

Development of Genetic Linkage Maps and Identification of Quantitative Trait
Loci Influencing Seed Oil Content, Fatty Acid Profile and Flowering Time in

Brassica napus L.

by

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ABBREVIATIONS

A genome	<i>Brassica rapa</i> genome
ACCase	Acetyl-CoA carboxylase
ACP	Acyl carrier protein
ADD	Additive effects
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
BCCP	Biotin carboxyl carrier protein
<i>BoGLS-ALK-1</i>	Gene marker for alkenyl glucosinolates of <i>B. oleracea</i>
<i>Bzh-1</i>	The dwarf Breizh gene
C genome	<i>Brassica oleracea</i> genome
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
C20:0	Arachidic acid
CAP	Cleaved amplified polymorphic sequences
CIM	Composite interval mapping
CL	Cardiolipin
cM	Centimorgan
DAG	Diacylglycerol
DArT	Diversity array technology
DF	Degree of freedom
DH	Doubled haploid
DNA	Deoxyribonucleic acid
ECR	3-enoyl-CoA reductase
ENR	Enoyl-ACP reductase
ER	Endoplasmic reticulum
ESTP	Expressed sequence tag polymorphism
FAD	Fatty acid desaturase (1-8)
FAE	Fatty acid elongase

FAS	Fatty acid synthase
FAT	Acyl-ACP theioesterases (A&B)
FLR	Flowering
G3P	Glycerol-3-phosphate
GE	Genotype x environment interaction
GPAT	Glycerol-3-phosphate acyltransferase
HAD	3-Hydroxyacyl-ACP dehydratase
HCD	3-Hydroxyacyl-CoA dehydratase
HEAR	High erucic acid rapeseed
HO	High in oleic acid
HOLLi	High in oleic acid, low linoleic acid and linolenic acid
Indels	Insertion/deletion polymorphisms
ISSR	Inter simple sequence repeat
KAR	3-Ketoacyl-ACP reductase
KAS	3-Ketoacyl-ACP synthases (I, II, III)
KCS	β -Ketoacyl-CoA synthase
LD	Linkage disequilibrium
LEAR	Low erucic acid rapeseed
LEC1	LEAFY COTYLEDON1
LG	Linkage group
LOD	Logarithm of odds
LPA	2-Lysophosphatidic acid
LPAAT	Lysophosphatidic acid acyltransferase
MAS	Marker assisted selection
MCMT	Malonyl-CoA: acyl carrier protein malonyltransferase
NIL	Near isogenic lines
NIR	Near infrared spectroscopy
PA	Phosphatidic acid
PC	Phosphatidylcholine
PCR	Polymerase chain reaction
PDAT	Phospholipid: diacylglycerol acyltransferase
QTL	Quantitative trait locus or loci
R ²	Phenotypic variance

RAPD	Random amplified polymorphic DNA
RIL	Recombinant inbred lines
SAD	Stearoyl-ACP desaturase
SAS	Statistical analysis software
SCAR	Sequence characterized amplified region
SHEAR	Super high erucic acid rapeseed
SNP	Single nucleotide polymorphism
SRAP	Sequence related amplified polymorphism
SSR	Simple sequence repeat
STS	Sequence target sites
TAG	Triacylglycerol
TFs	Transcription factors
TRAP	Target region amplified polymorphism
VLCFA	Very long chain fatty acid
WRI1	Wrinkle 1
χ^2	Chi square test

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ABSTRACT

Identification of allelic variation through quantitative trait loci (QTL) mapping offers possibilities for the improvement of quantitatively inherited traits. This requires a genetic map along with the phenotypic characterization of a mapping population. A doubled haploid (DH) Polo X Topas population consisting of 194 lines and a recombinant inbred line population of 92 lines was developed. Individual genetic maps derived from each population were integrated into a consensus map. The DH-based genetic map was used for QTL mapping. The DH-based map was comprised of 620 loci that were assembled into 19 linkage groups that were anchored to the *B. napus* chromosomes. The DH-based map covered 2244.1 cM genomic distance with an average marker interval of 3.7 cM.

The DH population was phenotyped in four environments with each line replicated twice in a randomized complete block design. Days to flowering was recorded and oil content and fatty acid composition were determined using Near Infrared spectroscopy (NIR) and Gas Chromatography, respectively.

Fourteen QTL were identified for oil content, 33 QTL for palmitic acid content, 18 QTL for stearic acid content, 21 QTL for oleic acid content, 20 QTL for linoleic acid content, 23 QTL for linolenic acid content, 16 QTL for arachidic acid content and 14 QTL for flowering time.

Oil content QTL were identified on five linkage groups, A3, A10, C1, C5, and C6. An oil content QTL, *qOIL-A10c* appeared in all four environments, whereas *qOIL-A10a* appeared in only one environment but explained 26.99% variation. The oil content in the population ranged from 35% to 55.5% with the parents having values of 42% to 46%.

Two genomic regions on C3, with map positions at 147.83 cM and 154.55 cM harbored QTL (*rQTL*) for all the fatty acids studied. The additive effects of the *rQTL* reveal a correlation pattern which is supported by the phenotypic correlation observed between the fatty acids. This suggests *rQTL* have role in the fatty acid composition and possibly determine total seed oil content. The *rQTL* and flanking markers of the identified QTL offer utility in further development of *B. napus*.

FOREWARD

This thesis has been written in manuscript style under the guidelines issued by the Faculty of Graduate Studies at the University of Manitoba. The thesis begins with a general introduction, followed by a literature review, which is followed by three manuscripts that comprise the main part of the thesis. The first and the third manuscript, individually contain an abstract, introduction, materials and methods, results, and discussion. The manuscript entitled “An Integrated Genetic Map for *Brassica napus* derived from Doubled Haploid and Recombinant Inbred Populations” (Chapter 3) has been published in the journal of Hereditary Genetics. The second manuscript is an overview of results obtained in this study. This second manuscript was prepared on a prescribed format given by Green Crop Network for publication in a special issue of the Canadian Journal of Plant Science. The second manuscript consists of an abstract, material and methods, general findings and conclusion. The third manuscript is to be submitted to a peer-reviewed journal in the near future. A general discussion, summary/conclusion, recommendations for future study, and a list of references cited follow the manuscripts.

1. GENERAL INTRODUCTION

The plant family Brassicaceae is comprised of 338 genera and 3,709 species (Warwick et al. 2006). The genus Brassica consists of about 35 species, which are mostly annual and perennial herbs. Six cultivated species of Brassica, *Brassica rapa*, *B. oleracea*, *B. nigra*, *B. napus*, *B. juncea* and *B. carinata*, are of agronomic interest as they carry significant economic value being important oilseed crops or vegetables. These species are genetically inter-related as depicted in the famous “triangle of U”, (U 1935). According to the triangle of U, *B. rapa*, *B. oleracea*, and *B. nigra* are diploids and the spontaneous hybridization among them has resulted in the evolution of amphidiploid or polyploid species i.e. *B. napus*, *B. juncea* and *B. carinata*. The polyploid species are generally more productive agronomically and thus more important economically (Pires and Gaeta 2011). *B. napus* is the most important in this group due to its worldwide cultivation as an oilseed crop and ranks second after soybean in global oilseed production (Iniguez-Luy and Federico 2011).

Brassica species are grown globally except in Antarctica (Lysak and Koch 2011). *B. napus* is ideally suited for cultivation in the temperate Canadian climate. As an oilseed crop, *B. napus* contributes \$19.3 billion to the Canadian economy (Canola Council of Canada 2014a). The economic value of *B. napus* as oilseed crop lies in its high seed oil content (Smooker et al. 2011). *B. napus* oil has broad-spectrum utilization in the modern world (Gupta and Pratap 2007). For edible oil purposes, erucic acid, a constituent fatty acid of the oil, must be less than 2%, whereas glucosinolate content in the seed meal must be less than 30 micromoles per gram of seed (Canola Council of Canada 2014a). This quality of Brassica oil is mainly available in seeds of varieties of *B. napus* and such varieties are termed ‘Canola’, trademarked by Canadian Oilseed Processors Association (Canola

Council of Canada 2014a). Two significant discoveries contributed to the development of the first canola variety, Tower by the University of Manitoba (Stefansson and Kondra 1975). First, almost zero erucic acid content in some of the selections of cv. Liho were reported by Stefansson et al. (1961) and secondly, low glucosinolate contents in cv. Bronowski were discovered by the Agriculture Canada Research Station in Saskatoon (Kondra and Stefansson 1970). These two characteristics were introgressed in a common genetic background of cv. Turret to develop the world's first canola variety (Stefansson and Kondra 1975). The objective of this breeding was to reduce the concentration of above-mentioned compounds, which are believed to be unsuitable for human and animal consumption.

The oil produced from non-canola type varieties of *B. napus* have high levels of erucic acid and are suitable for industrial utilization. Different Brassica oil characteristics are preferred for industrial use. Mainly, oils high in erucic acid content, because they are stable under intense heat and were historically used in lubricating steam engines (Gupta and Pratap 2007). The industrial utilization of Brassica oil has diversified over the time (Abbadi and Leckband 2011). The derivatives of erucic acid are now used in the production of cosmetics and other industrial products such as, lubricants, slip agents, plasticizers, coatings, and nylon (Luhs and Friedt 1993). Recently, Brassica oil to produce biodiesel is increasingly becoming popular (Abbadi and Leckband 2011; Smooker et al. 2011). Brassica oil is chemically similar to fossil oil (Durrett et al. 2008) therefore; it constitutes an excellent resource as a feedstock for renewable energy production (Durrett et al. 2008).

The diversity in the utilization of Brassica oil may be creating its further demand (Gupta and Pratap 2007). According to the Food and Agriculture Organization of the United

Nations (Fig. 1.1), the edible oil price index has increased three-fold in the past three decades and global oilseed production is expected to fall short of demand. Since canola/rapeseed is the second most important oilseed crop in the world, there is a large economic and scientific interest to understand and manipulate quality and quantity of seed oil content (Iniguez-Luy and Federico 2011; Nesi et al. 2008; Weselake et al. 2009). Additionally, a one percent increase in seed oil content equals an increase in seed yield of approximately 2.5% (Wang 2004). Improvement in seed oil content would add to the economic feasibility of Brassica crop production and therefore, its continuous enhancement remains a prime focus of Brassica breeding programs.

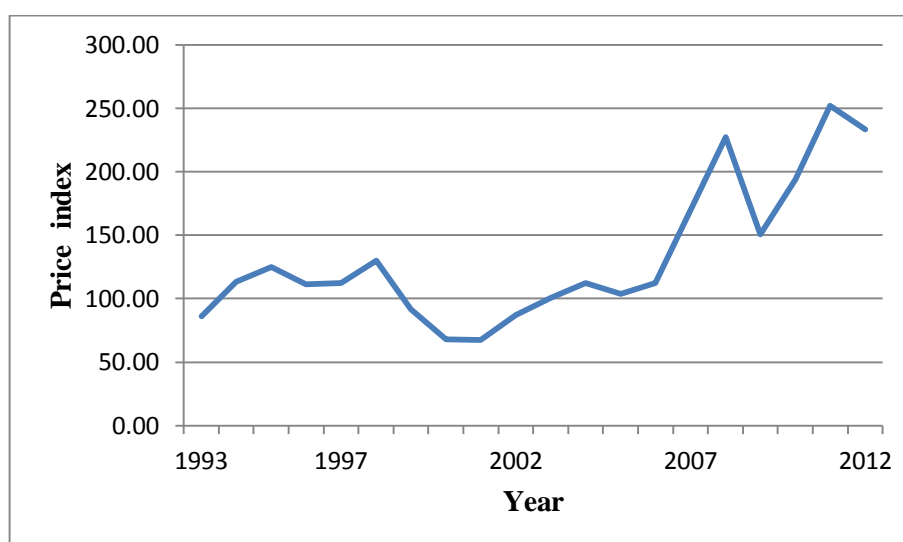


Fig. 1.1 World vegetable oil price index (adapted from FAO (2012))

Factors that determine the content of seed oil include the flow of metabolites between the endosperm and cotyledon, as well as between cellular compartments such as plastids, the cytoplasm, and mitochondria (Abbadi and Leckband 2011; Baud and Lepiniec 2010). The heritability of seed oil content in Brassicaceae is high and accurate measurement using Near-infrared reflectance spectroscopy has facilitated accelerated genetic gain (Engqvist

and Becker 1991; Wu et al. 2006). In the past three decades, gain in oil content in *B. napus* ranged from 41% to 56% (Grami and Stefansson 1977; Wang et al. 2013) with an average content of 43.5% in Canadian exported canola seed (Canadian Grain Commission 2013). However, the oil content in Brassica is a complex quantitative character that is correlated with other quality traits of the seed, plant developmental stages and the environment. The seed quality traits that affect oil content are protein content, seed coat color, and fatty acid composition. Plant developmental characteristics such as time to flowering, coupled with micro and macro environmental conditions of the plant, impact the variation seen for seed oil content. Genetic variation in seed oil content is regulated by multiple genes related to lipid metabolism, which are either sensitive or insensitive to environmental growing conditions (Li et al. 2011; Sun et al. 2012; Yan et al. 2009). These genes display additive gene action, where epistasis is absent and dominance is not significant (Abbadi and Leckband 2011; Li et al. 2011). Since the oil content phenotype is affected by many factors, selection based on the genotype using molecular markers should be more fruitful than using phenotypic data for further improvement of seed oil content.

Enhancement of seed oil content can be accomplished using two strategies, i.e., reverse genetic approaches or conventional breeding methods. The former technique requires in-depth knowledge of genes involved in specific biosynthetic pathways (Uelker and Weisshaar 2011). Many of the genes encoding enzymes for fatty acid biosynthesis have been characterized. Based on *Arabidopsis thaliana* genome sequence information, six hundred genes regulate acyl lipid metabolism (Beisson et al. 2003). Numerous studies under controlled conditions have employed gene over-expression and gene silencing techniques to manipulate the critical steps of lipid biosynthesis with varying results (Baud

et al. 2007; Bouvier-Nave et al. 2000; Jain et al. 2000; Jako et al. 2001; Lardizabal et al. 2008; Marillia et al. 2003; Roesler et al. 1997; Vigeolas et al. 2007; Zheng et al. 2008; Zou et al. 1997; Zou et al. 1999a). Under field conditions, Taylor et al. (2009) over-expressed *AtDGAT1* and *BnDGAT1* that encode the endoplasmic reticulum specific oil synthesis enzyme in *B. napus*, and reported a small gain from 2.5% to 7.0% in oil content in transgenic plants with maximum oil content of 47.5%.

Conventional plant breeding has been instrumental in increasing the content of seed oil and seed yield in *B. napus* (Weselake et al. 2009). However, increases in oil content beyond 50% seems difficult with conventional breeding because of its inability to estimate the effect of a single gene on a quantitative trait (Mather and Jinks 1971). With the advent of molecular markers, it has become possible to determine the underlying genes or genomic regions affecting a quantitative character, termed quantitative trait loci (QTL). Detection of QTL can also help to devise and optimize selection strategies for plant improvement (Dudley 1994).

Brassica napus has genomic co-linearity with *Arabidopsis* (i.e corresponding genes maintain similar order in both the species) (Parkin et al. 2005). It contains up to six orthologues of any given ancestral gene (Parkin et al. 2005; Smooker et al. 2011). However, the numbers of functional gene copies vary for the lipid biosynthesis pathway (O'Neill and Bancroft 2000; Smooker et al. 2011). Deletion of the expected orthologues of *A. thaliana* from the genome of the Brassicaceae is frequently reported which indicates the plant's adaptive response to the environment (Scheffler et al. 1997). Therefore, genetically, the composition of fatty acids in Brassicaceae is more variable than any other oilseed crop, and diversity studies have confirmed the presence of genes and allelic variation regulating seed

lipid synthesis (Barker et al. 2007; Mandal et al. 2002). QTL mapping studies in *B. napus* frequently report seed oil QTL specific/unique to distinct genetic backgrounds (Barker et al. 2007; Chen et al. 2010; Delourme et al. 2006). Unique seed oil content QTL identified in different genetic backgrounds represent allelic variation that can be harnessed for further seed oil enhancement.

The long-term goal of the research reported in this thesis is to enhance the understanding of the genetic control of seed oil content in *B. napus*. The knowledge gained from such studies should enable plant breeders to manipulate oil content in Brassica species. The specific objectives of this research are:

- a) To develop molecular marker-based genetic linkage maps of *B. napus*-derived from a doubled haploid line and recombinant inbred line population with identical pedigree.
- b) To utilize the developed saturated linkage maps to identify major and minor QTL for flowering time, seed oil content and fatty acid composition.

The prerequisite for QTL detection for any quantitative trait is the availability of a linkage map, derived from a structured population. In this study, two different types of populations, a recombinant inbred line (RIL) population, and a doubled haploid (DH) population of the same pedigree were developed. Individual genetic maps developed from these two populations were then integrated and the genetic structures of the two populations were compared (Geng et al. 2012). This study revealed synteny between the two populations where common markers occupied similar map positions and order on the same linkage groups. However, the DH-based map was derived from a much larger population (190 individuals) and it is a highly saturated SSR-based map. Therefore, the DH map is

more suited to the objectives of this study, i.e. to reliably locate QTL controlling flowering time, seed oil content and fatty acid profile in *B. napus*.

This study identified consistent or reproducible QTL across all environments for all traits studied. The QTL identified are comparable with published results for oil content and fatty acid composition, and are located on chromosomal regions associated with respective elongase and desaturation pathways for fatty acid biosynthesis. This study is the first one to report two QTL on the C3 at 147.83 cM and 154.55 cM, which synergistically may be modulating the correlations between the component fatty acids. Transgressive segregation for oil content was also identified in this study. The oil content values among the progeny were up to 9% higher than the high oil parent (46.2% oil content). The highest *B. napus* oil content (55.5%) reported in the current study supports the hypothesis that favourable alleles for oil content exist in the germplasm of *B. napus*.

