Identification of QTL for Seed Weight, Flowering Time, and Oil Content in Oilseed Rape (*Brassica napus* L.)

By

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ABSTRACT

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Rapeseed (*Brassica napus* L.) is cultivated worldwide as an oil crop, which is used as edible vegetable oil, animal feed and industrial oil (Bailey et al., 2006). Quantitative trait loci (QTL) controlling three yield traits, seed weight, flowering time and oil content were identified using a double haploid (DH) population. This population has 147 lines derived from the F_1 of the spring type rapeseed, Topas, and the semi-winter type rapeseed, Zhongyou821. A linkage map was constructed using 599 SRAP and 595 SNP markers, which covers a size of 2060.62 cM with an average resolution of 1.73 cM. Six QTL for three traits were detected using the data gathered from field trials in two years. Among these mapped QTL, two QTL for seed weight were located on chromosome C03 (*qSWC03*) in 2015 and on chromosome A03 (*qSWA03*) in 2016, two major QTL for flowering time on chromosome A02 (*qFTA02-1* and *qFTA02-2/3*) in two years, two QTL for oil content on chromosome C07 (*qOILC07*) in 2015 and chromosome C03 (*qOILC03*) in 2016, respectively.

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ABBREVIATIONS

ABI – Applied Biosystems Incorporated

AFLP – Amplified fragment length polymorphism

Bzh-1 – The dwarf Breizh gene

CIM – Composite interval mapping

cM – centiMorgan

DH – Doubled haploid

DNA – Deoxyribonucleic acid

EST – Expressed sequence tag

FLC – FLOWERING LOCUS

FRI – FRIGIDA

GBS – Genotyping by sequencing

IFLP – Intron fragment length polymorphism

INDEL – Insertion-deletion

ISSR – Inter-simple sequence repeats

LOD – Logarithm of the odds

LG – Linkage group

NGS – Next generation sequencing

NIR – Near infrared reflectance spectroscopy

NIL – Near-isogenic lines

QTL – Quantitative trait loci

RAPD – Random amplification of polymorphic DNA

RFLP – Restriction fragment length polymorphism

SCAR – Sequence characterized amplified region

SNP – Single nucleotide polymorphism

SRAP – Sequence-related amplified polymorphism

SSR – Simple sequence repeats

STS – Sequence target sites

TSW – Thousand seed weight

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1.0 GENERAL INTRODUCTION

Introduction

Brassica napus L. originated from an early allopolyploid evolution, resulted from the interspecific hybridization between *B. rapa* and *B. oleracea* (Nagaharu, 1935). Studies have indicated that the *Brassica* species and *Arabidopsis thaliana* L. are in the same family Brassicaceae (Schmidt & Bancroft, 2011).

Brassica napus is commonly known as rapeseed, which is used for making edible oil, protein-rich feed, industrial lubricant and biodiesel (Bailey et al., 2006). It is an important oilseed crop that contributes high economic value in Canada. It has been grown since 1960s and the production reached 21.3 million tons in Canada (Canola Council of Canada, 2017b) In addition, according to the Canola Council of Canada (2017a) the production of rapeseed makes $26.7 billion contributions to the Canadian economy.

As the demand for rapeseed oil is growing, new strategies are being explored for increasing the seed oil content. Many studies revealed that the oil yield of rapeseed could be improved by increasing seed yield and the essential agronomic traits, such as seed weight and flowering time (Zhou et al., 2014). Seed weight, flowering time, and oil content traits of *B. napus* are controlled by quantitative trait loci (QTL) (Yang et al., 2012). This genetic complexity can be addressed by QTL mapping. High-density linkage maps are developed using single or multiple molecular markers, which are selected on efficiency and costs (Chen et al., 2010; Landry et al., 1991). The ability of

detecting QTL could optimize the marker-assisted selection for crop improvement (Collard et al., 2005).

Research objectives

The long term goal of this research is to enhance the knowledge of the genetic determination of seed weight, flowering time, and oil content in *B. napus*. Many studies have identified QTL controlling these traits. The knowledge gained from such studies can be used for marker-assisted selection for crop improvement in breeding programs. The short term objectives of the research are:

1. To create linkage maps using SNP and SRAP molecular markers of ZT doubled haploid (DH) population derived from crosses among cultivars Zhongyou821 and Topas.
2. To utilize the developed linkage maps and phenotypes of field trial to identify QTL controlling seed weight, flowering time and oil content.

Research hypothesis

In this research, we hypothesize that QTL of the doubled haploid population (ZT) that derived from crosses between semi-winter type cultivar Zhongyou821 and spring type cultivar Topas would be identified for seed weight, flowering time and oil content. The identified QTL would be located on the position corresponds to other studies or on novel location.

2.0 LITERATURE REVIEW

2.1 *Brassica napus* and its progenitor diploid genome

The Brassicaceae family contains 338 genera and 3700 species that include crop plants and ornamentals (Bailey et al., 2006). The Brassicaceae family contains the cruciferous vegetables, which consist of the model plant *Arabidopsis* (thale cress), *Brassica napus* L. (rapeseed), *Brassica oleracea* L.(broccoli, cabbage, kale, etc.) and *Brassica rapa* L. (Chinese cabbage, etc.) (Schmidt & Bancroft, 2011).

Brassica napus originated from the Mediterranean area and considered as a new species formed approximately 500-1000 years ago (Gupta & Pratap, 2007; Kimber & McGregor, 1995; Prakash & Hinata, 1980).

According to the triangle of U theory, three allotetraploid species, *B. carinata*, *B. juncea*, and *B. napus* resulted from inbreeding of three ancestral diploid species of *B. rapa*, *B. nigra* and *B. oleracea* (Nagaharu, 1935).

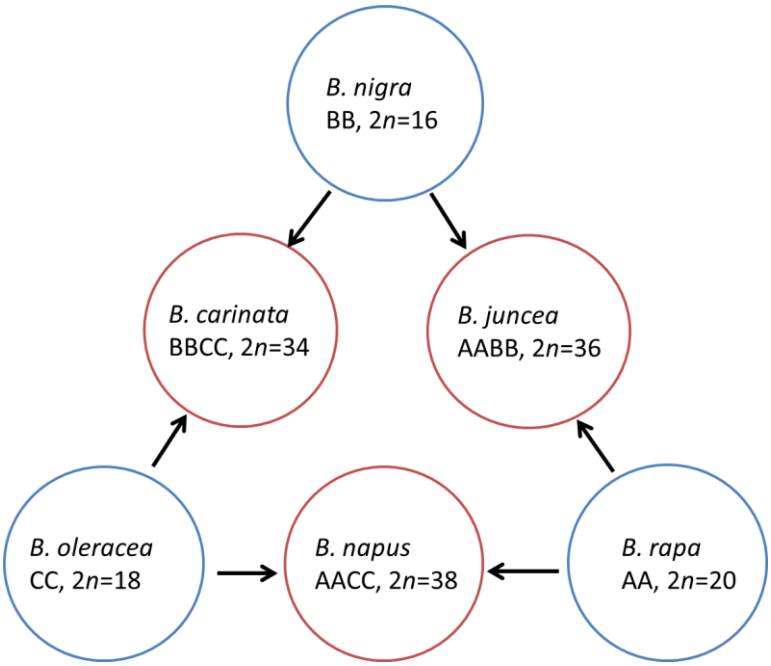

Figure 2. 1 Genetic relationship of six *Brassica* species in red (tetraploid) and blue (diploid) circles showing the triangle of U (adapted from U 1935).

*Brassica napus (*AACC) is generated by interspecific hybridization between two ancestral *Brassica* species, *B. rapa* (AA) and *B. oleracea* (CC) (Parkin et al., 1995; Prakash & Hinata, 1980). In addition, *Brassica juncea* (AABB) is the result of interbreeding from *B. rapa* (AA) and *B. nigra* (BB). *B. carinata* (BBCC) is formed from interspecific crossing of *B. nigra* (BB) and *B. oleracea* (CC) (Gómez-Campo & Prakash, 1999).

2.2 Economic importance

Brassica napus is commonly known as rapeseed and is used as edible vegetable oil, animal feed, and biodiesel used for on-road and off-road equipment (Bailey et al., 2006). Furthermore, the high erucic acid rapeseed (HEAR), containing 50-55% of erucic acid, has been used commercially for producing lubricants and preparing nitrogen derivatives (Nieschlag & Wolff, 1971).

Brassica napus makes up 13% of the global oilseed crop production and is used as vegetables such as rutabagas and fodder (Zhou et al., 2014). Rapeseed is widely grown in Canada and the production has reached 21.3 million tons in 2017, which was due to the development of new varieties and use of technological advances (Canola Council of Canada, 2017b). According to Canadian Oilseed Processors Association, 3 million tons of rapeseed oil was produced in Canada and approximately 70% of this oil was exported to the United States in 2016. In addition, Canadian rapeseed industry makes $26.7 billion contributions to the Canadian economy and $11.2 billion in wages in 2016 (Canola Council of Canada, 2017a).

2.3 Molecular Markers

For decades, people have used morphological markers to trace the desired traits in plant breeding (Collard et al., 2005). However, most agronomical traits are controlled by multiple genes, therefore, cannot be easily improved through conventional phenotypic evaluation (Collard & Mackill, 2008). The detection of polymorphisms on a molecular basis can help to better understand genetic diversity associated with agronomic traits (Agarwal et al., 2008). The linkage map is the graphic depiction of the relative distance of molecular markers that is measured in recombination frequencies between two markers (Kosambi, 1944; Lombard & Delourme, 2001).

Marker-assisted selection (MAS) is widely applied in plant breeding when the molecular markers are closely linked to the genes controlling important agronomical traits (Collard et al., 2005). MAS is efficient, reliable, and cost-effective when using proper types of molecular markers (Collard & Mackill, 2008). Therefore, it is an assistance or substitution to the traditional phenotypic selection (Ribaut & Hoisington, 1998). Different types of molecular markers are widely applied to reveal plant genetic complexity based on their own strengths as well as limitations (Agarwal et al., 2008). Molecular markers are classified into three types according to the detecting methods. The first-generation markers are based on hybridization, and the detection throughput is low (White et al., 2007). The most widely used marker for this type is restriction fragment length polymorphism analysis (RFLP) (Bostein et al., 1980). The second-generation markers are based on polymerase chain reaction (PCR) with medium throughput (White et al., 2007). There are a number of PCR based molecular

marker detecting methods. For instance, simple sequence repeats (SSR) (Tautz, 1989), random amplified polymorphic DNA (RAPD) (Williams et al., 1993), sequence characterized amplification region (SCAR) (Paran & Michelmore, 1993), amplified fragment length polymorphism (AFLP) (Vos et al., 1995), and variable number tandem repeat (VNTR) (Shin et al., 2000). Single nucleotide polymorphism (SNP) is the new generation markers, which are detected through DNA sequencing and bead array technologies with high throughput capacity. SNP have become a new generation of molecular markers as they are abundant in genomes (Li & Quiros, 2001; Tautz & Renz, 1984; Vignal et al., 2002).

2.3.1 Sequence-related amplified polymorphism (SRAP) markers

Li and Quiros (2001) developed sequence-related amplified polymorphism (SRAP) for construction of *Brassica oleracea* genetic map using AFLP. SRAP is developed using two primers that have a main purpose of amplifying open reading frames (ORFs) (Li & Quiros, 2001). SRAP markers have been applied in many other crops to amplify DNA and develop QTL to assess genetic diversity (Robarts and Wolfie, 2014). These agronomic crops include potato (He et al., 2007), Chinese cabbage (Li et al., 2012), rapeseed (Rahman et al., 2007), citrus (Amar, 2012) and wheat (Dong et al., 2010).

