University of Alberta

Understanding the flowering gene complex in hexaploid spring wheat (*Triticum aestivum* L.)

by

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Abstract

Understanding the flowering gene complex in bread wheat is important to utilize the maximum production potential in a time-limited growing season. Vernalization (*Vrn*), photoperiod (*Ppd*) and earliness *per se* are the three genetic constituents of the flowering gene complex. A series of experiments were conducted to explore the role of each genetic constituent in Canadian spring wheat germplasm. The first experiment revealed vernalization and photoperiod gene frequency and effects in Canadian germplasm released between 1885 and 2008. Vernalization insensitive *Vrn-A1a* is the most frequent allele in the Canadian hard red spring class and *Vrn-B1* is more frequent in Canadian soft white spring class. The photoperiod sensitive allele (*Ppd-D1)* is being replaced with the insensitive allele, *Ppd-D1a,* in all modern Canadian germplasm. An overall analysis of the data for the CWRS class revealed an average reduction of 0.04 days/year in maturity and an increase of about 8 kg/ha/year in grain yield. A second study was conducted on the Cutler × AC Barrie population to map and characterize the earliness *per se* QTL. Three QTLs of earliness *per se* affecting days to flowering and maturity were mapped on chromosomes 1B (*QEps.dms-1B1* and *QEps.dms-1B2*) and 5B (*QEps.dms-5B1*). Two grain yield QTLs were mapped on chromosome 5B. The photoperiod insensitive allele *Ppd-D1a* interacted in an additive fashion with QTLs for flowering and maturity times. The earliness *per se* QTL *QFlt.dms-5B.1* together with *Ppd-D1a* can induce earlier flowering and could help to elongate grain filling duration for higher grain yields. A comparative study of *Vrn* alleles suggests that the presence of *Vrn-D1* along with *Vrn-A1a* and *Vrn-B1* confers higher grain yield with similar maturity compared to all other *Vrn* gene groups in soft white spring wheat and we suggested the inclusion of *Vrn-D1* allele in elite Canadian germplasm, which has been overlooked in the past. In a final study we found that early flowering and maturity conferred by insensitive vernalization alleles *Vrn-A1a* and/or *Vrn-B1* has not conferred any yield advantage under organic conditions. The results also revealed that there is significant genotype × environment interaction and breeding for organic production should be practiced under organic conditions.

Preaface

For reproductive success and maximum yield the time of flowering plays a central role in wheat. Therefore, it is really important to better understand the underlying genetics in order to manipulate the time to flowering. I collected the germplasm studied in chapter 2 during April 2009, and packed the seed for sowing after testing their germination percentage and 1000 grain weight. I participated in all the planning steps and sowing of the experiment with the cereal staff at Edmonton research station (ERS), University of Alberta. I was responsible for taking notes for days to flowering and maturity, which were recorded every alternate day, throughout the flowering and maturity season. I helped in recording plant height and harvesting the experiment. I did plant yield per plot and grain protein analysis myself. I conducted the experiment during 2010 and 2011 with almost similar responsibilities at ERS as described above. I was responsible to pack and ship the seed for sowing at Agriculture Agri-Food Canada (AAFC) Lethbridge research station, Lethbridge. Notes on days to flowering and days to maturity at AAFC Lethbridge, were taken by Dr. Harpinder Singh Randhawa during 2009 and 2011.

I was responsible for laying out the experiment and planting it in greenhouse, and taking notes on all the traits under study during 2009 and 2010. Greenhouse staff at University of Alberta was responsible for watering and fertigation. I used to get occasional help in harvesting the greenhouse experiment. I was responsible for screening the three vernalization genes (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*) and a photoperiod allele (*Ppd-D1*) for all 102 lines/cultivars under study.

I joined the Cutler x AC Barrie population study presented in chapter 3 during 2009. The population development, field and greenhouse phenotyping for the years 2007 and 2008, and SSR marker screening was done before I joined the study. I was responsible for field experiment during 2011 and 2012 and extracting and shipping the DNA to Diversity Array Technology (DArT) for high throughput genotyping. I was responsible for data analysis and QTL mapping and was kindly guided by Dr. Rong-Cai Yang.

For comparative study of different vernalization genes presented in chapter 4, I was responsible for helping the seeding and harvesting at ERS and Ellerslie research station, University of Alberta. The seeds were packed and provided by Dr. Harpinder Singh Randhawa during 2010, 2011 and 2012. I recorded days to flowering and maturity

at ERS only, and grain yield per plot, test weight and grain protein content for both Edmonton and Ellerslie locations. The experiment at AAFC Lethbridge was managed by Dr. Harpinder Singh Randhawa.

The breeder seed collection for the study presented in chapter 5, on Canadian germplasm studied at organic and conventional field was kindly done by Klaus Strenzke. For seed packing, layout and randomization I got help from cereal group at University of Alberta. I was responsible for taking notes on all the traits under study in both conventional and organically managed lands during 2010, 2011 and 2012, however, I got help in seeding, harvesting and recording plant height.

I was responsible for all the data analysis and interpretation presented in all the studies of this thesis.

Atif Kamran

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1.1 Introduction

Wheat (*Triticum aestivum* L.*)*, the staple of more than 40 countries of the world, is grown from South America to Australia and from northern Canada to China (Bushuk, 1998). Wheat is cultivated on 225 million ha globally and occupies the largest area under any single crop in the world (FAO stat, 2009). Total wheat production in 2009 was 681 million t with an average yield of 2.5 t/ha (FAO stat, 2009). Generally, most successful crops are grown in between 30˚ to 60˚ N and 27˚ to 40˚ S and from sea level to altitudes of about 3000 meters above sea level (masl) (Nuttonson, 1955; Percival, 1921 as cited by Curtis et al. 2002). Optimum growth temperature for wheat is 25 ˚C, but satisfactory yield can also be achieved from temperature ranges of 3 ˚C to 32 ˚C during different wheat growth phases. Wheat is adapted to a wide range of soil moisture; from xerophytic to littoral soil conditions (Curtis et al. 2002). Wheat has enormous genetic potential to time its flowering, and this characteristic is the central pivot for its global adoptability. Wheat provides more than 20% of the calories and protein to almost 35% of the world's population (Bushuk, 1998).

Hexaploid or bread wheat has a very large genome, with an estimated size of about 16.4 Gb (Arumuganathan and Earle 1991). This allo-hexaploid species was formed by a two-step natural hybridization of three species. In the first step, allo-tetraploid *Triticum turgidum* L. evolved as a result of hybridization between *Triticum urartu* Tumanian ex Gandilyan (2*n* = 2*x* = 14 AA) (Dvorak et al. 1993) and *Aegilops speltoides* (Tausch) (2*n* = 2*x* = 14 BB) (Dvork and Zhang 1990). In the second step, *Triticum turgidum* L. (2*n* = 4*x* = 28 AABB) hybridized with *Aegilops tauschii* Coss. (2*n* = 2*x* = 14 DD) and resulted in the bread wheat genome (2n = 6x = 42 AABBDD). The hexaploid wheat genome is highly redundant with more than 80% of its content consisting of repetitive DNA

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sequences (Moore 1995). The three genomes (A, B and D) share substantial co-linearity among each other and with other members of *Poaceae* family (Paterson et al. 2000; Devos 2005). This synteny among the different species has helped scientists to construct high density maps and to perform positional cloning of some major genes (Yan et al., 2003, 2006). The physical map construction in bread wheat remains a challenge and lags behind other members of the poaceae family like rice (*Oryza sativa*) and maize (*Zea mays* L.).

1.2 Genetic constituents of flowering and maturity

Wide adaptation with disease resistance, high yield, and good bread making quality have been the general goals of wheat breeders for many years. Adaptation to diverse environmental conditions depends mostly on flowering time (Whitechurch and Slafer, 2002). Wheat has marvelous genetic potential to flower over a wide range which enables breeders to exploit this potential by defining the flowering time of wheat cultivars according to environmental conditions of a specific geographical region. This genetic potential is due to three main gene groups: vernalization response genes (*Vrn* genes), photoperiod response genes (*Ppd* genes) and earliness *per se* (*Eps* genes) (Snape et al. 2001). Genes responsible for flowering time are thought to be carried on all chromosomes (Law and Worland 1997). Vernalization genes have a major contribution in flowering time and are carried by group 5 chromosomes. Photoperiod genes are located on group 2 chromosomes. Earliness *per se* genes are inferred by detection of QTLs with small effect that are found on many chromosomes including 3A, 5A, 2B, 5B and 2D.

The present objective is to comprehensively review the physiological, molecular and biological aspects of the three genetic constituents of flowering and maturity time in wheat. Reviews written in the past have generally focused on either one of the aspects; and generally are focused one of the three genetic constituents of the flowering time. The current review provides a) an overview of all three flowering gene groups, *i.e* vernalization, photoperiod and earliness *per se*; b) the primer sequences and annealing temperatures for all known

vernalization and photoperiod alleles and c) the QTLs affecting flowering and/or maturity time in wheat reported to date.

1.2.1 Vernalization Response

1.2.1.1 Definition

Vernalization is a plant response to non-freezing low temperature to accelerate the induction of reproductive growth. The response of vernalization sensitive plants depends upon two factors: intensity of temperature and duration of exposure (Wang et al. 1995; Rawson, et al. 1998). Temperature intensity consists of a range of low temperatures, and vernalization requirement may be satisfied between -1.3 to 15.7 °C (Porter and Gawith 1999). Data on duration of exposure, reported in previous studies, implies a linear response of wheat plants to vernalization days (VD) (Streck et al. 2003), which suggests that vernalization response is quantitative in nature and increases with duration.

1.2.1.Wheat growth habits and vernalization

Winter, facultative and spring growth habits are conferred by different vernalization alleles. Winter wheat requires vernalization for the induction of reproductive growth and sensitive cultivars do not flower until their vernalization requirement is satisfied. A vernalization insensitive gene *VRN2* is reported to confer winter growth habit in diploid wheat (Dubcovsky et al. 1998; Yan et al. 2004) and has been characterized as a 'floral repressor'. The gene *VRN2* transcribes a protein with a zinc-finger motif, which inhibits flowering gene expression (Trevaskis et al. 2007). Studies on reciprocal gene expression suggest a decrease in expression level of *VRN2* at low temperatures (Yan et al. 2004). This eventually allows flowering genes (*VRN1*) to express and induce the flowering, after being exposed to continuous low temperature or vernalization. Another theory is that it is actually the expression of *VRN1* that represses *VRN2* expression for flowering induction (Loukoianov et al. 2005).

Facultative and spring growth habits are induced by insensitive vernalization genes and are termed as *Vrn-1* or *Vrn-2* series genes in the literature. *Vrn-1* or *VRN1* series genes are three orthologous genes: *Vrn-A1, Vrn-B1,* and *Vrn-D1* located on chromosome 5A, 5B and 5D, respectively (Law et al. 1976; Dubcovsky et al. 1998). *Vrn-2* series genes are *Vrn-D4* and *Vrn-D5* on chromosome 5D, and *Vrn-B3* on chromosome 7B (Goncharov 2004; Yoshida et al. 2010). The vernalization insensitive genes have a dominant and epistatic effect on vernalization sensitive genes and the presence of any single insensitive allele will confer facultative or spring growth habit. Emmer wheat (*Triticum dicoccoides*) is considered to be the ancestral donor parent for *Vrn-A1* only (Tsunewaki, 1962,) and/or for *Vrn-A1* and *Vrn-B1* both (Rigin et al. 1994); whereas *Vrn-D1* derives from *Aegilops tauschii* (Goncharov and Chikida, 1995).

1.2.1.2 Molecular basis of vernalization

The molecular basis of vernalization insensitivity was characterized by Yan et al. (2003, 2004) and Fu et al. (2005). Yan et al. (2004) studied and sequenced isogenic lines and accessions from hexaploid and tetraploid wheat, and revealed at least five different *Vrn* alleles due to insertion and/or deletion mutations at the *Vrn-A1* locus. *Vrn-A1a* was characterized as an insertion of a large (222-bp) and a small (131-bp) foldback fragment at the same site within the promoter region which created a host direct duplication (HDD). *Vrn-A1b* evolved from two mutations in HDD and a deletion of 20-bp in the 5´ Un-Translated Region (UTR), and *Vrn-A1c* due to a large deletion in intron 1 (Table 1-1). The alleles *Vrn-A1d* and *Vrn-A1e* were characterized in tetraploid wheat accessions only, and were from *dicoccoides* and *dicoccum* species respectively. These alleles resulted from two different 32-bp and 54-bp deletions in the HDD and CArG Box in the promoter region. The spring growth habit conferred by these two alleles is unclear and requires experimental demonstration (Yan et al. 2004a). At the *Vrn-B1* locus, the insensitive allele *Vrn-B1a* was characterized as the result of a 440-bp deletion in Intron 1 of the winter allele *vrn-B1* (Table 1-1) (Santra et al. 2009). The allele *Vrn-B1b* emerged due to further deletion of 36-bp and a single

nucleotide polymorphism (SNP) (G-C) in *Vrn-B1a* (Table 1-1) (Santra et al. 2009). The point mutation in *Vrn-B1b* is reported to be a silent mutation and hence is unlikely to have any direct role to play in altering vernalization response. Similarly, at the *vrn-D1* locus, apart from the intron 1 deletion, a single nucleotide polymorphism (C-A) has been recently characterized to mediate the spring and facultative growth habit conferred by *Vrn-D1a* and *Vrn-D1b* respectively (Zhang et al. 2012). The facultative growth habit allele *Vrn-D1b* delayed flowering 32d due to the disruption in the binding site of the MADS box transcription factor, which is believed to accelerate flowering in cereals (Zhang et al. 2012). The description of different vernalization alleles, marker name and sequences, expected PCR product size and annealing temperature to screen for these alleles are presented in Table 1-1.

To understand the physiological basis of vernalization insensitivity, changes in morphology of the shoot apex have been studied in relation to the change in transcriptional levels of insensitive vernalization genes (Shimada et al. 2009). During vegetative growth, the apical meristem produces leaf primordia. At a certain *VRN1* transcriptional level, instead of leaf primordia, it produces spikelet primordia, the very first step of reproductive growth (Zadoks et al. 1974). *VRN1* genes encode the MADS box transcription factor, which has substantial sequence similarity to *APETALA1*-like or *FRUITFULL* gene in *Arabidopsis;* this then triggers reproductive growth (Yan et al. 2004). A number of abbreviations for this gene *e.g WAP1* (Wheat *APETALA-1*) and *TaVRT-1*(*Triticum aestivum vegetative to reproductive Transition-1*) have been synonymously used in the literature (Danyluk et al. 2003; Trevaskis et al. 2003). This MADS box gene is believed to regulate the identity of the apical meristem in a number of species (Ferrandiz et al. 2000). Increase in the duration of vernalization results in a linear increase in the transcriptional level of the *VRN1* gene (Murai et al. 2003). The *VRN1* genes are translated in the leaves, and the transcriptome produced is transported to the shoot apical meristem to induce reproductive growth. Any disruption at the binding site for the transcription factor will decrease the basal transcriptome level of the genes and hence will delay the induction of reproductive growth. Zhang et

al. (2012) characterized *Vrn-D1a* and *Vrn-D1b* and found that a single nucleotide polymorphism in CArG box has altered the binding site for MADS box transcription factor, resulting in a flowering delay of 32d in Chinese germplasm. Similar deletion and/or mutation events in *VRN1* series genes have been identified to alter the gene action and are believed to have altered a critical region near the transcriptional start site (Yan et al. 2004b; Pidal et al. 2009). Another possible reason for spring growth habit in barley is attributed to the suppression of *VRN2* genes. A mutant non-functional copy of *VRN2* gene has an amino acid substitution in the CCT domain which obstructs *VRN2* transcription; resulting in earlier flowering (Dubcovsky et al. 2005; Karsai et al. 2005). Oliver et al. (2009) also correlated the state of DNA packing protein (histone) with *VRN-1* gene activity; where an active protein state confers earlier flowering and an inactive state is attributed to long term repression. Active histone state is possibly inherited by cell division and can have a role in cellular memory (Trevaskis 2010). Interestingly, vernalization is 'remembered' by cells or plants. A vernalization treatment to cells during tissue culture, germinating and/or maturing seeds reportedly accelerates flowering without any further *Vrn* treatment (Marcinska et al. 1995; Sasani et al. 2009).

1.2.1.3 Differential effect of vernalization genes

Vernalization genes confer varying potency of insensitivity to cold treatment, ranging from complete insensitivity to partial or weak sensitivity, depending upon the nature of the *Vrn* allele (Pugsley, 1971, 1972). *Vrn-A1a* is the strongest allele to induce complete insensitivity among the *Vrn* genes (Iqbal et al. 2007; Kamran et al. 2013). Loukoianove et al. (2005) reported differential expression of the three *Vrn* alleles (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*) at the one to six leaf stage of isogenic wheat lines. Transcripts of *Vrn-A1* were detected at the first leaf stage, whereas the *Vrn-B1* and *Vrn-D1* transcripts were only detectable at the 2nd and 3rd leaf stages. These findings suggest that the timing of expression for differing vernalization genes is different and *Vrn-A1* has a higher level of expression at 2nd or 3rd leaf stage compared to *Vrn-B1* and *Vrn-D1*. This

differential expression of *Vrn* alleles could possibly be attributed to the potency of *Vrn* allele. A relatively high basal level of *Vrn-A1* transcripts is thought to be the reason for being the most potent insensitive vernalization gene followed by *Vrn-B1* and *Vrn-D1* (Loukoianove et al. 2005). This varying degree of insensitivity conferred by different vernalization alleles offers enormous potential to fine tune the flowering time of cultivars for a specific geographical region. Kamran et al. (2013) reported the presence of *Vrn-A1a* either alone or in combination with *Vrn-B1* in more than 90% cultivars adapted in limited growing season of the Canadian prairies. Similarly, van Beem et al. 2005 reported that CIMMYT derived cultivars bred for relative longer growth season pre-dominantly possess *Vrn-D1* gene. Stelmakh (1993) reported high yield coupled with early maturity in Ukrainian cultivars resulting from a combination of dominant *Vrn-A1* and *Vrn-B1* or *Vrn-D1*. The presence of such double dominant genes resulted in a phenotype more tolerant of heat shock and drought stress during grain filling without compromising grain yield.

1.2.1.4 Frequency distribution of vernalization genes

Of all the environmental factors, temperature has a dictating role in plant development and adaptation, and is responsible for the geographical and frequency distribution of vernalization genes as well. In other words, the degree of winter coldness prevailing in a specific geographical region seems to mediate the distribution pattern of different vernalization genes (Iwaki, et al. 2000). Spring wheat is cultivated in those parts of the world where average January temperature is less than -12.2 ˚C (Wilsie, 1962 as cited by Iwaki et al. 2000). Therefore, complete insensitive cultivars with the *Vrn-A1* allele are cultivated in two extremes of winter coldness: either in areas of intense cold where average January temperature is below -7 ˚C or areas with average winter temperature is above 10 ˚C (Iwaki et al. 2001). *Vrn-A1* is frequent in Europe and Siberia (Stelmakh, 1990), and early maturity induced in 98% of modern wheat cultivars in Russia is by virtue of this allele (Goncharov and Shitova, 1999 as cited by Leonova et al. 2003). Iqbal et al. 2007 reported an 85% occurrence of *Vrn-A1* allele in a study on

40 Canadian Western spring wheat cultivars with 35% in combination of *Vrn-B1*, with no dominant *Vrn-D1*. Another point of view about the distribution of *Vrn* genes is that the cultivars grown at high altitude usually possess *Vrn-A1* followed by *Vrn-B1* (Stelmakh, 1998; Iqbal et al. 2007).

Vrn-D1 is predominantly found in cultivars from Asia (Stelmakh, 1990), Japanese and Chinese land races (Gotoh, 1979) and particularly in the sub-tropical regions where the growing season is longer. Facultative growth habit or the partial vernalization requirement of *Vrn-D1* plays a role in wider adaptability of wheat cultivars. In a study on CIMMYT derived wheat cultivars bred for wider adaptability, *Vrn-D1* was found to be the most frequent allele; 66% of these cultivars possessed *Vrn-D1* either alone or in combination with *Vrn-A1*, *Vrn-B1*, and *Vrn-4*, which occurred at a lesser frequency of about 41, 39, and 8% respectively (van Beem et al. 2005).

'Akakomugi' a Japanese cultivar is thought to be the ancestral parent of the *Vrn-D1* allele (Stelmakh 1990) which was transmitted to cultivars like Lerma Rojo 64 and Sonora 64. These two cultivars are thought to be the potential source of the *Vrn-D1* allele in South and Southeast Asian cultivars (van Beem et al. 2005; Stelmakh, 1990). *Vrn-D1* found in globally adopted wheat cultivars has a key role in yield advantage, by accelerating or delaying flowering time in order to avoid abiotic stresses such as heat, drought and frost injury (Stelmakh, 1993). The three most widely (8-9 million hectares globally) grown cultivars (Poster, Attila, and Kauz) possess *Vrn-D1* as the sole *Vrn* allele (van Beem et al. 2005).

Dominant vernalization genes accelerate vegetative growth from emergence to floral initiation (Whitechurch and Snape, 2003), with a consequent decrease in final leaf number (Hay and Kirby, 1991; Slafer and Rawson, 1994; Wang, et al. 1995); and recessive *Vrn* alleles prolong the vegetative phase with a higher number of leaves due to a constant growth rate of leaf primordia (Miglietta, 1989) which increases final leaf number and delays flag leaf appearance (Kirby, 1990) and heading (Amir and Sinclair, 1991). Whitechurch and Snape, (2003) reported *Vrn-A1* and *Vrn-D1* increase the number of spikelet

whereas *Vrn-D1* was reported to decrease the number of spikelets by Kato et al. (2001). The effect of *Vrn* genes is reported to be more pronounced during the vegetative phase *i.e.* emergence to floral initiation (Whitechurch and Snape, 2003), after which no developmental response is reported (Wang and Engel, 1998).

1.2.2 Photoperiod Response

1.2.2.1 Definition

Plants and animals have a remarkable capability to sense change in duration of light and initiate certain developmental changes. Many plant species sense and respond to altering day and/or night length by receiving signals in the form of cryptochrome or phytochrome to induce flowering (Fosket, 1994). This phenomenon is called photoperiodism. Cryptochromes or blue light receptors are found in the cell nucleus and play a role in flowering (Cashmore et al. 1999). Different types of phytochromes have been discovered in plants that sense and react to red light and can initiate their response independently or together with other chromoproteins (Briggs and Onley 2001). The phytochrome (A and B) mediated signal transduction pathway comprises not only the signaling components but also has transcriptional regulators. These transcriptional regulators are attributed to phenological changes induced in plants (Neff et al. 2000).

1.2.2.2 Mechanism and nomenclature of photoperiod genes

Light induced regulation of gene expression has been studied since the beginning of the 20th century (Garner and Allard 1920). Certain photoperiod alleles are light responsive and their expression can be up regulated only under certain day length; and hence are called photoperiod sensitive. Photoperiod sensitive genotypes continue growing vegetatively unless they attain desired day length and thereafter induce flowering or reproductive growth (Dyck et al. 2004). Genotypes with insensitive photoperiod alleles can induce flowering regardless of day length. A series of studies over the years have located and confirmed the

presence of photoperiod response genes on the short arm of group 2 chromosomes in wheat. These genes are *Ppd-A1* on chromosome 2A, *Ppd-B1* on chromosome 2B, and *Ppd-D1* on chromosome 2D (Law et al. 1978; Snape et al. 2001). Insensitive alleles are written by putting the suffix 'a' at the end and sensitive ones are written by putting 'b' at the end as: *Ppd-D1a*, *Ppd-B1a* and *Ppd-A1a* (insensitive) the alleles *Ppd-D1b, Ppd-B1b* and *Ppd-A1b* (sensitive) (McIntosh et al. 2003). Varying degree of photoperiod insensitivity has been reported with *Ppd-D1a* ranked as the strongest allele conferring photoperiod insensitivity, followed by *Ppd-B1a* and *Ppd-A1a* (Worland, 1996), which exhibit a smaller comparative degree of response. The description of different photoperiod alleles, marker name and sequences, expected PCR product size and annealing temperature to screen these alleles are presented in table 1-1

1.2.2.3 Molecular basis of photoperiod effect

Studies on hexaploid wheat (*Triticum aestivum*) revealed that a semi-dominant type of mutation occurred in the upstream direction of the coding region of photoperiod sensitive allele *Ppd-D1b.* A deletion mutation of 2089 bp, in *Ppd-D1b* has evolved as a pseudo response regulator (PRR) allele *Ppd-D1a*; and converted photoperiod sensitive cultivars to photoperiod insensitive ones. Response regulation (RR) is a basic signal transduction mechanism in prokaryotes (Mizuno 1998). Histidine kinase (HK) acts as a sensor, *i.e.*, senses external signals and releases a phosphate group. In response, a phospho-receiver turns on or off a cellular event at the transcriptional level (Mizuno and Nakamichi 2005). In higher plants (*Arabidopsis thaliana*) certain genes encode a pseudo HK activity and hence are called the pseudo response regulator (PRR) family. The photoperiod insensitive allele *Ppd-D1a* is considered to be a member of the PRR family. The photoperiod insensitive allele *Ppd-D1a* in hexaploid wheat is actually mis-expressed *i.e* up regulated without environmental signal or day length; and alters the expression of key floral regulator *FT* to induce earlier flowering (Beales et al. 2007). A deletion mutation may have changed the transcription start site or caused removal of the regulatory element from photoperiod sensitive wheat

genotypes (Beales et al. 2007). The 2D region of wheat chromosome is collinear with the region of the 2HS barley (*Hordeum vulgare L.*) that contains the photoperiod genes *Ppd-H1* (Laurie, 1995; Borner et al. 1998). The 2HS barley allele has been cloned and identified as a member of the pseudo-response regulator (*PRR*) family (Turner et al. 2005).

1.2.2.4 Effect of photoperiod on plant development

The acceleration in flowering time due to insensitive photoperiod alleles has been reported in a number of studies. Kamran et al. (2013) reported earlier flowering due to *Ppd-D1a* in spring wheat by 5.8-6.1d under greenhouse and field conditions respectively. Foulkes et al. (2004) reported *Ppd-D1a* triggers early flowering by 9-12 days in near isogenic lines in winter wheat, and Worland (1996) and Worland et al. (1998) reported 4-8 days or 6-14 days earlier flowering in UK. Earliness induced by insensitive *Ppd* genes is mediated through developmental acceleration, which may be either from emergence to floral initiation (Davidson et al. 1985), and/or by shortening the spikelet primordial initiation (Rawson and Richards, 1993), terminal spikelet to flowering time (Snape et al. 2001), or short thermal duration from crop emergence to stem extension (Foulkes et al. 2004).

The response of a wheat plant to photoperiod would be the transition of vegetative to a reproductive growth phase. During this transition two important phenological processes (spikelet initiation and stem elongation) overlap and compete for total assimilate supply (Kirby, 1988; Miralles et al. 1998). The assimilate supply and duration of this phase is critical in determining the total number of fertile florets and eventually grain yield. The duration of this phase can be manipulated by different *Ppd* alleles as this phase is photoperiod sensitive (Slafer and Rawson, 1996; Miralles et al. 2000; Whitechurch and Slafer, 2002). The selection of particular *Ppd* alleles or combination thereof can elongate the duration of this phase, which theoretically can result in enhanced supply of assimilate to spikelet growth, and to become more fertile and productive (Slafer et al. 2005). A higher yield is attributed to higher number of grains per unit area

(Slafer, 2003). This yield increase is reported as a result of a longer growth period and more availability of assimilates, not due to increased growth rate (Gonzalez et al. 2003 a,b). However, Serrago et al. (2008) reported decreased spikelet fertility when plants are exposed to long photoperiods during the stem elongation period, and found that the losses could be reversed by trimming the spikes and increasing the assimilate supply.

Environmental conditions in a particular growing season play a critical role in final grain yield of photoperiod responsive or non-responsive cultivars grown in a specific region and/or in that particular geographical area. A higher yield due to photoperiod insensitive alleles have been reported only under certain conditions. For example yield advantages with photoperiod insensitive cultivars were reported in hot dry summers in the UK, which were incurred probably due to escape from hot desiccating conditions through earlier maturation. The hot dry conditions are associated with decreased tiller number and decreased grain weight (Musick and Dusek, 1980). Photoperiod insensitivity conferred increased grain yield in Southern Europe up to 35% (Worland, 1996). This yield advantage was 7.7% in Germany and 33% in former Yugoslavia (Worland et al. 1998). Whereas in U.K the northern part of Europe photoperiod insensitivity has varying results in each year depending upon environmental conditions. A yield disadvantage of -1.8% in conventional cool humid summers, 5% yield advantage in hot dry summers, and no yield advantage were reported in different studies over the years (Worland et al. 1994; Worland et al. 1996; Worland et al. 1998).

A number of pleiotropic effects have been attributed to insensitive *Ppd* alleles, for example, reduced plant height (Marshall et al. 1989; Worland, 1996), reduced final leaf number (Miralles and Richards, 2000; Dyck et al. 2004), earlier spikelet primordial initiation (Rawson and Richards, 1993), reduced tillering (Miralles and Richards, 2000; Dyck et al. 2004), reduced spikelet per spike (Snape et al. 2001; Dyck et al. 2004), and a lesser green leaf area (Foulkes et al. 2004).

A novel photoperiod response allele designated as *Ppd-B2* has been mapped on wheat chromosome arm 7BS (Khlestkina et al. 2009). This allele

accelerates flowering only under long photoperiods in contrast to the well-known *Ppd-1* genes that are supposed to induce earlier flowering under short photoperiods. The findings were proposed based on a study of 46 single chromosomes recombinant lines (SCRLs) that exhibited flowering acceleration only under long photoperiods and no acceleration under short photoperiods (Khlestkina et al. 2009).