2. LITERATURE REVIEW

2.1.1 Brassicaceae

Recent studies based on molecular dating methods using a mitochondrial genome marker, the first intron of *nad4*, indicate that Brassicaceae may have evolved 19 million years ago in the eastern Mediterranean region from a common ancestor of its sister family Cleomaceae (Franzke et al. 2009). Brassicaceae have immense scientific significance due to the adoption of *Arabidopsis thaliana* as a model plant for studies (Franzke et al. 2009; Uelker and Weisshaar 2011). The Brassicaceae family consists of 330 genera and about 3,700 species, consisting of economically important edible and industrial oilseed, vegetable, condiment, and fodder crop species (Warwick et al. 2006). *Brassica napus* is the most important species for edible oil production (Iniguez-Luy and Federico 2011). *B. juncea* may be suited for cultivation in drier regions and its seed oil is used as condiment oil in Southeast Asia (Mishra et al. 1999; Pradhan and Pental 2011). Cole crops (*B. oleracea*) are important vegetables such as kale, cabbage, broccoli, cauliflower, kai-lan, brussels sprouts and kohlrabi (Quiros and Farnham 2011). Several other species such as *B. carinata*, *Camelina sativa*, *Crambe abyssinica*, *Eruca vesicaria*, are suited for edible oil/protein crops, feedstock for biodiesel, or for molecular farming (Warwick et al. 2007). Globally there exists general confusion on nomenclature in the Brassicaceae. Bailey (1922) listed three reasons which led to this confusion: 1) many forms included in one species, which weakened the definitions, 2) efforts to distinguish oriental forms from European forms, and 3) seed mixing.

Among the six cultivated species of Brassicaceae (Fig. 2.1), the subspecies in *B. napus* are cultivated for seed oil production (Iniguez-Luy and Federico 2011). Genetically, *B.*

napus is an amphidiploid of recent origin (5 to 10 million years ago) (Gupta and Pratap 2007; Olsson 1960). Many morphotypes of rapeseed have variations due to their origin, cultivation and use. Other commonly known names of *B. napus* are rape, oilseed rape, Argentine rape, Swede rape, colza, raps, or rapeseed (Iniguez-Luy and Federico 2011).

2.1.2 Genetic relationship among Brassica species

U (1935) utilized taxonomic studies to explain the genetic relationship among the six cultivated species of Brassicaceae (Fig. 2.1). According to the model commonly known as the “triangle of U” there are three diploid species, *B. rapa* ($n = 10$, genome AA), *B. oleracea* ($n = 9$, genome CC), and *B. nigra* ($n = 8$, genome BB). Natural hybridization and polyploidization of two of the diploid taxa created the three amphidiploid species, *B. napus* ($n = 19$, AACC), *B. juncea* ($n = 18$, AABB), and *B. carinata* ($n = 17$, BBCC). This relationship has been confirmed through chromosome pairing, artificial resynthesis of the amphidiploids, sequence analysis, and the use of genome specific markers (Kimber and McGregor 1995; Warwick 2011).

Divergence, based on nuclear sequence data and chloroplast restriction sites between diploid progenitors of *B. napus* have demonstrated their distinct evolutionary path (Warwick and Sauder 2005). The cultivated *Brassica* species have extensive duplication or triplication of the putative ancestral genome i.e. *Arabidopsis thaliana* (Town et al. 2006). Town et al. (2006) reported that for every *A. thaliana* gene there are three homologous gene copies found in *B. oleracea* and each gene copy under goes local adaptive pressure resulting in divergence or complete or partial gene loss.

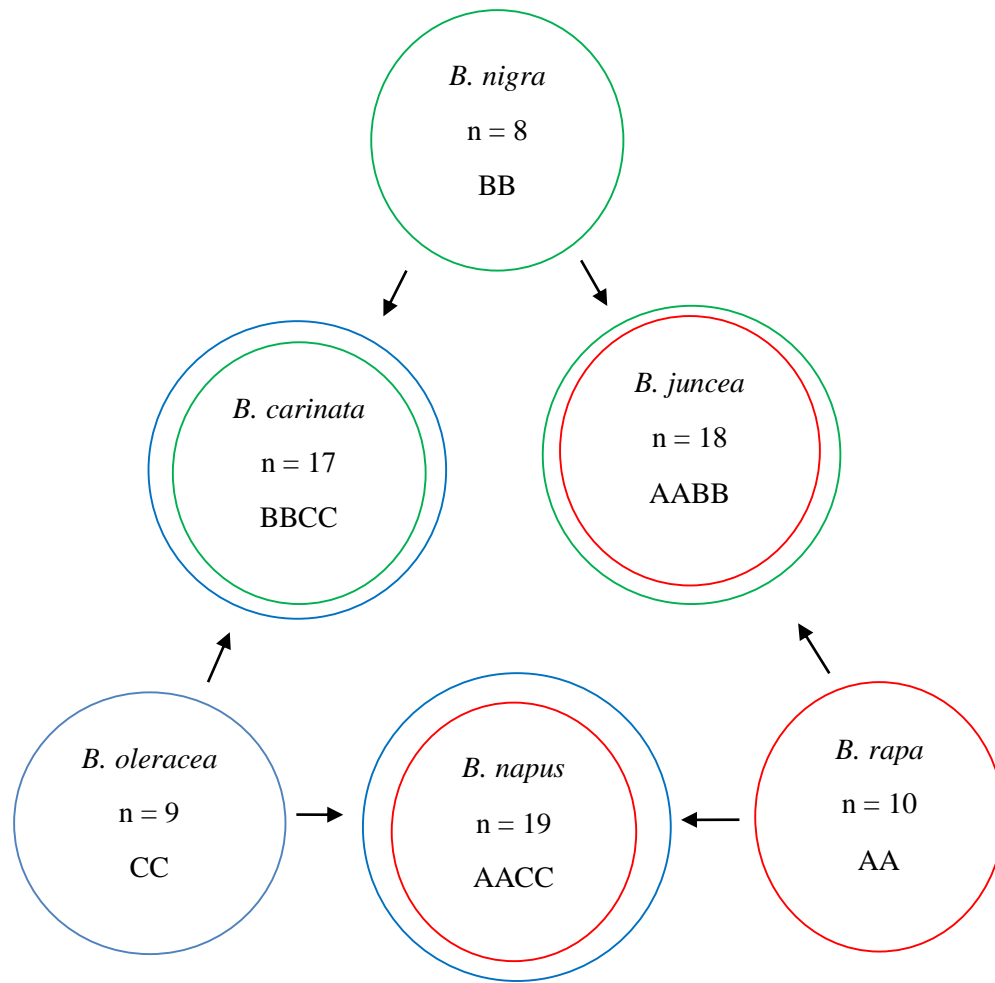


Fig. 2.1 Triangle of U, modified from U (1935)

2.1.3 Types of *Brassica napus*

Flowering initiation in biennial cultivars (winter types) in contrast to annuals (spring types) require exposure to cold (vernalization) which can be achieved in the field by overwintering or maintaining cold temperatures of 4°C for about zero to eight weeks in controlled conditions (Iniguez-Luy and Federico 2011; Johanson et al. 2000). Both types are grown for vegetable oil production; however, winter cultivars are generally more productive in terms of seed yield, under favorable conditions (Kimber and McGregor

1995). The annual growth habit has dominance over the biennial (Pelofske and Baggett 1979). Comparatively there exists greater genetic variation in annual types (Choi et al. 2007) and essentially all cultivars grown in Canada (Australia and Indian subcontinents) are annual types. Biennials are generally grown in Europe and China (Mendham and Salisbury 1995).

Recent use of *B. napus* oil pertaining to specific fatty acid combination has resulted into coining of further terminologies. For example, *Canola* (Canadian oil low acid) is a form of rapeseed or mustard specific in oil quality characteristics that are considered to be the most desirable among all the vegetable oils (Beare et al. 1963; Gupta and Pratap 2007; Stringam et al. 2003). Canola was first developed in Canada in 1974 and it contains less than 2% erucic acid and meal glucosinolate content under 30 micromoles/g seed (Canola Council of Canada 2014a; Stefansson and Kondra 1975). Rapeseed oil for industrial usage has distinct oil attributes yet also has low glucosinolate content. The term HEAR relates to High Erucic Acid Rapeseed containing up to 66% erucic acid while SHEAR relates to Super High Erucic Acid Rapeseed with erucic acid concentrations over 66% (McVetty et al. 2009). Many breeding programs are developing canola oil with custom fatty acid profiles to cater to various niche markets of the oleo-chemical industry (Abbadi and Leckband 2011). The development of “*HO*” (High in Oleic acid content) and “*HOLLi*” (High in Oleic acid content, Low in Linoleic acid content and Low in Linolenic acid content) canola cultivars is in demand offering stability and better oxidation resistance during food processing due to higher oleic acid and lower linolenic acid content, respectively (Abbadi and Leckband 2011).

2.2 History of rapeseed and development of canola

The word rape originated from Latin word “rapum” meaning turnip. The earliest reference to rapeseed cultivation comes from India, China and Japan. Sanskrit scripts of 2000-1500 BC directly refers rapeseed to its prevailing name *sarson* (Gupta and Pratap 2007). The archeological records for rapeseed in China go back to 5000 BC (Yan 1990). Before petroleum based products became popular in Europe, rapeseed oil was preliminary used as lamp oil. Historically, it has been part of south Asian diet as well (Mehra 1966). Its commercial cultivation started in the Netherlands in the sixteenth century with the discovery that rapeseed oil is an excellent lubricant for steam engines (Gupta and Pratap 2007). For similar reasons, its first cultivation in Canada was promoted by support pricing during the Second World War (Downey 2006). However, its cultivation in Canada after the war remained marginal till the development of canola (McVetty et al. 2009).

Reports from the European Economic Community (EEC) comprised of France, West Germany, Italy, the Netherlands, Belgium, and Luxemburg in 1960 recommended the edible use of rapeseed oil containing less than 5% erucic acid due to its role in heart abnormalities (Gupta and Pratap 2007). Charlton et al. (1975) reported incidence of focal myocardial necrosis and fibrosis in male rats that were fed rapeseed oil with varying erucic acid levels. Extraction of oil from rapeseed leads to an important byproduct, rapeseed meal, which is used for the production of animal feed. Rapeseed meal contains glucosinolates, which are plant secondary metabolites (Rodman et al. 1998). Glucosinolates affect the functioning of thyroid glands of animals if they are fed a diet high in glucosinolate contents (Uppstrom 1995).

A screening of *B. napus* germplasm resulted in the discovery of a low erucic acid source, a forage rape cultivar “Liho” (Stefansson et al. 1961) and a gene source for low glucosinolate content, cultivar Bronowski in 1967 (Stefansson and Downey 1995). Both of these traits were introgressed into *B. napus* for the development of first canola quality cultivar, “Tower” by the University of Manitoba in 1974 (Stefansson and Downey 1995). Present day canola grown worldwide; therefore shares a common source of origin for the low erucic acid and glucosinolate traits (McVetty et al. 2009). Intensive selection for specific oil and seed quality traits from a limited genetic resource is further limiting the genetic diversity among the elite canola cultivars (Hasan et al. 2006).

2.3 Economic importance of canola

The development of canola encouraged its cultivation worldwide as a source of healthy edible oil. There is a consistently increasing demand for vegetable oils as reflected by the oil and fat price index which has increased more than three fold in the last 12 years, up from a value of 68 points to a current value of 225 points (Fig. 1.1; FAO 2012). Rapeseed is the second largest oilseed crop in the world after soybean with an estimated annual production of 60.6 million tonnes in 2011-12 (FAO 2012).

In the past three decades, canola cultivation in Canada has generally witnessed an upward trend [Fig. 2.2; Statistics Canada (2013)]. In the year 2013, it was seeded to an area of 19.7 million acres (Fig. 2.2), down by 8.3% from the previous year; however; still yielding a record domestic production of 18.00 million metric tonnes, up by 29.5%. In Manitoba, canola was seeded to an area of 3.1 million acres in 2013, down by 12.1% from 2012. This decline in area produced increased yield of 2.9 million tonnes, representing a 36.7% increase from 2012. In 2013, a study was commissioned by Canola Council of

Canada to estimate the impact of Canadian grown canola and its end products on Canadian economy. According to this study conducted by L.M.C International Ltd (2013), canola contributes \$19.3 billion a year to the national economy and it provides 228,000 Canadian jobs mostly in sectors such as seed supply, farming, canola seed handling (elevator and port), transportation, crushing and refining. Furthermore, most of the economic benefits have been realized in the western provinces of Canada where canola cultivation predominates. The economic impact of canola in Manitoba has been reported to be in the range of \$3.3 billion (L. M. C. International Ltd 2013).

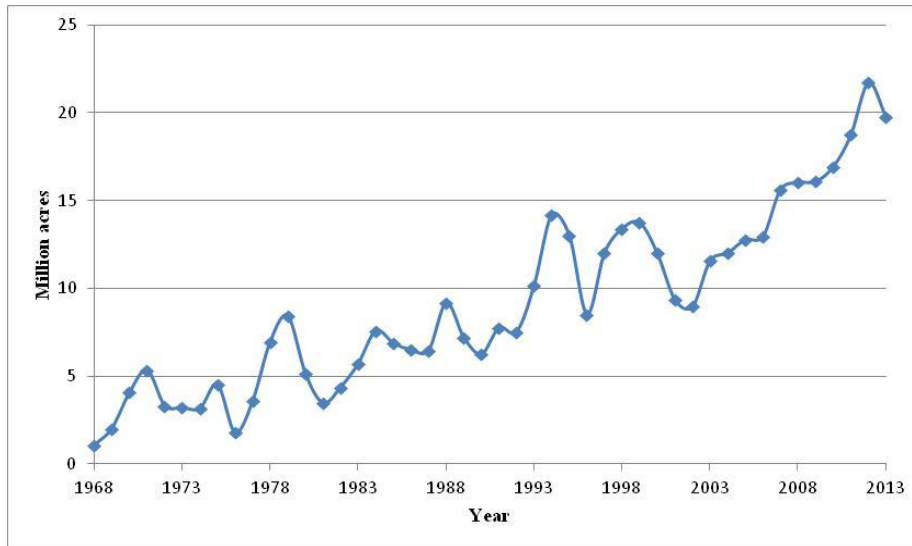


Fig. 2.2 Area under canola cultivation in Canada (Statistics Canada 2013)

Diminishing fossil fuel reserves and their role in global warming is a global concern (IPCC 2013; Soetaert and Vandamme 2009). Individuals and various levels of government are combining efforts to reduce their carbon footprints (IPCC 2013; Yan 2009). The urban transportation sector is exclusively fuelled by fossil fuel, and currently accounts for about 21 percent of world total energy consumption which will increase to 29 to 32% by 2050 (Nakicenovic and Swart 2000). In order to mitigate the effects of anthropogenic activities

related to the use of fossil fuels, the Canadian Government mandated the production and sale of B2 (a blend of 2% biodiesel with conventional fossil fuel) (Government of Canada 2011). This measure alone would result in reduction of 1.8 million tonnes of greenhouse gas emissions per year in Canada (Canola Council of Canada 2014a; Hill et al. 2006). Compared to energy utilized in the generation of biofuels, there is only 25% net energy gain from bioethanol produced from corn; however, the net return is 93% for soybean-derived biodiesel (Hill et al. 2006). Environmental impact analysis conducted through life cycle analysis methodology on canola oil have indicated a positive energy balance and its use as biofuel influences climate change positively (Requena et al. 2011). Although, with sufficient merits of using biofuel as renewable energy source, they inherently compete with food supplies (Hill et al. 2006). The Canadian B2 mandate should create an additional demand for about 430,000 tonnes of canola oil or approximately one million tonnes of canola seed annually. In order to cater to the growing domestic and international demands for canola oil, development of high oil content canola cultivars with improved agronomic performance remains a prime focus.

2.4 Brassica oil composition

The major oil producing crops such as soybean, oil palm, rapeseed, sunflower, cotton seed, and groundnut account for 84% of vegetable oil production in the world (Luhs and Friedt 1993). The major constituting fatty acids of most vegetable oils consist of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) as shown in Fig. 2.3 (Topfer et al. 1995). Rapeseed or canola oil may also contain arachidic acid (C20:0), gondoic acid (C20:1), behenic acid (C22:0), erucic acid (C22:1), lignoceric acid (C24:0) and nervonic acid (C24:1) (Ohlrogge and Browse 1995;

Uppstrom 1995). The end utilization as edible or industrial oil of Brassica depends upon its component fatty acids. Canola oil has the ideal fatty acid profile for human consumption compared to other vegetable oil such as olive oil that has 15% saturated fat, 75% oleic acid, 9% linoleic acid and 1% linolenic acid. (Canola Council of Canada 2014b; Stringam et al. 2003). It has the lowest level of saturated fatty acids (7.35%) mainly contributed by palmitic acid and stearic acid. Elevated levels of saturated fat are linked to coronary diseases (Nesi et al. 2008). Canola oil contains more than 65% oleic acid (Scarath et al. 1988), which is a monounsaturated fatty acid. It is thermo-stable and ideally suited as cooking oil (Tanhuanpaa et al. 1998). Linoleic acid (omega 6) and linolenic acid (omega 3) are essential fatty acids and their typical level in canola ranges between 21% and 11%, respectively (Scarath et al. 1988). These fatty acids offer numerous health benefits by inhibiting inflammation, cardio-vascular disease, and blood pressure by reducing the levels of low density lipids (LDL) (Kaur and Das 2011; Kelley et al. 1991). However, both linoleic acid and linolenic acid are polyunsaturated fatty acids, prone to oxidation, rancidity and reduced oil shelf-life (Scarath et al. 1988). Scarath and McVetty (1999) stated that canola cultivars with 75% oleic acid and 4% linolenic acid are suitable for oleo-chemical needs as well as edible oil end utilization.