2.3.2 Single nucleotide polymorphism (SNP) markers

The abundance and intensity of single nucleotide polymorphism (SNP) enable it the most feasible marker for automated, which is performed by different techniques in plant breeding studies (Alkan et al., 2011; Steemers & Gunderson, 2007). The high frequency of SNP is located in coding and mostly in non-coding regions (Vignal et al., 2002). The nature of SNP makes them an ideal tool to construct genetic maps (Kruglyak, 1997).

The selection of the SNP detecting techniques depends on their availability, reliability, cost, and time (Vignal et al., 2002). Sequencing-based techniques were used since the late 1970s, and several next generation sequencing (NGS)-based technologies have been established for SNP detection. These include Roche/454, Illumina, and SOLiD (Kircher & Kelso, 2010; Sanger et al., 1977).

2.4 Genotyping by sequencing (GBS)

MAS is widely used for crop improvement in breeding programs; however, traditional marker discovery is costly, time consuming and laborious (Collard & Mackill, 2008). Therefore, revolutionary high-throughput genome sequencing technologies were developed, and collectively named next-generation sequencing (NGS). As a novel application of NGS, the genotyping by sequencing (GBS) approach can be used to discover SNP in plant genomes (He et al., 2014). In GBS, restriction enzymes are used to prepare high-throughput NGS libraries (Elshire et al., 2011). GBS is now becoming an efficient and unique tool for MAS. It is used to determine SNP in many crops such as, wheat (Poland et al., 2012), maize (Crossa et

al., 2013) and rapeseed (Bayer et al., 2015). In addition, GBS is used to simultaneously identify SNP markers and perform genotyping (Elshire et al., 2011).

The procedure of GBS involves preparation of DNA samples and adapter selection, preparation of libraries for NGS, filtering raw sequence data, DNA sequence alignments and mapping (Elshire et al., 2011). Elshire et al. (2011) first developed the GBS method, which digests high molecular weight DNA using a restriction enzyme, mostly *ApeKI*. This enzyme is type II restriction endonuclease, which is used to reduce genome complexity (Elshire et al., 2011). In addition, the mapping of SNP is done using software like Tassel (Elshire et al., 2011). Later, a double enzyme digestion (*Pst*I/*Msp*I) GBS protocol was applied to wheat and barley and created a more stable library for sequencing (Poland et al., 2012).

Overall, GBS is useful because a large number of SNP are discovered and it offers a fast and low-cost tool to breeders to implement genome-wide association studies (GWAS) (Narum et al., 2013; Poland & Rife, 2012).

2.5 Linkage map construction using molecular markers

Linkage maps are like road maps that represent the genome using molecular markers and expressed as centiMorgan (cM) (Kosambi, 1944; Sturtevant, 1913). One cM is defined as one genetic recombination occurring in 100 meiotic divisions between two loci or markers (Sturtevant, 1913). Therefore, markers are the landmarks that can reveal the location and relative distance of genes according to genetic recombination of markers or loci in a population (Xu, 2010). Linkage maps are the perfect tool to locate

QTL for complex traits, for example, seed weight, flowering time and oil content (Cai et al., 2012; Liu et al., 2016; Qiu et al., 2006) .

2.6 Linkage mapping in *Brassica napus*

The linkage maps for *B.napus* are constructed by molecular markers such as SSR, RAPD, SCAR, AFLP, RFLP, and SRAP (Geng et al., 2012; Lombard & Delourme, 2001; Lowe et al., 2004; Piquemal et al., 2005; Qiu et al., 2006; Sun et al., 2007; Uzunova et al., 1995; Zhao et al., 2005). Recently, SNP markers are being used for developing more saturated maps to identify genes of interest or QTL (Delourme et al., 2013; Liu et al., 2013; Wang et al., 2015). A list of representative linkage maps is summarized in Table 2.1, which indicates two essential features: 1) map length, and 2) the average marker to marker distance (Tanksley et al., 1992; Xu, 2010). Different types of populations are selected for developing linkage maps, and each population has its own advantages and limitations. Population types such as F_2, backcross (BC), doubled haploid (DH) lines, near-isogenic lines (NIL), and recombinant inbred lines (RIL) are used for developing genetic maps in *B. napus* (Kumar, 1999).

Table 2. 1 Summarization of markers and linkage maps developed in *Brassica napus**.

Type of population	Marker Type	Map length (cM)	Marker interval (cM)	References
DH	RFLP	1016	7.70	(Ferreira et al., 1995)
DH	RFLP, RAPD, phenotypic	1441	6.96	(Uzunova et al., 1995)
Re-synthesized	RFLP	1656	4.15	(Parkin et al., 1995)
DH	RFLP, RAPD, Isozyme	1765	7.00	(Foisset et al., 1996)
DH	RFLP, RAPD, AFLP, Isozyme, SSR, *Bzh-1*	2429	4.49	(Lombard & Delourme, 2001)
Substitution lines	RFLP	1238	7.84	(Burns et al., 2003)
DH	SSR	1957	20.18	(Lowe et al., 2004)
F2	SSR, SCAR	2619	8.97	(Piquemal et al., 2005)
DH	SSR	1196	9.57	(Zhao et al., 2005)
F2	AFLP	1250	5.43	(Peleman et al., 2005)
DH	SSR	2690	8.81	(Delourme et al., 2006)
DH	AFLP, RFLP, SSR, STS	1685	6.08	(Qiu et al., 2006)
DH	SSR, SRAP	1747	4.40	(Chen et al., 2007)
DH	SRAP	1605	0.12	(Sun et al., 2007)
DH	SRAP, SSR	1868	4.83	(Chen et al., 2010)
DH	EST, SSR, SRAP, SCAR	1949	5.20	(Zhao et al., 2012)
DH	SSR, IFLP, INDEL,SNP	2266	4.30	(Sun et al., 2012)
DH	SSR, SRAP, ISSR, SCAR	2244	3.61	(Geng et al., 2012)
DH	SSR, SRAP, STS, IFLP	1784	4.40	(Wang et al., 2013)
RIL	SNP	1833	0.66	(Liu et al., 2013)
DH	SNP	2250	0.31	(Delourme et al., 2013)
DH	SSR, ISSR, SRAP, SCAR	2241	3.71	(Javed et al., 2016)
DH	SNP, SRAP	2598	0.47	(Liu et al., 2016)

*: DH, doubled haploid; RFLP, restriction fragment length polymorphism; RAPD, random amplification of polymorphic DNA; AFLP, amplified fragment length polymorphism; SSR, simple sequence repeats; *Bzh-1*, the dwarf Breizh gene; SCAR, sequence characterized amplified polymorphism; STS, sequence target sites; SRAP, sequence-related amplified polymorphism; EST, expressed sequence tag; IFLP, intron fragment length polymorphism; INDEL, insertion-deletion; ISSR, inter simple sequence repeat; SNP, single nucleotide polymorphism.

2.7 Quantitative trait loci (QTL) mapping

QTL mapping is used to detect chromosome regions for the traits of interest through QTL analysis and the construction of linkage maps (Collard et al., 2005). The identification of QTL is performed using DNA markers. The principle of mapping is based on the genes in segregation through crossing-over during meiosis that allows identification of the marker frequency of recombinant genotypes (Collard et al., 2005).

The predominantly used gene mapping methods are QTL mapping. However, parental diversity is a major drawback for QTL mapping (Breseghello & Sorrells, 2006; Yu & Buckler, 2006). Accurate results in genetic mapping require a rich genetic variance, high-density markers, a large population size, greater phenotypic variance and proper statistical methods with a combination of QTL mapping and association mapping (Fu et al., 2017).

There are two steps necessary for QTL mapping: genotyping and phenotyping (Mccough & Doerge, 1995). Genotyping determines genetic relevance to its parents via the use of molecular markers and phenotyping is to measure traits of interest for the same set of genotyped progeny with their parents in replicated trials (McCough & Doerge, 1995; Mohan et al., 1997).

Identification of QTL includes single-marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM), multiple interval mapping (MIM), and Bayesian interval mapping (BIM) (Chandra & Pandey, 2017). The SMA uses single markers for detecting QTL, which is the simplest method. Basic statistics methods including t-test and analysis of variance are used (Liu, 1997; Tanksley, 1993). In general, SMA does not determine the association of molecular markers with one or

more QTL and the detection is compromised when the distance between the marker and QTL increases (Chandra & Pandey, 2017).

The simple interval mapping is used to test the presence of a QTL at multiple locations between pair of loci (Lander & Bolstein, 1989). In comparison, this method is considered statistically more accurate than SMA (Chandra & Pandey, 2017). The output of the simple interval and CIM is given in logarithm of odds (LOD) values or a likelihood ratio (LR) (Collard et al., 2005). A QTL position is identified on the linkage map if the LOD value exceeds a threshold value (Liu, 1997). The QTL with minor effects on the phenotype may be hard to detect due to higher LOD threshold values (Collard et al., 2005). For instance, LOD values or scores exceeding 3 are considered to be significant in QTL mapping (Nyholt, 2000).

Composite interval mapping is a more robust approach, which combines multiple regression with interval mapping to isolate individual QTL effects (Jansen and Stam, 1994). It is more accurate when linked QTL are involved. Composite interval mapping has its advantage by employing background markers, which provides sensitivity for detecting QTL linked to background markers. Additionally, it aids in the separation of multiple linked QTL (Zeng, 1994).

Multiple interval mapping is used for multiple QTL via simultaneously searching multiple marker intervals and for detecting QTL and QTL interaction (Chandra & Pandey, 2017). Bayesian interval mapping (BIM) provides a model for QTL mapping, and provides the number, position, and effects of QTL. It can estimate QTL effect and position seperately (Satagopan et al., 1996).

A number of limitations are present in QTL mapping since QTL detection depending on statistical interference errors in statistical analysis may result in the biological insignificance of QTL. Traits that are hard to be precisely phenotyped could hamper accurate QTL analysis (Shi et al., 2009). Moreover, quantitative traits are sensitive to the environment, which requires researchers to do multi-location field trials (Maheswaran, 1998; Tanksley, 1993). In general, a large population size is required for accurate detection of QTL across environments (Maheswaran, 1998).

2.7.1 Seed weight and QTL mapping

Seed weight and seed sizes are important traits in crop improvement since the beginning of agriculture (Li et al., 2015). In general, larger and heavier seeds are selected in the agricultural sector because they are important components in yield (Ali et al., 2003). In addition, seeds store oil and proteins, which are the predominant products of crops (Ali et al., 2003). In natural settings, small seeds are dispersed widely, and larger seeds have good adaptability. However, depending on the climate, the seed number and weight are highly variable (Bodnaryk & Lamb, 1991).

The final size of a seed is influenced by an array of genes that regulate seed size through endosperm proliferation, maternal regulation and paternal imprinting (Haig & Westoby, 1991). For instance, *APETALA2* (*AP2*) is a gene member of the *AP2/EREBP* family that controls seed embryo mass via inhibits endosperm growth in early seed development (Ohto et al., 2005). The seed size of *Arabidopsis Ap2* mutants showed significantly increased seed mass ranging from 27% to 104% (Jofuku et al.,

2005). Through comparative mapping, the homologous gene of the *Arabidopsis AP2* (*AtAP*$_2$) was identified in *Brassica* species. There are three copies identified in *B. rapa*, two copies in *B. oleracea* and one copy from *B. napus* with a gene-specific marker for *BnAP2* (Cai et al., 2012).

In seed yield components, seed weight has comparatively high heritability (Gnan et al., 2014). Quijada et al. (2006) detected three QTL for seed weight, which are found on N7, N17, and N19 linkage groups. Udall et al. (2006) located six QTL in the Hua DH population, four QTL in the SYN DH population, and one major QTL on N14 in all environments. One recent study detected QTL for 1000-seed weight (TSW) on chromosome A09 from a DH population crossed between G-42 and 7-9 (Geng et al., 2016).