1.2.2.5 Role of photoperiod genes in adaptability

For reproductive success, it is important that wheat flowers in suitable environmental conditions. Spring wheat breeders in North America and Canada have deliberately bred for photoperiod insensitive alleles, in order to make use of winter season and carry on contra nurseries breeding activities in southern latitudes during the winter (Dyck et al. 2004). Similarly in North African, Mediterranean regions and Asia, almost all high yielding improved cultivars are photoperiod insensitive while all the landraces are sensitive to photoperiod (Ortiz-Ferrara et al. 1998). These results suggest that the selection for photoperiod insensitivity has enhanced the adoptability of improved cultivars in a wide range of environments in comparison with landraces (sensitive to photoperiod) in these regions (Gororo et al. 2001). Photoperiod response is associated with adaptability and grain yield in European and Canadian wheat cultivars (Martinı´c, 1975; Hunt, 1979; Worland et al. 1994, 1998a, b). Kamran et al. (2013) reported 72% of the Canadian spring wheat cultivars carrying insensitive allele *Ppd-D1a*, and 91% (58 out of 64) of the insensitive lines belonged to high yielding Canadian Western Soft White class. The allele *Ppd-D1a* is also wide spread in most of the European and Chinese insensitive cultivars. In a study of 926 of Chinese wheat improved cultivars and landraces collected from nine wheat growing zones differing in their environmental conditions were genotyped by using allele specific markers. 38.6% of the land races and 90.6% improved cultivars possessed *Ppd-D1a*, which was present in almost all improved cultivars released after 1970 (Yang et al. 2009).

1.2.3 Earliness *per se*

1.2.3.1 Definition

Earliness *per se* is an inherent ability of wheat to flower earlier without a known environmental stimuli (Miura and Worland, 1994). Ford et al. (1981) found some 'other genes' affecting the flowering time in wheat after vernalization and photoperiod requirements were satisfied and called them "earliness genes". Basic development rate, intrinsic earliness, and earliness in narrow sense are synonymous terms used for earliness *per se* in the literature (Hanocq et al. 2004; Lewis et al. 2008). Law (1966) reported these genes to be of quantitative nature and responsible for continuous variation in ear emergence time in wheat. Compared to the two flowering gene groups (vernalization and photoperiod) earliness *per se* genes have smaller affect. However, the effect is quantifiable and strong enough to induce early flowering even in the presence of major flowering (*Vrn* and *Ppd)* genes (van Beem et al. 2005).

1.2.3.2 Earliness *per se* in *monoploid* wheat

Due to progress in genetic marker technology, QTL mapping software and improved analytical abilities, a substantial number of the QTL have been reported to alter the flowering time in wheat during the last decade. However, a very large and redundant genome of hexaploid wheat has hampered the physical mapping of a number of genes. To overcome these complexities, diploid and tetraploid wheat genome has been studied. Bullrich et al. (2002) conducted a study on *Triticum monococum* accessions G3116 (wild) and DV92 (Cultivated) to characterise and map earliness *per se* QTL without confounding effects. They found G3116 as early flowering and DV 92 as late flowering with a major *Eps* QTL located close to *Xwg 241* on the long arm of chromosome 1A. The alleles were named as *Eps-Am1-e* G3116 allele for early flowering and *Eps-Am1-l* DV 92 allele for late flowering by Lewis et al. (2008). These alleles were considered as major *Eps* genes due to their larger effects on flowering date. Early flowering of 49 days was

reported in G3116 homozygous families compared to DV92 homozygous families by producing non-overlapping and clearly segregating heading dates in 24h light and vernalized plants. The effect was reduced to 8 days under natural conditions. Previously only a few days flowering differences were reported due to *Eps* genes by Laurie et al. (1995), Worland (1996) and Kato et al. (1999).

1.2.3.3 Role of earliness *per se* QTL in plant development

Acceleration in developmental rate has been reported due to earliness *per se* genes mediated by growth temperature (Slafer and Rawson, 1995; Bullrich et al. 2002). To re-examine the assumption that the expression of *Eps* genes is coupled with growing temperature van Beem (2005) crossed fifty one genotypes with near-isogenic triple dirk (TD) tester with known *Vrn* and *Ppd* genes. F_2's and parents were vernalized at 4 $^{\circ}$C for eight weeks and were exposed to 24h light in order to eliminate the confounding effect of vernalization and photoperiod from earliness *per se* genes. The results of the study suggest that there might be some genes which are thermo-sensitive and could accelerate the developmental response with increase in temperature. Similar results were found in another study conducted by Bullrich et al. (2002) on diploid wheat *Triticum monococum.* In this greenhouse study, plants with early and late flowering allele from G3116 flowering alleles were studied at 16 $^{\circ}$C and 23 $^{\circ}$C to detect the interaction of *Eps* genes with growth temperature. Genotype and genotype x temperature interactions were reported highly significant. Plants carrying *Eps-A^m1-e* allele flowered 80 days earlier than plants with DV92 allele, when the material was studied at 16 $^{\circ}$C, however, this flowering difference was reduce to 41 days when the material was studied at 23 $^{\circ}$C. They concluded that there was a genotype x temperature interaction and the earliness per se allele *Eps-A^m1-e* interacts with temperature. Further, it was revealed that the effect of temperature was more pronounced on the plant material with the *Eps-A^m1-l* (DV92 late flowering) allele. Fifty days flowering time difference was recorded when the lines with only *Eps-A^m1-l* (DV92 late flowering) allele were studied at 16 $^{\circ}$C and 23 $^{\circ}$C. While, the lines with *Eps-A^m1-e* (G3116 early flowering allele) and exhibited a difference of

11 days of flowering when studied at similar temperatures. However, Bullrich et al. (2002) reported there are some genes which are not temperature dependent and confer early flowering.

Earliness *per se* genes can accelerate developmental rate at any particular growth phase (Slafer, 1996). Earliness *per se* genes can induce early flowering by initiating floral primordial with minimal vegetative growth (Kato and Wada, 1999). A prominent effect of *Eps* genes was reported on ear emergence time by Miura and Worland (1994). Most of the earliness *per se* studies have quantified the effect of these QTL based on differences in flowering and/or maturity time. However, Lewis et al. (2008) studied the effect of *Eps* alleles on different phases of early reproductive and vegetative growth. They found that the near isogenic lines (NILs) with *Eps-A^{m}1-e* induced 35 days earlier transition of vegetative to reproductive apices than *Eps-A^{m}1-l*. Similarly, time taken from double ridge to terminal spikelet was significantly longer in NILs carrying *Eps-A^{m}1-l* allele. No significant effect of the QTL was found on the developmental phase between terminal spikelet initiation to heading. Lines with the late allele produced 8.7 more spikelets per spike than the early flowering allele *Eps-A^{m}1-e* (Lewis et al. 2008). Shah et al. (1999) also reported significant variation for number of grains per plant due to *Eps* QTL on chromosome 3A in a study on hexaploid wheat recombinant inbred chromosomal lines.

1.2.3.4 Location of earliness *per se* QTL

Eps QTL are reported to be present on all the wheat chromosomes and are found more frequently on chromosomes: 5A (Griffiths et al. 2009; Kato et al. 1999; Bennett et al. 2012) 2B (Scarth and Law, 1983; Maccaferri et al. 2008; Sourdille et al. 2003; Shindo et al. 2003), 3A (Hoogendoorn, 1985; Miura and Worland, 1994), 5B, 7B and 4D (Hoogendoorn, 1985; Kulwal et al. 2003; Lin et al. 2008). Earliness *per se* QTL are mapped and characterized on tetraploid and hexaploid wheat populations as well. Kamran et al. (2013b) reported three earliness *per se* QTL affecting both flowering and maturity in a hexaploid spring

wheat population. Maccaferri et al. (2008) reported QTLs affecting flowering time in tetraploid wheat.

A comprehensive literature search presented in this review has shown that the QTLs affecting flowering time are present on all the 21 wheat chromosomes. The B genome carries 41.5% of the QTLs found and reported in this review while the A and D genome harbor 30% and 28.5% of the QTLs affecting flowering or maturity times (Table 1-2 and 1-3). A relative large number of QTLs were found at group 2 and group 5 chromosomes (34 and 28 respectively). This suggests that these two groups of chromosomes carry the most QTLs along with vernalization and photoperiod genes. In Table 1-2, the QTLs affecting both flowering and maturity times were considered as earliness *per se* QTLs, without changing the name of the QTL. The meta-QTLs reported in the study conducted by Griffiths et al. (2009) were given arbitrary names for the sake of convenience. Marker interval for some of the QTLs reported in Table 1-2 was given based on the visual observation of the map provided by the author and might not be accurate as the manuscript does not provide the information in tabular form. Table 1-3 reflects the quality attributes of the reported QTL as the studies varied for the population size, number of phenotypic data sets, genome coverage map density and mapping technique. Some of the studies reported in Table 1-2 were not summarized for quality attributes due to the lack of information.

1.3 Conclusion

The time of flowering in wheat plays a central role for its adaptability in diverse conditions. Wheat has enormous genetic potential to synchronize its time of flowering with favorable environmental conditions and hence is being grown in all parts of the world. The time of flowering has an imperative role in determining the grain yield and grain protein content, and thus has more important role in global food security. A better understanding of flowering time will aid world wheat production.

1.4 Thesis research progression

I started my PhD program at the University of Alberta in February 2009 and started phenotyping the historical and elite Canadian spring wheat cultivars in summer 2009. Phenotypic differences in flowering, maturity, plant height and plant structure among the historical and elite Canadian germplasm led me to explore the flowering gene underlying the phenotypic differences. Relative higher frequencies of *Vrn-A1a* and *Vrn-B1* were observed in CWRS and CWSWS classes respectively (Chapter 2). *Vrn-A1a* conferred earlier maturity and had a positive correlation with grain protein content; while *Vrn-B1* was found in higher yielding but low protein cultivars. Based on these results I conducted two more experiments. One experiment was based on CWSWS class cultivars and was grown in multi-locational yield trail to verify the role of vernalization genes in altering the time to maturity and its effect on grain yield (Chapter 4). The second experiment was based on CWRS cultivars, studying the role of *Vrn-A1a* in accelerating the flowering time and increasing the grain protein content under organic and conventional conditions (Chapter 5). To explore the earliness *per se* QTL a study based on the Cutler × AC Barrie population was conducted. The population was segregating for photoperiod allele *Ppd-D1a*, which yielded an opportunity to study the interaction between the earliness *per se* QTL found in the study and *Ppd-D1a*. We found three earliness *per se* QTLs (Two novel and one in the region already reported in literature) accelerating or delaying the flowering/maturity times and interacting additively with *Ppd-D1a* (Chapter 3).

1.5 Objectives and hypothesis

1- Phenotype hard red and soft white cultivars/lines with different *Vrn* and *Ppd* genes and their combination; to quantify the effect of each *Vrn* and *Ppd* allele in terms of days to flowering, maturity and grain yield in the field and the greenhouse; and to study the interaction of flowering genes and their pleiotropic effects on agronomic traits.

2- Mapping the genome for earliness *per se* (*Eps*) genes on segregating Cutler x Barrie recombinant inbred lines (RILs) having known vernalization *(Vrn)* and

photoperiod *(Ppd)* genes; identify QTLs affecting flowering, maturity, plant height and grain yield in a Canadian spring wheat population derived from a cross between early and relatively late maturing cultivars; studying the effect of *Ppd-D1a* in a population where RILs share similar genetic background substantially; and to investigate the interaction between the *Ppd-D1a* and earliness *per se* QTLs to improve the understanding of flowering gene complex.

3- Study how individual vernalization genes and combination thereof alter agronomic traits in the high yielding low protein soft white spring wheat class in Northern growing regions; to quantify and compare the individual effects of vernalization gene on days to flowering and maturity and on yield and yield components in this class of wheat.

4- Investigate the flowering gene complex in recent Canadian wheat cultivars by screening them for vernalization and photoperiod genes and studying their phenotypic behavior under organic and conventional conditions; to study the differential behavior of vernalization genes in flowering and maturity time under the two systems (organic and conventional); whether reduction in days to maturity due to insensitive *Vrn* genes confers grain yield advantage under organic field conditions; to identify the genotypes/cultivars that are better suited to organic production.

Hypothesis # 1: 1-1) Soft white and hard red cultivars/lines have different *Vrn* and *Ppd* genes and flower and mature at different times; 1-2) the flowering gene interaction can be antagonistic or synergistic and result in either acceleration or delay in time of flowering; 1-3) there is a gradual increase in the grain yield and a gradual decrease in maturity times in Canadian spring wheat cultivars; 1-4) modern Canadian cultivars are photoperiod insensitive and are more adaptive compared to the cultivars released before 1950s.

Hypothesis # 2: 2-1)There are certain genomic regions involved in inducing early maturity apart from *Vrn* and *Ppd* genes; 2-2) Cutler × AC Barrie population has some genomic regions involved in affecting plant height and grain yield; the photoperiod insensitive allele *Ppd-D1a* interacts with earliness per se alleles; 2-3) a better understanding of the photoperiod insensitive allele and earliness per se

QTL will help to alter flowering and maturity times in Canadian spring wheat and will result in increased grain yield.

Hypothesis # 3: 3-1)Significant differences are induced in yield, flowering and maturity by different *Vrn* genes when grown under similar climatic conditions; 3-2) certain vernalization genes could be found in higher frequencies in different Canadian spring wheat classes; 3-3) vernalization insensitive allele *Vrn-D1a* has a direct or indirect role in increasing grain yield and is absent from Canadian Western Hard Red spring class

Hypothesis # 4: 4-1) Canadian cultivars, released in last ten years, possess different flowering genes and have differential behavior under organic conditions; 4-2) early maturity induced by most potent vernalization alleles will result in rapid plant growth and early maturity; 4-3) and will confer yield advantage; the cultivars exhibit significant genotype × environment interaction under organic conditions; 4-4) the organic breeding should be practiced under organic conditions.

1.4 Tables

Table 1-1. Vernalization and photoperiod alleles and their primer sequences with expected PCR product size and annealing temperatures

Allele	Primer name	Primer Sequence	PCR product Size (bp)	Annealing Temp (°C)	Comment	Reference
Vrn-A1a	VRN1AF VRN1R	5'-GAAAGGAAAAATTCTGCTCG-3' 5'-TGCACCTTCCC(C/G)CGCCCCAT-3'	650 or 750	55	222 and 113 bp insertion in promoter	Iqbal et al. 2007; Yan et al. 2004
Vrn-A1b	Ex1/C/F Intr1/A/R3	5'-GTTCTCCACCGAGTCATGGT-3' 5'- AAGTAAGACAACACGAATGTGAGA-3'	522	55.6	Two mutations in HDD and a 20-bp deletion in 5'UTR	Fu et al. 2005; Yan et al. 2004
Vrn-A1c	Intr1/As/F2 Intr1/A/R3	5'- AGCCTCCACGGTTTGAAAGTAA -3' 5'-AAGTAAGACAACACGAATGTGAGA-3'	1170	58.9	Large deletion in first intron	Fu et al. 2005
vrn-A1	Intr1/C/F Intr1/AB/R	5'-GCACTCCTAACCCACTAACC -3' 5'- TCATCCATCATCAAGGCAAA-3'	1068	56	no insertion/deletion	Fu et al. 2005
Vrn-B1a	Intr1/B/F Intr1/B/R3	5'-CAAGTGGAACGGTTAGGACA -3' 5'- CTCATGCCAAAAATTGAAGATGA-3'	709	58	440-bp deletion in first intron of winter allele	Santra et al. 2009
Vrn-B1b	Intr1/B/F Intr1/B/R3	5'-CAAGTGGAACGGTTAGGACA -3' 5'-CTCATGCCAAAAATTGAAGATGA-3'	673	58	36-bp deletion and a SNP of *Vrn-B1a*	Santra et al. 2009
vrn-B1	Intr1/B/F Intr1/B/R4	5'-CAAGTGGAACGGTTAGGACA -3' 5'-CAAATGAAAAGGAATGAGAGCA-3'	1149	56.4	no insertion/deletion	Fu et al. 2005
Vrn-D1a	Intr1/D/F VRN-SNP161CR	5'-GTTGTCTGCCTCATCAAATCC -3' 5'-CAAGATCAGACTCAGCCTCAAACA-3'	612	61	Deletion in the intron 1	Zhang et al. 2012

Vrn-D1b	Intr1/D/F VRN-SNP161AR	5'-GTTGTCTGCCTCATCAAATCC 5'-TAGCAACCGCAACATACACCAGA-3'	612	61	A single nucleotide polymorphism (C-A) from *Vrn-D1a*	Zhang et al. 2012
Vrn-D1	Intr1/D/F Intr1/D/R3	5'-GTTGTCTGCCTCATCAAATCC -3' 5'-GGTCACTGGTGGTCTGTGC-3'	1671	61	Deletion in the intron 1	Fu et al. 2005
vrn-D1	Intr1/D/F Intr1/D/R4	5'- GTTGTCTGCCTCATCAAATCC -3' 5'-AAATGAAAAGGAACGAGAGCG-3'	997	61	no insertion/deletion	Fu et al. 2005
Vrn-B3	VRN4-B-INS-F VRN4-B-INS-R	5'- CAT AAT GCC AAG CCG GTG AGT AC -3' 5'- ATG TCT GCC AAT TAG CTA GC -3'	1200	55	Retrotransposon insertion in the promoter region	Wheat Applied Genomics 2013
vrn-B3	VRN4-B-NOINS-F VRN4-B-NOINS-R	5'- ATG CTT TCG CTT GCC ATC C -3' 5'- CTA TCC CTA CCG GCC ATT AG -3'	1140	55	no insertion/deletion	Wheat Applied Genomics 2013
Ppd-D1a	Ppd-D1_F1 Ppd-D1_R2	5'-ACGCCTCCCACTACACTG-3' 5'-CACTGGTGGTAGCTGAGATT-3'	288	54	2089bp deletion	Beals et al. 2007
Ppd-D1b	Ppd-D1_F1 Ppd-D1_R1	5'-ACGCCTCCCACTACACTG-3' 5'-GTTGGTTCAAACAGAGAGC-3'	414	54	no insertion/deletion	Beals et al. 2007
Ppd-B1a *Ppd-B1b*	Ppd-B1exon3SNP_F1 Ppd-B1exon3SNP_R1	5'-AGACGATTCATTCCGCTCC-3' 5'-TCTGAATGATGAATACACCATG-3'	955 955	55	SNP	Beals et al. 2007
Ppd-A1a	Ag5del_F1 Ag5del_F2	5'-GTATGCGATTCGCCTGAAGT-3' 5'-TGTCACCCATGCACTCTCTTT-3'	381	55	deletion mutation	Bently et al. 2010
Ppd-A1b	Ag5del_R1 Ag5del_R2	5'-GAGCAAGGGATTGAGACTGC-3' 5'-CTGGCTCCAAGAGGAAACAC-3'	453	55	no insertion/deletion	Bently et al. 2010

Table 1- 2 .List of quantitative trait loci (QTL) affecting flowering time on hexaploid wheat genome reported so far in the literature.

Chro	Trait name	QTL name	Marker Interval / QTL position(cM)	R^2	Ploidy	Population type	RILs	Reference
1A	Ear emergence time	*Qeet.aww.1A*	*wpt-7541-wpt-6709*	-	Hexaploid	SSD RILS	380	Bennett et al. 2012
	Earliness *per se*	-	*X1Bamyl*	11.5	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Heading NS	-	*Xcdo1160*	14.7	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Earliness *per se*	*Eps-A^{m}1-e*	*Xcdo393-Xwg-241*	47	diploid	SSD RILs	68	Bullrich et al. 2002
2A	Heading date	*QHd.idw-2A.1*	*Xgwm636 − Xwmc177*	35.3	Tetraploid	SSD RILs	249	Maccaferri et al. 2008
	Heading date	*QHd.idw-2A.2*	*Xwmc177 − Xcfa2201*	18.1	Tetraploid	SSD RILs	249	Maccaferri et al. 2008
	Heading date	*Meta-QTL.2A.1*	*gwm636–wmc474*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Maturity time	*QMt.nfcri-2A*	*xbarc124-xgwm636*	8.4	Hexaploid	SSD RILs	142	Wang et al. 2009
	Vrn requirement	-	*Xpsr901*	11	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Heading in Field	-	*XksuF11b*	10.9	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Heading date	*Meta-QTL.2A.2*	*wmc827–cfd168*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
3A	Heading date	*Meta-QTL.3A*	*wmc505–wmc527*	25.4	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Vrn requirement	-	*Xbcd1823b*	10.8	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Flowering time	*QFlt.ipk-3A*	*Xcdo482-Xcdo451*	-	Hexaploid	SSD RILs	114	Borner et al. 2002
	Anthesis Date	*Qad.unl.3A.2*	*Xgwm5-Xpsp3047*	13.5	Hexaploid	RICLs	223	Ali et al. 2011
	Anthesis Date	*Qad.unl.3A.4*	*Xhbg491-Xgwm155*		Hexaploid	RICLs	223	Ali et al. 2011
	Anthesis Date	*Qad.unl.3A.5*	*Xwmc338-Xbarc1021*		Hexaploid	RICLs	223	Ali et al. 2011
4A	Flowering	*QFlt.dms-4A.1*	*Wpt-8841*	5.6	Hexaploid	SSD RILs	177	Kamran et al. 2013
	Days to heading	*QDh.ccsu-4A.1*	*Xbcd1670*	7.72	Hexaploid	Synthetic	110	Kulwal et al. 2003
	Flowering time	*QFlt.nau-4A.1*	*Xbc1.2–Xwmc420d*	9.6-10.6	Hexaploid	SSD RILs	230	Lin et al. 2008

	Flowering time	*QFlt.nau-4A.2*	*Xwmc161–Xmag3886*	18.1-19.1	Hexaploid	SSD RILs	230	Lin et al. 2008
5A	Heading date	*Meta-QTL.5A.1*	*wmc489–cfa2155*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Heading date	*Meta-QTL.5A.2*	*wmc110–gwm595*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Ear emergence time	*QEet.ocs.5A.1*	*Xcdo584*	37-51	Hexaploid	RICLs	120	Kato et al. 1999
	Heading date		*Xglk407*	6.9	Hexaploid	DH lines	187	Sourdille et al. 2003
	Days to heading	*QEet.fcu-5A*	*Xgdm132–Xcfa2155*	41	Hexaploid	DH Lines	120	Chu et al. 2008
	Ear emergence time	*Qeet.aww.5A*	*gwm0186-wpt-1370*		Hexaploid	SSD RILS	380	Bennett et al. 2012
	Ppd sensitivity	-	*Xpsr574a*	19.1	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Ppd sensitivity	-	*Xcdo412a*	11	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Vernalization	*VRQTL_5A*	*Xgwm271b*	21.8-39.6	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Photoperiod	*PSQTL_5A*	*Xgwm264c*	5.9	Hexaploid	SSD RILs	194	Hanocq et al. 2004
6A	Heading date	*Meta-QTL.6A*	*barc23–Sbarc113*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
7A	Heading date	*Meta-QTL.7A.1*	*wmc646–gwm60*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Heading date	*Meta-QTL.7A.2*	*wmc83–wPt7299*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Ear emergence time	*Qeet.aww.7A.1*	*cfa-2023-barc282*		Hexaploid	SSD RILS	380	Bennett et al. 2012
	Ear emergence time	*Qeet.aww.7A.1*	*barc0108-barc0281*		Hexaploid	SSD RILS	380	Bennett et al. 2012
	Ear emergence time	*Qeet.aww.7A.1*	*wpt-4744-wpt-4796*		Hexaploid	SSD RILS	380	Bennett et al. 2012
	Ppd sensitivity	-	*Xpsr103*	16.7	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Heading NS	-	*Xcdo1160b*	10.7	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Earliness *per se*	*IEQTL_7A*	*Xrz995*	8	Hexaploid	SSD RILs	194	Hanocq et al. 2004
1B	Earliness *per se*	*QEps.dms-1B1*	*Wpt-2744*	9.8	Hexaploid	SSD RILs	177	Kamran et al. 2013b
	Earliness *per se*	*QEps.dms-1B2*	*Wpt-2694*	12.2	Hexaploid	SSD RILs	177	Kamran et al. 2013b
	Heading date	*Qhd1B*	*Xwmc406-Xbarc156*	0.71	Hexaploid	DH Progeny lines	168	Zhang et al. 2009

	Heading date	*Meta-QTL.1B.1*	*wPt4129–gwm140*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Heading date	*Meta-QTL.1B.2*	*cfd48–wmc611*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Flowering time	*QFt.nfcri-1B*	*xbarc312-xbarc61*	15.77	Hexaploid	SSD RILs	142	Wang et al. 2009
	Maturity time	*QMt.nfcri-1B*	*xgwm33-xbarc312*	9.61	Hexaploid	SSD RILs	142	Wang et al. 2009
	Flowering time	*QFlt.nau-1B*	*Xcfa2292–Xbarc80*	10.8-21.9	Hexaploid	SSD RILs	230	Lin et al. 2008
	Heading date	*QHd.idw-2B.1*	*Xwmc332 − Xgwm1070*	3.2	Tetraploid	SSD RILs	249	Maccaferri et al. 2008
	Heading date	*QHd.idw-2B.2*	*Xgwm1300–Xwmc332*	9.2	Tetraploid	SSD RILs	249	Maccaferri et al. 2008
	Heading date		*Xfbb121*	44.4	Hexaploid	DH lines	187	Sourdille et al. 2003
	Photoperiod sensitivity		*Xfbb121*	25.9	Hexaploid	DH lines	187	Sourdille et al. 2003
	Flowering time	*QFt.nfcri-2B*	*xbarc373*	10.97	Hexaploid	SSD RILs	142	Wang et al. 2009
	Maturity time	*QMt.nfcri-2B*	*xbarc373-xbarc160*	8.28	Hexaploid	SSD RILs	142	Wang et al. 2009
	Heading date	*QHd.idw-2B.3*	*Xgwm1027-Xwmc361*	15.2	Tetraploid	SSD RILs	249	Maccaferri et al. 2008
2B	Earliness *per se and Ppd*	-	*Xpsr135b*	19.2	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Earliness *per se*	-	*Xabc451*	18.1	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Ppd sensitivity	-	*Xpsr126b*	22.5	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Ppd sensitivity	-	*Xpsr131*	20	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Photoperiod	*PSQTL_2B*	*Xgwm148*	13.1-20.5	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Vernalization	*VRQTL_2B*	*Xgwm374*	4.1	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Earliness *per se*	*IEQTL_2B*	*Xgwm148*	7.2-12.4	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Flowering time	*QFlt.ipk-2B*	*Xrz444-Xwmg950*	-	Hexaploid	SSD RILs	114	Borner et al. 2002
	Flowering time	*QFlt.nau-2B*	*Xwmc35–Xmag3319c*	10	Hexaploid	SSD RILs	230	Lin et al. 2008
	Heading date	*Meta-QTL.3B.1*	*wPt7225–wmc500*	3.4 − 12.4	Hexaploid	DH Lines	550	Griffiths et al. 2009
3B	Heading date	*Meta-QTL.3B.2*	*wmc540–wmc787*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Flowering time	*QFt.nfcri-3B*	*wgwm533*	12.9	Hexaploid	SSD RILs	142	Wang et al. 2009

4B	Heading date	*Meta-QTL.4B*	*wPt3608–cfd39*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Ppd sensitivity	-	*Xpsr584*	11.5	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Photoperiod sensitivity		*Xglk556*	6.3	Hexaploid	DH lines	187	Sourdille et al. 2003
	Maturity time	*QMt.nfcri-4B*	*xgwm495*	7.14	Hexaploid	SSD RILs	142	Wang et al. 2009
5B	Earliness *per se*	*QEps.dms-5B1*	*Wpt-6135*	11.2	Hexaploid	SSD RILs	177	Kamran et al. 2013b
	Heading date	*Meta-QTL.5B*	*wmc745–cfa21215B.2*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Earliness *per se*	*Eps-5BL1*	*Xwmc73*	8.2	Hexaploid	SCRILs	137	Toth et al. 2003
	Earliness *per se*	*Eps-5BL2*	*Xgwm499*	5.7	Hexaploid	SCRILs	137	Toth et al. 2003
	Vrn requirement	-	*Xcdo1326b*	13.8	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Ppd sensitivity	-	*Xrz630b*	14.9	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Ppd sensitivity	-	*Xbcd1030b*	13.8	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Heading in Field	-	*Xwec78*	19.2	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Heading in Field	-	*Xgwm234*	12.7	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Days to heading	*QEet.fcu-5B*	*Xbarc73–Xfcp593*	15	Hexaploid	DH Lines	120	Chu et al. 2008
	Ear emergence time	*Qeet.aww.5B*	*gwm0234b-wpt-8604*	-	Hexaploid	SSD RILS	380	Bennett et al. 2012
	Earliness *per se*	*IEQTL_5B*	*Xgwm371*	8.6	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Vernalization	*VRQTL_5B*	*Xgwm639a*	6.8	Hexaploid	SSD RILs	194	Hanocq et al. 2004
6B	Heading date	*Meta-QTL.6B*	*wPt3309–wmc152*	7.2 – 13.4	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Earliness *per se*	-	*Xcdo202*	13.8	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Heading NS	-	*Xcdo202*	14.5	Hexaploid	SSD RILs	66	Shindo et al. 2003
7B	Heading date	*QHd.idw-7B*	*Xgwm569–Xbarc1005*	17.8	Tetraploid	SSD RILs	249	Maccaferri et al. 2008
	Heading date		*XksuD18*	7.3	Hexaploid	DH lines	187	Sourdille et al. 2003
	Heading date	*Meta-QTL.7B*	*gwm344–wPt9746*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Heading date		*XksuD2*	5.3	Hexaploid	DH lines	187	Sourdille et al. 2003

