCCCTCATCATGAA-3’
Restriction enzyme: *Bam*HI (5’-G/GATCC-3’)
Expected product size = Tm53 (917-bp), G3116 (510-bp + 284-bp)

Flowering time gene marker: *TmVRN2*

Tm53

TCGTTCGTACCCGCACCGATCGACCGCGAGGACTCTTCTCCCCCAAGCTTAACCGGCCTCCTGGATCGCCGTTGTCGAC
TCCGGAGGTGCCCACGCTGCTACACCCAACCCTCCCCCCACCAGCCACCCAACCCCTCGCTGTTGCGAGAGGAAACCAC
CGGTGGCCGGACGGCGGCGGCAGGATGCTTCCGCATCCCATCTTTTTTTTTCGTTTGGCATATTCTAATCCTCTCATCC
TGCGGGTTGTGGCTATGTTTATCTTTCTTAGTTTTTACTTATTTTTGCTCCTTTTGCGAGATATATTTGGATACCAGAT
CTTGTTTTTTGCTGCAACTGCTTTAATAATATGGTCACATGCATCTTTTGGATGCACACGCTGGGGGATATCCCCCTTT
TCAAAAAAAAATATTTATATTCACATCCTCTCGTCCCTCTACCGCTCCCTTGTCATGGGAACCATATGTCCCATCCTAC
AGCAACCGGGAGCACGCATATTTCTTCACGGCAGGTGAGACTGGTTGTGCGGGTCACTCGCTACCATGGCTAGAGGCGC
GTCCCTTATTGTGGTGGTGACTCACTATGTGGGTGGACTCCGGCAAGATCCGATCGGATTTGGCGTCGGTCTTTCTACT
GGATGAGGACTATTGCTGCGAGGTGCTGGCTTTCCACGTGTGCGGTGGCTGGCTGGCGGCGCTAAGATCCATGGGTTGG
TGGTGGTAATCCAGATCTGACCTGCCAGATGGTGTTGCTGCAAACTATACTGCAACTACTTCCTAGGGCTCCACGACGG
CAGTGGTCATTGAATGGTCCCTGGGAGGGGTCATCTCTCCTGGATCCAAAAATTTGGAATCGAACCGGGAGGAACTTTC
TTCCCCAAGCTAACCGGCCTCTGGAACGCGTTGTCAACTCGGAGGTGCCCTCTTCTCCAACCCCCCCCCAACAACAAAG
CCTTTTTTTTTTTAGAAAAAAAAACAACTGCGGGCCCGCGGGGCGGGAGAACACCACCCCATTT

G3116

AGTATCCCTAAGTCCATAGACCGCGAGGACTCTTCTCCCCCAAGCTTAACCGGCCTCCTGGATCGCCGTTGTCGACTCC
GGAGGTGCCCACGCTGCCACACCCAACCCTCCCCCCACCAGCCACCCAACCCCTCGCTGTTGCGAGAGGAAACCACCGG
TGGCCGGACGGCGGCGGCAGGATGCTTCCGCATCCCATCTTTTTTTCGTTTGGCATATTCTAATCCTCTCATCCTGCGG
GTTGTGGCTATGTTTATCTTTCTTAGCTTTTACTTATTTTTGCTCCTTTTGCGAGATATATTTGGATACCAGACCTTGT
TTTTTGCTGCAACTGCTTTAATAATATGGTCACATGCATCTTTTGGATGCACACGCTGGGGGATATCCCCTTTTCAAAA
AAAAATATTTATATTCACATCCTCTCGTCCCTCTACCGCTCCCTTGTCATGGGAACCATATGTCCCATCCTACAGCAAC
TGGGAGCACGCATATTTCTTCACGGCAGGTGAGACTGGTTGTGCGGGTCACTCGCTACCATGGCTAGAGGCGCGTCCCT
TATTGTGGTGGTGACTCACTATGTGGGTGGACTCCGGCAAGATCCGATCGGATTTGGTGTCAGTCTTTCTACTGGATGA
GGACTGTTGCTGCGAGGTGCTGGCTTTCCGCGTGTGCGGTGGCTGGCTGGCGGCGCTAAGATCCATGGGTTGGTGGTGG
TGATCCAGATCTGACCTGCCAAATGGTGTTGCTGCAAACTATACTGCAACTACTTCCTAGGGCTCCACGACGGCAGTGG
TCATTGAATGGTCCCTGGGAGGGGTCATCTCTCCTGGATCCACTAACACTCAATTAGACCAGCGAGGACTTTCTCCCAA
GCTTAACGGCCTCTGGATCGCTTTGTCACTCGGATGTGCCCGGCTCACCAACCTCCCCACCAGCA