2.7.2 Flowering time and QTL mapping

The optimal yield of crops relies on the adaptation of reproductive systems and responses of plants to environmental stresses (Ferreira et al., 1995). The climatic adaptation of a reproductive system involves the conversion of the vegetative phase to the generative phase, which is achieved by the setting of flowering time (Méndez-Vigo et al., 2011). The investment of plants on the transition is controlled by leaf and root architecture and its synchronization with photoperiod, nutrients, temperature, water availability and light quality and quantity (Jaeger et al., 2006; Jung & Müller, 2009). The optimal time for flowering is determined by the flowering gene

network, transcription factors, photosensors, enzymes and miRNAs (Jaeger et al., 2006; Jung & Müller, 2009; Khan et al., 2014).

There are two types of rapeseed based on the difference in growing seasons, annual and bi-annual (Schmidt & Bancroft, 2011). Annuals flower in the first growing season and take about 40 to 60 days (Schmidt & Bancroft, 2011). In comparison, biennials require vernalization and flower after 150 to 185 days after planting (Schmidt & Bancroft, 2011). Temperature and photoperiod are major factors affecting flowering time, which later have an effect on net yield (Fraszczak et al., 2011). Annuals are growing in Canada where winter temperatures are below freezing, and spring is short. Therefore, annuals in Canada are referred to spring-type rapeseed. Biennial plants are exposed to the low-temperature environmental conditions and they are called winter type rapeseed. Both types of rapeseed undergo the same physiological and developmental process to flower (Schmidt & Bancroft, 2011). In a previous study, QTL for flowering time in *B. napus* were determined by analyzing the variations in plant materials formed from crosses of winter and spring types of varieties (Mei et al., 2009).

Flowering time is controlled by multiple loci. The QTL for flowering time in a given population can vary since the complexity of this trait makes it hard to detect the position of the QTL accurately (Bohuon et al., 1998). Analysis of mapping populations by Osborn et al. (1997) identified six QTL related to flowering time, where QTL *ft13* located on N3 of the general map had a strong influence on flowering time. Butruille et al. (1999) detected other QTL such as *ft17* located on N12.

Furthermore, Udall et al. (2006) and Quijada et al. (2006) reported *ft2a* and *ft3* correspondingly. In addition, Luo et al. (2014) identified four major loci localized on four chromosomes of A06, A07, C08, and C09. Moreover, Raman et al. (2013) detected five significant QTL on A02, A03, C02, C06, and C08 from a DH population. In addition, segregation distortions were observed in an evaluated population of *B. napus*, which could be caused by a reduction in the number of the male and female gametes (Mano et al., 2005). The integration of molecular markers linkage maps in segregating populations provides valuable information for identifying QTL locations in many life forms (Cloutier et al., 1995).

Comparative genetic analysis revealed that three loci characterized in *Brassica* species correspond to single locus in *Arabidopsis* genome (Osborn et al., 1997). Pathways that control flowering time of *Arabidopsis thaliana* include photoperiod and vernalization (Mouradov et al., 2002). The genetic analysis of the pathways between *A. thaliana* and three allotetraploid *Brassica* species identified *Flowering locus* (*FLC*) homologs, *BnFRI.A*$_3$ and *BnFT* paralogs (Tadege et al., 2001; Wang et al., 2014; Zou et al., 2012).

Genetic analysis of *Arabidopsis* shows the presence of 80 loci controlling flowering loci and some of these had epistatic interactions and additive effects on flowering time; however, only two loci including *FRIGIDA (FRI)* and *FLC* are responsible for flowering time differences (Burn et al., 1993; Clarke & Dean, 1994). These two loci act as repressors for flowering. *Flowering locus* encodes a MADS-box floral repressor while *FRI* upregulates *FLC* by increasing methylation of histone 3

lysine 4 (H3K4) and enhances the efficient splicing of the *FLC* locus. Evidence shows that with a reduced level of *FLC*, vernalization induces flowering (Choi et al., 2011; Geraldo et al., 2009; Michaels et al., 2003; Sheldon et al., 1999). Vernalization is an adaptive response of flowering to exposure of plants to the cold of winter (Tadege et al., 2001). The role of *FLC* in both spring and winter type rapeseed has been investigated. According to Osborn et al. (1997), the vernalization-responsive *FRI* and *FLC* found in *Arabidopsis* were also detected in *B. napus*, which contained five *FLC* homologs (*BnFLC1* to *BnFLC5*) to delay flowering. These homologs showed regression of flowering at different levels (Tadege et al., 2001). According to Tadege et al. (2001), *BnFLC1*, *BnFLC2,* and *BnFLC3* caused a late flowering in *B. napus*.

2.7.3 Oil content and QTL mapping

Rapeseed is an economically important oil crop, which contributes 48% of plant oil production in Canada, so the primary goal of the industry is to increase rapeseed oil content (Han-zhong, 2010; Seberry et al., 2010). In accessions such as Major (France), Zephyr (Canada), and Zhongyou0361 (China), oil comprises 50 to 55% of the seeds (Han-zhong, 2010).

Oil content of rapeseed is controlled by multiple loci. Therefore, identification and mapping of QTL have become an important strategy to improve oil content (Ecke et al., 1995). Highly variable environments affect oil content in rapeseed, which is not yet fully explored (Delourme et al., 2006; Zhao et al., 2008). Many QTL for oil content have been located via many types of markers (Sun et al., 2016). For example,

RFLP was applied to map QTL for seed erucic acid and oil content (Ecke et al., 1995), SSR markers for genetic control of seed oil content (Delourme et al., 2006), and combinations of SRAP, SSR, and AFLP for linkage analysis of *B. napus* oil content (Yan et al., 2009). Recent studies aided by advanced genotyping technology are able to construct linkage maps. These different markers such as SNP, SSR, and AFLP resulted in various QTL numbers, QTL effects, and QTL distributions (Burns et al., 2003; Chen et al., 2010; Delourme et al., 2006; Ecke et al., 1995; Qiu et al., 2006; Sun et al., 2012; Teh & Möllers, 2016; Yan et al., 2009; Zou et al., 2010).

Sums of transcription factors (TF), which regulate seed oil accumulation, were identified in *Arabidopsis* (Riechmann et al., 2000). The *GLABRA2 (GL2)* gene regulates seed oil content and trichome development in *Arabidopsis* (Rerie et al., 1994; Shen et al., 2006). The *GL2* orthologues have been identified in *Brassica* species and the orthologue (*BnaC.GL2.b*) recognized from *B.napus* C-genome sequence is most analogous to *Arabidopsis GL2* when analysed phylogenetically (Chai et al., 2010). The seed oil content of *GL2*-mutant-like *B.napus* transgenic line had increased consistently when overexpressed and supressed, but the wild-type-like overexpression did not show detectable differences (Chai et al., 2010). The mechanism behind how transcription factor *GL2* regulates seed oil accumulation is unclear (Shen et al., 2006).

QTL for oil content in rapeseed varied among studies, ranging from three to 27 QTL and distributed on 17 out of the 19 chromosomes, from chromosome A01-A10, C01-C03 and C05-C08 (Burns et al., 2003; Delourme et al., 2006; Ecke et al., 1995; Javed et al., 2016; Qiu et al., 2006; Sun et al., 2012; Teh & Möllers, 2016;

Wang et al., 2013; Yan et al., 2009). However, variation in environmental conditions can reduce the applicability of QTL. According to Chen et al. (2010) 23 out of the 27 QTL for oil content were associated with only a single environment. In addition, among 18 QTL identified in Rapid x NSL25 and Rapid x NSL96 DH populations, only three were reproducible in three trials. Therefore, the identification of stable QTL with small confidence intervals is valuable for plant breeding (Chen et al., 2010).

3.0 IDENTIFICATION OF QTL CONTROLLING SEED WEIGHT, FLOWERING TIME, AND SEED OIL CONTENT IN *Brassica napus*

3.1 Abstract

To identify QTL for seed weight (SW), flowering time (FT), and seed oil content (OC) in *Brassica napus* L., a doubled haploid (DH) population with 147 lines was developed by crossing cultivar Topas with Zhongyou821. A linkage map was constructed using 599 SRAPs and 595 SNP, covering a map distance of 2060.6 cM with a mean resolution of 1.7 cM. In addition, six QTL for seed weight, flowering time, and oil content were identified. QTL for seed weight were located on chromosome C03 (*qSWC03*) in 2015, and on chromosome A03 (*qSWA03*) in 2016. Two major QTL for flowering time were located on chromosome A02. Moreover, two QTL for oil content were detected on chromosome C07 (*qOILC07*) in 2015 and chromosome C03 (*qOILC03*) in 2016. The QTL identified in this study can be used for further study in cloning QTL of the three traits.

3.2 Introduction

Brassica napus L. (rapeseed) is an amphidiploid, which contains two subgenomes (AACC) from two different ancestral *Brassica* species through interspecific hybridization (Olsson, 1960). It consists one diploid genome from *B. rapa* (AA) and one diploid genome from *B. oleracea* (CC) (Nagaharu, 1935).

Brassica napus makes up 13% of the global production and is commonly used as animal feed, edible vegetable oil, and biodiesel (Bailey et al., 2006). Due to the enhanced economic importance of this oil crop, there is considerable interest in increasing oil content in rapeseed. Many studies have revealed that oil yield of rapeseed could be increased by improving seed weight and flowering time (Bailey et al., 2006). However, it is hard to address the genetic complexity since these traits are quantitative traits (Yang et al., 2012).

QTL mapping is a method to detect genomic sections related to a trait of interest (Yang et al., 2012). As an important part of QTL mapping, high-density linkage maps are considered a critical tool for understanding genome structure and are extensively used to tag genes of important traits (Paterson et al., 1996). Such linkage maps are developed using molecular markers detected in segregating populations. Therefore, constructing linkage maps is the one of the most important components in QTL mapping (Cui et al., 2015).

There are many types of molecular markers established for linkage maps. The first molecular marker detection method used is RFLP, which is challenging due to variable hybridization, radioactivity, and the limited number of available probes (Bernatzky & Tanksley, 1986). Later, PCR-based markers were developed including

SSR (Tautz, 1989), CAPS (Konieczny & Ausubel, 1993), RAPD (Williams et al., 1993), SCAR (Paran & Michelmore, 1993), AFLP (Vos et al., 1995), and DALP (Desmarais et al., 1998). The PCR-based markers are amplified from a single copy of a DNA segment, which is relatively inexpensive and less time-consuming than RFLP (Semagn et al., 2006). Recently, SNP markers have been adopted in many studies. In comparison to other markers, SNP markers are more efficient and used to improve map density, reduce costs and time significantly (He et al., 2014).

Numerous linkage maps in *Brassica* species have been constructed based on a single molecular marker (Landry et al., 1991) while some other maps are generated via several types of molecular markers (Chen et al., 2010; Cheng et al., 2009; Delourme et al., 2006; Suwabe et al., 2008). In this study, relatively high-density linkage maps were constructed by combining SRAP and SNP markers

The objective of the study is to identify QTL controlling seed weight, flowering time and oil content using a DH population formed from a F_1 result from a cross between Zhongyou 821 and Topas.

3.3 Materials and methods

3.3.1 Plant materials

The DH population was developed using a cross Zhongyou821 x Topas (ZT) between a spring type, small seed size and high oil content canola cultivar Topas (Svalof A.B., Sweden) and a semi-winter type, large seed size and low oil content rapeseed cultivar Zhongyou821 (He et al, 1987). One hundred and forty-seven DH lines of the ZT population were used to identify QTL for seed weight, flowering time,

and oil content (Liu, 2015). The DH lines were planted in growth chambers and greenhouses to increase seeds for field trials.

