Evaluation of oilseed *Brassica napus* germplasm for days to flowering under a short-day condition and QTL mapping of the trait

by

Karanjot Gill

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Plant Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Canola (*Brassica napus*, AACC = 2*n* = 38) is an important oilseed crop in Canada. Among the different agronomic traits, the earliness of flowering and maturity are important for growing this crop on the Canadian prairies. The earliness of flowering can be improved by using the genes/alleles capable of promoting flowering under a short-day condition. In the present study, a genome wide association study (GWAS) was carried out by using SNP marker data and phenotypic data of days to flowering of 184 inbred lines, derived from six *B. napus* × *B. oleracea* interspecific crosses, grown under a 10 h photoperiod condition. Three QTL affecting days to flowering under this short-day condition were identified on chromosomes C1, C5 and C9. The effect of the C9 QTL was further confirmed by designing SSR markers from this QTL region and testing the markers on this inbred population. The early flowering C9 QTL allele could also be confirmed through evaluation of a set of *B. napus* lines under a short-day condition (10 h photoperiod) and genotyping the lines with these SSR markers. Near-isogenic lines (NILs) were also developed in this thesis research for C1 and C9 QTL, which have been mapped previously by using a population derived from Hi-Q × RIL-144 (carrying genome contents introgressed from *B. oleracea* var. *alboglabra*). Marker-assisted backcrossing enabled the introgression of these QTL alleles in the genetic background of Hi-Q. This is also evident from phenotypic evaluation of the NILs under a 10 h photoperiod condition where the NILs carrying the C1 or C9 QTL allele of RIL-144 flowered significantly earlier than the NILs carrying the Hi-Q allele. Thus, the results from this study provided evidence for the existence of diverse and valuable alleles in the C genome of *B. oleracea* which can be exploited in the breeding of *B. napus* canola.

Preface

This thesis is submitted by Karanjot Gill for the degree of Master of Science in Plant science. Dr. Habibur Rahman prepared all research plans, helped in interpretation of the results and provided valuable comments and suggestions for writing all thesis chapters.

For the chapter II, Karanjot Gill conducted all experiments in growth chamber for phenotyping the mapping population for days to flowering, DNA extraction, designing primers, molecular marker analysis, association mapping by using phenotypic data and SNP marker data. The inbred lines used in this experiment were developed by the Canola program of the University of Alberta under supervision of Dr. Habibur Rahman. SNP genotyping of the inbred lines by using tGBS technique was done by Azam Nikzad in the Canola program of the University of Alberta under supervision of Dr. Habibur Rahman. Dr. Berisso Kebede also provided guidance for conducting different experiments and helped in data analysis and association mapping analysis. Kawalpreet Kaur helped in DNA extraction of the inbred lines.

For the chapter III, Karanjot Gill carried out all experiments, such as phenotyping the cultivars/lines in growth chamber for days to flowering under 10 h photoperiod condition, DNA extraction, designing primers, molecular marker analysis and statistical analysis. The cultivars/lines used in this experiment were available with Canola program of University of Alberta. Kawalpreet Kaur helped in DNA extraction of the cultivars/lines.

For the development of NILs in chapter IV, the F_1, BC_1 and BC_2 generation populations were produced by the Canola Program of the University of Alberta. I was responsible for the development of BC_3, BC_4, BC_4F_2 and BC_4F_3 NIL populations. I carried out all other experiments such as DNA extraction, designing primers, molecular marker analysis, phenotyping the NILs for

days to flowering, seed increase of the NILs, and statistical analysis of data. Dr. Berisso Kebede also provided suggestions for conducting this experiment.

Acknowledgements

Foremostly, I bow with all humility and reverence to the Almighty God for bestowing me with confidence, patience, hard work and blessing me with skills so as to be all what I am today.

It feel privileged to express my deepest sense of gratitude, obligation and thanks to my supervisor Dr. Habibur Rahman for giving me an opportunity to work with the Canola breeding program of the University of Alberta. His constructive guidance, invaluable criticism, thoughtful suggestions and polite dealing helped me to realize my potential and broaden my horizon. I am also thankful to Dr. Urmila Basu, my committee member, for her able guidance, valuable suggestions and ever willing help during the course of my research work.

Words at my command are inadequate in form and spirit to convey the depth of feelings to my family whose infinite love, silent prayers, positive attitude and encouragement enabled me to put this tireless venture to a fruitful result.

I take this opportunity to extend my gratitude to all members of Canola breeding program- Kawalpreet Kaur, Berisso Kebede, Rubeena Sheikh, An Vo, Salvador Lopez, Mehdi Farid, Mousam Dhar, Azam Nikzad as well as Harleen Kaur for their ever willing help and support for the research work. Without them, it would be hard to complete this whole investigation.

I would like to acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support for conducting this research.

I may not have mentioned all but none is forgotten. Heart-felt thanks to all for providing me even a minuscule of help during this period of investigation. It's my honest conviction and sincerest prayer that the Almighty God blesses you all abundantly.

Table of Contents

List of Tables

List of Figures

List of Symbols and Abbreviations

±	Plus/minus
=	Equal
>	Greater than
<	Less than
%	Percent
°C	Degrees Celsius
2n	Diploid number of chromosomes
ABI	Applied Biosystems
AFLP	Amplified fragment length polymorphism
AM	Association mapping
BC_1	First backcross generation
BC_1F_1	First filial generation after first backcross
BC_1F_x	x^{th} generation of BC_1-derived population
bp	Base pair
Canola	Canadian oil low acid
cm	Centimeter
cM	Centimorgan
CTAB	Cetyl trimethylammonium bromide
cv.	Cultivar
cvs.	Cultivars
df	Degree of freedom
DH	Doubled haploid

DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleotide triphosphate
DTF	Days to flowering
e.g.	For example
F_1	First generation
FarmCPU	Fixed and random model circulating probability unification
Fig.	Figure
F_x	x^{th} generation of F_2-derived population
g	Gram
GAPIT	Genome Association and Prediction Integrated Tool
GLM	Generalized linear model
GWAS	Genome-wide association study
Hi-Di	Highly Deionized Formamide
i.e.	That is
LG	Linkage group
LOD	Logarithm of odds
LSmeans	Least-squares means
Mb	Mega base pair
mM	Millimole
µMol	Micromole
µl	Microlitre
ml	Millilitre
min	Minute

MLM	Mixed linear model
Ng	Nanogram
n	Haploid number of chromosomes
n	Number of observations
NIL	Near- isogenic line
RIL	Recombinant inbred line
Rpm	Revolutions per minute
Ol.alb.nrc	*B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI
Ol.bot.cau	*B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1
Ol.cap.bad	*B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper
Ol.cap.bal	*B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro
Ol.cap.bin	*B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener
Ol.ita.pre	*B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop
PCA	Principle component analysis
PCR	Polymerase chain reaction
P-value	Probability value
QTL	Quantitative trait loci
r	Pearson's correlation coefficient
R	R project for statistical computing
RAPD	Random amplified polymorphism DNA
RFLP	Restriction fragment length polymorphism
SDS	Sodium dodecyl sulfate

S.E.	Standard Error
SNP	Single nucleotide polymorphism
SRAP	Sequence related amplified polymorphism
SSR	Simple sequence repeat
TASSEL	Trait analysis by association, evolution and linkage
tGBS	Tunable genotyping-by-sequencing
t-test	Test statistic for t-test
USDA	United States Department of Agriculture
var.	Variety

Chapter 1

Literature Review

1.1 Introduction

The family Brassicaceae (Cruciferae or mustard) includes 372 genera of which the genus *Brassica* includes several economically important agricultural and horticultural crop species ; they are predominantly grown on the northern side of the equator (Canola council of Canada, 2017). The cultivation of *Brassica* oilseed crops dates back to 2000 B.C. in India; it was brought into China and Japan around 30 B.C. (Canola council of Canada, 2017). *B. napus*, which is commonly called rapeseed or canola, is one of the most important oilseed crops grown in the temperate regions due to its ability to survive at low temperature as compared to other oilseed crops.

The terms rapeseed and canola differ for their seed quality traits. Oils of traditional *Brassica* oilseed crops contain a high proportion of polyunsaturated long-chain fatty acid, erucic acid; this fatty acid is undesirable for human nutrition (Cartea et al. 2019). The seed meal of this oilseed is a good source of protein; however, its use as feed for animal is limited by the presence of high amount of glucosinolates (sulfur containing compounds), which is considered anti-nutritional for animals i.e. reduce palatabilty, reduced growth of animals (Tripathi and Mishra 2017). This type of traditional *Brassica* oilseeds, containing a high level of erucic acid in oil and a high level of glucosinolates in seed meal is called rapeseed. Plant breeding efforts in the 1960-1970's eliminated or reduced the level of these two seed constituents (Maheshwari et al. 1981). The low erucic acid trait was introgressed into Canadian *B. napus* by crossing the traditional high erucic acid type with low erucic acid German cultivar 'Liho', and the first low erucic acid cultivar 'Oro' was released in 1968 and the first double low (low erucic and low glucosinolates) cultivar 'Tower' was released in 1974 (Eskin and Przybylski 2003). The low glucosinolate genes in these double low or canola

quality type *B. napus* was introduced from the Polish winter type forage *B. napus* cultivar Bronowski (Stefansson and Downey 1995). The low erucic acid low glucosinolate or double low cultivars are also known as canola, which is an abbreviation of Canadian Oil Low Acid. Canola oil is almost free from erucic acid and its seed meal contains less than 30 µmol glucosinolates/g defatted meal (Canola council of Canada, 2017). *Brassica* oilseed crops, which includes *B. napus*, *B. juncea* and *B. rapa*, is the second largest oilseed crop in the world after soybean (USDA 2018/19). In Canada, *B. napus* canola is the second largest field crop contributing about $26.7 billion annually to the economic activity of this country (Canola council of Canada, 2017).

1.2 Types of *B. napus* on the basis of growth habit

The oilseed crop *B. napus* can be classified into three categories: spring type, semi-winter type and winter type. The winter type plants require cold (4 °C) treatment (vernalization) for about eight weeks to induce flowering and are grown in areas having low temperature in winter, mainly in central Europe. The semi-winter type plants need a short period (about 4 weeks) of vernalization and are mainly grown in China. The spring type plants flowers without vernalization and are grown in summer in Canada, Europe and Australia (for review, see Leijten et al. 2018).

1.3 *Brassica* genome relationship and genome evolution

Polyploidy is an important mechanism of genome evolution; it involves either a single genome (autopolyploid) or two or more genomes (allopolyploid). There are substantial evidences to support that allopolyploidy can result in changes in genome organization and gene expression (for review, see Chen and Ni, 2006). Among different crop species, the six species of the genus *Brassica* constituting the well-known U's triangle (U 1935) is a good example of polyploidy. Among these, three species, viz. *B. rapa* (n = 10, A genome), *B. oleracea* (n = 9, C genome) and

B. nigra (*n* = 8, B genome) are diploids and three species, viz. *B. napus* (*n* = 19, AC genome), *B. carinata* (*n* = 17, BC genome) and *B. juncea* (*n* = 18, AB genome) are the allotetraploids which originated from the three diploid species (Fig 1-1). The amphidiploid *B. napus*, the largest *Brassica* oilseed crop in the world, originated about 7500 years ago and carries the A genome of *B. rapa* and the C genome of *B. oleracea* (Chalhoub et al. 2014). Sequencing of *B. napus* genome (Chalhoub et al. 2014) disclosed abundant occurrence of homoeologous exchange between the A and C sub-genomes, and re-sequencing of 588 *B. napus* accessions (Lu et al. 2019) disclosed that the A genome of this amphidiploid originated from European turnip and the C genome from the common ancestor of Chinese kale, cauliflower, broccoli and Kohlrabi. Of the different growth habit types of *B. napus*, the winter oilseed type probably evolved first, from which the other growth habit types evolved.

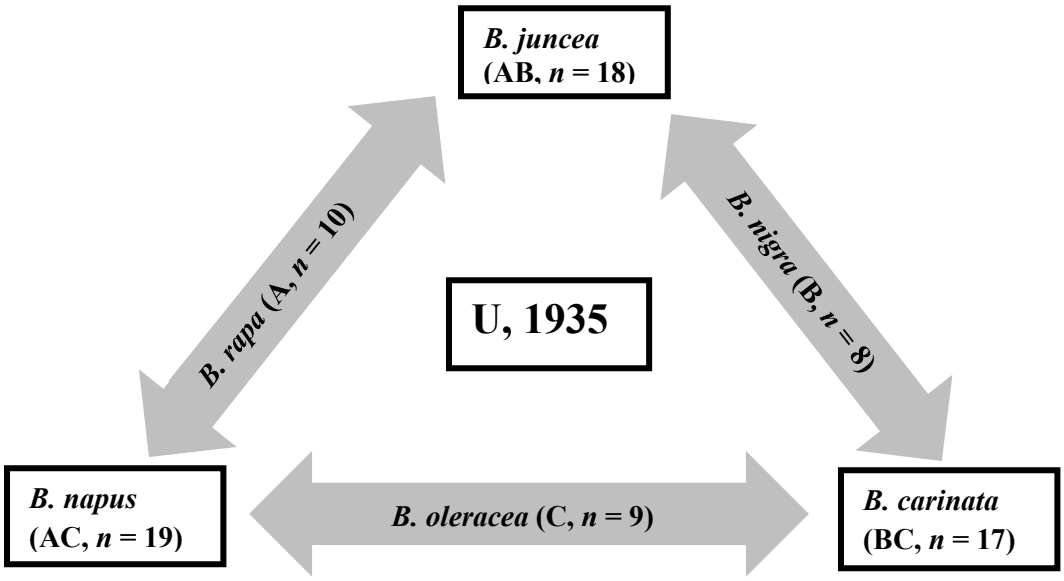


Figure 1-1. U's triangle representing genomic relationship between *Brassica* species (Nagaharu U 1935)

1.4 Flowering in plants

Flowering time plays an important role in domestication and adaptation of a crop in a certain region. The transition from vegetative to reproductive phase is important in plant's life cycle. Plants have developed several mechanisms, such as response to photoperiod and cold temperature or vernalization, to regulate the time of their flowering (Amasino and Micheals 2010). They usually interact with environmental conditions to regulate their various mechanisms. Flowering is one of the important phenomena responding to change in environmental cues. Controlling the timing of flowering in plants is beneficial for many reasons, which include the adaptation of the plants under continuously changing climatic conditions, expanding the area of cultivation through breeding cultivars tailored for flowering and maturity traits for better adaptation in the cropping system in a new geographical region (for review, see Jung and Muller 2009). On an average, the summer or spring type *B. napus* in Europe flowers for about 26 days and 75% of the siliques which are capable to reach maturity result from the flowers which bloom within 14 days of anthesis (Tayo and Morgan 1975); however, the duration of flowering and silique set can be influenced by genotype and environment (Robertson et al. 2002). The duration of flowering period in *B. napus* is negatively correlated (r = -0.883) with grain filling period (Chaghakaboodi et al. 2012) while the duration of grain filling period is positively correlated with seed yield (Gan et al. 2016; Nikzad et al. 2019).

1.5 Flowering time pathways

Genes are involved in the control of different physiological and plant development traits; however, environmental conditions, such as temperature, photoperiod, soil moisture and nutrients, and many phytohormones like gibberellins plays an important role in the transition of a plant from vegetative

to reproductive phase (for review, see Cho et al. 2017). The genetically controlled pathways involved in flowering time variation include vernalization, photoperiod and autonomous pathways (for review, see Mouradov et al. 2002; Jung and Muller 2009).

1.5.1 Vernalization pathway: The shift of vegetative to reproductive phase in plant occurs in response to various environmental cues. Prolonged exposure to low temperature (about 4 ºC) is one of the important factors responsible for initiation of flowering. Winter type plants often require exposure to cold temperature, the process known as vernalization, to induce flowering (Schmitz and Amasino 2007). Vernalization responsive plants are seeded before the onset of winter and they start to flower in spring. In *Brassicas*, the requirement of vernalization varies depending on the species and type. The spring type flowers without receiving any cold treatment, the semi-winter type require a short period of chilling treatment, while the winter type require a long exposure to low temperature (about 4 ºC) (for review, see Jung and Miller 2009).

Two genes, viz. *FLOWERING LOCUS C* (*FLC*) and *FRIGIDA* (*FRI*), has been reported to be main genes involved in regulation of flowering in vernalization-responsive pathway in *Arabidopsis* (Werner et al. 2005). Among these, *FLC* is the major repressor of flowering (Gu et al. 2013), and this gene is regulated by *FRI* (Micheals and Amasino 1999). The *FLC* encodes a MADS-box transcription factor that represses flowering (Michaels and Amasino, 1999; Sheldon et al., 1999). Vernalization represses the expression of *FLC* (Michaels and Amasino 1999; Sheldon et al. 1999, 2000) and promotes flowering. The expression of *FLC* can be placed under the following categories: (i) full expression of *FLC* in absence of cold temperature (vernalization), (ii) repression of *FLC* under prolonged low temperature, and (iii) continued repression of *FLC* even after vernalization due to epigenetic changes occurred during vernalization (for review, see Song et al. 2012). In the first stage, the protein products of *FRI* increase the expression of *FLC* mainly

by modification in chromatin structure (Liu et al. 2010). Oh et al. 2004 reported that *VERNALIZATION INDEPENDENCE* (*VIP*) gene complex also results upregulation of the expression of *FLC*. The expression of cold regulated gene, *VERNALIZATION INSENSITIVE3* (*VIN3*), result repression of the *FLC* and, thus, result early flowering in *Arabidopsis* (Hepworth et al. 2018). Swiezewski et al. (2009) reported that increased expression of cold induced long antisense intragenic RNA (*COOLAIR*) under cool temperature result low expression of *FLC* gene.

The *FLOWERING LOCUS C* (*FLC2*) has also been reported one of the important genes involved in the control of vernalization-responsive flowering in *B. rapa* (Zhao et al. 2010; Xiao et al. 2013; Huang et al. 2018). Kawanabe et al. (2016) found a reduced expression of *FLC* in *B. rapa* after vernalization, and this reduced expression seems to correlate with the extent of histone modifications. Tadege et al. (2001) isolated five *FLC* genes from *B. napus* and showed that the expression of *BnFLC* genes is negatively correlated with flowering time. Irwin et al. (2012) identified and characterized two *FRI* genes viz. *BolC.FRI.a* and *BolC.FRI.b* in *B. oleracea*. The homologues of *FRI* have also been reported in *B. napus* (Wang et al. 2011) which are known to differentiate the winter and spring growth habit types. Vernalization was also reported to regulate the expression of circadian clock gene (*BrCCA1*) by demethylation of casein kinase subunits in *B. rapa* (Duan et al. 2017).

1.5.2 Photoperiod pathway: Photoperiod is one of the important factors which influence the induction of flowering. Plants can be categorised into three groups on the basis of their responses to day length and includes: (i) long day plants which usually flower when day length exceeds a critical threshold of about 16 h light/ 8 h dark, (ii) short day plants which starts flowering when day length drops below a critical length (8 h light/16 h dark), and (iii) day neutral plants in which

flowering is not influenced by day length (for review, see Jung and Miller 2009; Andres and Coupland, 2012).

The plant leaves perceive the changes in day length and they send a signal to the shoot apical meristem which is responsible for initiation of flowering. *CONSTANS* gene was reported to be major gene regulating flowering under photoperiod mediated pathway and the CO protein degrades in darkness (Liu et al. 2008). During the morning hours, the expression of *CO* has been found to be repressed by CDFs (CYCLING DOF FACTORs) (Imaizumi et al. 2005). The stability of the CDF is controlled by a complex formed by *FKF1* (*FLAVIN-BINDING KELCH REPEAT F-BOX 1*) and *GIGANTEA* (*GI*) gene, and the *FKF1-GI* complex forms on the promoror of *CO* in late afternoon and regulate the expression of this gene (*CO*) in Arabidopsis (Sawa et al. 2007, Song et al. 2014). The expression of the *CO* gene under long day condition was reported to be regulated by *COP1* (*CONSTITUTIVE PHOTOMORPHOGENESIS*), and this gene also repress the *CO* under darkness (Liu et al. 2008). The *COP1* works in concert with *SUPPRESSOR of phyA-105* (*SPA*) proteins to repress photoperiod-responsive flowering by regulating the degradation of *CO*. According to Laubinger et al. (2006), the SPA proteins are essential for photoperiod-responsive flowering by controlling the stability of CO, and mutation in SPA result early flowering under short day but not long day condition. Sheerin et al. (2015) demonstrated that the light-regulated phytochromes A (phyA) and B (phyB) interact with the SPA proteins, and they also disrupt the formation of COP1/SPA protein complex, which is known to repress the *CO*, and thus maintain the expression of *CO*. The *CO* initiates the transcription of the floral integrator genes *FLOWERING LOCUS T* (*FT*), *FLOWERING LOCUS D* (*FD*) and *TWIN SISTER OF FT* (*TSF*) (for review, see Leijten et al. (2018). The *FT* gene after activation by *CO* forms a complex with *FD* (*FLOWERING LOCUS D*) in the meristem to initiate flowering through activitation of the

gene *APETALA1* (*AP1*) (Abe et al. 2005). Four *CO* genes have been reported to be present in *B. napus* (Robert et al. 1998). Many photoperiod genes (*CO*, *FKF*) were also reported in *B. rapa* by Schiessl et al. (2017). Robertson et al. (2002) studied the relationship between photoperiod, vernalization and flowering time and found that the late flowering spring *B. napus* lines respond to vernalization and photoperiod, while the early flowering lines does not show significant response to these two environmental conditions.

1.5.3 Autonomous pathway: Autonomous pathway refers to the internal factors of the plant which regulate flowering other than photoperiod and vernalization pathways (Srikanth and Schmid, 2011). This pathway includes factors involved in the processes like epigenetic regulation, RNA processing etc. (Simpson 2004). The autonomous pathway genes including *FCA* (*FLOWERING LOCUS CA*), *FPA* (*FLOWERING LOCUS PA*) *FY* (*FLOWERING LOCUS Y*) and *FLK* (*FLOWERING LOCUS KH DOMAIN*) were reported to repress the expression of *FLC* while interacting with RNA processing (Macknight et al. 1997, Schomburg et al. 2001, Simpson et al. 2003, Lim et al. 2004). Other autonomous pathway genes, such as *FLD* (*FLOWERING LOCUS D*) was reported to regulate flowering by chromatin remodeling or acetylation of histones in *Arabidopsis* (He et al. 2003).

1.6 Significance of flowering time in adaptation of crops

Flowering time is important for adaptation of a crop to different climatic conditions and geographical regions. The narrow genetic base of many crop species, including the *Brassica* oilseed crops (for review, see Rahman 2013), may impose a restriction for further improvement of the crops; therefore, there is a need of using new approaches to increase allelic diversity in our crop germplasm and breeding materials for the development of improved cultivars to meet the

food demand of the growing world population (Huang et al. 2002). Ni et al. (2009) showed that by regulating the physiological and metabolic pathways associated with flowering time, an increased growth and biomass yield can be achieved in allopolyploids. According to Blümel et al. (2015), a change in the expression of a single flowering time regulator can influence the flowering time to a great extent, and an improvement can be made in our crop plants through controlling the flowering time network.

1.7 Factors influencing flowering time

As the plant advances from vegetative to reproductive phase, several physiological changes occur in the plants which result from the effect of the genes and environmental conditions. Several environmental factors, such as temperature, light, moisture and nutrients play an important role in flowering time variation (Cho et al. 2017). Among these, the effect of temperature and light can be better predicted during the change in the crop growing season (Ausin et al. 2005). Every plant species has its own optimum temperature range within which they will flower; temperature below and above the optimum range is detrimental to the plants. Higher temperature often reduces the vegetative phase and results in early flowering and maturity which ultimately reduce the yield of the crop plants. For example, Angadi et al. (2000) and Gan et al. (2004) found that high temperature (35 °C) is injurious to reproductive parts of the plants and reduces seed yield in canola. Winter and semi-winter type require low temperature to induce flowering.

Light, especially the quality, quantity and duration, plays an important role in the control of flowering. This also affects the pigment contents in plants. For example, Zhu et al. (2017) reported that the duration of light hour can affect the level of anthocyanin and chlorophyll contents and

photosynthesis in *B. rapa.* Yeh et al. (2016) found that a combination of natural light and supplementary red light increases the fresh weight in *B. rapa* ssp. *chinensis*.

Exogenously applied hormones have been found to play an important role in the regulation of growth and development of crop plants. Bonhomme et al. (2000) found that application of cytokinin and gibberellins can result in early flowering in *Sinapis alba* by reducing the expression of the MADS box gene, *SaMADS A*. Cytokinin has also been reported to affect different components of flower, such as elongation of the stamen, growth of the petals and maturity of the anthers, and can cause epigenetic modifications (Zuniga-Mayo et al. 2018).

1.8 Photo-insensitivity and earliness in canola

B. napus is considered a long day plant which requires about 16 – 18 h photoperiod for flowering; therefore, this crop is largely grown in temperate regions like Canada under a long day condition (King and Kondra, 1986; Robertson et al. 2002). However, the maximum photoperiod often reaches about 15-17 h at flowering stage of the crop in these regions. Spring canola usually takes about four months for growing in North America and Europe, while it takes about a couple of months longer where day length at crop growing season is about 12 h, e.g. in India. Therefore, identification of the genes which are not affected by photoperiod or the genes involved in the control of flowering under a short-day condition is not only important for the improvement of earliness in canola for the temperate regions, but also for growing canola under a short-day condition. Seed yield in *B. napus* can be increased by combining the earliness of flowering with delayed maturity traits, i.e., by increasing the duration of grain-filling period (Habekotte 1997; Gan et al. 2016). Nikzad et al. (2019) found a significant positive correlation between the duration

of grain-filling period and seed yield in *B. napus* lines carrying genome content introgressed from *B. oleracea*.

1.9 Quantitative trait loci (QTL) mapping for flowering time in *Brassica*

Table 1-1. List of QTL affecting flowering time in *Brassica*

Cross	Mapping population	Population size	Marker type	No. markers	No. QTL and the linkage group(s)	Reference
Brassica napus:						
Winter × Spring	DH	104	RFLP	132	Three: LG9, LG12, LG16	Ferreira et al. 1995
Winter × semi-winter	DH, reconstructed F_2	606	SSR, RFLP, SNP,MS-AFLP, STS	621	Forty two: N1, N2, N3, N5, N9, N10, N12, N16, N18, N19	Long et al. 2007
Spring × spring	DH	40-45	SSR, SRAP, AFLP	248	Three main effect QTL and 5 pairs of epistatic QTL: N2, , N5, N6, N11, N18 (Total seven main effect QTL)	Cai et al. 2008
Winter × winter	$F_{2:3}$	145	AFLP, SSR	241	Six: LG2, LG3, LG6, LG13, LG17 (Total 13)	Mei et al. 2008
Spring × spring	DH	150	SRAP, SSR	387	Twenty two: LG1, LG2, LG3, LG6, LG7, LG8, LG11, LG13, LG14, LG16, LG17, LG18	Chen et al. 2010
Elite breeding lines	DH	391	SNP	253	Multiple-line Cross QTL mapping- two QTL on 2 linkage groups (A2, C6), Joint Linkage Association Mapping- four QTL on 4 linkage groups (A2, A7, A10, C6)	Wurschum et al. 2012
Semi - spring × semi- spring	DH	160 DH (field test), 186 DH (glasshouse)	SSR, SRAP, SCAR, DArT, candidate gene based markers	674	Twenty: A2, A3, A4, A6, A7, C2, C3, C5,C6, C8	Raman et al. 2013
Spring × Spring	DH	207	SSR, SRAP and AFLP	386	Four: A6, A7, C8 and C9	Luo et al. 2014
Summer × Summer	DH	131	SSR, DArT, intron polymorphism and gene-based markers	584	Three: A02, A07, C3 (Total ten QTL)	Nelson et al. 2014
Spring × Winter	F_2	93	SNP	658	Two: C04, C08	ArifuzZaman et al. 2016
Spring × Spring	DH	217	SSR, AFLP	495	Twenty two QTL: A2, A3. A5, A6, A7, A10, C2, C8	Liu et al. 2016
Spring, winter, semi-winter rapeseed core collection	Inbreds	448	SNP	20,342	Fourty: A1, A2, A3, A04, A06, A07, A10, C02, C03, C04, C09	Wang et al. 2016

Spring, winter, semi-winter diversity panel cultivars or inbreds	Inbreds or cultivars	523	Brassica 60K illumina infinium SNP array	-	Fourty one: A2, A3, A4, A5, A7, A9, A10, C1, C2, C3, C4, C5, C6, C9	Xu et al. 2016
Spring × Spring	DH	92	AFLP, SSR, and flowering gene based markers	79	Five: C1, C2, C3, C6	Rahman et al. 2017
Spring × winter	F_2	166	SNP	2,021	Two: A10, C06	Li et al. 2018
Spring × Spring	DH	95	AFLP, SSR and flowering gene based markers	-	Six: C1, C2, C8, C9	Rahman et al. 2018
Semi winter × Semi winter	RIL	172	SNP	2795	Twenty seven: A02, A05, A06, A07, A08, A10, C02, C04	Jian et al. 2019
Brassica rapa:						
Biennial × Annual	F_2 (phenotyping F_3)	85	RFLP	143	Two: LG2, LG8	Teutonica and Osborn 1995
Late flowering × early flowering	F_2	150	RFLP	31	Two: R2, R3	Axelsson et al. 2001
Late bolting DH line × Chinese cabbage DH line	DH	200	AFLP	248	Ten: LG1, LG2, LG3, LG5, LG9, LG10	Nishioka et al. 2005
Rapid cycling × vegetable type Chinese cabbage	DH, F_2 and BC_1	178 F_2, 135 DH, 136 BC_1	SSR	-	Eight: R01, R02, R03, R06, R07, R08, R10 (27 total)	Lou et al. 2007
Chinese cabbage × Chinese cabbage	DH	172	SNP	22,747	Six: A01, A08	Jing et al. 2016
Brassica oleracea:						
Cabbage × Broccoli	F_2, (phenotyping- F_3)	124	RFLP	112	Three: LG2, LG6, LG8	Camargo and Osborn 1996
var. *alboglabra* × var. *italica*	DH	149	RFLP	82	Six: O2, O3, O5, O9	Bohuon et al. 1998
var. *italica* × var. *alboglabra*	Recombinant backcross substitution lines	79	RFLP, AFLP	197	Eleven: O1, O2, O3, O5, O9	Rae et al. 1999
Late flowering × early flowering	F_2	150	RFLP	31	Two: O3, O9	Axelsson et al. 2001
Annual × Biennial	F_2	134	RFLP, SRAP, CAPS, SSR, AFLP	187	Six: O2, O3, O6, O8, O9	Okazaki et al. 2007

Quantitative trait loci (QTL) analysis is one of the important ways to understand the genetic variation of a quantitative trait; this method has been used by several researchers for mapping flowering time genes in *Brassica* (Table 1-1). In *B. napus*, QTL mapping of flowering time has

been carried out by several researchers (for example, Ferreira et al. 1995; Long et al. 2007; Chen et al 2010; Würschum et al. 2012; ArifuzZaman et al. 2016; Liu et al. 2016; Wang et al. 2016; Rahman et al. 2017, 2018; Jian et al 2019) and by using populations of different growth habits. By using a doubled haploid (DH) population derived from cross between annual and biennial *B. napus*, Ferreira et al. (1995) mapped a genomic region with major effect and two genomic regions with minor effect controlling flowering time. Similarly, ArifuzZaman et al. (2016) detected two QTL affecting days to flowering by using a segregating F_2 population derived from cross between winter and spring *B. napus*; one of these QTL located on chromosome C8 and the other on C4, and these two QTLs explained 21.7% and 15.0% of the total phenotypic variance, respectively. On the other hand, Long et al. (2007) detected 42 QTL affecting days to flowering under different environmental conditions.

Cai et al. (2008), by using a DH population of *B. napus* and growing under a long- and a short-day conditions, reported seven main effect QTL (QTL independently exert significant effect) and 11 pairs of epistatic QTL (QTL having no effect by its own but exert significant effect in combination with other QTL) on 13 linkage groups; among these three main effect and five pairs of epistatic QTL reported to affect days to flowering under a short-day and long conditions. Nelson et al. (2014) reported three QTL for days to flowering in *B. napus* under both long- and short-day conditions. Mei et al. (2008) reported six QTL affecting days to flowering by using $F_{2:3}$ segregating population. Jian et al. (2019) detected 27 QTL in *B. napus* from eight chromosomes of which the major QTL found to be located on chromosome C2; this QTL explained 11-25% of the total phenotypic variance for flowering.

By using a spring type *B. napus* population, Chen et al. (2010) identified 22 QTL under different environments where the individual QTL explained 4.41-48.28% of the total phenotypic

variance. Similarly, Liu et al. (2016) used a DH population of spring *B. napus* and reported 22 QTL associated with flowering time under varying environments; among these, four QTL exerting major effect were found to be located on the chromosomes A7, C2 and C8. By using a DH population derived from crossing of Australian spring canola cultivars, Raman et al. (2013) detected more than 20 QTL located on ten A and C genome chromosomes and Luo et al. (2014) reported four QTL on A6, A7, C8 and C9 chromosomes. By using a *B. napus* mapping population carrying genome content introgressed from *B. oleracea*, Rahman et al. (2017, 2018) mapped two flowering time QTL on C1 and C9. The C1 QTL is responsible for earliness of flowering in spring *B. napus* without being affected by photoperiod while the C9 QTL is significantly affected by photoperiod (10h).