Left primer: 5’-CATCCATGCTATTATCATGG-3’
Right primer: 5’-TGGATCAGGAGAGATGACCC-3’
Restriction enzyme: *Bgl*II (5’-A/GATCT-3’)
Expected product size = Tm53 (333-bp + 411-bp + 308-bp), G3116 (974-bp)

Flowering time gene marker: *TmVRN3*

Tm53

AGGGGGATTGTGGGGAGTGCTGGACCCTTTGTCCGGACCCAACCTCAGGGTGACCTTCGGGAACAGGACCGTGTCCAAC
GGCTGCGAGCTCAAGCCGTCCATGGTCGCCCAGCAGCCCAGGGTTGAGGTGGGCGGCAATGAGATGAGGACCTTCTACA
CACTCGTACGTACACAGTCACTATCTAATGCCTATATGTTAAGCTCTGAAAGTGCTCGCCACACGCACATGATCGATCG
GGCTCTATATATAGTACGTGCGGGAAGATGATTATCGATGCTTCTGTTCACAGCATGTTTGTCTTGGCAGGCACATGAC
TAATGCTCCATCTTGCATATGGCTCTGTGCTAGCTCTCTGCTGTTCATCATGATTTTCTATGCTTCTTTTCTATTCGGG
GAACACTGATTTTCGATGCTTCTGTTGACATGTTTTATGTTTGTTCTGGCAAGCACACGACTAATTAAAGCTCGATCTT
AAATATATGCTTATGCACGTAGTACTCTCTACATCTCTAGTATTGATCATGAT[GTGCAC]GCGTGTACTGCCCGCAGGTG
ATGGTAGACCCAGATGCTCCAAGTCCAAGCGATCCCAACCTTAGGGAGTATCTCCACTGGTAAGTAAATTTGTAGCTCA
GTTGAATAATTTCTCTTTCCCTAGATATACACACTAGCTCATGTGTGTGTGT[GTGCAC]GCGCGTGTGCATCTACATGTG
TGTGCAGGCTTGTGACAGATATCCCCGGTACAACTGGTGCCTCGTTCGGGCAGGAAGTGATGTGCTAGAGAGCCCTCGC
GATCCAGGG

G3116

TCGGGACTTGGGGAGTGCTGGGCCCCTTTGTCCGGACCCAACCTCAGGGTGACCTTCGGGAACAGGACCGTGTCCAACG
GCTGCGAGCTCAAGCCGTCCATGGTCGCCCAGCAGCCCAGGGTTGAGGTGGGCGGCAATGAGATGAGGACCTTCTACAC
ACTCGTACGTACACAGTCACTATCTAATGCCTATATGTTAAGCTCTGAAAGTGCTCGCCACACGCACATGATCGATCGG
GCTCTATATATAGTACGTGCGGGAAGATGATTATCGATGCTTCTGTTCACAGCATGTTTGTCTTGGCAGGCACATGACT
AATGCTCCATCTTGCATATGGCTCTGTGCTAGCTCTCTGGTGTTCATCATGATTTTCTATGCTTCTTTTCTATTCGGGG
AACACTGATTTTCGATGCTTCTGTTGACATGTTTTATGTTTGTTCTGGCAAGCACACGACTAATTAAAGCTCGATCTTA
AATATATGCTTATGCACGTAGTACTCTCTACATCTCTAGTATTGATCATGAT[GTGCAC]GCGTGTACTGCCCGCAGGTGA
TGGTAGACCCAGATGCTCCAAGTCCAAGCGATCCCAACCTTAGGGAGTATCTCCACTGGTAAGTAAATTTGTAGCTCAG
TTGAATAATTTCTCTTTCCCTAGATATACACACTAGCTCATGTGTGTGTGT[GCGCGC]GCGCGTGTGCATCTACATGTGT
GTGCAGGCTTGTGACAGATATCCCCGGTACAACTGGTGCCTCGTTCGGGCAGGAAGGATGTGCTAGAGAGCCCTCGCGA
CCAAGGGCCA