3.3.2 Seed reproduction

All DH and parental genotypes were planted in 96-cell tray flats filled with Sunshine #4 Mix (Sun Gro Horticulture, MA, USA) for seed germination and kept at 22 ℃ during the day and at 18 ℃ in the night in a growth chamber for 16-h artificial lighting for each day before transplanting into 15 cm diameter plastic pots. All pots were filled with a potting mixture containing peat moss, sand and soil with a ratio of 2 : 2 : 1. Two pots per DH and parental genotypes were watered daily and placed in the greenhouse (Agriculture Building, University of Manitoba) at 25 ℃ during the day and at 18 ℃ in the night with a 16-h photoperiod. Liquid fertilizer N-P- K in 20 : 20 : 20 ratio at 1.1 g / L of concentration was applied every second week (Plant-Prod, Leamington, Canada). In addition, pesticides, imidacloprid and spinosad, were used to control aphids and thrips by soil injection and spraying during reproductive development (Bayer Corporation, Robinson Township, USA; Dow AgroSciences, Indianapolis, USA).

3.3.3 Field trials

In the fall of the previous year of research, Edge® granular herbicide (Gowan Canada, Winnipeg, MB, CA) and granular fertilizer were applied on the field. The field was disked twice and cultivated in spring to break up clumps.

The DH population ZT was planted in late May of 2015 and mid-June of 2016 at the Point Research Station of the University of Manitoba (49.82 ˚N / 97.12 ˚W) under

rain fed conditions. The soil at this location is characterized as a sandy clay loam. Two parents and all individual DH lines were evaluated in a randomized complete block design with two replicates in each year. In addition, the seeds were planted in a 3 m row with 0.4 m row spacing for each replicate.

The insecticide, helix-treated corn grit (Syngenta Basel, Switzerland) was planted in row with seeds to control flea beetles and other pests. Once the plants reached the proper leaf stage, a herbicide mixture of Poast® (BASF, Ludwigshafen, Germany), Muster® (DuPont, Wilmington, DE, USA), and Lontrel® (Dow Agroscience, Midland, MI, USA) was used to control weeds.

At the flowering stage, the Lance® fungicide (BASF Canada Inc, Mississauga, ON, CA) was applied. The flowering time was recorded when more than 50% of the plants in a row had initiated flowering. Each row of the ZT population was harvested by hand when the plants were at full maturity stage in the fall of each year.

3.3.4 Seed weight and oil content measurement

One thousand seed weight (TSW) in grams in the replicates was used for seed weight for a DH line. After seed threshing by hand, seed cleaned using 1.4 mm and 1.0 mm sieves and then 1,000 well-filled seeds were selected randomly and weighed with a scale for TWS recording. The oil content of the ZT population was measured in the seed quality lab certified by the Canadian Grain Commision at the Department of Plant Science within the University of Manitoba using a FOSS 6500 near-infrared-reflectance-spectroscopy (NIR) with a standard NIR protocol (Daun et al.,

1994). Ten grams of seeds for each genotype were evaluated, and the overall oil content was expressed on WinISI® v.1.04a (ISI, Port. Matilda, PA, USA).

3.3.5 Statistical evaluation of field data

Phenotypic data for seed weight, flowering time, and oil content of the ZT population and parental lines were analyzed by ANOVA through the Generalized Linear Model Procedure (PROC GLM) from SAS v9.4 (SAS Institute Inc., NC, and USA). The threshold significance level was set at a P value equals to 0.0001 so the results would show either significant or non-significant differences.

3.3.6 DNA extraction

Fresh leaf tissue (1.0 g) was collected from one plant from each DH line that grew in the growth chamber for DNA extraction following a modified CTAB method (Li & Quiros, 2001) with an additional step of RNase treatment to purify the DNA. 0.5 g of fresh leaf tissue was macerated in liquid nitrogen for 30 s in a 1.5 ml tube, and then 0.5 ml of 2 x CTAB buffer, which contains 2% CTAB, 20 mM EDTA, 100 mM Tris and 1.4 M NaCl (pH 7.5), were added to the tube. It was incubated at 65˚C for 90 min and the 0.4 ml of chloroform was added to the tube, which was centrifuged at 14,000 rpm for 3 min for removal of proteins and plant materials. The supernatant then was transferred into a new tube where the genomic DNA was precipitated using 0.6 volume isopropanol. The remaining supernatant was thrown out and the pellet was rinsed with 70% alcohol. The DNA was then dissolved in dH_2O.

NanoDrop 2000 spectrophotometer (Fisher Scientific Company, Ottawa, ON, CA) was used to analyze the DNA concentration and purity. The pedestal surface was

cleaned with dH_2O prior to and after measurement. Two μL of DNA sample was directly applied onto the pedestal, and then the measurement was recorded. The purity of DNA was checked through a 260/280 ratio that falls within the range of 1.8 to 2.2.

3.3.7 Generation of molecular markers

Both SNP and SRAP molecular markers were used to construct linkage maps of ZT population. The genotypes of all 147 ZT DH lines were analyzed using SRAP markers, whereas SNP genotyping was only performed for 93 DH lines due to limitation.

SRAP molecular markers were developed from Liu (2015) as described by Li and Quiros (2001). Twenty-nine primer pairs were selected from 8 fluorescently labeled forward primers and 24 unlabeled reverse primers. The fluorescently labeled primers were FAM-blue, NED-yellow, PET-red, and VIC-green. The PCR reactions for SRAP markers were performed in 1.5 ml Eppendorf® PCR tubes (Eppendorf®, ON, CA) and programmed as designated by Sun et al. (2007). The PCR products were mixed and denatured in formamide (Hi-Di™, Life technologies, USA), and mixed with Liz-500 (Life technologies, USA). It was loaded onto the Genetic Analyzer (3130x1 Genetic analyzer, Life technologies, USA) and the data were analyzed with ABI GenScan 3.7 (Life technologies, USA) and Genographer® v1.6.0 (Genographer).

SNP molecular markers generated from genotype by sequencing (GBS), which was performed by Buckler Lab at Cornell University, Ithaca, New York, USA. RNA-free DNA of two parents and 93 DH lines, adapted pairs, and barcode adapters were plated in a 96 well microplate for sequencing. Each well was with unique barcode

adapter and the well was dried down. Prior to the placement, these barcode adapters and common adapters were diluted separately in TE followed by annealing in thermocycler. The barcode adapters were quantified via an intercalating dye, and then diluted in water in 1:1 ratio. The samples were digested with the restriction enzyme for 2 h at 37˚C and adaptors were ligated by adding ligase buffer with ATP and T4 ligase. The mixture was incubated at 22˚C for 1 h, and then transferred to the incubator set at 65 ˚C for 30 min to deactivate the T4 ligase. Primers with binding sites were added and PCR was performed. PCR products were cleaned and the sequencing library was purified and loaded on a DNA analyzer (BioRad, Hercules, CA) for evaluation. Single-end sequencing was performed in HiSeq 2500 system (Illumina, San Diego, CA). The raw data analyzed through the unfiltered qseq files. Burrows-Wheeler alignment tool (BWA) was used as a tool for aligning the filtered reads to the reference genome (Li & Durbin, 2009).

3.3.8 Linkage map construction and QTL analyses

The linkage map used for QTL identification was developed by combining SNP and SRAP molecular markers data. In total, 595 SNP and 599 SRAP markers were used to genotype the ZT population. Finally, all molecular markers were analyzed with JoinMap ® 4.0 (Van Ooijen, 2006) using the parameters set for the DH population type.

QTL mapping for seed weight, flowering time, and oil content was performed using Qgene ® 4.3.10 with the default setting (Joehanes & Nelson, 2008). The composite interval mapping was applied, and the threshold LOD (logarithm of odds) was calculated through running 1,000-fold permutations test ($\alpha = 0.05$) (Churchill &

Doerge, 1994). In addition, if a QTL was detected above the threshold LOD, it is considered as significant (Kosambi, 1944). The window size of QTL intervals was related to a 1-LOD reduction on both sides of the LOD peak. The QTL identification for seed weight, flowering time, and oil content was performed.

3.4 Results

3.4.1 Seed weight, flowering time, and oil content phenotypic variation

The seed weight of the ZT DH population sowed a range from 2.5-4.8 g/1000 seeds in 2015 (Fig. 3.1), and 2.7-5.2 g/1000 seeds in 2016 (Fig. 3.2). The mean value of TSW in the ZT population was 3.4 g and 3.9 g in 2015 and 2016, respectively. As shown in Figure 3.1 and 3.2, the overall distribution of seed weight followed a normal distribution skewed to the lower seed weight.

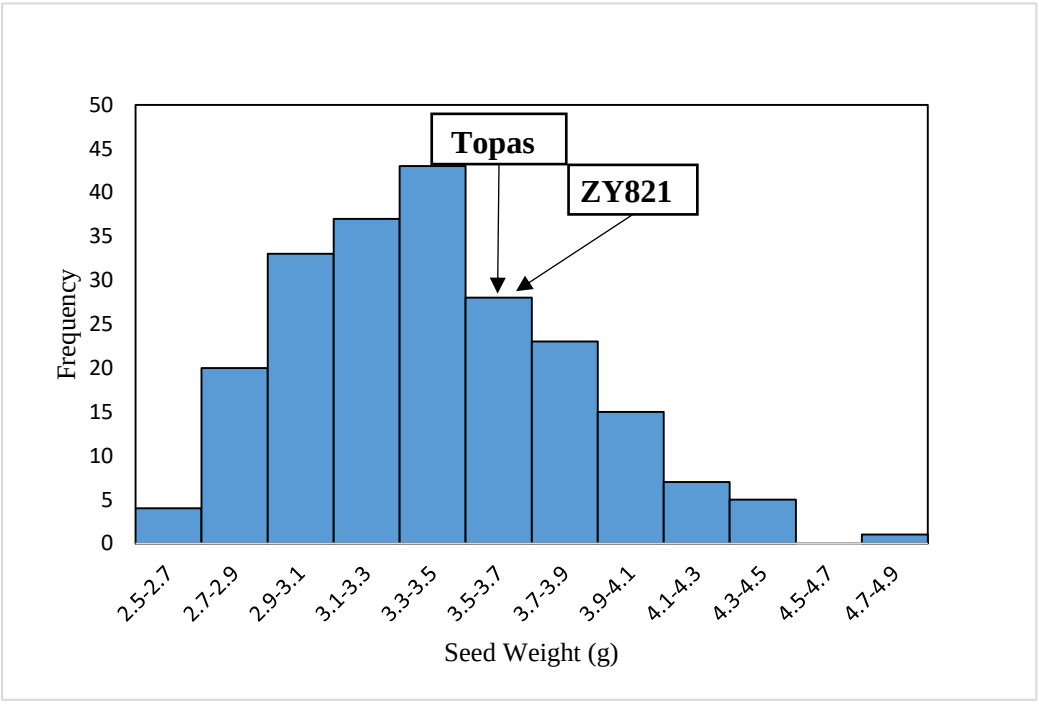

Figure 3. 1 Distribution of seed weight in ZT population in 2015 at the University of Manitoba