Xu et al. (2016) carried out a genome wide association study (GWAS) by using 523 *B. napus* cultivars and lines and reported 41 SNPs from 14 chromosomes associated with flowering time. Following similar approach and by using 448 *B. napus* lines collected from different parts of the world, Wang et al. (2016) detected 40 QTL associated with days to flowering under varying environments. Würschum et al. (2012) identified two QTL explaining 20.6% of the total genotypic variance by using multi-line cross QTL mapping (MC-QTL) approach and four QTL explaining 17.4% of the total genetic variation by using joint linkage association mapping (JLAM) approach; this research group also reported one epistatic interaction affecting days to flowering. Thus, both approaches were found to be suitable for detection of QTL in segregating populations.

Illumina deep sequencing of two *B. rapa* and two *B. oleracea* accessions and 35 paralogs of flowering regulators of *Arabidopsis thaliana* has been done by Schiessl et al. (2017) to understand the genetic network of flowering time in *Brassica.* They identified SNPs, insertion/deletions and copy number variation in the genes regulating flowering time; this research

group also found a rearrangement in the *B. napus* genome which probably occurred after the allopolyploidization event. Li et al. (2018) reported two QTL controlling days to flowering by using F_2 population derived from cross between spring introgression line and winter synthetic *B. napus* genotype.

QTL mapping of the genes controlling flowering time has also been carried out in the two diploid progenitor species *B. rapa* and *B. oleracea*. In case of *B. rapa*, Teutonica and Osborn (1995) reported two QTL and Nishioka et al. (2005) reported ten QTL affecting days to flowering in *B. rapa*. Lou et al. (2007) detected eight QTL affecting flowering time and the major QTL found to be located on chromosome R02, and this QTL co-localized with *BrFLC2*. Jing et al. (2016) reported six QTL affecting flowering time by using a DH population of *B. rapa* and growing under different environmental conditions. By using F_2 population of *B. rapa* and *B. oleracea*, Axelsson et al. (2001) detected two QTL each in *B. rapa* and *B. oleracea* controlling days to flowering.

In case of *B. oleracea*, Camargo and Osborn (1996) reported three QTL on three linkage groups; and these three QTL explained about 54% of the total phenotypic variance for flowering time. By using a DH population of *B. oleracea* var. *alboglabra* × var. *italica*, Bohuon et al. (1998) detected six QTL on four linkage groups, O2, O3, O5 and O9, affecting flowering time in this population. On the other hand, Rae et al. (1999) reported 11 QTL on five linkage groups, O1, O2, O3, O5 and O9, by using a recombinant backcross substitution population of *B. oleracea*. Okazaki et al. (2007) constructed a linkage map by using F_2 population of *B. oleracea* and identified six QTL affecting flowering time where the QTL from C2 play a major role in vernalization-responsive flowering.

Fine mapping of a gene and our knowledge of the molecular basis of the gene can be extended by using near-isogenic lines (NILs). NILs in *Brassica* crops has been developed by Zhao et al. (2010) and have been used for fine mapping and cloning of boron-efficient genes in *B. napus*. Zhao et al. (2019) also developed NILs of *B. rapa* and used to fine map the major QTL affecting seed coat colour. Zhu et al. (2020) developed NILs of *B. napus* for mapping QTL associated with seed number per silique.

From the literature reviewed above, it is apparent that a large number of the QTL/genes from both A and C genome chromosomes are involved in the control of flowering time in *B. napus* and its parental species *B. rapa* and *B. oleracea*, and the genes involved in different flowering pathways can be found in the *Brassica* genomes. Most of the studies with flowering time have been conducted by using natural population. It is well reported that interspecific cross in *Brassica* can introduce beneficial alleles into our *Brassica* crop species (for review see Rahman 2013). Therefore, QTL mapping of flowering time by using population derived from *Brassica* interspecific crosses can disclose novel QTL/allele for use in molecular breeding.

1.10 Research objectives

The long-term objectives of this study are to fine map and sequence the genes involved in the control of flowering under a short-day condition, and identify the candidate genes involved in the control of this trait. However, in short term, following studies were conducted in this MSc thesis research project:

Study I: Identify QTL for days to flowering under a short day (10 h) condition by using an advanced generation *B. napus* population derived from six *B. napus* × *B. oleracea* interspecific crosses.

Study II: Evaluate oilseed *B. napus* germplasm collected from gene bank and different breeding programs for earliness of flowering under a short-day condition (10 h and 18 °C temperature) for validation of the QTL alleles identified in Study I.

Study III: Develop near-isogenic lines of two flowering time QTL which has been introgressed from *B. oleracea* into *B. napus*.

Chapter 2

Identification of QTL for days to flowering under a short-day condition by using *Brassica napus* lines derived from *B. napus* × *B. oleracea* interspecific crosses

2.1 Introduction

The mustard family Brassicaceae includes more than 3,000 plant species which can be found predominantly on the northern side of the equator (Canola council of Canada, 2017). Among these *Brassica napus*, which is commonly called rapeseed or canola, is one of the most important oilseed crops in the temperate regions.

Days to flowering time plays an important role in adaptation of a crop in certain regions. In spring growth habit plants, photoperiod plays an important role for initiation of flowering. Plants can be categorised into three groups on the basis of their response to day length, and this includes: (i) long-day plants, which usually flower when the day length exceeds a critical threshold of about 16 h light/8 h dark, (ii) short-day plants, which flower when day length drops below a critical length of about 8 h light/16 h dark, and (iii) day neutral plants, in which flowering is not influenced by day length (for review, see Jung and Miller 2009 and Andres and Coupland 2012). *B. napus* is considered as a long-day plant and the spring type of this crop is grown in Canada in summer under a day-length of about 15-17 h prior to flowering; however, the minimum optimum photoperiod for this crop is about 18 h (King and Kondra, 1986; Robertson et al. 2002). Crop production time of spring canola in Canada is about four months; delayed maturity in this crop or early snowfall often affects its production. Spring oilseed *B. napus* is also grown in a limited acreage in Indian sub-continent where day length during the crop growing season is about 12 h. In this region, the crop usually requires a couple of months longer time to harvest; therefore, this crop

does not fit well in the cropping system of this region. Therefore, identification of the genes capable of inducing flowering under a short-day condition is important for the improvement of this crop for growing in temperate regions as well as for expansion of its cultivation under a short-day condition.

To date, several studies have been conducted to identify the genes or the genomic regions affecting days to flowering in *B. napus*. A quantitative trait locus (QTL) is a genomic region that influence a phenotypic trait, and QTL analysis is a statistical method which correlates the observed phenotype of a trait to genotype to understand the genetic basis of phenotypic variation for the quantitative traits (Kearsey 1998). A large number of QTL mapping studies conducted in *Brassica* on flowering time focused on the identification of the genes involved in vernalization-responsive flowering or QTL affecting days to flowering under a long-day condition; not much research has been conducted to identify QTL affecting days to flowering under a short-day condition. For example, Cai et al. (2008), by using a DH population of *B. napus*, reported three main effect QTL and 5 pairs of epistatic QTL affecting days to flowering under short-day and long-day conditions. Nelson et al. (2014) reported three QTL affecting days to flowering under both long- and short-day conditions in *B. napus*. Rahman et al. (2011) reported that the C genome of *B. oleracea* var. *alboglabra* carry alleles that can be utilized for the improvement of earliness of flowering in *B. napus,* and mapped (Rahman et al. 2017, 2018) a QTL on C1 regulating flowering without being affected by photoperiod and a QTL on C9 affecting flowering under a short-day condition. The species *B. oleracea* exhibits wide genetic as well as morphological diversity (Nikzad 2020). Therefore, it is probable that the other varieties of *B. oleracea* also carry alleles which are capable of inducing flowering in *B. napus* under a short-day condition.

Different approaches have been applied for molecular mapping and identification of QTL of a trait. Association mapping or linkage disequilibrium (LD) mapping is one of the important methods used to establish the relationship between a marker and a trait (Mackay and Powell, 2007). This method generally uses a large natural population, which capture the recombination events occurred in an extended period of time, and, thus, eliminates the drawback of the use of a bi-parental population which include only a limited amount of genetic variation (Flint-Garcia et al. 2003). Various populations such as diverse germplasm of natural population, homozygous lines from multiple crosses (Cockram and Mackay 2018) as well as from bi-parental cross (Li et al. 2016) have been used for association mapping. These types of populations need to be genotyped once; however, they can be phenotyped multiple times for multiple traits, and thus makes this efficient and cost-effective method for analyzing multiple traits over multiple environments. The success of association mapping relies on the quality of phenotypic data, population size and the occurrence of linkage disequilibrium between marker and trait (Flint-Garcia et al. 2005). Genome wide association study (GWAS) was first initiated to understand the genetic basis of common human disorders (Ozaki et al. 2002). However, rapid advances in sequencing technologies and SNP genotyping have enabled dissection of complex traits of agricultural crops by using this technique. Annotation of the crop genomes allows precise identification of the location of the causal gene. Among the different SNP detection techniques, the tunable genotype by sequencing (tGBS) technique is an important one. tGBS is an improved version of conventional GBS technique; this technique generates a greater number of reads per site. In this, two restriction enzymes are used while a single restriction enzyme is used in conventional GBS technique. Also, instead of ligating adapters to the digested ends, two single-stranded oligos are attached to 3' and 5' overhangs, and this simplifies the process of creating libraries for sequencing (Ott et al. 2017).

This technique also eliminates some of the drawbacks associated with conventional GBS technique, such as error rate and missing data, and detects SNPs in the genome more accurately, especially for the heterozygous sites which is difficult when using many other methods (Ott et al. 2017).

Thus, the objective of this study was to identify the QTL controlling days to flowering under a short-day condition by using an inbred population carrying genome contents introgressed from different varieties of *B. oleracea*, and SNP markers obtained by using tGBS technique.

2.2 Material and methods

2.2.1 Plant materials

A total of 184 advanced generation inbred *B. napus* lines derived from six *B. napus* × *B. oleracea* interspecific crosses involving one *B. napus* line A04-73NA (zero erucic acid in oil and low glucosinolates in seed meal) and six *B. oleracea* accessions (high erucic acid in oil and high glucosinolates in seed meal), viz. *B. oleracea* var. *alboglabra* line NRC-PBI, var. *botrytis* cv. BARI cauliflower, var. *capitata* cvs. Badger Shipper, Bindsachsener and Balbro, var. *italica* cv. Premium Crop, were used in this study (Appendix 2-1). In an earlier study, Rahman et al. (2011, 2017, 2018) demonstrated that the diploid species *B. oleracea*, despite it flower later than spring *B. napus*, carry alleles which can promote flowering in *B. napus* under a short-day condition. Therefore, the inbred lines derived from these six interspecific crosses were used in this study to identify QTL affecting days to flowering under a short-day condition. The detail of the development of the inbred lines from these interspecific crosses is reported elsewhere (Iftikhar et al. 2018; Nikzad et al. 2019). In brief, the *B. napus* line A04-73NA was used as female parent and the six *B. oleracea* accessions were used as male parent. *In vitro* ovule culture technique, described

by Bennett et al. (2008), was used to produce the F_1 plants, and the F_1 plants were self-pollinated to produce F_2 as well as were backcrossed to A04-73NA to produce backcross populations. The F_2 and BC_1 populations were subjected to pedigree breeding with selection for canola quality traits (zero erucic acid in oil and low glucosinolate in seed meal) and spring growth habit from where the F_{11} and BC_1F_{10} generation inbred *B. napus* lines were developed.

2.2.2 Phenotyping the population for days to flowering

The above mentioned 184 F_{11} and BC_1F_{10} lines and the *B. napus* parent A04-73NA were grown in a growth chamber set at 10 h photoperiod and 18°C constant temperature with photosynthetic flux density of 450 µE (mV) m^2 s^{-1} for evaluation of days to flowering. The plants were grown in 32-cell tray with pot size of 7 cm × 7 cm × 9 cm (L × W × D) filled with Sunshine Professional Growing Mix (Sunshine Horticulture, 15831 N.E. Bellevue, USA). Due to the size of the experimental population and limitation in the size of growth chamber, 2 to 3 plants of each line were grown, and the average value of these plants was used for statistical analysis. The experiment was repeated five times which constituted the number of replications. Data on days to flowering was recorded at first open flower stage. To avoid shading of a plant by the other, the lower leaves were removed at rosette stage, and the experiments were monitored on a daily basis.

Normally, most of the spring canola lines in a growth chamber at 14 or 18 h photoperiod with 18°C constant temperature flowers within 30 to 40 days; however, at 10 h photoperiod and 18°C temperature, some lines require about 100 days to flower (Rahman et al. 2018). Therefore, in the present study, the experiments were terminated at 160 days after seeding. At this stage, the non-flowering plants were recorded as being flowered at some time after 160 days of seeding.

The inbred *B. napus* lines derived from the six *B. napus* × *B. oleracea* interspecific crosses were also evaluated in 10 field trials in Alberta by Nikzad et al. (2019) for days to flowering and

other agronomic and seed quality traits. This days to flowering data was also used in this study to understand the effect of photoperiod on days to flowering.

2.2.3 Genotyping

2.2.3.1 ***Genomic DNA extraction*****:** Leaf sample of the inbred lines were collected at 25-30 days after seeding in 2 ml micro centrifuge tubes and were stored at -80 °C until use. The genomic DNA was extracted following modified SDS DNA extraction protocol. For this, leaves were grinded into fine powder by crushing with Qiagen tissue-lyzer, and 600 µl pre-warmed (65 °C) CTAB buffer was added into each micro centrifuge tube. The samples were kept at 65 °C for one hour with intermittent gentle mixing after every 15 min. The emulsion was cooled to room temperature for 15 min after the incubation and 600 µl chloroform was added to each, and the samples were centrifuged at 8,000 rpm for 10 min and the supernatant was transferred to a fresh 1.5 ml micro centrifuge tube. An equal volume (800 µl) of chilled isopropanol was added to the centrifuge tubes for precipitation of the DNA, and the tubes were kept at 4 °C for 60 min, and the precipitated DNA was spooled out in a fresh Eppendorf tube of 1.5 ml size. The residual debris was removed by washing the DNA pellet twice with 70% ethanol. The DNA was allowed to air dry at 37 °C for an hour and dissolved in 100 µl 1X TE buffer and stored at -20 °C until use.

2.2.3.2 ***DNA quantification*****:** The DNA quantity and quality was accessed by using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware, USA) and the concentration was adjusted to 25 ng/µl for polymerase chain reaction (PCR).

2.2.3.3 ***SNP Genotyping***: The inbred population was genotyped by Data2Bio (Ames, IW, USA) using tGBS technique; the detail of this technique and SNP detection is reported by Nikzad (2020). In brief, genomic DNA was quantified after extraction, and digested by two restriction enzymes (NSpI and BfuCI/Sau3AI) which creates 3’ and 5’ overhanging ends. Ligation of two single

stranded oligos (sample specific barcode and a universal oligo) was done to create tGBS libraries for sequencing. Trimming of the raw sequence data was done to remove internal barcode and low quality reads and the trimmed sequence data was aligned to reference *B. napus* genome (GCA_000751015.1; Chalhoub et al. 2014) to carry out SNP calling (Fig. 2-1).

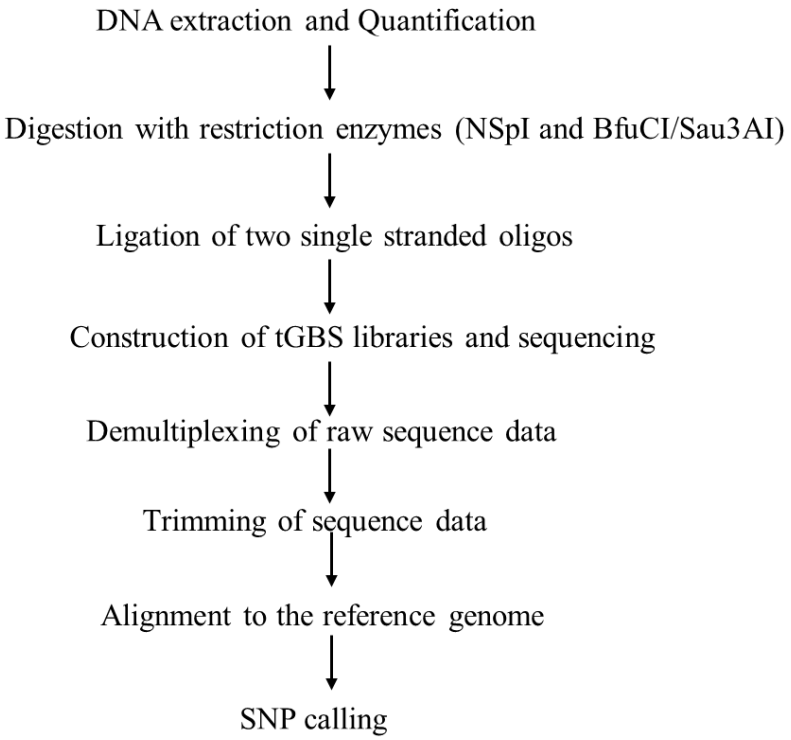


Figure 2-1. Schematic representation of tunable genotyping by sequencing (tGBS) technique

2.2.3.4 Designing SSR markers and genotyping

Simple sequence repeat (SSR) markers were designed from the C9 chromosome, which was found to be associated with days to flowering, by using *Brassica* genome sequence information available in NCBI (www.ncbi.nlm.nih.gov/nuccore/NC_027767.2) and Genoscope (www.genoscope.cns.fr/brassicanapus/) and using primer designing tool of WEBSAT (bioinfo.inf.ufg.br/websat/).

2.2.3.5 ***Polymerase Chain Reaction (PCR)*****:** PCR was carried out in a total volume of 12 µl, where each PCR reaction mixture comprised of 50 ng DNA (25 ng/µl × 2), 1.25 µl of 10X PCR buffer, 0.125 µl of 20 mM dNTPs, 1.25 µl of 50 mM $MgCl_2$, 0.5 µl of each of 5 µM forward and reverse primer, 6.225 µl of nuclease free water and 0.15 µl (0.75U) of GoTaq DNA polymerase enzyme. Amplification reaction was performed by using PCR profile which included three stages. The first stage included one initial denaturation cycle at 95 ℃ for 5 minutes. The second stage comprised of 95 ℃ for 30 seconds, 53 ℃ for 30 seconds and 72 ℃ for 45 seconds for 35 cycles. The third stage included the final extension at 72 ℃ for 10 minutes.

2.2.3.6 ***Gel electrophoresis separation of amplicons*****:** The genotyping of the PCR amplified products was done by electrophoresing on agarose gel (2.5%). The 2.5 % agarose gels were prepared by using 300 ml of 1 X TBE buffer, 7.5 grams of agarose (Invitrogen, Carlsbad, CA) and 9 µl SYBR safe. Each of the PCR amplified product was dissolved with loading dye and loaded into the agarose gel along with λ DNA of known concentration (400 ng/µl); electrophoresis was carried out in TBE buffer at 100 volts for one hour. Typhoon FLA 9500 scanner (GE Healthcare Bio-Sciences AB, Piscataway, NJ) was used to take gel image after electrophoresis.

For genotyping, in the first step, the SSR markers were tested on 10 most early and 10 most late flowering plants for polymorphism, and the polymorphic markers were used to genotype the whole mapping population. The genotypic data was used for single marker analysis for association of the markers with the trait. Furthermore, the mapping population was partitioned for the marker alleles and the two groups were tested for significant difference for days to flowering to understand the association of the markers with the trait.

2.2.4 Statistical analysis

Least square mean (LSmean) values were calculated for each replication as well as for pooled data of all replications by using the software program R (R studio team, 2016). Other statistical analysis, such as simple mean and standard error were calculated in Microsoft Excel and Pearson's correlation coefficients were calculated by using models available in R v1.1.463.

2.2.5 Association mapping: Association mapping analysis was carried out by using Genomic Association and Prediction Integrated Tool (GAPIT) version 3 (Lipka et al., 2012) in software program R version 3.5.2 (R Core Team, 2018) for identification of flowering time QTL. For this, Principal Component Analysis (PCA) was performed using TASSEL (Bradbury et al. 2007) and the population structure based on PCA was used for association mapping in GAPIT. In GAPIT, different models, such as GLM, MLM, CMLM, FarmCPU and BLINK, can be used to carry out genome-wide association study (GWAS) (Wang and Zhang 2020). All these models were evaluated in this study for their ability to control the false positives and the false negatives on the basis of uniformity between the observed *p*-values and the expected *p*-values in quantile-quantile (Q-Q) plots. In addition to Q-Q plots, a series of Manhattan plots were also obtained as output, where the FarmCPU was found to give the best results and was used in this study.

FarmCPU analysis uses a modified version of MLM method, the MLMM (Multiple Loci Linear Mixed Model), which incorporates multiple markers simultaneously as covariates in a stepwise MLM to partially remove the confounding between testing markers and kinship. This analysis controls the false positives more precisely as compared to any other methods of GWAS by taking the associated markers as covariates (Liu et al. 2016), and the analysis was performed by using R version 1.1.463. The SNPs significantly associated with the trait were identified on the

basis of Bonferroni threshold level of 3.5. In addition to this, association mapping was also performed by using the software program TASSEL, and the significant SNPs identified from the analysis in FarmCPU were compared with the results from TASSEL for final selection of the SNPs to be associated with the trait. TASSEL uses different methods of association mapping including GLM, MLM and weighted-MLM; it is an important tool for association mapping and detection of linkage pattern at higher resolution (Bradbury et al. 2007).

2.2.6 *Single marker analysis*: Single marker analysis of the SSR genotype data was performed by using the software program QTL cartographer (Basten et al. 1994) and using regression effect of one marker at a time. The regression model used was y = b0 + b1x + e, where, b0 is the overall mean, b1 is the single marker effect and e is the residual effect.

2.3 Results

2.3.1 Phenotyping the population

The 184 advanced generation inbred *B. napus* lines derived from six *B. napus* × *B. oleracea* interspecific crosses were evaluated for days to flowering in a growth chamber in five experiments (five replications); therefore, the reliability of the phenotypic data was tested through correlation analysis of the data sets. Coefficient of correlations from all 10 pair-wise comparisons of the five experiments were positive (r = 0.94 to 0.98) and highly significant (p<0.001) suggesting that the inbred lines behaved similarly for this trait in the five experiments (Fig. 2-2).

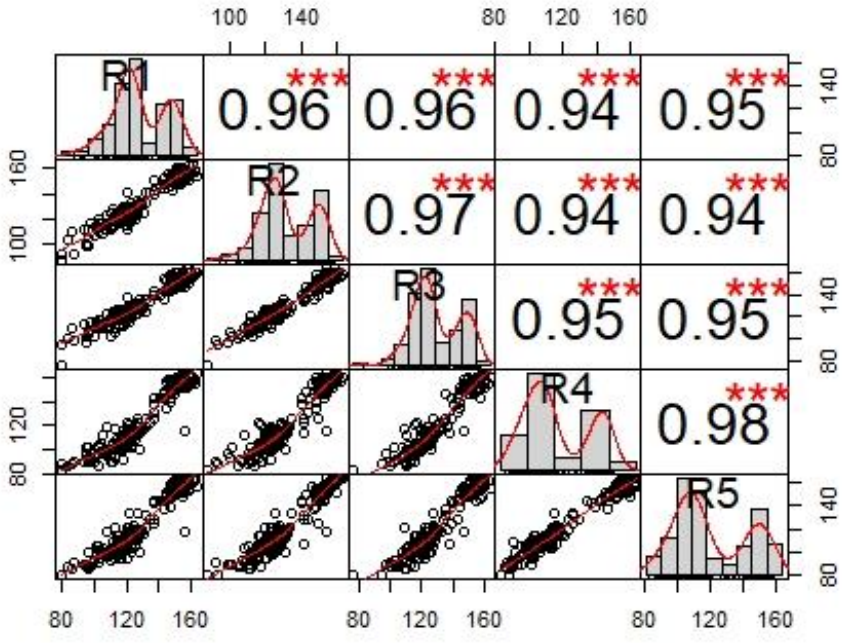


Figure 2-2. Correlation of days to flowering of the inbred lines, derived from six *Brassica napus* × *B. oleracea* interspecific crosses, in five experiments conducted in a growth chamber at 10 h photoperiod and 18 °C constant temperature conditions. R1 to R5 indicates the five experiments.

Of the total 184 lines, about 70% (129/184) lines flowered before termination of the experiment at 160 days after seeding. Among the inbred lines of the six crosses, all lines of the cross *B. napus* × *B. oleracea* var. *italica* broccoli flowered; however, 41% and 47% lines of the crosses involving var. *capitata* cvs. Badger Shipper and Balbro flowered within 160 days under this short-day condition (Fig. 2-3). In general, compared to the F_2-derived population, a greater proportion of the BC_1-derived lines flowered before termination of the experiment (Fig. 2-4).

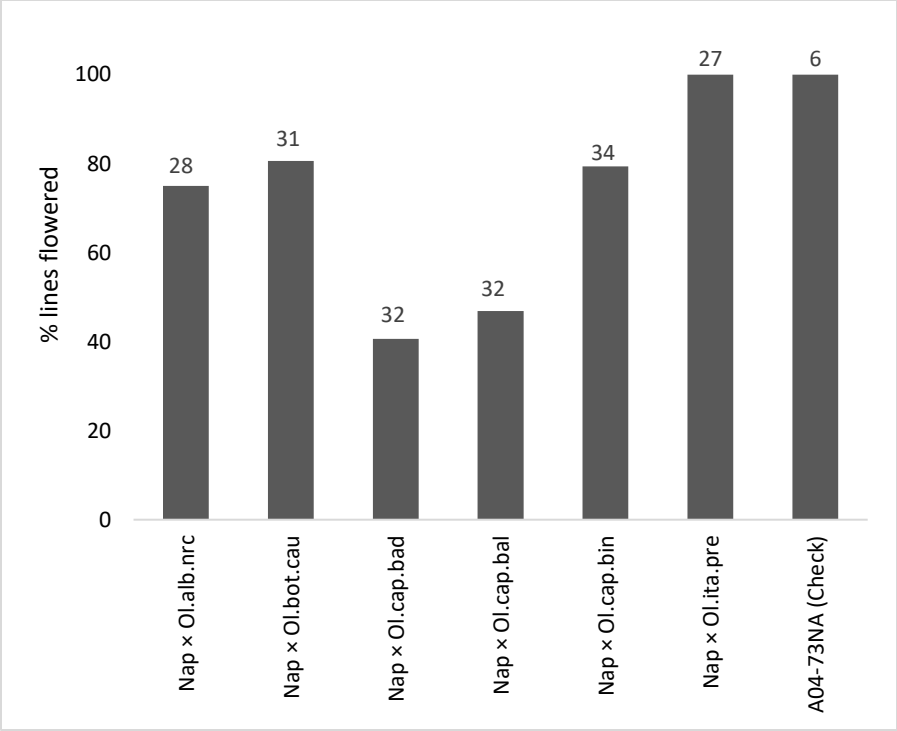


Figure 2-3. Frequency distribution of six inbred *Brassica napus* populations (pooled data of the lines derived from F_2 and from BC_1), derived from six *B. napus* × *B. oleracea* interspecific crosses, for the proportion of the lines flowered in a growth chamber at 10 h photoperiod and 18 °C constant temperature conditions. The number of inbred lines evaluated is shown on the top of the bar. Nap × Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Nap × Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1; Nap × Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Nap × Ol.cap.bal = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro; Nap × Ol.cap.bin = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener; Nap × Ol.ita.pre = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop.

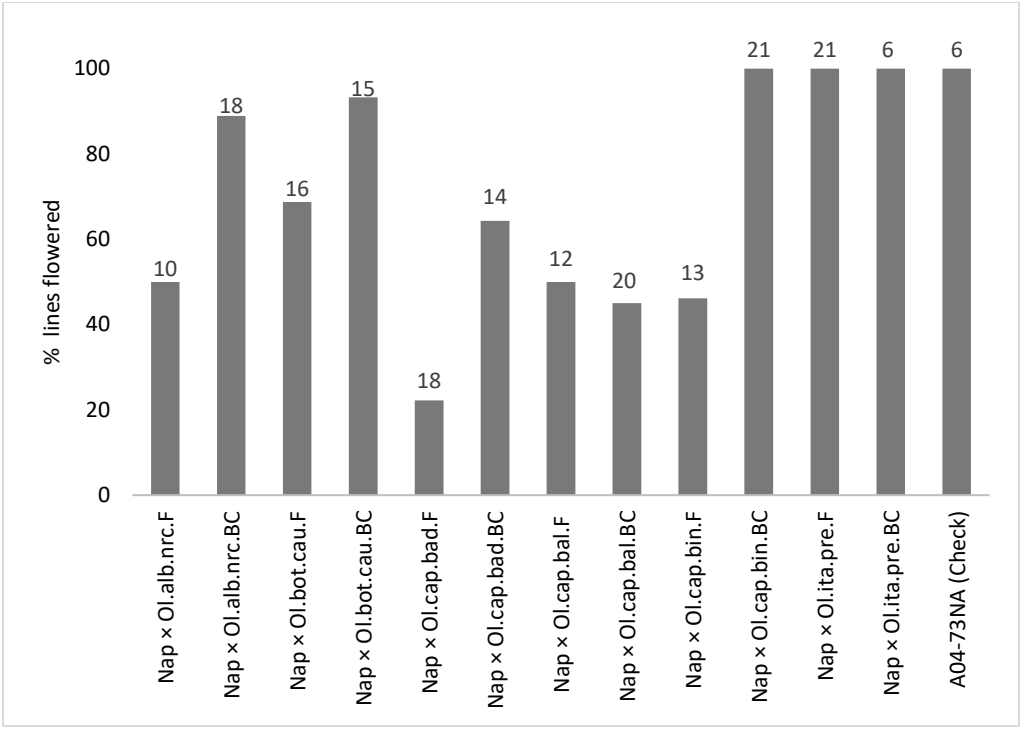


Figure 2-4. Frequency distribution of 12 inbred *Brassica napus* populations (six derived from F_2 and six derived from BC_1) derived from six *B. napus* × *B. oleracea* interspecific crosses for the proportion of the lines flowered in a growth chamber at 10 h photoperiod and 18 °C constant temperature conditions. The number of inbred lines evaluated is shown on the top of the bar. Nap × Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Nap × Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1; Nap × Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Nap × Ol.cap.bal = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro; Nap × Ol.cap.bin = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener; Nap × Ol.ita.pre = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop. F and BC indicates the lines derived from F_2, and BC_1, respectively.

Days to flowering in this inbred population showed a continuous variation ranging from 76 days to more than 160 days, where the *B. napus* parent took about 125 days to flower (Fig. 2-5, Appendix 2-3). Several inbred lines flowered earlier than the *B. napus* parent suggesting that alleles contributing to earliness of flowering under a short-day condition has been introgressed from *B. oleracea*. Of the 10 most early flowering lines, one derived from *B. napus* × *B. oleracea* var. *alboglabra* NRC-PBI cross and nine derived from *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener cross, and they were 5CA1300-363, 5CA1363-177, 5CA1682-128, 5CA1682-130,

5CA1682-133, 5CA1682-138, 5CA1682-140, 5CA1682-143, 5CA1682-150, 5CA1682-152 (Appendix 2-1).