Left primer: 5’-AGAGACCCGCTGGTGGTT-3’
Right primer: 5’-CACGAGCACGAAGCGATG-3’
Restriction enzyme: *Apa*LI (5’-G/TGCAC-3’)
Expected product size = Tm53 (546-bp + 157-bp + 132-bp), G3116 (545-bp + 289-bp)

Flowering time gene marker: *TmPPD1*

Tm53

AACGTTGTACATCATCGGGTCCGGTGGAGGTCTGCTGCCACTATGGTAGCAACTCTGGCAGTAATAACAACACCAACAA
TGGGAGCACCGCAGCTACTGCTTCTGGTGCTGCTGCTGCTGTACATGCTGAGACCGGTGGCATCGACAAAAGAAGCAAC
ATGATGCACATGAAACGGGAGCGCCGGGTGGCCGCCGTGAACAAGTTCAGAGAGAAGAGAAAAGAGAGGAACTTCGGGA
AGAAGGTACTGGGTTTTTCCGAAAGCCGATTCCGTCTGTTCTCTGTTCTTCGTTTCATTCTTCTGATTGGGTTTTGTTC
GTGATAGCTGATGAAAAATGGGTAACTCATTTTGCAGGTGCGTTACCAGAGCAGGAAGAGACTGGCCGAGCAGCGCCCG
CGGGTGCGCGGGCAGTTCGTGCGGCAGCCGCCACCGCCGGCTGCCGTGGAGAGATAACCTCCCGCCACACACCTAGCTA
TACCTAGTACGTAATACTATTTAGACAGCTGATTCTTGTGTCTTTGGCCATGGATCATTCAGAGACGGTCCTCATGCAT
CTCGTCTCATCTCCTGGAAGAAGAAGAATAAC[AAGCTACTCCTC]CCGCCTGCCTTAGCTACCTACCATAGAACATGCCG
GCGGAGGAGGACGACTGTAGAGTACACAGTACGTAGAAGTGCGGAGTCGGTCGATCAGACAGGCTCCCGAGCGCTAAGT
AGACGTTAAAA

G3116

CGACTGCTCTACGCGAATCTATAAGATAAGTAAGGTATGCTAAGAACTATGGTAGCAACTCTGGCAGTAATAACAACAC
CAACAATGGGAGCACCGCAGCTACTGCTTCTGGTGCTGCTGCTGCTGTACATGCTGAGACCGGTGGCATCGACAAAAGA
AGCAACATGATGCACATGAAACGGGAGCGCCGGGTGGCCGCCGTGAACAAGTTCAGAGAGAAGAGAAAAGAGAGGAACT
TCGGGAAGAAGGTACTGGGTTTTTCCGAAAGCCGATTCCGTCTGTTCTCTGTTCTTCGTTTCATTCTTCTGATTGGGTT
TTGTTCGTGATAGCTGATGAAAAATGGGTAACTCATTTTGCAGGTGCGTTACCAGAGCAGGAAGAGACTGGCCGAGCAG
CGCCCGCGGGTGCGCGGGCAGTTCGTGCGGCAGCCGCCACCGCCGGCTGCCGTGGAGAGATAACCTCCCGCCACACACC
TAGCTATACCTAGTACGTAATACTATTTAGACAGCTGATTCTTGTGTCTTTGGCCATGGATCATTCAGAGACGGTCCTC
ATGCATCTCGTCTCATCTCCTGGAAGAAGAAGAATAAC[AAGCTACTCCTCCTC]CCGCCTGCCTTAGCTACCTACCATAG
AACATGCCGGCGGAGGAGGACGACTGTAGAGTACACAGTACGTAGAAGTGCGGAGTCGGTCGATCAGACAGGCTCCCGA
GCGCTAAGTAGTACGTAAGA

Left primer: 5’-GTCTTTGGCCATGGATCATT-3’
Right primer: 5’-GACCGACTCCGCACTTCTAC-3’
Polymorphism: 3-bp deletion in Tm53
Expected product size: Tm53 (171-bp), G3116 (174-bp)