	Heading date		*Xfbb53*	9.4	Hexaploid	DH lines	187	Sourdille et al. 2003
	Days to maturity	*QDh.ccsu-7B.1*	*XksuD2*	8.57	Hexaploid	Synthetic	110	Kulwal et al. 2003
	Ppd sensitivity	-	*Xgwm57*	10.5	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Flowering time	*QFlt.nau-7B*	*Xgwm537–Xgwm333c*	9.9-18.2	Hexaploid	SSD RILs	230	Lin et al. 2008
	Heading date	*Meta-QTL.1D*	*wmc405–barc62*	10 – 27	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Days to heading	*QDh.ccsu-1D.1*	*Xcdo89*	6.8	Hexaploid	Synthetic	110	Kulwal et al. 2003
1D	Days to maturity	*QDh.ccsu-1D.1*	*Xcdo89*	13.21	Hexaploid	Synthetic	110	Kulwal et al. 2003
	Days to maturity	*QDh.ccsu-1D.2*	*Xcdo89*	13.21	Hexaploid	Synthetic	110	Kulwal et al. 2003
	Flowering time	*QFlt.nau-1D*	*Xgwm232–Xbarc62c*	6.2-13.1	Hexaploid	SSD RILs	230	Lin et al. 2008
	Days to heading	*QDh.ccsu-2D.1*	*Xcdo1379*	32.52	Hexaploid	Synthetic	110	Kulwal et al. 2003
	Days to heading	*QDh.ccsu-2D.2*	*Xcdo1379*	32.52	Hexaploid	Synthetic	110	Kulwal et al. 2003
	Days to heading	*QDh.ccsu-2D.3*	*Xgwm515*	11.48	Hexaploid	Synthetic	110	Kulwal et al. 2003
	Days to maturity	*QDh.ccsu-2D.1*	*Xgwm296*	15.63	Hexaploid	Synthetic	110	Kulwal et al. 2003
	Days to maturity	*QDh.ccsu-2D.2*	*Xcdo1379*	18.77	Hexaploid	Synthetic	110	Kulwal et al. 2003
2D	Photoperiod	*PSQTL_2D*	*Xgwm484*	16.1-19.8	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Earliness *per se*	*IEQTL_2D*	*Xgwm261*	8.2-11.4	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Ear Emergence time	*QEet.ipk-2D*	*Xbcd18-Xbcd1970*	-	Hexaploid	SSD RILs	114	Borner et al. 2002
	Flowering time	*QFlt.ipk-2D*	*Xfba400-Xcdo1379*	-	Hexaploid	SSD RILs	114	Borner et al. 2002
	Flowering time	*QFlt.nau-2D*	*Xwmc18–Xwmc181.1c*	5.4	Hexaploid	SSD RILs	230	Lin et al. 2008
	Days to maturity	*QDtm.crc-2D*	*Xwmc112*	5	Hexaploid	DH population	185	Huang et al. 2006
3D	Maturity time	*QMt.nfcri-3D*	*xgdm72-tx23-24*	8.74	Hexaploid	SSD RILs	142	Wang et al. 2009
4D	Heading date	*Meta-QTL.4D*	*wPt8836–gwm165*	-	Hexaploid	DH Lines	550	Griffiths et al. 2009
5D	Heading date	*Qhd5D*	*Xbarc320-Xwmc215*	-2.77	Hexaploid	DH Progeny lines	168	Zhang et al. 2009

	Heading date		*Xgwm174*	9.4	Hexaploid	DH lines	187	Sourdille et al. 2003
	Vrn requirement	-	*Xwec70*	10.6	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Flowering time	*QFt.nfcri-5D*	*xbarc93-xgdm63*	10.77	Hexaploid	SSD RILs	142	Wang et al. 2009
	Vernalization	*VRQTL_5D*	*Xbcd1421*	4.7	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Ear Emergence time	*QEet.ipk-5D*	*Xmwg970-Xfba209*	-	Hexaploid	SSD RILs	114	Borner et al. 2002
	Flowering time	*QFlt.ipk-5D*	*Xbcd450-Xmwg900*	-	Hexaploid	SSD RILs	114	Borner et al. 2002
	Days to maturity	*QDtm.crc-5D*	*Xwmc6409*	6.8	Hexaploid	DH population	185	Huang et al. 2006
	Flowering time	*QFt.nfcri-6D*	*xgdm132-xcfd42*	8.18	Hexaploid	SSD RILs	142	Wang et al. 2009
	Maturity time	*QMt.nfcri-6D*	*xbarc301*	7.82	Hexaploid	SSD RILs	142	Wang et al. 2009
6D	Heading in Field	-	*Xgdm132*	16.3	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Heading in Field	-	*Xgwm469*	15.8	Hexaploid	SSD RILs	66	Shindo et al. 2003
	Vernalization	*VRQTL_6D*	*Xcfd42*	4.5	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Heading date	*Meta-QTL.7D*	*wPt2054–cfd175*	14.3	Hexaploid	DH Lines	550	Griffiths et al. 2009
	Heading date		*Xfbb366*	5.8	Hexaploid	DH lines	187	Sourdille et al. 2003
	Heading in Field	-	*Xgwm428*	12.8	Hexaploid	SSD RILs	66	Shindo et al. 2003
7D	Earliness *per se*	-	*Xgwm130-Xcfd21*	10	Hexaploid	SSD RILs	188	Carter et al. 2011
	Photoperiod	*PSQTL_7D*	*pch1*	7.8	Hexaploid	SSD RILs	194	Hanocq et al. 2004
	Ear Emergence time	*QEet.ipk-7D*	*Xbcd1438-Xcnl6*	-	Hexaploid	SSD RILs	114	Borner et al. 2002
	Days to maturity	*QDtm.crc-7D*	*Xwmc4059*	31.6	Hexaploid	DH population	185	Huang et al. 2006

Table 1-3. List of the studies reporting flowering time QTL in wheat with the mapping quality parameters, mapping techniques and software used

Study	Genome coverage	Total Markers	Map density	Phenotypic data sets	Flowering QTL reported	Mapping Technique	Software	Population size
Maccaferri et al. 2008	2347	232	10.2	32	6	CIM[1]	JoinMap and QTL Cartographer	249
Bennett et al. 2012	3372	456	8.5	-	6	CIM	Map Manager/QTL network	368
Shindo et al. 2003	-	322	-	4	26	SIM[2]	Qgene	66
Griffiths et al. 2009	-	-	-	23	19	CIM/MIM[3]	JoinMap and QTL Cartographer	550
Wang et al. 2009	1821	270	5.5	7	11	MQM[4]	JoinMap and Map QTL	164
Borner et al. 2002	-	-	-	11	7	-	Map Maker/QTL	114
Ali et al. 2011	106 (3A)	-	-	6	3	CIM	Map Maker/QTL Cartographer	233
Kamran et al. 2013b	2279	488	4.67	6	4	CIM	QTL Cartographer	177
Kulwal et al. 2003	-	358	-	2	10	CIM/MCIM[5]	QTL Cartographer	110
Lin et al. 2008	3360	408		5	7	SIM/CIM/MQTL	Map Maker/QTL	230
Sourdille et al. 2003	3685	200	18.4	7	9	Marker Regression	Map Maker	187
Chu et al. 2008	3811.5	632	6.03	-	2	CIM	Map Maker/QTL Cartographer	120
Hanocq et al. 2004	2639	254	12	3	13	CIM	PlabQTL	194
Zhang et al. 2009	2141.7	305	7.02	6	2	CIM	QTL network	168
Toth et al. 2003	-	-	-	-	2	SIM/MQM	JoinMap /QTL Café	137
Huang et al. 2006	-	-	-	4	3	CIM	Map Maker/ QTL Cartographer	185
Carter et al. 2011	-	297	9	4	1	CIM	Map Maker/QTL Cartographer	188

Note: studies lacking most of the information were not included in the table.

[1]Composite Interval Mapping; [2]Simple Interval Mapping; [3]Multiple Interval Mapping; [4]Multiple trait QTL mapping; [5]Multiple Composite Interval mapping

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[2]2 Phenotypic effects of the flowering gene complex in Canadian spring wheat germplasm

2.1 Introduction

Wheat (*Triticum aestivum* L.) forms the staple diet in more than 40 countries and is grown in a wide range of temperatures, moisture levels, latitudes, altitudes and soils (Curtis et al. 2002). Wheat is cultivated on 225 million ha globally and occupies the largest area under any single crop, with a total global production of 681 million t (FAO stat, 2009).Wheat has enormous genetic potential to synchronize its flowering in favorable environmental conditions either by accelerating or delaying flowering; and this key feature is central to its global adaptability.

In the Canadian Western Prairie region, three types of wheat are produced: spring hexaploid (*Triticum aestivum* L.), winter hexaploid (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L.). Through the course of time and more specified market demands, Canadian wheat has been further classified into the following nine classes: Canada Western Red Spring (CWRS), Canada Western Amber Durum (CWAD), Canada Western Extra Strong (CWES), Canada Prairie Spring Red (CPSR), Canada Prairie Spring White (CPSW), Canada Western Red Winter (CWRW), Canada Western Soft White Spring (CWSWS), Canada Western Hard White Spring (CWHWS), and Canada Western General Purpose (CWGP). Hard Red Spring is the largest class, cultivated on about 92% of the area under spring wheat, in the Western Prairies. Total production was estimated to be 17.8 million t in 2011 (Statistics Canada 2011) with an average annual production of 15 million t. The second and third largest spring wheat classes in the Western Prairies are CPS and CWSWS, with 4% and 2.5% area under spring wheat, with a total production of about 9.6 and 5.5 million t annually (Statistics Canada 2011).

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Wheat adaptation to diverse environmental conditions depends mostly on flowering time, as frost can damage the sensitive floral organs and heat stress can reduce fertility (King and Heide 2009). Genetically, wheat can flower over a wide time range, which enables it to adapt to the specific environmental conditions of a geographical region. This is primarily due to three main constituent gene groups: vernalization response (*Vrn* genes), photoperiod response (*Ppd* genes) and earliness *per se* genes (*Eps* genes) (Snape et al. 2001). Vernalization is the plant response to non-freezing low temperature for transition from vegetative growth phase to the reproductive growth phase with the induction of flowering. A series of studies have allocated -1.3°C, 4.9°C, and 15.7°C as minimum, optimum and maximum vernalization temperatures, respectively (Porter and Gawith 1999). To estimate the effective duration of exposure, Streck et al. (2003) analysed data reported in previous literature and concluded that a *Vrn* sensitive wheat plant exhibits acceleration in flowering after 15-20 days of exposure to vernalization. This acceleration increased up to 35 days after which it decreased gradually, and appeared to be fully satisfied after 50 days. Hence, the vernalization effect is considered as cumulative (Trevaskis et al. 2010), as the longer the cold exposure; the faster the floral development (Sasani et al. 2009). Transcriptome of *Vrn-genes* apparently governs this transition by modifying the shoot apex to reproductive primordia instead of leaf primordia (Hemming et al. 2008) leading to the double ridge stage; the first visible sign of reproductive growth.

To resolve the underlying genetic mechanism of vernalization sensitivity or insensitivity, *Vrn*-1 alleles were cloned and characterized by Yan et al. (2003, 2004) and Fu et al. (2005). DNA sequencing revealed a deletion mutation in the transcriptional start site of *Vrn-genes*, resulting in vernalization insensitivity (Pidal 2009). This naturally deleted segment in the promoter region is suspected to retain a binding site for a repressor protein, which limits the rate of transcriptional initiation (Trevaskis 2010). Hence alleles lacking this segment are actively transcribed and exhibit vernalization insensitivity (Hemming et al. 2009). Recently, sequencing at the *Vrn-A1* locus has uncovered three alleles of the*Vrn-*

A1 gene due to: insertion within the promoter region (*Vrn-A1a*), a deletion mutation in the promoter region (*Vrn-A1b*), and a large deletion in the first intron (*Vrn-A1c*) (McIntosh et al. 2007).

Vernalization response in hexaploid wheat is governed by *Vrn-A1* on chromosome 5A (Storlie et al. 1998), *Vrn-B1* on 5B (Leonova, et al. 2003), and *Vrn-D1* on 5D (Law, et al. 1976). This series of orthologous genes is collectively referred as *Vrn-1* genes (Santra et al. 2009). Other vernalization genes have been mapped on chromosomes 3B, 4B, 4D, *Vrn-D5* on 5D and *Vrn-B3* on 7B (Iwaki et al. 2001) and these genes are considered as part of *Vrn-2* series. Vernalization genes confer varying potency, ranging from complete insensitivity to partial or week sensitivity depending upon the *Vrn* allele possessed and the size of deletion. A larger deletion can induce earlier flowering compared to smaller deletions (Hemming et al. 2009).

Many plants sense and respond to the altering length of day and night by receiving signals in the form of cryptochrome or phytochrome to induce flowering. In *Arabidopsis,* expression of the long-day flowering gene *FT* has a linear response to day length (Kobayashi et al. 1999). Higher expression levels were noted when day light exceeded a critical length due to an interaction between the circadian clock and light receptors (An et al. 2004). The *FT* protein is then transported to the shoot apex which accelerates reproductive growth (Corbesier et al. 2007). This phenomenon is called photoperiodism. Genotypes with dominant genes flower earlier, exhibit photoperiod insensitivity and thus shift to the reproductive phase in the absence of long days. Photoperiod sensitive plants remain vegetative until they attain the desired day length (Dyck et al. 2004). In hexaploid wheat, numerous studies have located and confirmed the presence of photoperiod response genes on the short arm of group 2 chromosomes. These genes are *Ppd-A1* on 2A, *Ppd-B1* on 2B, and *Ppd-D1* on 2D chromosomes (Snape et al. 2001). Varying degrees of photoperiod insensitivity have been reported, with *Ppd-D1a* ranked as the strongest allele conferring photoperiod insensitivity, followed by *Ppd-B1a* and *Ppd-A1a* (Worland, 1996).

Studies on hexaploid wheat revealed that a semi dominant type of mutation occurred in the upstream direction of the coding region of the photoperiod insensitive allele *Ppd-D1a.* A deletion mutation of 2089 bp, in the 2D PRR gene resulted in a change of photoperiod sensitive cultivars to photoperiod insensitive ones. A deletion mutation may have changed the transcription start site or caused removal of the regulatory element from wheat genotypes. (Beales et al. 2007). The 2D region of wheat chromosome is collinear with the region of the barley (*Hordeum vulgare* L.) 2HS that contains the photoperiod genes *Ppd-H1* (Borner et al. 1998). The 2HS barley allele has been cloned and identified as a member of the pseudo-response regulator (*PRR*) family (Turner et al. 2005).The response of a wheat plant to photoperiod would be the transition of vegetative growth phase to a reproductive growth phase. In this transition, spikelet initiation and growth overlaps with the stem elongation period which results in partitioning of total assimilate supply (Miralles et al. 1998). This overlapping period, which is sensitive to photoperiod (Whitechurch and Slafer, 2002), if elongated by manipulating specific *Ppd* genes in different combination can result in enhanced partitioning of assimilate supply to spikelet growth. They become more fertile and productive, which in turn increases the number of grains (Slafer et al. 2005) and high yield is attributed to more grains per unit area (Slafer, 2003).

Frost damage due to the short growing season on the Prairies magnifies the importance of early maturity. High yield and quality in a limited growing season (95-125days) poses a challenge to wheat breeders, as early maturity is negatively correlated with yield and yield with protein content (Iqbal et al. 2007a).

The objectives of the study were to: i) to screen Canadian germplasm for different *Vrn* and *Ppd* genes, ii) to quantify the effect of each *Vrn* and *Ppd* gene in terms of days to flowering, maturity and grain yield in the field and the greenhouse, and iii) to study the interaction of flowering genes and their pleiotropic effects on agronomic traits.

2.2 Materials and Methods

This study involved a random population of 102 Canadian spring wheat lines/cultivars developed in Canada from 1885 to 2008 in the Canadian Western Red Spring (CWRS), Canadian Prairie Spring (CPS) and Canada Western Soft White Spring (CWSWS) classes. The lines could be considered a random collection of Canadian germplasm over the last 120 years from two classes: Canadian Western Red Spring (CWRS) and CWSWS. The lines were screened for vernalization (*Vrn-A1a, Vrn-A1b, Vrn-B1, Vrn-D1)* and photoperiod (*Ppd-D1)* genes. Tissue samples from 7-10 day old seedling were used to extract DNA by common CTAB protocol proposed by Anderson et al. (1992). For vernalization gene screening the primers sequences were adopted as reported by Yan et al. (2004) and Fu et al. (2005). PCRs were done in GeneAmp® 9700 PCR systems (Applied Biosystems) using a total volume of 20µL. Generally, the reaction mixture recipes contained 0.5 µL of each forward and reverse primers of 5µM concentration, 10 µL of Extract-N-Amp^{TM}PCR ready mix (Sigma-Aldrich, Cat#E3004), 1-4 µL DNA with a concentration of 25-100 ng/µL and 5-8µL sterile water to make total reaction volume to 20µL. The PCR cycling duration and temperatures followed Yan et al. (2004) and Fu et al. (2005). The primer sequences and PCR conditions for the photoperiod allele (*Ppd-D1a*) were adopted from Bently et al. (2011). All PCR products were visualized in gel concentrations of 1-3% using ethidium bromide.

Experiments were designed to study these genes and their interactions both in the field and a greenhouse. Two row 2m long plots were grown with two replications, in randomized incomplete blocks with two blocks, at two locations: University of Alberta, South campus, Edmonton (53˚ 32'N, 113˚32'W) and the Agriculture and Agri-Food Canada, Lethbridge (49˚ 41'N, 112˚49'W) in 2009, 2010 and 2011 (except for 2010 South Campus, where single rows were used). Recommended production practices for fertilizer and herbicide were practiced. The greenhouse experiment was grown with two replications in 5 inch pots with each pot having two plants of a given genotype. Growing conditions were set to a

16hour light period and an average temperature of 21°C and 18°C the light and dark periods, respectively. Plants were irrigated regularly and fertilized with water soluble commercial fertilizer (15-30-15: N-P_2O_5-K_2O).The experiment was replicated in 2010.

Data on days to flowering, days to maturity, and plant height were recorded at all three locations, while grain yields were estimated at greenhouse and Edmonton location. Protein content was recorded from the Edmonton field samples. Days to flowering was recorded when 50% of the spikes in a plot had clearly emerged from the flag leaf. Physiological maturity was determined when 50% of the peduncles in a plot had completely lost their green color. Grain protein content was estimated by Near Infrared Reflectance (NIR) spectroscopy using *SpectraStar RTW* apparatus by Unity Scientific. Grain yield estimates were recorded by harvesting total plants in a plot, and were multiplied by a conversion factor to estimate yield per hectare.

2.2.1 Statistical Analysis

The data were analyzed by PROC MIXED in SAS statistical software package version 9.2 (SAS Institute Inc., Cary NC). The data for Edmonton and Lethbridge were analyzed separately, mainly because of different moisture conditions (as the sites are 500km apart, in different soil zone with the Lethbridge trial being irrigated), to avoid any confounding genotype x location effect. Secondly, within each location, the data were analyzed by class. To estimate the least square mean for each line, PROC MIXED was used with lines/cultivars, as fixed effects while year, blocks and incomplete blocks nested within blocks, as random effects. The lines with similar vernalization or photoperiod allele combinations were grouped together and were considered a treatment. To estimate the effect of each treatment PROC MIXED was used and LSMEANS were estimated with treatment, as fixed effects; while year, blocks and incomplete blocks nested within blocks, as random effects. As the number of lines in each group varied, the denominator degree of freedom was adjusted utilizing the

Kenward-Rogers method to avoid small sample inferences (Kenward and Rogers 1997).The significance of difference between the treatments was tested to compute the effect of different vernalization genes, by using a CONTRAST statement. For example to quantify the effect of *Vrn-A1a*, the group of lines having *Vrn-A1a* and *Vrn-B1* genes were compared with groups of lines with only *Vrn-B1* gene. Similarly, the effect of *Vrn-B1* was estimated by comparing groups of lines having *Vrn-A1a* and *Vrn-B1*, with those having only *Vrn-A1a* and the significance of the quantified effects were calculated by ESTIMATE statements. The photoperiod allele (*Ppd-D1a*) effect was estimated by using *Vrn* genes as a covariate, to remove any confounding effect. The number of cultivars in the CPSR class was quite low (only six), and were merged with the CWRS class to retain enough statistical power while estimating the individual gene effect. A general model to estimate the effect of *Vrn* or *Ppd* genes was as follows:

$$Trait = Treatment + BLOCK + iBLOCK(BLOCK) + YEAR + error$$

$$Y_{ijkl} = T_i + B_j + IB_k + Y_l + e_{ijkl}$$

Genotypic and phenotypic correlation coefficients were estimated using multivariate Restricted Estimation of Maximum Likelihood (REML) using PROC MIXED in SAS to avoid drawbacks related to traditional methods (Holland 2006). The genetic ($\hat{r}_{g(xy)}$) and phenotypic ($\hat{r}_{p(xy)}$) correlations between the trait *x* and *y* were estimated as follow:

$$\hat{r}_{g(xy)} = \frac{\hat{\sigma}_{G(xy)}}{\sqrt{\hat{\sigma}^2_{G(x)} \cdot \hat{\sigma}^2_{G(y)}}},$$

and

$$\hat{r}_{P(xy)} = \frac{\hat{\sigma}_{P(xy)}}{\sqrt{\hat{\sigma}^2_{P(x)} \cdot \hat{\sigma}^2_{P(y)}}} = \frac{\hat{\sigma}_{G(xy)} + \hat{\sigma}_{GE(xy)} + \hat{\sigma}_{e(xy)}}{\sqrt{\hat{\sigma}^2{}_{G(x)} + \hat{\sigma}^2{}_{GE(x)} + \hat{\sigma}^2{}_{e(x)}} \cdot \sqrt{\hat{\sigma}^2{}_{G(y)} + \hat{\sigma}^2{}_{GE(y)} + \hat{\sigma}^2{}_{e(y)}}},$$

where $\hat{\sigma}_{G(xy)}$, $\hat{\sigma}_{P(xy)}$, $\hat{\sigma}_{GE(xy)}$ and $\hat{\sigma}_{e(xy)}$ are the estimated genetic, phenotypic, genotype x environment and error co-variances, respectively, between the two traits (*x* and *y)*; While $\hat{\sigma}_G^2$, $\hat{\sigma}_P^2$, $\hat{\sigma}_{GE}^2$ and $\hat{\sigma}_e^2$ are the estimated genetic, phenotypic, genotype x environment and error variances calculated for both traits. To test whether genotypic and phenotypic correlation coefficients were significantly different from zero, the coefficients were Z transformed as suggested by (Fisher, 1925): $Z_{xy} = \left[\ln(1+r_{xy}) - \ln(1-r_{xy})\right]/2$. The Z_{xy} variable was checked under the null hypothesis of no correlation with $Z' = \frac{Z_{xy}}{1/\sqrt{n-3}}$ where n is the number of lines.

2.3 Results

Canadian wheat germplasm in this study exhibited a wide range of days to flowering (51-67 days), maturity (100 -118 days), yield (4.10-9.20 t/ha) and protein content (9.2-13.6%) for both classes under field conditions (Table 2-11a, b). An overall analysis of the data for the CWRS class revealed a declining trend of 0.04 days/year in cultivars released from 1885 to 2008, and an increase of 8 kg/ha/year in grain yield (Fig 1).

Within hard red spring wheat, 94% (30 out of 32) possessed *Vrn-A1a* either alone or in combination with *Vrn-B1* (60% of the lines possessed *Vrn-A1a* alone and 40% of the lines have *Vrn-A1a* in combination with *Vrn-B1*) (Table 2-2). *Vrn-A1a* and *Vrn-B1* were the only *Vrn* genes present in the CWRS class except one cultivar (Rescue and a sister line RC5D), which have *Vrn-A1b* in combination with *Vrn-B1*. In soft white spring wheat the overall frequency of *Vrn-A1a* was 36%, one line out of 64 possessed *Vrn-A1a* alone; 14 lines in combination with *Vrn-B1*, and 8 lines in combination with *Vrn-B1* and *Vrn-D1* (Table 2-2). In this class the *Vrn-B1* allele frequency was high (74%) as 10 lines have *Vrn-B1* alone, 14 lines in combination with *Vrn-A1a*, 22 lines in combination with *Vrn-D1* and 8 lines with both *Vrn-A1a* and *Vrn-D1*. In the CPS class, only two out of six varieties harbored *Vrn-A1a* while four varieties retained

only *Vrn-B1* allele (Table 2-2). *Vrn-D1* allele was not found in hard red and Canadian Prairie Spring classes, but its frequency in soft white wheat was 48% : one line possessed *Vrn-D1* alone; 22 lines in combination with *Vrn-B1* and 8 lines in combination with *Vrn-A1a* and *Vrn-B1* (Table 2-2).

Vrn-A1a, alone or in combination with *Vrn-B1*, was found to be the most potent allele to confer early flowering in the material under study (Table 2-3a, b). It induced earlier flowering (4.5 days) in Edmonton, 4.0 days in Lethbridge, and 11.0 days earlier in the greenhouse in hard red spring wheat (Fig 2a). In soft white spring wheat *Vrn-A1a* induced flowering 1.9 days earlier in Edmonton, 1.4 days earlier in Lethbridge, and 9.0 days earlier in the greenhouse, respectively (Fig 2b, table 2-3a, b). A similar trend was observed for days to maturity in hard red wheat as *Vrn-A1a* induced earlier maturity of 8.8 days in Edmonton, 2.0 days in Lethbridge, and 13.3 days in greenhouse (Fig 2a, table 3a). *Vrn-A1a* did not alter days to maturity for CWSWS in the field but did ($P<0.05$) in the green house (4.5 days) (Fig 2b, table 2-3b). *Vrn-A1a* did not alter yield in field conditions in Edmonton. Grain protein content increased 1.1% due to the *Vrn-A1a* pleiotropic effect in the CWRS class, while this decreased 0.41% in the CWSWS class. *Vrn-B1* ranked second in its potency to induce early flowering of 1.4 days in Edmonton, 1.2 days in Lethbridge and 3.3 days in greenhouse (Fig 2a table 2-3a). Contrary to the effect of *Vrn-A1a*, *Vrn-A1b* delayed flowering times in the CWSWS class by 1.6 days in Edmonton and 1.5 days in Lethbridge (Fig 2b, table 2-3b). The *Vrn-D1* allele did not alter flowering and maturity, but increase in grain yield under field (0.64 t/ha) and green house (0.45 g/plant) conditions in the CWSWS class. The presence of this allele also increased grain protein content by 0.4% in CWSWS.

Only 12% (2 out of 16) of the CWRS varieties registered between1885 to 1986 carried the insensitive *Ppd* gene, *Ppd-D1a*; whereas 43% (7 out of 16) of the varieties released after 1991, had the insensitive *Ppd* allele in the CWRS class. Ninety one percent of the lines in the CWSWS class and 100% of the varieties in the CPSR class retained the insensitive *Ppd* allele (Table 2-2).

2.4 Discussion

The present study presents four main findings about *Vrn* and *Ppd* gene effects and the change in their relative frequencies through the course of time in western Canada. 1) *Vrn-A1a* and *Vrn-B1* genes are assembled in relatively higher frequencies in CWRS and CWSWS classes, respectively; 2) The higher yielding Canadian cultivars, generally, possess a less potent vernalization allele (*Vrn-B1*) and an elongated reproductive growth. These cultivars were classified in CWSWS class. On the contrary, cultivars in the CWRS class (with higher protein content) strictly retained the most potent vernalization allele (*Vrn-A1a*), conferring a shorter reproductive growth; 3) the photoperiod sensitive allele (*Ppd-D1)* is being replaced with the insensitive gene, *Ppd-D1a*, in all modern Canadian germplasm, which suggests its adaptive advantages in the Prairies, and ; 4) An overall analysis of the data for the CWRS class revealed a declining trend of 0.04 days/year in days to maturity in cultivars released from 1885 to 2008, and an increase of 8 kg/ha/year in grain yield.

Temperature among all the environmental factors has a dictating role in plant growth, development and adaptation, thereby deciding the geographical distribution of most vernalization genes as well. In other words, the degree of winter coldness prevailing in a specific geographical region seems to mediate the distribution pattern of different vernalization genes (Iwaki, et al. 2000). Spring wheat is cultivated in those parts of the world where average January temperature is less than -12.2°C (Wilsie, 1962 as cited by Iwaki et al. 2000). Therefore, complete insensitive cultivars with the *Vrn-A1* allele are cultivated in two extremes of winter coldness: either in the areas of intense cold where average January temperature is below -7°C or areas with average winter temperature above 10°C; and thereby failing to satisfy the vernalization requirement (Iwaki et al. 2001). *Vrn-A1* is frequent in Europe and Siberia (Stelmakh, 1990), and early maturity induced in 98% of modern wheat cultivars in Russia is by virtue of this allele (Goncharov and Shitova, 1999 as cited by Leonova et al. 2003).

The current study was conducted on a germplasm adapted to high latitude Canadian Prairies. Almost all the lines in CWRS class possessed *Vrn-A1a* allele either alone or in combination with *Vrn-B1*. The frequency distribution of *Vrn* genes in this class seems to agree the view point that the cultivars grown at high latitude usually possess *Vrn-A1a* followed by *Vrn*-B1 (Iqbal et al. 2007).*Vrn-A1a* reduces the pre anthesis period and grain yield. This actually is a trade-off. Early flowering and maturity, significantly reduce grain yield but often ensure higher protein content. Therefore, a less potent vernalization allele *Vrn-B1*, is more adapted in CWSWS cultivars, which are actually bred for higher grain yields, leaving out a concern about protein content. The prevalence of *Vrn-B1* allele in higher frequencies elaborates why this class is generally considered as late maturing and higher yielding than the CWRS class. Overall, most of the lines in the CWRS class have *Vrn-A1a*; whilst in the CWSWS class, the majority of lines tend to have *Vrn-B1*. In the CWSWS class, *Vrn-D1* was the second most frequent gene. *Vrn-D1* is more frequent in Asia (Stelmakh, 1990), Japan and China (Gotoh, 1979). Partial vernalization requirement of *Vrn-D1* confers broad diversity in adaptability of wheat cultivars. *Vrn-D1* is found to be the most frequent allele in CIMMYT derived wheat cultivars as, 66% possess this allele (van Beem et al. 2005).

The frequency distribution analysis implies that different *Vrn* allele frequencies have played their role to categorize Canadian wheat into different classes with varying potential of maturity times, grain yield and protein content *e.g* the CWRS class was earliest maturing as the class average was 105.7days to maturity followed by the CPS class (111.8) and the CWSWS class (114.1) being the latest. As early maturity is negatively correlated with yield, the CWSWS class was the highest yielding among the classes. Average yield for the CWSWS class was 6.38 t/ha, followed by the CWRS at 5.25 t/ha and the CPS at 5.24 t/ha. Similarly, as protein is negatively correlated with yield, the highest protein was found in the CWRS class (12.79%) followed by the CPS (11.63%) and the CWSWS (10.25%).