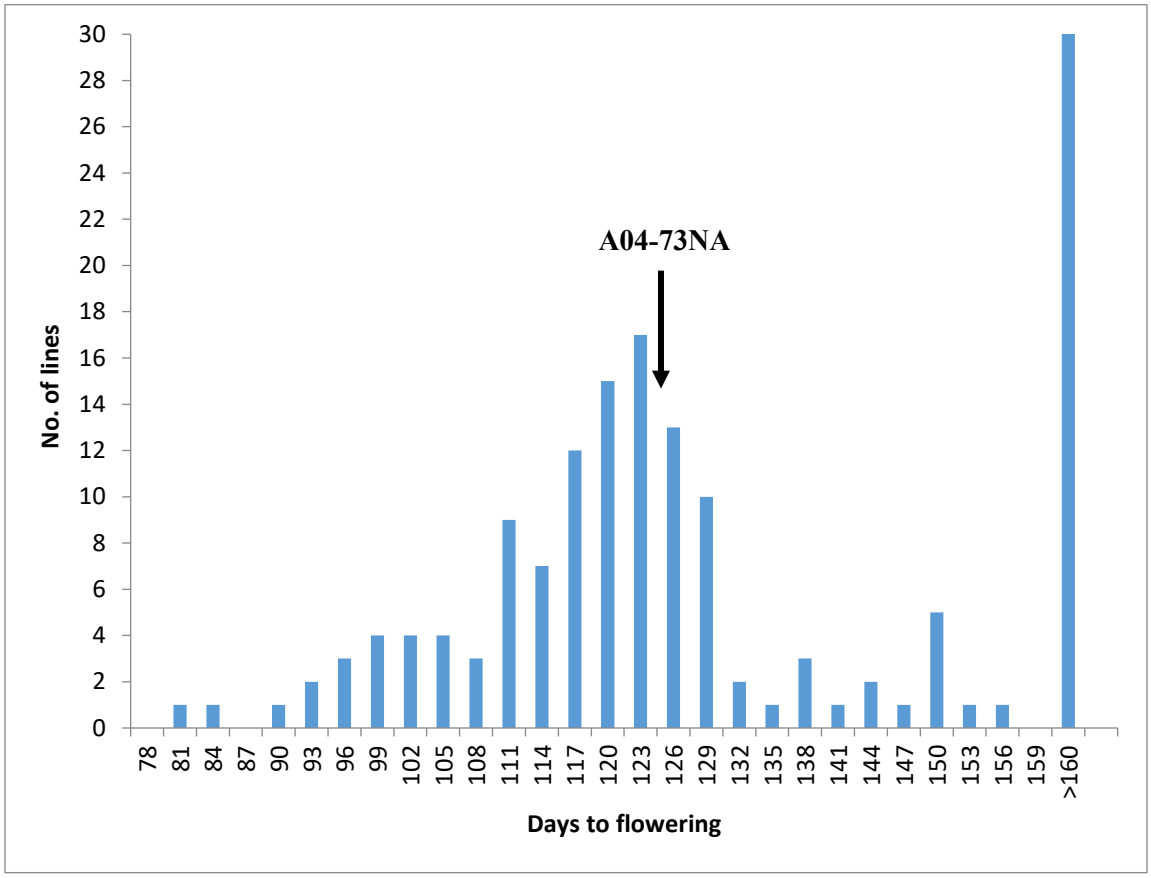


Figure 2-5. Frequency distribution of the *Brassica napus* inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses for days to flowering in a growth chamber at 10 h photoperiod and 18 °C constant temperature. LSmean values from five experiments presented. The *B. napus* parent A04-73NA took about 125 days to flower, which is indicated by vertical arrow.

Correlation between data from the five growth chamber experiments and data from 10 field trials conducted in Alberta (Nikzad et al. 2019) was calculated to understand the effect of photoperiod on days to flowering in this population. The coefficient of correlation values varied from r = 0.14 to 0.53 while using data from the individual experiments, r = 0.42 to 0.48 while using LSmean data of the 10 field trials and individual data of from the five growth chamber experiments, r = 0.18 to 0.52 while using LSmean data of the five growth chamber experiments

and individual data from the 10 field trials, and $r = 0.46$ based on LSmean data from the field trials and growth chamber experiments (Table 2-1). Thus, the occurrence of a weak to moderate correlation between days to flowering under growth chamber and field conditions suggests that the inbred lines did not behave similarly under these two growth conditions. This indicates that a different set of genes or interaction of the flowering genes with photoperiod as well as other environmental factors played an important role in the regulation of this trait under short-day condition.

Table 2-1. Coefficient of correlation of days to flowering of the inbred lines derived from *B. napus* × *B. oleracea* interspecific crosses under field and short-day (10 h) photoperiod (18 °C constant temperature) conditions.

Trials[1]	R1[2]	R2	R3	R4	R5	LSmean
2016ERS1	0.36	0.34	0.35	0.37	0.38	0.37
2016ERS2	0.28	0.29	0.30	0.30	0.32	0.31
2016KIL	0.30	0.25	0.25	0.28	0.29	0.29
2016STA	0.27	0.27	0.26	0.25	0.27	0.27
2017ERS1	0.42	0.39	0.39	0.43	0.44	0.43
2017ERS2	0.42	0.41	0.41	0.43	0.44	0.44
2017STA1	0.21	0.23	0.22	0.24	0.26	0.24
2017STA2	0.15	0.16	0.14	0.19	0.21	0.18
2018ERS1	0.49	0.49	0.48	0.52	0.53	0.52
2018ERS2	0.41	0.43	0.42	0.46	0.48	0.46
LSmean	0.43	0.43	0.42	0.46	0.48	0.46

[1] Ten field trials conducted in Alberta (Nikzad et al. 2019); [2] R1 to R5 are the five experiments conducted in growth chamber (10 h photoperiod)

2.3.2 Association mapping

Five different models in GAPIT, viz. GLM, MLM, CMLM, FarmCPU and BLINK, were evaluated through Q-Q plot analysis to determine if the models control false positives and false negative and, thus, to choose the correct model for association mapping. As evident from the Q-Q plots based on the data from five replications and LSmean data, the *p*-values for GLM and MLM (the GLM line completely overlapped the MLM line in the Q-Q plots) were highly inflated and showed the

greatest deviations from the expected *p*-values; therefore, these models were not chosen (Fig. 2-6). The Q-Q plots from BLINK were close to 1:1 line but showed deviation from the expected *p*-values with greater deviation in the tail as compared to FarmCPU. In case of CMLM model, the observed *p*-values were close to the expected *p*-values and followed the 1:1 straight line without any deviated tail. FarmCPU Q-Q plots were close to the 1:1 straight line with sharp upward tail from expected *p*-values indicating that this model was found to be the best for explaining the associations and also for controlling false positives and false negatives and was used for further analysis for identification of the QTL and SNP markers.

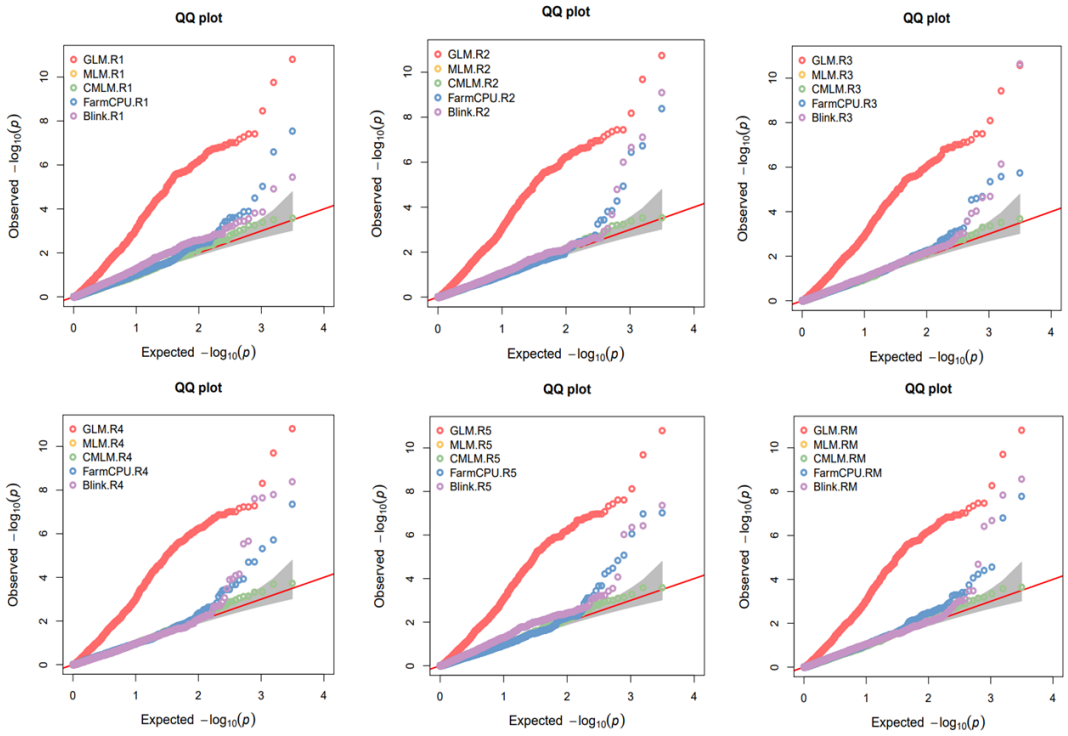


Figure 2-6. Comparison of Quantile-Quantile (Q-Q) plots obtained from GAPIT analysis of the inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses by using different models (GLM, MLM, CMLM, FarmCPU, and BLINK). After the model names, R1 to R5 indicates the five experiments conducted in the growth chamber, and RM indicates the LSmeans of five replications.

Association mapping identified three QTL on chromosomes C1, C5 and C9 with probability above Bonferroni-corrected significance threshold $\log_{10}(1/3.12 \times 10^{-4}) = 3.5$ (Fig. 2-

7; Table 2-2). These three QTL could be identified in individual trials as well as based on LSmean data of the five trials. The SNP markers of the C1 QTL are located at 43.1 Mb position, C5 at 22.5 – 39.2 Mb position and C9 at 24.6 – 39.7 Mb position (Table 2-2). Among these, the majority of the SNPs of C1 are located at about 43 Mb position, C5 SNPs at 30 Mb and C9 SNPs at 29-32 Mb positions (Fig. 2-8). One to two SNP markers from the chromosomes A1, A6, C2, C6 and C7 could also be detected with probability above Bonferroni-corrected significance threshold $\log_{10}$ (1/5.97 $\times$ 10^{-4}) = 3.2; however, with this limited number of markers they cannot be considered as reliable QTL.

Table 2-2. List of SNP markers associated with days to flowering identified at 10 h photoperiod and 18 ºC constant temperature through genome-wide association mapping by using 184 inbred lines, derived from six *Brassica napus* × *B. oleracea* interspecific crosses, and SNP data obtained from tunable genotype-by-sequencing (tGBS) technique.

SNP	LG[1]	Alleles	Position	*p*-value	Lod/-log10(p)	Effect[2]
SNP116996	11	G/T	43198577	5.41× 10-5	4.266793	-11.0406
SNP116997	11	C/T	43198580	0.000143	3.846021	-10.661
SNP116998	11	T/C	43198605	0.000242	3.616904	-10.5362
SNP193534	15	A/C	22505432	0.000312	3.506311	8.923782
SNP193712	15	C/T	23083393	0.00012	3.92076	9.519254
SNP196126	15	G/A	28650014	0.000238	3.623169	9.286394
SNP196199	15	A/C	28831071	1.87 × 10-5	4.729046	-10.8479
SNP196344	15	G/T	29181038	0.000186	3.73004	-9.45156
SNP196356	15	G/A	29181190	0.000193	3.713886	9.359087
SNP196745	15	C/T	30654857	0.000251	3.600095	10.25194
SNP196746	15	C/T	30654865	0.000251	3.600095	10.25194
SNP196748	15	T/C	30654885	0.000158	3.802278	-10.745
SNP196749	15	G/A	30654890	0.000251	3.600095	-10.2519
SNP196751	15	G/A	30654907	0.000251	3.600095	-10.2519
SNP196814	15	C/G	30796374	0.000224	3.649225	10.2834
SNP196815	15	A/G	30796387	0.000224	3.649225	10.2834
SNP196816	15	A/C	30796390	0.000224	3.649225	10.2834
SNP196819	15	A/T	30796417	0.000258	3.588037	9.030286
SNP196821	15	G/C	30796451	0.000224	3.649225	-10.2834
SNP196822	15	C/G	30796463	0.000224	3.649225	10.2834
SNP196824	15	G/A	30796497	0.000224	3.649225	-10.2834
SNP196903	15	A/G	30911303	0.00022	3.658486	-12.795
SNP196907	15	T/G	30911328	0.000215	3.668264	-10.1047

SNP196960	15	T/C	31011319	5.66 × 10-5	4.246856	-16.0539
SNP196961	15	G/C	31011366	5.66 × 10-5	4.246856	-16.0539
SNP196964	15	A/C	31011381	5.66 × 10-5	4.246856	16.05391
SNP197552	15	G/T	32621065	0.000234	3.629992	10.60277
SNP198031	15	C/T	33852644	0.00031	3.508844	9.04665
SNP199038	15	G/A	36402833	0.000251	3.599858	-10.2767
SNP200086	15	G/A	39223424	0.000239	3.621234	-9.62748
SNP200090	15	A/G	39223452	0.000134	3.871497	-9.96607
SNP200092	15	A/G	39223474	0.000134	3.871497	-9.96607
SNP200097	15	T/A	39223499	0.000156	3.807747	-16.11
SNP200100	15	A/G	39223523	0.000301	3.521514	-9.19391
SNP266665	19	G/T	24619729	0.000294	3.53107	8.721721
SNP267708	19	C/T	28192578	9.96 × 10-5	4.001938	10.53195
SNP267709	19	A/G	28192602	9.96 × 10-5	4.001938	10.53195
SNP268001	19	C/T	29013262	7.90 × 10-5	4.102451	9.470329
SNP268006	19	A/G	29013317	7.90 × 10-5	4.102451	9.470329
SNP268007	19	A/T	29013344	7.90 × 10-5	4.102451	9.470329
SNP268009	19	T/C	29013349	9.93 × 10-5	4.003038	8.874301
SNP268011	19	C/T	29013354	7.90 × 10-5	4.102451	9.470329
SNP268012	19	G/A	29013393	7.90 × 10-5	4.102451	9.470329
SNP268063	19	A/C	29145985	1.27 × 10-5	4.895522	10.44396
SNP268064	19	C/A	29146031	1.86 × 10-5	4.729866	10.21585
SNP268188	19	T/C	29401536	2.96 × 10-5	4.529316	9.837131
SNP268190	19	C/T	29401552	2.96 × 10-5	4.529316	9.837131
SNP268192	19	A/G	29401582	2.96 × 10-5	4.529316	9.837131
SNP268193	19	A/G	29401607	6.69 × 10-6	5.17441	10.75455
SNP268296	19	C/T	29552449	1.09 × 10-5	4.964111	-12.1911
SNP268820	19	C/T	30527412	7.35 × 10-5	4.13355	9.765192
SNP268824	19	T/C	30527465	7.35 × 10-5	4.13355	9.765192
SNP268825	19	C/A	30527478	7.35 × 10-5	4.13355	9.765192
SNP268910	19	T/A	30665429	6.12 × 10-5	4.213571	9.776782
SNP268913	19	A/G	30665459	6.12 × 10-5	4.213571	9.776782
SNP268938	19	G/A	30689977	0.000227	3.643032	-16.9702
SNP269050	19	T/A	30910167	0.000132	3.880371	9.313294
SNP269623	19	A/C	32088997	4.52 × 10-6	5.344384	11.01767
SNP269626	19	C/A	32089090	1.37 × 10-5	4.862094	10.8901
SNP269629	19	A/T	32089125	1.37 × 10-5	4.862094	10.8901
SNP269703	19	T/G	32231038	4.19 × 10-5	4.377678	15.69237
SNP269704	19	T/C	32231052	5.21 × 10-5	4.283572	16.83006
SNP269705	19	C/T	32231070	6.48 × 10-6	5.188243	10.75475
SNP270496	19	C/T	33519478	0.000184	3.734486	9.704797

SNP270497	19	T/C	33519479	0.000264	3.577623	9.639169
SNP270498	19	A/G	33519484	0.000152	3.816949	9.672632
SNP270499	19	T/C	33519521	0.000152	3.816949	9.672632
SNP270882	19	G/T	34460281	0.000113	3.94624	12.16642
SNP271158	19	A/G	35320092	2.42 × 10-5	4.617018	10.72826
SNP271313	19	A/G	35845457	0.000134	3.871289	8.400863
SNP271482	19	C/T	36373356	3.73 × 10-5	4.428861	10.00094
SNP271532	19	C/G	36461884	0.000142	3.846624	-9.04378
SNP271533	19	G/T	36461889	0.000142	3.846624	-9.04378
SNP272643	19	G/C	39676185	0.000284	3.547014	8.060851

[1] Linkage group; [2] Allelic effect (this could be positive and negative)

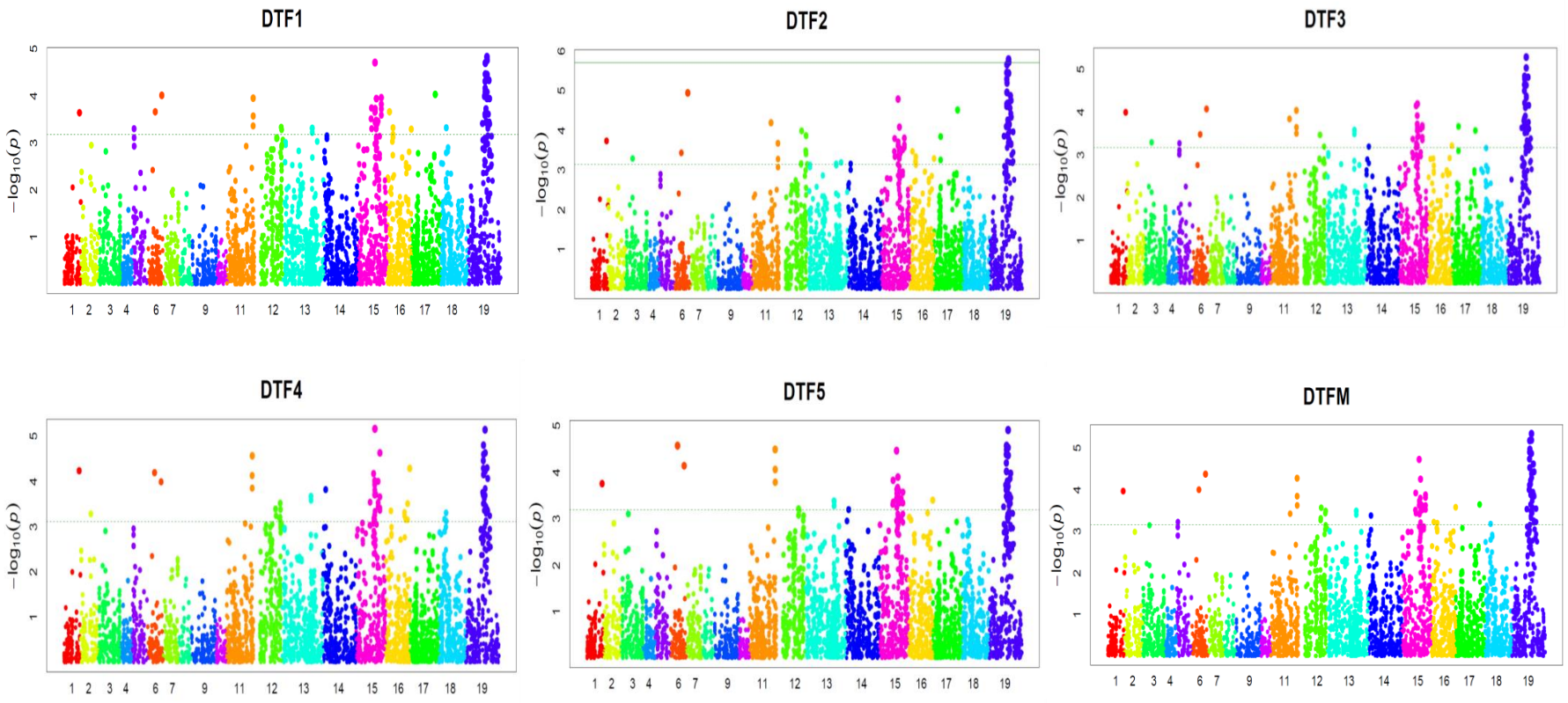


Figure 2-7. Manhattan plots of genome-wide association analysis of days to flowering by using inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses and SNP data obtained from tunable genotype-by-sequencing (tGBS) technique. Analysis carried out using phenotypic data from five experiments conducted in a growth chamber under 10 h photoperiod and 18 °C constant temperature conditions and LSmeans data of the five experiments. The horizontal solid line indicates the Bonferroni-corrected significance threshold $\log_{10}(1/5.97 \times 10^{-4}) = 3.2$. DTF1 to DTF5 indicates the five replications of the growth chamber experiment and DTFM indicates the LSmeans of the five replications.

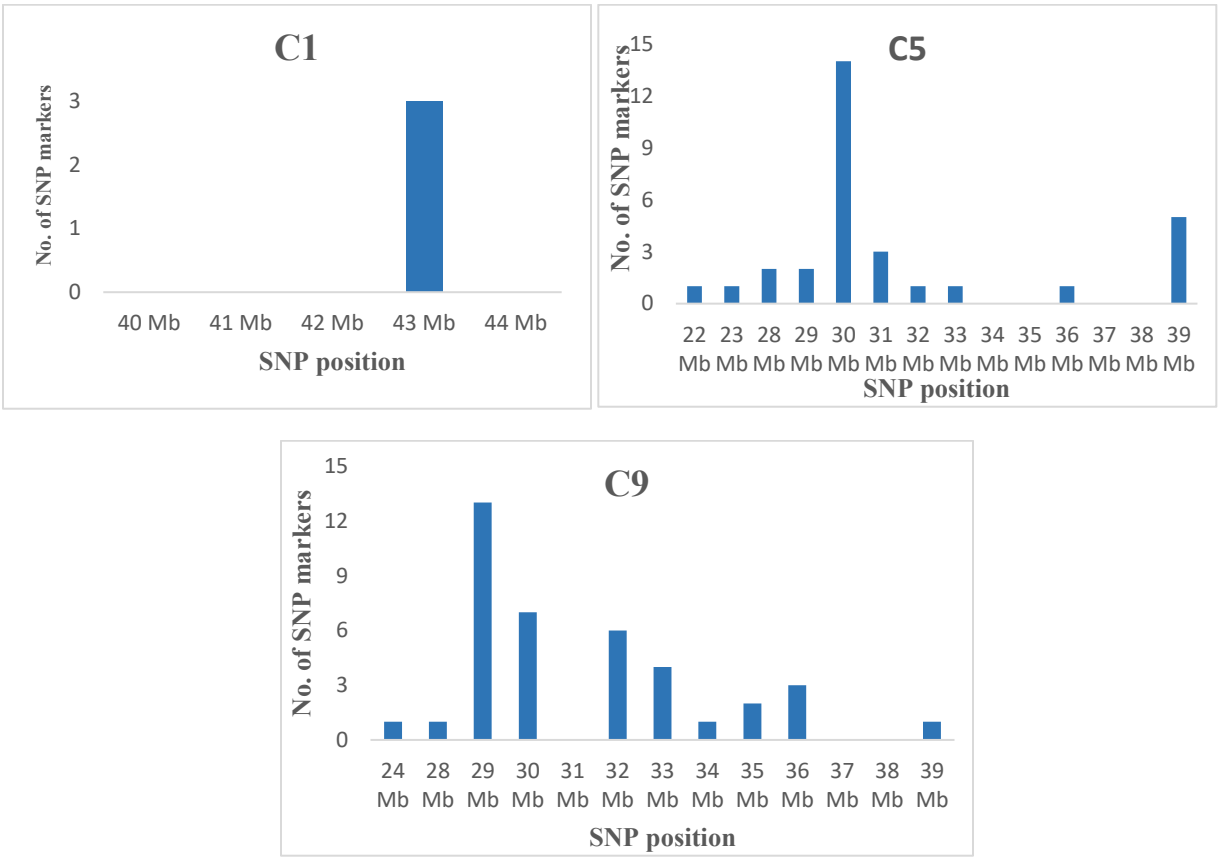


Figure 2-8. Number and position of the SNP markers identified for the QTL detected on chromosome C1, C5 and C9 of *B. napus* for days to flowering under a short-day condition (10 h photoperiod and 18 °C constant temperature).

2.3.3 SSR marker analysis

A total of 15 SSR markers were designed from the C9 QTL region of which two markers, 4928 and 4929 (Appendix 2-2), showed polymorphism in the inbred population. Single marker analysis also showed an association of the SSR markers from the C9 QTL region to be associated with days to flowering under short-day condition (Table 2-3). The C9 QTL markers SSR_4928 and SSR_4929 located at 30128941 – 30129189 bp and 31681068 – 31681420 bp positions, respectively. The effect of these markers varied from about 8-9 days, estimated through single marker analysis (Table 2-3).

Table 2-3. Single marker analysis of the association of the SSR markers from the C9 QTL region with days to flowering in an inbred *Brassica napus* population derived from six *Brassica napus* × *B. oleracea* interspecific crosses and grown at 10 h photoperiod and 18 °C constant temperature condition.

Marker	LG[1]	Genome position (bp)	b0[2]	b1[3]	-2ln(L0/L1)[4]	F(1,n-2)	pr(F)
SSR_4928	C9	30128941 – 30129189	127.91	8.80	51.575	62.522	0.000000001****
SSR_4929	C9	31681068 – 31681420	131.14	8.45	33.162	37.262	0.000000012****

[1] LG = Linkage group; [2] b0 = overall mean; [3] b1 = effect of the allele; [4] -2ln(L0/L1) = likelihood Ratio test statistic

To further confirm the effect of the C9 QTL on days to flowering, the inbred population was partitioned into two groups based on the alleles of the SSR markers and *t*-test was done for significant difference between these groups for days to flowering (Table 2-4). The marker 4928 produced a band of 250 bp size in late flowering lines but a band of 300 bp size in the early flowering lines. The mean days to flowering of the lines carrying the 300 bp band was 112.47 ± 1.98 (range: 83 to 123 days) and the lines carrying the 250 bp band it was 136.90 ± 0.99 (range: 124 to 155 days); these two groups were statistically different for days to flowering (t = -8.135, p < 0.001) (Table 2-4). In case of the marker 4929, it produced 250 bp size band in late flowering lines but a 250 bp and 350 bp size bands in the early flowering lines. Thus, the marker allele 250 bp was found to be monomorphic, while the marker allele 350 was found to be polymorphic in this population. The mean days to flowering of the lines carrying the 350 bp band was 124.38 ± 1.16 (range: 83 to 140 days) and the lines lacking this marker allele was 147.53 ± 0.82 (range: 140.3 to 155 days); these two groups were also statistically different for days to flowering (t = -3.024, p <0.001) (Table 2-4).

Table 2-4. *t-t*est for significant difference between the inbred *Brassica napus* lines, derived from six *Brassica napus* × *B. oleracea* interspecific crosses, partitioned based on SSR marker alleles, for days to flowering (DTF) at 10 h photoperiod and 18 °C constant temperature condition.

Chromosome (marker start to end position, bp)	Marker name	Early flowering lines			Late flowering lines			*t*-test	
		Allele size (bp)	DTF range	DTF Mean ± SE	Allele size (bp)	DTF range	DTF Mean ± SE	*t*-value	*p*-value
C9 (30128941 – 30129189)	4928	300	83 - 123	112.47 ± 1.98	250	124 - 155	136.90 ± 0.99	- 8.135	< 0.001
C9 (31681068 – 31681420)	4929	250, 350	83 - 140	124.38 ± 1.16	250	140.3 - 155	147.53 ± 0.82	- 3.024	< 0.001

2.3.4 Identification of candidate genes

Two genes, *zinc finger protein CONSTANS-LIKE-12-like* and *ELF3* (*EARLY FLOWERING3) like protein 2*, were found in C1 QTL region to be associated with days to flowering. The gene *zinc finger protein CONSTANS-like-12-like* (gene ID106374859) is located at 42,786,450 bp (start position) to 42,789,007 bp (end position) and the gene *ELF3 like protein 2* (gene ID106376922) to be located at 43,352,821 bp (start position) to 43,355,581 bp (end position) positions. In case of the C5, three genes to be playing a role in flowering time were found in this QTL region. This includes *AP2-like (APETALA2-like) ethylene-responsive transcription factor PLT2* (27,255,609 to 27,261733 bp, gene ID 111206225), *zinc finger CONSTANS-LIKE 15-like* (33,781,290 to 33,783,327 bp, gene ID 106416779), and *COP1 (CONSTITUTIVE PHOTOMORPHOGENIC1) interating protein 7* (37,990,345 to 37,996,811 bp, gene ID 106452244). In case of the QTL region of C9, a single gene, *COP1 interating protein 7* (28,804,639 to 28,806,327 bp, gene ID 106431586) was found.

Table 2-5. List of candidate genes, which probably play a role in days to flowering, identified in the QTL regions of the chromosomes C1, C5 and C9 of *Brassica napus*.

Chromosome	GeneID	Description	Other designations	Genomic nucleotide accession version	Start position on the genomic accession	End position on the genomic accession	Exon count
C1	106374859	zinc finger protein CONSTANS-LIKE 12-like	zinc finger protein CONSTANS-LIKE 12-like	NC_027767.2	42786450	42789007	6
C1	106376922	ELF3-like protein 2	ELF3-like protein 2	NC_027767.2	43352821	43355581	5
C5	111206225	AP2-like ethylene-responsive transcription factor PLT2	AP2-like ethylene-responsive transcription factor PLT2	NC_027771.2	27255609	27261733	9
C5	106416779	zinc finger protein CONSTANS-LIKE 15-like		NC_027771.2	33781290	33783327	0
C5	106452244	COP1-interacting protein 7	COP1-interacting protein 7	NC_027771.2	37990345	37996811	12
C9	106431586	COP1-interacting protein 7-like	COP1-interacting protein 7-like	NC_027775.2	28804639	28806327	7

2.4 Discussion

Quantitative trait loci (QTL) analysis has been carried out by several researchers for mapping of flowering time genes in *Brassica* by using populations of different growth habits. QTL affecting flowering time have been reported on all chromosomes of the A and C genomes of *B. napus* (Ferreira et al. 1995; Long et al. 2007; Cai et al. 2008; Mei et al. 2008; Chen et al. 2010; Würschum et al. 2012; Raman et al. 2013; Lou et al. 2014; Nelson et al. 2014; ArifuzZaman et al. 2016; Liu et al. 2016; Wang et al. 2016; Xu et al. 2016; Rahman et al. 2017; Li et al. 2017; Rahman et al. 2018; Jian et al. 2019). For example, Long et al. (2007) detected 42 QTL by using a population derived from winter × spring *B. napus* cross, and Chen et al. (2010) reported 22 QTL by using population derived from spring × spring *B. napus* cross and evaluating under different

environmental conditions. Raman et al. (2013) detected more than 20 QTL from ten A and C genome chromosomes by using a DH population derived from Australian spring canola cultivars. Xu et al. (2016) reported 41 QTL and Wang et al. (2016) reported 40 QTL affecting days to flowering in *B. napus* through genome-wide association study (GWAS). In case of the C genome of *B. oleracea*, several QTL affecting days to flowering have been reported; for example, three QTL by Camargo and Osborn (1996), six QTL by Bohuon et al. (1998), 11 QTL by Rae et al. (1999) and six QTL by Okazaki et al. (2007). Among these, the QTL on C2 has been reported by all these three studies.

The study was conducted to identify QTL affecting days to flowering under a short-day condition by using *B. napus* population carrying genome contents introgressed from six *B. oleracea* accessions belonging to four varieties of this species. To develop these inbred lines, a single *B. napus* line was crossed to the above-mentioned six *B. oleracea* accessions. Assuming no allosyndetic pairing of the A and C genome chromosomes occurred in the *B. napus* × *B. oleracea* interspecific hybrids, the A genome of these inbred lines was expected to be similar to the A genome of the *B. napus* parent, while the C genome of these lines was expected to be a blend of the C genome contents of the *B. napus* and *B. oleracea* parents. This is also evident from the detection of QTL primarily from the C genome. Rahman et al. (2017, 2018) reported QTL on C1 and C9 under 10 h photoperiod and 18 °C temperature conditions by using a *B. napus* mapping population carrying genome content introgressed from *B. oleracea* var. *alboglabra*, and these QTL exerted effect on days to flowering under a short-day condition.

In this study, we identified three QTL on the C genome chromosomes C1, C5 and C9 affecting days to flowering under a short-day condition by following GWAS and single marker analysis approaches. Study on QTL mapping of flowering time in *Brassica* under short-day

condition is limited. By using a DH population of *B. napus* derived from Canadian spring type canola Hyola 401 × Q2 cross, Cai et al. (2008) reported seven main effect QTL and 11 pairs of epistatic QTL affecting days to flowering and photoperiod sensitivity where the major QTL of N18 (C8) was found to affect both days to flowering and photoperiod sensitivity. Nelson et al. (2014) used a DH population derived from spring type European × Australian *B. napus* and reported three QTL affecting days to flowering on chromosomes A02, A07 and C3; these QTL explained a total of 52.7 % of the variation under both long- and short-day conditions. Thus, none of the three QTL that we detected in the present study under a short-day condition could be detected by using natural *B. napus* population. However, by using a spring *B. napus* population carrying genome contents of *B. oleracea* var. *alboglabra*, Rahman et al. (2017, 2018) mapped two flowering time QTL on C1 and C9. The C1 QTL affects flowering time without being affected by photoperiod while the C9 affect flowering time only under a short-day (10 h) condition. The C1 QTL is located in the genomic region of 21.1 Mb to 41.1 Mb (Rahman et al. 2017); however, single marker analysis located this QTL at about 41.1 Mb position (Rahman et al. 2018). In the present study, the QTL on C1 is detected to be present at about 43 Mb position and can be considered as the same QTL reported by Rahman et al (2017, 2018). In case of the C9 QTL reported by Rahman et al. (2017, 2018); the two flanking markers has been found to be located about 18 cM apart with the physical position of one of these two markers at about 0.7 Mb region. However, the SNP markers which we detected in this study for the C9 QTL are located at 24.6 – 39.7 Mb regions (Table 2-2). Therefore, it was not possible to deduce whether the C9 QTL reported by Rahman et al. (2017, 2018) and the QTL detected in this study is the same or to be different. Fine mapping of these QTL will be needed to determine this.