Flowering time gene marker: *TmVRT2*

Tm53

CGTACATGTTTAGCAAGAGTCCATCGTCATGCACTCTGTTACTACAATGGATAAGTCCAGAGAGTTGGCTAGACCGACT
GACATGGAGGAGTCCATAAGAAGATTGAGACTGAGTATCACCAGACTAGGCCATGGACAGGGGCATGTGCAGCTACTAT
CCATGTGCAGAACTATGAGTTGGTTGCGAGATCTGTTGGGTTTCACCTCTAGCCTACCCCAACTTGTTGGGACTAAAGG
CTTTGTTGTTGTTGTTGTTGGTGGTGTTGTTGTATATTGAATGGAATGTTATCAGCAATATATCCCTTTATGTGTGGTT
TTAAAATTTTATTATTTATTTAAGTGGAAGAAATTATTCTTTTACCACCAACACAGTCAATGGAGTTGCTCCCTGATTG
CATTATTTTTATTATAAACTATTCAGCAACACAAAATTGACCAGAGTTTATTGGTTTTGAGAATATTTTTGGTGTACTT
TTACAAGGTGAACATTTGATAATGTAATTTCTCTTTTCCTTGTAGGGAACACAGCTGGCAGAGGAAAATATGCGCTTGA
AAAACCAAGTAATAAGATTTTAGATGCATTCGTAGTCCTGCACTTAATCAATCTTTATAAGAAGTGCAGCTTGAACAAA
AGTCCAATAAAAGTTGGGGTGGCAGAGCACACATTTACATCATGTTCGTGTGCTTGGATGTAGATGCATGAGGTGCCAA
CTGCTAGCACGGTGGCCGTTGCCGAAGCCGAAAATGTTGTCCCTGAAGATGCTCATTCATCTGACTCTGTGATGACGGC
AGTACATTCGGGAAGCTCACAGGACAATGATGACGGTTCTGATATATCCTTGAAACTTGCGTGAGTTTCGCCCAGAAAC
CCTTGCTCATCATCTTTATCCTGATCATATGCTAGTCTCAAAATGCCTTAATAGAATCTGCGACATGAGAAAAAATATA
TATAGTAGTTACTCCCTTCGTTCCCAAATTGTATATTTTGTACTCCCTCTGTACTATACTTTTGGGACGGAGGGAGTAC
TATTATGGGACGGAGGGAGTAGTTAGCTTTGATAATTGGAGATGAGTCTGACAACTCTAATTTCAATGTGTGTTTGTCG
GTTTTTATTTTTGAGGGAACCAATGTGTGTTTGTCACCTGACTGAGTTACCAACAGTTTCTATCATCAATTCTGAAATG
TGGCAGATTCTTCTTCACTCAACAGGTTACCTGGAAGTAAGGACCCTGGGGAGTATCACAGTTTAAATGGGCACTAACA
GAGCAAGTATTCATTAGTT

G3116

CTTCCTGAACTTGCAGTTATGTAACCAGGTCAATGTCATGGATCTTTTTCTTCCCATGGATATGTCAGAGAGGTGGGTA
ACGACTGACATGAAGAGTTCATAAGGAAGATCGAGACTGAGTATCACCAAGAACTAGGCATGGACAGGGCATGTGGAGC
TACTATCATGTGCCAGACTATGAGTTGGTGCGAGATCTGTGGGTTTCACTCTAGCTACCCCACTAGTTTGGACTAAAGG
CTTGTGTTGTGGTGGTGGTGGTGTTGTTGTATATTTGAATGGAATGTTATCAGCAATATATCCCTTTATGTGTGGTTTT
AAAATTTTATTATTTATTTAAGTGGAAGAAATTATTCTTTTACCACCAACACAGTCAATGGAGTTGCTCCCTGATTGCA
TTATTTTTATTATAAACTATTCAGCAACACAAAATTGACCAGAGTTTATTGGTTTTGAGAATATTTTTTGGTGTACTTT
TACAAGGTGAACATTTGATAATGTAATTTCTCTTTTCCTTGTAGGGAACACAGCTGGCAGAGGAAAATATGTGCTTGAA
AAACCAAGTAATAAGATTTTAGATGCATTCGTAGTCCTGCACTTAATCAATCTTTATAAGAAGTGCAGCTTGAACAAAA
GTCCAATAAAAGTTGGGGTGGCAGAGCACACATTTACATCATGTTCGTGTGCTTGGATGTAGATGCATGAGGTGCCAAC
TGCTAGCACGGTGGCCGTTGCCGAAGCCGAAAATGTTGTCCCTGAAGATGCTCATTCATCTGACTCTGTGATGACGGCA
GTACATTCGGGAAGCTCACAGGACAATGATGACGGTTCTGATATATCCCTGAAACTTGCGTGAGTTTCGCCCAGAAACC
CTTGCTCATCATCTTTATCCTGATCATATGCTAGTCTCAAAATGCCTTAATAGAATCTGCGACATGAGAAAAAATATAT
ATAGTAGTTACTCCCTCCGTTCCCAAATTGTATATTTTGTACTCCCTCTGTACTATACTTTTGGGACGGAGGGAGTACT
ATTATGGGACGGAGGGAGTAGTTAGCTTTGATAATTGGAGATGAGTCTGACAACTCTAATTTCAATGTGTGTTTGTCGG
TTTTTATTTTTGAGGGAACCAATGTGTGTTTGTCACCTGACTGAGTTACCAACAGTTTCTATCATCAATTCTGAAATGT
GGCAGATTCTTCTTCACTCAACAGGTTATCTGCAAGTAGACAGCTGCAAGTATCACAGATATATATGTAATATAGCGCA
AGTATTGCATAAGTT