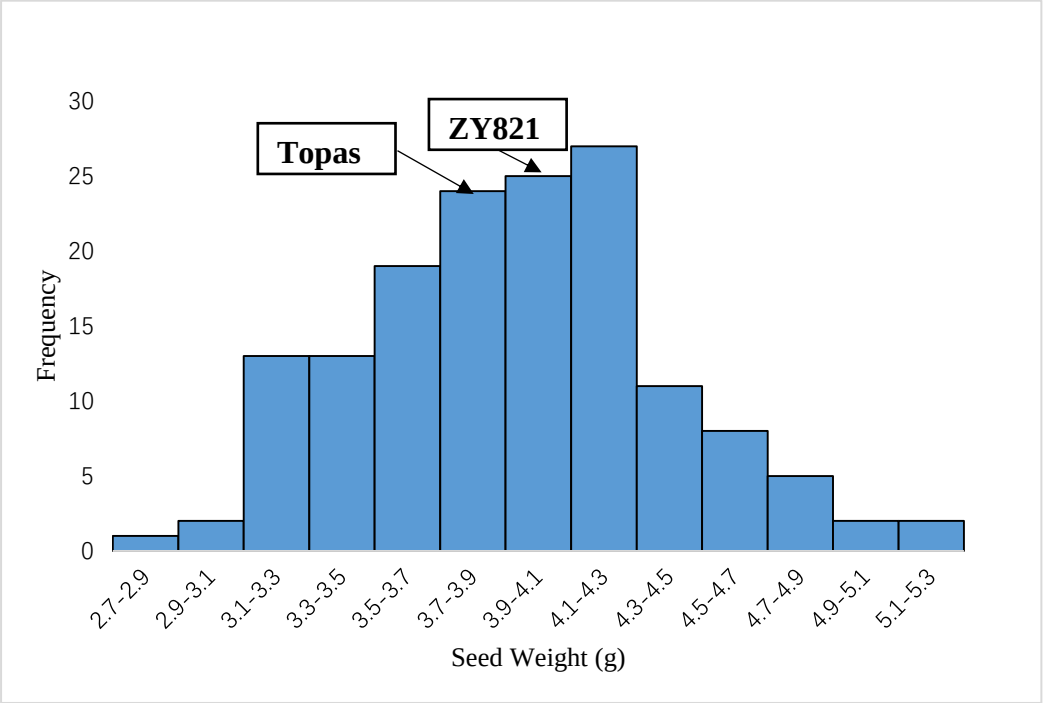

Figure 3. 2 Distribution of seed weight in ZT population in 2016 at the University of Manitoba

ZY821 had a mean seed weight of 3.6 g, which was same as the parental line Topas in 2015 (Table 3.1). In addition, ZY821 and Topas had an average seed weight of 4.1 g and 3.9 g in the year 2016 (Table 3.2).

Table 3. 1 Statistical analysis of the seed weight, flowering time, and seed oil content for the two parental lines of ZT population in the year 2015 (n = 147)

Variable	Line	Mean	SE	SD	Minimum	Maximum
Seed weight (g)	ZY821	3.6	0.1	0.4	3.0	4.1
	Topas	3.6	0.1	0.3	3.3	3.8
Flowering time	ZY821	61.5	3.2	9.2	53.0	70.0
(days)	Topas	42.0	0	0	42.0	42.0
Oil content (%)	ZY821	34.9	0.8	2.4	32.3	39.0
	Topas	43.2	0.4	0.3	42.4	44.2

Table 3. 2 Statistical analysis of the seed weight, flowering time, and seed oil content for the two parental lines of ZT population in the year 2016 (n = 135).

Variable	Line	Mean	SE	SD	Minimum	Maximum
Seed weight (g)	ZY821	4.1	0.2	0.5	3.4	4.4
	Topas	3.9	0.2	0.4	3.2	4.5
Flowering time	ZY821	54.4	1.8	5.2	50.0	64.0
(days)	Topas	43.0	0	0	43.0	43.0
Oil content (%)	ZY821	37.0	0.4	0.8	36.3	38.2
	Topas	39.4	0.8	1.9	37.0	42.7

The non-normal distribution of flowering time was displayed in the ZT population in both years (Fig. 3.3, 3.4). The mean flowering time was 47 days, ranging from 36 to 64 days, in 2015 (Table 3.1); the mean was slightly longer (52.3 days), ranging from 42 to 70 days in 2016 (Table 3.2).

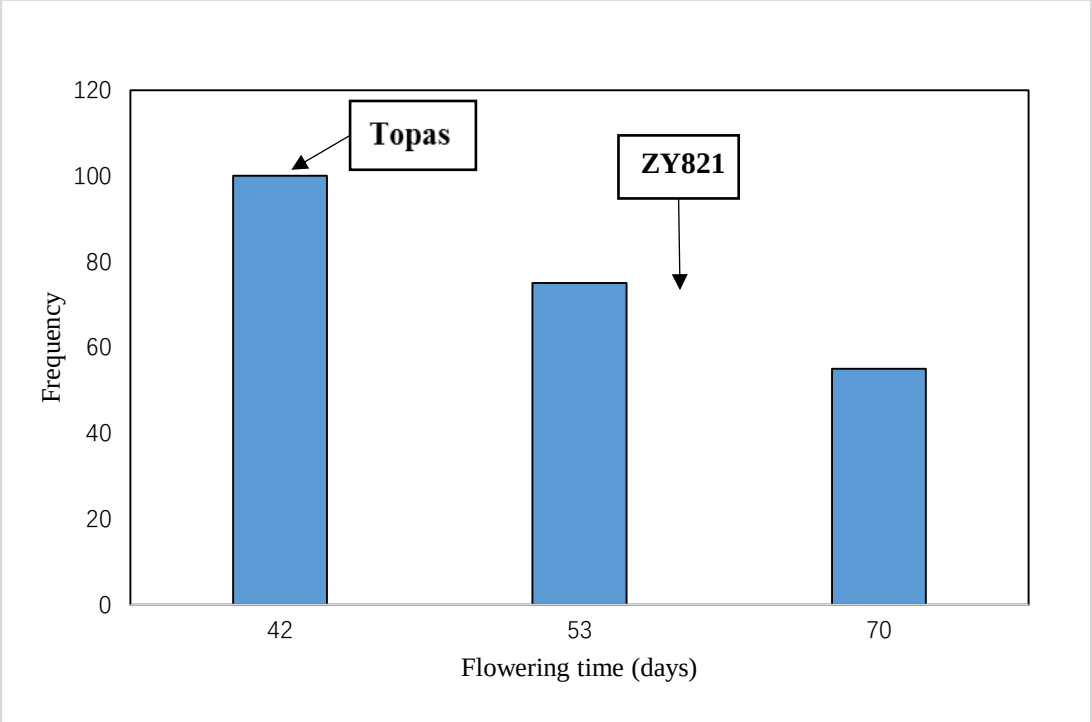

Figure 3. 3 Distribution of flowering time in ZT population in 2015 at the University of Manitoba

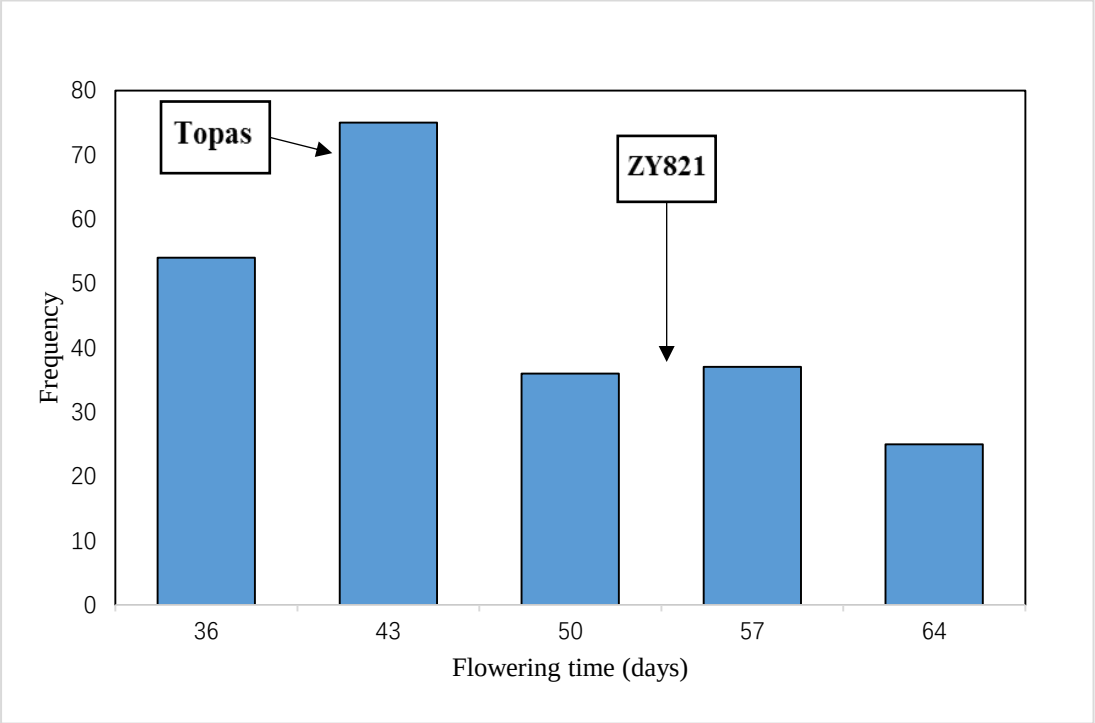

Figure 3. 4 Distribution of flowering time in ZT population in 2016 at the University of Manitoba

The distribution of oil content in the ZT population in two years showed a normal distribution with a mean value of 39.2 %, ranging from 29.8 % to 45.5 %, in 2015 (Fig. 3.5), and an average of 38.3 % , ranging from 31.0 % to 46.9 %, in 2016 (Fig. 3.6). The parental lines ZY821 and Topas had mean oil content of 34.9 % and 43.2%, respectively, in 2015 (Table 3.1), and 37.0 % and 39.4 %, respectively, in 2016 (Table 3.2). Both the seed weight and oil contents showed normal distribution in two years of field trials by performing the Shapiro-Wilks normality test, but flowering time had slight deviations from normality.

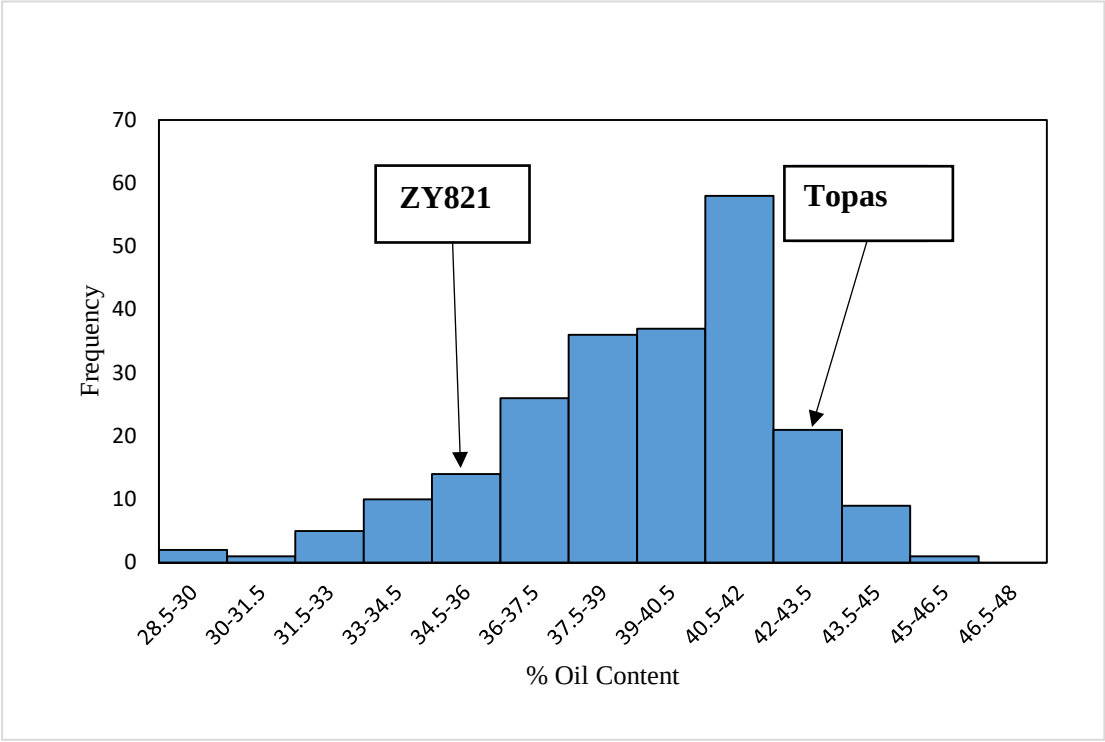

Figure 3. 5 Distribution of the percentage of oil content in ZT population in 2015 at the University of Manitoba