In *B. oleracea*, Bohuon et al. (1998) reported QTL affecting days to flowering on chromosome C9 at 38 cM, 46 cM, 78 cM, 100 cM positions of their linkage map. Axelsson et al. (2001) and Okazaki et al. (2007) also reported QTL on chromosome C9 in *B. oleracea*. Robert et al. (1998) mapped one of the homologue of *CO* gene on chromosome C9 of *B. napus*. Luo et al (2014) mapped a QTL at 2.00- 9.00 cM position of C9 by using a DH population of *B. napus*. Xu et al. (2016) also reported two QTL on C9, however, at about 11 Mb and 44 Mb positions; therefore, it is highly likely that the QTL detected in our study is different from the QTL reported by the above-mentioned researchers.

The presence of QTL on C5, affecting flowering time in *Brassica*, has also been reported previously by other researchers. For example, by using a DH population derived from *B. oleracea* var. *alboglabra* × *B. oleracea* var. *italica* and testing this under field conditions, Bohuon et al. (1998) reported two QTL at 16 cM and 46 cM positions of O5 (≈C5) of their linkage map constructed by using RFLP markers. Rae et al. (1999) also reported a QTL on O5 (≈C5) by using a set of recombinant backcross substitution lines derived from crossing of *B. oleracea* var. *italica* and var. *alboglabra* and testing them under field conditions. This QTL is located at 0 to 34.2 cM position of their RFLP and AFLP marker-based linkage map. The C5 QTL detected in the present study could not be aligned with these QTL due to the lack of common marker or sequence information of the QTL markers.

In *B. napus*, by using a DH population derived from crossing of vernalization responsive semi-spring Australian cultivars, Raman et al. (2013) reported two QTL on C5 under field and glasshouse conditions (vernalized and unvernalized); these two QTL were found to be responsive to vernalization, and were mapped at 44.1-44.7 cM and 224.6-227.7 cM positions of their linkage map constructed by using DArT, SRAP, SSR, SCAR and candidate gene-based markers. However,

the physical position of these C5 QTL was not provided by Raman et al. (2013). Xu et al. (2016) carried out a GWAS by using 523 *B. napus* cultivars and lines and evaluating under semi-winter growth conditions, and identified SNPs at 0.1 Mb, 0.2 Mb, 3.6 Mb and 42.8 Mb positions of C5 to be affecting flowering time. However, the C5 QTL detected in our study is located at about 30 Mb position; therefore, it could be a novel QTL which has not been reported previously and has been introgressed from *B. oleracea*. Based on the occurrence of SNP alleles in about top 10% early-flowering line, it could be anticipated that this QTL allele is derived from *B. oleracea* var. *capitata* cv. Bindsachsener.

BLASTn search in the C1, C5 and C9 QTL region identified a few genes, viz. *zinc finger protein CONSTANS-LIKE 12-like*, *ELF3* (*EARLY FLOWERING3) like protein 2, AP2-like (APETALA2-like) ethylene-responsive transcription factor PLT2, zinc finger CONSTANS-LIKE 15-like, COP1 (CONSTITUTIVE PHOTOMORPHOGENIC1) interating protein 7* to be involved in the regulation of flowering time in plants. It is well reported that the *CO* (*CONSTANS*) gene plays a key role in the regulation of flowering in response to photoperiod (Yanovsky and Kay 2002). *CO* gene initiates the transcription of floral integrator genes i.e. *FLOWERING LOCUS T* (*FT*), *FLOWERING LOCUS D* (FD) (for review, see Leijten et al. (2018) to initiate flowering. Lagercrantz and Axelsson (2000) reported multiple copies of *CO* in *Brassica* which evolved through gene duplication during evolution of these genomes. *COL4*, member of *CO* family, was reported to repress the expression of *FT* gene in *Arabidopsis* (Steinbach 2019). In cotton, the *CO*-like genes have been reported to play an important role in draught and salt stress response (Qin et al. 2018).

Circadian clock consisted of three main complexes and includes morning, evening and central (for review, see Harmer 2009). *EFL3* is an important component of the evening complex

(Nusinow et al. 2011). The *EARLY FLOWERING 3* (*ELF3)* has been reported to play an important role in the regulation of circadian pathway and flowering in *Arabidopsis* (Hicks et al. 2001). A variant of *ELF3* has also been reported to induce flowering in rice under short- and long-day conditions (Saito et al. 2012) and *Efl1* (variant of Arabidopsis *EFL3*) was reported to increase earliness of flowering in chick pea (Ridge et al. 2017). Lin et al. (2019) overexpressed the *EFL* proteins in *Arabidopsis* and found that *EFL1* and *EFL3* delayed flowering by repressing the expression of the *CO* and *FT* genes. Silva et al. (2020) reported that binding the Evening Complex of the circadian clock, which composed of *LUX* (*LUX ARRHYTHMO*), *ELF3* and *ELF4*, with DNA occur strongly at 4°C temperature and weakly at 27°C temperature.

The *COP1* gene play an important role in photoperiod-responsive flowering in *Arabidopsis* by regulating the expression of *CO* (Liu et al. 2008). This gene acts upstream of the *CO* and delay flowering by promoting the proteolysis of *CO* in darkness (Liu et al. 2008). The *COP1* works in concert with *SUPPRESSOR of phyA-105* (*SPA*) proteins to repress photoperiod-responsive flowering by regulating the degradation of *CO*. Jang et al. (2008) reported that mutation in *COP1* results early flowering in *Arabidopsis* under short day conditions by increasing transcription of the *CO*. *COP1* also act as a repressor of *GIGANTEA* gene which reduce the expression *FT* and, thus, result a delayed flowering under low temperature in *Arabidopsis* (Jang et al. 2015). Rolauffs et al. (2015) showed that *COP1* promotes petiole and hypocotyl elongation under low light conditions, and this gene has also been reported to positively regulate the defense against turnip crinkle virus (Lim et al. 2018). Wang et al. (2015) reported that *COP1* also repress the *ELF3* in *Arabidopsis* by interacting with the transcription factor BBX (B-Box family of proteins).

APETELA2 is an important gene found to be present only in plants and its expression can be regulated by a microRNA (Chen 2004). Ogawa et al. (2007) reported that expression of *AP2* gene

is upregulated by *Arabodopsis ethylene binding protein* (A*tEBP*) but downregulated by *ethylene-insensitive2* (*EIN2*) gene. In *Arabidopsis*, the *TARGET OF EAT1* (*TOE)* gene, a member of *AP2* (Wang et al. 2016), was reported to regulate flowering by interacting with *CO* (Zhang et al. 2015). Debernardi et al. (2020) showed that the expression of *AP2* gene is also required for proper development of florets and spikelets in wheat. Thus, it is evident that the genes identified in the three QTL regions might play a role in days to flowering under short day conditions.

In conclusion, by using an inbred population derived from six *B. napus* × *B. oleracea* interspecific crosses, we identified three QTL on C1, C5 and C9 affecting days to flowering under a short-day condition. Among these, the C5 QTL affecting days to flowering under a short-day condition has not been reported previously. The SNP and SSR markers developed in this study can be used in breeding to improve the earliness of canola for growing under a short-day condition as well as for the improvement of earliness of this crop for growing under a long-day condition, e.g. on the Canadian prairies.

Chapter 3

Evaluation of a diverse panel of *Brassica napus* lines under a short-day condition for identification of early-flowering lines and validation of the QTL

3.1 Introduction

In a plant, the transition from vegetative to reproductive phase is determined by interaction of numerous external factors, such as temperature and photoperiod, and a network of different genes (Cho et al. 2017). Days to flowering is one of the key factors playing an important role in adaptation of a crop under continuously changing climatic conditions (for review, see Jung and Muller 2009). In Canada, the crop growing season is short; therefore, early flowering and maturity remain the most important agronomic traits for different field crops including *Brassica* oilseed crops (Robertson et al. 2002). In *B. napus*, days to flowering often correlate well with days to maturity (Nikzad et al. 2019; Shiranifar et al. 2020); therefore, earliness of maturity of this crop can be improved through the improvement of the earliness of flowering. Flowering in a plant is often determined by genes involved in different pathways, such as vernalization, photoperiod and autonomous pathways, and interaction of the genes involved in these pathways (Mouradov et al. 2002 and Jung and Muller 2009). Among these, the genes involved in vernalization pathway, primarily play an important role in flowering of the winter growth habit types. Therefore, identification of the genes involved in the photoperiod and autonomous pathways is important for the improvement of days to flowering in spring *B. napus* canola. The optimum photoperiod for spring canola is about 18 h; however, canola in Canada receives a photoperiod of about 15-17 h prior to flowering, i.e. the crop does not receive the minimum optimum photoperiod. In this regard,

it is important to identify the genes involved in the photoperiod and autonomous pathways to improve the earliness of this crop.

It is well reported that days to flowering in *B. napus* is a quantitative trait controlled by several loci (Ringdahl et al. 1986; Long et al. 2007; Raman et al. 2013; Luo et al. 2014). QTL alleles for earliness of flowering under a short-day condition can be found in *B. oleracea* (Rahman et al. 2017, 2018) and *B. rapa* (Kubik et al. 1999), and these two parental species have been used by several researchers for the improvement of *B. napus* (Miller 2001; Qian et al. 2005; Rahman et al. 2011; Bennett et al. 2012; Iftikhar et al. 2018). *B. napus* is known to have polyphyletic origin involving multiple parental genotypes in its evolution (Song and Osborn, 1992); therefore, it can be hypothesized that the alleles contributing to earliness in flowering under a short-day condition can be found in available oilseed *B. napus* germplasm.

In this MSc study, by using a recombinant inbred *B. napus* population derived from *B. napus* × *B. oleracea* interspecific crosses, I identified three QTL on C1, C5 and C9 affecting days to flowering under a short-day condition and developed simple sequence repeat (SSR) markers from one of the three QTL regions. SSR markers have been found to be efficient for use in breeding due to their reproducibility and co-dominance nature (Powell 1996). For example, Hearne et al. (1992) demonstrated the efficiency of SSR markers in developing linkage maps for genetic studies. Plieske and Struss (2001) demonstrated the efficiency of SSR markers to distinguish the spring and winter *B. napus,* and Bus et al. (2011) used SSR markers to analyse genetic diversity in *B. napus* germplasm.

Thus, the objectives of this study were to evaluate a set of diverse *B. napus* lines, collected from gene bank and different breeding programs, under a short-day condition (10 h photoperiod

and 18 °C constant temperature) for days to flowering to identify the lines carrying earliness of flowering trait under this condition, as well as to investigate the occurrence of the early flowering allele by using SSR markers designed from one (C9) of the three QTL identified in this study.

3.2 Material and methods

3.2.1 Plant material

A total of 280 diverse canola cultivars and lines (Appendix 3-1) were used in this study. This includes 38 cultivars collected from the gene bank Plant Genetic Resources of Canada, Saskatoon, 215 lines and cultivars from the canola breeding program of the University of Alberta (U of A), and 29 lines and cultivars from Nutrien Ag Solutions (NAS), Saskatoon.

3.2.2 Phenotyping for days to flowering

The 280 canola lines and cultivars were grown in growth chamber under 10 h photoperiod and 18℃ constant temperature conditions for evaluation of days to flowering. For this, these accessions were grown in 32-cell tray with pot size of 7 cm × 7 cm × 9 cm (L × W × D) filled with Sunshine Professional Growing Mix (Sunshine Horticulture, 15831 N.E. Bellevue, USA). Three plants of each accession were grown and the average value of these plants was used for statistical analysis. To avoid shading of a plant by the other, the lower leaves were removed at rosette stage, and the experiments were monitored on a daily basis. Data on days to flowering was recorded at first open-flower stage. The experiment was repeated twice which constituted two replications.

Normally, most of the spring canola lines in a growth chamber at 10 h photoperiod and 18 ℃ temperature flowers within 100 days after seeding (Rahman et al. 2018). In the present study, the experiments were terminated at 180 days after seeding. At this stage, the non-flowering plants were recorded as being flowered at some time after 180 days of seeding.

3.2.3 Leaf sample collection and DNA extraction: Leaf samples of all accessions was collected at 25-30 days after seeding and was stored at -80°C until use. DNA was extracted following SDS DNA extraction protocol. For this, two to three leaves from each plant at the age of 20-25 days after seeding were collected in 2 ml micro centrifuge tubes and kept in liquid nitrogen. Leaves were grinded into fine powder by crushing with Qiagen tissue-lyzer, and 600 µl pre-warmed (65 °C) CTAB buffer was added into each micro centrifuge tube. The samples were kept at 65 °C for one hour with intermittent gentle mixing after every 15 min. The emulsion was cooled to room temperature for 15 minutes after the incubation and 600 µl chloroform was added to each, and the samples were centrifuged at 8,000 rpm for 10 min and the supernatant was transferred to a fresh 1.5 ml micro centrifuge tube. An equal volume (800 µl) of chilled isopropanol was added to the centrifuge tubes for precipitation of the DNA, and the tubes were kept at 4 °C for 60 min, and the precipitated DNA was spooled out in a fresh Eppendorf tube of 1.5 ml. The residual debris was removed by washing the DNA pellet twice with 70% ethanol. The DNA was allowed to air dry at 37 °C for an hour and dissolved in 100 µl 1X TE buffer and stored at -20°C until use.

3.2.4 DNA quantification

The DNA quality and quantity was accessed by using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware, USA) and the concentration was adjusted to 25 ng/µl for polymerase chain reaction (PCR).

3.2.5 Molecular marker analysis

SSR markers from the C9 QTL region identified in Chapter 2 were used in this study. For this, the markers were tested on 75 most early- and 75 most late-flowering accessions of this population. Based on the occurrence of the marker alleles, this population was partitioned for the presence and

absence of the marker alleles, and these two groups were tested for significant difference (*t*-test) for days to flowering.

3.2.6 Polymerase Chain Reaction (PCR)

PCR was carried out in a total volume of 12 µl, where each PCR reaction mixture comprised of 50 ng DNA (25 ng/µl × 2), 1.25 µl of 10X PCR buffer, 0.125 µl of 20 mM dNTPs, 1.25 µl of 50 mM $MgCl_2$, 0.5 µl of each of 5 µM forward and reverse primer, 6.225 µl of nuclease free water and 0.15 µl (0.75U) of GoTaq DNA polymerase enzyme. Amplification reaction was performed by using PCR profile which comprised three stages. The first stage included one initial denaturation cycle at 95 ℃ for 5 minutes. The second stage included 35 cycles where each cycle included 95 ℃ for 30 seconds, 53 ℃ for 30 seconds and 72 ℃ for 45 seconds. The third stage comprised the final extension at 72 ℃ for 10 minutes.

3.2.7 Gel electrophoresis separation of amplicons

The PCR amplified products were visualized through agarose gel (2.5%) electrophoresis. For this, the 2.5 % agarose gels were prepared by using 300 ml of 1 X TBE buffer, 7.5 grams of agarose (Invitrogen, Carlsbad, CA) and 9 µl SYBR safe. The PCR amplified products were dissolved with a loading dye and were loaded onto the agarose gel; λ DNA of known concentration (400 ng/µl) was also used for size estimation. Electrophoresis was carried out in TBE buffer at 100 volts for one hour. Typhoon FLA 9500 scanner (GE Healthcare Bio-Sciences AB, Piscataway, NJ) was used to take the gel image after electrophoresis.

3.2.8 Statistical analysis

Least square mean (LSmean) values were calculated by using the software program R (R studio team, 2016). Other statistical analysis, such as simple mean and standard error were calculated in MS-Excel and Pearson's correlation coefficients were calculated by using models available in R v1.1.463.

3.3 Results

3.3.1 Phenotyping the population for days to flowering

3.3.1.1 Correlation between the replications for days to flowering

Highly significant positive correlation ($r = 0.96$, $p < 0.001$) was found between the replications for days to flowering (Fig. 3-1). This suggests that the accessions flowered similarly in the two replications.

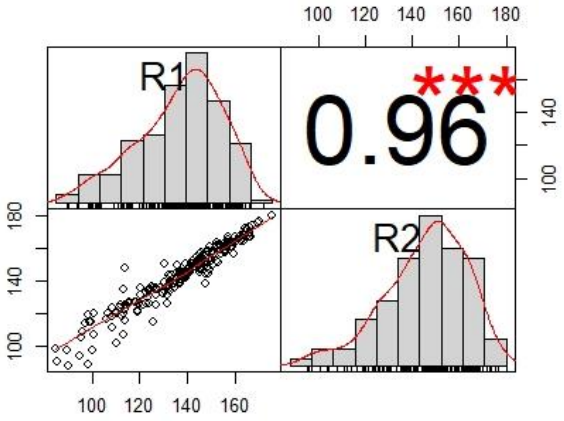


Figure 3-1. Correlation of days to flowering data of the spring oilseed *Brassica napus* lines and cultivars from two growth chamber experiments conducted at 10 h photoperiod and 18 °C constant temperature. R1 and R2 indicate the two experiments/replications.

Days to flowering in this population varied from 81 days to more than 180 days. Of the 280 lines, 202 (72.1%) flowered within 180 days after seeding, while 78 (27.9%) lines did not flower at the time when the experiment was terminated. The Canadian canola cultivars VT-Barrier

flowered at about 131 days after seeding and A07-26NR flowered at about 140 after seeding. Several lines flowered earlier than these checks (Fig. 3-2).The 10 most early-flowering lines/cultivars were: (Hylite 201; Gene bank; 88 days), (CC08084; NAS; 89.2 days), (A03-22805NA; U of A; 93.3 days), (CC08149; NAS; 93.7), (1RA1443.058-A1202; U of A; 94.5 days), (1RA1444.007-A1202; U of A; 98.7 days), (NBC12-00771; NAS; 100.3 days), (Apollo, LL; Gene bank; 102.3 days), (Westar; Gene bank; 106.7 days), (1RA1443.072-A1202; U of A; 107.2 days) (Appendix 3-1).

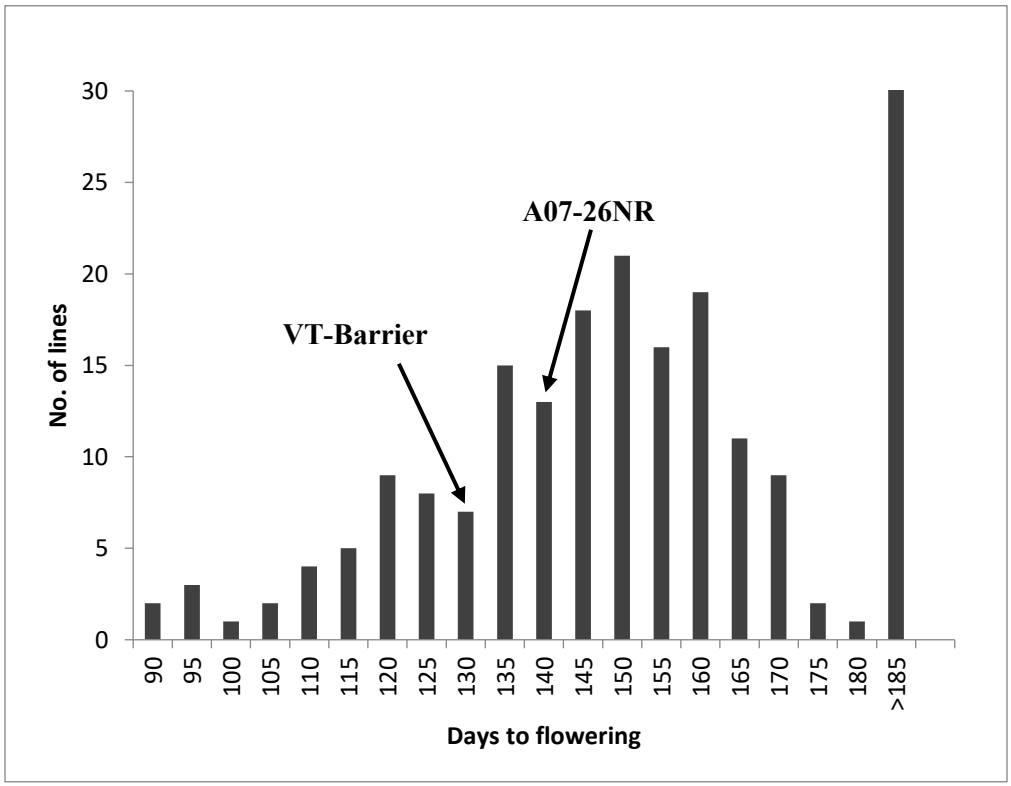


Figure 3-2. Frequency distribution of the diverse lines and cultivars of *Brassica napus* for days to flowering in a growth chamber at 10 h photoperiod and 18 °C temperature. LSmean data of two replications presented. VT-Barrier and A07-26NR are shown as Canadian standard canola cultivars.

3.3.2 SSR markers designed from QTL regions

Two SSR markers, viz. 4928 and 4929, from the C9 QTL region, which showed co-segregation with days to flowering in 184 inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses (Chapter 2) were tested on 75 most early flowering and 75 most late flowering accessions of the diverse *B. napus* population used in this study. The marker 4928 produced a band of 250 bp size in the late flowering accessions but a band of 300 bp size in the early flowering accessions. The mean days to flowering of the accessions carrying the 300 bp band was 100.06 ± 0.86 (range: 88 to 111 days) and the accessions carrying the 250 bp band was 160.39 ± 1.95 (range: 135 to 175 days); these two groups were statistically different for days to flowering (t = -4.57, p < 0.001) (Table 3-1). In case of the marker 4929, it produced a band of 250 bp size in late flowering accessions and a band of 250 bp and 350 bp sizes in the early flowering accessions; thus, the marker allele 250 bp was monomorphic in this population while the marker allele 350 was found to be polymorphic. Mean days to flowering of the accessions carrying the 350 bp band was 102.36 ± 1.22 (range: 88 to 135 days) and the accessions lacking this marker allele was 163.61 ± 0.98 (range: 157 to 175 days); these two groups were statistically different for days to flowering (t = -5.215, p <0.001) (Table 3-1).

Table 3-1. *t-t*est for significant difference between the diverse *Brassica napus* cultivars and lines, collected from different sources and partitioned based on SSR marker alleles, for days to flowering (DTF) at 10 h photoperiod condition.

Chromosome (marker start to end position, bp)	Marker name	Early flowering lines			Late flowering lines			*t*-test	
		Allele size (bp)	DTF range	DTF Mean ± SE	Allele size (bp)	DTF range	DTF Mean ± SE	*t*-value	*p*-value
C9 (30128941 – 30129189)	4928	300	88 – 111	100.06 ± 0.86	250	135 - 175	160.39 ± 1.95	- 4.57	< 0.001
C9 (31681068 – 31681420)	4929	250, 350	88 – 135	102.36 ± 1.22	250	157 - 175	163.61 ± 0.98	- 5.215	< 0.001

3.4 Discussion

In this study, phenotyping of total 280 diverse canola cultivars and lines for days to flowering under 10 h photoperiod and 18 °C constant temperature identified a few lines which flowered much earlier than the standard Canadian canola cultivars. This suggests that significant genetic diversity exists in this germplasm for days to flowering under a short-day condition, and the early-flowering lines can be used in breeding for the improvement of earliness of flowering and maturity traits in this crop for growing in Canada as well as under short-day conditions, e.g. in Indian sub-continent.

Being a long-day plant, canola requires about 16 – 18 h photoperiod for flowering (King and Kondra, 1986; Robertson et al. 2002). However, in countries like Canada, the crop growing season is short and the crop does not receive the optimum photoperiod; therefore, plant breeding efforts are aimed at developing early flowering and maturing cultivars. Most of the breeders often use the natural genetic variation for the development of early flowering/maturing cultivars. However, interspecific hybridization of *B. napus* with its diploid parental species, viz. *B. rapa* and *B. oleracea,* have been performed by several researchers to develop early flowering/maturing *B. napus* (Miller 2001; Qian et al. 2005; Rahman et al. 2011; Bennett et al. 2012; Iftikhar et al. 2018). The occurrence of canola lines/cultivars, carrying the early flowering trait under a 10 h photoperiod condition, in breeding population developed for growing under a long-day condition suggests that canola breeders might have unintentionally retained the QTL affecting earliness of flowering under a short-day condition while breeding cultivars for Canada.

Flowering time is a quantitative trait controlled by many loci (Long et al. 2007; Raman et al. 2013; Luo et al. 2014), therefore QTL alleles for earliness of flowering can be dispersed in different cultivars, as has been reported for quantitative traits like seed oil content (for review, see

Rahman et al. 2013). Evaluation of the SSR markers from the C9 QTL region on this diverse *B. napus* cultivars and lines provided evidence for the occurrence of this QTL allele in this population. SSR markers have been used in different crop species by different researchers for construction of genetic linkage map for QTL mapping (Long et al. 2007; Cai et al. 2008; Chen et al. 2010; Raman et al. 2013; Guo et al. 2017; Daware et al. 2016; Kim et al. 2017) as well as in marker-assisted selection (Liang et al. 2004; Sahin et al. 2018; Chandran et al. 2019; Long et al. 2019). The development of SSR markers from the C9 QTL region (Chapter 2) and genotyping the population used in this study provided evidence for potential use of these markers in marker-assisted selection for earliness of flowering.

In conclusion, based on phenotypic and genotypic data, this study demonstrated that significant variation for days to flowering under short-day condition exists in Canadian spring oilseed *B. napus*. The early-flowering elite lines/cultivars identified in this study expected to be superior as compared to the early-flowering lines identified in Chapter 2 for agronomic and seed quality traits; therefore, they can be effectively used for the improvement of earliness of the Canadian canola, as well as for the development of cultivars for growing under a short-day condition, such as in Indian sub-continent. The SSR markers used in this study may have potential for use in molecular breeding.

Chapter 4

Development of near-isogenic lines of *Brassica napus* for flowering time QTL

4.1 Introduction

The genus *Brassica* of the family Brassicaceae includes several economically important agricultural and horticultural crop species. For example, *B. oleracea* (*n* = 9, C genome) is grown as a vegetable crop, *B. rapa* (*n* = 10, A genome) as vegetable or oilseed, *B. juncea* (*n* = 18, AB genome) as oilseed or condiment, and *B. napus* (*n* = 19, AC genome) as oilseed crop. Among the six species of U's triangle (U 1935), *B. rapa*, *B. oleracea* and *B. nigra* (*n* = 8, B genome) are diploids and *B. napus*, *B. carinata* (*n* = 17, BC genome) and *B. juncea* are allotetraploids which originated from the three diploid species. Among these, the spring type *B. napus* is the most important oilseed crops in Canada.

Different agronomic and plant development traits, such as early vigor, days to flowering and maturity, number of siliques per plant, number of seeds per silique, resistance to lodging, and seed size (1000-seed weight), and environmental conditions such as temperature, moisture and soil nutrients plays an important role in the productivity of this crop (Borges et al. 2018). Among the different agronomic traits, early flowering and maturity are one of the most important ones for growing this crop in Canada. Tailoring the time of flowering is important for various purposes including adaptation of the crops under changing climatic conditions and expanding the area of their cultivation by breeding cultivars adapted to new geographical regions (for review, see Jung and Muller 2009). The environmental conditions required for flowering often vary depending on the plant species, as different plants respond differently to environmental conditions. Plants have

developed several mechanisms, such as response to photoperiod and temperature, to induce flowering (Amasino and Micheals 2010).

In *B. napus*, seed yield can be increased by combining the earliness of flowering with delayed maturity traits, i.e. by increasing the duration of grain-filling period (Habekotte 1997; Gan et al. 2016). Nikzad et al. (2019) found a significant positive correlation between the duration of grain-filling period and seed yield in *B. napus* lines carrying genome content introgressed from *B. oleracea*. Also, Shiranifar et al. (2020) reported that the early flowering spring canola lines derived from spring canola × rutabaga crosses give greater seed yield and also contain higher oil content in the seeds. Therefore, improvement of the earliness of flowering is an important breeding objective in many parts of the world where the growing season of this crop is short, e.g. in Canada (Robertson et al 2002), or for the regions where crop growth is disrupted by high temperature (> 25 °C) at grain filling period, e.g. in Indian sub-continent. Angadi et al. (2000) and Gan et al. (2004) found that high temperature (35 ℃) at reproductive stage is injurious to canola and this can reduce seed yield. *B. napus* is considered a long day plant which requires about 16 – 18 h photoperiod for flowering (King and Kondra, 1986).

Spring type *B. napus* takes about 4-5 months for growing in North America and Europe, while it takes about two months longer time where day length at crop growing season is about 12 hours, e.g. in India, due to its photosensitivity. Therefore, earliness of flowering and maturity in *B. napus* can be improved through the development of photo-insensitive cultivars (Akbar 1987; Zaman 1989). The knowledge of the *Brassica* genomes and the availability of molecular breeding tools are expected to accelerate the identification of the genes involved in photo-insensitivity and the development of molecular markers for use in breeding for the improvement of earliness in canola for growing this crop under a short-day condition as well as in temperate regions. Rahman

et al. (2011, 2017, 2018) demonstrated that, despite *B. oleracea* flowers later than *B. napus*, the C genome of this species carry early flowering alleles which can be utilized to improve the earliness of *B. napus*.

Quantitative trait loci (QTL) analysis is one of the important ways to understand the genetic variation of a quantitative trait; this has been carried out by several researchers (e.g. Ferreira et al. 1995; Long et al. 2007; Chen et al 2010; Würschum et al. 2012; ArifuzZaman et al. 2016; Liu et al. 2016; Wang et al. 2016; Rahman et al. 2017, 2018; Jian et al. 2019) for mapping of flowering time genes in *B. napus*. Rahman et al. (2011, 2017, 2018) introgressed genome content of *B. oleracea* into *B. napus* and mapped a major flowering time QTL on chromosome C1; the *B. oleracea* allele of this QTL improved the earliness of flowering in *B. napus*, and this QTL was found not to be affected by photoperiod. This research group also mapped a locus on C9 which is sensitive to photoperiod. However, the interval of the flanking markers of the C1 QTL is about 30 cM and the markers for C9 QTL is about 18 cM; this is an impediment for use of these markers in molecular breeding; fine mapping of these genomic regions is, therefore, needed.

Among the different mapping populations, near-isogenic lines (NILs) is an important population for fine mapping of the genes. NILs have been developed by several researchers and used for fine mapping of different traits in different crop species. For example, Habib et al. (2017) used NILs of barley for transcriptome sequencing to identify the gene on chromosome 4HL responsible for fusarium rot resistance, and following a similar approach, Gao et al. (2019) delineated a resistance locus on 1HL and also developed SNP markers for use in breeding. Oiested et al. (2017) used two NILs of wheat differing for QTL alleles controlling solidness of stem to identify the candidate genes involved in the control of this trait; this trait is also associated with resistance to wheat stem sawfly. Mia et al. (2019) reported that NILs are one of the most important

resources for identification and mapping of QTL controlling water stress in wheat. By performing transcriptome analysis on NILs of rice, Zhang et al. (2018) identified the genes involved in the regulation of panicle growth and development. NILs have also been developed in *Brassica* for different purposes, for example, to identify the genes involved in clubroot resistance (Chen et al. 2016), seed color (Zhao et al. 2018), silique development (Chai et al. 2018), fertility restoration (Long et al. 2019) and number of seeds per silique (Zhu et al. 2020). Thus, it is apparent that NILs can also be used for fine mapping of QTL and identification of the candidate genes for days to flowering, as well as for the development of molecular markers for use in breeding.

The objective of this study was to develop NILs for the C1 and C9 QTL for fine mapping of these genomic regions and to identify the candidate genes involved in the control of days to flowering under a short-day (10 h photoperiod) condition.

4.2 Material and methods

4.2.1 Plant material and development of NILs

The parent lines used in this study were one spring *B. napus* canola cultivar Hi-Q, developed by University of Alberta, and an early flowering *B. napus* line RIL 144 carrying genome content of *B. oleracea* var. *alboglabra*. A doubled haploid (DH) population, derived from the cross Hi-Q × RIL-144, has been used for mapping the photo-insensitive and photosensitive QTL on C1 and C9, respectively (Rahman et al. 2017, 2018). To develop NILs for these two QTL, the F_1 plants of Hi-Q × RIL 144 were backcrossed by using Hi-Q as the recurrent parent. Two separate backcrossing programs were carried out for the development of the NILs for the two QTL of C1 and C9. In each backcross generation, marker assisted selection for the QTL marker allele of RIL-144 was performed. For this, the backcross generation populations were grown in a growth chamber set at

16/8 h day/night photoperiod and 22 ℃ constant temperature with photosynthetic flux density of 450 μE (mV) m^2 s^{-1}. The plants were grown in 32-cell tray with pot size of 7 cm × 7 cm × 9 cm (L × W × D) filled with Sunshine Professional Growing Mix (Sunshine Horticulture, 15831 N.E. Bellevue, USA) and were analysed before flowering by the C1 and C9 QTL markers for the presence of the RIL 144 alleles, i.e. plants heterozygous for the RIL-144 and Hi-Q alleles. The heterozygous BC_1 plants were further backcrossed to Hi-Q to produce BC_2 seeds and the selected BC_2 plants were backcrossed to Hi-Q to produce BC_3 seeds. This marker-assisted selection and recurrent backcrossing was performed until production of BC_4 seeds. The BC_4 plants heterozygous for the QTL marker alleles were self-pollinated to produce BC_4F_2 seeds, and the BC_4F_2 plants carrying the RIL-144 or Hi-Q allele in homozygous condition were identified through marker analysis. The homozygous plants were self-pollinated to produce BC_4F_3 generations families carrying either RIL-144 or Hi-Q allele. In addition to self-pollination of the homozygous plants, a few heterozygous BC_4F_2 plants were also self-pollinated to develop NILs from these plants. For each QTL, multiple homozygous plants for RIL-144 or Hi-Q alleles were developed which were designated as NIL families. The crossings, BC_1 and BC_2 generation populations were developed by the Canola Program of the University of Alberta, and I developed the BC_3 to BC_4F_3 generation populations.