Left primer: 5’-CATGAGGTGCCAACTGCTAG-3’
Right primer: 5’-GCGAAACTCACGCAAGTCTCA-3’
Restriction enzyme: *Dde*I (5’-C/TNAG-3’)
Expected product size: Tm53 (164-bp), G3116 (142-bp + 22-bp)

Flowering time gene marker: *TmGI*

Tm53

GGCTAGCATGGGGGTATCGAGGCTCTCGATCTCCTAAGATCTGTTATCTCCAAAAACAGGAACTATGCTTCTCTGTTGT
CTCATTGCTCTGGCATAAGCTTATTGCATCTCCTGAAACGCAGATGTCTGCAGAAAGTACATCAGCTCATCAAGGTTGG
AGAAAGGTATGATTCGTGTGGCCATTGGCATAACACAACAATGCTTCAGTGACATTCTAATTTTGTTATCTTATTAACT
GTATATCTGACATTTTTTTTACGTATAGGTTGTAGATGCACTTTGTGATGTTGTTTCAGCCTCACCAGCCAAGGCTTCA
ACTGCTATTGTTCTGCAGGTAAAGATTTATTCAGACATTTGGTCGCAATGAAATTTGGTTCAGATTTTGGCTTCTTCTC
CCTTGTTCATTTCTCACGTGGGAACATACATTATCTTCATGACGTATAAATATTTCCTGTCATATTCATGGACTCTTGT
ATGCAGGCTGAGAAGGACCTACAGCCCTGGATTGCTCGAGATGACGAGGAAGGTCAGAAGATGTGGAGAGTCAACCAGC
GAATAGTTAAACTGATAGCTGAGCTTATGAGGAACCATGATAGCCCAGAAGCATTGATAATTCTTGCTAGTGCTTCAGA
CCTTCTGCTTCGTGCTACAGATGGGATGCTTGTCGACGGTGAAGCTTGTACCTTGCCTCAGTTAGAGGTAAATACATAT
ACAAACTTCCTTCACAGAATACCACAAACATATCATCTATCGTACTTTGTTGTCGTTAGAGCAGCATCGACTCATTTTC
TTTCTTGAAAGTTGCATGTCATCGATTCTTTCTGACAACATTGCTTCCTGTGATTGAATATGCACAGCTCCTGGAAGTA
ACTGCTAGAGCTATTCATCTCATCGTTGAATGGGGAGACCCAGGCGTAGCAGTTGCTGATGGCCTCTCAAATCTGTTAA
AGGTATGCCCTGCTCAGATGTCGCCCCAATTCTGTTCATCAGAAACACACCAACGTTCAGAATGCTAACATTCATGTGC
CGCATCCTTTACCCGG