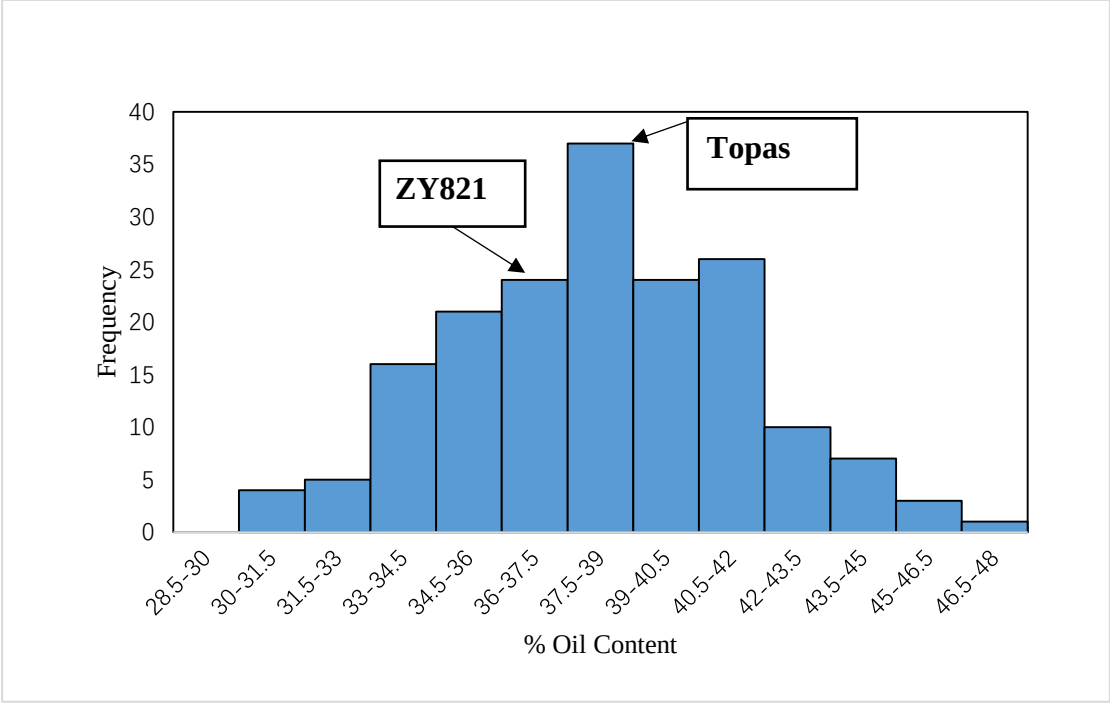

Figure 3. 6 Distribution of the percentage of oil content in ZT population in 2016 at the University of Manitoba

3.4.2 ANOVA analysis

ANOVA analysis indicated that genotypic effects were statistically significant for all three traits. The replicate effects were not significant for flowering time and oil content, but significant for seed weight in 2015 (Table 3.3). In 2016, both genotypic and replicate effects were significant for flowering time and oil content, while genotypic and replicate effects were not significant for seed weight (Table 3.4).

Table 3. 3 ANOVA for seed weight, flowering time, oil content of the ZT Population and two parental lines in two replicates in 2015.

Traits	Source	DF	Mean Square	F value	P value
Seed weight	Genotype	142	0.2	3.6	$<1.0x10^{-4}$
	Rep	1	1.7	26.2	$<1.0x10^{-4}$
	Error	126	0.1		
Flowering time	Genotype	149	212.4	7.3	$<1.0x10^{-4}$
	Rep	1	30.5	1.0	3.1×10^{-1}
	Error	144	29.2		
Oil content	Genotype	141	15.0	4.7	$<1.0x10^{-4}$
	Rep	1	0.0	0.0	9.8×10^{-1}
	Error	127	3.2		

Table 3. 4 ANOVA for seed weight, flowering time, oil content of the ZT Population and two parental lines in two replicates in 2016.

Traits	Source	DF	Mean Square	F value	P value
Seed weight	Genotype	119	0.3	2.1	$1.0x10^{-4}$
	Rep	1	1.8	12.9	$5.0x10^{-4}$
	Error	90	0.1		
Flowering time	Genotype	142	155.5	20.2	$<1.0x10^{-4}$
	Rep	1	262.4	34.1	$<1.0x10^{-4}$
	Error	141	7.7		
Oil content	Genotype	126	15.1	5.2	$<1.0x10^{-4}$
	Rep	1	145.6	50.5	$<1.0x10^{-4}$
	Error	93	2.9		

3.4.3 Linkage map of the ZT DH population

The linkage map of the ZT population was created using 595 SNP and 599 SRAP molecular markers. A total of 1194 polymorphic loci were analyzed with JoinMap ®4.0 using the parameters set for the DH population type and LOD values ranging from 2.0 to 7.0 (Fig. 3.7).

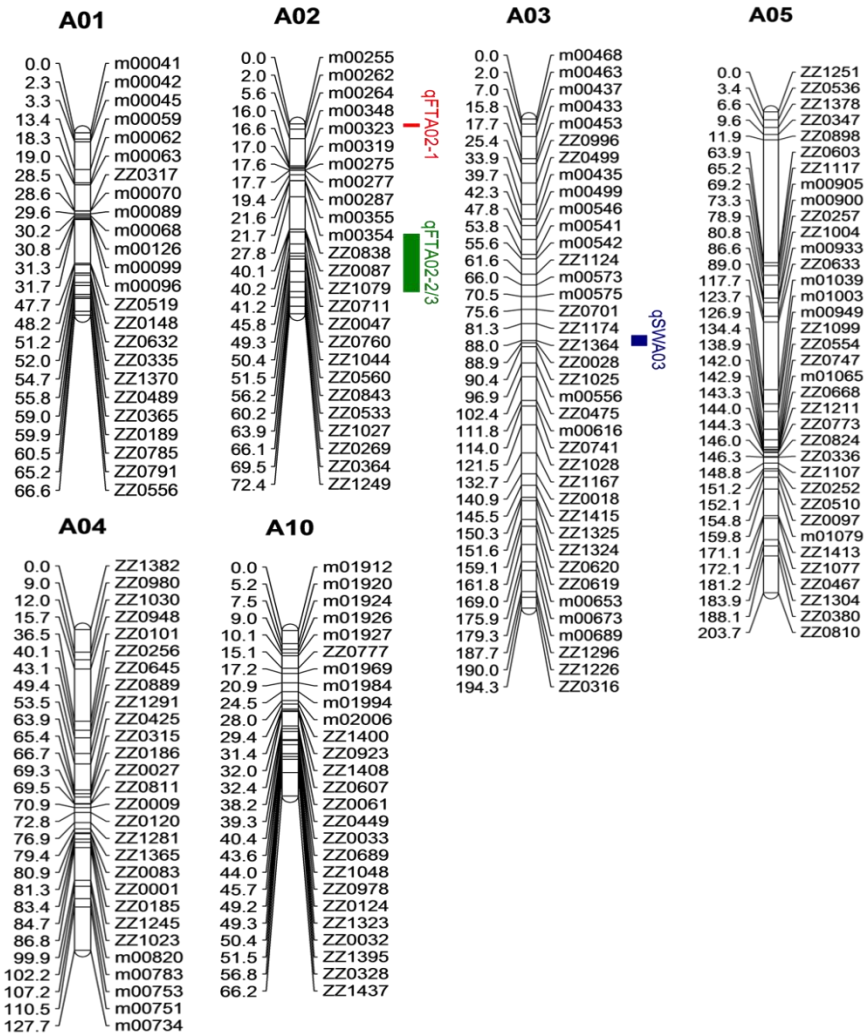

Figure 3. 7 The linkage maps developed using SRAP and SNP markers with six QTL on chromosomes A02, A03, C03 and C07.

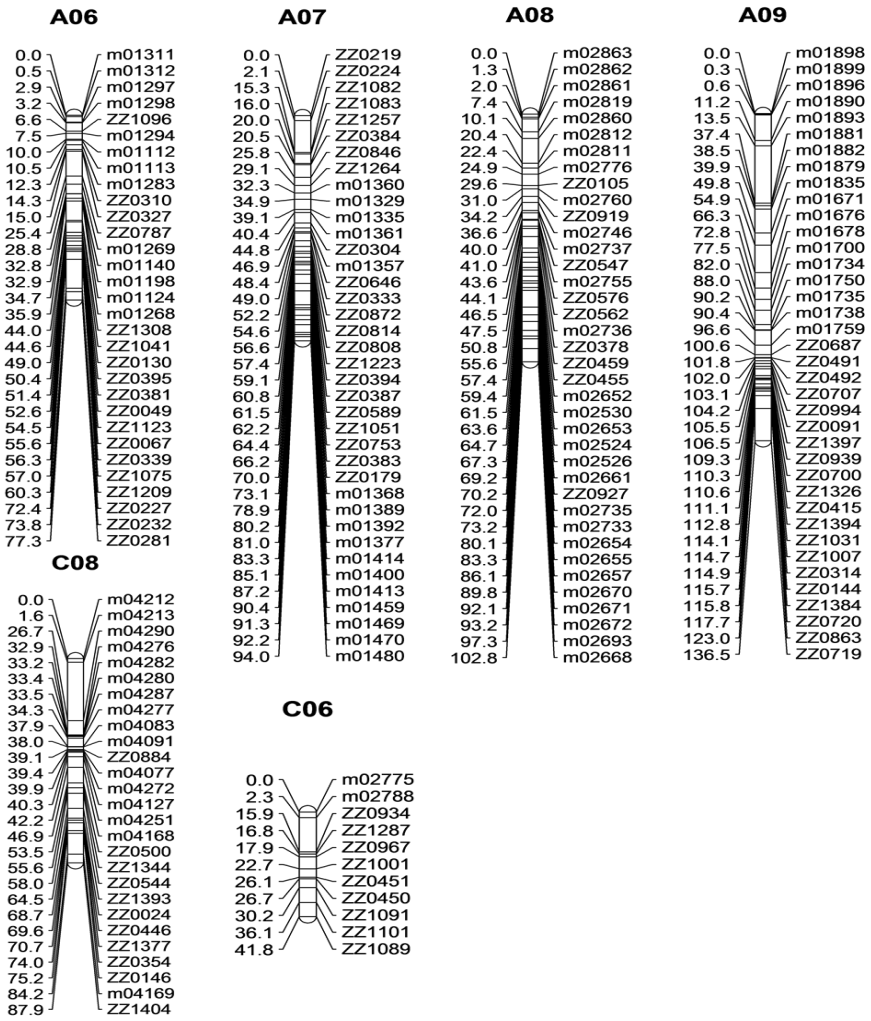

Figure 3. 7 Continued.