Abiotic stresses had aggravated the response of *Vrn*-genes. This was revealed when moisture stress (Edmonton) and non-stress (Lethbridge) environments were compared; as an enhanced delay or a rapid acceleration in flowering, and maturity was observed under field conditions. The effects were greater in the Edmonton location, where experiments were rain fed, compared to Lethbridge station, where the experiment was irrigated at regular interval. So, this augmented effect of *Vrn* genes could probably be attributed to irregular moisture supply in Edmonton. This differential effect was also noted among the classes.*Vrn-A1a* in combination with *Vrn-B1* induced earliest maturity in the CWRS class, whereas *Vrn-A1a* alone was the earliest in the CWSWS class. This inconsistency indicates that these classes also possess some other flowering genes, which are not accounted for in this study, and those genes interact with *Vrn-A1a,* and/or *Vrn-B1.*The pleiotropic effect of *Vrn-A1a* in CWRS resulted in tall plants with a significant increase in plant height in this study, which is probably a skewed estimate as number of cultivars in this class were released before the green revolution; and may not have any dwarfing gene. The effect of *Vrn-A1b* on height was similar to *Vrn-A1a.* The *Vrn-B1* allele was found to reduce the plant height significantly, whereas *Vrn-D1* had a non significant effect under both field and green house conditions.

The frequency of photoperiod insensitive genes was quite low in varieties released during 1885 to 1986, whereas almost all the varieties released after 1990 possess a *Ppd* gene, which exhibits a mega shift of sensitive *Ppd* allele to insensitive one during the last two decades in Canadian germplasm. In North African, Mediterranean regions and Asia, almost all landraces are sensitive to photoperiod, whereas all high yielding improved cultivars are insensitive (Ortiz Ferrara et al. 1998). Therefore, photoperiod insensitivity could be considered as one of the traits that enhances the adoptability of the improved cultivars in a wide range of environments in comparison with landraces (sensitive to photoperiod) in these regions. Photoperiod insensitivity is one of the better adaptability attributes and grain yield in European and Canadian wheat cultivars (Worland et al. 1994, 1998a, b). *Ppd-D1a* being the most potent allele to confer insensitivity is also

wide spread in most of the European and Chinese insensitive cultivars. In a study of 926 of Chinese wheat cultivars and landraces collected from nine wheat growing zones differing in their environmental conditions, the average frequency of the photoperiod-insensitive *Ppd-D1a* allele was 66.0% (442 Cultivars), with the frequencies of 38.6 and 90.6% in landraces and improved cultivars, respectively. However, the *Ppd-D1a* allele was present in almost all improved cultivars released after 1970 (Yang et al. 2009).

The effect of *Ppd-D1a* gene, in this study, was found significant in reducing the time to flowering by 1.57, 1.52 and 6.13 days in Edmonton, Lethbridge and greenhouse, respectively (Table 2-4). But this was only true in CWSWS class, as this effect was non-significant in CWRS class under field conditions. However in greenhouse, this allele has reduced the days to flowering and maturity by 5.87, and 5.83 days respectively (Table 2-4). Different studies have shown that *Ppd* genes triggers early flowering by 9-12 d, (Foulkes et al. 2004) or 4-8 d (Worland, 1996) or 6-14 d (Worland et al. 1998). Earliness induced by dominant *Ppd* genes is mediated through developmental acceleration, which may be either from emergence to floral initiation (Davidson et al. 1985), and/or by shortening the spikelet primordial initiation, (Rawson and Richards, 1993) terminal spikelet to flowering (Snape et al. 2001) or short thermal duration from crop emergence to stem extension (Foulkes et al. 2004).

The insensitivity allele *Ppd-D1a* showed a pleiotropic, non-significant increase in grain yield in this study. In the literature, photoperiod insensitivity is reported to have contrasting effects on the grain yield of hexaploid wheat. In fact, the environmental conditions, in a particular growing season, have critical role to play in describing the grain yield of responsive or non-responsive cultivars grown in that particular geographical area. Different studies proved the photoperiod non-responsive cultivars have an increased yield compared to the photoperiod responsive cultivars and *vice versa*. Photoperiod insensitivity dictated increased grain yield in Southern Europe up to 35% (Worland, 1996). This yield advantage was 7.7% in Germany and 33% in the former Yugoslavia (Worland et al. 1998).

Whereas in U.K and the northern part of Europe photoperiod insensitivity has varying results from year to year depending upon varying environmental conditions. A yield disadvantage of -1.8% in conventional cool humid summer, 5% of yield advantage in hot dry summer, and no yield advantage were reported in different studies over the years (Law et al. 1994; Worland et al. 1994; 1996; 1998). Yield advantages with photoperiod insensitive cultivars were actually escape from hot dry summer by maturing earlier as hot dry conditions are associated with decreased tiller number and decreased grain weight.

The pleiotropic effects of the *Ppd* genes have been reported on various agronomic traits of wheat in different studies. Dominant *Ppd* genes are known to reduce plant height (Marshall et al. 1989; Worland, 1996), final leaf number (Miralles and Richards, 2000; Dyck et al. 2004), spikelet primordial initiation (Rawson and Richards, 1993), tillering (Miralles and Richards, 2000; Dyck et al. 2004), spikelet per spike (Snape et al. 2001; Dyck et al. 2004), and green leaf area index (Foulkes et al. 2004).

A strong positive genetic correlation between grain yield and days to flowering ($r = 0.64^{**}$), and days to maturity ($r = 0.68^{**}$) were observed which suggests that a negative correlation exists between early maturity and grain yield. Similarly, a strong negative genetic correlation between protein content and days to flowering ($r = -0.55^{**}$), days to maturity ($r = -0.90^{**}$) and grain yield ($r = -0.76^{**}$) were observed which suggests that protein content increases with early maturity and declines with high grain yield (Table 2-5). These results are in agreement with the findings of Iqbal et al. (2007) and Reid et al. (2009).

Canadian germplasm under study revealed that the *Vrn-A1a* allele is most frequent in the CWRS class; whereas the *Vrn-B1* allele is more frequent in CWSWS class. *Vrn-A1a* either alone or in combination with *Vrn-B1* confers earliest flowering; followed by *Vrn-B1* and *Vrn-D1*. Positive correlation between grain yield and days to flowering, and maturity suggests that higher grain yields are attributed to longer growth period, whereas negative correlation between

protein content and days to flowering suggests that longer growth periods reduce the grain quality.

2.5 Conclusion

The plant material comprised of 102 Canadian spring wheat cultivars/lines. These lines/cultivars were developed between 1885 and 2008, from three Canadian wheat classes: Canada Western Red Spring (CWRS), Canada Prairie Spring Red (CPSR) and Canada Western Soft White Spring (CWSWS) classes. Almost all the hard red cultivars possessed *Vrn-A1a* allele either alone or in combination with dominant *Vrn-B1* while 74% of the higher yielding soft white lines possessed *Vrn-B1* allele alone or in combination with other *Vrn* genes. The *Vrn-A1a* alone or in combination with *Vrn-B1* allele was the most potent allele conferring early maturity. *Ppd-D1a* did not alter flowering and maturity in the hard red cultivars tested in the field, but did in the greenhouse. *Ppd-D1a* reduced days to flowering for soft white spring wheat but did not alter maturity. The photoperiod sensitive allele (*Ppd-D1b)* is being replaced with the insensitive gene, *Ppd-D1a*, in most modern Canadian germplasm, suggesting its adaptive advantages in the Prairies. An overall analysis of the data for the hard red class revealed a declining trend of 0.04 days/year in days to maturity in cultivars released from 1885 to 2008, and an increase of 8 kg/ha/year in grain yield. The findings of this study may aid wheat breeders selecting parents with appropriate vernalization and photoperiod gene complexes.

Table 2-1a Overall mean, standard error of the mean, range, heritability and standard error of heritability for days to flowering, maturity, plant height (cm), grain yield (t/ha) and protein content (WPRO) in Canada Western Red Spring (CWRS) class

		FLOWERING (Days)			MATURITY (Days)			HEIGHT (cm)		YIELD (t/ha)		WPRO (%)
		Edm†	Leth‡	GH§	Edm	Leth	GH	Edm	GH	Edm[a]	GH[b]	Edm
Mean		54.7	59.4	64.4	106.9	106.8	120.8	90	97.1	4.97	5.99	12.55
SE(Mean)		0.24	0.38	0.52	0.63	0.25	0.7	1.1	0.73	0.11	0.12	0.09
Range	Mini.	51	56	52	100	102	105	70	79	4.10	3.16	11.0
	Maxi.	60	65	78	115	113	143	116	120	5.96	8.61	13.6
Heritability		0.26	0.25	0.57	0.05	0.51	0.61	0.2	0.61	0.12	0.19	0.15
SE(Heritability)		0.07	0.1	0.07	0.04	0.08	0.06	0.06	0.06	0.04	0.06	0.08

Table 2-1b Overall mean, standard error of the mean, range, heritability and standard error of heritability for days to flowering, maturity, plant height (cm), grain yield (t/ha) and protein content (WPRO) in Canada Western Soft White Spring (CWSWS) class

		FLOWERING (Days)			MATURITY (Days)			HEIGHT (cm)		YIELD (t/ha)		WPRO (%)
		Edm†	Leth‡	GH§	Edm	Leth	GH	Edm	GH	Edm	GH	Edm
Mean		59.9	62.3	69.7	114.2	112.4	136.1	80.4	84.1	6.08	6.41	10.19
SE(Mean)		0.18	0.25	0.46	0.43	0.21	0.51	0.64	0.39	0.13	0.11	0.06
Range	Mini.	54	58	57	110	105	120	66	69	4.4	3.76	9.2
	Maxi.	63	67	93	118	118	154	96	101	8.87	9.20	11.7
Heritability		0.21	0.27	0.47	0.07	0.55	0.34	0.53	0.41	0.24	0.08	0.09
SE(Heritability)		0.07	0.09	0.05	0.04	0.06	0.05	0.05	0.05	0.05	0.04	0.06

†Edmonton; ‡Lethbridge; §Green House

Table 2-2. Frequency distribution of different vernalization and photoperiod genes in 102 lines/cultivars under study in Canada Western Soft White Spring (CWSWS), Canada Western Red Spring (CWRS) and Canada Prairie Spring Red (CPSR) classes.

	CWSWS			CWRS			CPS		
	Vrn **Genes frequency**	***Ppd*** **frequency**		***Vrn*** **Genes frequency**	***Ppd*** **frequency**		***Vrn*** **Genes frequency**	***Ppd*** **frequency**	
		PpdD1a	*PpdD1*		*PpdD1a*	*PpdD1*		*PpdD1a*	*PpdD1*
Vrn-A1a	1	1	.	18	7	11	2	2	.
Vrn-A1a, VrnB1	14	11	3	12	2	10	.	.	.
Vrn-A1b	1	1	.	.	.	.	.	.	.
Vrn-A1b, VrnB1	7	6	1	2	0	2	.	.	.
Vrn-B1	10	10	.	.	.	.	4	4	
Vrn-B1 , Vrn-D1	22	21	1	.	.	.	.	.	.
Vrn-D1	1	1		.	.	.	.	.	.
Vrn-A1a,Vrn-B1, Vrn-D1	8	7	1	.	.	.	.	.	.
Total	64	58	6	32	9	23	6	6	0

Table 2-3a. Least square means estimates of different *Vrn* gene groups and effect of *Vrn-A1a* and *Vrn-B1* for days to flowering, maturity, plant height (cm), grain yield (t/ha) and protein content (WPRO) in Canada Western Red Spring class.

		FLOWERING (Days)			MATURITY (Days)			HEIGHT (cm)		YIELD (t/ha)		WPRO (%)
	n†	Edm‡	Leth§	GH¶	Edm	Leth	GH	Edm	GH	Edm[a]	GH[b]	Edm
Vrn-A1a	18	55	60	65	106	106	119	93	100	5.12	5.84	12.8
Vrn-A1a , Vrn-B1	12	54	58	61	106	106	121	88	95	4.91	6.00	12.8
Vrn-A1b , Vrn-B1	2	56	60	66	106	107	113	97	107	4.70	6.94	13.1
Vrn-B1	4	59	62	72	114	108	134	76	85	5.12	6.21	11.7
Effect of Vrn-A1a		**-4.5**	**-4.0**	**-11.0**	**-8.8**	**-2.0**	**-13.3**	**12.8**	**10.3**	**-0.21**	**-0.21**	**1.1**
(Vrn-A1a, Vrn-B1) vs Vrn-B1	**Pr > F**	<0.0001	0.005	<0.0001	<0.0001	0.02	<0.0001	<0.0001	<0.0001	0.32	0.29	<0.0001
Effect of Vrn-B1		**-1.4**	**-1.2**	**-3.3**	**-0.4**	**0.0**	**1.9**	**-4.2**	**-4.8**	**-0.21**	**-0.16**	**0.0**
(Vrn-A1a, Vrn-B1) vs Vrn-A1a	**Pr > F**	<0.0001	0.04	0.0019	0.54	0.94	0.15	0.006	0.0016	0.1	0.21	0.93

†Number of lines

‡Edmonton; §Lethbridge; ¶Green House

Table 2-3b. Least square means estimates of different *Vrn* gene groups and effect of *Vrn-A1a,Vrn-A1b* and *Vrn-D1* for days to flowering, maturity, plant height, grain yield and protein content (WPRO) in Canada Western Soft White Spring class.

		FLOWERING (Days)			MATURITY (Days)			HEIGHT (cm)		YIELD (t/ha)		WPRO (%)
	n†	Edm‡	Leth§	GH¶	Edm	Leth	GH	Edm	GH	Edm	GH	Edm
Vrn-A1a , Vrn-B1	14	57	61	64	113	110	133	79	84	6.10	6.38	10.1
Vrn-A1a	1	56	60	61	112	111	139	79	90	5.98	5.33	10.2
Vrn-A1b , Vrn-B1	7	61	64	75	114	114	138	83	86	6.49	6.57	9.9
Vrn-B1	10	59	62	73	114	112	138	79	83	5.91	6.32	10.5
Vrn-B1 , Vrn-D1	22	59	62	72	114	111	137	79	85	6.54	6.77	10.2
Vrn-A1a , Vrn-B1 , Vrn-D1	8	571	61	64	114	110	133	81	84	6.66	5.94	10.0
Effect of Vrn-A1a		**-1.9**	**-1.4**	**-9.0**	**-1.3**	**-1.1**	**-4.5**	**-0.1**	**1.2**	**0.19**	**0.06**	**-0.4**
(Vrn-A1a, Vrn-B1) vs Vrn-B1	**Pr > F**	<0.0001	0.0026	<0.0001	0.0261	0.1118	0.0056	0.92	0.33	0.37	0.088	0.01
Effect of Vrn-A1b		**1.6**	**1.5**	**2.3**	**-0.2**	**2.6**	**0.2**	**4.0**	**3.2**	**0.58**	**0.25**	**-0.6**
(Vrn-A1b, Vrn-B1) vs Vrn-B1	**Pr > F**	0.003	0.0062	0.1598	0.816	0.0008	0.91	0.0067	0.04	0.021	0.007	0.002
Effect of Vrn-D1		**-0.6**	**-0.3**	**-0.5**	**-0.4**	**-0.1**	**-0.3**	**0.3**	**2.1**	**0.64**	**0.45**	**0.4**
(Vrn-B1, Vrn-D1) vs Vrn-B1	**Pr > F**	0.0959	0.486	0.65	0.5137	0.9867	0.82	0.7855	0.07	0.0012	0.008	0.026

†Number of lines

‡Edmonton; §Lethbridge¶Green House

Table 2-4 *Ppd-D1a* gene effect on days to flowering, maturity, height, yield and protein content (WPRO) in Canada Western Red Spring and Canada Western Soft White Spring with all three locations.

	CWRS					CSWSW				
	Flowering	**Maturity**	**Height**	**Yield**	**WPRO**	**Flowering**	**Maturity**	**Height**	**Yield**	**WPRO**
Edmonton	NS†	NS	-4.3	NS	NS	-1.5	NS	3.2	NS	NS
Lethbridge	NS	NS	NS	.‡	.	-1.5	NS	NS	.	.
Greenhouse	-5.8	-5.8	-4.6	-0.86	.	-6.1	NS	2.8	NS	.

†Non significant

‡Data not available

Negative sign means insensitive *Ppd* gene has reducing effect

Table 2-5. Phenotypic and genetic correlation coefficients among days to flowering, days to, days to maturity, plant height, grain yield and protein content (WPRO).

	Flowering	Maturity	Height	Yield	WPRO
Flowering		0.61*** ±0.04	NS	NS	-0.39*** ±0.06
Maturity	0.83*** ±0.04		NS	0.26*** ±0.05	-0.57*** ±0.04
Height	NS	NS		NS	0.47*** ±0.05
Yield	0.64*** ±0.06	0.68*** ±0.11	NS		-0.37*** ±0.04
WPRO	-0.55*** ±0.08	-0.9 ±0.03	0.62*** ±0.07	-0.76*** ±0.1	

***Indicates Significance at P < 0.001, NS indicates non significant

Values above the line are phenotypic correlations and below the line are genotypic correlations.

Figure 2-1. Changes in days to maturity and grain yield in cultivars released under Canada Western Red Spring class from 1885 to 2008.

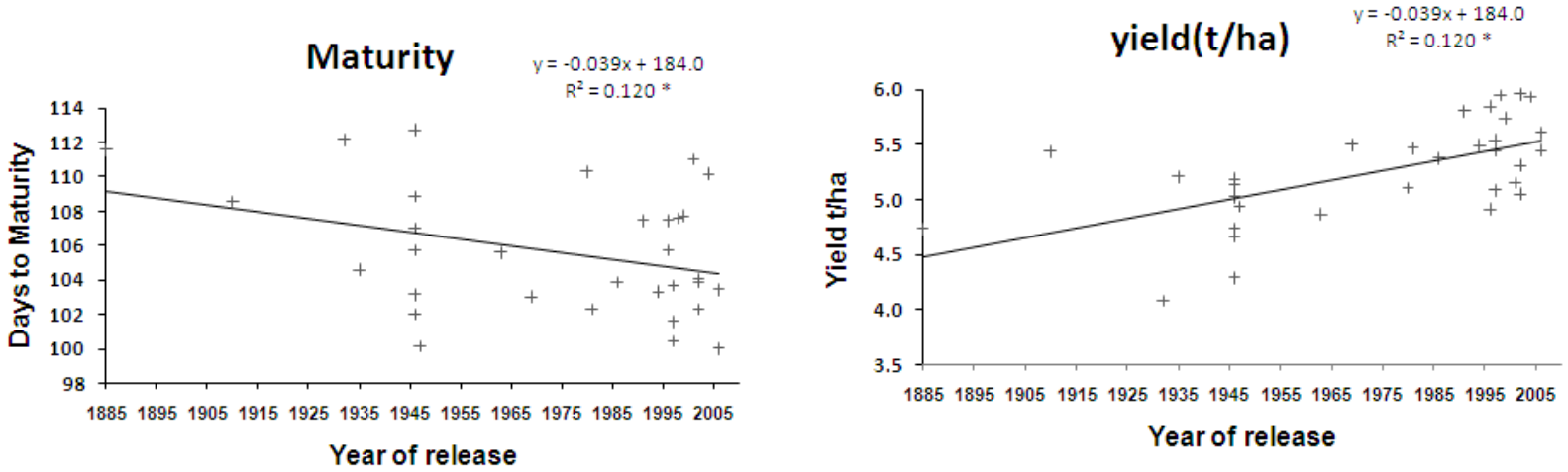


Figure 2-2 (a) The effect of the *Vrn* gene on flowering, maturity, plant height, grain yield, and protein content in Canadian Western Red Spring class

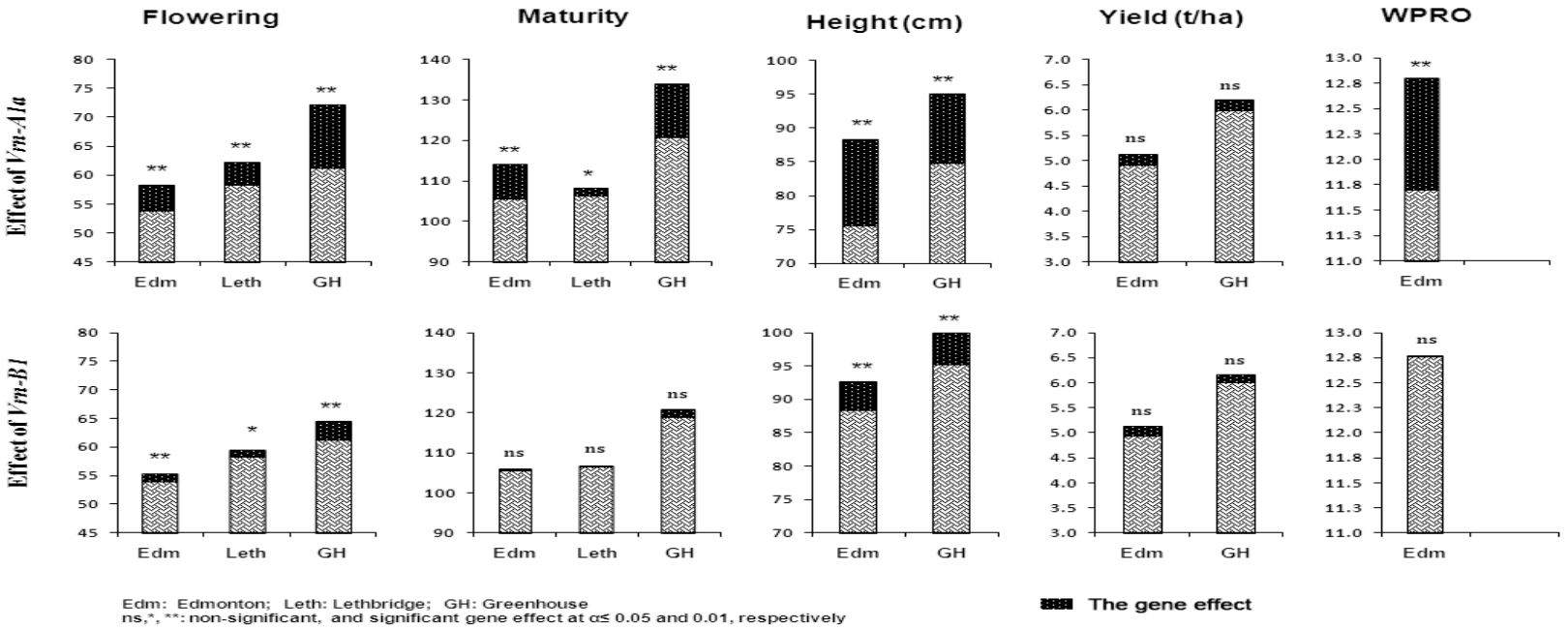
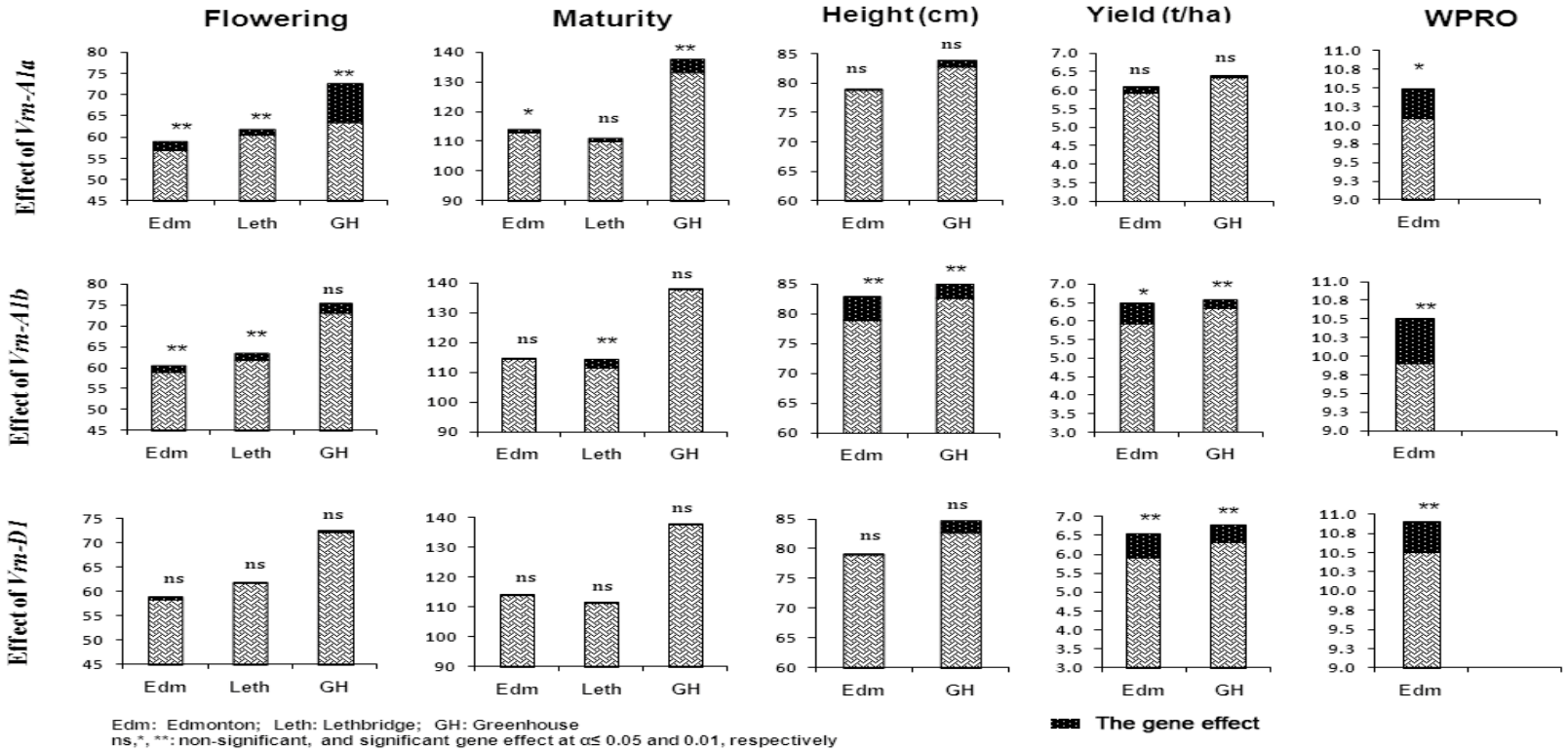


Figure 2-2 (b) The effect of the *Vrn* gene on flowering, maturity, plant height, grain yield, and protein content in Canadian Western Soft White Spring wheat.



Edm: Edmonton; Leth: Lethbridge; GH: Greenhouse
ns,*, **: non-significant, and significant gene effect at α≤ 0.05 and 0.01, respectively

2.7 References

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[3]3 Earliness *per se* QTLs and their interaction with the photoperiod insensitive allele *Ppd-D1a* in the Cutler × AC Barrie spring wheat population

3.1 Introduction

Early maturity in wheat (*Triticum aestivum* L.) is an important breeding objective in regions where the growing season is short and days are long (>14 h), such as the Northern Great Plains of Canada and the USA. The development of early maturing cultivars is also important to avoid frost damage, which can affect both yield and grain quality (Iqbal et al. 2007). Flowering time of wheat is the outcome of a complex interaction of genes that regulate growth habit and earliness. Major gene classes that determine flowering time include vernalization (*Vrn*), photoperiod (*Ppd*) and earliness *per se* (*Eps*). Different alleles at the *Ppd* loci divide cereals into photoperiod sensitive and insensitive, while *Vrn* genes divide them into winter and spring types (Distelfeld et al. 2009). Insensitivity to photoperiod and vernalization is due to deletion mutations in the genomic regions involved in plant responses to environmental signals (Yan et al. 2004; Beales et al. 2007; Santra et al. 2009). A two Kbp deletion mutation upstream of the *Ppd-D1* coding region is supposed to have altered the transcriptional start site or caused removal of the regulatory element (Beales et al. 2007) changing the photoperiod sensitive cultivars to insensitive ones.

Earliness *per se* (*Eps*) genes are considered to be of smaller effect and are not involved in the *Vrn* and *Ppd* complexes (Miura and Worland, 1994). Ford et al. (1981) reported some 'other genes' controlling flowering time in wheat apart from vernalization and photoperiod genes and called them "earliness genes". Earliness

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per se genes has been reported as being strong enough to induce earlier flowering, even in the presence of *Vrn* and *Ppd* genes (van Beem et al. 2005).

The intricate flowering gene network and allo-hexaploid nature of bread wheat has challenged scientists attempting to quantify individual gene effects. To avoid confounding effects of different genomes, studies on *monococum* wheat have been conducted. Bullrich et al. (2002) mapped a major QTL in a *Triticum monococum* accession located close to the SSR marker *Xwg 241* on the long arm of chromosome 1A. Minor flowering differences due to *Eps* genes have been reported by Laurie et al. (1995), Worland (1996) and Kato et al. (1999).

Earliness *per se* genes can induce early flowering by initiating floral primordia with minimum vegetative growth (Kato and Wada, 1999). The effect of *Eps* genes on different phases of early reproductive and vegetative growth was investigated by Lewis et al. (2008). Their study revealed that *Eps-A^{m}1-e* can activate transition of vegetative apices to reproductive apices 35 days earlier than *Eps-A^{m}1-l*. Time from double ridge formation to terminal spikelet was longer in the lines carrying the *Eps-A^{m}1-l* allele. This prolonged duration resulted in increased grain yield as the lines with late allele *Eps-A^{m}1-l* produced more spikelets per spike than those with the early flowering allele *Eps-A^{m}1-e* (Lewis et al. 2008). Hence earliness *per se* genes can alter different growth phases and some other pleiotropic effects are attributed to these genes. In another study, a major *Eps* gene was reported on chromosome 3A that was responsible for significant variation in plant height, 1000 grain weight and number of grains per plant (Shah et al. 1999). Similar findings of pleiotropic effect of vernalization, photoperiod and earliness *per se* genes have been reported (Worland and Snape, 2001). Earliness *per se* genes are also considered to play their role in adaptability (Snape et al. 2001). The *Eps* genes are important from the practical breeding viewpoint due to their high broad sense heritability (0.90-0.99) (Kato and Wada, 1999) and additive type of gene action (Bullrich et al. 2002).

This study was designed to map QTLs for earliness *per se* by crossing two spring wheat cultivars maturing significantly apart from each other (Iqbal et al.