G3116

GTCATCATGGGGGTATCGGGCGCTAGATCTCCTAACAGCTGTTATCTCAAAAAACAGGAACTATGCTTCTCTGTTGTCT
CATTGCTCTGGCATAAGCTTATTGCATCTCCTGAAACGCAGATGTCTGCAGAAAGTACATCAGCTCATCAAGGTTGGAG
AAAGGTATGATTCGTGTGGCCATTGGCATAACACAACAATGCTTCAGTGACATTCTAATTTTGTTATCTTATTAACTGT
ATATCTGACATTTTTTTTACGTATAGGTTGTAGATGCACTTTGTGATGTTGTTTCAGCCTCACCAGCCAAGGCTTCAAC
TGCTATTGTTCTGCAGGTAAAGATTTATTCAGACATTTGGTCGCAATGAAATTTGGTTAAGATTTTGGCTTCTTCTCCC
TTGTTCATTTCTCACGTGGGAACATACATTATCTTCATGACGTATAAATATTTCCTGTCATATTCATGGACTCTTGTAT
GCAGGCTGAGAAGGACCTACAGCCCTGGATTGCTCGAGATGACGAGGAAGGTCAGAAGATGTGGAGAGTCAACCAGCGA
ATAGTTAAACTGATAGCTGAGCTTATGAGGAACCATGATAGCCCAGAAGCATTGATAATTCTTGCTAGTGCTTCAGACC
TTCTGCTTCGTGCTACAGATGGGATGCTTGTCGACGGTGAAGCTTGTACCTTGCCTCAGTTAGAGGTAAATACATATAC
AAACTTCCTTCACAGAATACCACAAACATATCATCTATCGTACTTTGTTGTCGTTAAAGCAGCATCGACTCATTTTCTT
TCTTGAAAGTTGCATGTCATCGATTCTTTCTGACAACATTGCTTCCTGTGATTGAATATGCACAGCTCCTGGAAGTAAC
TGCTAGAGCTATTCATCTCATCGTTGAATGGGGAGACCCAGGCGTAGCAGTTGCTGATGGCCTCTCAAATCTGTTAAAG
GTATGCCCTGCTCAGATCTCGCCCCAATTCTGTTCATCAGAAACACACCAACGTTCAGAATGCTAACATTCATGTGCCG
CAGGCTTTCCGCGG

Left primer: 5’-TTTGGCGAACTTCCTTACCATG-3’
Right primer: 5’-TCCCACGTGAGAAATGAACA-3’
Restriction enzyme: *Mse*I (5’-T/TAA-3’)
Expected product size: Tm53 (254-bp + 186-BP), G3116 (252-bp + 143-bp + 43-bp)

VITA

Chor Tee Tan

Candidate for the Degree of

Master of Science

Thesis: GENETIC LOCI FOR VERNALIZATION REQUIREMENT DURATION IN WINTER WHEAT

Major Field: Plant and Soil Sciences

Biographical:

Education:
Received Bachelor of Science degree in Plant and Soil Sciences from Oklahoma State University, Stillwater, Oklahoma in Spring 2006. Completed the requirements for the Master of Science in Plant and Soil Sciences at Oklahoma State University, Stillwater, Oklahoma in December, 2008.

Experience:
Received OSU Wentz Project Award for the 2005-2006 academic Year to conduct independent research with the guidance of Dr. Shipeng Deng; Employed by Oklahoma State University, Department of Plant and Soil Sciences, as a graduate research assistant; Oklahoma State University, Department of Plant and Soil Sciences, 2006 – present.

Professional Memberships: CSSA

Name: Chor Tee Tan Date of Degree: December, 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: GENETIC LOCI FOR VERNALIZATION REQUIREMENT DURATION IN WINTER WHEAT

Pages in Study: 57 Candidate for the Degree of Master of Science

Major Field: Plant and Soil Sciences

Scope and Method of Study:

The purpose of this study was to identify key genetic loci responsible for vernalization requirement duration in winter wheat. Mapping information from diploid species will be used to make a cross-map in common wheat, which should facilitate our long-term goal to eventually clone genes responsible for vernalization requirement duration.

Findings and Conclusions:

In this study, an F_2 population was generated from a cross between two diploid winter wheat *T. monococcum* accessions G3116 and Tm53 that respectively required 2 weeks and 5 weeks of low temperatures to attain the vernalization saturation point. Each of 94 F_2 plants was split to produce two populations having identical genetic backgrounds: one was vernalized for 2 weeks to satisfy a full vernalization requirement for G3116 but not for Tm53, and the other was not vernalized as a control. A total of 114 markers were mapped, including 104 SSR markers transferred from hexaploid wheat and 10 PCR markers developed for 6 known flowering time genes and 4 RFLP loci. A major QTL *QVrd.osu.7A*^{m} on the long arm of chromosome 7A^m explained 22.8% of the total phenotypic variation in the 2 weeks vernalized population. *TmVRN2* but not five other flowering time genes (*TmVRN1*, *TmVRN3*, *TmPPD1*, *TmGI*, and *TmVRT2*) was detected a significant genetic effect on the phenotypic variation in the vernalized population. A genetic model was established to understand the variation in vernalization requirement duration in winter wheat.

ADVISER'S APPROVAL: Dr. Liuling Yan