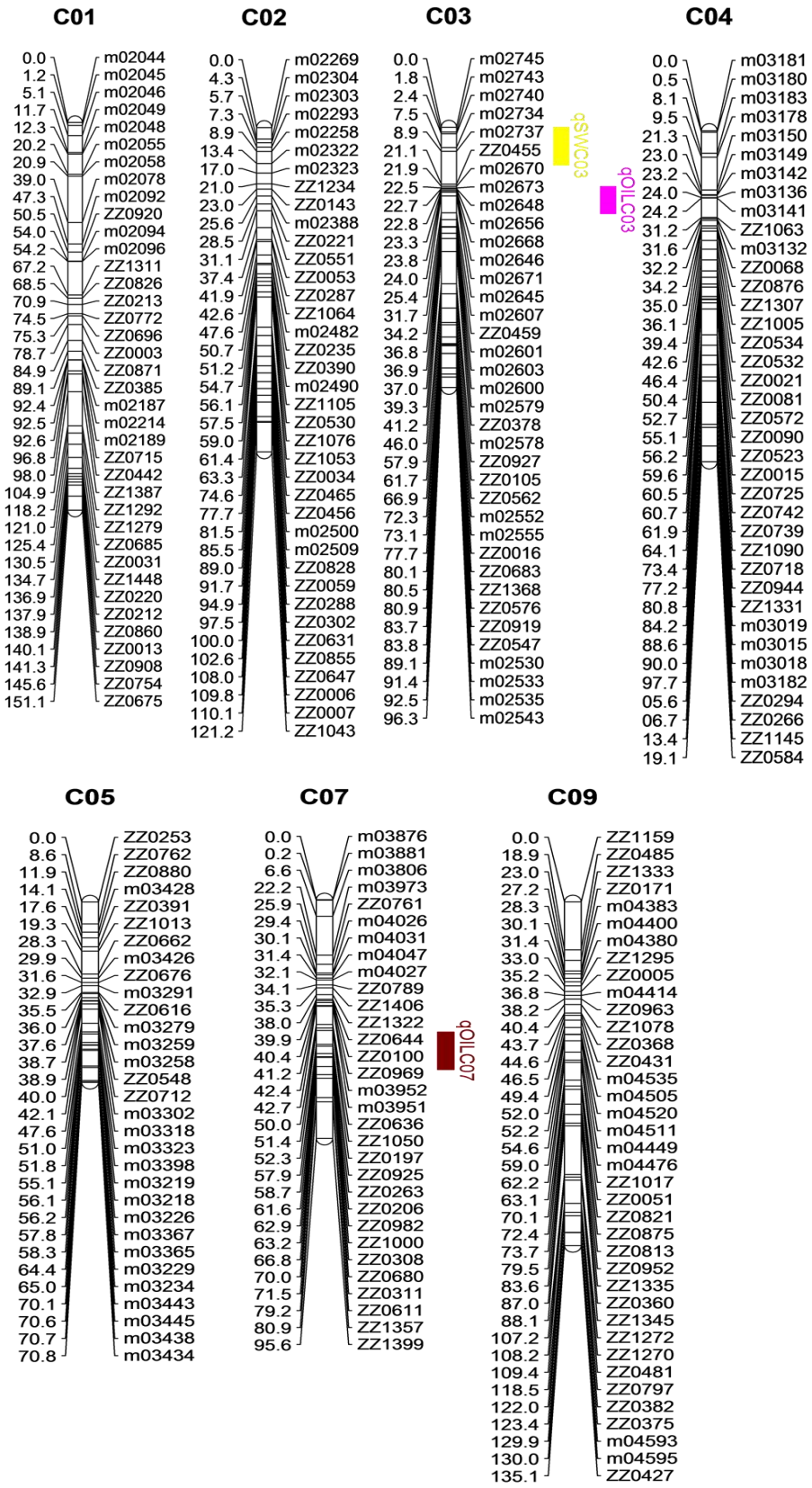

Figure 3. 7 Continued.

The linkage map was 2060.6 cM long with a mean interval of 1.7 cM between molecular markers (Table 3.5).

Table 3. 5 Summary of the map length, average interval, LOD score, and number of SRAP, and GBS markers of the chromosomes for the ZT population*

Chromosome	Length (cM)	Average interval (cM)	LOD	# of SRAP markers	# of GBS markers	Number of molecular markers
A1	66.6	1.7	5.0	18	21	39
A2	72.4	1.8	5.0	21	20	41
A3	194.3	2.9	3.0	40	26	66
A4	127.7	2.2	5.0	36	21	57
A5	203.7	2.2	3.0	64	27	91
A6	77.3	1.2	7.0	34	29	63
A7	94.0	0.8	4.0	57	55	112
A8	102.8	3.1	3.0	12	44	56
A9	136.5	1.5	3.0	27	67	94
A10	66.2	1.5	6.0	27	17	44
C1	151.1	2.5	5.0	33	27	60
C2	121.2	1.0	6.0	73	46	119
C3	96.3	1.5	3.0	13	51	64
C4	119.1	2.1	5.0	34	22	56
C5	70.8	1.4	2.0	14	38	52
C6	41.9	3.2	7.0	11	2	13
C7	95.6	2.2	6.0	26	18	44
C8	87.9	1.9	3.0	15	31	46
C9	135.1	1.8	5.0	44	33	77
Total	**2060.6**	**1.7**	**-**	**599**	**595**	**1194**

*: cM, centiMorgan; LOD, logarithm of the odds; SRAP, sequence-related amplified polymorphism; GBS, genotyping by sequencing.

3.4.4 QTL analysis

Since significant effects for all three traits in this study showed quantitative phenotypic distribution, QTL analysis was performed individually using field trial data

for the seed weight, flowering time, and oil content traits respectively. According to ANOVA analysis, specific traits without a significant difference in two replicates were combined and mean values were used to perform QTL mapping, while data from the two replicates were run separately if the ANOVA analysis showed a significant replicate difference. The results of the CIM analysis for seed weight, flowering time, and oil content traits are summarized in Table 3.6.

3.4.5 QTL mapping for seed weight

Based on ANOVA, two replicates for seed weight in 2015 showed a significant difference. Therefore, Qgene ® 4.3.10 was performed for each of the replicates. The results showed a peak LOD value of 2.4 on chromosome A05 for replicate one, but it was lower than the threshold LOD score (alpha 0.05 = 3.0). In the second replication, one QTL peak *qSWC03* had a LOD value of 3.0 (alpha 0.05 = 2.9) (Fig. 3.7). This QTL had a negative effect on seed weight, and an interval from 0 to 14 cM on chromosome C03, and explained 9.5 % of the genotypic variance. In 2016, one QTL for seed weight located on chromosome A03 (*qSWA03*) was identified based on the average seed weight values of the two replications (Fig. 3.7). The QTL *qSWA03* showed a negative effect, with a LOD value of 3.2 (alpha 0.05 = 2.9) in the trial. This QTL with an interval from 86 to 90 cM explained 10.4 % of the genotypic variance. Both of the two QTL identified in two years had a negative effect on the mean thousand seed weight of 0.1 g.

3.4.6 QTL mapping for flowering time

Two QTL for flowering time on chromosome A02 were identified based on the mean of two replications of the field trial in 2015. The results of CIM analysis showed that two QTL, *qFTA02-1* and *qFTA02-2* on chromosome A02 had a LOD score of 9.3 (alpha 0.05 = 3.2) and 3.3 (alpha 0.05 = 3.2) (Fig. 3.7). In addition, the genotypic variance ranged from 10.8 to 27.1 % with negative effects. The QTL intervals were 0 to 1 cM for *qFTA02-1* and 42 to 64 cM for *qFTA02-2* on chromosome A02. Since there was a significant difference for flowering time in two replicates in 2016, the CIM analysis was performed individually for each replicate. In replicate one, two QTL, *qFTA02-1* and *qFTA02-3* had LOD scores of 14.0 (alpha 0.05 = 3.2), and 6.4 (alpha 0.05 = 3.2), respectively (Fig. 3.7). Both QTL were located on chromosome A02, and had negative effects and explained genotypic variance of and 37.9 % and 19.7 %, respectively. The QTL intervals were 0 to 1 cM for *qFTA02-1* and 46 to 60 cM for *qFTA02-3*. In the second replicate, two QTL on chromosome A02 were identified as observed in replication one. The QTL, *qFTA02-1* and *qFTA02-3* had LOD scores of 13.3 (alpha 0.05 = 3.1) and5.3 (alpha 0.05 = 3.1), respectively. Both QTL had negative effects and explained a genotypic variance of 36.5 % and 16.6 %, respectively. Moreover, the QTL intervals were 0 to 1 cM for *qFTA02-1* and 44 to 62 cM for *qFTA02-3*. The QTL *qFTA02-1* for flowering time was located at the same location, and the same QTL intervals were identified in 2015 and 2016. The QTL, *qFTA02-2* and *qFTA02-3* had similar locations and QTL intervals in two years. Although the highest LOD scores were located on different marker positions, they might be the same QTL with no significant difference. The two QTL, *qFTA02-1* and *qFTA02-2/3*, for flowering

time on chromosome A02 had average negative effects of 5.5 days and 3.9 days, respectively.

3.4.7 QTL mapping for oil content

One QTL on chromosome C07 (*qOILC07*) was identified based on the mean value of two replicates of the field trial in 2015 (Fig. 3.7). Composite interval mapping analysis showed that *qOILC07* had a LOD score of 3.3 (alpha 0.05 = 2.9) with an additive effect of 0.92 % on the oil content. The QTL interval was from 53 to 68 cM, explaining 10.7 % of the genotypic variance. Since there was a significant effect between the two replicates in 2016, CIM analysis was performed for each of the replicates separately. In the second replicate, one QTL *qOILC03* with a LOD value of 2.8 (alpha 0.05 = 2.7) was identified (Fig. 3.7). The QTL had additive effect on oil content, an interval from 22 to 32 cM on chromosome C03, and explained 9.0 % of the genotypic variance. It was worth mentioning that both of the two replications had a similar trend in CIM analysis on chromosome C07. Though the peak values of LOD were lower than the threshold LOD score, similar peak shapes on chromosome C07 were identified in 2015.

Table 3. 6 Significant QTL for seed weight, flowering time, and oil content in the ZT DH population (n=147) in 2015 and 2016*.

Trait	QTL #	QTL	Year	CN	QTL interval (cM)	Marker	LOD score	Additive effect	R^2 (%)
Seed weight (g)	1	*qSWC03*	2015 Rep2	C03	0-14	m02745	2.9	-0.1	9.5
	2	*qSWA03*	2016	A03	86-90	ZZ1364	3.2	-0.1	10.4
Flowering time (days)	3	*qFTA02-1*	2015	A02	0-1	m00255	9.3	-5.5	27.1
	4	*qFTA02-2***	2015	A02	42-64	ZZ0843	3.3	-3.6	10.8
	3	*qFTA02-1*	2016 Rep1	A02	0-1	m00255	14.0	-5.5	37.9
	4	*qFTA02-3***	2016 Rep1	A02	46-60	ZZ0560	6.4	-4.2	19.7
	3	*qFTA02-1*	2016 Rep2	A02	0-1	m00255	13.3	-5.6	36.5
	4	*qFTA02-3***	2016 Rep2	A02	44-62	ZZ0560	5.3	-4.0	16.6
Oil content (%)	5	*qOILC07*	2015	C07	53-68	ZZ0925	3.3	0.9	10.7
	6	*qOILC03*	2016 Rep2	C03	22-32	M02645	2.8	0.8	9.0

*: QTL, quantitative trait loci; CN, chromosome number; LOD, logarithm of the odds; Rep, replicate.