2006). The earlier maturing parent Cutler possessed the photoperiod insensitive allele (*Ppd-D1a*), while the late maturing parent AC Barrie possessed the photoperiod sensitive allele (*Ppd-D1b*) (Iqbal et al. 2006). However, both the parents are vernalization insensitive and possess similar vernalization alleles at the three *Vrn-1* loci (Iqbal et al. 2006; Iqbal et al. 2007). This yielded an opportunity to study the interaction between the photoperiod and earliness *per se* genes. In this study, we aimed to: (i) identify QTLs affecting flowering, maturity, plant height and grain yield in a Canadian spring wheat population derived from a cross between early and relatively late maturing cultivars, (ii) study the effect of *Ppd-D1a* in a population where RILs share substantially similar genetic background (iii) investigate the interaction between the *Ppd-D1a* and earliness *per se* QTLs to improve the understanding of flowering gene complex. This information will help to develop elite breeding material aimed for early maturing and high yielding cultivars in the region.

3.2 Materials and Methods

3.2.1 Development of Recombinant Inbred Lines

A population was developed by crossing two Canadian spring wheat cultivars, AC Barrie and Cutler. AC Barrie was one of the most widely grown and high yielding cultivars of Canadian Western Red Spring wheat. It has been characterized as having high grain yield and protein content, late maturing (compared to Cutler) and resistant to some diseases (McCaig et al. 1995). Cutler is an early maturing, semi dwarf cultivar from the Canadian Prairie Spring class. Cutler was bred for regions where early maturity was of prime concern (Briggs et al. 1991) and possesses the vernalization genes *Vrn-A1a-vrn-B1-vrn-D1* and the photoperiod insensitive allele *Ppd-D1a*. AC Barrie, the comparative late parent possesses *Vrn-A1a-vrn-B1-vrn-D1* genes and the photoperiod sensitive allele *Ppd-D1b*. Cutler and AC Barrie were crossed to produce F_1 seeds, and to subsequently develop a population of 177 $F_{6:7}$ recombinant inbred lines (RILs). The population

($F_{6:7}$) was originally used in the first field and green house experiment in 2007. Thereafter heads were taken and re-grown for experimental use annually.

3.2.2 Greenhouse Evaluation

Phenotypic analysis of parents and RILs was completed after satisfying the vernalization and photoperiod requirements. The sprouted seeds were vernalized for 42 days at 1˚C in the dark. At the end of the vernalization treatment, seedlings of similar size were transplanted into 12.5 cm diameter pots (two plants per pot). The experiment was arranged as a randomized complete design with four replications, each consisting of one pot per treatment. The population was grown in a greenhouse maintained at 25˚C and 18 hr photoperiod (plants received both natural light and artificial illumination). Plants were watered every second day and fertilized biweekly with a 200 ppm solution of a water soluble commercial fertilizer (15-30-15: N-P_2O_5-K_2O). To remove any confounding effects of differential growth during vernalization treatment, the date of 2nd leaf unfolding was recorded for all plants. The date of heading was subsequently recorded for all plants as the date when the spike completely emerged from the flag leaf. Days to heading was recorded as the number of days from 2nd leaf unfolding to the date of heading.

3.2.3 Field Evaluation

Field experiments were conducted at or near the University of Alberta South Campus Crops Research facility in Edmonton, Canada, (53˚19’ N, 113˚35’W and 723.3 m elevation) in 2007, 2008 and 2011. In 2007 and 2011, the experiment was seeded on May, 27 and May 14, respectively, while in 2008, the complete experiment was grown twice, one planted on May, 07 (early) and one on June 04 (late) to assess any confounding effects of vernalization requirement. Each experiment was grown in a randomized complete block design with two replications. Plots consisted of 2 m long double rows with a row spacing of 22.5 cm in 2007 and 2008, while in 2011 the plot size was 1.35x1.8 m with six rows each spacing 22.5 cm. The parents, RILs and ten check cultivars Superb, AC

Intrepid, CDC Go, AC Foremost, Lovitt, AC Mckenzie, AC Crystal, AC Splendor, and Peace were planted. Data were recorded on days to flowering, maturity and grain yield. Flowering was recorded when 50% of the spikes had emerged out of the flag leaf. Physiological maturity was determined when 50% of the peduncles in a plot had completely lost green color. The days to flowering and maturity were converted into growing degree days by summing the average daily temperatures (over a base temperature of 0˚C) from the date of seeding to the date when flowering or maturity was recorded. Plant height was measured as height (cm) from ground level to the tip of spike (excluding awns) at maturity. Grain yield per plot was converted to t/ha. Fertilizers (N-P_2O_5-K_2O:11-52-0) were applied at the rate of 36 kg/ha at the time of sowing and other standard agronomic practices were carried throughout the growing season to obtain even crop stands.

3.2.4 DNA Extraction

Seed was sown in trays containing commercial soil (Sunshine-LA4 Sun Grow Horticulture, Canada) and placed in a growth chamber for 7-10 days. Growing conditions were maintained at 21 ˚C day and 19 ˚C night and a 16h photoperiod. Young plant tissue (100-150 mg.) was harvested and flash frozen in liquid nitrogen. Tissue was stored at -80°C until DNA extracted. Leaf tissue from a single plant was ground in liquid nitrogen. DNA of 177 RILs along with parents was extracted according to the protocol suggested by Diversity Arrays Technology (DArT) (http://www.diversityarrays.com). DNA was quantified by NanoDrop® (ND-1000). DNA was diluted to 50-100 ng/µl. 100 µl DNA solution was aliquoted to 96 well microtitreplates and sent to DArT, Pty Ltd. Australia for marker genotyping with high density arrays around 7000 cloned sequences. The Diversity Arrays Technology genotyping followed protocols previously described by Akbari et al (2006).

3.2.5 SSR Genotyping

Three to four plants from selected RILs, parents and F_1 were grown in a controlled environment chamber. Genomic DNA was extracted from leaves of 7-10 day old plants using the Extract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, Oakville, Canada; Cat# XNAP), following the protocol provided by the manufacturer. One hundred and two SSR loci from all 21 chromosomes of wheat were selected for genotyping. These were selected based on polymorphic differences when tested against the parents AC Barrie and Cutler. The forward primer of each primer pair was fluorescently labeled using either 6-FAM, NED or VIC (Applied Biosystems). Polymerase chain reaction was performed in a 20µL volume in a GeneAmp® 9700 thermal cycler (Applied Biosystems; Foster City, CA). The reaction mixture contained 0.5 µL each of the 5 µM forward and reverse primers (2 primer pairs were used in the same reaction to amplify 2 loci), 10 µL Extract-N-Amp™ PCR ReadyMix (Sigma-Aldrich, Cat# E3004), 4 µL sterile water and 4 µL DNA extract. After initial denaturation at 94˚C for 2 min, 35 cycles at 94˚C for 1 min., 47-66˚C (depending on the primer pair used) for 1 min. and 72˚C for 2 min. were performed, followed by a final extension at 72˚C for 10 min. The PCR products (0.5 µL) were mixed with 0.10 µL Gene Scan™ 500-LIZ (Applied Biosystems) size standard and 9.4 µL Hi-Di Formamide, denatured at 94˚C for 2 min. and chilled on ice for 2 min. Capillary electrophoresis was performed using ABI 3730 DNA Analyzer and product sizes were determined using Gene Mapper v. 3.7 software (Applied Biosystems).

3.2.6 Statistical Analysis

The data were analyzed by using PROC MIXED in SAS statistical software package version 9.2 (SAS Institute Inc., Cary NC). The lines were considered as a fixed effect, whereas the effect of the block, incomplete block nested in block and seeding environments were considered as random effects.

Multivariate Restricted Estimation of Maximum Likelihood (REML) method was used for estimation of genetic and phenotypic correlation coefficients in PROC MIXED of SAS (Holland et al. 2006). In the correlation analysis, the lines

were considered as a random effect along with year, block and incomplete block. The genetic ($\hat{r}_{g(xy)}$) and phenotypic ($\hat{r}_{p(xy)}$) correlations between the trait *x* and *y* were estimated as follow:

$$\hat{r}_{g(xy)} = \frac{\hat{\sigma}_{G(xy)}}{\sqrt{\hat{\sigma}^2_{G(x)} \cdot \hat{\sigma}^2_{G(y)}}},$$

and

$$\hat{r}_{P(xy)} = \frac{\hat{\sigma}_{P(xy)}}{\sqrt{\hat{\sigma}^2_{P(x)} \cdot \hat{\sigma}^2_{P(y)}}} = \frac{\hat{\sigma}_{G(xy)} + \hat{\sigma}_{GE(xy)} + \hat{\sigma}_{e(xy)}}{\sqrt{\hat{\sigma}^2{}_{G(x)} + \hat{\sigma}^2{}_{GE(x)} + \hat{\sigma}^2{}_{e(x)}} . \sqrt{\hat{\sigma}^2{}_{G(y)} + \hat{\sigma}^2{}_{GE(y)} + \hat{\sigma}^2{}_{e(y)}}},$$

where $\hat{\sigma}_{G(xy)}$, $\hat{\sigma}_{P(xy)}$, $\hat{\sigma}_{GE(xy)}$ and $\hat{\sigma}_{e(xy)}$ are the estimated genetic, phenotypic, genotype x environment and error covariances, respectively, between the two traits (*x* and *y)*; While $\hat{\sigma}^2_G$ is genetic variance, $\hat{\sigma}^2_P$ is phenotypic variance, and $\hat{\sigma}^2_{GE}$ and $\hat{\sigma}^2_e$ are the estimated genotype x environment and error variances calculated for both traits (Holland et al. 2006). To test whether genetic and phenotypic correlation coefficients, differed from zero significantly, the coefficients were Z transformed as suggested by (Fisher, 1925): $Z_{xy} = \left[\ln(1 + r_{xy}) - \ln(1 - r_{xy})\right]/2$. The Z_{xy} variable was examined with $Z' = \frac{Z_{xy}}{1/\sqrt{n-3}}$ where n is the total number of recombinant inbred lines in the study.

PROC MIXED of SAS (SAS Institute Inc. 2003) was used for better estimation of lsmeans (Yang, 2010) for QTL analysis using four traits of interest, days to flowering, days to maturity, plant height and grain yield. Incomplete block nested within block was considered as random effects in estimating the individual seeding environment while incomplete block nested within block and seeding environment were considered as random effects to estimate lsmeans for pooling the seeding environments.

The QTL and *Ppd-D1a* allele interaction was studied by identifying the lines having the QTL linked marker and *Ppd-D1a* scores present versus absent. The CONTRAST and ESTIMATE commands were used in PROC MIXED analysis of SAS by comparing the lines with alternate molecular variants at each QTL and *Ppd-D1a* allele with the lines: a) having both QTL linked marker and *Ppd-D1a*, b) having *Ppd-D1a* allele only, and, c) having QTL linked marker only. The coefficients for CONTRAST and ESTIMATE were orthogonal and hence the alpha values were not adjusted.

3.2.7 QTL Analysis

One hundred and seventy seven recombinant inbred lines (RILs) were genotyped using SSR and DArT markers. The RILs were grown in four different environments, 2007, 2008 early, 2008 late and 2011. QTL analysis was performed on least square means for individual years and on combined least square means over all sites. The lines were treated as fixed effects. For QTL analysis, WinQTL Cart 2.5 software (Wang et al. 2010) was used to perform composite interval mapping.

To determine QTL threshold levels, 1000 permutations were carried out using WinQTL prior to QTL analysis at the 0.05 significance level to avoid any obvious Type II error, and the walking distance was one centimorgan (cM). The QTL were named according to a catalogue of gene symbols given by McIntosh (2003).

3.3 Results

Overall, the environmental conditions varied significantly in terms of growth temperature and rainfall quantity and distribution in all four seeding environments (Table 3-1). Therefore, a higher proportional contribution of environmental variation was noted in analysis of variance, followed by the genotypes and genotype × environment interaction (data not shown).

As expected, the earlier flowering parent Cutler flowered and matured earlier, and was shorter than AC Barrie. It also produced less grain yield (1.06 t/ha) than the higher yielding parent AC Barrie. Transgressive segregation was observed in the population as some of the lines flowered and matured earlier than the early maturing parent Cutler, and others later than AC Barrie. This was also found true for plant height, as some of the lines were shorter than Cutler and others were taller than AC Barrie (Table 3-2). The overall contribution of environmental variation was higher, indicating significant environmental effects on the traits under study (data not shown). Broad sense heritability estimates were high for days to flowering, maturity and plant height and were medium for grain yield (Table 3-2). Days to flowering was strongly correlated with days to maturity and moderately correlated with grain yield (Table 3-3). The photoperiod insensitive allele (*Ppd-D1a*) induced earlier flowering and maturity by 0.7d and 1.3d, respectively compared to the photoperiod sensitive allele (*Ppd-D1b*) (Table 3-4).

In total, 488 markers (102 Simple sequence repeat (SSR) markers and 386 DArT markers (of 7000 clones) were used for mapping the population. The linkage map was constructed by DArT (http://www.diversityarrays.com). Initially, 566 DArT markers were polymorphic for the population; however, 180 DArT markers were either distorted or redundant and were discarded. The map spanned a distance of 2279.13 cM and covered all 21 wheat chromosomes with an average distance of 4.67 cM between the markers. A total of seven QTLs were identified in this study (Table 3-5). Three QTLs of earliness *per se* affecting both days to flowering and maturity were found on chromosomes 1B and 5B, in individual and combined environments. The QTL found on chromosome 1B (*QEps.dms-1B1*) was mapped at 31.8 cM and its late allele delayed flowering by 0.6d in the field and 2.57d in the greenhouse. This corresponded to a maturity delay of 0.8-2d. The second QTL on 1B *QEps.dms-1B2* was detected only when the data was converted to degree days. This QTL was mapped at 35.8 cM and the effect was delayed flowering by approximately 0.8d (12.9 heat units) and maturity by approximately 0.8 to 3d (21.2 to 43.1 heat units) (Table 3-5). The earliness *per se* QTL found on chromosome 5B was positioned on 72.1 to 76.1 cM in different environments, and was mapped on

76.1 cM in combined data. The early allele of this QTL *QEps.dms-5B1* induced earlier flowering by 0.93d in the field and 1.99d in greenhouse; and earlier maturity by 0.74 to 1.63d. A flowering time QTL was detected on chromosomes 4A (*QFlt.dms-4A1)* and induced an earlier flowering by 0.5d (Table 3-5). The first grain yield QTL was found on chromosome 1D at 62.2 cM and increased grain yield by 0.38 t/ha. The other two grain yield QTLs (*QYld.dms-5B1*and *QYld.dms-5B2)* were found on chromosome 5B at 29.5, 43.1 cM positions respectively. The two yield QTLs, increased grain yield by 0.29, 0.31 t/ha, respectively (Table 3-5).

The combined effect of the earliness *per se* QTL *QEps.dms-5B1* and *Ppd-D1a* on days to flowering and maturity was almost equal to their sum (Table 3-6), suggesting that the *Ppd-D1a* and earliness *per se* QTLs interacted in an additive fashion. The interaction followed the similar trend when the QTLs with delaying affect (*QEps.dms-1B1*and *QEps.dms-1B2*) interacted with *Ppd-D1a* additively and the interaction effects on flowering and maturity were equal to their sum and non-significant due to the opposite direction of effect. The specific alleles at QTLs (*QEps.dms-1B1* and *QEps.dms-1B2*) delayed the time to flowering and maturity while the *Ppd-D1a* induced earlier flowering and maturity. Similar results were found for the interaction between the flowering time QTL *QFlt.dms-4A1* and *Ppd-D1a* (Table 3-6).

3.4 Discussion

The Cutler × AC Barrie population was developed for a better understanding of the flowering gene complex. Here we report four main findings: 1) three earliness *per se* and one flowering time QTLs were mapped on chromosomes 1B, 4A, and 5B 2) earliness *per se* and flowering time QTLs interact in an additive fashion with photoperiod insensitive allele *Ppd-D1a*, 3) photoperiod insensitive allele *Ppd-D1a* reduced the days to flowering and maturity but did not alter plant height and grain yield, 4) there is a positive genetic correlation between days to flowering days to maturity and grain yield, while a negative correlation exists between days to maturity and plant height.

The QTL *QEps.dms-5B.1* found in this study is positioned between72.6 to 76.1 cM and most probably is the same QTL that has already been reported in previous studies. Hanocq et al. (2007) mapped a meta QTL (MQTL) on chromosome 5B having a position of 76.5 cM on bread wheat consensus map (Somers et al. 2004) with a confidence interval from 71.6 cM to 81.4 cM. This meta QTL was declared as one of the most accurate MQTL by pooling eight QTLs. The QTL (*QFlt.dms-5B.1*) identified in this study is located at 72.6 cM having a distance of 0.2 cM from the closest DArT marker (*wPt-666939*) and about 0.4 cM from the SSR marker *Xgwm-371* and is therefore, mapped within the confidence interval described by Hanocq et al. (2007). They also claimed the MQTL location without ambiguity in this region on chromosome 5B. Some other earliness *per se* QTLs have also been reported on 5B chromosome *e.g.,* a QTL reported by Toth et al. (2003) is located in a close proximity of SSR marker *Xgwm49* between 16-21 cM on long arm of the 5B chromosome while another QTL on the same chromosome detected by Hanocq et al. (2004) is positioned between 38-48 cM region explaining 6.8% of the total variation explained in the population under study. Shindo et al. (2003) stated three QTL inducing early flowering of 1.7, 1.6 and 1.4 days which are linked with SSR markers *Xwec78, Xrz630b* and *Xgwm234* on chromosome 5B. Two of the earliness *per se* QTLs found in this study, on chromosome 1B are located in close proximity to each other on 31.8 and 35.8 cM close to SSR marker *Xbarc187,* and are involved in delaying days to flowering and maturity. Wang et al. (2008) reported QTLs delaying the days to flowering and maturity on chromosome 1B in a Chinese winter wheat population. Lin et al. (2008) found flowering time QTLs at about 106 cM on long arm of chromosome 1B close to SSR marker *Xbarc-80*. They reported an earlier flowering by 0.9 to 2.4 days induced by the QTL in the population developed by crossing 'Nanda 2419' and 'Wangshuibai'. They also reported a flowering time QTL on chromosome 1D close to SSR marker *Xbarc-62* or *Xgwm-232*. However, QTL reported on chromosome 1B in this study are novel and, to the best of our knowledge, have not been reported previously.

The parents of the QTL mapping population we used differ in their time of flowering and maturity, in part, due to different photoperiod alleles on *Ppd-D1* locus, as AC Barrie carries photoperiod sensitive (*Ppd-D1b*) allele, while Cutler is photoperiod insensitive. Different plant growth stages differ in their response to photoperiod insensitivity (Slafer and Rawson, 1994). Therefore, developmental acceleration induced by insensitive photoperiod genes could be either from emergence to floral initiation (Davidson et al. 1985), and/or reduced spikelet primordial initiation period (Rawson and Richards, 1993) or accelerated terminal spikelet to flowering stage (Slafer and Rawson, 1996; Snape et al. 2001). In this study, the dominant photoperiod allele (*Ppd-D1a*) induced 0.7d earlier flowering and 1.3d earlier maturity (Table 3-4). Foulkes et al. (2004) reported 9-12 days earlier flowering in the lines with *Ppd-D1a* in winter wheat. Similarly, Worland (1996) and Worland and Sayers (1996) reported 8d and 6-14 d earlier flowering, respectively, in British germplasm. In spring wheat, the photoperiod insensitive near isogenic lines headed 1.3-3.1d earlier in Northern Great Plains of America and Canada (Lanning et al. 2012). In another study on Canadian germplasm, the earlier flowering and maturity of 1.5-5.8d was reported due to photoperiod insensitive allele *Ppd-D1a* (Kamran et al. 2013). Earliness *per se* QTLs and *Ppd-D1a* interacted in an additive type of gene action. This suggests that accumulation of the earliness *per se* QTLs together with *Ppd-D1a* allele can help to further reduce the days to flowering and thus elongate the grain filling duration for higher yields. This information will add to our understanding about the interaction between flowering gene complexes.

In general, earliness *per se* QTLs affected the days to flowering and maturity in both directions by reducing or delaying the flowering and maturity times without environmental signals. These QTLs interacted with *Ppd-D1a* in an additive fashion, and *Ppd-D1a* reduced the days to flowering and maturity. The mapping results also indicate that the B genome contributed most of the genetic variation in this population; as a majority of the QTLs identified in this study were found on this genome. Hence, this population can be further explored for B genome

mapping studies, and the parents Cutler and AC Barrie possess polymorphic regions especially for flowering and maturity times.

3.5 Conclusion

The study was carried out to map and characterize earliness *per se* quantitative trait loci (QTLs) affecting days to flowering and maturity in a population developed by crossing two spring wheat cultivars Cutler and AC Barrie. The population of 177 recombinant inbred lines (RILs) was genotyped for a total of 488 SSR and DArT polymorphic markers on all 21 chromosomes. Three QTLs of earliness per se affecting days to flowering and maturity were mapped on chromosomes 1B (*QEps.dms-1B1* and *QEps.dms-1B2*) and 5B (*QEps.dms-5B1*), in individual environments and when all the environments were combined. A QTLs affecting flowering time (*QFlt.dms-4A1*) was identified on chromosome 4A. Two grain yield QTLs were mapped on chromosome 5B while one QTL was mapped on chromosome 1D. The population segregated for photoperiod insensitive gene, and *Ppd-D1a*, induced earlier flowering by 0.69 days and maturity by 1.28 days. The photoperiod insensitive allele *Ppd-D1a* interacted in an additive fashion with QTLs for flowering and maturity times. The earliness per se *QTL QFlt.dms-5B.1* inducing earlier flowering could help to elongate grain filling duration for higher grain yields. Hence, chromosome 5B possesses promising genomic regions that may be introgressed for higher grain yields with earlier maturity through marker assisted selection in bread wheat.

Table 3-1. Mean temperature, precipitation and degree days data for the year 2007, 2008 and 2011 growing season, Edmonton AB, Canada.

	Year	May	June	July	August	September
Temperature (°C)	2007[a]	11	16	21	15	11
	2008	13	16	18	18	12
	2011	13	15	17	17	15
	30 Year Average	**12**	**16**	**18**	**17**	**11**
Precipitation (mm)	2007	58	55	45	23	29
	2008	47	34	80	16	21
	2011	11	139	113	21	15
	30 Year Average	**49**	**87**	**92**	**69**	**44**
Degree Days	2007	354	489	660	469	322
	2008	394	481	557	562	372
	2011	400	452	529	531	435
	30 Year Average	**363**	**465**	**542**	**515**	**340**

[a]Degree days: sum of average daily temperatures over a base temperature as 0˚C

Note: Data from Environment Canada.

Table 3-2. Mean and ranges of days to flowering, days to maturity, plant height and grain yield among parents, checks and recombinant inbred lines (RILs) from the Cutler x AC Barrie population during six seeding environments

Trait	Environment	Parents		Checks (n=10)	RILs population (n=177)			
		Cutler	Barrie					
		Mean	Mean		Mean	Minimum	Maximum	Heritability
Flowering (days)	2007	48	48	49	50 ± 0.11	45	55	-
	2008 Early	53	55	55	55 ± 0.08	51	58	-
	2008 Late	43	47	46	46 ± 0.13	41	56	-
	2011	56	61	59	58 ± 0.14	51	71	-
	Greenhouse 2007	36	43	39	41 ± 0.09	30	47	-
	Greenhouse 2008	36	47	38	41 ± 0.10	31	63	-
	Overall	**51**	**53**	**53**	**52 ± 0.13**	**41**	**71**	**0.67**
Flowering (degree days[a])	2007	810	810	829	850 ± 2.2	742	958	-
	2008 Early	780	814	817	815 ± 1.8	735	889	-
	2008 Late	708	739	754	758 ± 2.3	678	945	-
	2011	735	814	785	773 ± 2.53	662	974	-
	Overall	**756**	**806**	**795**	**796 ± 1.4**	**662**	**974**	**0.68**
Maturity (Days)	2007	77	83	83	85 ± 0.22	77	99	-
	2008 Early	94	95	96	95 ± 0.13	79	103	-
	2008 Late	87	90	91	91 ± 0.24	78	107	-
	2011	114	118	115	119 ± 2.6	106	125	-
	Overall	**95**	**98**	**99**	**100 ± 0.92**	**77**	**125**	**0.61**
	2007	1311	1402	1400	1418 ± 2.8	1311	1616	-

Maturity (degree days)	2008 Early	1509	1534	1544	1535 ± 2.52	1424	1698	-
	2008 Late	1560	1647	1617	1622 ± 5.4	1043	1852	-
	2011	1598	1652	1616	1617 ± 4.97	1717	1783	-
	Overall	**1506**	**1569**	**1551**	**1556 ± 2.9**	**1043**	**1852**	**0.51**
Height (cm)	2007	73	80	78	75 ± 0.43	56	96	-
	2008 Early	88	96	88	89 ± 0.51	65	112	-
	2008 Late	78	96	88	90 ± 0.48	68	111	-
	2011	84	101	92	94 ± 0.81	62	126	-
	Overall	**81**	**94**	**87**	**87 ± 0.29**	**56**	**126**	**0.83**
Yield (t/ha)	2007	3.74	5.38	4.31	3.42 ± 0.04	1.41	5.48	-
	2008 Early	8.37	8.83	9.47	9.27 ± 0.06	2.94	12.47	-
	2008 Late	4.57	5.93	5.83	5.42 ± 0.05	1.98	8.43	-
	2011	6.84	6.38	7.22	7.48 ± 0.05	2.92	9.84	-
	Overall	**5.69**	**6.75**	**6.72**	**6.08 ± 0.05**	**1.41**	**12.47**	**0.24**

[a]Degree days: sum of average daily temperatures over a base temperature as 0˚C

Table 3-3. Phenotypic and genotypic correlation coefficients among days to flowering, days to maturity, plant height and grain yield (seeding environments combined).

	Flowering (days)	Flowering Heat Units	Height (cm)	Maturity (days)	Maturity Heat Units	Yield (t/ha)
Flowering (days)		0.99**	0.05	0.87**	0.87**	0.45**
		±0.06	±0.09	±0.03	±0.04	±0.03
Flowering (Degree days)	0.98**		0.05	0.86**	0.88**	0.11
	±0.03		±0.03	±0.03	±0.03	±0.03
Height (cm)	0.07	0.07		-0.28**	-0.22**	0.34**
	±0.05	±0.06		±0.09	±0.05	±0.10
Maturity(days)	0.67**	0.67**	-0.17**		0.99**	0.47
	±0.03	±0.03	±0.05		±0.01	0.12
Maturity (Degree days)	0.63**	0.84**	-0.15*	0.93**		0.07
	±0.03	±0.03	±0.03	±0.01		±0.08
Yield (t/ha)	0.13**	0.16**	0.25**	0.08	0.09	
	±0.01	±0.03	±0.04	±0.04	±0.04	

***Indicates Significance at P < 0.001, ns Indicates non significant

Values above the line are genotypic correlations and below the line are phenotypic correlations.

Table 3-4. Effect of the photoperiod (*Ppd-D1*) dominant and recessive alleles on various agronomic traits

Trait	Insensitive Allele (*Ppd-D1a*)	Sensitive Allele (*Ppd-D1*)	Difference
Days to Flowering (days)	49.8 ± 0.28	50.3 ± 0.27	-0.7*
Days to Flowering (Degree days)	799.7 ± 2.1	813.5 ± 2.3	-13.8**
Days to Maturity (days)	89.5 ± 0.38	90.8 ± 0,39	-1.3**
Days to Maturity (Degree days)	1517.3 ± 3.2	1537.9 ± 3.2	-20.6**
Height (cm)	87.2 ± 0.96	85.0 ± 0.94	2.2^{ns}
Yield (t/ha)	5.92 ± 0.18	6.10 ± 0.17	-0.18^{ns}

**Indicates Significance at $P < 0.01$

* Indicates Significance at $P < 0.05$

Table 3-5. Summary of quantitative trait loci (QTL) identified for days to flowering and days to maturity, plant height and grain yield of 177 recombinant inbred lines evaluated in combined and individual seeding environments during 2007, 2008 (early and late) and 2011 and greenhouse.

	QTL	Trait	Year	Chromosome	Map Position	Closest DArT Marker	[a]d1	Closest SSR marker	[a]d2	LOD[b]	R^2 (%)[c]	Additive effect
1	***QEps.dms-1B1***	Flowering	Combined	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	4.79	9.8	0.64
		Flowering	Greenhouse	1B	32.5	Wpt-2744	0.54	*barc187*	2.0	3.52	9.8	2.57
		FLWHT[d]	2011	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	4.07	11.3	11.17
		FLWHT	Combined	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	3.66	7.3	10.31
		Maturity	Combined	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	4.40	8.5	0.85
		Maturity	2007-08	1B	32.9	Wpt-2744	1.17	*barc187*	2.6	4.58	15.8	1.96
		MATHT[e]	2008L	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	5.20	10.7	34.49
		MATHT	Combined	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	5.38	11	17.44
		MATHT	2008L	1B	31.8	Wpt-2744	0.16	*barc187*	2.7	4.98	10.2	32.09
2	***QEps.dms-1B2***	FLWHT	2011	1B	35.8	Wpt-2694	0.79	*barc187*	1.3	3.69	12.2	12.88
		MATHT	2008L	1B	35.8	Wpt-2694	0.79	*barc187*	1.3	5.23	18.1	43.11
		MATHT	Combined	1B	35.8	Wpt-2694	0.79	*barc187*	1.3	5.84	9.8	21.23

3	***QEps.dms-5B1***	Flowering	2011	5B	76.1	Wpt-6135	0.34	*gwm371*	3.1	3.81	9.87	-0.93
		Flowering	Greenhouse	5B	72.1	Wpt-666939	0.10	*gwm371*	0.9	3.88	15.3	-1.99
		FLWHT	Combined	5B	76.1	Wpt-6135	0.34	*gwm371*	3.1	4.71	11.2	-17.75
		FLWHT	2007-08	5B	72.1	Wpt-666939	0.10	*gwm371*	0.9	4.83	8.3	-12.68
		Maturity	2007	5B	72.4	Wpt-666939	0.20	*gwm371*	0.6	7.03	12.7	-1.63
		Maturity	2007-08	5B	72.4	Wpt-666939	0.20	*gwm371*	0.6	3.8	4.4	-0.74
		MATHT	2007	5B	72.2	Wpt-666939	0.01	*gwm371*	0.8	7.22	13.4	-17.87
4	***QFlt.dms-4A.1***	Flowering	2007-08	4A	61.2	Wpt-8841	0.10	*wmc262*	0.2	3.44	5.6	-0.51
5	***QYld.dms-1D1***	Yield	2008early	1D	62.2	Wpt-0413	0.11	*wmc590*	5.4	4.16	9.1	0.38
6	***QYld.dms-5B1***	Yield	2011	5B	29.5	Wpt-5120	0.08	*gwm234*	8.5	3.74	8.1	0.29
7	***QYld.dms-5B2***	Yield	2011	5B	43.1	Wpt-1457	0.05	*wmc73*	8.1	4.92	9.5	0.31

[a]d1,2: distance from the closest DArT/SSR marker; [b]LOD: Logrithm of odds; [c]R^2: total variation explained (%)

[d]FLWHT: Flowering degree days; [e]MATHT: Maturity degree days;

Table 3-6. The comparison of the lines with *Ppd-D1a* and flowering QTLs to explain interaction between the photoperiod insensitive allele *Ppd-D1a* and the identified QTLs in the study for days to flowering, days to maturity, plant height, and grain yield.