Erucic acid is an important constituent of rapeseed oil. Its derivatives have numerous end uses such as high and low-temperature lubricants, heat transfer oils, plasticizers, perfumes and cosmetics, surfactants, slip and coating agents, and pharmaceutical products (Ecke et al. 1995; Luhs and Friedt 1993). Erucic acid can also be used to commercially produce nylon-13,13 which is a light-weight material for the manufacturing of engine automotive parts (Sonntag 1995), and its economic feasibility depends upon 90% content

of erucic acid in oil (Sonntag 1995). This potential product alone would consume all current supply of erucic acid (Sonntag 1995).

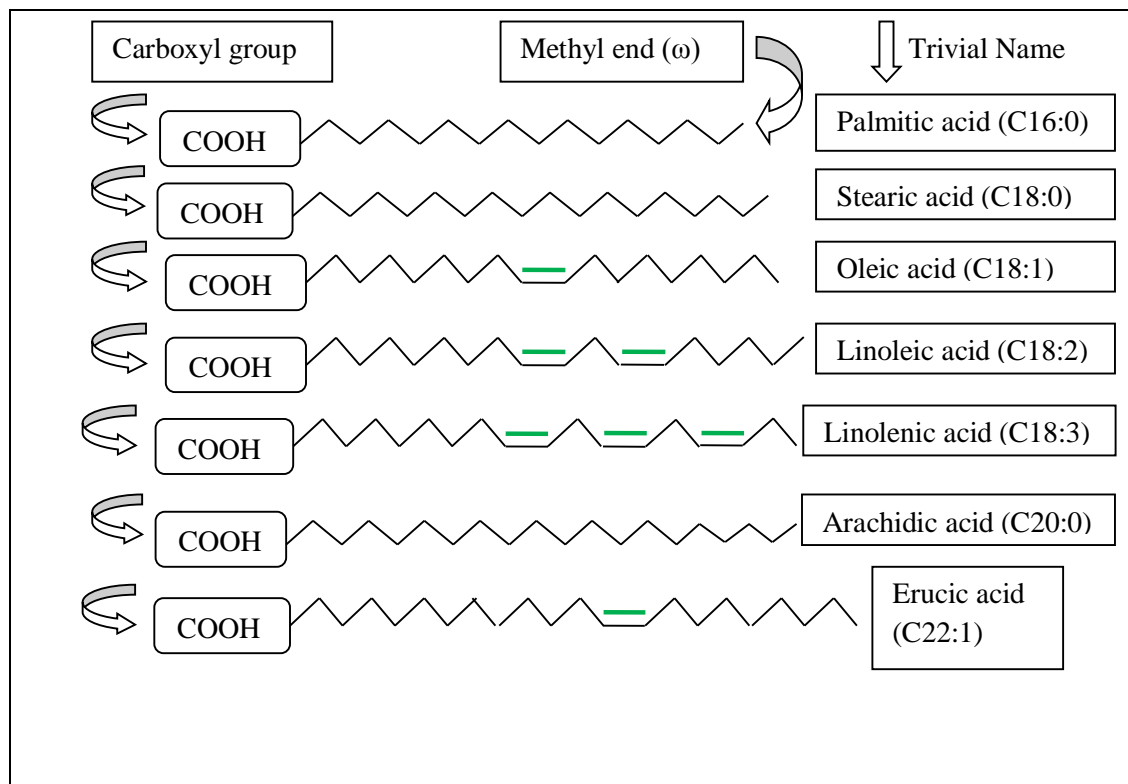


Fig. 2.3 Chemical formula, trivial names and symbols of fatty acids found in canola, modified from (Topfer et al. 1995).

2.5 Fatty acids

Fatty acids are carboxylic acids with hydrocarbon chains with a methyl group at one end (referred to as omega: ω). Fatty acids can be saturated or unsaturated and typically contain 16 or 18 carbons (Somerville et al. 2000). Plants also produce fatty acids containing 8-14 carbon atoms termed medium chain fatty acids, long chain fatty acids (C16-C18), very long chain fatty acids (VLCFA) (C20-C24) and epicuticular waxes (Graham 1989; Lu et al. 2009; Samuels et al. 2008). Lipids are derived from fatty acids and preferentially soluble in non-aqueous solvents like chloroform. They are constituents of biological

membranes such as the membrane of chloroplasts that are internally composed of galactolipids and externally of phospholipids (Somerville et al. 2000). They create a hydrophobic barrier and prevent diffusion of plant cell contents thus, are critical to the normal functioning of plant cells (Somerville et al. 2000). Seed oil is the most reduced form of carbon and serves as an energy source for germination and post-germination growth in plants. On a per weight basis, compared to starch and protein, oil has two times more energy (Huang 1992). Plant oil is a triacylglycerol (TAG), consisting of three fatty acid esterified to hydroxyl positions (sn-1, sn-2, sn-3) of the glycerol molecule (Kinney 1994). Triacylglycerol are stored in subcellular bodies called oil bodies (Huang 1992; Somerville et al. 2000). The precise process of oil body production is unknown (Baud et al. 2008). Huang (1992) proposed a budding mechanism, as TAGs are sequestered from the endoplasmic reticulum (ER) and accumulate within the bilayer of the ER from where they eventually bud off with a protein bearing phospholipid layer that is also synthesized within the ER. Oil bodies are spherical in shape with a diameter 1 μm (0.6 μm in *B. napus*) surrounded by a monolayer of phospholipid with their hydrophobic end interacting with TAGs and hydrophilic ends facing cytosol (Huang 1996). Each oil body is shielded with a layer of oleosin protein providing structural stability during seed desiccation, in addition to maintaining the surface-to-volume ratio of oil bodies for efficient lipase activity (Somerville et al. 2000). In mature embryos of *Arabidopsis*, the peripheral region and 60% of the cell volume of cotyledons is occupied by oil bodies (Mansfield and Briarty 1992).

2.5.1 *De novo* fatty acid biosynthesis

In plants, fatty acid synthesis occurring in the plastids is known as prokaryotic type (Type II) while in the cytosol, it is called eukaryotic type (Fig. 2.4) (Ohlrogge and Jaworski

1997). The *de novo* fatty acid synthesis in plastid occurs in a multi-enzyme complex termed fatty acid synthase (FAS) (Li-Beisson et al. 2010). The building block of fatty acid synthesis is acetyl-CoA, a two carbon molecule which is produced only in the plastids with no evidence of inter-sub-cellular movement and its concentration is low but constant (Ohlrogge and Browse 1995; Post-Beittenmiller et al. 1992). Acetyl-CoA is derived from pyruvate (Johnston et al. 1997) and its conversion to malonyl-CoA is catalyzed by acetyl-CoA carboxylase (ACCase) (Konishi et al. 1996). The incorporation of malonyl-CoA into FAS requires the malonyl group moiety be converted to a acyl carrier protein forming malonyl ACP by the catalyzation of malonyl-CoA: acyl carrier protein malonyltransferase (MCMT) (Nikolau et al. 2003). In FAS, seven cycles of reactions where each consists of a condensation, two reductions and a dehydration reaction, lead to the formation of 16:0-ACP (Li-Beisson et al. 2010; Ohlrogge and Jaworski 1997). The first condensation reaction is catalyzed by 3-ketoacyl-ACP synthase (KAS III) resulting in the formation of 4:0-ACP (Ohlrogge and Browse 1995). The second condensation reaction forms 6:0-ACP, which is catalyzed by KAS I and the final condensation from 16:0-ACP to 18:0-ACP is catalyzed by KAS II enzyme (Pidkowich et al. 2007). Each condensation reaction is followed by two reductions and a dehydration reaction to add two carbon molecules to the elongating fatty acyl chain. The first reduction of 4:0-ACP is catalyzed by 3-Ketoacyl-ACP reductase (KAR) followed by a dehydration reaction catalyzed by enzyme 3-Hydroxyacyl-ACP dehydratase (HAD) to form enoyl-ACP which is finally reduced by the enzyme enoyl-ACP

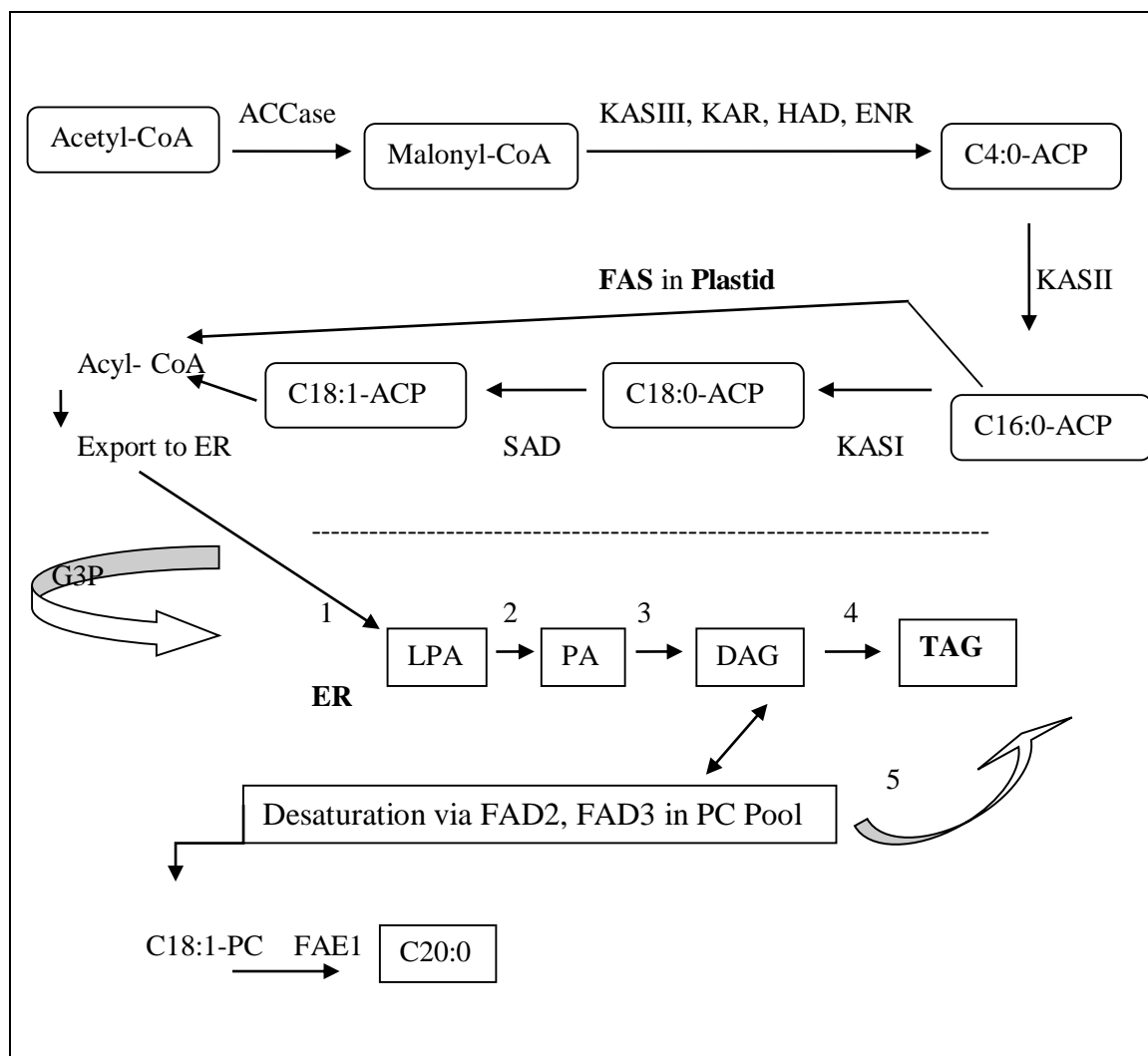


Fig. 2.4 De novo fatty acid and triacylglycerol synthesis in *Arabidopsis*.

This diagram and description is modified from Li-Beisson et al. (2010). Newly formed acyl-CoA from plastid (Enzymes involved, ACCase: acetyl-CoA carboxylase, KAS [I-III]: Ketoacyl-ACP synthase, KAR: 3-Ketoacyl-ACP reductase, HAD: Hydroxyacyl-ACP dehydratase, ENR: Enoyl-ACP reductase, ACP: Acyl carrier protein, SAD: Stearoyl-ACP desaturase) are exported to ER where they are assembled onto G3P molecule through a series of reactions forming products given in the boxes and adjacently, the enzymes catalyzing the relevant reactions are given in numeric format. Abbreviations: LPA: 2-lysophos-phatidic acid, PA: Phosphatidic acid, DAG: Diacylglycerol, TAG: Triacylglycerol, 1: glycerol-3-phosphate acyltransferase (GPAT), 2: 2-lysophosphatidic acid acyltransferase (LPAAT), 3: phosphatidate phosphatase (PP), 4: Diacylglycerol acyltransferase (DGAT), 5: Phospholipid-diacylglycerol acyltransferase (PDAT), C16:0: palmitic acid, C18:0: Stearic acid, C18:1: oleic acid, FAD [2-3]: Fatty acid desaturase, FAE1: Fatty acid elongase, C20:0: Arachidic acid.

reductase (ENR) to yield a saturated fatty acid (Mou et al. 2000). FAS may release some 16:0-ACP and other molecules may be elongated to 18:0-ACP, of which some proportionally are desaturated by stearoyl-ACP desaturase (SAD) to form 18:1-ACP (Browse and Somerville 1991). This marks the first desaturation event of a long chain acyl group that occurs in the plastid. However, acyl-ACPs are also further subjected to desaturation by fatty acid desaturases, *FAD5*, *FAD6*, *FAD7* and *FAD8* for the synthesis of thylakoid membranes of chloroplasts (Hugly and Somerville 1992; Li-Beisson et al. 2010). *FAD4* was recently reported, which inserts a trans-3 double bond at the carboxyl end of palmitic acid in *Arabidopsis* (Gao et al. 2009; Li-Beisson et al. 2010).

Long-chain acyl groups are hydrolyzed by two kinds of acyl-ACP thioesterases and their activity releases fatty acids from the FAS machinery (Kjellberg et al. 2000). FatA and FatB are two main types of acyl-ACP's thioesterases that cleave specific acyl-ACP. The former type acts upon 18:1-ACP while the latter acts on saturated acyl-ACP. The free fatty acids formed are then converted into acyl-CoA (16:0-CoA or 18:1-CoA) by catalyzation by acyl-CoA synthetases. A portion of nascent fatty acid is utilized for glycerolipid synthesis within the plastid (Ohlrogge and Jaworski 1997). Distinctively, phosphatidic acid formed in the prokaryotic pathway has the palmitate esterified at *sn*-2 position (Ohlrogge and Jaworski 1997). A major portion of acyl-CoA is exported to the cytosol for further elongation and desaturation in the eukaryotic pathway (Pollard and Ohlrogge 1999).

2.5.2 Oil accumulation in endoplasmic reticulum

Fatty acids synthesized in plastids are assembled into TAG or seed oil in the ER of the *Arabidopsis* cell (Fig. 2.4) (Huang 1992). First, acylation of the glycerol-3-phosphate molecule with acyl-CoA, in a series of reactions (also known as Kennedy pathway)

produces 2-lysophosphatidic acid (LPA) by the catalyzation using glycerol-3-phosphate acyltransferase (GPAT) (Beisson et al. 2007). The second acylation of G3P catalyzed by 2-lysophosphatidic acid acyltransferase (LPAAT) produces phosphatidic acid (PA) (Kim and Huang 2004). The *sn*-2 position of PA produced in the eukaryotic pathway is always occupied by C₁₈ fatty acids, which differ from the prokaryotic pathway that has a C₁₆ fatty acid occupying this position (Heemskerk and Wintermans 1987; Somerville et al. 2000). After the second acylation, the subsequent dephosphorylation of PA produces diacylglycerol (DAG). This activity is catalyzed by phosphatidate phosphatase (PP) (Carman and Han 2006). DAG to TAG synthesis is catalyzed by ER-specific diacylglycerol acyltransferase (DGAT) (Zou et al. 1999b). DAG synthesis is an important branch point (Bates et al. 2009). It can either be directly converted to TAG or can be acylated with phosphatidylcholine (PC) to form a PC pool where subsequent desaturation or saturation of fatty acids can occur (Li-Beisson et al. 2010). The PC pool and DAG are interchangeable and the former can also serve as an acyl donor for TAG synthesis through the activity of phospholipid: diacylglycerol acyltransferase (PDAT) (Durrett et al. 2008; Li-Beisson et al. 2010). Recently it has been proposed that in addition to the sequential acylation of G3P, a direct acylation of acyl-CoA onto the *sn*-2 position of PC may occur (Bates et al. 2009). Moreover, DAG required for TAG synthesis is accessed from the PC pool and newly synthesised DAG is converted into the PC pool (Bates et al. 2009).

2.5.3 Mitochondrial lipid synthesis

As mentioned earlier, ACPs are important protein cofactors for *de novo* plastidial fatty acid synthesis. Investigation on the presence of ACP in the mitochondria is linked to mitochondrial lipid synthesis (Wada et al. 1997). However, mitochondria lack acetyl-CoA

carboxylase but malonyl-CoA is produced from malonate, which is then transported from the cytosol and the conversion is catalyzed by malonyl-CoA synthetase (Gueguen et al. 2000). Subsequently, malonyl-ACP is produced by malonyl-ACP synthase (Gueguen et al. 2000). Contrary to plastidial fatty acid synthesis, the first condensation and the subsequent condensations of malonyl-ACP are catalyzed by 3-ketoacyl-ACP synthase (KAS), followed by similar reduction, dehydration and reduction cycles catalyzed by KAR, HAD and ENR enzymes, respectively (Yasuno et al. 2004). Among the major glycerolipids constituting mitochondrial membranes, the cardiolipin (CL) is exclusive to mitochondria. While other glycerolipids can be imported from the ER, CL is *de novo* synthesised in mitochondria (Jouhet et al. 2004). Using acyl-ACP produced in mitochondria, six enzymes are required for acylation of G3P to produce CL, of which only two have been identified (Babiychuk et al. 2003).