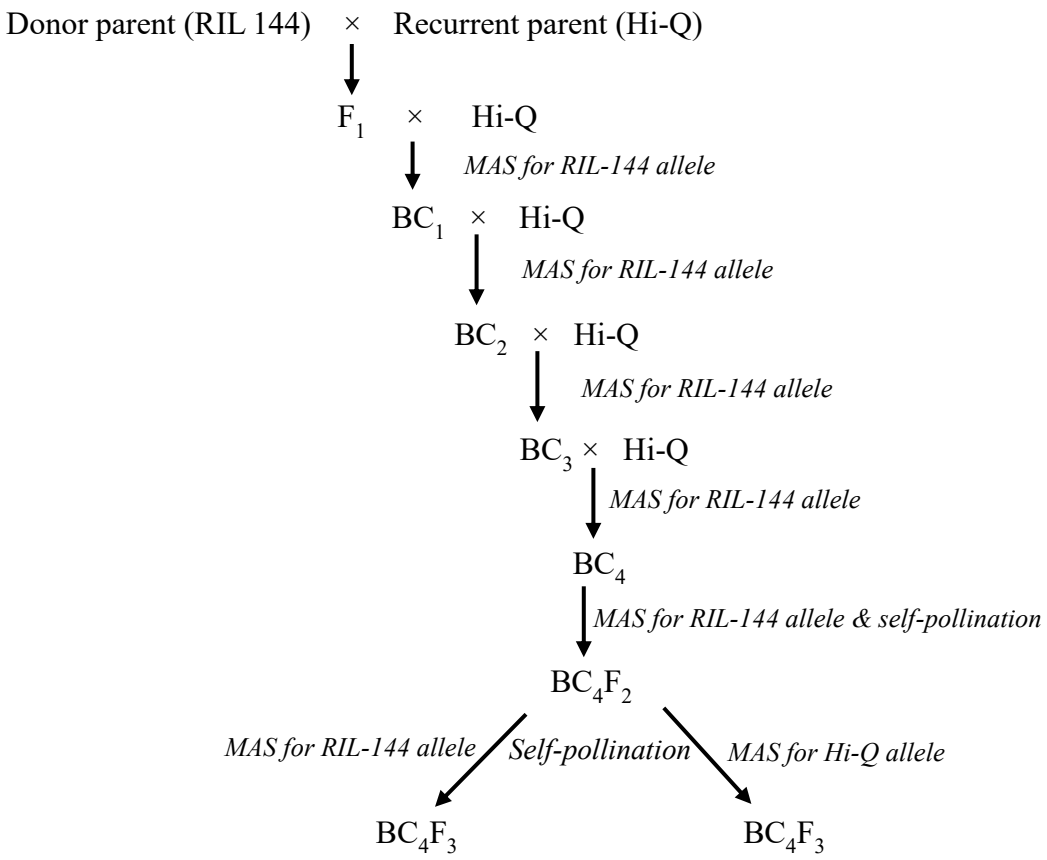


Figure 4-1 A schematic diagram of marker assisted backcrossing in *Brassica napus* for the development of near-isogenic lines (NILs) carrying flowering time QTL alleles of RIL-144 or Hi-Q.

4.3 Molecular analysis

Three SSR (simple sequence repeat) markers from the C1 QTL region and three markers from the C9 QTL regions (Rahman et al. 2017, 2018) were used for genotyping the backcross generation plants for further backcrossing or self-pollination (Table 4-1).

Table 4-1. C1 and C9 QTL markers used for molecular marker analysis for the development of the near isogenic lines (NILs).

Primer name (primer no.)	Primer type	Primer sequence	Hi-Q allele (bp)	RIL-144-allele (bp)
C1 QTL markers:				
sN0248Ia (210)	Forward primer	CACGACGTTGTAAAACGACGTCGCTACACCTGCCAAAAT	118	113
	Reverse primer	CTGGTTACTCCGGTACGCAT		
sN1035R (2282)	Forward primer	CACGACGTTGTAAAACCATTGTGTAGATTCACTTCCCC	249	254
	Reverse primer	TGGTGAACTTGTTCTGCCTG		
sN0842 (2311)	Forward primer	CACGACGTTGTAAAACAAGCCTGACAATCCAAAACG	388	391
	Reverse primer	CGATTTCATGGCAAATTCCT		
C9 QTL markers:				
sNRG42 (313)	Forward primer	CACGACGTTGTAAAACGACTCGTGGGGATTAGTCTGAGC	146	138
	Reverse primer	ATCCCGAGTGACAAAAATTG		
sN4029 (129)	Forward primer	CACGACGTTGTAAAACGACCGATTCCTTCTCCTTGTCCA	336	340
	Reverse primer	GAGCCGTTCTTGTTTCTTGC		
BRAS050 (726)	Forward primer	CACGACGTTGTAAAACGACCTTTGTGGTGGGTAGTGG	156	138
	Reverse primer	ACTTAGCCTCAATACGGTCTT		

Note: The Forward primer contains a M13 primer sequence, which is a universal primer sequence 5′-CACGACGTTGTAAAACGAC-3′ labelled with fluorescent dyes FAM, VIC, NED and PET (Applied Biosystems, Foster City, CA).

4.3.1 Genomic DNA Extraction

The genomic DNA of the different generation plants was extracted following modified SDS DNA extraction protocol. For this, two to three leaves from each plant at the age of 20-25 days after seeding were collected in 2 ml micro centrifuge tubes and kept in liquid nitrogen. The leaves were grinded into fine powder by crushing with Qiagen tissue-lyzer and 600 µl pre-warmed (65 °C) CTAB buffer was added into each micro centrifuge tube. The samples were kept at 65 °C for one hour with intermittent gentle mixing after every 15 min. The emulsion was cooled to room temperature for 15 minutes after the incubation and 600 µl chloroform was added to each, and the samples were centrifuged at 8,000 rpm for 10 min and the supernatant was transferred to a fresh 1.5 ml micro centrifuge tube. An equal volume (800 µl) of chilled isopropanol was added to the centrifuge tubes for precipitation of the DNA, and the tubes were kept at 4 °C for 60 min, and the

precipitated DNA was spooled out in a fresh Eppendorf tube of 1.5 ml. The residual debris was removed by washing the DNA pellet twice with 70% ethanol. DNA was allowed to air dry at 37°C.

4.3.2 DNA quantification

The DNA quantity and quality was accessed by using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, Delaware, USA) and the concentration was adjusted to 25 ng/µl for polymerase chain reaction (PCR).

4.3.3 Polymerase Chain Reaction (PCR)

PCR plate was prepared with a total volume of 12 µl, where each well comprised of 50 ng DNA (25 ng/µl × 2), 1.25 µl of 10X PCR buffer, 0.125 µl of 20 mM dNTPs, 1.25 µl of 50 mM $MgCl_2$, 0.5 µl of each of 5 µM forward and reverse primer, 6.225 µl of nuclease free water, and 0.15 µl (0.75U) of GoTaq DNA polymerase enzyme. Amplification reaction was performed by using PCR profile which included four stages. The first stage included one initial denaturation cycle at 95 ℃ for 5 minutes. The second stage comprised of 95 ℃ for 30 seconds, 57 ℃ for 45 seconds and 72 ℃ for 45 seconds for 30 cycles. The third stage comprised of 94 ℃ for 30 seconds, 53 ℃ for 45 seconds and 72 ℃ for 45 seconds for eight cycles. The fourth stage comprised the final extension at 72 ℃ for 10 minutes. Four individual PCRs were performed using four different fluorescent dyes (FAM, VIC, NED, PET).

4.3.4 Detection of amplicons by using ABI sequencer

After PCR, a 96-well plate was prepared following the same sequence of the PCR plate and numbered from 1 to 96 for sequencing. For the 96 samples, 8 µl size standard (GeneScan™ 500 LIZ®) was mixed in 700 µl Hi-Di™ Formamide. To prepare the ABI plate, 7 µl of this mixture and 1 µl PCR product of each sample from the four PCR plates was mixed together. The samples

in the loaded ABI plate were denatured by incubating at 95 ºC for 2 minutes followed by cooling on ice block for 2 minutes. After that, size-based separation of the amplified DNA fragments was done by capillary electrophoresis using ABI Genetic Analyzer 3730 (Life Technologies, Foster City, CA).

The software program GeneMarker version 2.4.0 (SoftGenetics, State College, Pennsylvania, USA) was used to score the polymorphic bands based on ABI sequence data, where score "1" was given for the presence of the allele or peak and "0" was used for absence of the allele or peak.

4.3.5 Additional marker development from the flowering time QTL regions

To develop additional markers from the QTL regions, SSR and random amplified polymorphic DNA (RAPD) markers were designed from the C1 QTL region by using *Brassica* genome sequence information (ncbi.nlm.nih.gov/nuccore/NC_027767.2) and employing primer designing tools of NCBI (ncbi.nlm.nih.gov/tools/primer-blast/) and WEBSAT (bioinfo.inf.ufg.br/websat/). RAPD markers were also designed from the two gene regions, viz. *protein EARLY FLOWERING 3-like* and *zinc finger CONSTANS-LIKE 12-like*, which have been reported by Rahman et al. (2017) to be located in the C1 QTL region. The polymorphic markers were tested on the DH population (Rahman et al. 2017, 2018) for linkage association with flowering time; DNA of this DH population was available in the canola program for use in this study. To carry out genotyping by the ABI sequencer, M-13 tail was attached to each of the forward primer.

4.3.6 Genotyping of the NILs

The C1 and C9 NILs were genotyped by using the available three C1 and three C9 QTL markers (Table 4-1). On the basis of genotypic data, the NILs were classified into homozygous for RIL-

144 allele, heterozygous for RIL-144 and Hi-Q alleles, and homozygous for Hi-Q allele.

4.3.7 Phenotypic evaluation of the NILs

The NILs, developed through recurrent backcrossing followed by self-pollination, were evaluated in growth chamber set at 10 h photoperiod and 20 ℃ constant temperature with photosynthetic flux density of 450 µE (mV) m^2 s^{-1} for days to flowering. For this, the plants were grown in 32-cell tray with pot size of 7 cm × 7 cm × 9 cm (L × W × D) filled with Sunshine Professional Growing Mix (Sunshine Horticulture, 15831 N.E. Bellevue, USA). Four plants of each NIL family were grown and the average value of these plants was used for statistical analysis. The experiment was repeated twice which constituted the number of replications. Data on days to flowering was recorded at first open flower stage. While growing the populations in growth chamber, to avoid shading of a plant by the other, the lower leaves were removed at rosette stage, and the experiments were monitored on a daily basis.

The NILs were also evaluated in one replication trial in a growth chamber set at 22 h photoperiod and 20 ℃ constant temperature with photosynthetic flux density of 450 µE (mV) m^2 s^{-1} for days to flowering. For this, four plants of each NIL were grown and these four plants were considered as four replications for statistical analysis.

4.3.8 Statistical analysis

Least square means (LSmeans) were calculated for phenotypic data of each replication as well as for all replications by using LSmeans package in R (Lenth 2016). Other statistical analyses, such as frequency distribution, and calculation of mean, range and standard error were performed in MS-excel and Pearson's correlation coefficients were calculated by using different R packages. Significant difference between the means was calculated using fisher's least square difference.

4.4 Results

4.4.1 Development of NILs

4.4.1.1 C1 QTL

Twenty to 40 plants of each of the backcross generation (BC_1 to BC_4) populations were analysed by three SSR markers from the C1 QTL region (Table 4-1). In most cases, segregation of the marker alleles followed a 1:1 ratio (Table 4-2). In case of BC_4F_2, 96 plants were analysed by the QTL markers where expected 1:2:1 segregation for the marker alleles was found for the markers sN0248Ia (210) and sN0842 (2311); segregation for the marker sN1035R (2282) deviated significantly from the expected ratio. From this population, a total of 22 NIL families homozygous for RIL-144 allele, 16 homozygous for Hi-Q allele and 8 heterozygous for Hi-Q and RIL-144 alleles were retained for phenotypic evaluation (Appendix 4-1).

Table 4-2. Segregation for simple sequence repeats (SSR) marker alleles in different backcross generation populations for the development of near-isogenic lines of *Brassica napus* for C1 QTL

Generation	Marker name	Total plants	No. plants with			Segregation		
			Hi-Q allele	Heterozygous	RIL-144 allele	Ratio	Chi-square	*p*-value
BC_1	sN0248Ia	20	13	-	7	1 : 1	1.80	0.18
	sN1035R	20	12	-	8	1 : 1	0.80	0.37
	sN0842	20	11	-	9	1 : 1	0.20	0.65
BC_2	sN0248Ia	40	17	-	23	1 : 1	0.90	0.34
	sN1035R	40	22	-	18	1 : 1	0.40	0.53
	sN0842	40	25	-	15	1 : 1	2.50	0.11
BC_3	sN0248Ia	40	23	-	17	1 : 1	0.90	0.34
	sN1035R	40	21	-	19	1 : 1	0.10	0.75
	sN0842	40	28	-	12	1 : 1	6.40	0.011*
BC_4	sN0248Ia	40	21	-	19	1 : 1	0.10	0.75
	sN1035R	40	27	-	13	1 : 1	4.90	0.027*
	sN0842	40	18	-	22	1 : 1	0.40	0.53
BC_4F_2	sN0248Ia	96	25	47	24	1 : 2 : 1	0.06	0.97
	sN1035R	96	20	41	35	1 : 2 : 1	6.73	0.034*
	sN0842	96	23	48	25	1 : 2 : 1	0.08	0.96

* indicate observed segregation deviated significantly from the expected segregation

4.4.1.2 C9 QTL

Similar to the C1 QTL, 20-40 plants of each of the BC_1 to BC_4 generation populations were evaluated for the three C9 QTL markers (Table 4-1). In this case also, a goodness of fit of the marker data to a 1:1 segregation was found in most cases (Table 4-3). In BC_4F_2, the expected 1:2:1 segregation for the RIL-144 and Hi-Q marker alleles was found for the markers sNRG42 (313) and sN4029 (129), but not for the marker BRAS050 (726). From this population, a total of 9 NIL families homozygous for RIL-144 allele, 11 homozygous for Hi-Q allele and 3 heterozygous for Hi-Q and RIL-144 alleles were retained for phenotypic evaluation (Appendix 4-1).

Table 4-3. Segregation for simple sequence repeats (SSR) marker alleles in different backcross generation populations for the development of near-isogenic lines of *Brassica napus* for C9 QTL

Generation	Marker name	Total plants	No. plants with			Segregation		
			Hi-Q allele	Heterozygous	RIL-144 allele	Ratio	Chi-square	p-value (0.05)
BC_1	sNRG42	20	12	-	8	1 : 1	0.80	0.37
	sN4029	20	11	-	9	1 : 1	0.20	0.65
	BRAS050	20	8	-	12	1 : 1	0.80	0.37
BC_2	sNRG42	40	16	-	24	1 : 1	1.60	0.21
	sN4029	40	23	-	17	1 : 1	0.90	0.34
	BRAS050	40	21	-	19	1 : 1	0.10	0.75
BC_3	sNRG42	20	12	-	8	1 : 1	0.80	0.37
	sN4029	20	15	-	5	1 : 1	5.00	0.025*
	BRAS050	20	7	-	13	1 : 1	1.80	0.18
BC_4	sNRG42	20	13	-	7	1 : 1	1.80	0.18
	sN4029	20	16	-	4	1 : 1	7.20	0.007*
	BRAS050	20	8	-	12	1 : 1	0.80	0.37
BC_4F_2	sNRG42	96	27	45	24	1 : 2 : 1	0.56	0.75
	sN4029	96	21	47	28	1 : 2 : 1	0.77	0.68
	BRAS050	96	17	61	18	1 : 2 : 1	7.06	0.029*

* indicate observed segregation deviated significantly from the expected segregation

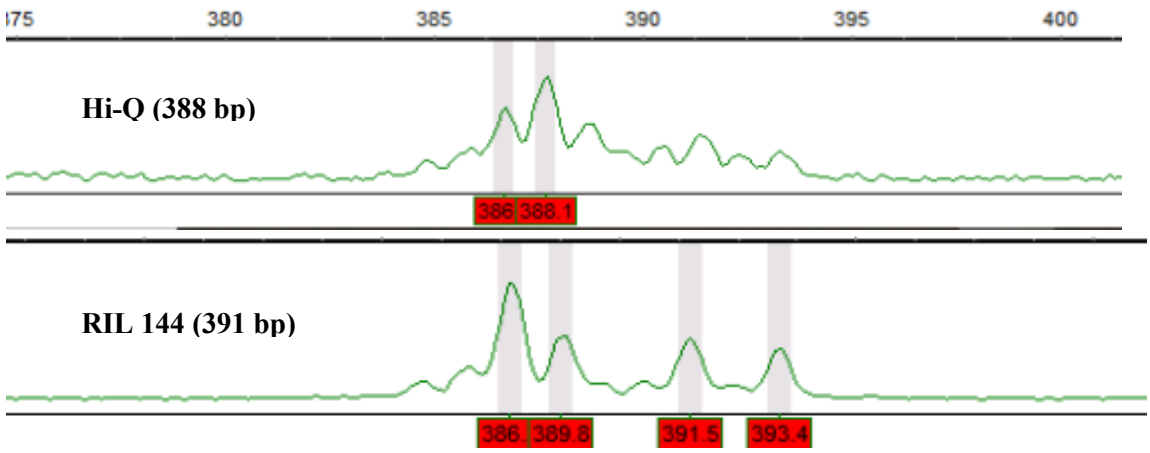


Figure 4-2. DNA fragment analysis using ABI sequencer 3730 of the *Brassica napus* plants for SSR marker sN0842 from the QTL located on C1 chromosome. ABI electropherogram of the two parents Hi-Q and RIL-144 are shown.

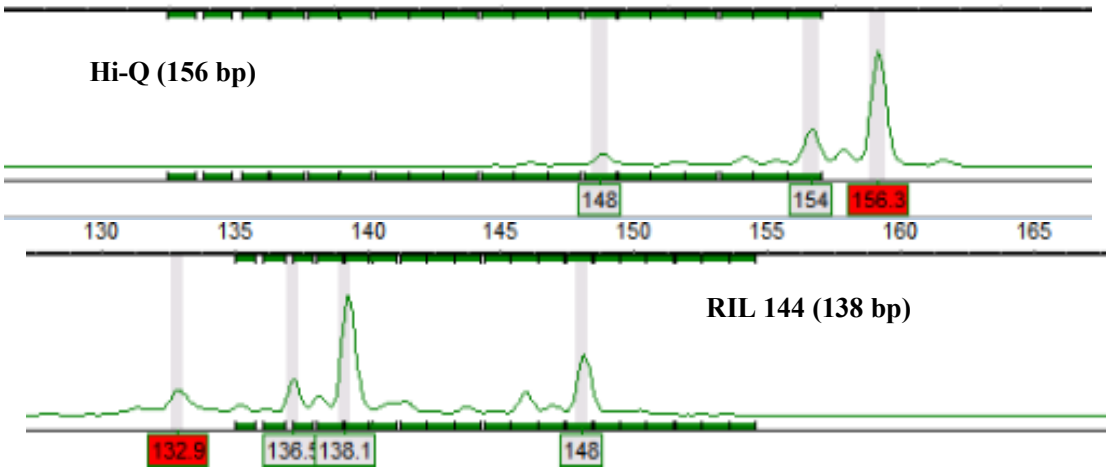


Figure 4-3. DNA fragment analysis by ABI sequencer 3730 of the *Brassica napus* plants by using SSR marker BRAS050 from the QTL located on C9 chromosome. ABI electropherogram of the two parents Hi-Q and RIL-144 are shown.

4.4.2 Phenotypic evaluation of the NILs

4.4.2.1 Correlation between two replications for days to flowering

Correlations of days to flowering data from the two experiments (replications) conducted in growth chamber was positive (r = 0.98 for C1, r = 0.93 for C9) and highly significant ($p<0.001$) (Fig. 4-4). This indicates that the individual lines behaved similarly for this trait in the two experiments.

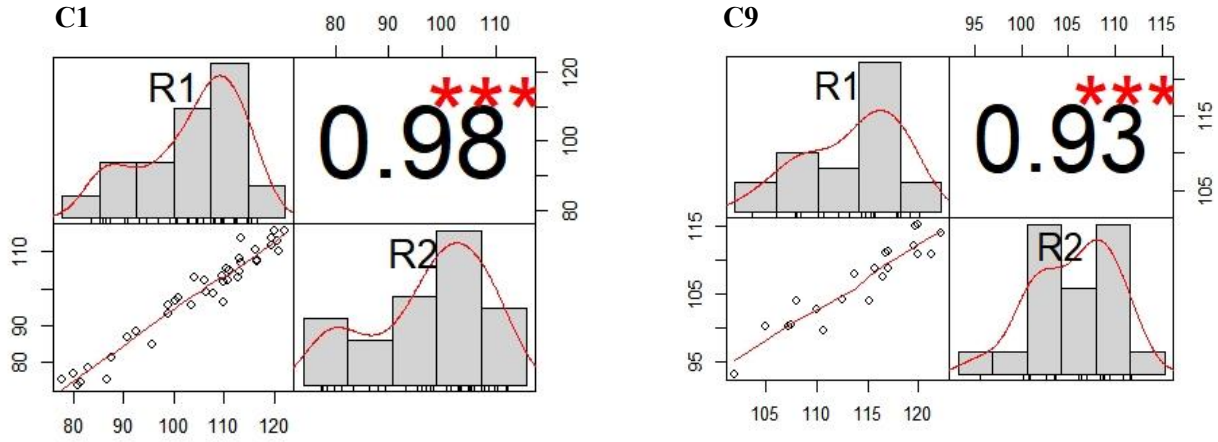


Figure 4-4. Correlation between days to flowering between two replications of the near isogenic lines (NILs) carrying Hi-Q or RIL-144 allele. The experiments conducted in a growth chamber at 10 photoperiod and 20 °C temperature. C1 and C9 indicate the NILs for two different QTL; R1 and R2 indicates the two growth chamber experiments.

4.4.2.2 C1 QTL lines

LSmean data of days to flowering, collected from two replications, followed a continuous distribution (Fig. 4-5). The NILs carrying the RIL-144 allele flowered at 75 to 103 days after seeding, while the NILs carrying the Hi-Q allele flowered at 106 to 121 days after seeding (Fig. 4-5 C1; Table 4-4). The parent lines, RIL-144 flowered at about 87 days after seeding and Hi-Q flowered at about 114 days after seeding. The NILs carrying the Hi-Q or RIL-144 alleles differed significantly for days to flowering; however, the difference between the NILs carrying the Hi-Q allele and Hi-Q and between the NILs carrying the RIL-144 allele and RIL-144 were not statistically significant (Table 4-4).

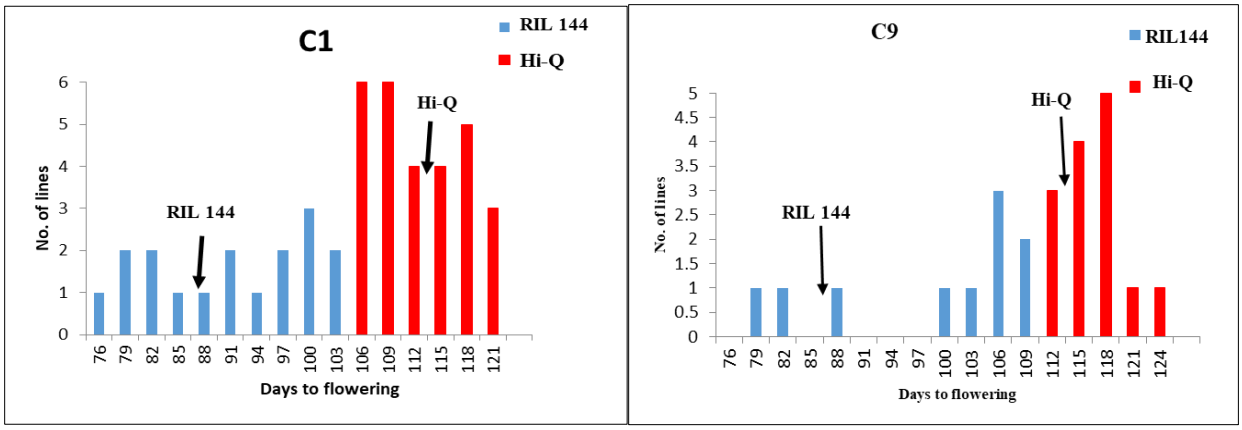


Figure 4-5. Frequency distribution of the near-isogenic lines (NILs) of *Brassica napus* for days to flowering in a growth chamber at 10 photoperiod and 20 °C constant temperature conditions. C1 and C9 indicates the NILs for the two QTL; the NILs carrying RIL-144 allele is indicated by blue colour and the NILs carrying Hi-Q allele is indicated by red color. LSmean data of two replications is presented.

4.4.2.3 C9 QTL lines

In case of the C9 QTL, the NILs carrying the RIL-144 allele flowered at 78 to 109 days after seeding while the lines carrying the Hi-Q allele flowered at 112 to 124 days after seeding (Fig. 4-5). In this case also, the NILs carrying the Hi-Q or RIL-144 alleles differed significantly for days to flowering (Table 4-4).

Table 4-4. Days to flowering of the near-isogenic lines (NILs) of *Brassica napus* for C1 and C9 QTL alleles and evaluated under 10 h photoperiod and constant 20 ℃ temperature conditions.

QTL	Allele	No. fam	Range	Mean ± SE	Allele	No. fam	Range	Mean ± SE	Allele	No. fam	Range	Mean ± SE
C1	**sN0842 (2311)**				**sN1035R (2282)**				**sN0248I(a) (210)**			
	Hi-Q (388 bp)	14	108.0 – 119.0	114.4 ± 1.1 a	Hi-Q (249 bp)	13	108.0 – 119.0	114.3 ± 1.0 a	Hi-Q (118 bp)	15	109.0 – 119.0	115.1 ± 0.8 a
	RIL-144 (391 bp)	14	76.5 - 103.7	90.4 ± 2.8 c	RIL-144 (254 bp)	13	76.5 – 103.75	92.8 ± 2.7 c	RIL-144 (113bp)	14	76.5 – 103.5	90.7 ± 2.8 c
	Het (388, 391 bp)	7	95.87 – 106.5	103.8 ± 1.4 b	Het (249, 254 bp)	6	102.9 – 107.9	105.5 ± 0.8 b	Het (113, 118 bp)	6	95.9 – 107.9	104.8 ± 1.8 b
	Hi-Q (RP)	1	113.5	113.5 ± 0 ab	Hi-Q (RP)	1	113.5	113.5 ± 0 ab	Hi-Q (RP)	1	113.5	113.5 ± 0 ab
	RIL-144 (DP)	1	86.7	86.7 ±0 c	RIL-144 (DP)	1	86.7	86.7 ± 0 c	RIL-144 (DP)	1	86.7	86.7 ± 0 c
C9	**sNRG42 (313)**				**BRAS050 (726)**				**sN4029 (129)**			
	Hi-Q (146 bp)	6	113.9 – 121.8	116.7 ± 1.4 a	Hi-Q (156 bp)	5	113.9 – 121.8	116.7 ± 1.4 a	Hi-Q (336 bp)	7	113.9 – 121.8	116.9 ±1.2 a
	RIL-144 (138 bp)	8	78 – 112.2	103.9 ± 4.2 bc	RIL-144 (138 bp)	8	78 – 112.2	101.9 ± 3.8 bc	RIL-144 (340 bp)	10	78 – 112.2	103.9 ± 3.2 bc
	Het (138, 146 bp)	2	112.9 – 113.4	113.1 ± 0.31 ab	Het (156, 138 bp)	1	112.9	112.9 ± 0 abc	Het (336, 340 bp)	2	112.9 – 113.4	113.1 ± 0.31 ab
	Hi-Q (RP)	1	113.5	113.5 ± 0 ab	Hi-Q (RP)	1	113.5	113.5 ± 0 ab	Hi-Q (RP)	1	113.5	113.5 ± 0 ab
	RIL-144 (DP)	1	86.7	86.7 ± 0 c	RIL-144 (DP)	1	86.7	86.7 ± 0 c	RIL-144 (DP)	1	86.7	86.7 ± 0 c

Note: Mean ± SE values followed by the same letter are not significantly different.
Hi-Q with allele fragment size = NIL carrying Hi-Q allele; RIL-144 with allele fragment size = NIL carrying RIL-144 allele; Hi-Q (RP) = Recurrent parent, RIL-144 (DP) = Donor parent; Het = Heterozygous for the two alleles

Table 4-5. Mean days to flowering of the near-isogenic lines (NILs) of *Brassica napus* for C1 and C9 QTL alleles and evaluated under 22 h photoperiod and 20 ℃ constant temperature conditions.

	Allele	No. fam	Range	Mean ± SE	Allele	No. fam	Range	Mean ± SE	Allele	No. fam	Range	Mean ± SE
C1	**sN0842 (2311)**				**sN1035R (2282)**				**sN0248I(a) (210)**			
	Hi-Q (388 bp)	14	31.3 – 39.0	35.1 ± 0.6 a	Hi-Q (249 bp)	13	31.3 – 39.0	34.9 ± 0.5 a	Hi-Q (118 bp)	15	31.3 – 39.0	35.1 ± 0.5 a
	RIL-144 (391 bp)	14	30.8 – 35.0	32.7 ± 0.5 b	RIL-144 (254 bp)	13	30.8 – 36.5	33.4 ± 0.5 ab	RIL-144 (113 bp)	14	30.8 – 36.5	33.2 ± 0.5 ab
	Het (388, 391 bp)	7	32.3 – 36.0	34.1 ± 0.6 ab	Het (249, 254 bp)	6	33.3 – 38.8	35.6 ± 0.8 a	Het (113, 118 bp)	6	32.3 – 38.8	34.3 ± 1.0 ab
	Hi-Q (RP)	1	37.0	37.0 ± 0 a	Hi-Q (RP)	1	37.0	37.0 ± 0 a	Hi-Q (RP)	1	37.0	37.0 ± 0 a
	RIL-144 (DP)	1	32.0	32.0 ±0 b	RIL-144 (DP)	1	32.0	32.0 ± 0 ab	RIL-144 (DP)	1	32.0	32.0 ± 0 ab
C9	**sNRG42 (313)**				**BRAS050 (726)**				**sN4029 (129)**			
	Hi-Q (146 bp)	6	31.0 – 37.5	34.9 ± 0.9 a	Hi-Q (156 bp)	5	34.8 – 37.3	35.6 ± 0.4 a	Hi-Q (336 bp)	7	31.0 – 37.5	35.1 ± 0.8 ab
	RIL-144 (138 bp)	8	30.5 – 36.0	32.8 ± 0.8 a	RIL-144 (138 bp)	8	30.5 – 35.5	32.7 ± 0.6 b	RIL-144 (340 bp)	10	30.5 – 36.0	32.6 ± 0.5 b
	Het (138, 146 bp)	2	34.8 – 36.5	35.1 ± 0.4 a	Het (156, 138 bp)	1	34.8	34.8 ± 0 ab	Het (336, 340 bp)	2	34.8 – 36.5	35.1 ± 0.4 ab
	Hi-Q (RP)	1	37.0	37.0 ± 0 a	Hi-Q (RP)	1	37.0	37.0 ± 0 a	Hi-Q (RP)	1	37.0	37.0 ± 0 a
	RIL-144 (DP)	1	32.0	32.0 ± 0 b	RIL-144 (DP)	1	32.0	32.0 ± 0 b	RIL-144 (DP)	1	32.0	32.0 ± 0 b

Note: Mean ± SE values followed by the same letter are not significantly different.
Hi-Q with allele fragment size = NIL carrying Hi-Q allele; RIL-144 with allele fragment size = NIL carrying RIL-144 allele; Hi-Q (RP) = Recurrent parent, RIL-144 (DP) = Donor parent; Het = Heterozygous for the two alleles

The above-mentioned NILs were also evaluated at 22 h photoperiod and 20 ℃ constant temperature conditions for days to flowering. Under this condition, no significant difference between the NILs carrying the Hi-Q allele or RIL-144 allele could be found; however, the NILs carrying the Hi-Q allele tended to flower later than the NILs carrying the RIL-144 allele (Table 4-5).