QTL	Comparison	Flowering (Days)	FLWHT[a]	Height (cm)	Maturity (Days)	MATHT[b]	Yield (t/ha)
QEps.dms-1B1	**Effect of The QTL only[c]**	0.6	10.1**	0.7	1.7	40.0**	0.08
	Effect of *Ppd-D1a* only[d]	-0.7	-13.4**	-1.8	-0.9	-17.2*	-0.12
	Effect of Both *Ppd-D1a* and QTL[5]	0.4	6.0	-0.3	0.4	13.1	-0.14
QEps.dms-1B2	**Effect of The QTL**	0.7	13.7**	0.5	1.6	29.6**	0.1
	Effect of *Ppd-D1a*	-0.5	-10.7**	-1	-0.3	-17.5*	-0.09
	Effect of Both *Ppd-D1a* and QTL	0.3	3.5	-1.4	0.9	12.3	-0.23
QEps.dms-5B1	**Effect of The QTL**	-0.8**	-12.9**	-4.5	-0.6	-9.4	-0.34
	Effect of *Ppd-D1a*	-0.3	-5.1	-3.8	-0.2	-0.9	-0.13
	Effect of Both *Ppd-D1a* and QTL	-1.3**	-23.7**	-4.7	-0.7	-29.1**	-0.49
QFlt.dms-4A1	**Effect of The QTL**	-0.8*	-12.6**	-3.4	-0.3	-6.7	-0.17
	Effect of The *Ppd-D1a*	-0.3	-11.6*	-4.9	-0.9	-15.5*	-0.24
	Effect of Both *Ppd-D1a* and QTL	-1.1**	-21.4**	-1.9	-1.3	-24.0**	-0.31

[a]FLWHT: Flowering degree days; [b]MATHT: Maturity degree days; Contrast coefficients: (QTL only, *Ppd-D1a* only, QTL and *Ppd-D1a* both, no QTL and *Ppd-D1a*) [c]The effect of QTL was estimated by comparing the lines with QTL present vs absent (1 0 0 -1), [d]The effect of Ppd-D1a was estimated by comparing the lines with Ppd-D1a present vs absent (0 1 0 -1) The Interaction effect was estimated by comparing the lines with both Ppd-D1a and The QTL present vs absent (0 0 1 -1).

Figure 3-1 (a) DArT and SSR linkage map for Cutler × AC Barrie population with chromosomal regions showing the genomic regions involved in controlling days to flowering, maturity and grain yield

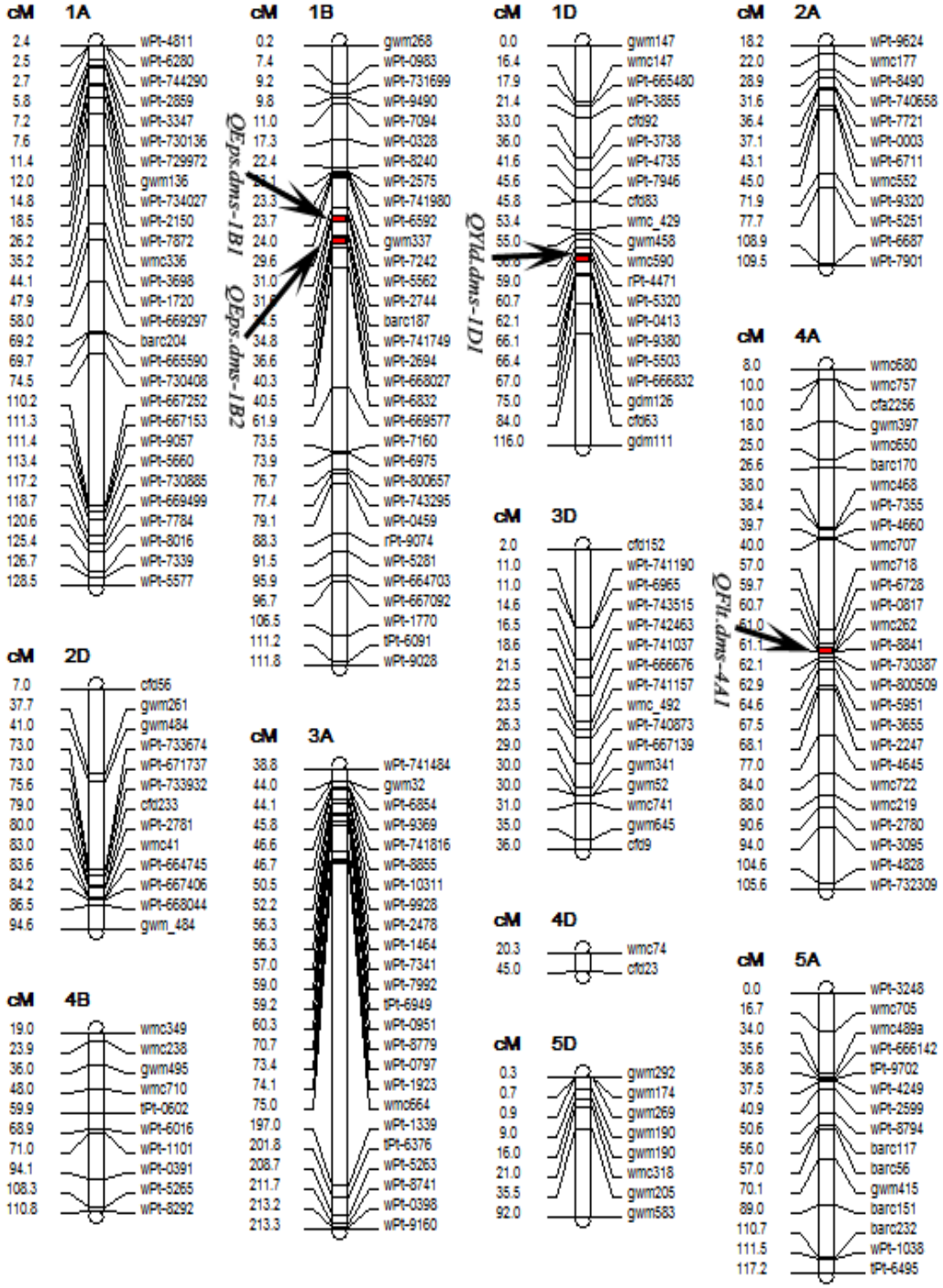


Figure 3-1(b) DArT and SSR linkage map for Cutler × AC Barrie population with chromosomal regions showing the genomic regions involved in controlling days to flowering, maturity and grain yield

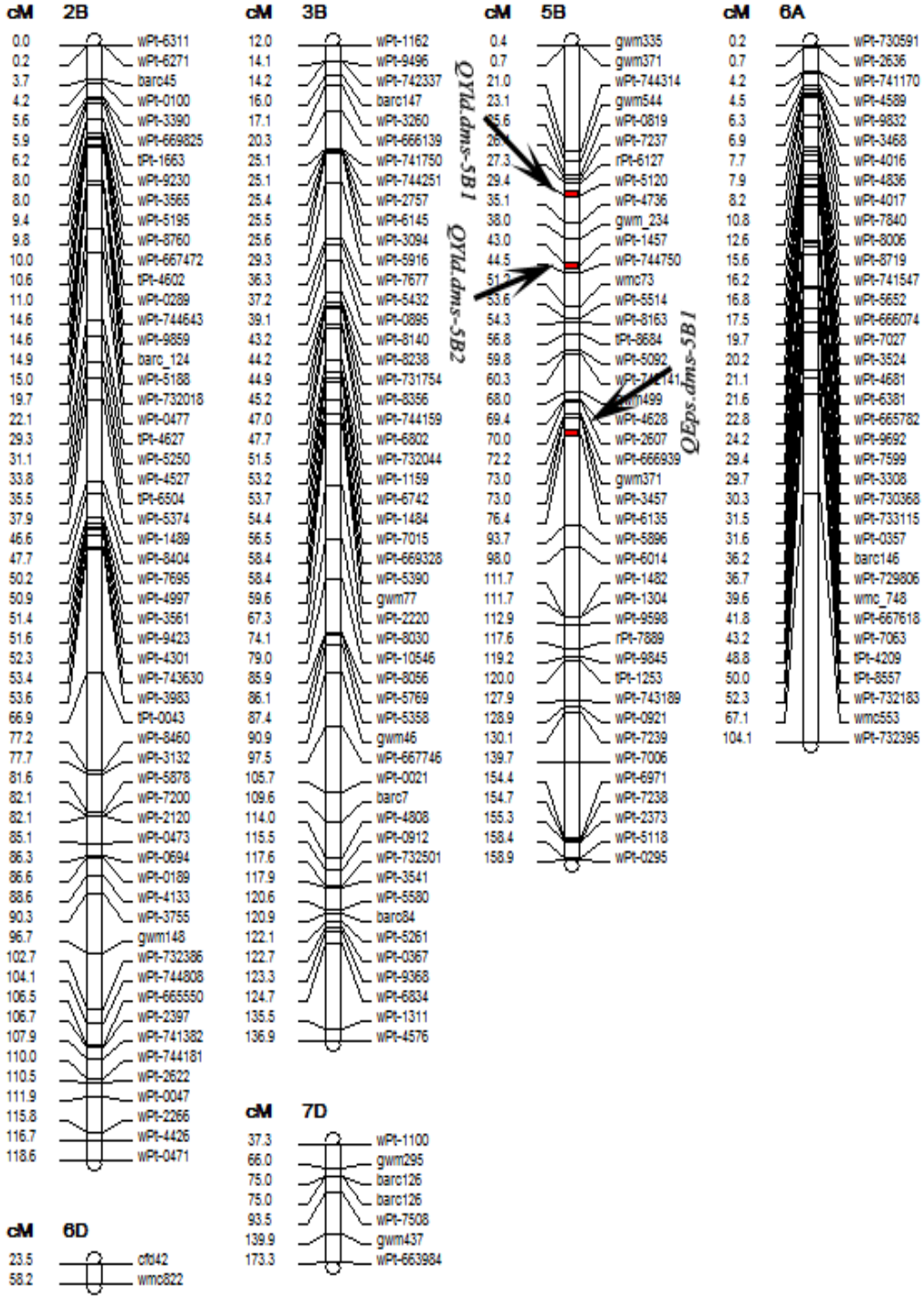
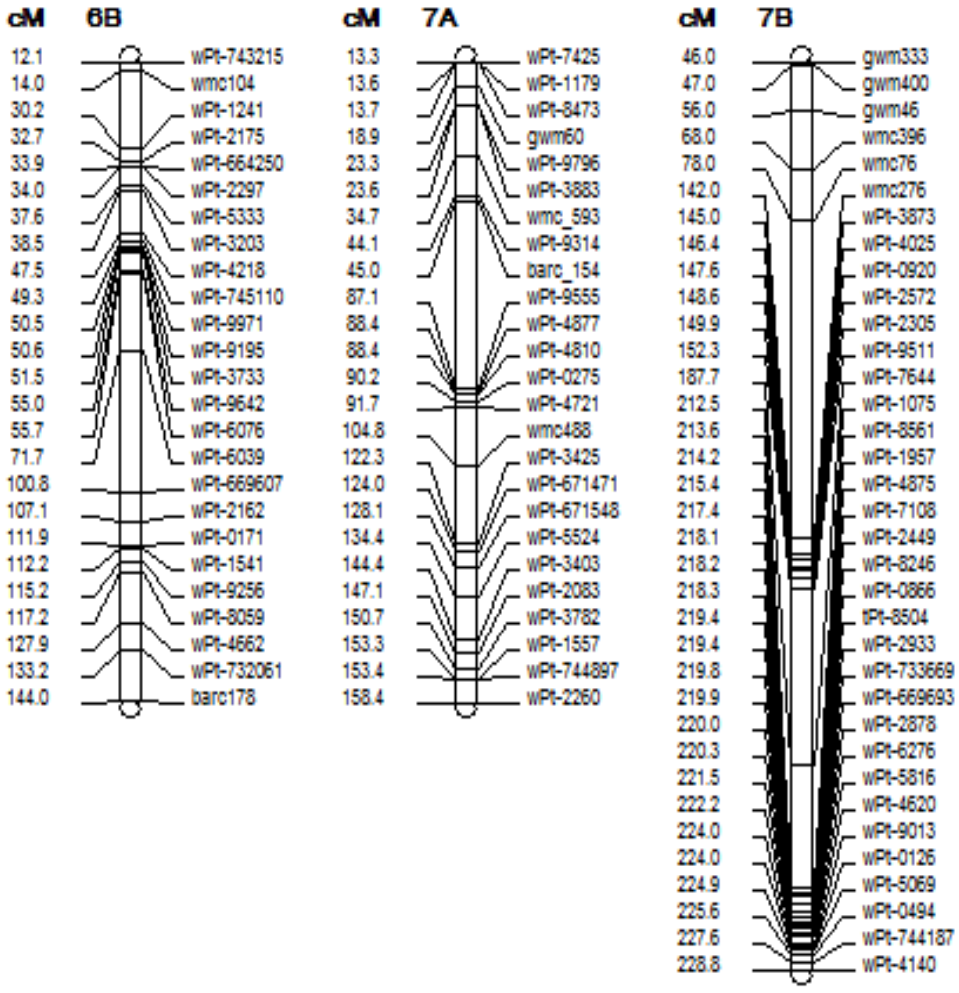


Figure 3-1 (c) DArT and SSR linkage map for Cutler × AC Barrie population.



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[4]4 The effect of vernalization genes on important agronomic traits in high yielding Canadian soft white spring wheat

4.1 Introduction

An important phenological step in the plant life cycle is the switch to reproductive growth or flowering. This stage must synchronize with favourable environmental conditions for reproductive success. Plants make this shift by signal perception and transduction pathways triggered by exogenous (temperature and day-length) and endogenous cues (genes controlling developmental hormones) (Jung and Muller 2009). Flowering time in cereal crops is governed by three genetic constituents: vernalization, photoperiod and earliness *per se*. Vernalization is a plant response to non-freezing low temperature resulting in a switch from vegetative to reproductive growth phase. The main exogenous or environmental cue is temperature; where plant response depends on degree and duration of exposure to low temperature (Rawson et al. 1998). Endogenous response cues comprise the genetic composition of plants (*i.e.*, type and number of vernalization alleles possessed) and the regulatory elements (*i.e.*, activators and repressors, and/or the magnitude of transcriptome in the cells) (Jarillo and Pineiro 2011, Jung and Muller 2009).

Studying flowering time in the hexaploid wheat can be challenging due to its large and redundant genome and a series of molecular intricacies in the flowering network (Cockram et al. 2007). Mapping and understanding these genes is easier in the model plant *Arabidopsis thaliana*, or other cereals with relatively smaller genome sizes. A comparative analysis of the flowering behaviour in these plants has helped to map and understand flowering genes in hexaploid wheat (Baurle and Dean 2006, Cockram et al. 2007). The series of orthologous *VRN1* genes have been located on the long arm of the group 5 chromosome (Worland et al. 1996). These genes are *Vrn-A1* on chromosome 5A (Law et al. 1976), *Vrn-B1*

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on 5B (Hoongendoorn 1985), *Vrn-D1* on 5D (Law et al. 1976), *Vrn-D5* on 5D (Law et al. 1976). To understand the molecular basis of vernalization insensitivity Yan et al. (2003, 2004) and Fu et al. (2005) characterised isogenic lines and accessions from hexaploid and tertraploid wheat, and revealed insertion and/or deletion mutations at the *Vrn-A1* locus. *Vrn-A1a* allele evolved as result of a large (222-bp) and a small (131-bp) foldback fragment insertion within the promoter region. *Vrn-A1b* emerged from two mutations in host direct duplication (HDD) and a deletion of 20-bp in the 5´ Un-Translated Region (UTR), and *Vrn-A1c* due to a large deletion in intron 1. Similarly, the allele *Vrn-B1b* is the result of a single nucleotide polymorphism and a deletion of 36 bp (Santra et al. 2009).

Vernalization genes vary in their potency of effect in conferring vernalization insensitivity, and can result in complete insensitivity to partial or weekly sensitive plants. This varying degree of sensitivity confers a differential genetic potential to alter flowering time through different combinations of vernalization genes. For example, *Vrn-A1a* gene, which is the most potent vernalization gene to eliminate the vernalization requirement, was found in very high frequencies in high latitude regions (Iqbal et al. 2007) where the germplasm was continuously bred for earlier maturity and higher protein in the hard red spring wheat class. In tropical and sub-tropical regions of Asia, where the growing season is longer, the germplasm primarily possesses the less potent *Vrn-D1*.

Different vernalization alleles confer winter, facultative and spring growth habits. Absence of any vernalization insensitive alleles confers winter growth habit and sensitive cultivars necessarily require vernalization treatment for the induction of reproductive growth. Studies on diploid wheat have shown that the vernalization insensitive gene *VRN2* confers winter growth habit (Dubcovsky et al. 1998; Yan et al. 2004) and is considered to be a 'floral repressor'. The *VRN2* transcriptome (a protein with a zinc-finger motif), inhibits flowering gene expression (Trevaskis et al. 2007) and results in a delayed flowering induction. Reciprocal gene expression studies suggest a decrease in the expression level of

VRN2 after a continuous exposure to low temperature or vernalization treatment (Yan et al. 2004). This eventually allows flowering genes (*VRN1*) to express and induce the flowering. Another possible hypothesis is, it is actually the expression of *VRN1* that represses *VRN2* expression for flowering induction (Loukoianov et al. 2005).

Vrn genes have direct or indirect effects on grain yield and grain protein content. Kamran et al. (2013) reported comparatively low yield and high grain protein content in earlier maturing spring wheat cultivars of the hard red spring wheat class. A negative correlation existing between days to maturity and grain yield was reported by Iqbal et al. (2007) and Reid et al. (2009). Insensitive vernalization genes accelerate vegetative growth from emergence to floral initiation, hence results in a decrease in the final leaf number (Wang et al. 1995). Sensitive vernalization genes prolong the vegetative phase and result in higher leaf numbers due to the constant growth rate of leaf primordia (Miglietta 1989). This increase in final leaf number eventually delays flag leaf appearance and heading (Amir and Sinclair 1991). Whitechurch and Snape (2003) reported *Vrn-A1* and *Vrn-D1* increase the number of spikelets per spike and the effect of *Vrn* genes is reported to be more pronounced during the vegetative phase (Whitechurch and Snape 2003).

The objectives of the present study were: a) to study how individual *VRN1* genes and combination thereof alter agronomic traits in the high yielding low protein soft white spring wheat class in Northern growing regions b) to quantify and compare the individual effects of *VRN1* gene on days to flowering and maturity and on yield and yield components in this class of wheat.

4.2 Materials and methods

The experimental material consisted of 30 soft white spring wheat lines. The lines were divided in five groups and each group comprised of six lines that possess similar *VRN1* alleles at all *VRN1* loci: 1) *vrn-A1-Vrn-B1- vrn-D1,* 2) *Vrn-A1a-Vrn-B1-vrn-D1,* 3) *Vrn-A1b* and *Vrn-B1- vrn-D1,* 4) *vrn-A1-Vrn-B1-Vrn-D1,* and

5) *Vrn-A1a, Vrn-B1-Vrn-D1.* These 5 groups of 30 lines were selected from a group of 102 lines previously screened for *Vrn* genes in Canadian spring wheat germplasm (Kamran et al. 2013). The *VRN1* genes were screened by using the primers sequences reported by Yan et al. (2004) and Fu et al. (2005). The *Vrn-A1* was screened by using the primers VRN1AF (GAAAGGA AAAATTCTGCTCG) and VRN1R (TGCACCTTCCC(C/G)CGCCCCAT). *Vrn-A1a* and *Vrn-A1b* were differentiated based on the product size. *Vrn-B1* and *Vrn-D1* were screened by using the primers: Intr1/B/F (CAAGTGGAACGGTTAGG ACA), Intr1/B/R3 (CTCATGCCAAAAATTGAAGATGA), Intr1/D/F (GTTGT CTGCCTCATCAAATCC) and Intr1/D/R3 (GGTCACTGGTGGTCTGTGC) respectively. Polymerase chain reactions were conducted in GeneAmp® 9700 PCR systems (Applied Biosystems). Generally, the reaction mixture contained 10 µL of Extract-N-Amp^{TM}PCR ready mix (Sigma-Aldrich, Cat#E3004), 0.5 µL of each forward and reverse primers of 5µM concentration, 1-4 µL DNA with a concentration of 25-100 ng/µL. The total volume was made upto 20µL by adding 5-8µL sterile water. The PCR cycling conditions were adapted from Yan et al. (2004) and Fu et al. (2005). PCR products were ran and visualized on 1-3% agarose gel stained with ethidium bromide.

All the lines belong to the Canadian Western Soft White (CWSWS) class. The CWSWS class is bred for higher grain yields and low protein content and is primarily pastry wheat flour and also used for animal feed and or ethanol production (Canadian Grain Commission 2008). The experimental material was grown in 2010, 2011 and 2012 at four sites: University of Alberta, South campus, Edmonton (53° 32'N, 113°32'W) and Ellerslie research station, Ellerslie (53°42'N, 113°45'W) and on irrigated and dry-land sites of Agriculture and Agri-Food Canada, Lethbridge (49°41'N, 112°49'W). The irrigated 2010 experiment was flooded, and data were discarded. The experiment was not planted in Lethbridge in 2012. In total, then, nine site-years of experimental data were used for this study. The experiment was planted with three replications in a randomized complete block design with incomplete sub-blocks within blocks in 2 meters plots with six rows and 23 cm distance between the rows. Plots were seeded at 350

seeds per m^2. At seeding granular fertilizer (N-P_2O_5-K_2O:11-52-0) was applied at a rate of 36 kg ha^{-1}. Post emergence herbicides were sprayed at an appropriate time to ensure weed free crop stands.

Data were recorded for days to flowering, days to maturity, grain yield (t ha^{-1}), grain protein (%) and test weight (kg hL^{-1}) at Edmonton and Ellerslie. At Lethbridge, days to flowering, days to maturity, and grain yield (t ha^{-1}) were recorded. Days to flowering were recorded when 50% of the spikes had completely emerged above the flag leaf. Physiological maturity was recorded when 50% of the peduncles in a plot had completely lost their green color. Plant height was measured in cm from the tip of spike excluding awns to ground level. Grain yield per plot was weighed and yield in tha^{-1} were estimated on a dry weight basis. Test weights were estimated by using Ohaus test weight apparatus manufactured by Ohaus, USA. Near Infrared Reflectance (NIR) spectroscopy was used to estimate grain protein content using *SpectraStar RTW* apparatus manufactured by Unity Scientific.

4.2.1 Statistical analysis

Following initial data exploration, Ellerslie 2011 was removed from further analyses. This was because low soil moisture at seeding followed by a dry spring resulted in a very uneven crop stand and a very high coefficient of variation for that site. Lines with similar genes were considered as a fixed treatment. Data were thereafter pooled over the years and location, and the treatment lsmeans were estimated in PROC MIXED with location, year, block, and incomplete sub-block, individual lines and all the interactions considered as random effects. The LSMEANS of different gene combinations were compared with CONTRAST and ESTIMATE commands in SAS version 9.2 (SAS Institute Inc. Cary NC). Different group of lines were compared to estimate the effect of a particular *Vrn* gene *e.g* the group of lines having two insensitive *VRN1* genes: *Vrn-A1a* and *Vrn-B1*were compared with the group of lines having *Vrn-B1*only. The comparison was done to quantify the effect of *Vrn-A1a* gene. The multiple

comparisons among the treatments were orthogonal and hence the alpha values were not adjusted. To investigate the strength of relationship among the traits, multivariate Restricted Estimation of Maximum Likelihood (REML) was used in SAS PROC MIXED (Holland 2006). The genetic ($\hat{r}_g$) and phenotypic ($\hat{r}_p$) correlations between the trait *x* and *y* were estimated as follow:

$$\hat{r}_{g(xy)} = \frac{\hat{\sigma}_{G(xy)}}{\sqrt{\hat{\sigma}^2_{G(x)} \cdot \hat{\sigma}^2_{G(y)}}},$$

and

$$\hat{r}_{P(xy)} = \frac{\hat{\sigma}_{P(xy)}}{\sqrt{\hat{\sigma}^2_{P(x)} \cdot \hat{\sigma}^2_{P(y)}}} = \frac{\hat{\sigma}_{G(xy)} + \hat{\sigma}_{GE(xy)} + \hat{\sigma}_{e(xy)}}{\sqrt{\hat{\sigma}^2{}_{G(x)} + \hat{\sigma}^2{}_{GE(x)} + \hat{\sigma}^2{}_{e(x)}} . \sqrt{\hat{\sigma}^2{}_{G(y)} + \hat{\sigma}^2{}_{GE(y)} + \hat{\sigma}^2{}_{e(y)}}},$$

where $\hat{\sigma}_{G(xy)}$, $\hat{\sigma}_{P(xy)}$, $\hat{\sigma}_{GE(xy)}$ and $\hat{\sigma}_{e(xy)}$ are the co-variances estimates of genetic, phenotypic, genotype x environment and error, between the two traits (*x* and *y)*; While $\hat{\sigma}^2_G$, $\hat{\sigma}^2_P$, $\hat{\sigma}^2_{GE}$ and $\hat{\sigma}^2_e$ are the estimated genetic, phenotypic, genotype x environment and error variances. For correlation analysis the data from Edmonton and Ellerslie location were used.

4.3 Results

Overall, *VRN1* allele groups differed ($P < 0.05$) for days to flowering, maturity, grain yield, test weight and protein content (Table 4-1). The lines with the *Vrn-A1a-Vrn-B1* allele group were the earliest to flower and mature, while *Vrn-A1b-Vrn-B1* was the latest to flower and mature (Table 4-1). The *Vrn-B1-Vrn-D1* group was the highest yielding group followed by *Vrn-A1a-Vrn-B1- Vrn-D1*, and *Vrn-B1* had the lowest yield (Table 4-1). The *Vrn-A1a-Vrn-B1* group flowered and matured 1.7d and 1.6d earlier, and yielded 0.2 t ha^{-1} higher than *Vrn-B1*, and the test weight was higher by about 0.9kg hL^{-1} in *Vrn-B1* only (Table 4-1).

Both genetic (r_g) and phenotypic (r_p) correlations between days to flowering or maturity were positively correlated with grain yield (r_g =0.37, r_p = 0.32 and r_g =0.42, r_p =0.41 respectively), while days to flowering or maturity were negatively correlated with test weight ($r_g = -0.53, r_p = -0.47$and $r_g = -0.31$, r_p =-0.28 respectively) and grain protein content ($r_g = -0.13, r_p = -0.16$and $r_g = -0.22, r_p = -0.23$respectively) (Table 4-3). We also found a moderate negative correlation between the test weight and grain yield ($r_g = -0.22, r_p = -0.42$) (Table 4-3).

To quantify the effect of *Vrn-A1b*, the lines having *Vrn-A1b-Vrn-B1* were compared with the lines having *vrn-A1-Vrn-B1*. *Vrn-A1b* delayed days to flowering and maturity by 2.7d and 2.5d, and had a lower test weight (0.93 kg hL^{-1}) than *vrn-A1* (Table 4-1). In another comparison, *Vrn-B1-Vrn-D1* was compared with *Vrn-B1* to estimate the effect of *Vrn-D1*. The *Vrn-B1-Vrn-D1* flowered half a day later than the *Vrn-B1*; however, produced 0.54 t ha^{-1} more grain yield than the *Vrn-B1*. This finding suggests that the partial insensitivity conferred by *Vrn-D1* allele plays an indirect role in increasing yield in soft white spring wheat germplasm. These results confirm our previous findings in Kamran et al. (2013).

Different gene groups were also compared to compare their cumulative effects on days to flowering and maturity, and their consequential effects on grain yield, test weight and grain protein content. For example, the cumulative effect of two insensitive *Vrn* genes: *Vrn-A1a-Vrn-B1* was compared with the cumulative effect of the *Vrn-A1b-Vrn-B1*. The former group flowered and matured 2.7d and 2.5d earlier, had higher test weight of about 0.92kghL^{-1}compared to the latter group (Table 4-2). We further found that the inclusion of *Vrn-A1a* in the presence of *Vrn-B1*, and *Vrn-D1* has an advantage of inducing earlier flowering and maturity (1.2d), a higher test weight (0.8kghL^{-1}), with similar ($P < 0.05$) grain yield and grain protein (Table 4-2).

4.4 Discussion

We report four main results from this study: a) the highest yielding lines possessed *Vrn-D1;* the gene group *Vrn-B1- Vrn-D1* yielded the most grain, followed by *Vrn-A1a-Vrn-B1-Vrn-D1,* b) *Vrn-A1b* takes longer to flower and mature and has an epistatic effect on *Vrn-B1,* c) *Vrn-A1a-Vrn-B1,* which is the most frequent gene combination in CWSWS class has the earliest flowering and maturity, d) a negative correlation exists between test weight and grain yield; and test weight and grain protein content.