**: *qFTA02-2* and *qFTA02-3* are considered as same QTL with no difference.

3.5 Discussion

In this study, QTL for seed weight, flowering time, and oil content of *B. napus* were evaluated in a DH population constructed from a cross between ZY821 and Topas. ZY821 is a Chinese semi-winter type variety of rapeseed that has a 39.7 % oil content, 29.8 % protein content and 19.7 % fiber content (Qu et al., 2013). Topas is a spring type cultivar that originated in France. It consists of 41.9% oil content and 22.1 % protein content (Mekki, 2007).

Seed weight is one of the yield-related traits in crops, which is regulated by polygenes and highly influenced by silique-associated traits (Wang et al., 2016). In this study, two QTL for seed weight were detected on chromosome C03 in 2015, and on chromosome A03 in 2016, respectively. In a recent study (Fu et al., 2015), a major QTL was detected on chromosome A09 of a DH population by using SNP markers. The DH population was developed from a F_1 hybrid between Express and SWU07 that are winter and semi-winter, respectively. In another study, QTL on chromosomes A02, A03, and A04 were recognized in a DH population from the F_1 hybrid of KenC-8 and N53-2 (Wang et al., 2013). Furthermore, it showed a positive correlation between seed weight and six silique related traits, which include silique volume, silique thickness, silique breadth, and seed density. This suggests that same genes are shared in these traits. However, the silique length is negatively correlated with seed weight (Wang et al., 2016). Also, four major QTL for seed weight in TN DH (Tapidor x Ningyou7) and RC-F_2 populations of *B. napus* were identified in ten natural environments (Shi et al., 2009).

QTL for flowering time with large phenotypic effects were identified and two major QTL of flowering time on A02 (qFTA02-1, qFTA02-3) in the ZT DH population explained 37.9 % and 19.7 % of phenotypic variation for late flowering, respectively. In comparison, Javed et al. (2016) localized a QTL on A02 that explained 43.2 % of phenotypic variation for early flowering. A major QTL was also detected on A02 from a DH population of spring and winter type rapeseed (Ferreira et al., 1995; Osborn et al., 1997). In addition, Luo et al. (2014) identified four major loci on four chromosomes A06, A07, C08, and C09. Moreover, Butruille et al. (1999) described a major QTL on A02 for vernalization requirement in an inbred backcross population. Another study on winter x spring crosses showed 20 QTL on A02, A03, A04, A06, A07, C02, C03, C05, C06, and C08. In addition, significant QTL on A02, A03, C02, C06, and C08 were identified by Raman et al. (2013), and 36 major QTL and six minor QTL for flowering time were detected in a DH population from an F_1 of Tapidor x Ningyou7 (TN). The major QTL identified on chromosome A10 in this TN DH population explained 50 % of the phenotypic variance (Long et al.,2007)..

The QTL for flowering time showed a good segregation ratio and detected a major QTL on chromosome A02 that is consistent with other study (Raman et al., 2013); this is due to the ultimate difference of flowering time between the two parental genotypes. Although the detected QTL is corresponding to other studies, the recording method is less likely quantitative because the flowering time was only rated three times in 2015, and five times in 2016. This can be improved in the future by record the flowering time on a daily basis (Long et al., 2007).

Flowering time refers to the stage of flowering, which is influenced strongly by climate (Bohuon et al., 1998). There are three ecotypes of *B. napus* that are classified by various systems, including spring, semi-winter, and winter types (Diers & Osborn, 1994). Spring type rapeseed grows in Canada and in some areas of Australia where subtropical climates exist (Wang et al., 2011). In comparison, the winter type requires vernalization and grows in moderate temperate climates, for instance in Western European countries. The semi-winter type flowers after winter, but is sown before winter, similar to the winter type. The frost hardiness of the semi-winter type rapeseed is not as strong as the winter type so it is cultivated in the areas with temperate climate (Wang et al., 2011). Different varieties of *B. napus* show different flowering times and may vary in the identification of QTL for the trait. In addition, photoperiod in a winter climate is the second major environmental cue affecting flowering time, since cold temperature and longer day light promote flowering (Wang et al., 2011). According to Qian et al. (2006), the DH populations from the crosses between spring and semi-winter types of *B. napus* were non-adaptable to the corresponding growing environments, especially the flowering time trait. Moreover, *B. napus* has homologs of a main regulator *FRI* in *Arabidopsis* such as *BnaA.FRI.a*, which inhibits floral transition and causes flowering time variation through activation of a flowering repressor, *FLC* that controls the vernalization requirement, which is strong in winter type rapeseed (Burn et al., 1993; Johanson et al., 2000).

In previous QTL analyses, different *FLC* and *FT* flowering time regulation candidate genes were identified in *Brassica* species (Lin, 2005; Zhang et al. 2015; Zhao

et al., 2010). The QTL (*qFTA02-1, qFTA02-2, and qFTA02-3*) for flowering time detected in this study were on chromosome A02 and ranged at 0-1 cM, 42-64 cM, and 44-62 cM. This result was similar to the flowering time gene homologs found in *B. rapa* reported from other studies. Zhao et al. (2010) found two QTL for flowering time (FLQTL) on top of A02, which is distributed at 34.8 cM, and Liu et al. (2016) identified three QTL on chromosome A02 under three growth conditions (long day, short day, and non-vernalized) located on the region 31.2-33.7 cM.

Oil content is an important trait within *B.napus*. In this study, QTL on chromosome C07 in 2015 and C03 in 2016 were identified. Among other studies, QTL were found on 17 out of 19 chromosomes, i.e. from chromosome A01 to A10, C01 to C03 and C05-C08 with QTL ranging from three to 27 (Burns et al., 2003; Chen et al., 2010; Delourme et al., 2006; Qiu et al., 2006; Sun et al., 2012; Teh & Möllers, 2016; Wang et al., 2013; Yan et al., 2009).

In this study, QTL *qOILC03* (LOD = 2.8) and *qSWC03* (LOD = 2.9) showed lower LOD score than the general threshold LOD score of 3.0, however they are still statistically significant according to the result of permutation test. A permutation test can establish a maximum LOD score through shuffling genotypes and phenotypes, and the identified LOD score can be used as a threshold of statistical significance (Churchill & Doerge, 1994). Other studies also identified QTL with lower LOD score (> 2.0), but they considered it as micro-real QTL (MR-QTL), which is used to protect the minor QTL (Long et al., 2007; Wang et al., 2013). MR-QTL describes a QTL which is detected in multiple environment locations under the threshold LOD score

but above standard value ($p \leq 0.5$) (Long et al., 2007). Among the identified six QTL in this study, QTL for flowering time in ZT population were repeatedly detected in 2015 and 2016 replicates, which makes them reliable. In comparison, QTL detected for seed weight and oil content are less reliable because they were only found in one year or even in one replicate. It is ideal to perform field trials in multiple locations and multiple years to avoid environmental effects (Guo et al., 2017).

The field trial of the ZT DH population was performed in two years at one location. Due to to the non-uniform maturation of the individual ZT DH lines, as some matured in September and others matured in October; it was hard to use machinary to harvest the plants all at one time. Thus, hand harvesting was required. This was time consuming and laborous and it was difficult to carry the research in multiple locations. If the study had contrasted environments, the results may become more reliable due to the allelic varation between the parents. In order to increase the likelihood to detect loci contributing to the trait, variation from numerous lines is required (Breseghello & Sorrells, 2006; Yan et al., 2011; Yu & Buckler, 2006). Moreover, high-density linkage maps and large population sizes combined with appropriate statistical methods may contribute to more reliable results (Fu et al., 2017).

In this study, the variation of the climate in different years affected phenotypic data evaluation in the field trials. In 2016, precipitation in June (PPT = 128.28 mm) was 2.5 times greater than in June of 2015 (PPT = 52.06 mm) at the Point of agricultural research land that is located at the east end of the University of Manitoba Fort-Garry campus. A large amount of precipitation delayed flowering and resulted in the late

maturation of seeds. Immature seeds led to missing data or lower oil content in the semi-winter type DH lines. In addition, the increased precipitation triggered sclerotinia stem rot, which is a lethal fungal disease, induced by *Sclerotinia sclerotiorum* (Lib.) de Bary that resulted in yield loss. Therefore, a large number of missing phenotypic data in 2016 affected the identification of QTL for seed weight and oil content.

A linkage map of a *B. napus* ZT DH population was developed using SNP markers from GBS, because GBS improves marker density and significantly reduces costs and time than other markers (He et al., 2014). SRAP markers were also used since it is easily replicated in many crops. Although QTL mapping provides useful information on trait of interests, the location and effect is specific to a certain population that is tested. Because of each population is polymorphic at different sets of loci, it is hard to compare QTL at marker level (Remington & Purugganan, 2003).

In this study, linkage maps showed lower density with high interval between markers when compared to other study conducted by Javed et al. (2016). The number of available SNP markers were reduced and showed poor quality, which may be caused by genotyping errors in PCR amplification of GBS (He et al., 2014).

In conclusion, a linkage map was constructed by using SNP from GBS and SRAP markers and QTL for seed weight, flowering time, and oil content of the ZT DH population of *B. napus* were identified. Through combining the genotypic and phenotypic data together, six QTL for three traits were detected in two years. Two QTL for seed weight were located on chromosome C03 in 2015 and on chromosome A03 in 2016, and two major QTL for flowering time on chromosome A02 in 2015 and 2016. In

addition, two QTL for oil content on chromosome C07 in 2015 and chromosome C03 in 2016 were identified. The QTL identified for the three traits could be used to facilitate map-based QTL cloning and dissecting the genetics of these traits.

4.0 GENERAL DISCUSSION

Rapeseed (*B. napus*, AACC, 2n=38) is widely used as edible oil, animal feed, and biofuel. As the demand for *B. napus* production increases, developing a new breed with elevated oil content becomes a major goal in breeding programs (Bailey et al., 2006). Seed weight, flowering time and seed oil content trait is complex and quantitatively controlled by polygenes (Shi et al., 2009). In addition, it is highly influenced by growth environments including light intensity, precipitation, and temperature (Maheswaran, 1998).

For quantitative traits, QTL mapping is an adequate tool to reveal the complexity. Molecular markers such as PCR-based markers are used to construct linkage maps with low marker densities. Since high-density genetic maps enhance the detection of QTL, high throughput single nucleotide polymorphism (SNP) markers via bead arrays is widely used in plant breeding programs (He et al., 2014).

In this study, QTL for seed weight, flowering time, and oil content of rapeseed were analyzed in a DH population (Zhongyou821 x Topas) with 147 lines. QTL for seed weight were localized on chromosome C03 in 2015, and on chromosome A03 in 2016. However, other studies identified significant QTL for seed weight on chromosome A09 (Fu et al., 2017). Two major QTL (qFTA02-1, qFTA02-3) for flowering time in the ZT DH population were detected on A02 and explained 37.9% and 19.7% of phenotypic variation for late flowering, respectively. Other studies detected major QTL on A02 and significant QTL for flowering time (FL) on A03, A06, A07, A10, C02, C06, C08 and C09 (Butruille et al., 1999; Ferreira et al., 1995; Long et al., 2007; Luo et al., 2014; Osborn et al., 1997; Raman et al., 2013). QTL for oil content

on C07 in 2015 and on C03 in 2016 were identified. Various studies have located QTL for seed oil content in rapeseed that are distributed on most chromosomes, except C04 and C09 (Burns et al., 2003; Chen et al., 2010; Delourme et al., 2006; Ecke et al., 1995; Qiu et al., 2006; Sun et al., 2012; Teh & Möllers, 2016; Wang et al., 2013; Yan et al., 2009). Since the oil content trait is easily affected by environmental conditions, it is best to have research done in contrasting environments.

In this study, QTL for three important seed yield related traits seed weight, flowering time, and oil content were identified and can be used for future research in QTL cloning.

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