2.6 Elongation and desaturation pathways

C16 and C18 fatty acids are produced in plastids (Brown et al. 2006). However, most oilseed rape species of Brassicaceae accumulate appreciable levels of C20 to C24 fatty acids (Snowdon et al. 2007). All plants produce waxes that require fatty acids containing carbon chains ranging from C26 to C32 (Somerville et al. 2000). Synthesis of longer than C18 fatty acid occurs in the elongation pathway mediated by ER-associated fatty acid elongase. Elongase reactions share some commonality with FAS as two carbons from malonyl-CoA are used as an acyl primer, followed by reduction, dehydration and final reduction reactions (Li-Beisson et al. 2010; Somerville et al. 2000). Distinctive from FAS, this fatty acid elongase localizes in the cytosol. ACP is not involved in these reactions and

the condensation of malonyl-CoA is catalyzed by elongase-3 ketoacyl-CoA synthase (elongase KCS).

Synthesis of very long chain fatty acids (VLCFA) takes place in four reactions as mentioned above, catalyzed by four distinct enzymes (Puyaubert et al. 2005). In the first reaction, the substrate preference of elongase KCS is either stearoyl-CoA or oleoyl-CoA to form 3-ketoacyl-CoA, which is then reduced by 3-ketoacyl-CoA reductase (KCR) to generate 3-hydroxyacyl-CoA in the second reaction. The third reaction is the dehydration of 3-hydroxyacyl-CoA catalyzed by 3-hydroxyacyl-CoA dehydratase (HCD) to form a trans-2, 3-enoyl-CoA which is finally reduced by trans-2, 3-enoyl-CoA reductase (ECR) to yield two carbon atoms added to acyl-CoA while the repetition of cycles results in the formation VLCFA (Joubes et al. 2008; Li-Beisson et al. 2010; Millar and Kunst 1997). Elongase reactions have been biochemically characterized but the genes involved still remain unknown (Beaudoin et al. 2009). Recently, *Arabidopsis* genes encoding elongase moieties (KCR, HCD, and ECR) have been identified and their mutations have been reported to have pleiotropic effects on sphingolipids and seed triacylglycerols (Beaudoin et al. 2009). KCS for fatty acid elongation is encoded by *FAE* in *Arabidopsis* (Millar and Kunst 1997; Puyaubert et al. 2005). The cloning of KCS genes in *Arabidopsis* and jojoba (*Simmondsia chinensis*) revealed that the encoded proteins of these genes share very little sequence similarity with any other condensing enzyme. There may be up to 25 genes in *Arabidopsis* encoding elongase KCS, which indicates these genes may have narrow substrate specificity for the synthesis of fatty acids up to C30 (Somerville et al. 2000). Fatty acid elongase, *Bn-FAE1.1* and *Bn-FAE1.2* are the two homologs in *B. napus* (Puyaubert et al. 2005). They have been characterized in embryos and are reported to encode proteins

with 98.2% homology (Puyaubert et al. 2005). Genes encoding 3-ketoacyl-CoA reductase have been cloned from the seeds of *B. napus* (Puyaubert et al. 2005). The genes designated as *Bn-kcr1* and *Bn-kcr2* have been demonstrated to have different temporal expression of mRNA between HEAR and low erucic acid rapeseed plants (LEAR) (Puyaubert et al. 2005).

Desaturation is the incorporation of a double bond in the acyl chain in an enzymatic reaction involving reduction of a dioxygen molecule to water (Shanklin and Cahoon 1998). Unsaturated fatty acids have one or more double bonds, and relative to a saturated fatty acid have two hydrogen atoms less per double bond (Shanklin and Cahoon 1998). Both physiological and physical properties of fatty acids are affected by the number and position of the double bond (Shanklin and Cahoon 1998). Polyunsaturated fatty acids in plants are produced by two independent pathways with specific enzymes catalyzing the reactions, one in the plastid and the other in the microsomes or ER (Sperling et al. 1993). Desaturation of fatty acids in plastid is mediated by *FAD5* (C16:0 to 16:1), *FAD6* (C16:1 to C16:2), *FAD7* (C18:2 to C18:3) and *FAD8* (C16:2 to C16:3) genes, whereas, *FAD4* inserts a trans-3 double bond in palmitic acid (Gao et al. 2009; Li-Beisson et al. 2010).

The first desaturation of C18:0-ACP occurs in the plastid and the subsequent addition of double bonds to the hydrocarbon chain occurs in the ER and most prominently in the PC pool (Li-Beisson et al. 2010; Somerville et al. 2000). Oleate desaturase (*FAD2*) converts C18:1-PC into C18:2-PC whereas linoleate desaturase (*FAD3*) converts C18:2-PC to C18:3-PC (Baud et al. 2008; Somerville et al. 2000). The vast majority of polyunsaturated fatty acid synthesis routes through oleate desaturase (Okuley et al. 1994). Moreover, this enzyme is responsible for 90% of the polyunsaturated fatty acid synthesis

in non-synthetic tissues such as roots and seeds (Okuley et al. 1994). Linoleic acid and linolenic acid are important membrane components and essential fatty acids for human nutrition (Okuley et al. 1994).

2.7 Genes related to fatty acid biosynthesis in *B. napus*

ACCase activity is tightly regulated (Nikolau et al. 2003) and may be a limiting factor in attempts to increase the rate of fatty acid synthesis (Somerville et al. 2000). ACCase has two forms with plants utilizing both the prokaryotic-type (heteromeric coenzyme) in plastids and the multifunctional (homomeric coenzyme) eukaryotic-type in the cytosol except for members of grass family that have only the eukaryotic type (Roesler et al. 1997). Plastidial ACCase is comprised of four subunits of which β -carboxyltransferase (β -CT) is encoded by the chloroplast genome whereas biotin carboxylase (BC), biotin carboxyl carrier protein (BCCP) and α -carboxyltransferase (α -CT) are coded by the nuclear genome (Somerville et al. 2000).

Six hundred genes are involved in acyl lipid metabolism in *Arabidopsis* (Beisson et al. 2003). Since *B. napus* is an amphidiploid originated from the duplication or triplication of the *Arabidopsis* genome, therefore for each *Arabidopsis* gene, 4-6 orthologues are expected in *B. napus* (Parkin et al. 2005). Identification of genes controlling pivotal enzymatic reactions in the model crop *B. napus* has commercial implications. Elborough et al. (1996) cloned six *BCCP* genes and Li et al. (2010b) reported four carboxyltransferases (α -CT) genes in *B. napus* and two in each diploid parent. Genes encoding enzymes for TAG accumulation in the ER have been recently reported in *B. napus*. Chen et al. (2011) identified three *BnGPAT4* homologs, two in the C genome and one in the A genome but they exhibited differences in expression patterns and functional divergence. Maisonneuve

et al. (2010) detected four isoforms of LPAAT, whereas Fourmann et al. (1998) identified two *FAE* orthologues designated as *BN-FAE1.1* and *BN-FAE1.2* and respectively mapped them on A8 and C3 chromosomes. Scheffler et al. (1997) reported the estimated number of gene copies for *FAD2* and *FAD3* and mapped their position with RFLP markers. There are 4-6 copies of the former and the latter had 6-8 copies distributed over A1, A3, A5, C1, C3, and C5.

2.8 Molecular markers

The difference between two individuals at a particular segment of DNA constitutes a genetic or molecular marker that may or may not manifest a phenotype (Agarwal et al. 2008). Molecular markers do not influence the represented trait and therefore, serve as tags or signs to identify an association between the marker and the genetic region controlling the trait of interest (Xu 2010). Genetic markers can be categorized as a morphological marker, a biochemical marker and a DNA marker (Kole and Abbott 2008).

Differential phenotypes of plants are produced by differences at the genetic level with the modifying effect of environments (Agarwal et al. 2008; Xu 2010). Contrasting phenotypes or morphological types in a genotypic analysis can potentially be mapped to a particular chromosome (Xu 2010). Thus, polymorphism observed in plant height, flower color, seed size and color can be used as morphological markers for respective traits (Kole and Abbott 2008; Xu 2010). Gregor J. Mendel in the late 1800s used morphological markers and investigated their transmission through subsequent generations of *Pisum sativum* and laid the foundation of modern genetics (Xu 2010). Currently, in rice, more than 300 morphological markers are available for genetic studies (Khush 1987). Morphological markers influence the phenotype they represent; however, they

inadequately distinguish the polymorphism and are available at only specific plant developmental stages. Hence, morphological markers have limited use in genetic studies.

Variation in chromosome structure and number can serve as cytological markers (Xu 2010). Chromosomal karyotyping reveals information about chromosome structure, which serves as landmarks to characterize them. Cytological markers are limited in numbers and are not suitable for diversity or mapping studies (Xu 2010).

Protein markers depict variation in enzymatic properties. For example, isozymes are variants of an original enzyme and may possess different molecular weights but similar catalytic properties (Xu 2010). These variations of isozymes are the result of different alleles; therefore, they can be traced back to genes and can be used as genetic markers. There are approximately 57 isozyme markers available in plants (Vallejos and Chase 1991), an insufficient number to develop a saturated linkage map. The saturated linkage map relative to any other genetic map has as many polymorphic loci marked and linked with each other. An ultra-dense genetic map targets to link all genes of interest with a marker, mostly < 1 cM apart (Mihovilovich et al. 2008). Since their advent, molecular markers have played an increasingly important role in the evaluation of plant germplasm (Newbury 2003). They determine differences at the DNA level, generate genotypic data, and are capable of distinguishing miniscule genetic differences, beyond the capabilities of other categories of markers (Avisé 1994; Xu 2010). Genome sequence information of many plant species is available, and this information has increased the possibility of generating large volumes of DNA markers. DNA markers have thus overcome the limitation of both morphological and biochemical markers and therefore, are preferred tools for the study of genetic inheritance, variation, mapping and marker-assisted selection (MAS) (Dudley

1994; Paterson and Wing 1993; Xu 2010). Many types and increasingly complicated acronyms are used to describe the array of DNA markers today (Xu 2010).

Based on how DNA markers are detected, they can be divided into two categories, hybridization-based and Polymerase Chain Reaction-based (PCR) markers (Agarwal et al. 2008; Collard et al. 2005). Hybridization-based markers such as Restriction Fragment Length Polymorphism (RFLP) markers reveal genetic dissimilarity as the radioactively labelled probe is hybridized with the target DNA. Initially, reported by Grodzicker et al. (1975) and later Botstein et al. (1980) pioneered the use of this kind of marker in genetic analysis. Restriction endonuclease can cleave the DNA at specific sites, which differ due to insertion and deletions of nucleotides during evolutionary changes among members of species. This differential length of restricted DNA fragments reveals DNA polymorphism between two individuals (Xu 2010). Although RFLP markers are co-dominant and reproducible, their detection process is time consuming and requires high quality and abundant DNA quantities (50 ug) and also involves exposure to toxic radioactive chemicals; therefore, PCR based markers have gained more attention (Agarwal et al. 2008; Erlich 2012).

The invention of PCR by Mullis and Faloona (1987) led to more efficient ways to generate molecular markers such as Random Amplified Length Polymorphic DNA or RAPDS (Welsh and McClelland 1990; Williams et al. 1990), Inter Simple Sequence repeats or ISSRs (Zietkiewicz et al. 1994) and Amplified Fragment Length Polymorphisms or AFLPs (Vos et al. 1995) which employ arbitrary primers to amplify the target DNA in a thermocycler. This combines the advantages of RFLPs and eliminates the need for prior sequence information for target DNA. However, markers based on sequence-specific

primers for example Simple Sequence Repeats or SSRs (Tautz and Renz 1984), Sequence Characterized Amplified Region or SCAR (Paran and Michelmore 1993), Sequence Related Amplified Polymorphism or SRAP (Li and Quiros 2001) and single nucleotide polymorphisms or SNPs (Vignal et al. 2002) are a new generation of molecular markers distinguishing individuals on sequence dissimilarity.

Each marker system has its strengths and weaknesses. RAPDS have poor repeatability across labs whereas SSR are expensive to develop, SNP detection may require expensive equipment while SRAP though generating multiple loci data may have to be converted to other marker systems. While investigating the genetic differences between 24 broccoli (*B. oleracea*) inbreds (Hale and Farnham 2006), SSR markers produced on average 2 bands per primer and 25% of the primers were monomorphic. While AFLP markers produced 24 bands per primer but revealed 63% less polymorphism compared to SSR. SRAP markers produced 14 bands per primer and revealed 80% more polymorphism. Some of the considerations on the selection of a particular DNA markers system include how they generate genotypic information, the resources required to detect such markers and the transferability of generated information across labs (Agarwal et al. 2008; Avise 1994).

SRAP markers are detected by developing 17 to 18 nucleotide long primers (Li and Quiros 2001). Each primer consists of a core sequence in which the first 10 to 11 bases are non-specific known as fillers followed by CCGG at the 5' end of forward primer and AATT at the reverse primer. Both of the primers have differential filler lengths that can be either 10 or 11 bases. Moreover, the core sequence in the forward primers has selective three bases at 3' end. Genotyping through SRAP offers simplicity, reliability and moderate

throughput and has been used for mapping studies in rice, Chinese cabbage (*B. rapa* L.), and rapeseed (*B. napus* L.) (Chen et al. 2010; Li and Quiros 2001).

SSR markers are highly polymorphic and for a period were the most preferred and effective Mendelian genetic markers (Li et al. 2002). SSR loci consist of repeated small sequences from 1 to 6 nucleotides for example (CA), (AAT) and (GATA). The slippage of a strand during DNA synthesis creates the variation in SSR repeat numbers (Levinson and Gutman 1987). The polymorphism in SSR repeats is detected through PCR analysis using discriminatory oligonucleotide primers that are developed using the unique sequences adjacent to SSR motif (Agarwal et al. 2008; Hamada et al. 1982). For analyzing the PCR product with the ABI 3100 genetic analyzer (Applied Biosystem, California, USA) one of the primers must be fluorescently labeled which makes SSR assays expensive (Agarwal et al. 2008). However, Schuelke (2000) developed a cost-effective procedure involving three primer combinations in which forward and reverse primers are sequence specific while the 5' end of the forward primer has an M13(-21) tail. The third primer is a universal fluorescent-labelled M13(-21) primer (Schuelke 2000). The specific PCR conditions promote annealing of the forward primer first, which is then taken over by a universal primer thereby labeling the entire PCR product. With a wealth of sequence information data available on the model plant *Arabidopsis* and various crops, the pace of SSR development has accelerated, making it a preferred marker system for genotyping across commercial breeding programs (Lamkey and Lee 2006; Uzunova and Ecke 1999).

SNP markers are based on single nucleotide base difference in DNA fragments of two individuals (Xu 2010). SNP markers target the smallest unit of inheritance in a genome, they constitute the ultimate form of a molecular marker (Xu 2010). Frequency of one SNP

in every 100-300 bp in plants (Cassava, barley, maize, potato, soybean and sugarbeet) has been reported by Edwards et al. (2007). Technologies such as Pyrosequencing from Royal Institute of Technology (Sweden), BeadXpress™ and GoldenGate™ from Illumina (<http://www.illumina.com>) perform SNP genotyping using different chemistries for allele discrimination and SNP detection methods such as Fluorescence Resonance Energy Transfer (FRET) (Semagn et al. 2014; Xu 2010). With increased sequence information and availability of next generation genotyping platforms, SNP markers are replacing the use of other types of molecular markers for different genetic analyses studies such as QTL mapping, linkage mapping and germplasm characterization (Semagn et al. 2014). However, Hamblin et al. (2007) utilized 89 SSRs and 847 SNPs markers on 259 maize inbred lines and reported that SSR markers were comparatively more effective on clustering the germplasm into populations and discriminating relationships between individual inbred lines.

2.9 Linkage map

Like road maps, linkage maps elucidate genome structure and organization based on gene location and relative distance between loci on a chromosome, expressed as map units or centimorgan (cM). The change in map position occurs due to crossing-over during meiosis as the chromosome portions are exchanged between homologous chromosomes, and this process creates new genetic recombination. In a cross, 1% recombination is indicative of 1% crossing over, expressed as 1 cM, which represents the distance between two loci/markers (Xu 2010). However, this relationship between recombination frequency and crossing over becomes non-linear as the genetic distance increases beyond 10 cM (Hartl and Ruvolo 2001). As the distance between markers increases, it induces as well as

hinders the occurrence of double crossing over in the vicinity (Xu 2010). Kosambi (1944) and Haldane mapping functions (1919) are commonly utilized in mapping software to account for crossover interference (Hartl and Ruvolo 2001). The mapping software, JoinMap (Van and Voorrips 2001), establishes linkages between markers on the basis of logarithm of odds (LOD) value (Risch 1992). A LOD value of 3 is a commonly accepted threshold for linkage analysis meaning a linkage between two markers is 1000 times likely against the odds of one. Genes with lower recombination tend to remain on the same chromosome, forming a particular linkage group (LG) and the occurrence of the total number of linkage groups corresponds to species chromosome numbers provided the linkage map is saturated enough (Acquaah 2007; Lamkey and Lee 2006; Xu 2010).

Linkage groups in *B. napus* are arranged from N1-N10 for A genome and N11 to N19 for C genome (Parkin et al. 2003). The Multinational Brassica Genome Project has further proposed amendments in linkage group nomenclature based on canonical diploid Brassica genomes indicating A genome chromosomes from A1 to A10 and C genome from C1 to C9 (MBGP 2007). Linkage groups of A and C genomes share a close homology, particularly A1/C1, A2/C2 and A3/C3 are homologous for their entire lengths (Parkin et al. 2005).