4.4.3 Newly designed markers

A total of 69 RAPD markers were designed from the C1 QTL region; however, none of these showed polymorphisms between the parents Hi-Q and RIL-144. In case of the two flowering time genes, the *protein EARLY FLOWERING 3-like* and *zinc finger CONSTANS-LIKE 12-like*, located in the C1 QTL region, 32 RAPD markers were designed from the gene sequences; however, none of the markers showed polymorphism between the parents. In addition to this, a total of 16 SSR markers were designed from the C1 QTL region; none of these markers also showed polymorphism between the parents.

4.5 Discussion

Flowering time is one of the important agronomic traits that affects seed yield, and also plays an important role in adaptation of the plants in new areas as well as under changing climatic conditions (for review, see Jung and Muller 2009). The yield of many crop species can reach a plateau state due to the use of similar parents or narrow genetic base population in breeding while developing new cultivars. To overcome this, there is a need to introduce genetic variation in our crop species (Huang et al. 2002). Ni et al. (2009) and Blümel et al. (2015) showed that improving the flowering time network can contribute to increased growth and yield in crops.

Flowering time being a quantitative trait in *B. napus* and is governed by a complex network of both additive and non-additive effect genes (Ringdahl et al. 1986; Long et al. 2007). To better understand the genetic mechanisms resulting in phenotypic variation for a quantitative trait, identification and fine mapping of the QTL is needed. Different mapping populations including F_2, recombinant inbred lines (RILs), DH and NILs have been used for fine mapping of genes in many crop species (Schneider 2005; Zhao et al. 2010, 2018; Zhu et al. 2020).

In this study, NILs were developed for two flowering time QTL by using the cv. Hi-Q as recurrent parent and RIL-144 as donor parent which carrying the C genome content of *B. oleracea* var. *alboglabra.* Earliness of *B. rapa* has been utilized to some extent to improve the earliness of *B. napus* (Kubik et al. 1999; Miller 2001); however, not much effort has been made to improve the earliness of *B. napus* by using the genes/alleles from the C genome of *B. oleracea*. Pedigree information and QTL mapping of days to flowering demonstrated that the donor parent RIL-144 carry early flowering alleles on chromosomes C1 and C9 which have been introgressed from *B. oleracea* var. *alboglabra* (Rahman et al. 2011, 2017, 2018). The C1 QTL affected days to flowering without being affected by photoperiod, temperature, and field trial conditions; however, this QTL allele exerts 2- to 3-times greater magnitude of effects under a short-day (10 h) photoperiod condition; while the C9 QTL was sensitive to photoperiod and exert effect only under a short-day condition (Rahman et al. 2017, 2018). Marker-assisted backcrossing enabled the introgression of these early-flowering QTL alleles in the genetic background of Hi-Q. This is evident not only from marker genotype of the plants, but also from phenotypic evaluation of the C1 and C9 NILs under a 10 h photoperiod condition. Under this condition, the NILs carrying the C1 or C9 allele of RIL-144 flowered significantly earlier than the NILs carrying the Hi-Q allele. The greater effect of the C1 and C9 QTL alleles under a short-day condition is also evident from

the evaluation of the NILs under a long-day (22 h) condition (Table 4-5). Under this condition, no striking difference between the NILs carrying Hi-Q allele or RIL-144 allele could be found as compared to evaluation of the NILs under a short-day condition. This further confirms the results reported by Rahman et al. (2018) of the effect of these two QTL under different photoperiod conditions.

To develop additional markers from the C1 QTL region, SSR and RAPD markers were designed from this QTL region as well as from the genes *protein EARLY FLOWERING 3-like* and *zinc finger CONSTANS-LIKE 12-like* found in this QTL region (Rahman et al. 2017). However, none of the markers showed polymorphism between the parents to establish their association with days to flowering. This might be due to close similarity between the two parents Hi-Q and RIL-144 for this chromosome. This is also evident from the linkage map of the nine C genome chromosomes published by Rahman et al. (2017, 2018) by using a DH population derived from Hi-Q × RIL-144. Among the nine linkage groups, the lowest density of markers (23.2 cM/marker) was found for C1; the density of markers on the remaining eight linkage groups (C2 to C9) varied from 7.6 to 19.0 cM/marker.

Chapter 5

General discussion and conclusions

5.1 General discussion

Oilseed *Brassica* is the second largest oilseed crop in the world after soybean (USDA 2019/20). Among the six species of U's triangle (U 1935), *B. napus*, *B. juncea* and *B. rapa* are grown as oilseed crop. In Canada, spring growth habit *B. napus* canola contributes about $26.7 billion annually to the economic activity of this country. Days to flowering plays an important phenomena affecting the production of this crop (for review, see Jung and Muller 2009), especially for cultivation of this crop in short summer in Canada. *B. napus* is a long-day plant which usually requires about 16 – 18 h photoperiod for flowering; however, earliness of this crop for growing under a long-day condition can be improved through identification of QTL allele contributing to earliness under short-day condition (Rahman et al. 2018) as well as QTL insensitive to photoperiod. To date, very limited studies have been carried out to identify QTL affecting flowering time under short-day condition (Cai et al. 2008; Nelson et al. 2014; Rahman et al. 2018). This MSc thesis research was conducted to identify the QTL controlling flowering time under short-day conditions by using an inbred *B. napus* population derived from six *B. napus* × *B. oleracea* interspecific crosses involving one *B. napus* line A04-73NA and six *B. oleracea* accessions, viz. *B. oleracea var. alboglabra*, var. *botrytis* cv. BARI Cauliflower-1, var. *capitata* cvs. Badger Shipper, Bindsachsener and Balbro, and var. *italica* cv. Premium Crop developed by Iftikhar et al. (2018) and Nikzad et al. (2019). The crossing design (single *B. napus* AACC × several *B. oleracea* CC) that laid out for the development of this re-constituted *B. napus* (AACC)

inbred population suggests that the inbred lines would differ primarily for the make-up of their C genome, while a limited genetic variation would be expected in the A genome.

In this study, by using phenotypic data obtained from a short-day condition (10 h photoperiod) and SNP data obtained through tGBS technique (Nikzad 2020), I identified three QTL on chromosomes C1, C5 and C9. However, I was not able to detect a conclusive QTL in the A genome, which was apparently due to limited genetic variation among the inbred lines for their A genome, as explained above. The C9 QTL could be further confirmed by designing SSR markers from the QTL region and performing single marker analysis. Among the three QTL, the C1 QTL has also been reported by Rahman et al. (2017, 2018) by using a spring *B. napus* population carrying genome contents introgressed from *B. oleracea* var. *alboglabra*; the early-flowering allele of this QTL has been introgressed from var. *alboglabra*. This research group provided evidence that this QTL has not been reported previously. In this study, by using a *B. napus* population carrying genome contents of different varieties of *B. oleracea* including var. *alboglabra*, I further confirmed this QTL. A QTL on C9 has also been reported by Rahman et al. (2018) by evaluating the above-mentioned mapping population under a short-day condition; however, the position of this QTL could not be confirmed based on SSR marker sequence from this QTL. In case of C5, no QTL has been reported previously under a short-day condition (Cai et al. 2008; Nelson et al. 2014; Rahman et al. 2018); however, QTL on this chromosome affecting flowering time has also reported by Bohuon et al. (1998) and Rae et al. (1999) in *B. oleracea*, and Raman et al. (2013) and Xu et al. (2016) in *B. napus* under field or greenhouse conditions. However, the physical position of the QTL on C5 has only been reported by Xu et al. (2016); these QTL located at about 0.1 to 0.2 Mb, 3.6 Mb and 42.8 Mb positions. In contrast, the QTL that I detected on this chromosome is located at about 30 Mb position. Thus, based on this, it is probable that the C5 QTL that I

identified in this study is a novel one; this QTL has not been reported previously under a short-day condition.

The phenotyping of diverse *B. napus* lines collected from gene bank, University of Alberta and Nutrien Ag Solutions (NAS) under 10 h photoperiod conditions provided the evidence about existence of significant genetic diversity for earliness of flowering in spring *B. napus* that could be used for breeding cultivars suitable for short day conditions. Evaluation of the C9 QTL markers on this population provided evidence for the occurrence of this QTL in natural *B. napus* population.

Different mapping populations including NILs has been developed and used for fine mapping of different traits in different crop species including *Brassica* (Chen et al. 2016; Habib et al. 2017; Oiested et al. 2017; Zhang et al. 2018; Zhao et al. 2018; Chai et al. 2019; Gao et al. 2019; Mia et al. 2019; Zhu et al. 2020). In this study, NILs were developed for two QTL affecting days to flowering by using the cv. Hi-Q as recurrent parent, and RIL-144 as donor parent. The donor parent RIL-144 carried early flowering alleles on chromosomes C1 and C9 which have been introgressed from *B. oleracea* var. *alboglabra* (Rahman et al. 2011, 2017, 2018). Marker-assisted backcrossing enabled the introgression of these early-flowering QTL alleles in the genetic background of Hi-Q; this is also is evident from phenotypic evaluation of the C1 and C9 NILs under a 10 h photoperiod condition.

Thus, this MSc thesis research

- ✓ Identified QTL on chromosomes C1, C5 and C9 affecting days to flowering under a short-day condition by using inbred lines derived from *B. napus* × *B. oleracea* interspecific crosses.

- ✓ Developed SSR markers from the QTL region of the chromosome C9 for use in breeding for earliness of flowering.
- ✓ Identified naturally occurring oilseed *B. napus* germplasm which apparently carry alleles for earliness of flowering under a short-day condition.
- ✓ Developed NILs for days to flowering alleles of C1 and C9 QTL, where the early-flowering alleles derived from *B. oleracea*.

5.2 Conclusions

The main conclusions drawn from this research includes:

- ✓ The chromosomes C1, C5 and C9 carry QTL affecting days to flowering under a short-day condition.
- ✓ SSR markers developed from the QTL region of C9 can be used in breeding for earliness of flowering.
- ✓ The alleles for earliness of flowering under a short-day condition can be found in natural oilseed *B. napus* germplasm.
- ✓ Confirmed that the C1 and C9 QTL alleles of *B. oleracea* var. *alboglabra* contribute to earliness of flowering under a short-day condition.

5.3 Future research

- ➢ The NILs carrying the C1 and C9 QTL can be used for fine mapping of these genomic regions.
- ➢ The C1, C5 and C9 QTL and the molecular markers can be used for the improvement of earliness flowering and maturity of *B. napus*.

- The knowledge of the QTL regions and the early-flowering germplasm identified can be used for better understanding of the molecular basis of flowering under short-day conditions.

References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K and Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator *FT* at the shoot apex. Science 309:1052–1056.

Akbar M A (1987) Artificial *Brassica napus* flowering in Bangladesh. Theor Appl Genet 73:465-468.

Amasino R M, Michaels S D (2010) The timing of flowering. Plant Physiol 154: 516–20.

Andrés F and Coupland G (2012) The genetic basis of flowering responses to seasonal cues. Genetics 13: 627- 39.

Angadi S V, Cutforth H W, Miller P R, McConkey B G, Entz M H, Brandt S A, et al. (2000) Response of three *Brassica* species to high temperature stress during reproductive growth. Can J Plant Sci 80: 693 - 701.

Arifuzzaman M, Mamidi S, McClean P, Rahman M (2016). QTL mapping for root vigor and days to flowering in *Brassica napus* L. Can J of Plant Sci. 97.

Ausín I, Alonso-Blanco C, Martínez-Zapater J M (2005) Environmental regulation of flowering. Int J Dev Biol 49: 689-705.

Axelsson T, Shavorskaya O, Lagercrantz U (2001) Multiple flowering time QTL within several *Brassica* species could be the result of duplicated copies of one ancestral gene. Genome 44: 856–864.

Basten C J, Weir B S and Z.-B. Zeng Z B (1994) Zmap-a QTL cartographer. IN Proceedings of the 5th World Congress on Genetics Applied to Livestock Production: Computing Strategies and Software, edited by C. Smith, J. S. Gavora, B. Benkel, J. Chesnais, W. Fairfull, J. P. Gibson, B. W. Kennedy and E. B. Burnside. Volume 22, pages 65-66. Published by the

Organizing Committee, 5th World Congress on Genetics Applied to Livestock Production, Guelph, Ontario, Canada.

Bennett R A, Séguin-Swartz G and Rahman H (2012) Broadening genetic diversity in canola using C-genome species *Brassica oleracea* L. Crop Sci. 52:2030–2039. doi:10.2135/cropsci2011.11.0580

Bennett R A, Thiagarajah M R, King J R and Rahman M H (2008) Interspecific cross of *Brassica oleracea* var. *alboglabra* and *B. napus*: effects of growth condition and silique age on the efficiency of hybrid production, and inheritance of erucic acid in the self-pollinated backcross generation. Euphytica 164:593-601.

Blümel M, Dally N and Jung C (2015) Flowering time regulation in crops-what did we learn from *Arabidopsis*? Current opinion in biotechnology 32:121–129.

Bohuon E J R, Ramsay L D, Craft J A, Arthur A E, arshall D F, Lydiate D J et al. (1998) The association of flowering time quantitative trait loci with duplicated regions and candidate loci in *Brassica oleracea*. Genetics 150: 393–401.

Bonhomme F, Kurz B, Melzer S, Bernier G, Jacqmard A (2000) Cytokinin and gibberellin activate SaMADS A, a gene apparently involved in regulation of the floral transition in *Sinapis alba*. Plant J 24: 103-11.

Borges C V, Junior S S, Ponce F S, and Lima G P P (2018) Agronomic Factors Influencing *Brassica* Productivity and Phytochemical Quality. *Brassica* Germplasm - Characterization, Breeding and Utilization.

Bradbury P J, Zhang Z, Kroon D E, Casstevens T M, Ramdoss Y, Buckler E S (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23(19):2633-5.

Bus, A., N. Korber, R. J. Snowdon, and B. Stich, 2011: Patterns of molecular variation in a species-wide germplasm set of *Brassica napus*. Theor. Appl. Genet. 123: 1413-1423.

Canola Council of Canada 2017 (canolacouncil.org.).

Crop life Canada, 2019 (croplife.ca/canola-in-canada).

Cai C C, Tu J X, Fu T D, Chen B Y (2008) The genetic basis of flowering time and photoperiod sensitivity in rapeseed *Brassica napus* L. Russian J Genet. 44: 326–333.

Camargo L E A, Osborn T C (1996) Mapping loci controlling flowering time in *Brassica oleracea*. Theor Appl Genet 92: 610–616.

Cartea E, Haro-Bailón A D, Padilla G, Obregón-Cano S , Rio-Celestino M and Ordás A (2019) Seed Oil Quality of *Brassica napus* and *Brassica rapa* Germplasm from Northwestern Spain. Foods 8: 292.

Chaghakaboodi Z, Kahrizi D, Zebarjadi A (2012) Heritability and genetic advance in rapeseed *(Brassica napus* L.). Iranian Journal of Genetics and Plant Breeding 1(2): 16-21.

Chai L, Zhang J, Lu K, Li H, Wu L, Wan H, Zheng B, Cui C, Jiang J and Jiang L (2019) Identification of genomic regions associated with multi-silique trait in *Brassica napus*. BMC Genomics 20:304.

Chalhoub B, Denoeud F, Liu S, Parkin I A P, Tang H, Wang S, Chiquet J, Belcram H, Tong C, Samans B, Corréa M, Da Silva C, Just J, Falentin C, Koh C S, Le Clainche I, Bernard M, Bento P, Noel B, Labadie K, Alberti A, Charles M, Arnaud D, Guo H, Daviaud C, Alamery S, Jabbari K, Zhao M, Edger P P, Chelaifa H, Tack D, Lassalle G, Mestiri I, Schnel N, Le Paslier M-C, Fan G, Renault V, Bayer P E, Golicz A A, Manoli S, Lee T-H, Thi V H D, Chalabi S, Hu Q, Fan C, Tollenaere R, Lu Y, Battail C, Shen J, Sidebottom C H D,Wang X, Canaguier A, Chauveau A, Bérard A, Deniot G, Guan M, Liu Z, Sun F, Lim

Y P, Lyons E, Town CD,Bancroft I,Wang X, Meng J,Ma J, Pires J C, King GJ, Brunel D, Delourme R, Renard M, Aury J-M, Adams KL, Batley J, Snowdon RJ, Tost J, Edwards D, Zhou Y, Hua W, Sharpe A G, Paterson AH, Guan C,Wincker P (2014) Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science 345:950–953.

Chandran S, Pukalenthy B, Adhimoolam K, Manickam D, Sampathrajan V, Chocklingam V, Eswaran K, Arunachalam K, Joikumar meetei L, Rajasekaran R, Muthusamy V, Hossain F and Natesan S (2019) Marker-Assisted Selection to Pyramid the Opaque-2 (O2) and β-Carotene (crtRB1) Genes in Maize. Front. Genet. 10:859.

Chen J, Pang W, Chen B, Zhang C and Piao Z (2016) Transcriptome Analysis of *Brassica rapa* Near-Isogenic Lines Carrying Clubroot-Resistant and –Susceptible Alleles in Response to *Plasmodiophora brassicae* during Early Infection. Front. Plant Sci. 6:1183.

Chen G, Geng J, Rahman M, Liu X, Tu J, Fu T et al. (2010) Identification of QTL for oil content, seed yield, and flowering time in oilseed rape (*Brassica napus*). Euphytica 175: 161–174.

Chen Z J, Ni Z (2006) Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bioessays 28: 240-52.

Chen X A (2004) MicroRNA as a translational repressor of *APETALA2* in *Arabidopsis* flower development. Science. 26:303.

Cho L H, Yoon J and An G (2017) The control of flowering time by environmental factors. Plant J 90: 708-719.

Cockram J, Mackay I J (2018) Genetic mapping populations for conducting high-resolution trait mapping in plants. In: Varshney RK, Pandey MK, Chitikineni A (eds) Plant genetics and molecular biology. Springer, Cham, Switzerland, p 109–138.

Daware A, Das S, Srivastava R, Badoni S, Singh A K, Agarwal P, Parida S K and Tyagi A K (2016) An Efficient Strategy Combining SSR Markers- and Advanced QTL-seq-driven QTL Mapping Unravels Candidate Genes Regulating Grain Weight in Rice. Front. Plant Sci. 7:1535.

Debernardi J M, Greenwood J R, Jean Finnegan E, Jernstedt J, Dubcovsky J (2020) *APETALA 2*-like genes *AP2L2* and Q specify lemma identity and axillary floral meristem development in wheat. Plant J. 101(1):171-187.

Dixon G R (2006) Vegetable *Brassicas* and related crucifers. Crop production science in horticulture. Int J Veg Sci 14: 93.

Duan W, Zhang H, Zhang B, Wu X, Shao S, Li Y, Hou X, Liu T (2017) Role of vernalization-mediated demethylation in the floral transition of *Brassica rapa*. Planta. 245: 227–233.

Eskin N A M and Przybylski R (2003) Rapeseed oil/ Canola. Encyclopedia of Food Sciences and Nutrition. 4911-4916.

Ferreira M E, Satagopan J, Yandell B S, Williams P H, Osborn T C (1995) Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theor Appl Genet 90: 727–732.

Flint-Garcia S A, Thuillet A C, Yu J, Pressoir G, Romero S M, Mitchell S E, Doebley J, Kresovich S, Goodman M M and Buckler E S (2005) Maize association population: a high-resolution platform for quantitative trait locus dissection. Plant J. 44: 1054–1064.

Flint-Garcia S A, Thornsberry J M and Buckler E S (2003) Structure of linkage disequilibrium in plants. *Annu Rev Plant Biol* 54**:** 357-74

Gan Y, Harker K N, Kutcher H R, Gulden R H, Irvine B, May W E (2016) Canola seed yield and phenological responses to plant density. Can J Plant Sci. 96: 151–159.

Gan Y, Angadi S V, Cutforth H, Potts D, Angadi V V, McDonald C L (2004) Canola and mustard response to short periods of temperature and water stress at different developmental stages. Can J Plant Sci. 84:697–704.

Gao S, Zheng Z, Powell J, Habib A, Stiller J, Zhou M and Liu C (2019) Validation and delineation of a locus conferring Fusarium crown rot resistance on 1HL in barley by analysing transcriptomes from multiple pairs of near isogenic lines. BMC Genomics 20:650.

Gu X, Le C, Wang Y, Li Z, Jiang D, Wang Y and He Y (2013) *Arabidopsis* FLC clade members form flowering-repressor complexes coordinating responses to endogenous and environmental cues. Nat Commun 4:1947.

Guo Y, Wu Y, Anderson J A, Moss J Q, Zhu L, Fu J (2017) SSR Marker Development, Linkage Mapping, and QTL Analysis for Establishment Rate in Common Bermudagrass. Plant Genome. 10(1). doi: 10.3835/plantgenome2016.07.0074. PMID: 28464062.

Habekotte B (1997) Options for increasing seed yield of winter oilseed rape (*Brassica napus* L.): a simulation study. Field Crops Res. 54:109–126.

Habib A, Powell J J, Stiller J, Liu M, Shabala S, Zhou M, Gardiner D M, Liu C (2017) A multiple near isogenic line (multi-NIL) RNA-seq approach to identify candidate genes underpinning QTL. Theor Appl Genet. (doi.10.1007/s00122-017-3023-0).

Harmer S L (2009) The circadian system in higher plants. Annu Rev Plant Biol. 60, 357–377.

He Y, Michaels S D, Amasino R M (2003) Regulation of flowering time by histone acetylation in *Arabidopsis.* Science 302: 1751-4.

Hearne, C. M., S. Ghosh, and J. A. Todd, 1992: Microsatellites for linkage analysis of genetic traits. Trends Genet. 8: 288-294.

Hepworth J, Antoniou-Kourounioti R L, Bloomer R H, Selga C, Berggren K, Cox D, Collier Harris B R, Irwin J A, Holm S, Sall T (2018) Absence of warmth permits epigenetic memory of winter in *Arabidopsis*. Nat Commun. 9: 639.

Hicks K A, Albertson T M and Wagner D R (2001) EARLY FLOWERING3 encodes a novel protein that regulates circadian clock function and flowering in *Arabidopsis*. The Plant cell, 13(6), 1281–1292.

Huang F, Liu T, Wang J, Hou X (2018) Isolation and functional characterization of a floral repressor, *BcFLC2*, from Pak-choi (*Brassica rapa* ssp. *Chinensis*). Planta 248:423-435.

Huang J, Pray C and Rozelle S (2002) Enhancing the crops to feed the poor. Nature 418: 678–684.

Iftikhar R, Wang X and Rahman H (2018) Broadening the genetic base of *Brassica napus* canola by interspecific crosses with different variants of *B. oleracea*. Euphytica 214:133–149.

Imaizumi T, Schultz T F, Harmon F G, Ho L A, Kay S A (2005) FKF1F BOX protein mediates cyclic degradation of a repressor of *CONSTANS* in *Arabidopsis*. Science 309:293–297.

Irwin J A, Lister C, Soumpourou E, Zhang Y, Howell EC, Teakle G, Dean C (2012) Functional alleles of the flowering time regulator FRIGIDA in the *Brassica oleracea* genome. BMC Plant Biol 12:21.

Jang K, Lee H G, Jung S J, Paek N C, Seo P J (2015) The E3 Ubiquitin Ligase *COP1* Regulates Thermosensory Flowering by Triggering *GI* Degradation in *Arabidopsis*. Scientific Reports 5, 12071.

Jang S, Marchal V, Panigrahi K, Wenkel S, Soppe W, Deng X, Valverde F, Coupland G (2008) *Arabidopsis* COP1 shapes the temporal pattern of CO accumulation conferring a photoperiod flowering response. The EMBO journal. 27:1277-88.

Jian H, Zhang A, Ma J, Wang T, Yang B, Shuang L S, Liu M, Li J, Xu X, Paterson A H and Liu L (2019) Joint QTL mapping and transcriptome sequencing analysis reveal candidate flowering time genes in *Brassica napus* L. BMC Genomics 20: 21.

Jing L, Bo L, Feng C, Jianli L, Xiaowu W, and Jia W (2016) A High Density Linkage Map Facilitates QTL Mapping of Flowering Time in *Brassica rapa.* Hortic Plant J 2 (4)*:* 217–223.

Jung C and Muller A E (2009) Flowering time control and applications in plant breeding. Trends in Plant Sci 14: 563-573.

Kawanabe T, Osabe K, Itabashi E, Okazaki K, Dennis E S, Fujimoto R (2016) Development of primer sets that can verify the enrichment of histone modifications, and their application to examining vernalization-mediated chromatin changes in *Brassica rapa* L. Genes Genet. Syst. 91: 1–10.

Kearsey M J (1998) The principles of QTL analysis (a minimal mathematics approach). Journal of Experimental Botany 49: 1619–1623.

Kim J-H, Chung IK, Kim K-M (2017) Construction of a genetic map using EST-SSR markers and QTL analysis of major agronomic characters in hexaploid sweet potato (*Ipomoea batatas* (L.) Lam). PLoS ONE 12(10): e0185073.

King J R, Kondra Z P (1986) Photoperiod response of spring oilseed rape (*Brassica napus* L. and *B. campestris* L.). Field Crops Res 13: 367–373.

Kubik T J, Hawkins G P, Stringam G R (1999) Cytological stability of doubled haploid lines derived from interspecific crosses between *B. napus* L. and *B. rapa* L. Proc 10th Int Rapeseed Congr, Australia.

Lagercrantz U and Axelsson T (2000) Rapid evolution of the family of *CONSTANS LIKE* genes in plants. Mol Biol Evol. 17: 1499–1507.

Laubinger S, Marchal V, Gentilhomme J, Wenkel S, Adrian J, Jang S, Kulajta C, Braun H, Coupland G, Hoecker U (2006) *Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer *CONSTANS* to regulate its stability. Development 133:3213–3222.

Leijten W, Koes R, Roobeek I and Frugi G (2018) Translating Flowering Time from *Arabidopsis thaliana* to *Brassicaceae* and *Asteraceae* Crop Species. Plants 7: 111.

Lenth, R. V. (2016) Least-Squares Means: The R Package lsmeans. J. Stat. Softw. 69(1): 1–33.

Li R, Jeong K, Davis J T, Kim S, Lee S, Michelmore R W, Kim S, Maloof J N (2018) Integrated QTL and eQTL Mapping Provides Insights and Candidate Genes for Fatty Acid Composition, Flowering Time, and Growth Traits in a F_2 Population of a Novel Synthetic Allopolyploid *Brassica napus.* Frontiers in Plant Science 9: 16-32.

Li X, Zhou Z, Ding J, Wu Y, Zhou B, Wang R, Ma J, Wang S, Zhang X, Xia Z, Chen J and Wu J (2016) Combined Linkage and Association Mapping Reveals QTL and Candidate Genes for Plant and Ear Height in Maize. Front. Plant Sci. 7:833.

Liang F, Deng Q, Wang Y, Xiong Y, Jin D, Li J, Wang B (2004) Molecular marker-assisted selection for yield-enhancing genes in the progeny of "9311× *O. rufipogon*" using SSR. Euphytica. 139. 159-165. 10.1007/s10681-004-2560-1.

Lim G H, Hoey T, Zhu S, Clavel M, Yu K, Navarre D (2018) COP1, a negative regulator of photomorphogenesis, positively regulates plant disease resistance via double-stranded RNA binding proteins. PLoS Pathog 14(3): e1006894.

Lim M H, Kim J, Kim Y S, Chung K S, Seo Y H, Lee I, Kim J, Hong C B, Kim H J, Park C M (2004) A new *Arabidopsis* gene, FLK, encodes an RNA binding protein with K homology motifs and regulates flowering time via *FLOWERING LOCUS C*. Plant Cell.16:731-740.

Lin K, Zhao H, Gan S, Li G (2019) *Arabidopsis* ELF4-like proteins EFL1 and EFL3 influence flowering time. Gene 700:131-138.

Lipka A E, Tian F, Wang Q S, Peiffer J, Li M, Bradbury P J, Gore M A, Buckler E S and Zhang Z (2012) GAPIT: genome association and prediction integrated tool. Bioinformatics 28:2397– 2399.

Liu H, Du D, Guo S, Xiao L, Zhao Z, Zhao Z, Xing X, Tang G, Xu L, Fu Z, Yao Y, Duncan RW (2016) QTL analysis and the development of closely linked markers for days to flowering in spring oilseed rape (*Brassica napus* L.). Mol Breed 36: 52.

Liu X, Huang M, Fan B, Buckler ES, Zhang Z (2016) Iterative Usage of Fixed and Random Effect Models for Powerful and Efficient Genome-Wide Association Studies. PLoS Genet 12(2): e1005767.

Liu F, Marquardt S, Lister C, Swiezewski S, Dean C (2010) Targeted 3' processing of antisense transcripts triggers *Arabidopsis FLC* chromatin silencing. Science 327: 94–97.

Liu L J, Zhang Y C, Li Q H, Sang Y, Mao J, Lian H L, Wang L, Yang H Q (2008) COP1-mediated ubiquitination of *CONSTANS* is implicated in cryptochrome regulation of flowering in *Arabidopsis*. Plant Cell 20(2):292-306.

Long W, Hu M, Gao J, Sun L, Zhang J and Pu H (2019) Identification and application of markers closely linked to the restorer gene (Rfm) in rapeseed (*Brassica napus* L.) Breed Sci 69: 316–322.

Long Y, Shi J, Qiu D, Li R, Zhang C, Wang J et al. (2007) Flowering time quantitative trait loci analysis of oilseed *Brassica* in multiple environments and genome wide alignment with *Arabidopsis.* Genetics 177: 2433–2444.

Lou P, Zhao J, Kim J S, Shen S, Del Carpio D P, Song X, Jin M, Vreugdenhil D, Wang X, Koornneef M, Bonnema G (2007) Quantitative trait loci for flowering time and morphological traits in multiple populations of *Brassica rapa*. J Exp Bot 58: 4005–4016.

Lu K, Wei L, Li X, Wang Y, Wu J, Liu M , Zhang C , Chen Z , Xiao Z , Jian H , Cheng F, Zhang K , Du H, Cheng X, Qu C, Qian W, Liu L, Wang R, Zou Q , Ying J , Xu X, Mei J, Liang Y, Chai Y R, Tang Z, Wan H, Ni Y, He Y , Lin N , Fan Y , Sun W , Li N N, Zhou G, Zheng H, Wang X, Paterson A H and Li J (2019) Whole-genome resequencing reveals *Brassica napus* origin and genetic loci involved in its improvement. Nature Communications 10: 1154.

Luo Y X, Luo C Y, Du D Z, Fu Z, Yao Y M, Xu C C and Zhang H S (2014) Quantitative trait analysis of flowering time in spring rapeseed (*B. napus* L.). Euphytica 200:321–335.

Mackay L and Powell W (2007) Methods for linkage disequilibrium mapping in crops. Trends Plant Sci 12:57-63.

Macknight R, Bancroft I, Page T, Lister C, Schmidt R, Love K, Westphal L, Murphy G, Sherson S, Cobbett C, Dean C (1997) FCA, a gene controlling flowering time in *Arabidopsis*, encodes a protein containing RNA-binding domains. Cell 89:737-745.

Maheshwari P N, Stanley D W and Gray J I (1981) Detoxification of Rapeseed Products. J Food Prot. 44: 459-470.

Mei D S, Wang H Z, Hu Q, Li Y D, Xu Y S, Li Y C (2009) QTL analysis on plant height and flowering time in *Brassica napus*. Plant Breed 128:458–465.

Mia M S, Liu H, Wang X and Yan G (2019) Multiple Near-Isogenic Lines Targeting a QTL Hotspot of Drought Tolerance Showed Contrasting Performance Under Post-anthesis Water Stress. Front. Plant Sci. 10:271.

Michaels S D and Amasino R M (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell. 11(5):949-56.

Miller T A (2001) Agronomic and quality performance of three doubled haploid lines derived from a *Brassica napus/ Brassica rapa* interspecific cross. M.Sc. thesis, University of Alberta, Edmonton.

Mouradov A, Cremer F, Coupland G (2002) Control of flowering time: interacting pathways as a basis for diversity. Plant Cell 14: 111-30.

Nagaharu U (1935) Genome analysis in Brassica with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot 7: 389–452.

Nega T and Woldes Y (2018) Review on nutritional limitations and opportunities of using rapeseed meal and other rape seed by-products in animal feeding. *J Nutr Health Food Eng*. 8(1): 43-48.

Nelson M N, Rajasekaran R, Smith A, Chen S, Beeck C P, Siddique K H et al. (2014) Quantitative trait loci for thermal time to flowering and photoperiod responsiveness discovered in summer annual-type *Brassica napus* L. PLoS One 9:e102611.

Ni Z, Kim E D, Ha M, Lackey E, Liu J, Zhang Y, Sun Q, Chen Z J (2009) Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. Nature 457: 327-31.

Nikzad A (2020) Potential of the *Brassica oleracea* gene pool for the improvement of spring *B. napus* canola. Ph.d thesis, University of Alberta, Edmonton.