The three insensitive *Vrn* genes in combination (*Vrn-A1a-Vrn-B1-Vrn-D1)* result in similar maturity as the *Vrn-B1-Vrn-D1* group, with higher grain yield and similar grain protein content (Table 4-2). The presence of *Vrn-D1* apparently confers a yield advantage, as the two highest yielding groups possessed this gene. The presence of *Vrn-A1a* along with *Vrn-B1-Vrn-D1* significantly reduces the days to flowering and maturity with similar grain yield and grain protein content, while, its absence delays days to flowering and maturity by 1.2d approximately. Therefore, the authors suggest using three insensitive *Vrn* alleles (*Vrn-A1a-Vrn-B1-Vrn-D1*), in the areas where early maturity is of prime concern *e.g.,* northern region of the Great Plains. However, the areas where the crop production time permits, two insensitive *Vrn* alleles *i.e., Vrn-B1-Vrn-D1,* should be deployed, as this combination provides a relative higher test weight of about 0.8kghL^{-1}, and a marginal yield advantage compared to the three insensitive *Vrn* genes. Partial sensitivity to vernalization requirement of *Vrn-D1* found in globally adopted wheat cultivars has a key role in yield advantage. This yield advantage is attributed to the nature of this gene, which can delay or accelerate the flowering time in order to avoid the biotic and especially abiotic stresses of heat, drought and frost injury. A yield advantage conferred by *Vrn-D1* in heat or drought stress environment has been reported by Stelmakh (1993). Moreover, the three most widely (8-9 million hectares globally) grown cultivars (Poster, Attila, and Kauz), possess the *Vrn-D1* allele as the sole flowering gene (van Beem et al. 2005). Partial vernalization requirement of *Vrn-D1* can confer high adaptability of wheat cultivars (Nakai and Tsunewaki 1967).

Asian, Japanese and Chinese landraces predominantly possess *Vrn-D1* (Stelmakh 1990; Gotoh, 1979; Kato, et al. 1988). The Japanese cultivar 'Akakomugi' is considered to be the donor parent of *Vrn-D1* allele (Stelmakh 1990) and served as an ancestor of the cultivars like 'Lerma Rojo 64' and 'Sonora 64' that are the potential source of *Vrn-D1* allele in South and Southeast Asian cultivars (van Beem et al. 2005, Stelmakh 1990). *Vrn-D1* is the most frequent *Vrn* gene in CIMMYT derived wheat cultivars. van Beem et al. (2005), reported 66% of these cultivars possessed *Vrn-D1* allele as the sole spring growth habit gene, or in combination with *Vrn-A1, Vrn-B1 ,* and *Vrn-4* which occurred at a frequency of about 41%, 39%, and 8% of the total lines under study, respectively.

In a study on Canadian germplasm, Kamran et al. (2013) reported the presence of *Vrn-D1* allele up to 48% of the lines used in high yielding CWSWS class, and a complete absence of this allele from a comparative low yielding CWRS class. It is also believed that the cultivars grown at higher altitudes usually possess *Vrn-A1* followed by *Vrn-B1* (Iqbal et al. 2007). This means that *Vrn-D1* is lacking in germplasm from the regions with higher altitudes including the Canadian germplasm. From the results of this study it is evident that *Vrn-D1* aids to improve yield in the soft white wheat class without negatively affecting the already low protein status on this class. We found no *Vrn-D1* present in the Canadian hard red spring class characteristically having 3-4% higher protein than the soft Candian spring wheat (Kamran et al. 2013, Iqbal et al. 2006). It is unknown how *Vrn-D1* may alter yield, maturity, and grain protein in the hard red spring wheat class. Nevertheless, we suggest more frequent introgression of the *Vrn-D1* allele in elite breeding material along with *Vrn-A1a,* and *Vrn-B1,* for an added yield advantage and wider adaptability, without delaying time to maturity.

The *Vrn-A1b*, an allele resulting from the deletion in the promoter region of the locus *Vrn-A1,* delays days to flowering and maturity. This was found when *Vrn-B1* was compared with *Vrn-A1b-Vrn-B1*. This suggests that apart from its individual effect, it interacts antagonistically with *Vrn-B1* and serves to delay flowering and maturity (Table 4-3). We have reported similar results previously

(Kamran et al. 2013). Zhang et al. (2012) reported 32d delay in days to flowering due to a single nucleotide polymorphism (SNP) mutation in the promoter region of *Vrn-D1a* locus, and concluded that the SNP mutation has altered the binding site for MADS box transcription factor, and hence delayed the flowering time. A 20-bp deletion in the promoter region of *Vrn-A1b* might have resulted into similar kind of delay reported in the present study.

A moderate positive correlation between days to flowering/maturity and grain yield verifies the concept of higher grain yield with longer duration of growth. A negative correlation found between the grain protein content and days to flowering/maturity and grain yield also suggests that a higher grain protein content is incurred in relatively early maturity or a shorter growth period and lower grain yield. Among the group of lines studied in this experiment, the *VRN1* allele group *Vrn-A1a* and *Vrn-B1* were the earliest flowering and maturing and had highest grain protein content (Table 4-1). These results are in agreement with Iqbal et al. (2007), Reid et al. (2009) and Kamran et al. (2013).

4.5 Conclusion

The gene group *Vrn-A1a-Vrn-B1* flowers and matures the earliest. However, the findings of this study suggest that the presence of *Vrn-D1* along with *Vrn-A1a* and *Vrn-B1* confers higher grain yield with similar maturity to all other *Vrn* gene groups in the soft white spring wheat class in western Canada. The inclusion of *Vrn-D1* allele in elite Canadian germplasm has been overlooked in the past. Therefore, we suggest the introduction of this allele into the Canadian germplasm especially high yielding low protein soft white spring wheats, as it will not only help to improve the productivity but also will help to broaden the genetic base and to improve the adaptability. It would also be very interesting to study the introduction of *Vrn-D1*in the CWRS class which predominately possesses *Vrn-A1a-vrn-B1-vrn-D1* and *Vrn-A1a-Vrn-B1-vrn-D1* and to determine whether high protein levels may be maintained.

Table 4-1. Least square means for the groups with different vernalization genes and the net effect of these genes on days to flowering, days to maturity, grain yield (tha^{-1}), test weight ($kghL^{-1}$) and grain protein (%).

Vernalization genes	Flowering (Days)	Maturity (Days)	Grain Yield (tha^{-1})	Test Weight ($kghL^{-1}$)	Grain Protein (%)
Vrn-B1	62.7	109.9	4.79	71.4	9.74
Vrn-A1a-Vrn-B1	61.1	108.3	5.08	70.5	9.74
Vrn-A1b-Vrn-B1	63.8	110.8	4.97	70.0	9.51
Vrn-B1-Vrn-D1	63.2	110.1	5.21	71.4	9.70
Vrn-A1a-Vrn-B1-Vrn-D1	62.0	108.9	5.18	70.6	9.54
Treatment (Pr > F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SE (difference)	0.20	0.42	0.10	0.30	0.12
Effect of Vrn-A1a[1]	**-1.7*****	**-1.6*****	**0.21***	**-0.9****	**0.0** ns
Effect of Vrn-A1b[2]	**1.1*****	**0.9***	**0.19** ns	**-1.9*****	**-0.2** ns
Effect of Vrn-D1[3]	**0.5***	**0.3**ns	**0.54*****	**-0.1** ns	**0.0** ns

[1]Effect Estimated by Contrast (*Vrn-A1a-Vrn-B1*) vs (*Vrn-B1*) (1 -1)
[2] Effect Estimated by Contrast (*Vrn-A1b-Vrn-B1*) vs (*Vrn-B1*) (1 -1)
[3] Effect Estimated by Contrast (*Vrn-B1-Vrn-D1*) vs (*Vrn-B1*) (1 -1)
Pr > F represents the overall significance of the gene groups of being different
*Indicates Significance at P < 0.05,
**Indicates Significance at P < 0.01,
***Indicates Significance at P < 0.001,
ns indicates non-significant

Table 4-2. Comparison analysis based on the least square means of groups with different vernalization genes and their effect on days to flowering, days to maturity, grain yield (tha^{-1}), test weight (kghL^{-1}) and grain protein (%).

	Flowering (Days)	Maturity (Days)	Grain Yield (tha^{-1})	Test Weight (kghL^{-1})	Grain Protein (%)
Vrn-A1a-Vrn-B1	61.1	108.3	5.00	70.5	9.7
Vrn-A1b-Vrn-B1	63.8	110.8	4.98	69.6	9.6
Comparative effect[1]	**-2.7*****	**-2.5*****	**0.02** ns	**0.9****	**0.2** ns
Vrn-A1a-Vrn-B1-Vrn-D1	62.0	108.9	5.18	70.5	9.6
Vrn-A1a-Vrn-B1	61.1	108.3	4.98	70.5	9.7
Comparative effect[2]	**0.9*****	**0.6** ns	**0.22***	**0.0** ns	**-0.1** ns
Vrn-A1a-Vrn-B1-Vrn-D1	62.0	108.9	5.18	70.5	9.6
Vrn-B1-Vrn-D1	63.2	110.1	5.32	71.4	9.7
Comparative effect[3]	**-1.2*****	**-1.2****	**-0.10** ns	**-0.8***	**-0.1** ns

[1]Effect Estimated by Contrast (*Vrn-A1a-Vrn-B1*) vs (*Vrn-A1b-Vrn-B1*) (1 -1)

[2] Effect Estimated by Contrast (*Vrn-A1a-Vrn-B1-Vrn-D1*) vs (*Vrn-A1a-Vrn-B1*) (1 -1)

[3] Effect Estimated by Contrast (*Vrn-A1a-Vrn-B1-Vrn-D1*) vs (*Vrn-B1-Vrn-D1*) (1 -1)

Pr > F represents the overall significance of the gene groups of being different

*Indicates Significance at P < 0.05,

**Indicates Significance at P < 0.01,

***Indicates Significance at P < 0.001,

ns indicates non-significant

Table 4-3. Phenotypic and genetic correlation coefficients among days to flowering, days to maturity, grain yield (tha^{-1}), test weight (kghL^{-1}) and grain protein (%) and their confidence intervals (±C.I).

	Flowering (Days)	Maturity (Days)	Yield (t/ha)	Test Weight (kg/hL)	Protein (%)
Flowering		**0.61** ±0.07	**0.37** ±0.14	**-0.53** ±0.15	**-0.13** ±0.13
Maturity	**0.54** ±0.07		**0.42** ±0.09	**-0.31** ±0.14	**-0.22** ±0.04
Yield	**0.32** ±0.14	**0.41** ±0.08		**-0.22** ±0.22	**-0.12** ±0.11
Test Weight	**-0.47** ±0.11	**-0.28** ±0.09	**-0.42** ±0.11		**-0.11** ±0.11
Protein	**-0.16** ±0.11	**-0.23** ±0.06	**-0.10** ±0.09	**-0.17** ±0.09	

Values above the line are genetic correlations and below the line are phenotypic correlations

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[5]5 Relative performance of Canadian spring wheat cultivars under organic and conventional field conditions

5.1 Introduction

Crop production without the use of synthetic fertilizers and chemicals is called organic farming. International Federation of Organic Agriculture Movements (IFOAM) defines organic farming as "a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects". Extensive use of chemical inputs during the last century has raised numerous concerns over the human and environmental health, and agricultural sustainability. Due to the fact that, organic food production is safe and chemical-free, the demand for organic food has increased 4-5 times since 1990. Ever since, it has evolved as the most dynamic food industry worth $59 billion globally with approximately 37.2 million ha area under certified organic cultivation worldwide (Paull and Hennig 2011). Canadian contribution towards organic food production is relatively small and approximately 0.7 million ha are organically managed and wheat is the number one Canadian organic crop grown on about 102, 434 ha of land (Agriculture Agri-Food Canada 2009).

Organically managed lands differ substantially from their conventional counterparts for soil biodiversity, nitrogen level, soil moisture and retaining capacity, soil organic matter, weed intensity and types, and biotic and abiotic stresses (Hornick 1992; Gruber et al. 2000; Barberi 2002; Entz et al. 2001). This results in considerable differences in agronomic and quality traits of various crops grown and compared in organic and conventional management systems. Aside from the environmental stress, the cultivars exhibit significant genetic variation

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among each other to respond any biotic or abiotic stress (Romagosa and Fox 1993). Therefore, several studies reported inconsistencies in crop and/or cultivar performance from one location to other (Peterson et al. 1992; Sial et al. 2000).

In organic management system, the grain yields are generally low compared to conventional production system (Poutala et al., 1993; Mason et al. 2007; Reid et al. 2009) while a great deal of confusion exists around the quality of the produce. In fact the nutritionally important quality micronutrients are affected by a number of factors related to soil, environment and management (Davis et al. 2006). Nelson et al. (2011) concluded that the quality of the organic produce varies from one location to other and should not be universalized. A number of studies advocate the organic production to be superior and rich in their nutritional value (Nelson et al. 2011; Reid et al. 2009); however, there are studies reporting significant loss in grain protein content under organic conditions (Starling and Richards 1993; Ryan et al. 2004; Zorb et al. 2009). Mader et al. (2007) reported non-significant differences in nutritional value and baking quality in Eurpoean germplasm studied over 21 years.

Across the Canadian Prairies, the growing season or the number of frost free days are limited to 95-125 days in total. Earlier flowering and maturity are desirable traits to avoid yield and quality losses in this region. Under organic field conditions, earliness with better early season vigour is beneficial to compete for limited nutrient supply. Mason et al. (2007) reported higher grain yield of the early maturing cultivars with better early season vigour under organic field conditions. Early maturity also confers adaptive advantage by avoiding abiotic stress conditions of early or late season frost, and hence, most of the Canadian spring wheat cultivars possess the most potent vernalization genes (Kamran et al. 2013). Iqbal et al. (2007) suggested that simultaneous selection for higher grain yield and early maturity is possible by identifying the certain *Vrn* gene combination. In this context, it is important to study the how the different vernalization genes interact to induce early maturity without negatively affecting

the grain yield and whether the early maturity confers any yield advantage under organic field conditions.

The objectives of the study were: a) to study the differential behaviour of vernalization genes in days to flowering and maturity under organic and conventional management systems; b) if reduction in days to maturity due to insensitive *Vrn* genes confers grain yield advantage under organic field conditions; c) to identify the genotypes/cultivars that are better suited to organic production.

5.2 Materials and Methods

In this experiment we studied a set of 32 Canadian spring wheat cultivars in organic and conventional management systems. These cultivars were mostly released during the last ten years under the following four classes: Canada Western Red Spring (CWRS), Canada Western Soft White Spring (CWSWS), Canada Prairie Spring (CPS) and Canada Western Hard White spring (CWHWS). The four classes represent more than 96% of the area under cultivation in Canadian Prairies (Statistics Canada 2011).These cultivars were studied in 2010, 2011 and 2012 at the Edmonton Research Station (ERS) (53° 32'N, 113°32'W) University of Alberta, on conventional and organically managed fields located less than 1 km apart. The experiment was laid out in alpha lattice design. Plot dimension at each site was 2 × 1.38 m with six rows. Data on days to flowering, days to anthesis and maturity, plant height, grain yield, test weight, and grain protein content were recorded. The organic field experiment in 2011 was overwhelmed with weeds and data were recorded for days to flowering, anthesis and maturity only. Days to flowering was recorded when 50% of the spikes emerged out of the flag leaf. Days to anthesis was recorded when 50% of the spikes in a plot have protruded out anthers. Days to maturity were recorded when 50% of the peduncles had completely lost their green color. Grain protein content was estimated using SpectraStar RTW apparatus by Unity Scientific by Near Infrared Reflectance (NIR) spectroscopy. Grain yield per plot was weighed and

converted to yield t/ha dry basis. Grain fill duration (GFD) was estimated as the duration between anthesis and maturity and grain fill rate (GFR) was estimated by dividing the total gain yield to total grain fill days.

The cultivars were genotyped for vernalization (*Vrn-A1a, Vrn-A1b, Vrn-B1, Vrn-D1)* genes. 7-10 day old seedling leaf tissue was ground to extract DNA by standard CTAB protocol. Primer sequences for these *Vrn* genes were adopted from Yan *et al*., (2004) and Fu *et al*., (2005). PCRs were done in GeneAmp® 9700 PCR systems (Applied Biosystems). A total volume of 20µL of the reaction mixture was used for PCR containing: a) 1-4 µL of template DNA about at a concentration of 25-100 ng/µL, b) 0.5 µL of each forward and reverse primers at 5µM concentration, c) 10 µL of Extract-N-Amp^{TM}PCR ready mix (Sigma-Aldrich, Cat#E3004), and d) 5-8µL sterile water to make total reaction volume to 20µL. The PCR cycling conditions were followed exactly as described by Yan *et al*., (2004) and Fu *et al*., (2005). The PCR products were ran and visualized in a 3% agarose gel.

The cultivar least square means (LSMEANS) were estimated using SAS PROC MIXED (SAS Institute 2012) with the cultivar being the only fixed effect, and year, replication nested in year and block nested in replication and year being random effects in each of the two environments (organic and conventional management systems). The LSMEANS were ranked from earliest to latest in terms of time to flowering, anthesis, maturity and highest to lowest in terms of grain yield, grain protein content, test weight, grain fill duration and grain fill rate.

The combined analysis of variance (ANOVA) over the two management systems (organic and conventional) was carried out using SAS PROC MIXED (SAS Institute 2012). The model for the ANOVA included fixed effects (cultivar, system and cultivar x system interaction) and random effects (year, year x cultivar, year x system, year x cultivar x system, replication nested in year and block nested in replication and year). The identified significant interaction effects were further partitioned with SLICE command in PROC MIXED for both

cultivars and environments. To account for the number of comparisons, the alpha values (significance levels) were adjusted by BONFERRONI correction (Dunn 1961). The significance of difference between the conventional and organic management systems was estimated by comparing it with the critical range estimate provided by Rayan-Einot-Gabrial-Welsh test which adjusts the alpha value 'experiment-wise'. The cultivars were also sub-grouped based on their *Vrn* gene composition and class, and were considered as a treatment. To retain enough statistical power and to avoid the genetic background noise of the cultivars from different classes, only CWRS cultivars were taken into consideration for ranking and comparison under organic field conditions. The differences between the cultivars with single insensitive allele (*Vrn-A1a*) or two (*Vrn-A1a, Vrn-B1*) were estimated only in CWRS class. These treatments were dealt as a fixed effect to compare the organic and conventional environments.

5.3 Results

Most of the cultivars under study possessed insensitive vernalization alleles at two of the *Vrn-1* loci: 28 of 32 cultivars possessed insensitive *Vrn* allele *Vrn-A1a* either alone (*Vrn-A1a-vrn-b1-vrn-D1* 50% or 16 cultivars) or in combination with *Vrn-B1* (*Vrn-A1a-Vrn-b1-vrn-D1* 38% or 12 cultivars) (Table 5-1). The harvest from the organic plots were weighed with and without weed seeds. Overall, the weed seed contributed 16 to 43% of the total harvested yield during 2010 and 2012 (Data not shown). All the traits under study except days to maturity, plant height and grain protein content differed statistically between organic and conventional management in overall analysis of variance (Table 5-1). Generally, the cultivars under organic conditions were earlier flowering, lower yielding with a lower test weight compared to the conventional management system (Table 5-1). This trend was similar in sub-groups of cultivars belonging to CWRS class having either one or two insensitive alleles at *Vrn-1* locus (*Vrn-A1a-vrn-B1-vrn-D1 or Vrn-A1a-Vrn-B1-vrn-D1*) (Table 5-2). The only exception was the cultivars with single insensitive *Vrn* allele matured earlier under organic environment compared to the cultivars having two insensitive vernalization alleles (*Vrn-A1a*

and *Vrn-B1*) (Table 5-2). However, this reduction in days to maturity failed to translate into higher grain yields under organic field conditions.

Of the 22 CWRS cultivars, three out of the top six earliest maturing cultivars were among the lowest yielding (Lillian, Osler, and Alvena ranks: 21, 22 and 18); whereas, two out of the six latest maturing cultivars were higher yielding cultivars ranked as 6 and 5 (AC Barrie and Superb) (Table 5-3). These results suggest that there is no clear evidence to conclude whether earlier flowering and/or maturity have conferred any yield advantage. Rather, a strong positive correlation was found between grain yield and days to flowering and maturity (Table 5-6). These results also suggested that significant cultivar × environment interaction is more likely to impact the grain yield, rather than the maturity times. Cultivar × environment interaction effects were found significant for grain yield, grain protein content and grain fill rate (Table 5-4). The estimates of sliced LSMEANS of the cultivars and environment for the traits were ranked according to the minimum and maximum differences between organic and conventional environments (Table 5-1). Maximum differences between organic and conventional conditions for days to flowering, anthesis and maturity were observed in Sadash and Superb. Sadash has the maximum acceleration under organic conditions by 3.3, 4.1 and 6.7d in flowering, anthesis and maturity, while Superb had the maximum delay of 0.6 and 3.7d in flowering and maturity (Table 5-1, 5-5). A low negative correlation between plant height and grain yield was found in organic and non-significant positive correlation was found under conventional conditions (Table 5-6).

Marquis, the oldest studied cultivar, had higher yield and protein content under organic conditions than conventional. This was the only exception, as all other cultivars had lower yield under organic conditions. The minimum grain yield losses under organic conditions were incurred by Infinity (0.14 t ha^{-1}) while the maximum losses were noted in 5701PR (2.07 t ha^{-1}) (Table 5-1, 5-5). In terms of grain protein content, cross-over interaction among the cultivars and environments occurred: 15 of 32 cultivars studied exhibited higher grain protein

content under organic conditions while 17 cultivars had higher grain protein under conventional conditions (Table 5-1, 5-5). The top six cultivars with higher grain protein (>1.85%) under organic conditions were: Andrew, Bhishaj, 5700PR, 5701PR, and 5702PR (Table 5-1, 5-5), however, all of these cultivars except 5702PR incurred maximum grain yield losses under organic field conditions (Table 5-1). The cultivars like Fieldstar, Infinity, AC Barrie, Stettler, and Katepwa had significant loss of grain protein (>1.05%) under organic grown conditions (Table 5-5). The trend for loss in grain filling rate was quite similar to the trend in grain yield. The cultivar Infinity had almost no difference (3.38) in grain fill rate while cultivar Lillian had substantially low (56.2) grain fill rate under organic conditions (Table 5-1).

Cultivars grown under organic field conditions generally flowered earlier as compared to conventional conditions. As the nutrient supply under organic conditions is not as abundant, the cultivars under organic field conditions produced 21% less grain yield on an average; however, the cultivars like Marquis, Infinity, Park, Unity, Harvest and Minnedosa had comparable grain yields in both management systems (Table 5-5).

5.4 Discussion

We report the following four main results of this study: a) most of the cultivars studied possessed two insensitive *Vrn* alleles: *Vrn-A1a* either alone or in combination with *Vrn-B1* b) cultivars having single insensitive *Vrn* alleles, *i.e.* *Vrn-A1a* induced earlier maturity under organic conditions compared to *Vrn-A1a* and *Vrn-B1* in combination; however, this accelerated maturity did not confer any yield advantage; c) cultivars grown under organic management system were earlier flowering, lower yielding and had lower test weight compared to the conventional management system d) significant cultivar x environment interactions were noted for grain yield, grain protein content and grain fill rate.

The *Vrn* gene screening results of the cultivars under study reveals that most of the cultivars predominantly possessed two insensitive vernalization

alleles at either one or two loci (*Vrn-A1a* and *Vrn-B1*). These results are in line with previous findings in older Canadian spring wheat cultivars (Kamran et al. 2013). Mason et al. (2007) proposed a hypothesis that earlier flowering and maturity can help the plants outcompete weeds and produce better yields in organic systems. Based on this hypothesis, we studied modern Canadian germplasm to figure out if any of the particular *Vrn* genes or combination, governing the flowering and maturity time, is better suited for organic conditions. Based on the germplasm studied in this experiment, the proposed hypothesis does not stand true; the insensitive allele *Vrn-A1a* conferred earlier maturity compared to the two *Vrn* alleles *Vrn-A1a* and *Vrn-B1,* but this acceleration cannot be clearly attributed to any yield advantage. The earliest maturing cultivar 'Park' (94 days to maturity) ranked 11 followed by Lovit (96.7 days to maturity) at 16th position (Table 5-3). A strong positive correlation was also found between days to flowering and/or maturity and grain yield (Table 5-6); which also suggests that the grain yield increases with longer growth duration and delayed maturity. Apparently, cultivar × environment interaction seems to be responsible for higher grain yield under organic field conditions rather than the delay in days to maturity or *Vrn* gene combination. Kirk et al. (2012) found significant effects of the environments (both organic and conventional) on grain yield and protein content; and reported higher grain yield of the lines selected in organic environments. Another possibile reason for this discrepancy could be due to the QTL responsible for accelerated grain filling rate (GFR) (Nass and Reisser 1975). GFR is relatively less explored area of research, and is reported to play a significant role in determining the wheat grain yield (Yang and Zhang 2006). Wang et al. (2009) detected 17 QTL explaining 7.2-20.8% of the total phenotypic variation in GFR in a Chinese winter population. Kirigwi et al. (2007) also reported a QTL affecting the GFR on chromosome 4A. Therefore, a differential genetic background of the cultivars under study might have contributed towards a significant cultivar × environment interaction for grain yield. A strong positive correlation between GFR and grain yield found in this study (Table 5-6) further justifies the differential behaviour of the cultivar under stress conditions. A low negative

correlation between the grain yield and plant height found in organic conditions also suggest that the taller CWRS cultivars does not necessarily better compete with weeds for light interception. However, these results are not in agreement with Mason et al. (2007) and Wolfe et al. (2008). Similarly, a strong negative correlation between grain protein content and grain fill duration in organic conditions was found, and a moderate to low positive correlation between the same traits was found in conventional management systems (Table 5-6).

The significant cultivar × environment interaction for grain yield found in this study suggests that some cultivars can manage to tolerate nutrient, weed and disease pressure are able to adapt stress conditions better than other cultivars. Overall, the cultivars exhibited significant interaction with the organic and conventional management systems for grain yield, grain protein content and grain fill rate.

Cultivar interaction with environment for grain protein content was interesting, as some of the cultivars had higher grain protein content under organic conditions and some had higher grain protein content under conventional conditions. Some cultivars produced similar grain protein under both organic and conventional management systems (Table 5-5). As all the cultivars are flowering and maturing within the available growing time in Canadian Prairies, cultivars better suited to organic condition were selected based on minimum grain yield and grain protein losses. Selecting the top 6 cultivars (20%) that had minimum grain yield and grain protein losses among the systems resulted in three common cultivars (Table 5-5). These three cultivars *viz* Marquis, Unity and Minnedosa had comparable yield and grain protein contents in both production systems. Marquis, the oldest cultivar in the study, had higher yield and better protein content under organic conditions. Marquis was bred in early 20^{th} century, and the selection was practiced under organic conditions. The other two cultivars that had minimum grain yield and grain protein losses under organic conditions, were released

recently (Minnedosa 2008 and Unity 2009); and defy the particular hypothesis that older cultivars are better suited to organic breeding (Table 5-4). In a micronutrient analysis of five Canadian spring wheat cultivars released during 1910 to 2000 Nelson et al. (2011) found no particular trend in cultivar performance in relation to their year of release.

Better performance of certain cultivars under organic field conditions has been reported previously (Mason et al. 2007). Nass et al. (2003) reported significant higher grain yield by AC Barrie under organic conditions compared to check AC Walton, which generally outcompeted AC Barrie for grain yields under conventional conditions. Kitchen et al. (2003) found higher grain yields in organic conditions at 3 of the 14 paired sites; and found significant cultivar x environment interaction for grain yield. Carr et al. (2006) reported cultivar x environment interactions for grain yield, grain protein content and test weights in fifteen spring wheat cultivars of northern Great Plains. Murphy et al. (2007) reported different genotypic ranks between the organic and conventional management systems. Przystalski et al. (2008) reported cross-over interaction of the cultivars between the management systems and suggested trait and stress oriented breeding for organic crop production.

Overall, we found significant interaction of the cultivars with management system; therefore, breeding for organic production should be conducted on organically managed lands. When grown under organic field conditions the cultivars generally earlier flowering; produced lower grain yields and had lower test weights. Three cultivars: Marquis, Minnedosa, Harvest and Unity had minimum losses under organic conditions and can potentially serve as parents for organic breeding.

5.5 Conclusion

Producing higher yields under organic conditions is generally hampered by weeds and lesser nutrient supply. In wheat certain adaptive traits like early season vigour, taller plants, and shorter life cycle have been reported to help

plants to compete weeds and produce satisfactory yields. In this experiment we tested a hypothesis 'whether early flowering and maturity conferred by insensitive vernalization alleles *Vrn-A1a* and/or *Vrn-B1* has any yield advantage under organic conditions' in Canadian spring wheat germplasm. We genotyped 32 cultivars for their vernalization gene composition (*Vrn-A1a, Vrn-B1 and Vrn-D1*) and studied these cultivars in organic and conventional management systems. We found 88% of the cultivars possessed vernalization (*Vrn*) insensitive allele *Vrn-A1a* either alone or in combination with *Vrn-B1*. There were no differential affects between the cultivars having insensitive *Vrn* allele at either single locus (*Vrn-A1a*) or two (*Vrn-A1a, Vrn-B1*) under organic and conventional field conditions; except for days to maturity, where cultivars having only *Vrn-A1a* allele matured earlier. This earlier maturity did not translate to any yield advantage under organic field condition, notwithstanding the proposed hypothesis of the study. Overall, the cultivars under organic conditions were earlier flowering, lower yielding with lower test weight compared to the conventional management system. Significant cultivar × management system interactions were found for grain yield, grain protein content and grain fill rate. For grain protein content, cross-over interactions of the cultivars between the management systems were observed. Three CWRS cultivars (Marquis, Unity and Minnedosa) exhibited minimal comparative loss in grain yield and grain protein content under organic field conditions, and hence could potentially serve as parents for organic wheat breeding programs.

Table 5-1. Mean differences between the conventional and organic management system for days to flowering, anthesis, maturity, plant height (cm), grain yield (t ha^{-1}), test weight (Kg hL^{-1}), grain protein content (%), grain fill days and grain fill rate for all the cultivars under study grown at Edmonton Research station during 2010, 2011 and 2012.