In linkage analysis, recombination events are investigated in related progeny where the genes linked to markers with pronounced effect on phenotype are discovered (Terwilliger 1995). Association analysis determines the effect of an allele at a much finer resolution in a group of unrelated individuals by comparing the frequencies of associations or otherwise between two loci with respect to a trait of interest (Terwilliger 1995). Both mapping approaches rely on linkage disequilibrium (LD) which is the non-random association of

two loci, considered to be higher if the association between two loci is tight and no recombination occurs between them during meiosis (Gupta et al. 2005). The later approach requires more densely populated maps and more robust markers such as SNP for mapping. However, Wurschum et al. (2012) recently compared both mapping approaches by developing multiple segregating populations from 10 parental lines and then genotyped them with 253 SNP markers. The results indicated both approaches to be suitable in detecting QTL for traits of interests.

Among the essential features of a genetic map are its length and the average marker distance (Paterson 1996; Tanksley et al. 1992; Xu 2010). Linkage maps of unique characteristics have been developed in Brassicaceae for different genetic analysis; a list of representative genetic maps is given in Table 2.1. The length or genome coverage of the map is a direct function of recombinants present in the mapping population (Kole and Abbott 2008). Progeny developed from diverse parents would therefore yield more recombinants resulting in more polymorphic loci to produce more detailed/saturated linkage maps (Qiu et al. 2006). Such maps serve as useful tools to localize genes of interest for genetic analysis (Tanksley et al. 1992). Genetic maps are dependent on recombination events of the derived progeny and therefore differ in genome coverage and present genetic distance in approximation (Ablett et al. 2003) compared to physical maps which are expressed in base pairs. The increased numbers of molecular markers enable the demarcation of allelic variation present in the genome thereby detecting if a meiotic recombination occurred between markers which could affect the detection of the association between the marker and the trait (Tanksley 1993). The number of markers required to construct a saturated linkage map depends upon the genome size and

polymorphism of loci between parents. Foisset et al. (1996) and Sharma (2002) stated that 343 and 500 markers at 15 cM spacing would be required respectively in *B. juncea* and *B. napus* to generate a complete map. However, for effective marker-assisted selection, markers should ideally be 1 cM or at least 5 cM apart from the gene (Michelmore 1995). A combination of various marker types is preferred sometimes over a single marker system to get an even coverage of the genome as certain LG could be deficient for specific marker loci or due to clustering of markers over certain LG (Kesseli et al. 1992). Foisset et al. (1996) reported lack of RFLP loci in designated LG groups (DY3, DY7, DY-17 and DY-18).

Anchor markers or common markers from individual maps derived from different genetic backgrounds can be aligned together to create an integrated/reference or a consensus genetic map (Zhang et al. 2011a). Ablett et al. (2003) screened 259 SSR markers for polymorphism between 12 barley DH populations and used them to develop a consensus map. Out of 259 markers, 149 markers were mapped on one or more population/maps. Recently, Geng et al. (2012) developed an integrated map from two individual maps derived initially from DH line and RI line *B. napus* populations with the same genetic background. The consensus map found no effect of type of population on marker order. Markers from individual maps can be placed evenly across

Table 2.1 Representative genetic maps in Brassicaceae

Species	Type& Size of Pop	Marker systems-tally	Map length (cM)	Marker interval (cM)	Reference
<i>B. oleracea</i>	F2-96	RFLP-258	820	3.5	(Slocum et al. 1990)
<i>B. rapa</i>	F2-95	RFLP-280	1850	6.6	(Song et al. 1991)
<i>B. rapa</i>	F2-104	RFLP-360	1876	5.2	(Chyi et al. 1992)
<i>B.napus</i>	DH-105	RFLP-132	1016	7.7	(Ferreira et al. 1994)
<i>B. nigra</i>	F2-83	[Isozyme-4, RFLP-47 and RAPD-85]-136	677	5.5	(Truco and Quiros 1994)
<i>B. napus</i>	DH-151	[RFLP-204, RAPD-2, phenotypic-1]-207	1441	6.96	(Uzunova et al. 1995)
<i>B. napus</i>	Resy.-50	RFLP-399	1656	4.15	(Parkin et al. 1995)
<i>B. napus</i>	DH-152	[RFLP-61, RAPD-61, Isozyme-10]-254	1765	7.00	(Foisset et al. 1996)
<i>B. juncea</i>	DH-119	RFLP-343	2073	6.6	(Cheung et al. 1997)
<i>B. oleracea</i>	RIL-86	[SRAP-130, AFLP-120, BoGLS-ALK-1]-251	2165	8.62	(Li and Quiros 2001; Yan et al. 2009)
<i>B. napus</i>	DH-152	[RFLP-58, RAPD-269, AFLP-195, Isozyme-9, PCR specific-3, SSR-5, Bzh-1]-540	2429	4.49	(Lombard and Delourme 2001)
<i>B. juncea</i>	DH-131	[RFLP-264, RAPD-9]-273	1641	6.30	(Lionneton et al. 2002)
<i>B.juncea</i>	RIL-94	RAPD-114	790	6.93	(Sharma et al. 2002)
<i>B. napus</i>	DH-35	SSR-97	1957	20.0	(Lowe et al. 2004)
<i>B. napus</i>	F2-574	[SSR-240 and SCAR-52 Total]-292	2619	7.2	(Piquemal et al. 2005)
<i>B. napus</i> *	DH-282	SSR-125	1196	9.6	(Zhao et al. 2005)
<i>B. napus</i>	DH-445	SSR-305	2690	8.81	(Delourme et al. 2006)
<i>B. napus</i>	DH-188	[AFLP-115, RFLP-23, SSR-71 and STS-68]-277	1685	7.20	(Qiu et al. 2006)
<i>B. rapa</i>	DH-78	[AFLP-278, SSR-235, RAPD-25, & ESTP, STS & CAP -18]-56	1182	2.83	(Choi et al. 2007)
<i>B. napus</i> *	DH-258	[SSR-208, SRAP-189]-397	1747	4.4	(Chen et al. 2007)

* Linkage map developed to identify QTL for seed yield and other objectives.

Table 2.1 Continued

Species	Type& Size of Pop	Marker systems-tally	Map Length (cM)	Marker interval (cM)	Reference
<i>B. napus</i>	DH-58	SRAP-13551	1604.8	0.12	(Sun et al. 2007)
<i>B. napus</i> *	DH-160	RFLP-312	1668	6.0	(Udall et al. 2006)
<i>B. napus</i> *	DH-105	[AFLP-262, SSR-85]-347	1720.8	4.9	(Badani et al. 2006)
<i>B. napus</i> *	DH-150	RFLP-205	1453	7.5	(Quijada et al. 2006)
<i>B. napus</i>	F2-184	AFLP-230	1250	5.4	(Peleman et al. 2005)
<i>B. napus</i>	DH-150	[SRAP-353, SSR-34]-387	1868	4.8	(Chen et al. 2010)
<i>B. napus</i>	DH-88	SSR-598	1842.9	5.98	(Li et al. 2011)
<i>B. napus</i> *	F2-118	[SSR-73, AFLP-170]-243	1207.7	4.96	(Zhang et al. 2011b)
<i>B. napus</i>	Sub-200	RFLP-158	1238	7.8	(Burns et al. 2003)
<i>B. napus</i>	DH-105	RFLP-196	1506	7.68	(Thormann et al. 1996)
<i>B. napus</i> *	DH-235	AFLP-143	1141	7.92	(Dreyer et al. 2001)
<i>B. napus</i>	RIL-183	[SRAP-198, SSR-140, AFLP-107, TRAP-6]-451	1589	3.52	(Yan et al. 2009)
<i>B. rapa</i>	F2-94	[113-SSR, RFLP-87, RAPD-62]-262	1005.5	3.7	(Suwabe et al. 2006)
<i>B. rapa</i>	F2-134	[RFLP-525, PCR-25]-545	1287	2.4	(Kim et al. 2006)
<i>B. napus</i> *	DH-140	[SSR-150, AFLP-195]-345	1759.6	5.1	(Zhang et al. 2011a)
<i>B. juncea</i>	BC5F4-110	[AFLP-408, SSR-2, IP-10]-420	1614	3.84	(Jagannath et al. 2011)
<i>B. napus</i>	DH-282	[Gene-218, EST-50, SSR-87, SRAP-17, SCAR-2]-375	1948.6	4.05	(Zhao et al. 2012)
<i>B. napus</i>	DH-131	[DArT-437, SSR-135, IP-6 and gene -marker-6]-584	2288	3.91	(Raman et al. 2012a)
<i>B. napus</i>	DH-194	[SSR-383, SRAP-191, ISSR-29, SCAR-17]-620	2244.1	3.61	(Geng et al. 2012)
<i>B. napus</i>	DH-88	SSR-359	1821.3	5.07	(Cheng et al. 2009)
<i>B. rapa</i>	F2-94	[SSR-121, RFLP-87, RAPD-62, SNP-3] 273	743	3.6	(Suwabe et al. 2008)
<i>B. napus</i> *	RIL-185	[SSR-65, RAPD 65, SRAP 290]-420	1744	4.15	(Fu et al. 2007)
<i>B. napus</i>	DH-86	[SSR-340, Indels+SNP-17]-357	1381	3.86	(Smooker et al. 2011)

* Linkage map developed to identify QTL for seed yield and other objectives.

the genome in the form of a consensus map thereby efficiently filling the gaps and creating the possibility of tight linkage in genetic studies (Geng et al. 2012).

Similarly, linkage maps of different species can be used to develop comparative genetic maps between species using the common set of markers or sequences used in the development of individual maps (Kole and Abbott 2008; Xu 2010). Such maps highlight the difference and similarities of genomic regions to deduce insight about species evolution (Moore et al. 1995). Comparative mapping resulting from aligning the model monocot rice maps with other 11 species indicates that rice has 30 conserved genomic regions which can be rearranged or duplicated to form other grass genomes. The relatively unconserved genomic regions could be related to speciation in response to the environment, thus allowing the transfer of genetic resources between species (Devos and Gale 2000; Moore et al. 1995). Comparative genetic analysis between *B. napus* and *Arabidopsis* indicated genetic synteny of genomic regions and also opened the possibility of using model plant gene information in a commercial crop (Collard et al. 2005; Parkin et al. 2005; Xu 2010).

Linkage maps developed for specific traits based on structured populations offers the opportunity for selection of phenotypes based on marker position if both are closely linked (Dudley 1994). Such marker-based selection enables breeders to select phenotypes without the influence of the environment which results in accelerated trait development due to higher genetic gain (Dudley 1994; Edwards and Page 1994). This is of particular interest in traits of economic importance that are controlled by many genes such as flowering time, seed yield, oil content and component fatty acids in *B. napus*. Generally a population size of 50-250 individuals is sufficient to detect recombination between the marker and genes within 10-20 cM (Mohan et al. 1997). However, much larger population size is required

for fine mapping a gene at less than 1 cM. Principally, selection of parents is done based on two criteria, polymorphism and segregation for traits of interest. For example, Zhao et al. (2005) developed a mapping population which originated from parents belonging to Chinese and European origins. Such diversity in parental selection allows the detection of more polymorphic loci and results in better coverage of the map (Chyi et al. 1992). Secondly, the population should be segregating for traits of interest. Foisset et al. (1996) developed populations designated as *Darmour-bzh* and *Yudal* which segregated for oil content and component fatty acid, dwarfism, earliness of flowering, seed quality parameters (erucic acid and glucosinolate contents) and disease resistance to blackleg.

Selection based on the type of population such as backcross population, segregating F_2 population, doubled haploid (DH) line, recombinant inbred lines (RIL), and near isogenic lines (NIL) is based on the species' pollination mechanism and nature of the gene action controlling the trait of interest. Each population type has its advantages and disadvantages. F_2 , F_3 and backcross populations are convenient to construct but their genetic architecture changes with every cycle of production, consequently limited seed is available for testing in time and space (Collard et al. 2005; Xu 2010). DH line or RIL populations have the advantage of being able use smaller population sizes for detection of QTL with a better estimate of QTL position, lower variance and with lower trait heritability (Carbonell et al. 1993). In order to achieve similar experimental accuracy using F_2 and backcross populations, a minimum of 14% trait heritability is required (Carbonell et al. 1993). However, when working with traits that have no allelic dominance, an F_2 population is advantageous to using a backcross population (Carbonell et al. 1993). Whereas, production of RILS using single seed decent is time consuming since they are developed through

inbreeding of F_1 to the F_8 generation (Kole and Abbott 2008). Each line harbours multiple crossing over events and unique genetic information from the parents (Collard et al. 2005).

Members of the Brassicaceae family generally are outcrossing (Nasrallah 2011); however, the amphidiploids *B. napus* and *B. juncea* have a self-pollination mechanism (Kimber and McGregor 1995; Rakow and Woods 1987), allowing the use of inbreeding to construct populations with various genetic constitutions, in addition to being amenable to tissue culture. The critical step in haploid plantlet production from microspores is the selection of appropriate anther size (3-4 mm), which corresponds to the bi-nucleate stage of microsporogenesis (McCormick 2004). Grown at a temperature under 32°C in a media containing 17% sucrose, microspores are induced to form haploid embryos whose chromosomes are doubled with 0.2 % (w/v) colchicine (Coventry et al. 1988). The development of a DH line population is efficient and preferred where relevant facilities are available due to the quick period in which a homozygous population can be generated.

2.10 Quantitative trait loci (QTL) mapping

Biological variation in plants is enormous and can be classified into two types (Xu 2010). In the first type, the biological variation forms discrete groups such as cotyledon colour in pea or awned or awnless spikes in wheat. Plant traits displaying biological variation forming distinct classes are known as qualitative traits. Qualitative traits are controlled by a single gene or a few loci, and hence can be manipulated with relative ease (Acquaah 2007; Xu 2010). The second type of biological variation is continuous, which cannot be categorized into discrete classes. Plant traits displaying continuous variation are termed quantitative traits (Kole and Abbott 2008). They are controlled by many genes with each gene having only a small effect on phenotype contribute to continuous variation in

the trait (Kole and Abbott 2008). Classical breeding cannot resolve an individual effect of a gene underlying a polygenic character whose effect is further modified by environment which poses challenges in selection and further improvement (Grandillo et al. 2013; Kole and Abbott 2008; Xu 2010).

QTL mapping is a process of utilizing linkage maps to test the correlations between marker genotypes and quantitative phenotypes (Paterson 1995). For QTL mapping, two processes are necessary. The first is known as genotyping that determines the genetic relevance of a progeny in relation to both parents through the use of molecular markers. The whole progeny at a marker position is categorized into two groups containing the marker or otherwise. The second process is known as phenotyping in which the same genotyped progeny along with the parents is evaluated in replicated trials for the measurement of a trait of interest. Through applying appropriate statistical procedures or computer software a change in phenotype of the progeny is related to a change in a marker or genotype. A consistent change of a phenotype (qualitative or quantitative) with a marker is termed as linkage or association (Young 1996). Thus polymorphism in marker locus and underlying gene controlling a quantitative trait is essential to QTL mapping (Grandillo et al. 2013). The concept of linkage of a quantitative trait with a marker (phenotypic) was first introduced in a study on *Phaseolus vulgaris* by Sax (1923) in which he reported that the seed size was related to the seed coat color.

Methods to identify QTL include single marker analysis, interval mapping and composite interval mapping. Single marker analysis is the simplest method that utilizes common statistical procedures such as t-test, ANOVA and linear regression to determine the marker-QTL association. It is advantageous to use this method since it does not require

a complete linkage map; however, if the marker association with the phenotype is not strong, a QTL will not be detected (Liu 1998; Tanksley 1993). Interval or simple interval mapping requires a linkage map to determine the frequencies of two marker positions flanking a QTL location, thereby detecting recombination between the marker and the putative QTL (Lander and Botstein 1989). Thus, this method is more powerful than the single marker method. Since this method determines the frequency of each marker at a time, it is possible for QTL to go undetected, if multiple QTL are present between the two markers (Liu 1998; Maheswaran 1998). Composite interval mapping (CIM) is a modification of the simple interval mapping method (Jansen 1993), which is integrated with linear regression and additional genetic markers. Between each marker interval, CIM analyzes multiple points for the presence of QTL by employing additional background markers. There are two advantages to using background markers in addition to flanking markers. Firstly, in the event of non-linkage with a target interval, it provides sensitivity for the detection of QTL linked to background markers. Secondly, upon linkage with the target interval, it aids in the separation of multiple linked QTL (Zeng 1994). QTL Cartographer software (Wang et al. 2011c) has been used by many researchers to perform QTL analysis using the CIM method.

The output of simple interval and CIM is given in logarithmic of odds value (LOD) or a likelihood ratio (LR) (Collard et al. 2005; Liu 1998). A QTL position is identified on the linkage map if the LOD value exceeds a threshold value (Liu 1998). QTL detected with 1-2 LOD are interesting, 2-3 are suggestive, whereas QTL with > 3 LOD are in strong linkage with the marker (Botstein et al. 1980; Xu 2010). Other than the position of the QTL on the

map, the probability of the QTL presence is reported in LR. The LR and LOD statistics are inter-convertible with a conversion relationship of $\text{LOD} \times 4.6$ (Liu 1998).

There are a number of limitations associated with QTL mapping. QTL detection is based on statistical inference, and any inappropriate statistical operation or experimental error in genotyping and/or phenotyping may result in identification of QTL that may not have any biological significance. Accurate QTL analyses are further hampered for traits that are difficult to precisely phenotype such as crop yield (Shi et al. 2009). Moreover, quantitatively inherited traits as mentioned earlier are sensitive to the environment, which leads to the identification of different QTL at different locations in multi-location trials (Maheswaran 1998; Tanksley 1993). The QTL with minor effects on the phenotype may not be detected because of higher LOD threshold values (Collard et al. 2005). Generally larger population size is required for accuracy and identification of common QTL across different environments (Maheswaran 1998). Although QTL mapping is enriching the knowledge to dissect quantitatively inherited traits, they do not provide any immediate solution to the enhancement of traits. This is due to the fact that markers often lose association with the QTL in different genetic backgrounds, making some markers inefficient in certain selection situations (Maheswaran 1998; Reyna and Sneller 2001; Young 1999).