Nikzad A, Kebede B D, Pinzon J, Bhavikkumar J, Wang X, Yang R C and Rahman H (2019) Potential of the C genome of the different variants of *Brassica oleracea* for heterosis in spring *B. napus* canola. Front Plant Sci 10: 1691.

Nishioka M, Tamura K, Hayashi M, Fujimori Y, Ohkawa Y, Kuginuki Y, Harada K (2005) Mapping of QTLs for Bolting Time in *Brassica rapa* (syn. *campestris*) under Different Environmental Conditions. Breeding 55: 127-133.

Nusinow D A, Helfer A, Hamilton E E, King J J , Imaizumi T, Schultz T F, Farré E M, Kay S A (2011) The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature. 475(7356):398-402.

Ogawa T, Uchimiya H, Kawai-Yamada M (2007) Mutual regulation of *Arabidopsis thaliana* ethylene-responsive element binding protein and a plant floral homeotic gene, *APETALA2*. Ann Bot 99(2):239-44.

Oh S, Zhang H, Ludwig P, van Nocker S (2004) A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the *Arabidopsis FLC*/*MAF MADS* box gene family. Plant Cell. 16(11):2940-53.

Oiestad A J, Martin J M, Cook J, Varella A C and Giroux M J (2017) Identification of Candidate Genes Responsible for Stem Pith Production Using Expression Analysis in Solid-Stemmed Wheat. The plant genome 10: 2.

Okazaki K, Sakamoto K, Kikuchi R, Saito A, Togashi E, Kuginuki Y, Matsumoto S, Hirai M (2007) Mapping and characterization of FLC homologs and QTL analysis of flowering time in *Brassica oleracea*. Theor Appl Genet 114: 595–608.

Ott A, Liu S, Schnable J C, Yeh C T E, Wang K S and Schnable P S (2017) tGBS® genotyping-by sequencing enables reliable genotyping of heterozygous loci. Nucleic Acids Res. 45:e178.

Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T, Sato H, Sato H, Hori M, Nakamura Y, Tanaka T. Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. Nat Genet. 2002 Dec; 32(4):650-4.

Prakash, S., X. M. Wu, and S. R. Bhat, 2012: History, Evolution, and Domestication of *Brassica* Crops. Plant Breed. Rev. 35: 19-84.

Plieske, J., and D. Struss, 2001: Microsatellite markers for genome analysis in *Brassica*. I. Development in *Brassica napus* and abundance in Brassicaceae species. Theor. Appl. Genet. 102: 689-694.

Powell W, Machray G C and Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 1:215–222. doi:10.1016/S1360-1385(96)86898-0

Qian W, Chen X, Fu D, Zou J and Meng J (2005) Intersubgenomic heterosis in seed yield potential observed in a new type of *Brassica napus* introgressed with partial *Brassica rapa* genome. Theor. Appl. Genet. 110:1187–1194.

Qin W, Yu Y, Jin Y, Wang X, Liu J, Xi J, Li Z, Li H, Zhao G, Hu W, Chen C, Li F, Yang Z (2018) Genome-Wide Analysis Elucidates the Role of *CONSTANS-like* Genes in Stress Responses of Cotton. Int J Mol Sci. 19(9):2658.

R Studio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

Rae A M, Howell E C, Kearsey M J (1999) More QTL for flowering time revealed by substitution lines in *Brassica oleracea*. Heredity 83:586–596.

Rahman H, Bennett RA, Kebede B (2018) Molecular mapping of QTL alleles of *Brassica oleracea* affecting days to flowering and photosensitivity in spring *Brassica napus*. PLoS ONE 13(1): e0189723.

Rahman H, Bennett R A, Kebede B (2017) Mapping of days to flower and seed yield in spring oilseed *Brassica napus* carrying genome content introgressed from *Brassica oleracea*. Mol Breeding 37:5.

Rahman M H (2013) Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic Germplasm. Can. J. Plant Sci. 10: 24-29.

Rahman M H, Bennett R A, Yang R-C, Kebede B and Thiagarajah M R (2011) Exploitation of the late flowering species *Brassica oleracea* L. for the improvement of earliness in *B. napus* L.: an untraditional approach. *Euphytica* 177: 365-374.

Raman H, Raman R, Eckermann P, Coombes N,Manoli S, Zou X, Edwards D, Meng J, Prangnell R, Stiller J, Batley J, Luckett D, Wratten N, Dennis E (2013) Genetic and physical mapping of flowering time loci in canola (*Brassica napus* L.). Theor Appl Genet 126:119–132.

Ridge S, Deokar A, Lee R, Daba K, Macknight R C, Weller J L, Tar'an B (2017) The Chickpea *Early Flowering 1* (*Efl1*) Locus Is an Ortholog of *Arabidopsis ELF3*. Plant Physiol 175(2):802-815.

Ringdahl E A, McVetty P B E, Sernyk J L (1986) Inheritance of earliness, height, and leaf number in crosses of early maturing rapeseed. Can J Genet Cytol 28:1009–1015.

Robert L S, Robson F, Sharpe A, Lydiate D, Coupland G (1998) Conserved structure and function of the *Arabidopsis* flowering time gene *CONSTANS* in *Brassica napus*. Plant Mol Biol 37: 763-772.

Robertson M J, Watkinson A R, Kirkegaard J A, Holland J F, Potter T D, Burton W, et al. (2002) Environmental and genotypic control of time to flowering in canola and Indian mustard. Australian J Agric Res 53:793-809.

Rolauffs S, Fackendahl P, Sahm J, Fiene G, Hoecker U (2015) *Arabidopsis* COP1 and SPA genes are essential for plant elongation but not for acceleration of flowering time in response to a low red light to far-red light ratio. Plant Physiol 160(4):2015-27.

Sahin E C, Kalenderoglu, A, Aydın Y, Evci G and Uncuoglu A A (2018). SSR Markers Suitable for Marker Assisted Selection in Sunflower for Downy Mildew Resistance, *Open Life Sciences*, *13*(1), 319-326.

Saito H, Ogiso-Tanaka E, Okumoto Y, Yoshitake Y, Izumi H, Yokoo T, Matsubara K, Hori K, Yano M, Inoue H, Tanisaka T (2012) *Ef7* Encodes an ELF3-like Protein and Promotes Rice Flowering by Negatively Regulating the Floral Repressor Gene *Ghd7* under Both Short- and Long-Day Conditions, Plant and Cell Physiology 53(4): 717–728.

Sawa M, Nusinow D A, Kay S A, Imaizumi T (2007) FKF1 and GIGANTEA complex formation is required for day-length measurement in *Arabidopsis*. Science 318:261–265.

Schiessl S V, Huettel B, Kuehn D, Reinhardt R and Snowdon R J (2017) Flowering time gene variation in *Brassica* species shows Evolutionary principles. Front Plant Sci 8: 1742.

Schmitz R J, Amasino R M (2007) Vernalization: a model for investigating epigenetics and eukaryotic gene regulation in plants. Biochim Biophys Acta 1769: 269-75.

Schneider K (2005) Mapping populations and principles of Genetic mapping. doi. 10.1002/3527603514.ch1.

Schomburg F M, Patton D A, Meinke D W, Amasino R M (2001) FPA, a gene involved in floral induction in *Arabidopsis*, encodes a protein containing RNA-recognition motifs. Plant Cell 13:1427-1436.

Sheerin D J, Menon C, zur Oven-Krockhaus S, Enderle B, Zhu L, Johnen P, Schleifenbaum F, Stierhof YD, Huq E, Hiltbrunner A (2015) Light-activated phytochrome A and B interact with members of the SPA family to promote photomorphogenesis in *Arabidopsis* by reorganizing the COP1/SPA complex. Plant Cell 27:189–201

Sheldon C C, Rouse D T, Finnegan E J, Peacock W J, Dennis E S (2000) The molecular basis of vernalization: the central role of *FLOWERING LOCUS C* (*FLC*). Proc Natl Acad Sci USA. 97(7):3753-8.

Sheldon C C, Burn J E, Perez P P, Metzger J, Edwards J A, Peacock W J, Dennis E S (1999) The FLF MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. Plant Cell. 11(3):445-58.

Shiranifar B, Hobson N, Kebede B, Yang R-C, Rahman H (2020) Potential of rutabaga (*Brassica napus* var. *napobrassica*) gene pool for use in the breeding of *B. napus* canola. Crop Science. 60:157–171.

Silva C S, Nayak A, Lai X, Hutin S, Hugouvieux V, Jung J H, López-Vidriero I, Franco-Zorrilla J M, Panigrahi K C S, Nanao M H, Wigge P A, Zubieta C (2020) Molecular mechanisms of Evening Complex activity in *Arabidopsis*. Proc Natl Acad Sci U S A. 117(12):6901-6909.

Simpson G G (2004) The autonomous pathway: epigenetic and post-transcriptional gene regulation in the control of *Arabidopsis* flowering time. Curr Opin Plant Biol 7: 570-4.

Simpson G G, Dijkwel P P, Quesada V, Henderson I, Dean C (2003) FY is an RNA 3' end-processing factor that interacts with FCA to control the *Arabidopsis* floral transition. Cell 113:777-787.

Song Y H, Estrada D A, Johnson R S, Kim S K, Lee SY, MacCoss M J, Imaizumi T (2014) Distinct roles of FKF1, GIGANTEA, and ZEITLUPE proteins in the regulation of *CONSTANS* stability in *Arabidopsis* photoperiodic flowering. Proc Natl Acad Sci USA 111:17672–17677.

Song J, Angel A, Howard M and Dean C (2012) Vernalization - a cold-induced epigenetic switch. J Cell Sci. 125(Pt 16):3723-31.

Song K and Osborn TC (1992) Polyphyletic origins of *Brassica napus*: new evidence based on organelle and nuclear RFLP analyses. Genome 35:992–1101. doi:10.1139/g92-152.

Srikanth A, Schmid M (2011) Regulation of flowering time: all roads lead to Rome. Cell Mol Life Sci 68: 2013-37.

Stefansson BR and Downey RK (1995) Rapeseed. In Slinkard AE and Knott DR (Eds.) Harvest of gold: the history of field crop breeding in Canada. Saskatoon, Canada: University Extension Press, University of Saskatchewan. pp 140-152.

Steinbach Y (2019) The *Arabidopsis thaliana CONSTANS-LIKE 4 (COL4)* – A Modulator of Flowering Time. Front Plant Sci 10:651.

Swiezewski S, Liu F, Magusin A and Dean C (2009) Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. *Nature* 462**:** 799–802.

Tadege M, Sheldon C C, Helliwell C A, Stoutjesdijk P, Dennis E S, Peacock W J (2001) Control of flowering time by FLC orthologues in *Brassica napus*. The Plant J 28:545–553.

Tayo T O and Morgan D G (1975) Quantitative analysis of the growth, development and distribution of flowers and pods in oil seed rape (*Brassica napus* L.). J Agric Sci 85: 103-110.

Teutonica R A and Osborn T C (1995) Mapping loci controlling vernalization requirement in *Brassica rapa*. Theoretical and Applied Genetics 91: 1279 – 1283.

Wang J and Zhang Z (2020) GAPIT Version 3: Boosting Power and Accuracy for Genomic Association and Prediction. bioRxiv .403170; doi: /10.1101/2020.11.29.403170.

Wang N, Chen B, Xu K, Gao G, Li F, Qiao J, Yan G, Li J, Li H, Wu X (2016) Association mapping of flowering time QTLs and insight into their contributions to rapeseed growth habits. Front Plant Sci 7:338.

Wang P, Cheng T, Lu M, Liu G, Li M, Shi J, Lu Y, Laux T and Chen J (2016) Expansion and Functional Divergence of AP2 Group Genes in Spermatophytes Determined by Molecular Evolution and *Arabidopsis* Mutant Analysis. Front. Plant Sci. 7:1383.

Wang C Q, Sarmast M K, Jiang J, Dehesh K. 2015. The Transcriptional Regulator BBX19 Promotes Hypocotyl Growth by Facilitating *COP1*- Mediated *EARLY FLOWERING3* Degradation in *Arabidopsis*. The Plant Cell 27:1128–1139

Wang N, Qian W, Suppanz I, Wei L, Mao B, Long Y, Meng J, Müller A E, Jung C (2011) Flowering time variation in oilseed rape (*Brassica napus* L.) is associated with allelic variation in the FRIGIDA homologue *BnaA.FRI.a*. J Exp Bot 62:5641–5658.

Werner J D, Borevitz J O, Uhlenhaut N H, Ecker J R, Chory J, Weigel D (2005) *FRIGIDA*-independent variation in flowering time of natural *Arabidopsis thaliana* accessions. Genetics 170: 1197–1207.

Wurschum T, Liu W, Maurer H P, Abel S and Reif J C (2012) Dissecting the genetic architecture of agronomic traits in multiple segregating populations in rapeseed (*Brassica napus* L.). Theor Appl Genet 124: 153–161.

Xiao D, Zhao J J, Basnet R K, Carpio D P D, Zhang N W, Bucher J, Lin K, Cheng F, Wang X W and Bonnema G (2013) The *Brassica rapa FLC* homologue *FLC2* is a key regulator of flowering time, identified through transcriptional co-expression networks. J Exp Bot 64: 4503-4516.

Xu L, Hu K, Zhang Z, Guan C, Chen S, Hua W, Li J, Wen J, Yi B, Shen J, Ma C, Tu J, Fu T (2016) Genome-wide association study reveals the genetic architecture of flowering time in rapeseed (*Brassica napus* L.) *DNA Research* 23:43-52.

Xu D, Zhu D and Deng X W (2016) The role of COP1 in repression of photoperiodic flowering [version 1; referees: 2 approved] F1000Research 2016, 5(F1000 Faculty Rev):178.

Yanovsky M J, Kay S A (2003) Living by the calendar: how plants know when to flower. Nat Rev Mol Cell Biol 4:265-275.

Yeh S C, Lee H P, Kao S T, Lu J L (2016) Effects of supplementary lighting by natural light for growth of *Brassica chinensis*. Journal of Physics: Conference Series. 710. 012021.

Zaman M W (1989) Introgression in *Brassica napus* for adaptation to the growing conditions in Bangladesh. Theor Appl Genet 77:721-728.

Zhang W, Sun P, He Q, Shu F, Deng H (2018) Transcriptome analysis of near-isogenic line provides novel insights into genes associated with panicle traits regulation in rice. PLoS ONE 13 (6): e0199077.

Zhang, B., Wang, L., Zeng, L., Zhang, C., and Ma, H. (2015). Arabidopsis TOE proteins convey a photoperiodic signal to antagonize *CONSTANS* and regulate flowering time. Genes Dev. 29, 975–987.

Zhao H, Basu U, Kebede B, Qu C, Li J, Rahman H (2019) Fine mapping of the major QTL for seed coat color in *Brassica rapa* var. Yellow Sarson by use of NIL populations and transcriptome sequencing for identification of the candidate genes. PLoS ONE 14(2): e0209982.

Zhao H, Liu J, Shi L, Xu F and Wang Y (2010) Development of boron-efficient near isogenic lines of *Brassica napus* and their response to low boron stress at seedling stage. Russ J Genet 46, 57–63.

Zhao J, Kulkarni V, Liu N, Del Carpio D P, Bucher J and Bonnema G (2010) *BrFLC2* (*FLOWERING LOCUS C*) as a candidate gene for a vernalization response QTL in *Brassica rapa*. J Exp Bot 61: 1817–1825.

Zhu Y,Ye J,Zhan J,Zheng X,Zhang J, Shi J, Wang X, Liu G and Wang H (2020) Validation and Characterization of a Seed Number Per Silique Quantitative Trait Locus qSN.A7 in Rapeseed (*Brassica napus L.*). *Front Plant Sci*. 11:68.

Zhu H, Li X, Zhai W, Liu Y, Gao Q, Liu J, et al. (2017) Effects of low light on photosynthetic properties, antioxidant enzyme activity, and anthocyanin accumulation in purple pak-choi (*Brassica campestris* ssp. *Chinensis Makino*). PLoS ONE 12(6):e0179305.

Zuñiga-Mayo V M, Baños-Bayardo C R, Díaz-Ramírez D, Marsch-Martínez N and Folter S (2018) Conserved and novel responses to cytokinin treatments during flower and fruit development in *Brassica napus* and *Arabidopsis thaliana*. Scientific reports 8:6836.

Appendices

Appendix 2-1. List of the inbred *Brassica napus* lines derived from six different *B. napus* × *B. oleracea* interspecific crosses used in this study for QTL mapping of days to flowering at 10 h photoperiod. The 10 most early flowering lines marked with bold and blue font.

Inbred line number	Reg number	Cross	Gene-ration[1]	Days to flowering[2]
A_3	5CA1300-353	A04-73NA × *B. oleracea* var. *alboglabra*	F	162.4
A_4	5CA1300-355	A04-73NA × *B. oleracea* var. *alboglabra*	F	147.3
A_6	**5CA1300-363**	**A04-73NA × *B. oleracea* var. *alboglabra***	**F**	**87.8**
A_7	5CA1300-368	A04-73NA × *B. oleracea* var. *alboglabra*	F	133.8
A_14	5CA1300-401	A04-73NA × *B. oleracea* var. *alboglabra*	F	160.0
A_15	5CA1300-404	A04-73NA × *B. oleracea* var. *alboglabra*	F	138.7
A_16	5CA1300-410	A04-73NA × *B. oleracea* var. *alboglabra*	F	131.7
A_17	5CA1300-412	A04-73NA × *B. oleracea* var. *alboglabra*	F	117.0
A_19	5CA1300-416	A04-73NA × *B. oleracea* var. *alboglabra*	F	106.7
A_20	5CA1300-419	A04-73NA × *B. oleracea* var. *alboglabra*	F	124.1
A_41	5CA1676-361	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	118.3
A_42	5CA1676-363	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	121.0
A_43	5CA1676-365	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	124.2
A_44	5CA1676-377	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	160.0
A_45	5CA1676-380	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	106.0
A_46	5CA1676-389	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	105.1
A_48	5CA1676-402	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	160.0
A_49	5CA1676-405	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	117.3
A_51	5CA1676-409	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	142.9
A_52	5CA1676-412	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	122.3
A_53	5CA1676-413	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	115.7
A_54	5CA1676-416	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	103.9
A_56	5CA1676-422	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	117.4
A_57	5CA1676-423	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	124.2
A_58	5CA1676-427	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	105.7
A_59	5CA1676-429	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	112.7
A_61	5CA1676-438	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	118.4
A_63	5CA1676-442	(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA	BC	116.8
A_22	5CA1343-320	A04-73NA × *B. oleracea* var. *botrytis*	F	150.7
A_23	5CA1343-321	A04-73NA × *B. oleracea* var. *botrytis*	F	160.0
A_24	5CA1343-323	A04-73NA × *B. oleracea* var. *botrytis*	F	160.0
A_25	5CA1343-327	A04-73NA × *B. oleracea* var. *botrytis*	F	160.0
A_26	5CA1343-329	A04-73NA × *B. oleracea* var. *botrytis*	F	115.7
A_27	5CA1343-330	A04-73NA × *B. oleracea* var. *botrytis*	F	119.4
A_28	5CA1343-333	A04-73NA × *B. oleracea* var. *botrytis*	F	118.0

A_29	5CA1343-336	A04-73NA × *B. oleracea* var. *botrytis*	F	113.8
A_30	5CA1343-339	A04-73NA × *B. oleracea* var. *botrytis*	F	115.3
A_31	5CA1343-343	A04-73NA × *B. oleracea* var. *botrytis*	F	118.9
A_33	5CA1343-349	A04-73NA × *B. oleracea* var. *botrytis*	F	121.0
A_35	5CA1343-353	A04-73NA × *B. oleracea* var. *botrytis*	F	160.0
A_36	5CA1343-357	A04-73NA × *B. oleracea* var. *botrytis*	F	138.3
A_37	5CA1343-360	A04-73NA × *B. oleracea* var. *botrytis*	F	160.0
A_39	5CA1343-367	A04-73NA × *B. oleracea* var. *botrytis*	F	160.0
A_40	5CA1343-368	A04-73NA × *B. oleracea* var. *botrytis*	F	118.5
A_65	5CA1677-326	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	117.8
A_66	5CA1677-328	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	118.0
A_67	5CA1677-330	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	123.6
A_71	5CA1677-344	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	117.8
A_73	5CA1677-351	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	111.5
A_75	5CA1677-355	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	112.0
A_80	5CA1677-376	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	114.7
A_81	5CA1677-379	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	121.8
A_82	5CA1677-383	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	119.2
A_83	5CA1677-386	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	122.7
A_85	5CA1677-390	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	123.8
A_86	5CA1677-394	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	122.2
A_87	5CA1677-395	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	126.2
A_89	5CA1677-405	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	160.0
A_93	5CA1677-418	(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA	BC	160.0
J_2	5CA1362-152	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_4	5CA1362-156	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_6	5CA1362-161	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_7	5CA1362-162	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_8	5CA1362-164	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_9	5CA1362-165	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_10	5CA1362-166	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_11	5CA1362-167	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_12	5CA1362-169	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_13	5CA1362-170	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_14	5CA1362-171	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_15	5CA1362-173	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_16	5CA1362-174	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	124.8
J_17	5CA1362-175	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	122.0
J_18	5CA1362-176	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	107.5
J_19	5CA1362-177	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0
J_20	5CA1362-179	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	160.0

J_22	5CA1363-164	A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*	F	144.6
J_47	5CA1681-083	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	117.4
J_48	5CA1681-084	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	160.0
J_50	5CA1681-086	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	113.6
J_51	5CA1681-090	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	113.4
J_52	5CA1681-091	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	119.4.
J_53	5CA1681-092	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	109.1
J_54	5CA1681-096	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	108.6
J_55	5CA1681-097	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	160.0
J_57	5CA1681-100	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	160.0
J_58	5CA1681-101	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	160.0
J_59	5CA1681-102	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	153.9
J_60	5CA1681-103	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	135.0
J_61	5CA1681-104	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	142.3
J_62	5CA1681-105	(A04-73NA × *B. oleracea* var. *capitata* cv. *Badger Shipper*) × A04-73NA	BC	160.0
J_23	5CA1363-165	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	143.8
J_24	5CA1363-168	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	160.0
J_25	5CA1363-170	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	160.0
J_26	5CA1363-171	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	160.0
J_27	5CA1363-173	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	160.0
J_28	**5CA1363-177**	**A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener***	**F**	**79.9**
J_30	5CA1363-180	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	103.3
J_31	5CA1363-181	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	100.8
J_33	5CA1363-183	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	114.8
J_37	5CA1363-194	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	145.3
J_39	5CA1363-197	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	116.3
J_41	5CA1363-205	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	119.8
J_43	5CA1363-207	A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*	F	160.0
J_63	5CA1682-099	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	125.6
J_66	5CA1682-102	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	122.0
J_67	5CA1682-103	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	115.9
J_68	5CA1682-104	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	117.0
J_70	5CA1682-108	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	125.4
J_71	5CA1682-113	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	117.5
J_75	**5CA1682-128**	**(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA**	**BC**	**92.3**
J_76	**5CA1682-130**	**(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA**	**BC**	**91.9**
J_77	5CA1682-131	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	105.3
J_78	**5CA1682-133**	**(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA**	**BC**	**97.9**
J_80	**5CA1682-138**	**(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA**	**BC**	**96.7**
J_81	**5CA1682-140**	**(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA**	**BC**	**95.4**
J_82	**5CA1682-143**	**(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA**	**BC**	**97.1**

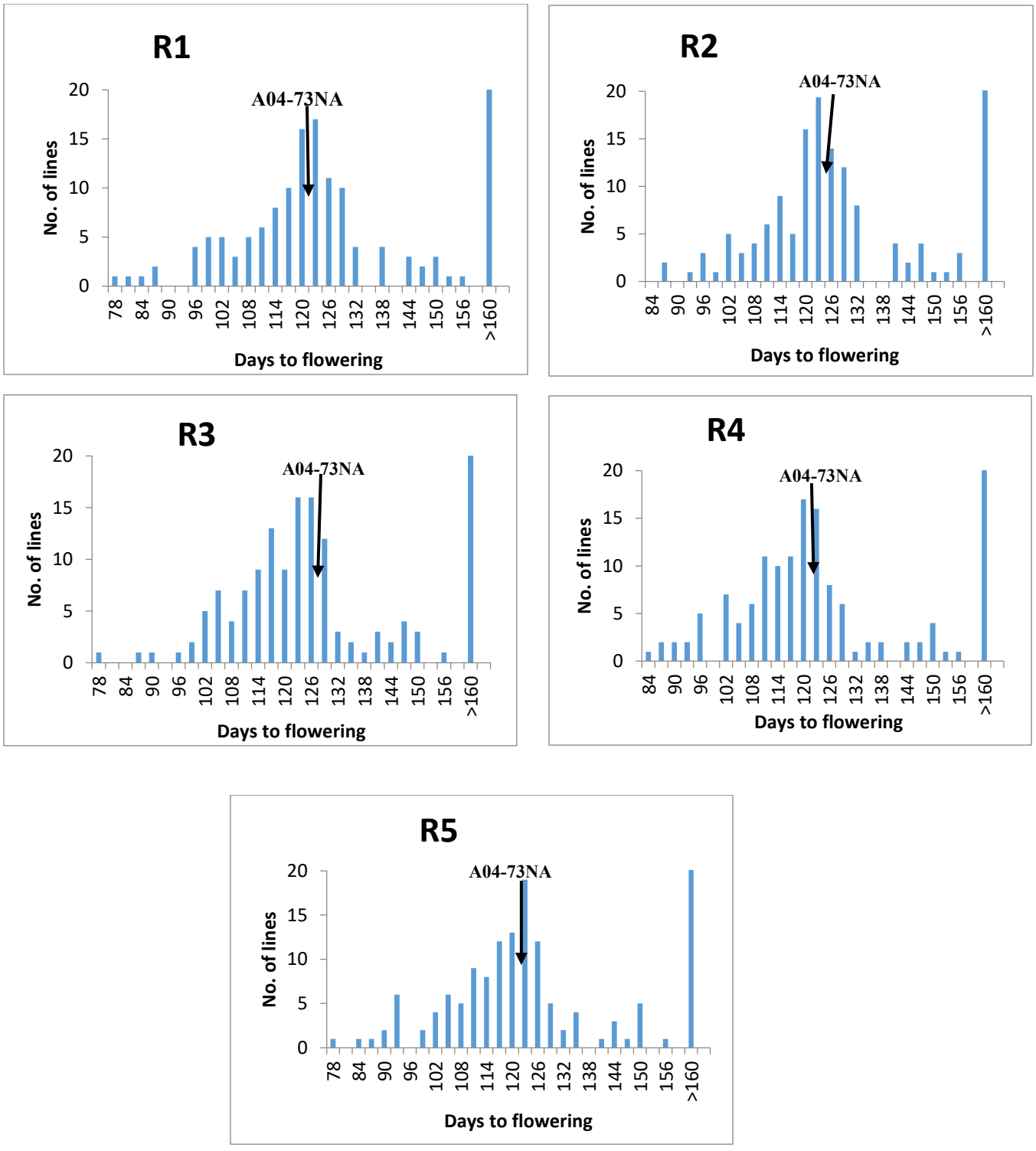
J_83	5CA1682-145	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	100.6
J_84	5CA1682-147	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	101.8
J_85	5CA1682-149	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	99.9
J_86	**5CA1682-150**	**(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA**	**BC**	**95.8**
J_87	**5CA1682-152**	**(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA**	**BC**	**97.7**
J_88	5CA1682-154	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	112.7
J_89	5CA1682-155	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	108.1
J_91	5CA1682-158	(A04-73NA × *B. oleracea* var. *capitata* cv. *Bindsachsener*) × A04-73NA	BC	118.0
S_1	5CA1358-594	A04-73NA × *B. oleracea* var. *italica*	F	160.0
S_3	5CA1358-615	A04-73NA × *B. oleracea* var. *italica*	F	121.3
S_4	5CA1358-616	A04-73NA × *B. oleracea* var. *italica*	F	117.2
S_5	5CA1358-620	A04-73NA × *B. oleracea* var. *italica*	F	115.2
S_6	5CA1358-623	A04-73NA × *B. oleracea* var. *italica*	F	109.0
S_8	5CA1358-634	A04-73NA × *B. oleracea* var. *italica*	F	112.3
S_9	5CA1358-635	A04-73NA × *B. oleracea* var. *italica*	F	115.0
S_10	5CA1358-640	A04-73NA × *B. oleracea* var. *italica*	F	111.0
S_11	5CA1358-652	A04-73NA × *B. oleracea* var. *italica*	F	117.5
S_12	5CA1358-656	A04-73NA × *B. oleracea* var. *italica*	F	113.9
S_14	5CA1358-667	A04-73NA × *B. oleracea* var. *italica*	F	111.3
S_15	5CA1358-679	A04-73NA × *B. oleracea* var. *italica*	F	117.5
S_16	5CA1358-685	A04-73NA × *B. oleracea* var. *italica*	F	114.5
S_17	5CA1358-688	A04-73NA × *B. oleracea* var. *italica*	F	112.8
S_19	5CA1358-701	A04-73NA × *B. oleracea* var. *italica*	F	118.4
S_20	5CA1358-703	A04-73NA × *B. oleracea* var. *italica*	F	122.7
S_21	5CA1358-705	A04-73NA × *B. oleracea* var. *italica*	F	122.5
S_24	5CA1358-719	A04-73NA × *B. oleracea* var. *italica*	F	122.2
S_25	5CA1358-720	A04-73NA × *B. oleracea* var. *italica*	F	118.8
S_27	5CA1358-731	A04-73NA × *B. oleracea* var. *italica*	F	116.2
S_29	5CA1358-747	A04-73NA × *B. oleracea* var. *italica*	F	109.9
S_51	5CA1678-263	(A04-73NA × *B. oleracea* var. *italica*) × A04-73NA	BC	104.9
S_52	5CA1678-264	(A04-73NA × *B. oleracea* var. *italica*) × A04-73NA	BC	98.4
S_56	5CA1678-277	(A04-73NA × *B. oleracea* var. *italica*) × A04-73NA	BC	121.7
S_57	5CA1678-281	(A04-73NA × *B. oleracea* var. *italica*) × A04-73NA	BC	150.2
S_58	5CA1678-285	(A04-73NA × *B. oleracea* var. *italica*) × A04-73NA	BC	109.7
S_61	5CA1678-309	(A04-73NA × *B. oleracea* var. *italica*) × A04-73NA	BC	112.3
S_34	5CA1392-300	A04-73NA × *B. oleracea* var. *capitata*	F	148.6
S_36	5CA1392-305	A04-73NA × *B. oleracea* var. *capitata*	F	124.4
S_37	5CA1392-306	A04-73NA × *B. oleracea* var. *capitata*	F	124.8
S_38	5CA1392-312	A04-73NA × *B. oleracea* var. *capitata*	F	107.4
S_39	5CA1392-313	A04-73NA × *B. oleracea* var. *capitata*	F	98.4
S_40	5CA1392-319	A04-73NA × *B. oleracea* var. *capitata*	F	160.0

S_43	5CA1392-324	A04-73NA × *B. oleracea* var. *capitata*	F	160.0
S_44	5CA1392-325	A04-73NA × *B. oleracea* var. *capitata*	F	160.0
S_45	5CA1392-327	A04-73NA × *B. oleracea* var. *capitata*	F	160.0
S_48	5CA1392-339	A04-73NA × *B. oleracea* var. *capitata*	F	107.3
S_49	5CA1392-342	A04-73NA × *B. oleracea* var. *capitata*	F	110.9
S_50	5CA1392-345	A04-73NA × *B. oleracea* var. *capitata*	F	160.0
S_64	5CA1679-354	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	132.2
S_65	5CA1679-357	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	126.6
S_67	5CA1679-377	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	160.0
S_70	5CA1679-382	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	129.8
S_72	5CA1679-399	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	160.0
S_76	5CA1679-437	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	114.9
S_77	5CA1679-440	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	160.0
S_78	5CA1679-442	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	143.3
S_79	5CA1679-460	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	136.4
S_80	5CA1679-465	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	146.1
S_81	5CA1679-470	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	151.9
S_83	5CA1679-474	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	124.2
S_84	5CA1679-483	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	121.0
S_85	5CA1679-486	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	149.5
S_86	5CA1679-497	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	150.2
S_87	5CA1679-502	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	151.8
S_89	5CA1679-506	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	160.0
S_91	5CA1679-535	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	125.8
S_92	5CA1679-541	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	112.4
S_93	5CA1679-543	(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	BC	160.0
Check	A04-73NA			125.0

[1] F = inbred lines derived from F_2 of the crosses; BC = inbred lines derived from BC_1 of the crosses
[2] LSmean of five replications presented

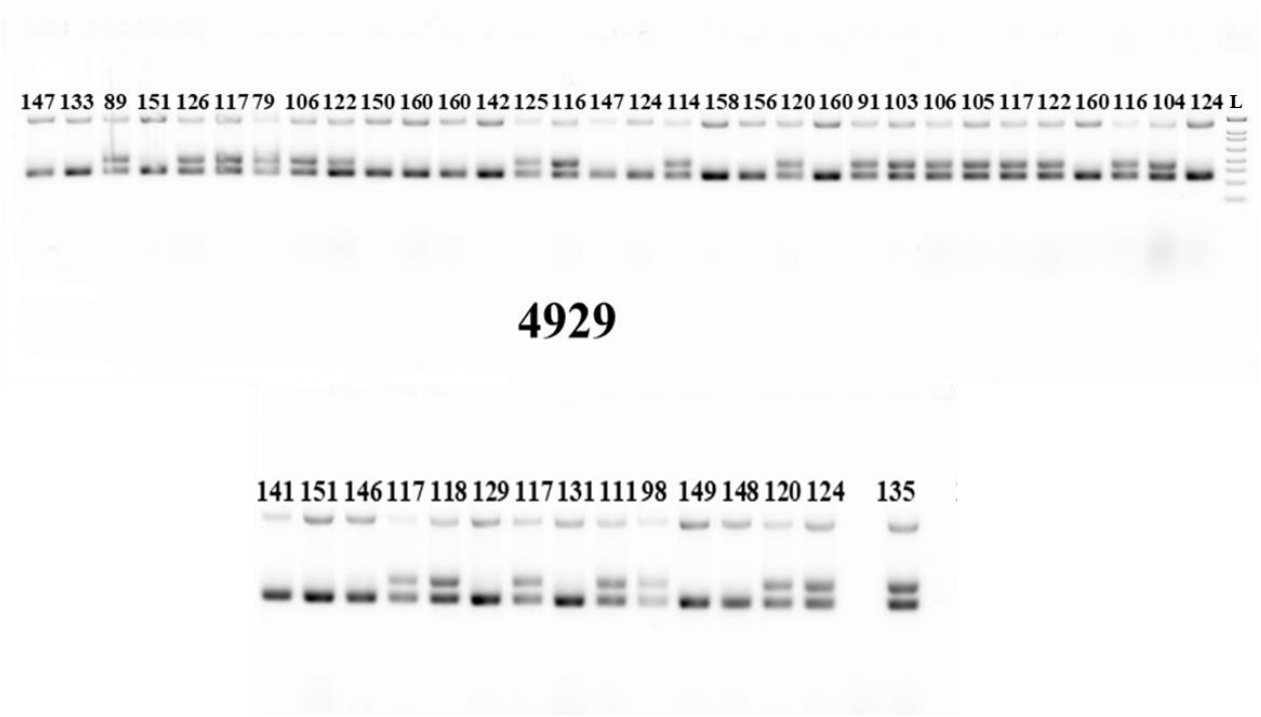
Appendix 2-2. Sequence of the SSR markers from C9 QTL affecting days to flowering under a short-day condition (10 h photoperiod and 18 ºC constant temperature).