Entry	Variety	YOR	class	*Vrn-A1a*	*Vrn-B1*	*Vrn-D1*	Flowering (Days)	Anthesis (Days)	Maturity (Days)	Height (cm)	Yield (t ha^{-1})	Test Weight (Kg hL^{-1})	Grain Protein (%)	Grain Fill Duration	Grain Fill Rate
1	5700PR	2000	CPS[a]	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	0.0	0.6	3.3	-2.5	1.77	2.00	-1.87	3.7	40.6
2	5701PR	2001	CPS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	1.2	1.5	6.5	-0.8	2.07	1.23	-1.87	6.4	36.7
3	5702PR	2010	CPS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	0.6	1.7	-0.4	-5.2	1.42	0.75	-1.85	-1.6	47.5
4	Ac Vista	1996	CPS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	0.9	0.4	2.7	-0.3	1.39	1.64	-1.11	3.0	33.1
5	Alvena	2007	CWRS[b]	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	1.3	1.5	2.1	0.8	1.60	3.6	0.66	1.6	47.6
6	Andrew	2000	CWSWS[c]	*vrn-A1*	*Vrn-B1*	*Vrn-D1*	1.4	2.2	2.6	-1.5	1.85	1.68	-2.13	1.3	48.2
7	Attila	-	CIMMYT	*vrn-A1*	*vrn-B1*	*Vrn-D1*	0.9	1.2	2.7	-1.2	0.54	2.9	-0.82	2.6	14.6
8	AC Barrie	1994	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	0.5	0.9	-4.1	2.9	0.63	2.75	1.24	-2.9	33.8
9	Bhishaj	2002	CWSWS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	1.5	2.0	3.8	0.1	1.17	-0.52	-1.99	3.3	28.9
10	Bounty	-	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	1.0	1.2	1.1	0.0	1.26	2.88	0.34	1.2	44.9
11	CDC Go	2004	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	-0.4	0.4	1.4	-2.2	1.03	3.43	-0.15	1.9	28.3
12	Fieldstar	2009	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	1.4	2.7	5.4	-3.9	0.64	-0.38	1.05	3.5	19.5
13	Goodeve	2008	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	1.1	2.4	0.8	1.9	1.24	2.62	-0.10	-1.0	46.1
14	Harvest	2002	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	0.8	1.0	0.3	0.7	0.34	1.51	-0.09	0.8	16.6
15	Infinity	2006	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	0.7	1.3	3.9	-2.4	0.14	0.46	1.10	3.9	3.4
16	Kane	2006	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	0.1	0.6	-0.5	0.3	1.17	3.94	0.96	-0.4	37.0
17	Katepwa	1981	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	-0.5	0.1	1.1	-0.6	1.11	3.62	1.59	1.9	36.0
18	Lillian	2004	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	1.5	1.5	3.2	2.6	2.05	1.74	0.51	2.5	56.2
19	Lovit	2003	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	1.3	1.5	2.7	-0.6	0.96	1.42	0.73	2.0	32.7
20	Marquis	1910	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	2.4	2.9	5.4	3.2	-0.41	2.25	-0.40	4.0	-6.3

21	Minnedosa	2008	GP	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	1.6	2.1	3.1	-1.1	0.53	0.79	-0.34	1.8	12.5
22	Osler	2003	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	0.2	1.0	2.7	2.1	2.01	3.64	0.89	2.9	53.2
23	Park	1963	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	0.3	0.9	5.6	6.3	0.29	2.70	0.67	5.8	6.6
24	Sadash	2008	CWSWS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	3.3	4.1	6.7	-0.4	1.91	0.76	-0.73	4.2	41.8
25	Snowwhite 475	2004	CWHWS[d]	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	0.7	2.6	2.1	-2.8	1.40	1.66	0.05	0.8	35.3
26	Snowwhite 476	2004	CWHWS	*vrn-A1*	*Vrn-B1*	*vrn-D1*	2.9	3.3	2.4	-1.8	1.09	1.14	0.14	0.6	31.6
27	Somerset	2005	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	2.2	2.1	1.0	5.1	1.07	3.52	0.49	0.6	33.5
28	Stettler	2008	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	0.1	0.2	1.0	0.0	1.11	4.66	1.47	2.2	30.6
29	Superb	2001	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	-0.6	0.2	-3.7	3.4	0.61	3.10	-1.12	-2.6	27.9
30	CDC Teal	1991	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	0.5	0.9	0.1	2.2	0.95	3.37	0.76	0.3	35.4
31	Unity	2009	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	-0.4	1.4	2.8	3.6	0.32	1.17	-0.28	2.0	14.5
32	Waskada	2008	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	1.0	1.5	0.1	-1.2	1.32	1.89	0.39	-0.2	42.4
															121.
				Mean (Conventional)			**56.4**	**59.5**	**102.9**	**94.5**	**5.30**	**76.00**	**10.50**	**43.4**	**2**
				S.E (Mean)			**0.17**	**0.10**	**0.50**	**0.50**	**0.11**	**0.15**	**0.09**	**0.49**	**2.17**
				Mean (Organic)			**55.5**	**58.0**	**100.8**	**94.3**	**4.20**	**73.50**	**10.60**	**41.7**	**89.7**
				S.E (Mean)			**0.20**	**0.20**	**0.80**	**0.50**	**0.13**	**0.40**	**0.35**	**0.97**	**2.29**
											<0.000	**<0.000**			**0.01**
				Prob > F			**<0.0001**	**<0.0001**	**0.119**	**0.78**	**1**	**1**	**0.87**	**0.0008**	**8**

[a]Canadian Prairie Spring
[b]Canada Western Red Spring
[c]Canada Western Soft White spring
[d]Canada Western Hard White Spring

Table 5-2. Differences in effects of vernalization genes in organic and conventional management systems for CWRS cultivars in the study, grown at Edmonton Research station during 2010, 2011 and 2012.

	Lines with *Vrn-A1a*			Lines with *Vrn-A1a* and *Vrn-B1*		
	Conventional	Organic	Pr > F	Conventional	Organic	Pr > F
Flowering (Days)	56.3 ± 0.3[a]	55.2 ± 0.4	0.0018	55.8 ± 0.3	55.3 ± 0.3	0.0834
Anthesis (Days)	59.4 ± 0.2	57.8 ± 0.4	<0.0001	58.9 ± 0.2	57.9 ± 0.3	0.0002
Maturity (Days)	100.7 ± 0.8	102.0 ± 1.2	0.1339	101.2 ± 0.8	103.5 ± 1.1	0.0073
Height (cm)	97.8 ± 1.1	97.0 ± 0.9	0.4701	94.9 ± 0.7	94.2 ± 0.7	0.5259
Yield (t ha^{-1})	5.03 ± 0.2	4.04 ± 0.3	<0.0001	5.08 ± 0.2	3.99 ± 0.2	<0.0001
Test Weight (kg hL^{-1})	76.5 ± 0.3	72.6 ± 1.7	0.001	77.6 ± 0.2	73.9 ± 0.4	<0.0001
Protein Content (%)	10.94 ± 0.2	10.61 ± 0.3	0.2669	10.64 ± 0.1	10.56 ± 0.2	0.7447
Grain Fill Duration	41.25 ± 0.8	43.41 ± 0.9	0.0186	42.4 ± 0.8	44.8 ± 0.9	0.0022
Grain Filling rate	123.3 ± 4.7	89.6 ± 5.2	<0.0001	120.8 ± 3.5	85.7 ± 3.3	<0.0001

[a]Standard error of the mean

Table 5-3. LSEAMNS for the CWRS cultivars in organic management system and their relative ranks for days to flowering, anthesis, maturity, plant height (cm), grain yield (t ha^{-1}), test weight (Kg hL^{-1}), grain protein content (%), grain fill days and grain fill rate at Edmonton Research station during 2010, 2011 and 2012.

Variety	Flowering (Days)	Rank	Anthesis (Days)	Rank	Maturity (Days)	Rank	Yield (t ha^{-1})	Rank	Test Weight (Kg hL^{-1})	Rank	Grain Protein (%)	Rank	Grain Fill Duration	Rank	Grain Fill Rate	Rank
Park	53.2	1	56.1	2	94.0	1	4.05	11	73.9	11	10.40	12	36.8	22	98.4	6
Lovit	55.1	10	58.1	14	96.7	2	3.72	16	74.6	8	10.12	14	37.8	19	84.7	15
Lillian	56.7	21	59.0	21	97.0	3	3.34	21	73.4	15	11.02	4	37.2	21	82.2	17
Osler	55.6	15	58.3	16	97.3	4	3.13	22	72.7	19	10.32	13	37.8	18	76.7	21
Alvena	55.1	11	57.9	11	97.5	5	3.72	18	73.7	22	10.66	7	38.7	16	84.9	14
Marquis	55.5	13	57.5	9	97.6	6	4.29	7	75.4	3	11.57	1	38.7	15	100.6	5
unity	54.7	6	56.8	5	97.7	7	4.62	3	76.1	2	10.84	6	40.3	13	104.6	3
Infinity	56.4	19	58.9	20	97.8	8	4.76	2	74.5	9	9.70	19	37.7	20	117.5	1
Field Star	53.5	2	56.1	1	97.9	9	4.29	8	76.7	1	9.87	18	41.0	9	93.0	7
Goodeve	54.8	7	57.0	7	98.5	10	4.16	9	73.8	12	11.02	5	40.8	10	90.4	8
Bounty	55.8	17	58.8	18	98.5	11	3.90	12	75.1	4	10.63	9	38.5	17	88.9	9
Harvest	54.0	5	56.4	3	98.6	12	4.55	4	75.0	6	10.63	10	40.7	11	102.7	4
Katepwa	55.7	16	58.2	15	99.8	13	3.48	20	72.7	18	9.60	22	40.7	12	77.6	20
Minnedosa	55.2	12	57.4	8	99.8	14	4.88	1	74.8	7	10.44	11	41.6	7	110.7	2
Somerset	56.2	18	58.8	19	100.0	15	3.72	17	72.4	21	11.12	3	39.5	14	85.9	13

Teal	55.0	8	57.5	10	100.0	16	3.85	14	73.6	14	10.05	16	41.3	8	82.7	16
Kane	55.0	9	57.9	12	101.6	17	3.69	19	73.7	13	9.68	21	43.2	5	77.9	19
CDC Go	53.7	4	56.8	6	101.7	18	3.89	13	73.3	16	11.23	2	43.8	3	78.8	18
Waskada	53.6	3	56.6	4	102.2	19	3.75	15	75.0	5	10.09	15	44.3	2	71.7	22
Stettler	56.9	22	59.2	22	102.9	20	4.15	10	73.1	17	9.69	20	42.3	6	86.3	12
Barrie	55.5	14	58.0	13	103.8	21	4.31	6	74.4	10	9.95	17	43.7	4	88.5	10
Superb	56.6	20	58.6	17	105.7	22	4.49	5	72.5	20	10.65	8	45.8	1	87.6	11

Note: the cultivars are ranked based on the days to maturity from earliest to latest maturing.

Table 5-4. ANOVA table of the probability of F-tests for cultivar, environment (Organic and conventional) and the interaction between cultivar and environment for the experiment grown at Edmonton Research station during 2010, 2011 and 2012.

	Cultivar	management system	Cultivar × management system
Flowering (Days)	<0.0001	<0.0001	0.3081
Anthesis (Days)	<0.0001	<0.0001	0.1879
Maturity (Days)	<0.0001	0.0616	0.7295
Height (cm)	<0.0001	0.7793	0.9922
Yield (t ha^{-1})	<0.0001	<0.0001	0.0002
Test Weight (kg hL^{-1})	0.0742	<0.0001	0.1455
Protein Content (%)	<0.0009	0.6967	<0.0001
Grain Fill Duration	<0.0001	<0.0010	0.8534
Grain Filling rate	0.0042	<0.0001	0.0100

Table 5-5. Relative ranking of the cultivars based on the differences between the conventional and organic management systems for grain yield (t ha^{-1}), grain protein content (%) for all the cultivars grown at Edmonton Research station during 2010, 2011 and 2012.

Rank		Yield (t ha^{-1})				Protein Content (%)		
	Cultivar	Conv[a]	Org[b]	Diff[c]	Cultivar	Conv	Org	Diff
1	Marquis	3.88	4.29	-0.41	Harvest	10.53	10.63	-0.09
2	Infinity	4.91	4.76	0.14	Goodeve	10.92	11.02	-0.10
3	Park	4.33	4.05	0.29	CDC Go	11.09	11.23	-0.15
4	Unity	4.94	4.62	0.32	Unity	10.55	10.84	-0.28
5	Harvest	4.88	4.55	0.34	Minnedosa	10.09	10.44	-0.34
6	Minnedosa	5.41	4.88	0.53	Marquis	11.17	11.57	-0.40
27	5700PR	5.90	4.12	1.77	Kane	10.64	9.68	0.96
28	Andrew	6.88	5.03	1.85	Field Star	10.92	9.87	1.05
29	Sadash	6.85	4.94	1.91	Infinity	10.80	9.70	1.10
30	Osler	5.14	3.13	2.01	AC Barrie	11.19	9.95	1.24
31	Lillian	5.39	3.34	2.05	Stettler	11.15	9.69	1.47
32	5701PR	5.71	3.64	2.07	Katepwa	11.19	9.60	1.59
	LSD (0.05)		**0.17**				**0.23**	

[a]Conventional
[b]Organic
[c]Difference

Table 5-6. Pearson correlation co-efficient for the conventional and organic management system for days to flowering, anthesis, maturity, plant height (cm), grain yield (t ha^{-1}), test weight (Kg hL^{-1}), grain protein content (%), grain fill days and grain fill rate for the CWRS cultivars only.

	Flowering (Days)	Anthesis (Days)	Maturity (Days)	Height (cm)	Yield (t ha^{-1})	Test Weight (Kg hL^{-1})	Protein Content (%)	Grain Fill Duration	Grain Filling rate
Flowering (Days)	1	**0.85** <.0001	**0.78** 0.001	**0.06** 0.4232	**0.42** <.0001	**-0.41** <.0001	**-0.01** 0.9158	**-0.22** 0.0023	**0.54** <.0001
Anthesis (Days)	0.94 <.0001	1	**0.15** 0.0293	**0.10** 0.1655	**0.40** <.0001	**-0.34** <.0001	**0.08** 0.342	**-0.11** 0.1363	**0.47** <.0001
Maturity (Days)	**0.73** <.0001	**0.72** <.0001	1	**0.19** 0.0051	**0.43** <.0001	**0.31** 0.0003	**0.45** <.0001	**0.97** <.0001	**-0.09** 0.1957
Height (cm)	**-0.16** 0.03	**-0.20** 0.01	**-0.23** 0.01	1	**0.07** 0.30	**0.47** <.0001	**0.47** <.0001	**0.17** 0.01	**0.03** 0.67
Yield (tha^{-1})	**0.66** <.0001	**0.58** <.0001	**0.79** <.0001	**-0.20** 0.02	1	**0.42** <.0001	**0.33** 0.00	**0.32** <.0001	**0.84** <.0001
Test Weight (kghL^{-1})	**0.20** 0.02	**0.16** 0.07	**0.19** 0.03	**0.01** 0.89	**0.21** 0.02	1	**0.26** 0.00	**0.38** <.0001	**0.19** 0.03
Protein Content (%)	**-0.69** <.0001	**-0.67** <.0001	**-0.78** <.0001	**0.30** 0.00	**-0.76** <.0001	**-0.15** 0.10	1	**0.43** <.0001	**0.01** 0.87
Grain Fill Duration	**0.56** <.0001	**0.52** <.0001	**0.97** <.0001	**-0.21** 0.02	**0.76** <.0001	**0.17** 0.05	**-0.72** <.0001	1	**-0.22** 0.00
Grain Filling rate	**0.59** <.0001	**0.50** <.0001	**0.52** <.0001	**-0.13** 0.12	**0.92** <.0001	**0.19** 0.03	**-0.63** <.0001	**0.46** <.0001	1

Note: Values above diagonal are conventional and below are organic data coefficients

Supplementary Table A. Nutrient analysis 0-12 inch soil depth of organic and conventional fields during the years 2010, 2011 and 2012, at Edmonton research station, Edmonton.

Nutrient	**Conventional**			**Organic**		
	2010	**2011**	**2012**	**2010**	**2011**	**2012**
NO_3 (Kg ha^{-1})	129	110	107	68	22	105
P (Kg ha^{-1})	62	170	103	60	57	120
K (Kg ha^{-1})	477	932	296	1111	481	578
SO_4 (Kg ha^{-1})	26	22	21	41	14	17

Note: The soil nutrient status in conventional includes the fertilizer added to the soil, and the organic site is boosted with 37 tha^{-1} every two years.

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6 General Discussion and Conclusion

6.1 Introduction

Wheat (*Triticum aestivum* L.) forms the staple diet in more than 40 countries and is grown in a wide range of temperatures, moisture levels, latitudes, altitudes and soils (Curtis *et al*., 2002). Wheat is cultivated on 225 million ha globally and occupies the largest area under any single crop, with a total global production of 681 million t (Faostat, 2009).Wheat has enormous genetic potential to synchronize its flowering in favorable environmental conditions either by accelerating or delaying flowering; and this key feature is central to its global adaptability.

Wheat adaptation to diverse environmental conditions depends mostly on flowering time, as frost can damage the sensitive floral organs and heat stress can reduce the floret fertility (King and Heide 2009). Genetically, wheat can flower over a wide time range, which enables it to adapt to the specific environmental conditions of a geographical region. This is primarily due to three main constituent gene groups: vernalization response (*Vrn* genes), photoperiod response (*Ppd* genes) and earliness *per se* genes (*Eps* genes) (Snape *et al*., 2001).

Winter, facultative and spring growth habits are conferred by different vernalization alleles. Winter wheat requires vernalization for the induction of reproductive growth and sensitive cultivars do not flower until their vernalization requirement is satisfied. By contrast, spring wheat cultivars induce flowering without being exposed to low temperature and hence are vernalization insensitive.

Plants and animals have remarkable ability to sense the change in duration of light and initiate certain developmental changes as a consequence. Many plant species sense and respond to altering day and/or night length by receiving signals in the form of cryptochrome or phytochrome to induce flowering (Fosket, 1994). This phenomenon is called photoperiodism.

Earliness *per se* is an inherent ability of wheat plants exhibited by flowering earlier apart from the environmental stimuli (Miura and Worland,

1994). Ford *et al*., (1981) found some 'other genes' affecting the flowering time in wheat after vernalization and photoperiod requirements were satisfied and called them "earliness genes". Basic development rate, intrinsic earliness, and earliness in narrow sense are synonymous terms used for earliness *per se* in literature. Therefore, flowering and maturity times in wheat is outcome of intricate network of these three genetic constituents.

6.2 Phenotypic effects of the flowering gene complex in Canadian spring wheat germplasm.

The first experimental study of this thesis project is to study the phenotypic effects of the two major flowering gene group *i.e* vernalization and photoperiod. One hundred and two cultivars from historical and elite Canadian spring wheat germplasm were selected to study the phenotypic effect and frequency of different vernalization genes. Almost all the hard red cultivars possessed *Vrn-A1a* allele either alone or in combination with *Vrn-B1* while 74% of the higher yielding soft white lines possessed *Vrn-B1* allele alone or in combination with other *Vrn* genes. The *Vrn-A1a* alone or in combination with *Vrn-B1* allele conferred earliest maturity. Contrary to the effect of *Vrn-A1a*, *Vrn-A1b* delayed flowering times in the CWSWS class by 1.6 days in Edmonton and 1.5 days in Lethbridge. *Ppd-D1a* did not alter flowering and maturity in the hard red cultivars tested in the field, but did in the greenhouse. *Ppd-D1a* reduced days to flowering for soft white spring wheat but did not alter maturity. The photoperiod sensitive allele (*Ppd-D1b)* is being replaced with the insensitive allele *Ppd-D1a,* in most modern Canadian germplasm, suggesting its adaptive advantages in the Prairies. An overall analysis of the data for the hard red class revealed a declining trend of 0.04 days/year in days to maturity in cultivars released from 1885 to 2008, and an increase of 8 kg/ha/year in grain yield.

6.3 Earliness *per se* QTLs and their interaction with the photoperiod insensitive allele *Ppd-D1a* in the Cutler × AC Barrie spring wheat population

The objective of the study was to map earliness *per se* quantitative trait loci (QTLs) affecting days to flowering and maturity and grain yield in a population developed by crossing two spring wheat cultivars Cutler and AC Barrie. We found three earliness *per se* QTLs affecting days to flowering and maturity, which were mapped on chromosomes 1B (*QEps.dms-1B1* and *QEps.dms-1B2*) and 5B (*QEps.dms-5B1*).We also found a QTL affecting flowering time only (*QFlt.dms-4A1*) on chromosome 4A. Two grain yield QTLs were mapped on chromosome 5B while one QTL was mapped on chromosome 1D. The population segregated for photoperiod insensitive allele *Ppd-D1a,* which induced earlier flowering by 0.69 days and maturity by 1.28 days. The photoperiod insensitive allele *Ppd-D1a* interacted in an additive fashion with QTLs for flowering and maturity times. The earliness *per se* QTL *QFlt.dms-5B.1* inducing earlier flowering could help to elongate grain filling duration for higher grain yields. Hence, chromosome 5B possesses promising genomic regions that may be introgressed for higher grain yields with earlier maturity through marker assisted selection in bread wheat.

6.4 The effect of vernalization genes on important agronomic traits in high yielding Canadian soft white spring wheat

In this experiment we aimed to study how different vernalization (*Vrn*) gene combinations alter the days to flowering and maturity; and how does this change in flowering and maturity time affect grain yield, and other agronomic traits. The experimental material comprised of 32 spring wheat lines belonging to the high yielding Canadian Western Soft White Spring class. We found that the *Vrn* gene group *Vrn-A1a-Vrn-B1-vrn-D1* was the earliest to flower and mature, while *Vrn-A1b-Vrn-B1-vrn-D1* was the latest to flower in this class. Spring wheat lines with *vrn-A1-Vrn-B1-Vrn-D1* were the highest yielding and matured at a similar time as those having vernalization genes *Vrn-A1a, Vrn-B1,* and *Vrn-D1*.

The findings of this study suggest that the presence of *Vrn-D1* has a direct or indirect role in producing higher grain yield. We therefore, suggest the introduction of *Vrn-D1* allele into higher yielding classes within Canadian spring wheat germplasm.

6.5 Relative performance of the Canadian spring wheat cultivars under organic and conventional field conditions

In wheat, certain adaptive traits like early season vigor, taller plants, and shorter life cycle are reported to help plants to compete weeds and produce satisfactory yields. In this experiment we tested the hypothesis whether early flowering and maturity conferred by insensitive vernalization alleles *Vrn-A1a* and/or *Vrn-B1* has any yield advantage under organic conditions' in Canadian spring wheat germplasm. We genotyped 32 cultivars for their vernalization gene composition (*Vrn-A1a, Vrn-B1 and Vrn-D1*) and studied those cultivars in organic and conventional management systems. We found 88% of the cultivars possessed vernalization (*Vrn*) insensitive allele *Vrn-A1a* either alone or in combination with *Vrn-B1*. There were no differential affects between the cultivars having insensitive *Vrn* allele at either single locus (*Vrn-A1a*) or two (*Vrn-A1a, Vrn-B1*) under organic and conventional field conditions; except for days to maturity, when the cultivars having single insensitive *Vrn* allele matured earlier under organic conditions. This earlier maturity had not conferred any yield advantage under organic field condition; and thus notwithstanding the proposed hypothesis of the study. Overall, the cultivars under organic conditions were earlier flowering, lower yielding with lower test weight compared to the conventional management system. Significant cultivars x environment interactions were found for grain yield, grain protein content and grain fill rate. For grain protein content, cross-over interactions of the cultivars between the management systems were observed. Here we report three CWRS cultivars (Marquis, Unity and Minnedosa) with minimum comparative losses in grain yields and grain protein content under organic field conditions, and hence could potentially serve as parents for organic wheat breeding programs.

6.6 My contribution to the research presented in this thesis

I collected the germplasm studied in chapter 2 during April 2009, and packed the seed for sowing after testing their germination percentage and 1000 grain weight. I participated in all the planning steps and sowing of the experiment with the cereal staff at Edmonton research station (ERS), University of Alberta. I was responsible for taking notes for days to flowering and maturity, which were recorded every alternate day, throughout the flowering and maturity season. I helped in recording plant height and harvesting the experiment. I did plant yield per plot and grain protein analysis myself. I conducted the experiment during 2010 and 2011 with almost similar responsibilities at ERS as described above. I was responsible to pack and ship the seed for sowing at Agriculture Agri-Food Canada (AAFC) Lethbridge research station, Lethbridge. Notes on days to flowering and days to maturity at AAFC Lethbridge, were taken by Dr. Harpinder Singh Randhawa during 2009 and 2011.

I was responsible for laying out the experiment and planting it in greenhouse, and taking notes on all the traits under study during 2009 and 2010. Greenhouse staff at University of Alberta was responsible for watering and fertigation. I used to get occasional help in harvesting the greenhouse experiment. I was responsible for screening the three vernalization genes (*Vrn-A1, Vrn-B1* and *Vrn-D1*) and a photoperiod allele (*Ppd-D1*) for all 102 lines/cultivars under study.

I joined the Cutler x AC Barrie population study presented in chapter 3 during 2009. The population development, field and greenhouse phenotyping for the years 2007 and 2008, and SSR marker screening was done before I joined the study. I was responsible for field experiment during 2011 and 2012 and extracting and shipping the DNA to Diversity Array Technology (DArT) for high throughput genotyping. I was responsible for data analysis and QTL mapping and was kindly guided by Dr. Rong-Cai Yang.

For comparative study of different vernalization genes presented in chapter 4, I was responsible for helping the seeding and harvesting at ERS and Ellerslie research station, University of Alberta. The seeds were packed and provided by Dr. Harpinder Singh Randhawa during 2010, 2011 and 2012. I recorded days to flowering and maturity at ERS only, and grain yield per plot, test weight and grain protein content for both

Edmonton and Ellerslie locations. The experiment at AAFC Lethbridge was managed by Dr. Harpinder Singh Randhawa.

The breeder seed collection for the study presented in chapter 5, on Canadian germplasm studied at organic and conventional field was kindly done by Klaus Strenzke. For seed packing, layout and randomization I got help from cereal group at University of Alberta. I was responsible for taking notes on all the traits under study in both conventional and organically managed lands during 2010, 2011 and 2012, however, I got help in seeding, harvesting and recording plant height.

I was responsible for all the data analysis and interpretation presented in all the studies of this thesis.

6.7 Contribution to Knowledge

Chapter 1 presented in the thesis provides comprehensive information on the physiological, molecular and biological aspects of all three genetic constituents of flowering and maturity time in wheat. Reviews written in the past (Trevaskis et al., 2007, 2010; Loukoianov et al., 2005) have covered either one of the aspects; and generally focused on one of the three genetic constituents of the flowering time. The current review provides a detailed description of all three flowering gene groups *i.e* vernalization, photoperiod and earliness *per se*; along with the primer sequences and annealing temperatures for all the vernalization and photoperiod alleles and an up to date list of QTLs affecting flowering and/or maturity time in wheat.

Chapter 2 reveals the flowering gene composition of over 100 historical and elite Canadian spring wheat germplasm released from 1885 to 2008. The study also impulses the changes in flowering gene composition of the Canadian germplasm in order to adapt the climate changes and to meet the shuttle breeding requirement of modern era. The phenotypic effect of vernalization allele *Vrn-A1b* has been quantified in the study, and to the best of our knowledge, has never been quantified before. The study also sheds light on the relative frequency distribution of vernalization genes and their role in high yielding Canadian class CWSWS and premium quality class CWRS produced in Canada.

In chapter 3, the Cutler x AC Barrie population study reveals two important genomic regions involved in affecting days to maturity and grain yield on chromosome 5B. The study also reports the additive nature of gene action between the insensitive photoperiod allele *Ppd-D1a* and the earliness *per se* QTL reported in the study. The additive gene action found in the study is important as it can elongate the grain filling duration without delaying the total days to maturity and resulting in a higher grain yield. These findings opens up new avenues for enhancing grain yield in a limited season.

Chapter 4 further verifies the results reported in chapter 2 and confirms the vernalization insensitive allele *Vrn-A1b* delays days to flowering and maturity compared to *Vrn-A1a*. We found *Vrn* gene group *Vrn-A1a-Vrn-B1-vrn-D1* was the earliest to flower and mature, while *Vrn-A1b-Vrn-B1-vrn-D1* was the latest to flower. We found that highest grain yield was incurred by the lines having *Vrn-D1* allele. This suggest that the presence of *Vrn-D1* has a direct or indirect role in producing higher grain yield. We therefore, suggested the introduction of *Vrn-D1* gene into higher yielding classes within Canadian spring wheat germplasm.

In chapter 5, we found accelerating the days to maturity in organic field conditions did not confer any yield advantage, and apparently the cultivars x environment interaction seems to be more important for higher grain yield under stress conditions. We identified three Canadian spring wheat cultivars with almost similar grain yield and grain protein content under both organic and conventional managed lands. The cross over cultivar x environment interaction for grain protein content found in the study also suggests the need for specified breeding under organic conditions for improved and higher grain yield.

6.8 Future Research

In a limited growing season, a single day change in time to maturity can significantly affect grain yield and grain protein content. Therefore, it is imperative to:

a) To screen the Canadian Spring wheat germplasm for all vernalization alleles (*Vrn-A1a, Vrn-A1b, Vrn-A1c, Vrn-A1d, Vrn-A1e, Vrn-B1a, Vrn-B1b, Vrn-D1a, Vrn-D1b, Vrn-B3*, and *Vrn-B5*) and photoperiod alleles (*Ppd-A1a, Ppd-A1b, Ppd-B1a, Ppd-B1b, Ppd-D1a, Ppd-D1b,*) identified so far, to fine tune the flowering and maturity times for maximum possible yield in Canadian Prairies.

b) To quantify the effect of each *Vrn* allele and to study the interaction among these alleles for a better understanding of flowering gene complex.

c) Introgression of the identified earliness *per se* QTLs and further exploration and practically:

- Utilization of earliness *per se* QTLs in addition with photoperiod insensitive alleles to elongate the grain fill duration for higher grain yields.
- Introgression of vernalization insensitive allele *Vrn-D1a* in Canadian Western Red Spring wheat class and its relative role in increasing the grain yield.
- Exploring the better suited cultivars under organic conditions for increased grain yield and better quality, to help meet the domestic organic market demand.
- Specific projects funded for organic breeding activities carried on organically managed lands and targeted for cultivar development and release.

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