As mentioned earlier, the essence of QTL mapping is to use the marker information in marker-assisted selection (MAS), which can accelerate genetic gain in the process of trait enhancement. If a strong linkage between the marker and trait is achieved and MAS is deployed for selection, it can result in increased gain per unit time and a reduction in cost per data point (Bernardo 2008; Ribaut and Hoisington 1998). For example, field screening

over multiple locations against Fusarium head blight resistance in wheat is time consuming and sometimes with conflicting results (Campbell and Lipps 1998). The same information can be achieved using the *Fhb1* QTL, although initial development of the *Fhb1* marker required concentrated efforts in the validation of the marker (Bernardo 2008). Effectiveness of MAS can be further illustrated from a study involving the phenotypic screening for soybean cyst nematode (SCN) that can take 30 days at the cost of \$1.50 to \$5.00 per sample. The same can be achieved in 1-2 days with reduced cost of \$ 0.25 to \$ 1.00 per sample using MAS (Concibido et al. 1994; Concibido et al. 2004). In theory, indirect selection using MAS should reduce the influence of the environment in systems where robust, consistent marker-trait associations are identified.

Following genetic mapping, it may be possible to physically isolate a genomic region, if that region is flanked by two markers that do not segregate in a large enough population. Various physical mapping techniques, for example constructing yeast artificial libraries (YAC) or using more stable, Bacterial Artificial Chromosome (BAC) libraries can be employed (Barry 2013). Genetic markers can be used to screen BAC libraries and their location on a chromosome can be determined through Fluorescence In-situ Hybridization (FISH) technique (Barry 2013).

2.10.1 Mapping of oil content QTL

Mostly, RIL (Hobbs et al. 2004; Singh et al. 2013; Yan et al. 2009) and DH populations (Chen et al. 2010; Delourme et al. 2006; Ecker et al. 1995; Mahmood et al. 2006; Qiu et al. 2006; Smooker et al. 2011; Sun et al. 2012; Wurschum et al. 2012; Zhao et al. 2005) have been used for the identification of oil content in Brassicaceae. Utilization of such population types is linked to previously discussed advantages. Additionally, they require a

large investment in time and resources, along with large population sizes, which are essential to detect QTL with minor effects. Increased population size also requires modification in designing field trials for phenotyping (Soller et al. 1976). To address this problem in QTL mapping, Burns et al. (2003) have also utilized substitution lines for the identification QTL controlling component fatty acids and oil content in *B. napus*.

Initial studies by Ecke et al. (1995) reported three QTL for oil content in *B. napus*. Later studies, for example by Mahmood et al (2006) in *B. juncea*, Qiu et al. (2006), Yan et al. (2009), Burns et al. (2003), Delourme et al. (2006), Zhao et al. (2005) and Chen et al. (2010) in *B. napus* respectively reported 6, 7, 11, 13, 14, 18 and 27 QTL for oil content. The increased number of QTL for oil content in recent studies compared to previous studies may be attributed to increased population size, type of molecular marker used and diversity in selection of parents to generate mapping populations (Abbadì and Leckband 2011).

Following examination of different studies on QTL identification for oil content in *B. napus*, presence of oil content QTL have been reported on almost all the linkage groups (Chen et al. 2013). However, QTL mapping studies for oil content in *Arabidopsis* such as by Hobbs et al. (2004), reported presence of four oil content QTL, two QTL on the bottom and top position of LG1, and one QTL on LG2 and LG3. Zhao et al. (2005) identified oil content QTL on all LG of *B. napus* except LG4, LG5, LG6 and LG8. Qiu et al. (2006) developed a comparative linkage map of *Arabidopsis* and *B. napus* for the genetic analysis and identification of QTL controlling oil content in *B. napus*. The study proportionally utilized published SSR and RFLP markers and also used sequence tagged loci (STS) of defined *Arabidopsis* genes, which allowed the alignment of the maps. The study identified seven oil content QTL on chromosomes, N1, N3, N4, N8 and N12, N13. Aligning the maps

of the two species indicated that chromosome 4 of *A. thaliana* and *B. napus* (N1, N8 and N13) contained QTL for oil content and erucic acid which corresponded to the *FAEI* locus that encodes fatty acid elongase (β -ketoacyl-CoA synthase) for the production of long-chain fatty acids. Delourme et al. (2006) identified oil content QTL through SSR markers in two *B. napus* population and identified QTL on N1, N3, N8 and N13. Only the oil content QTL on N3 seemed common between the two populations. Detection of different genomic regions controlling oil content across different studies may be due to lack of segregation of loci in distinct genetic backgrounds.

Quantitative trait loci can be labelled as minor or major, the latter explains more than 10% phenotypic variation for the trait of influence (Collard et al. 2005). QTL mapping studies on oil content have revealed a number of minor and major QTL. Maximum phenotypic variation of 19% was explained by an individual oil content in a study by Delourme et al. (2006). Qiu et al. (2006) identified a major oil content QTL explaining 15.7% variation, whereas Chen et al. (2010) reported a major QTL that explained 30% variation in oil content. For traits controlled by polygenes the magnitude of effect of an individual gene may vary and in many instances a few major genes can explain a major proportion of variation in a quantitative trait (Tanksley 1993). QTL explaining more than 20% variation are common (Tanksley 1993), and Doebley and Stec (1991) reported a genomic region explaining more than 40% of phenotypic variance while studying the morphological differences between maize and teosinte. To detect QTL with minor effect, a saturated linkage map is needed and the marker locus must be closely associated with a QTL (Tanksley 1993; Xu 2010). In addition to mapping distance, larger population sizes

are needed for detecting minor QTL. For example, Edwards et al. (1987) was able to detect a QTL explaining 0.3% of variation with a population size of $n = 1700$.

Previous research suggests that QTL of functionally correlated traits in Brassicaceae such as floral morphology and flowering characteristics (Song et al. 1995) or oil content, seed hull and seed coat color, colocalize or tend to occupy the same genomic positions (Yan et al. 2009). Yan et al. (2009) identified QTL for oil content, seed hull content and seed coat color in a RIL population of *B. napus* using SSR, SRAP, AFLP and TRAP markers. Seed hull content was negatively correlated with seed oil content and seed coat color. On N8, six QTL were detected at the same genomic regions in different environments of which three were related to seed hull content, one for oil content and two for seed coat color. Collocation of QTL could be due to tight linkage or pleiotrophic effects of genes (or by epistasy) controlling related traits (Jagannath et al. 2011).

2.10.2 Mapping of QTL for fatty acid profile

Component fatty acids affect the quantity and quality of seed oil content (Barker et al. 2007; Sanyal and Linder 2012). Major uses of Brassica oil falls into two categories, edible and industrial (Smooker et al. 2011). Developing cultivars specific to each use requires an in depth understanding of genetic determinism of fatty acid composition (Smooker et al. 2011). Three of the major fatty acids of canola (*B. napus*) are oleic acid, linoleic acid and linolenic acid (Smooker et al. 2011). *FAD2* encodes oleate desaturase in endoplasmic reticulum and is responsible for 90% of polyunsaturated fatty acid in seeds of oil crops (Okuley et al. 1994). A high oleic acid mutant had defects in *FAD2* (Miquel and Browse 1994). Markers linked to *FAD2* and *FAD3* genes can be utilized to create variations in content of linoleic acid and linolenic acid (Hu et al. 2006).

Varying number of QTL have been identified controlling different component fatty acids in Brassicaceae in the vicinity of fatty acid elongase and fatty desaturase genes. Hobbs et al. (2004) mapped a QTL for fatty acid in *Arabidopsis* specifically, C18:2 and C18:3 to a *FAD3* locus and C18:1 to *FAD2* locus. Sanyal and Linder (2012) identified 72 QTL controlling 12 different fatty acids in *Arabidopsis*. Most of the fatty acid QTL were either related with candidate genes of seed oil biosynthesis or transcription factors influencing lipid biosynthesis (Sanyal and Linder 2012).

In *B. napus*, using SSR, AFLP and allele specific markers, Hu et al. (2006) mapped a major QTL on N5 (15 cM), and a minor QTL on N1 (25 cM) for oleic acid content. The former corresponds to the *fad2* locus while the latter is likely a duplication of the locus. Such duplicates have been reported earlier on N1 (A1), N5 (A5), C1 and C5 (Scheffler et al. 1997). In the study by Hu et al. (2006), a major locus for linolenic acid was mapped on N4 that corresponded to *fad3a* (A genome) while the other was on C4 corresponded to *fad3c*. The markers associated with *fad2* and *fad3* could be used for direct selection for the development of high oleic acid and low linolenic acid cultivars (Hu et al. 2006).

Smooker et al. (2011) evaluated three methods of QTL analysis (interval mapping, multiple QTL mapping (modified CIM) and single marker regression method) on 92 DH resynthesised *B. napus* lines that segregated for genes controlling erucic acid content. The linkage map used was derived from SSR and SNP markers. The study revealed that allelic composition of progeny and method of QTL analysis affect the detection of QTL (Smooker et al. 2011). Thirty-two QTL were identified through IM, 7 in the A genome and 25 in the C genome. Using CIM, 23 QTL were identified, of which nine were in the A genome and 14 were in C genome. Using single marker regression, the authors were able to decouple

the effect of the erucic acid genes and 34 QTL were identified (14 in the A and 20 in the C genome). Thirteen novel alleles were identified of which one corresponded to the *FAD2* locus (Smooker et al. 2011).

2.10.3 Mapping of QTL for flowering time

In *Arabidopsis*, variation in flowering time helps in adaptation to local environmental conditions (Koornneef et al. 1998). Networks and genes controlling flowering time are well studied in *Arabidopsis* (Koornneef et al. 1998), and over 80 genes have been shown to influence flowering time (Levy and Dean 1998). In *Arabidopsis*, transition to flowering is regulated by several partially overlapping pathways: photoperiod, vernalization, gibberellic acid (GA), autonomous pathway, thermal clock, and plant endogenous developmental cues (Baurle and Dean 2006; Poethig 2003; Putterill et al. 2004; Reeves and Coupland 2001). Cross talk between pathways of floral transition of *Arabidopsis* results in the upregulation of *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) genes which subsequently converges in the upregulation of floral promoter genes, *LEAFY* (*LFY*) and floral identity genes, *APETALA1* (*API*) which induce flowering (Fig. 2.5) (Putterill et al. 2004).

Two floral transition pathways in plants, photoperiod and vernalization have major roles in the diversity of crop plants (Raman et al. 2013). In the photoperiod pathway, a transcription factor, *CONSTAN* (*CO*) responds to long days through combining light quality and clock signals to upregulate *FT* and *SOC1* expression. In the vernalization pathway, *FLOWERING LOCUS C* (*FLC*) mediate cold exposure requirements by suppressing *FT* and *SOC1* (Putterill et al. 2004). *FRIGIDA* (*FRI*) is an upstream

regulator of *FLC* and respective dominant alleles of these two transcription factors (*FRI* and *FLC*) work synergistically to confer vernalization (Johanson et al. 2000; Minorsky 2002; Putterill et al. 2004). *FRIGIDA* is an ancestral form of a late flowering gene and various deletion events in the gene have resulted in the development of early flowering types in *Arabidopsis* (Johanson et al. 2000; Koornneef et al. 2004).

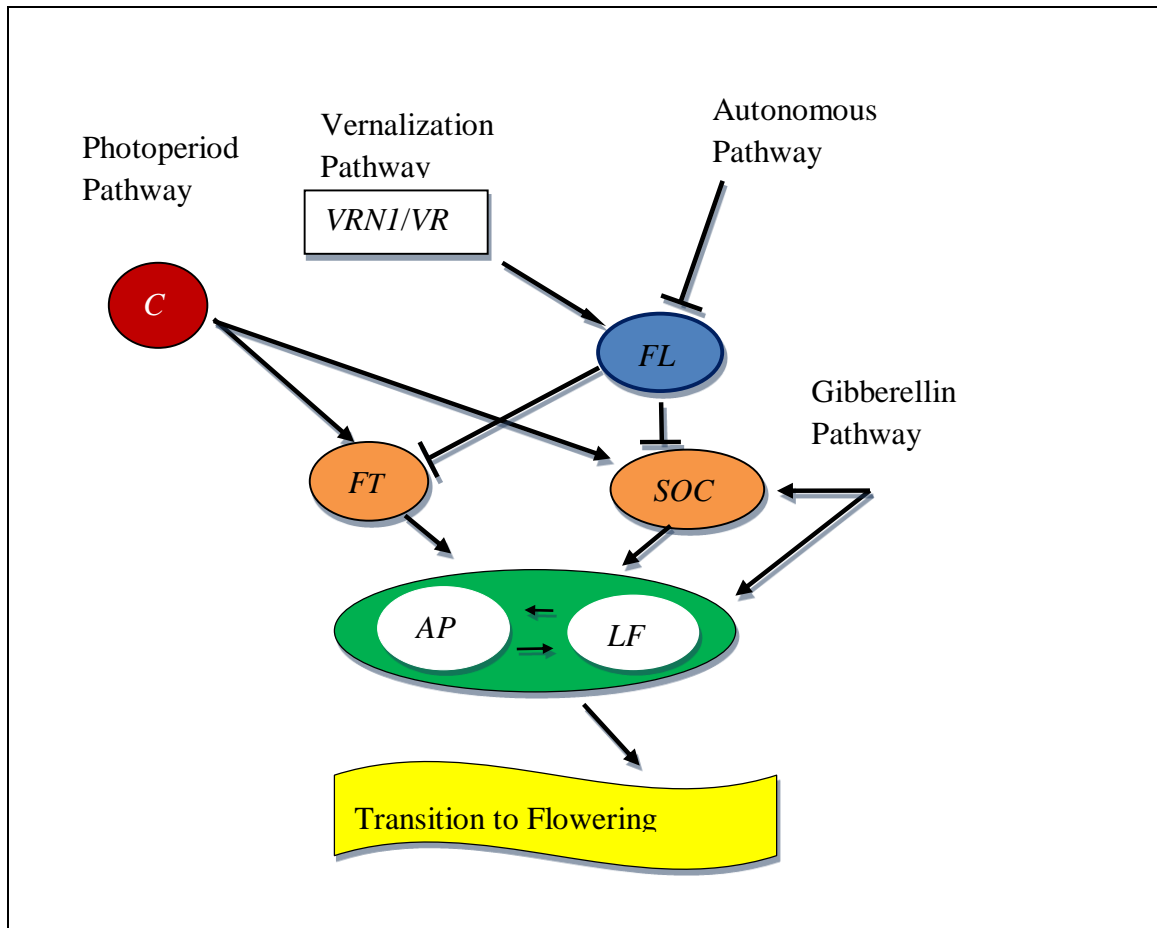


Fig. 2.5 Floral transition pathways in *Arabidopsis*, modified from (Putterill et al. 2004).

Arrow headed lines indicate upregulated gene expression; lines with bars indicate gene repression. *CONSTANS* (*CO*), *VERNALIZATION 1-2* (*VRN1/VRN2*), *FLOWERING LOCUS C* (*FLC*), *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), *APETALA1* (*API*), *LEAFY* (*LFY*).

Different loci that control variation in flowering time have been mapped in Brassicaceae (Koornneef et al. 2004). In addition to *FLC* and *FRI* loci, 25 QTL in *A. thaliana* have been reported to control flowering (Koornneef et al. 2004). *FRIGIDA* and other flowering time genes of *Arabidopsis* such as *CO*, *FLC* and *FY* have been identified on chromosome 4 and 5, respectively (Lister and Dean 1993). Since Brassica crops have genomic collinearity with *A. thaliana* (Lagercrantz 1998), many flowering time QTL in Brassica ssp. can be related to chromosome 4 and chromosome 5 of *Arabidopsis* (Irwin et al. 2012; Lagercrantz 1998; Osborn et al. 1997). Functional similarities of the *Arabidopsis* flowering locus, *FLC*, have been found with Brassica *FLC* and association of flowering time QTL with the *FLC* locus has been reported in *B. rapa* (Lou et al. 2007; Yuan et al. 2009), in Chinese cabbage (Kim et al. 2007) and in *B. napus* (Tadege et al. 2001; Zou et al. 2012).

Several researchers have identified four to 36 flowering time QTL in *B. napus* populations (Cai et al. 2008; Chen et al. 2010; Delourme et al. 2006; Long et al. 2007; Osborn et al. 1997; Shi et al. 2009; Wang et al. 2011b). Twenty-five of the 32 flowering time QTL identified by Shi et al. (2009) were syntenic to the *Arabidopsis* genome that control flowering time. Flowering time QTL are distributed on LG1, LG2, LG3, LG5, LG6, LG7, LG8, LG11, LG13, LG14, LG17, and LG18 in *B. napus* (Chen et al. 2010). Osborn et al. (1997) identified flowering time QTL in spring and winter types of *B. napus*. In spring type *B. napus*, flowering time QTL were identified on LG9 (A2), LG12 (A10) and LG16 that explained 6.4% to 46.9% phenotypic variation. In winter type *B. napus*, the QTL *FNI* was also identified on LG9, which explained 29.1% variation in flowering time. Major differences in flowering time between spring and winter types of *B. napus* and *B. rapa*

cultivars can be explained by genes controlling response to vernalization (Osborn et al. 1997).

Relationship between flowering time and seed oil content has been investigated in many studies (Chen et al. 2010; Delourme et al. 2006; Wurschum et al. 2012; Zhao et al. 2006). Chen et al. (2010) mapped QTL for oil content, flowering time and seed yield from 150 DH lines of *B. napus* using SRAP markers. On LG 7, QTL for low oil content collocated with early flowering and higher seed yield QTL. Whereas, on LG2, a high oil content QTL was located in the vicinity of an early flowering QTL on LG13, and a low seed yield QTL collocated with a later flowering QTL. These findings suggest breeding challenges for simultaneous trait enhancement. Zhao et al. (2006) used conditional mapping approaches that allowed independent analysis of oil content and flowering time QTL in a *B. napus* population and reported that these traits had no genetic relationship. Likewise, studies of Delourme et al. (2006) and (Wurschum et al. 2012) elude towards the possible relationship between oil content and flowering time, however, they concluded that the latter does not make a major contribution.