Primer name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
SSR_4928	AGTGACCAACTTTGAAGCGAAC	TCCTTCTTTGATAGGTCTGGGA
SSR_4929	TGTCTTACCGATGTTCCATGTC	CTTCGTCTTCTTCGCCTACTTC



Appendix 2-3. Frequency distribution of the *Brassica napus* inbred lines derived from *B. napus* × *B. oleracea* interspecific crosses for days to flowering in a growth chamber at 10 h photoperiod and 18 °C temperature. R1 to R5 indicates five experiments. The *B. napus* parent A04-73NA took about 122 - 127 days to flower between different experiments, which is indicated by vertical arrow.

Appendix 2-4. Electrophoretic separation of PCR amplicons of the SSR marker 4929 from C9 QTL region of an inbred *Brassica napus* population derived from six *Brassica napus* × *B. oleracea* interspecific crosses. Days to flowering data of the inbred lines shown on the top of the lanes. L indicates size standard.



Appendix 3-1. List of 280 diverse spring oilseed *Brassica napus* lines or cultivars used for phenotyping of days to flowering at 10 h photoperiod and 18 °C constant temperature conditions. The 10 most early flowering lines marked with bold and blue font.

Code	Entry name	Source	Days to flowering[1]
23	AC Elect	Gene bank: Agriculture and Agri-Food Canada	167.8
21	AC Excel	Gene bank: Agriculture and Agri-Food Canada	111.0
19	AC Tristar	Gene bank: Agriculture and Agri-Food Canada	130.7
31	Agassiz	Gene bank: Agriculture and Agri-Food Canada	185.0
29	**Apollo (LL)**	**Gene bank: Agriculture and Agri-Food Canada**	**102.3**
20	Argentine	Gene bank: Agriculture and Agri-Food Canada	176.3
26	Crusher	Gene bank: Agriculture and Agri-Food Canada	185.0
25	Global	Gene bank: Agriculture and Agri-Food Canada	115.0
8	Golden (non-CQ)	Gene bank: Agriculture and Agri-Food Canada	143.0
36	**Hylite 201 (Apetalous)**	**Gene bank: Agriculture and Agri-Food Canada**	**88.0**
24	Legend	Gene bank: Agriculture and Agri-Food Canada	185.0
5	Midas	Gene bank: Agriculture and Agri-Food Canada	168.3
33	Next 500	Gene bank: Agriculture and Agri-Food Canada	134.4
34	Next 700	Gene bank: Agriculture and Agri-Food Canada	185.0
9	Nugget	Gene bank: Agriculture and Agri-Food Canada	168.5
17	OAC Springfield	Gene bank: Agriculture and Agri-Food Canada	151.5
18	OAC Summit	Gene bank: Agriculture and Agri-Food Canada	139.2
12	OAC Triton	Gene bank: Agriculture and Agri-Food Canada	147.5
16	OAC Triumph	Gene bank: Agriculture and Agri-Food Canada	185.0
35	Option 501	Gene bank: Agriculture and Agri-Food Canada	141.5
1	Oro	Gene bank: Agriculture and Agri-Food Canada	185.0
13	Pivot	Gene bank: Agriculture and Agri-Food Canada	185.0
22	Profit	Gene bank: Agriculture and Agri-Food Canada	185.0
37	Prominent	Gene bank: Agriculture and Agri-Food Canada	185.0
10	Regent	Gene bank: Agriculture and Agri-Food Canada	148.40
14	Reston	Gene bank: Agriculture and Agri-Food Canada	147.5
30	Senator	Gene bank: Agriculture and Agri-Food Canada	140.3
32	Sentry	Gene bank: Agriculture and Agri-Food Canada	126.2
27	Stallion (TR tolerant)	Gene bank: Agriculture and Agri-Food Canada	185.0
2	Tanka	Gene bank: Agriculture and Agri-Food Canada	169.8
3	Target	Gene bank: Agriculture and Agri-Food Canada	164.9
38	Topas	Gene bank: Agriculture and Agri-Food Canada	170.3
7	Tower	Gene bank: Agriculture and Agri-Food Canada	185.0
15	Tribute	Gene bank: Agriculture and Agri-Food Canada	140.3
6	Turret	Gene bank: Agriculture and Agri-Food Canada	185.0
28	Vanguard	Gene bank: Agriculture and Agri-Food Canada	137.5
11	**Westar**	**Gene bank: Agriculture and Agri-Food Canada**	**106.7**

4	Zephyr	Gene bank: Agriculture and Agri-Food Canada	156.5
55	02-24557-4	University of Alberta	145.8
63	1CA0110.004-A2006	University of Alberta	185.0
85	1CA0591.361-A1299	University of Alberta	129.4
188	1CA1446.437-A2096	University of Alberta	147.9
189	1CA1446.441-A2096	University of Alberta	185.0
190	1CA1446.444-A2096	University of Alberta	155.8
191	1CA1446.445-A2096	University of Alberta	165.5
192	1CA1446.446-A2096	University of Alberta	147.5
193	1CA1446.447-A2096	University of Alberta	123.5
194	1CA1446.448-A2096	University of Alberta	158.0
195	1CA1446.449-A2096	University of Alberta	185.0
196	1CA1446.450-A2096	University of Alberta	160.2
197	1CA1446.458-A2096	University of Alberta	137.3
198	1CA1446.464-A2096	University of Alberta	128.8
199	1CA1866.258-A2295	University of Alberta	147.4
200	1CA1866.260-A2295	University of Alberta	185.0
259	1IA0944.124-A4073	University of Alberta	153.5
215	1IA0944.298-A2095	University of Alberta	185.0
211	1IA1078.064-A2095	University of Alberta	108.8
212	1IA1078.067-A2095	University of Alberta	116.0
213	1IA1082.068-A2095	University of Alberta	118.0
214	1IA1096.060-A2095	University of Alberta	119.5
216	1IA1287.109-A2065	University of Alberta	185.0
217	1IA1287.113-A2065	University of Alberta	185.0
218	1IA1287.116-A2065	University of Alberta	185.0
219	1IA1287.121-A2065	University of Alberta	185.0
220	1IA1288.097-A2065	University of Alberta	118.5
164	1RA0461.328-A3093	University of Alberta	166.2
86	1RA0999.177-A2293	University of Alberta	185.0
87	1RA1001.134-A4072	University of Alberta	134.5
88	1RA1002.106-A2282	University of Alberta	138.5
89	1RA1003.164-A2282	University of Alberta	185.0
165	1RA1003.170-A2282	University of Alberta	185.0
90	1RA1003.182-A2282	University of Alberta	185.0
91	1RA1003.189-A2282	University of Alberta	185.0
92	1RA1003.226-A2293	University of Alberta	135.6
93	1RA1003.267-A2273	University of Alberta	144.0
166	1RA1003.304-A2096	University of Alberta	185.0
94	1RA1124.059-A4082	University of Alberta	127.5
95	1RA1142.058-A4082	University of Alberta	141.0

167	1RA1143.141-A2282	University of Alberta	185.0
168	1RA1143.142-A3093	University of Alberta	145.0
169	1RA1143.147-A2282	University of Alberta	164.2
96	1RA1143.234-A2293	University of Alberta	146.5
97	1RA1305.717-A1272	University of Alberta	185.0
98	1RA1305.729-A1272	University of Alberta	156.5
99	1RA1443.040-A1202	University of Alberta	117.4
100	1RA1443.056-A1202	University of Alberta	115.7
101	**1RA1443.058-A1202**	**University of Alberta**	**94.5**
102	1RA1443.063-A1202	University of Alberta	125.8
103	1RA1443.071-A1202	University of Alberta	139.0
104	**1RA1443.072-A1202**	**University of Alberta**	**107.2**
105	1RA1443.177-A1202	University of Alberta	122.3
106	**1RA1444.007-A1202**	**University of Alberta**	**98.7**
107	1RA1444.541-A1202	University of Alberta	127.6
108	1RA1444.619-A1202	University of Alberta	109.5
173	1RA1475.027-A2242	University of Alberta	141.5
174	1RA1481.006-A2242	University of Alberta	141.7
260	1RA1483.021-A3053	University of Alberta	185.0
261	1RA1484.037-A3053	University of Alberta	167.3
175	1RA1484.038-A2242	University of Alberta	185.0
280	1RA1484.050-A2242	University of Alberta	169.5
109	1RA1484.080-A2253	University of Alberta	161.0
110	1RA1488.014-A1232	University of Alberta	148.8
111	1RA1488.023-A2242	University of Alberta	166.8
176	1RA1488.034-A3053	University of Alberta	185.0
177	1RA1498.014-A3053	University of Alberta	137.7
262	1RA1498.016-A2253	University of Alberta	154.3
178	1RA1503.020-A2242	University of Alberta	185.0
112	1RA1503.024-A2253	University of Alberta	185.0
113	1RA1584.049-A2253	University of Alberta	132.0
114	1RA1584.050-A2253	University of Alberta	133.3
115	1RA1584.051-A2253	University of Alberta	185.0
116	1RA1584.052-A2253	University of Alberta	185.0
117	1RA1584.054-A2253	University of Alberta	130.6
118	1RA1584.055-A2253	University of Alberta	185.0
119	1RA1584.056-A2253	University of Alberta	141.7
120	1RA1584.057-A2253	University of Alberta	159.5
121	1RA1584.058-A2253	University of Alberta	185.0
122	1RA1584.059-A2253	University of Alberta	157.9
205	1RA1584.116-A2086	University of Alberta	139.2

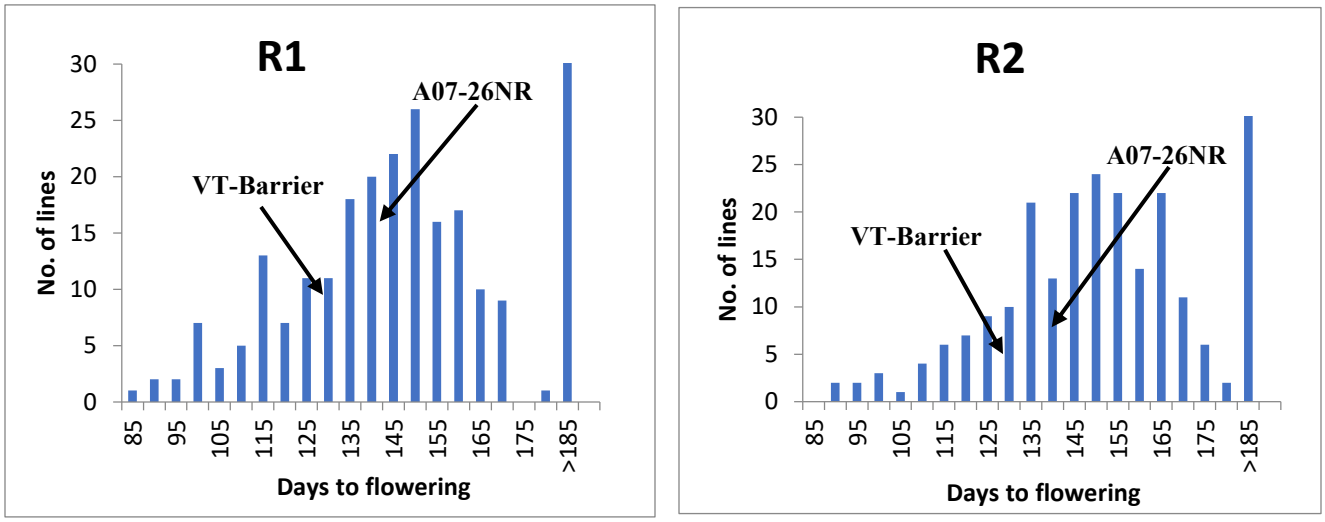
206	1RA1584.120-A2086	University of Alberta	135.8
207	1RA1584.129-A2086	University of Alberta	153.3
277	1RA1584.130-A2086	University of Alberta	173.3
208	1RA1584.132-A2086	University of Alberta	146.2
278	1RA1584.134-A2086	University of Alberta	185.0
209	1RA1584.135-A2086	University of Alberta	143.0
210	1RA1584.139-A2086	University of Alberta	151.2
279	1RA1584.140-A2086	University of Alberta	130.8
123	1RA1634.023-A2243	University of Alberta	155.0
224	1RA1634.032-A2076	University of Alberta	157.9
225	1RA1634.033-A2076	University of Alberta	157.6
226	1RA1634.034-A2076	University of Alberta	185.0
124	1RA1636.222-A2243	University of Alberta	185.0
125	1RA1636.223-A2243	University of Alberta	185.0
126	1RA1637.236-A2243	University of Alberta	147.1
127	1RA1638.076-A2243	University of Alberta	150.8
128	1RA1638.084-A2243	University of Alberta	157.5
129	1RA1638.085-A2243	University of Alberta	185.0
227	1RA1638.099-A2076	University of Alberta	185.0
201	1RA1638.100-A2076	University of Alberta	158.5
202	1RA1638.101-A2076	University of Alberta	158.0
203	1RA1638.102-A2076	University of Alberta	185.0
204	1RA1638.103-A2076	University of Alberta	185.0
228	1RA1638.104-A2076	University of Alberta	185.0
229	1RA1638.105-A2076	University of Alberta	185.0
230	1RA1638.106-A2076	University of Alberta	185.0
231	1RA1638.107-A2076	University of Alberta	185.0
263	1RA1741.006-A1204	University of Alberta	122.3
264	1RA1743.028-A1204	University of Alberta	117.5
170	1RA1745.009-A1204	University of Alberta	185.0
171	1RA1745.034-A1204	University of Alberta	185.0
172	1RA1745.046-A1204	University of Alberta	185.0
265	1RA1746.008-A1234	University of Alberta	163.5
266	1RA1746.009-A1234	University of Alberta	162.3
232	1RA1746.037-A2005	University of Alberta	185.0
253	1RA1746.039-A2005	University of Alberta	155.5
233	1RA1746.044-A2005	University of Alberta	148.2
234	1RA1746.045-A2005	University of Alberta	150.3
235	1RA1746.054-A2005	University of Alberta	136.1
236	1RA1746.066-A2005	University of Alberta	185.0
237	1RA1746.125-A2005	University of Alberta	185.0

254	1RA1746.134-A2005	University of Alberta	130.3
238	1RA1746.150-A2005	University of Alberta	130.5
158	1RA1746.173-A2066	University of Alberta	152.1
159	1RA1746.174-A2066	University of Alberta	185.0
271	1RA1746.175-A2066	University of Alberta	163.5
276	1RA1746.176-A2066	University of Alberta	162.5
179	1RA1746.177-A2066	University of Alberta	146.4
160	1RA1746.178-A2066	University of Alberta	147.8
267	1RA1751.003-A1234	University of Alberta	157.3
268	1RA1751.008-A1234	University of Alberta	185.0
269	1RA1751.012-A1234	University of Alberta	185.0
239	1RA1751.047-A2005	University of Alberta	150.7
240	1RA1751.049-A2005	University of Alberta	185.0
241	1RA1751.054-A2005	University of Alberta	185.0
242	1RA1751.064-A2005	University of Alberta	185.0
243	1RA1751.079-A2005	University of Alberta	151.5
244	1RA1751.088-A2005	University of Alberta	157.9
245	1RA1751.090-A2005	University of Alberta	185.0
246	1RA1751.124-A2005	University of Alberta	158.7
247	1RA1751.126-A2005	University of Alberta	185.0
248	1RA1751.166-A2005	University of Alberta	185.0
161	1RA1751.218-A2255	University of Alberta	114.5
162	1RA1751.220-A2066	University of Alberta	163.7
180	1RA1751.222-A2066	University of Alberta	185.0
181	1RA1751.230-A2066	University of Alberta	151.7
182	1RA1751.234-A2066	University of Alberta	150.2
163	1RA1751.235-A2066	University of Alberta	155.0
270	1RA1752.007-A1234	University of Alberta	159.3
249	1RA1752.052-A2005	University of Alberta	156.8
250	1RA1752.053-A2005	University of Alberta	185.0
255	1RA1752.078-A2005	University of Alberta	185.0
251	1RA1752.120-A2005	University of Alberta	156.2
252	1RA1752.131-A2005	University of Alberta	185.0
183	1RA1781.023-A2056	University of Alberta	158.7
187	1RA1781.026-A2056	University of Alberta	147.2
184	1RA1789.029-A2056	University of Alberta	144.5
272	1RA1789.030-A2056	University of Alberta	143.5
273	1RA1789.031-A2056	University of Alberta	147.3
185	1RA1789.034-A2056	University of Alberta	161.7
274	1RA1789.035-A2056	University of Alberta	185.0
186	1RA1789.037-A2056	University of Alberta	144.8

275	1RA1789.039-A2056	University of Alberta	158.3
257	1RA1869.197-A2096	University of Alberta	159.8
258	1RA1869.201-A2096	University of Alberta	137.7
256	1RA1869.202-A2096	University of Alberta	124.8
221	1RP1686.016-A2004	University of Alberta	124.8
222	1RP1686.022-A2004	University of Alberta	140.8
223	1RP1686.023-A2004	University of Alberta	130.8
47	A01-104NA	University of Alberta	131.2
80	A03-21367NI PM1+2	University of Alberta	129.4
82	A03-21449NI PM1+2	University of Alberta	134.0
81	A03-21659NI PM1+2	University of Alberta	103.5
56	A03-22307NA	University of Alberta	149.8
44	A03-22620NA	University of Alberta	113.2
65	A03-22758NA	University of Alberta	168.4
40	A03-22762NA	University of Alberta	146.0
50	**A03-22805NA**	**University of Alberta**	**93.3**
57	A03-22808NA	University of Alberta	143.6
68	A03-3NR	University of Alberta	185.0
52	A04-72NA (A02-22536)	University of Alberta	137.2
61	A04-73NA (A02-22606 NA)	University of Alberta	109.5
49	A04-74NA (A02-22561)	University of Alberta	119.5
42	A04-75NA (A02-22707)	University of Alberta	116.0
78	A05-10NI (A03-21292NI PM1+2)	University of Alberta	105.5
48	A05-22NA (A03-22564NA)	University of Alberta	123.8
76	A05-4NI (A03-21821NI PM1+2)	University of Alberta	91.5
77	A05-6NI	University of Alberta	110.4
41	A06-19NA	University of Alberta	120.5
51	A06-20NA	University of Alberta	129.2
69	A06-9NR	University of Alberta	185.0
70	A07-25NR	University of Alberta	144.5
282	A07-26NR	University of Alberta	140.0
58	A07-28NA	University of Alberta	129.4
84	A07-35NI	University of Alberta	138.5
71	A07-38NR	University of Alberta	185.0
72	A07-45NR	University of Alberta	185.0
73	A07-46NR	University of Alberta	185.0
74	A07-47NR	University of Alberta	185.0
67	A99-13NR(lobeless)	University of Alberta	153.2
62	Altex-1	University of Alberta	148.0
45	Alto	University of Alberta	144.9
43	Andor	University of Alberta	133.2

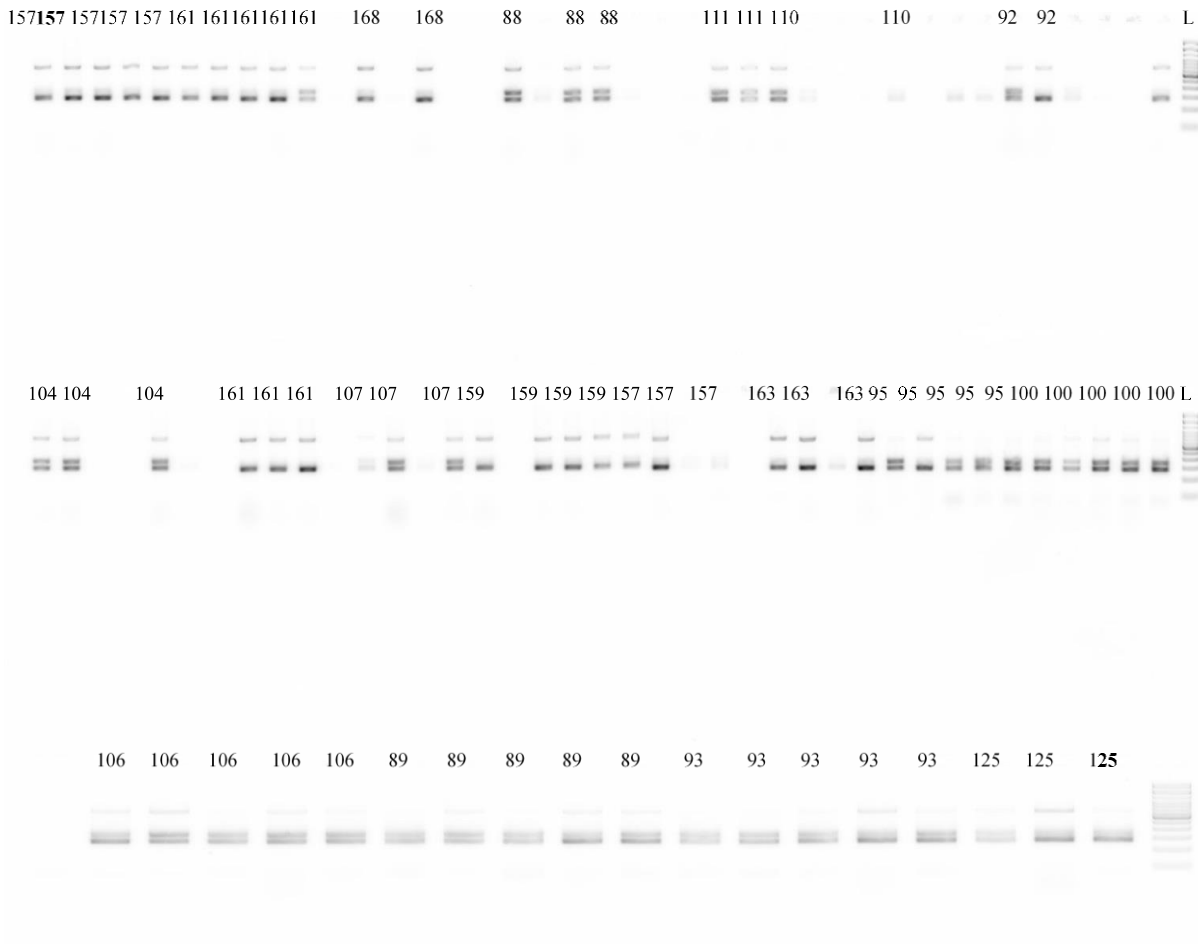
66	Conquest	University of Alberta	148.5
75	Cougar	University of Alberta	110.5
53	Hi-Q	University of Alberta	120.5
60	Kelsey	University of Alberta	121.0
39	Peace	University of Alberta	130.9
54	Q2	University of Alberta	130.7
59	Quantun	University of Alberta	143.4
46	Roper	University of Alberta	135.2
64	SILEX (A91-15026)	University of Alberta	151.5
83	UA AlfaGold (A07-29NI)	University of Alberta	148.5
79	UA BountyGold (A05-17NI)	University of Alberta	123.0
153	**CC08084**	**Nutrien Ag Solutions (NAS)**	**89.2**
154	**CC08149**	**Nutrien Ag Solutions (NAS)**	**93.7**
141	NBC11-04953	Nutrien Ag Solutions (NAS)	185.0
138	NBC11-82224	Nutrien Ag Solutions (NAS)	130.6
152	NBC11-82388	Nutrien Ag Solutions (NAS)	148.3
137	NBC11-87232	Nutrien Ag Solutions (NAS)	118.5
150	**NBC12-00771**	**Nutrien Ag Solutions (NAS)**	**100.3**
149	NBC12-02926	Nutrien Ag Solutions (NAS)	149.3
143	NBC13-42060	Nutrien Ag Solutions (NAS)	129.0
139	NBC13-44732	Nutrien Ag Solutions (NAS)	185.0
147	NBC15-07424	Nutrien Ag Solutions (NAS)	140.3
151	NBR12-00315	Nutrien Ag Solutions (NAS)	113.3
136	NBR12-07404	Nutrien Ag Solutions (NAS)	154.3
145	NGC11-01625	Nutrien Ag Solutions (NAS)	147.2
140	NGC11-13145	Nutrien Ag Solutions (NAS)	138.5
134	NGC14-29324	Nutrien Ag Solutions (NAS)	140.2
144	NGC14-29939	Nutrien Ag Solutions (NAS)	142.0
131	NGR12-00847	Nutrien Ag Solutions (NAS)	129.5
142	NGR14-16946	Nutrien Ag Solutions (NAS)	185.0
146	NGR14-40284	Nutrien Ag Solutions (NAS)	140.8
135	NGR15-04038	Nutrien Ag Solutions (NAS)	132.3
133	NR06-24122	Nutrien Ag Solutions (NAS)	124.2
156	NR09-60965	Nutrien Ag Solutions (NAS)	136.0
157	NRB10-77292	Nutrien Ag Solutions (NAS)	161.5
148	SP Banner	Nutrien Ag Solutions (NAS)	146.2
281	VT Barrier	Nutrien Ag Solutions (NAS)	130.0
132	SW 02100563 RR	Nutrien Ag Solutions (NAS)	185.0
155	SW 0927621 B	Nutrien Ag Solutions (NAS)	185.0
130	SW 0928729 B	Nutrien Ag Solutions (NAS)	166.5

[1] LSmean data of two replications presented



Appendix 3-2. Frequency distribution of the diverse lines and cultivars of *Brassica napus* for days to flowering in a growth chamber at 10 h photoperiod and 18 °C temperature. R1 and R2 indicate the two replications. VT-Barrier and A07-26NR are shown as Canadian standard canola cultivars.

Appendix 3-3. Electrophoretic separation of the PCR amplified products of the SSR marker 4929 in *Brassica napus* lines/cultivars. Days to flowering data of the lines/cultivars shown on the top of the lanes. L indicates size standard.



Appendix 4-1. List of the near-isogenic lines (NILs) of *Brassica napus* carrying Hi-Q or RIL-144 alleles evaluated for days to flowering at 10 h photoperiod and 20 ºC constant temperature.

Entry	QTL	Reg no.	Marker allele	Days to flowering[1]
1	C1	5CA1056.183-A1270	Hi-Q	115.6
2	C1	5CA1056.188-A1270	Hi-Q	113.5
3	C1	5CA1056.190-A1270	Hi-Q	117.7
4	C1	5CA1056.195-A1270	Hi-Q	119.0
5	C1	5CA1056.198-A1270	Hi-Q	116.8
10	C1	5CA1056.209-A1270	Hi-Q	112.1
13	C1	5CA1056.181-A1270	Hi-Q	115.5
15	C1	5CA1056.193-A1270	Hi-Q	109.0
19	C1	5CA1056.203-A1270	Hi-Q	116.5
25	C1	5CA1056.212-A1270	Hi-Q	110.2
26	C1	5CA1056.213-A1270	Hi-Q	108.0
49	C1	5CA1056.182-A1270	Hi-Q	114.5
50	C1	5CA1056.184-A1270	Hi-Q	121.7
51	C1	5CA1056.191-A1270	Hi-Q	116.5
52	C1	5CA1056.197-A1270	Hi-Q	120.5
66	C1	5CA1056.257-A1200	Hi-Q	110.6
7	C1	5CA1056.200-A1270	RIL 144	98.5
9	C1	5CA1056.205-A1270	RIL 144	103.0
11	C1	5CA1056.214-A1270	RIL 144	90.2
12	C1	5CA1056.215-A1270	RIL 144	99.5
18	C1	5CA1056.202-A1270	RIL 144	90.5
20	C1	5CA1056.206-A1270	RIL 144	97.2
22	C1	5CA1056.208-A1270	RIL 144	81.1
23	C1	5CA1056.210-A1270	RIL 144	99.2
24	C1	5CA1056.211-A1270	RIL 144	103.2
27	C1	5CA1056.217-A1270	RIL 144	84.3
53	C1	5CA1056.201-A1270	RIL-144	102.7
54	C1	5CA1056.216-A1270	RIL-144	102.2
55	C1	5CA1056.218-A1270	RIL-144	97.0
58	C1	5CA1056.245-A1200	RIL-144	103.0
60	C1	5CA1056.250-A1200	RIL-144	102.7
61	C1	5CA1056.251-A1200	RIL-144	103.0
62	C1	5CA1056.252-A1200	RIL-144	78.0
64	C1	5CA1056.255-A1200	RIL 144	88.7
65	C1	5CA1056.256-A1200	RIL 144	76.5
67	C1	5CA1056.258-A1200	RIL 144	78.5
68	C1	5CA1056.261-A1200	RIL 144	77.3
69	C1	5CA1056.263-A1200	RIL 144	80.6

6	C1	5CA1056.199-A1270	Hetero	105.5
8	C1	5CA1056.204-A1270	Hetero	105.5
14	C1	5CA1056.186-A1270	Hetero	103.2
16	C1	5CA1056.194-A1270	Hetero	105.8
17	C1	5CA1056.196-A1270	Hetero	106.0
21	C1	5CA1056.207-A1270	Hetero	105.8
59	C1	5CA1056.248-A1200	Hetero	104.5
63	C1	5CA1056.254-A1200	Hetero	103.0
72	C9	5CA1056.180-A1279	Hi-Q	122.5
28	C9	5CA1056.221-A1280	Hi-Q	115.7
30	C9	5CA1056.227-A1280	Hi-Q	117.3
34	C9	5CA1056.239-A1280	Hi-Q	118.1
35	C9	5CA1056.219-A1280	Hi-Q	109.6
36	C9	5CA1056.220-A1280	Hi-Q	117.6
37	C9	5CA1056.224-A1280	Hi-Q	110.9
43	C9	5CA1056.235-A1280	Hi-Q	116.0
46	C9	5CA1056.240-A1280	Hi-Q	115.3
47	C9	5CA1056.241-A1280	Hi-Q	113.8
48	C9	5CA1056.242-A1280	Hi-Q	114.1
74	C9	5CA1056.180-A1279	RIL-144	81.3
29	C9	5CA1056.222-A1280	RIL 144	78.2
31	C9	5CA1056.233-A1280	RIL 144	97.6
32	C9	5CA1056.234-A1280	RIL 144	102.6
33	C9	5CA1056.238-A1280	RIL 144	103.7
38	C9	5CA1056.228-A1280	RIL 144	106.0
40	C9	5CA1056.230-A1280	RIL-144	104.0
41	C9	5CA1056.231-A1280	RIL-144	108.3
45	C9	5CA1056.237-A1280	RIL-144	105.1
39	C9	5CA1056.229-A1280	Hetero	112.2
42	C9	5CA1056.232-A1280	Hetero	112.0
44	C9	5CA1056.236-A1280	Hetero	112.8

[1]LSmean data of two replications presented