2.11 Intellectual Property Rights

Various legal regimes/statutes such as the Copyright Act, the Patent Act, the Trademark Act in the USA and elsewhere provides exclusive rights to discoveries or arts known as intellectual properties. A novel idea, an invention or innovatively processing an already established process and plant variety protection certificates represent different forms of intellectual properties and are protected by law (Besen and Raskind 1991; Erbish and Velazqueze 1998). Patents must prescribe to standards of patentability such as uniqueness, usefulness and non-obviousness (CIPO 2014; Kimpel 1999). In Canada, patent

rights enable inventors to exclude others from using, selling, manufacturing or manufacturing products depending on the patents (CIPO 2014). This allows the inventors to recover the cost of inventions and promote further investment in innovative products and processes (Deardorff 1992; Tung 2013). Patents are granted for 20 years and their validity is jurisdictional with the possibility of global extension among member countries of World Trade Organization under the agreement of Trade Related Aspects of Intellectual Property Rights (TRIPS) (Blakeney 2011; Chi-Ham et al. 2012).

Historically, public universities and research institutes have led the developments in agricultural sector. The Bayh-Dole Act, passed in 1980, encouraged the universities of the United States to patent their research and licence it to private sector for their commercialization (Bennett and Boettiger 2009). With the passage of time, through the effective management of Intellectual Property Rights, the private sector's contribution has increased in US agriculture (Kowalski et al. 2002).

International Union for the Protection of New Varieties of Plants (UPOV) provides protection to cultivars under “ Plant Breeders Rights” in 71 member countries (UPOV 2014). Canada, became signatory to UPOV in 1991 and administers IP rights through Plant Breeders' Right (PBR) Office which is a part of Canadian Food Inspection Agency (CFIA). Amendments to PBR Act through the Agricultural Growth Act- Bill C-18 (Bill C-18 2013) have been ratified by the House of Commons of Canada. The amendments, now a law, further protect the rights of the PBR owner by allowing import and export from Canada of the propagating material of the protected variety. It also allows the PBR owner to claim royalty over the harvests if it was propagated from material that was not legitimately purchased. The Bill C-18 extends the protection of the PBR by two years and keeps the

research exemptions intact and further strengthens the farmers' privileges. The latter means farmers can save, clean, treat and store seed of a protected variety under the act for their own use.

In Canada, intellectual property rights pertaining to plants can be protected through various instruments of law as described previously such as patents and plant breeder's right act. The Seeds Act, however, deals with import, sale and export of seeds from Canada. The seeds act prohibits import/export or sale of a seed that is not registered in Canada, risk to public and environment and not conforms to prescribed standards of grading, labelling and germination (CFIA 2015).

Across all member countries of UPOV, respective individuals of countries are eligible to receive similar protection of rights under the UPOV convention. In the USA, IP rights on plants are granted through plant patents, plant variety protection certificates with farmer and research exemptions and utility plant patents (Kimpel 1999). For example, a US Patent No. 5,491,296 on a corn inbred line contains claims to a corn plant, pollen, ovules, tissue cultures, regenerated corn plants, hybrid corn seed developed using this inbred as a parent, and methods for producing such hybrid corn seed (Kimpel 1999). In another example, many utility plant patents revolve around the process of development of transgenic plants containing genes conferring resistance to insects, herbicides and diseases and on the vectors and promoters used for the expression of these genes (Kimpel 1999).

Patents laws are inconsistent on research (non-commercial) use exemptions, for example, in Europe such exemptions are embedded in the law, in Australia, these are uncertain and in Canada, reasonable use is permitted (McBratney et al. 2004). However, in USA, in *Madey v. Duke University*, the research-use defense has been practically

invalidated (McBratney et al. 2004). Although, universities are rarely sued for patent infringement, commercial use of proprietary technologies is considered patent infringement across all patent jurisdictions (Chi-Ham et al. 2012).

The IP landscape in agriculture is becoming increasingly more dynamic with the availability of genome sequence information on many organisms, resulting in the incremental growth of new gene patents (Chi-Ham et al. 2012). At the inception of a new scientific endeavor, a comprehensive scrutiny of relevant IP landscape is important not only to avoid IP infringements but to create sound freedom-to-operate strategy (FTO) which otherwise could hinder research to application translation (Kryder et al. 2000). For example, the Bill and Melinda Gates foundation requires an assessment of IP landscape as a condition of support to identify IP issues which may impede the commercialization of research results (Chi-Ham et al. 2012).

2.12 Patents related to fatty acids genes

The Canadian Intellectual Property Office (CIPO) accepted a patent application (CA 2638739) to which a patent # 7981677 was granted later by the USA on November 27, 2012 to Pioneer Hi-Bred International, Inc. of the USA. This patent claims a specific composition of maize seed oil in relation to a QTL designed as QTL6. The selection of genomic regions of maize through the marker loci of QTL6 results in enhanced oil content or increased oleic acid and/or an increased oleic acid to linoleic acid ratio in maize (Allen et al. 2012). These marker loci of QTL6 represent the DGAT 1-2 protein and could be used in selection to breed maize with increased oil content.

The Canadian Intellectual Property Office, on September 12, 2006 granted patent # CA 2180386 to Cargill Incorporated (United States of America). Through this patent, it was

claimed that *B. napus* seed can endogenously contain 86% of oleic acid, 2% linolenic acid and 7% linoleic acid (DeBonte and Hitz 2003). This patent describes the production of transgenic *B. napus* plants that have seed-specific inhibition of microsomal *FAD2* and *FAD3* gene expression.

On behalf of BAYER BIOSCIENCE N.V., a patent application CA 2592335, dated January 28, 2005 was filed for a patent over a new mutation in the *FAD2* desaturase gene. This mutation was induced through ethyl methanesulfonate (EMS) mutagenesis and identified via the TILLING method. This mutation was claimed to be a deletion in the coding sequence of the *FAD2* gene, which results in a frame shift mutation. This frame shift in nucleotides resulted in a nonsense translation and affected the functionality of *FAD2* desaturase gene. Consequently the conversion of oleic acid to linoleic acid was reduced which resulted in the increase of oleic acid content in plants containing this mutation. The introgression of *FAD2* mutation into *B. napus* produced transgenic plants containing 75% oleic acid.

MONSANTO S.A.S. (France) filed a patent application CA 2634000 (January 04, 2007) which has been examined and published recently (Despeghel et al. 2012). This invention is a mutant sequence related to delta-12 fatty acid desaturase (*FAD2*). The mutant sequence is referred to as SNP1540 and was induced through EMS mutagenesis and subsequently selected. Transgenic *B. napus* plants containing SNP1540 mutation can contain up to 70% oleic acid.

VITERRA INC. (Canada) filed a patent application (CA 2471884, dated July 15, 2004) on mutant alleles of *B. juncea* designated as MJ02-086-3/BjFAD2-a. Random mutations through EMS mutagenesis or mutations of specific nucleotides were employed to create a

FAD2 mutant. MJ02-086-3/BjFAD2-a mutant contains a premature stop codon in the *FAD2* locus which renders it non-functional and the *B. juncea* plants containing this mutation may have up to 70% oleic acid content.

AGRIGENETICS, INC. (United States of America) has now renounced interest in a claim filed initially via patent application CA 2133881. Under this application, a variety of *B. napus*, designated as AG019 was patented whose seed contained 71.4% to 77.4% oleic acid and 3% linolenic acid content. The oil extracted from AGO19 seed had better oxidative stability compared with normal rapeseed oil when both oils were treated with antioxidants.

As illustrated by the above mentioned patents and also given in Table 2.2, most oil-related patents are based on creating mutations in a few major fatty acid desaturases genes of the fatty acid biosynthesis pathway of *Brassica* spp. Using appropriate technologies such as gene antisense or gene over expression, desired phenotypes have been created and selected. Alternatively, target mutations have been created in target genes. Likewise, desired mutant alleles, novel marker loci related to target genes or the elite inbred lines containing such mutations or phenotypes have been patented. This legal and patenting environment will continue to advance and become more complex as biotechnology developments continue.

QTL identification if opted can lead to development of transgenic plants containing QTL of interest with desired phenotype. Genetic transformations of plants involve a number of enabling technologies such as *Agrobacteria*-mediated transformation, selectable markers, and gene promoters. These transformation enabling technologies have limited

FTO, requiring a careful review or license to use or alternatively resources available in public domain may be perused (Chi-Ham et al. 2012).

Table 2.2 List of selected patents from the patent data base of Canadian Intellectual Property office related to fatty acids and oil content of *B. napus*

Owner of the patent	Patent Application #	Filed/Issued Year	Invention
National Research Council Of Canada (Canada)	2355845	1999/2008	Isolation, purification, characterization and use of plant's <i>DGAT</i> to produce transgenic plants for altered oil content and carbon flux into seed components.
Bayer CropScience, Belgium	2451589	2003	Conferring in <i>B. napus</i> , resistance to Blackleg disease through <i>LEM-8-SYL</i> gene, derived from <i>B. rapa</i> spp. <i>sylvestris</i>
National Research Council of Canada (Canada)	2437478	2003	This invention involves isolation and characterization of a gene, <i>PDHK</i> and its utilization in Brassica spp. to regulate seed oil content and other plant development processes.
National Research Council of Canada (Canada)	2474906	2004/2007	Isolation and characterization and use of mitochondrial pyruvate dehydrogenase kinase (PDHK) gene from Brassica spp. to increase the content of seed oil in Brassica spp.
Max Planks Institute, Germany BASF Plant Science, Germany	2627780	2006	Methods expressing Glycerol-3-phosphate dehydrogenases (G3PDH) gene from <i>Saccharomyces cerevisiae</i> to increase seed oil content in plants.
Syngenta Participations AG, Switzerland	2690561	2008	New Hybrid System for <i>Brassica napus</i> comprising "pre-basic female lines" where sterility and reversion to fertility is mediated through environmental stimulus (temperature).
Bayer CropScience, Belgium	2692687	2008	Brassica plants encode mutant protein, fatty acyl-acyl carrier protein (ACP) thioesterase B proteins (FATB) and thereby the plant seed oil contains low saturated fatty acids.
Dow Agrosciences LL, USA	2741211	2009	Creation of mutations in <i>BjFAD2</i> and <i>BjFAD3</i> to produce OMEGA-9 quality <i>Brassic juncea</i> .

3. AN INTEGRATED GENETIC MAP FOR *Brassica napus* L. DERIVED FROM DOUBLED HAPLOID AND RECOMBINANT INBRED POPULATIONS

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In this research paper, Nasir Javed contributed to the development of the populations, genotyping, constructing the genetic map, writing, editing and the revisions of this published manuscript.

3.1 Abstract

A hybrid, developed from a cross between two diverse *Brassica napus* cultivars (“Polo” and “Topas”) was used to produce a microspore-derived doubled haploid (DH) population and a single seed descent-derived recombinant inbred (RI) population for genetic mapping. Each of the two populations consisting of 190 DH lines and 94 RI lines was characterized for various types of polymorphic molecular markers (SSR, SRAP, ISSR, and SCAR). The DH population was scored for 620 molecular markers while the RI population was scored for 349 molecular markers to construct two independent genetic maps. In both genetic maps, all of the molecular markers clustered in 19 linkage groups (LGs) and covered a total genome length of 2244.1 cM and 1649.1 cM for the DH and RI maps, respectively. The data from the two genetic maps was used to construct a consensus integrated genetic map covering a total genome length of 2464.9 cM. Previously published *Brassica* reference genetic maps were used to assign each of the nineteen LGs to corresponding *B. napus* chromosomes named N01 to N19. To our knowledge, this is the first integrated genetic map based on DH and RI populations developed from the same cross in *B. napus*.

Key words: *Brassica napus*, integrated genetic map

Abbreviations: DH, doubled haploid; RI, recombinant inbred; QTL, quantitative trait loci

3.2 Introduction

Genetic maps of crop plants are now considered standard tools or even “road maps” (Paterson 1996) not only to understand genome structure and organization but also to tag economically important traits or genes. Such maps are developed by following the inheritance of detectable markers or genes in segregating populations derived from crosses of diverse parents. Rapid development in the field of molecular biology has allowed the use of molecular markers for the construction of high density genetic maps by exploiting variations (polymorphism) at the DNA level. Since the first use of restriction fragment length polymorphism (RFLP) as molecular markers followed by several other types of first generation markers, more than 30 types of 2nd and 3rd generation molecular markers are now used for the construction of genetic maps (Doveri et al. 2008). Among different types of 2nd generation molecular markers, SSRs (simple sequence repeats) are becoming the preferred markers of choice for construction of genetic maps, tagging genes and assessing genetic diversity. This is largely due to the many useful features of SSRs such as co-dominant inheritance, multi-allelic nature with high polymorphism, abundance and even distribution in genomes, the low amount of DNA required for their detection by Polymerase Chain Reaction (PCR) and their suitability for high-throughput analysis (Gupta and Varshney 2000). The SSRs are also ideal for anchoring molecular linkage maps since they are readily transferable among mapping populations (Studer et al. 2010). This unique feature has been exploited to anchor genetic maps to physical maps in many important crop plants such as *Hordeum vulgare* (Kunzel and Waugh 2002), *Gossypium hirsutum* (Xu et al. 2008), *Brassica rapa* (Li et al. 2010a) and *Cucumis melo* (Gonzalez et al. 2010). Sequence-related amplified polymorphism (SRAP), inter-simple sequence repeat (ISSR)

and sequence-characterized amplified region (SCAR) are some of the new generation markers which are getting increased usage in the construction of genetic maps due to various desirable features (Flandez-Galvez et al. 2003; Gupta et al. 2008; Hashizume et al. 2003; Li and Quiros 2001; Rubeena et al. 2003; Sun et al. 2007; Zietkiewicz et al. 1994).

Development of integrated genetic maps have been facilitated with the ability to detect different types of molecular markers and to generate multiple types of segregating populations such as doubled haploid and recombinant inbred populations. This approach is becoming popular in map construction since a large number of potentially useful markers can be mapped and validated in various genetic backgrounds. Consequently, greater genome coverage is obtained (Piquemal et al. 2005). A number of integrated genetic maps using multiple segregating populations and multiple types of molecular markers were constructed in sorghum (Mace et al. 2009), red clover (Isobe et al. 2009), pepper (Lee et al. 2009), soybean (Hwang et al. 2009), ryegrass (Studer et al. 2010), and common bean (Cordoba et al. 2010).

Brassica napus is the second most important oilseed crop in the world after soybean (Basunanda et al. 2010) and there is tremendous interest to understand the genetic structure and genome organization of this plant species including the construction of genetic maps. Many genetic maps of *Brassica* species have been published in recent years which are mainly based on a single type of population (Chen et al. 2010; Cheung et al. 1997; Ferreira et al. 1994; Foisset et al. 1996; Landry et al. 1991; Sharpe et al. 1995; Uzunova et al. 1995). A number of studies (Cheng et al. 2009; Delourme et al. 2006; Li et al. 2010a; Lombard and Delourme 2001; Piquemal et al. 2005; Sun et al. 2007; Suwabe et al. 2008; Udall et al.

2005; Xu et al. 2008) have also reported genetic maps based on multiple molecular markers and population types.

In this study we have attempted to construct genetic maps of *B. napus* using two types of mapping populations (DH and RI) segregating for various types of molecular markers. These two maps were further combined into an integrated genetic map. The genetic maps are mainly populated with previously published SSR markers (Cheng et al. 2009; Geng et al. 2007; Lowe et al. 2004; Piquemal et al. 2005; Suwabe et al. 2002). However, the map was also saturated using newly developed SSR markers designed from the information of SSR sequences in the gene bank and by using other marker types such as SRAP, ISSR, EST-SSR and SCAR. These genetic maps will be a useful addition in understanding the *B. napus* genome and tagging the economically important genes in this important oil seed crop species.

3.3 Materials and Methods

Plant Material and DNA extraction

More than two hundred DH lines were developed by microspore culture from an F₁ hybrid generated from a cross between two Canadian canola (*B. napus*) cultivars called Polo and Topas. Randomly selected 190 fertile DH lines and two parents were used for the construction of a genetic map. One hundred and thirty-six F₆ RI lines were developed through continuous self-pollination from the same cross between Polo and Topas. Randomly selected 94 RI lines and two parents were used for the construction of a second genetic map. Considering the differences in parental cultivars, populations were found to be segregating for various agronomic traits such as plant height, yield and oil content. Approximately 0.7 gram of young leaves from each greenhouse grown DH and RI lines

were collected for genomic DNA extraction using a modified CTAB method (Li and Quiros 2001; Sun et al. 2007).

Molecular Markers

Simple Sequence Repeats (SSR): The sequences of 387 public SSR primer pairs were obtained from published papers (Cheng et al. 2009; Geng et al. 2007; Lowe et al. 2004; Piquemal et al. 2005; Suwabe et al. 2002). In addition, 130 unpublished sequences for SSR primer pairs, named SR+ hereafter, were kindly provided by the Molecular Genetics Laboratory at the University of Manitoba. Moreover, new SSRs were also developed in this study. For this purpose, an online SSR identification tool called SSRIT (<http://www.gramene.org/db/markers/ssrtool>) was applied to detect the di-, tri-, tetra- SSR sequences in 4563 *Brassica* genome survey sequences downloaded from the NCBI website. The SSR primers were then designed using a Primer3Plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>) program with options to generate primers with a length of 18-22 bp, GC content of 45-55%, and the predicted PCR products ranging from 100 to 500 bp. The newly developed SSR markers were named RaAC (represents *B. rapa* AC library), OlBH (represents *B. oleracea* BH library) and NaJB (represents *B. napus* JBnB BAC). Using the same tool, EST-SSR primers were detected and designed from sequences of *Brassica* ESTs downloaded from NCBI.

Sequence-Related Amplified Polymorphism (SRAP): The protocols to develop SRAP markers and sequences for primer pairs have been described previously (Li and Quiros 2001; Sun et al. 2007). The primers were kindly provided by the Molecular Genetics Laboratory at the University of Manitoba.

Intersimple Sequence Repeats (ISSR): The ISSRs are semi-arbitrary markers (Zietkiewicz et al. 1994) and consist of PCR amplification of DNA sequences delimited by two inserted microsatellites. The PCR amplifications are performed with only one primer composed of SSR units, with or without an anchoring end. Seventy-seven ISSR primers were obtained from Nanjing Sunshine Biotechnology Co., Ltd, China while 116 ISSR primers were designed in our lab.

Sequence-Characterized Amplified Region (SCAR): The published sequences of full length *B. napus* genes which are involved in fatty acid biosynthesis were used to design gene specific primers using Primer3Plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer-3plus.cgi>). The remaining sixteen primer sequences have been reported previously (Hu et al. 2009).

Genotyping for Molecular Markers

General Protocol: The DNA from individual lines in each of the mapping populations described earlier was used as a template to amplify the regions corresponding to various types of primers as described above. In order to separate the PCR products on the ABI 3100 Genetic analyzer, a common 19-base sequence of M13 (CACGACGTTGTAAAACGAC) was added before the 5' end of forward primers. Four-color fluorescent dyed M13 primers (FAM-blue, VIC-green, NET-yellow PET-red) were synthesized by ABI company (Foster City, California). The PCR reactions were performed in 384-well plates where each well contained 10 µl of mixture containing 20 ng of template DNA, 0.05 mM forward primer, 0.15 mM reverse primer, 0.1 mM labeled-M13 primer, 0.15mM dNTPs, 2.0 mM MgCl₂, 1× PCR buffer, and 0.5 Units of Taq DNA polymerase. The PCR cycling was programmed as i) 94 °C for 5 min; ii) 5 cycles of 94 °C for 50 s, 56 °C for 50 s, 72 °C for 1min, with a 0.8

°C decrease in annealing temperature at each cycle; iii) 30 cycles of 94 °C for 50 s, 51 °C for 50 s, 72 °C for 1min; iv) an elongation step of 7 min at 72°C. Samples from four different color (FAM-blue, VIC-green, NET-yellow PET-red) labeled primers were pooled together after running PCR reactions and 2.5 µl of the pooled samples was added to a 5.5 µl mixture of formamide and 500-LIZ size standard (Applied Biosystems, Foster City, California) and denatured at 95°C for 5 minutes. The plates containing the samples were loaded into the auto sampler of the ABI 3100 Genetic analyzer equipped with 36 cm 16-channel arrays with a 40 min running time. The array profiles were analyzed with Genscan (ABI) software and specific polymorphic loci were scored with Genographer software: <http://hordeum.oscs.montana.edu/~software/genographer/>. The ABI files were converted to gel-like images and scoring of polymorphic loci by the software was confirmed visually. Each of the polymorphic loci was scored as a dominant marker only one band was scored for codominant bands based on the maternal parent. The final molecular markers were scored as a primer name followed by the size of the amplified DNA fragment or visual band on the gel image. For example, OIBH001-234 represents the primer OIBH001 which amplified a 234 base pair DNA fragment.

Markers that were reproducibly polymorphic between the parental lines were scored in the DH and RI populations. Goodness of fit to expected ratios (1:1) of segregation of the markers was estimated by chi-square test (χ^2 test) at significance $P < 0.05$. Markers that deviated from the expected ratios were also incorporated into the linkage analysis. Linkage analysis and map construction were performed by using Joinmap 3.0 software (Baud and Lepiniec 2010; Van and Voorrips 2001) to assemble 19 linkage groups. The *Kosambi* map function (Kosambi 1944) was used for converting recombination frequency into genetic

distance with LOD values of 6 to 12 and a recombination rate of 0.4. Linkage groups containing more than two common markers in each map were selected and integrated using the 'Combine the Groups for Map Integration' function.

3.4 Results

Segregation analysis of molecular markers

Segregation analysis of molecular markers showing detectable allelic variation or polymorphism between parents is an inevitable step in map construction. A total of 1013 primer pairs or primers, corresponding to various types (SSR, SRAP, ISSR and SCAR) of molecular markers were used in this study. Of these primers, 698 (68.9%) amplified successfully, and were further used to screen polymorphism between parents. A total of 716 and 533 polymorphic bands were collected to construct genetic maps for the DH and RI populations, respectively. A test of goodness of fit to 1:1 (χ^2 test) revealed that 362 (50.6%) and 174 (32.6%) molecular markers (distorted markers) violated the expected Mendelian segregation ratio of 1:1 for the DH and RI populations, respectively. Among these 362 distorted markers in the DH population, 175 (48.3%) markers biased to the maternal parent Polo, and 187 (51.7%) markers showed bias to the paternal parent Topas; however, the second χ^2 test on distorted markers produced a Mendelian segregation ratio of 1:1, which indicates that the DH population was a normal population. Whereas, among the 174 distorted markers of the RI population, 113 (64.9%) markers biased to the maternal parent Polo, and 71 (35.1%) markers biased to the paternal parent Topas, the result of second χ^2 test showed a significant violation to Mendelian segregation ratio of 1:1.

Construction of individual linkage maps

The data collected from the genotyping for all the polymorphic molecular markers were used for linkage analysis and map construction. Out of 716 molecular markers in the DH population, a total of 620 (87.0%) molecular markers, including 383 SSR, 191 SRAP, 29 ISSR and 17 SCAR markers were assigned onto 19 linkage groups (Fig. 3.1). Using common SSR markers from existing *Brassica* reference linkage maps (Cheng et al. 2009; Piquemal et al. 2005) the LGs were anchored to chromosomes named N01 to N19 (Fig. 3.1). This map covered 2244.1 cM with an average marker spacing of 3.7 cM. The length of each LG ranged from 30.3 cM to 207.3 cM for N14 and N13, respectively, the average marker spacing ranged from 2.2 to 5.5 cM for N12 and N11, respectively. The overall number of markers on each chromosome ranged from 12 to 53 for N14 and N01, respectively. Individually, SSR markers ranged from 11 to 38, SRAP markers ranged from 1 to 26, 29 ISSR markers were mapped onto 13 chromosomes, ranged from 1 to 8 and 17 SCAR markers were mapped onto 8 chromosomes ranging from 1 to 8 (Table 3.1).

For the RI population, a total of 349 (65.5%) molecular markers, including 316 SSR, 27 ISSR and 6 SCAR markers were assembled onto 19 LGs, and similar public SSR markers for the DH population were used to anchor LGs to chromosomes (Fig. 3.2). The average distance between markers and the total genome coverage were 4.7 cM and 1649.1 cM, respectively. The length of each LG ranged from 43.4 cM to 132 cM for N08 and N05, respectively, the average marker spacing ranged from 2.7 to 9.4 cM for N01 and N17, respectively. The total number of markers on each chromosome ranged from 8 to 43 for N19 and N01, respectively. The number of SSR markers across LGs ranged from 8 to 43. The ISSR markers were mapped onto 12 chromosomes, ranging from 1 to 6 while SCAR

markers were mapped onto N09, N11 and N12 with numbers of 3, 2 and 1, respectively (Table 3.2).

Construction of an integrated genetic map

Allele data sets related to the same LGs with at least two loci in common were integrated into one data set by applying Joinmap software (Van and Voorrips 2001). Common markers, ranging in numbers from 2 to 23 with an average of 9.6 per LG were detected in the same linkage groups of the DH and RI populations, which allowed the construction of an integrated genetic map. A total number of 796 markers, including 539 SSR, 193 SRAP, 45 ISSR and 19 SCAR markers were combined into an integrated genetic map. This integrated genetic map was comprised of 19 LGs and covered 2464.9 cM with a marker density of 3.1 cM (Fig. 3.3). The length of each LG ranged from 83.7 cM to 209.4 cM for N14 and N13, respectively. The number of markers on each LG ranged from 18 to 73 for N14 and N01, respectively (Table 3.3).

Comparison of individual and integrated genetic maps

Common markers among homologous LGs allowed the comparison of marker order between these individual maps and the integrated map. One hundred and ninety-four molecular markers were found in common between two populations; however, just 183 (94.3%) were arranged onto the resulting 19 integrated LGs. Of these mapped common markers, 170 (92.9%) markers were assembled onto the same LGs of the DH and RIL maps, nine located onto N01 (A genome) or N11 (C genome), N03 (A genome) or N13 (C genome), N04 (A genome) or N 14 (C genome), N07 (A genome) or N17 (C genome), N08 (A genome) or N18 (C genome) and N09 (A genome) or N19 (C genome) in the DH and RI maps, which suggests synteny between the A and C genomes, while the remaining four

markers distributed on different LGs without any relationship. The integrated map is generally in agreement with the two individual maps, and the two individual maps complement each other on the integrated map with small translocations. Regarding the length of LGs, five LGs (N02, N03, N07, N18, N19) in the integrated map are 10 cM longer than in the individual maps, whereas eleven LGs (N01, N04, N05, N06, N08, N09, N10, N11, N13, N15, N17) in the integrated map are similar to the longer ones of the individual maps, while the remaining 3 LGs (N12, N14 and N16) were over 10 cM shorter than the longer ones of the individual maps. This result revealed that most of LGs (84.2%) in the integrated map were longer or similar to the related longer LG in individual maps, which resulted in a significantly longer coverage for the integrated map.

Regarding marker order, for example on LG 01 (Fig. 3.4), most of the markers on individual maps shared the similar order; however, there were five translocations (sa7/pm52-150, odd20/bg1-377, SR027-238, SR027-305 and SR027-338) detected between the DH and integrated maps. Similarly, between the RI and integrated maps, three markers (BRAS100-199, BRMS044-429 and Na12E09-404) translocated; however, only one translocation (SR027-380) was found between the DH and RI populations.

3.5 Discussion

Generating sharable and lab-to-lab reproducible results is becoming the most important and final purpose of genetic map construction. In this study, two individual genetic maps, based on two types of populations with similar pedigree, and using different types of markers, such as SSR, SRAP, ISSR and SCAR were constructed. Further, these individual maps were combined into an integrated genetic map using DH and RI populations in *B. napus*. With the public SSR markers (Cheng et al. 2009; Piquemal et al. 2005) found

between individual maps, all the LGs were anchored to the corresponding chromosomes of *B. napus*. A total of 796 loci could be mapped onto the integrated map, whereas, only 620 and 349 markers were assembled on the DH and RI map, respectively. The integrated map covered a total genetic distance of 2464.9 cM, which is similar to the mean confidence-interval estimates of genome length estimated as 2,127–2,480 cM Lombard and Delourme (2001), and thus, seemed to indicate near-complete genome coverage. The differences of map lengths in different studies are usually attributed to scoring errors, type of markers, population size, recombination frequency, LOD values, and the software employed (Gosselin et al. 2002). Previously, Qi et al. (1996) reported that the length and observed genome coverage in barley was greater with MAPMAKER than with JOINMAP. Pradhan (2003) also observed reduction in the total genetic length although more markers were mapped in comparison to other maps in *B. juncea*. In our study, we observed that the length of the DH-based map was longer than the RI-based map due to two reasons: first, one additional type of marker (SRAP) was used and secondly, the RI-population size was relatively smaller. The additional marker filled large gaps on LGs, and improved the genome coverage. The SRAP markers target ORF of the DNA and they consequently detected more polymorphism in DH populations that resulted in improved map coverage.

Marker segregation distortion is a common phenomenon when molecular markers do not segregate in ratios as expected (Foisset et al. 1996; Kim et al. 1999; Voorrips et al. 1997), especially in maps derived from DH populations regardless of marker types (Chen et al. 2010; Ferreira et al. 1994; Landry et al. 1991; Piquemal et al. 2005). This distortion probably results from gametic or zygotic selection, or from a specific selection derived from the production of plants using in vitro microspore culture. In the study by Lombard

and Delourme (2001) the segregation bias was towards certain parents, with a corresponding region for microspore culture responsiveness being identified in these parents. This finding suggests that distortion segregation is related to the genes controlling microspore responsiveness during haploid production *inter alia*. In our study, the result of goodness fit test (χ^2 test) revealed that 362 (50.6%) and 174 (32.6%) molecular markers violated the expected Mendelian segregation ratio of 1:1 for the DH and RI populations, respectively. The results suggested that the RI population is closer to normal than the DH population. However, when a χ^2 test was used on distorted markers for the DH population, 48.3% markers biased to Polo, and 51.7% markers biased to Topas, which suggests that this DH population is not distorted but rather a standard normal population. This conclusion is further supported by the similar response to microspore culture observed in both parents (data not published). Therefore, double χ^2 tests are recommended for fitting Mendelian segregation ratio of 1:1. However, among the distorted markers of the RI population, 64.9% markers biased to Polo, and 35.1% markers biased to Topas. The result of second χ^2 test showed a significant violation to the Mendelian segregation ratio of 1:1 and this could be due to the smaller population size.

Genetic maps based on multiple populations and multiple types of molecular markers offer many advantages over a map based on a single population and one type of molecular marker (Wang et al. 2011a). Likewise, in this study, a DH and a RI population were developed from the same cross, which provided an opportunity to compare these populations as well as to construct an integrated map from them. A higher percentage of markers (85.6%) were assembled onto the main 19 LGs in the DH map than in the RI map (65.5%). It could be due to two reasons, firstly, an additional type of marker (SRAP) was

used in the DH population and secondly, the DH population size was larger than RI population. Most common markers (94.3%) were mapped onto the same LGs and found at similar positions in individual maps, which suggests that DH and RI populations are both ideal for map constructions and complement each other.

Syntenic is the preserved order of genes on chromosomes of related species that results from descent from a common ancestor (Newbury 2003; Schranz et al. 2007; Xu 2010). A chromosomal region of one species is said to be syntenic with a chromosomal region in another species if the regions carry two or more homologous genes (Dominguez et al. 2003; Lukens et al. 2003). During evolution, chromosome rearrangements result in disruptions of synteny (McCouch et al. 2002). In our study, seventeen molecular markers showed synteny between the A genome and the C genome in the DH map, eight markers in the RI map and 26 markers in the integrated map.

Compared with other crops such as rice and soybean (Hwang et al. 2009; McCouch et al. 2002), there are relatively few public SSR markers available in *Brassica*, although several research groups have presented a number of SSR markers with different technology (Cheng et al. 2009; Lowe et al. 2004; Piquemal et al. 2005; Suwabe et al. 2002; Suwabe et al. 2006; Xu et al. 2010). SSR development has been improved since expensive probe hybridization technology to today's online comparison method. In our study, a large number of SSR sequences (including (AT)_n, (CT)_n, and (GA)_n) were downloaded from a website, and a number of SSR primers were developed, tested and utilized to construct the genetic map. For the newly developed and linked SSR markers, 92 markers were assigned onto the A genome and 45 markers were mapped onto the C genome, 9 distributed both on

the A and C genome LGs. We believe that this method is accessible and efficient for SSR development and map construction.

Similar to SSR based markers, ISSRs are semi-arbitrary markers and are easy and quick to develop and use. Two kinds of ISSR primer, with or without an anchoring end were used in this study. Seventy-seven successfully amplified, 36 primers detected polymorphism between the two parents, and 29 ISSR markers were integrated onto the DH map and distributed on 13 LGs. All the mapped ISSR markers filled gaps, and 3 of the markers, ISSR185, ISSR060 and ISSR44, were mapped onto the ends of N03, N04 and N17 respectively. This consequently improved map length by up to 43.4 cM. In the RI population, 7 ISSR markers were mapped onto the ends of N04, N05, N11, N14, N15 and N16, respectively, which improved the map length by up to 81.5 cM. This result indicates that ISSR markers are desirable and suitable for map construction in combination with SSR markers.

Sequence-related amplified polymorphisms (SRAP) have proved to be a simple approach and an efficient system for the framework construction of genetic maps (Li and Quiros 2001; Sun et al. 2007). We selected 64 primer pairs for map construction, and 199 polymorphic bands were detected from these primer pairs, with 191 of them assigned onto the DH map, distributed on 19 LGs, which saturated the map greatly. This result supported that SRAP could be valuable to saturate the genetic map.

Nineteen SCAR markers related to fatty acid synthesis pathway genes were integrated onto the present maps. The current maps and previously developed genetic maps could play an important role in QTL mapping and map-based gene cloning in *B. napus*.

Acknowledgements

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Table 3.1 Main characteristics of DH-based genetic map for *Brassica napus*

LGs	Total Markers	SSR	ISSR	SRAP	SCAR	Map Length (cM)	Marker density (cM/marker)
N01	53	38	1	14	-	134.3	2.5
N02	35	20	4	9	2	105.4	3.0
N03	46	31	3	12	-	182.9	4.0
N04	37	19	3	15	-	100.7	2.7
N05	44	25	8	11	-	138.8	3.2
N06	24	16	-	8	-	83.6	3.5
N07	36	22	1	13	-	124.5	3.5
N08	31	24	2	5	-	135.9	4.4
N09	29	14	1	12	2	83.7	2.9
N10	35	20	1	13	1	116.7	3.3
N11	27	21	-	6	-	148.4	5.5
N12	26	14	-	11	1	57.9	2.2
N13	51	22	2	26	1	207.3	4.1
N14	12	11	-	1	-	30.3	2.5
N15	29	20	1	7	1	109.5	3.8
N16	29	17	1	11	-	134.2	4.6
N17	30	18	1	11	-	164.2	5.5
N18	17	11	-	5	1	63.7	3.7
N19	29	20	-	1	8	122.1	4.2
Total	620	383	29	191	17	2244.1	3.7
Average	32.6	20.2	2.2	10.1	0.9	118.1	3.7

SSR: Simple Sequence Repeat markers, ISSR: Inter Simple Sequence Repeat markers, SCAR: Simple Characterized Amplified Region, cM: Centimorgan.

Table 3.2 Main characteristics of RI-based genetic map for *Brassica napus*

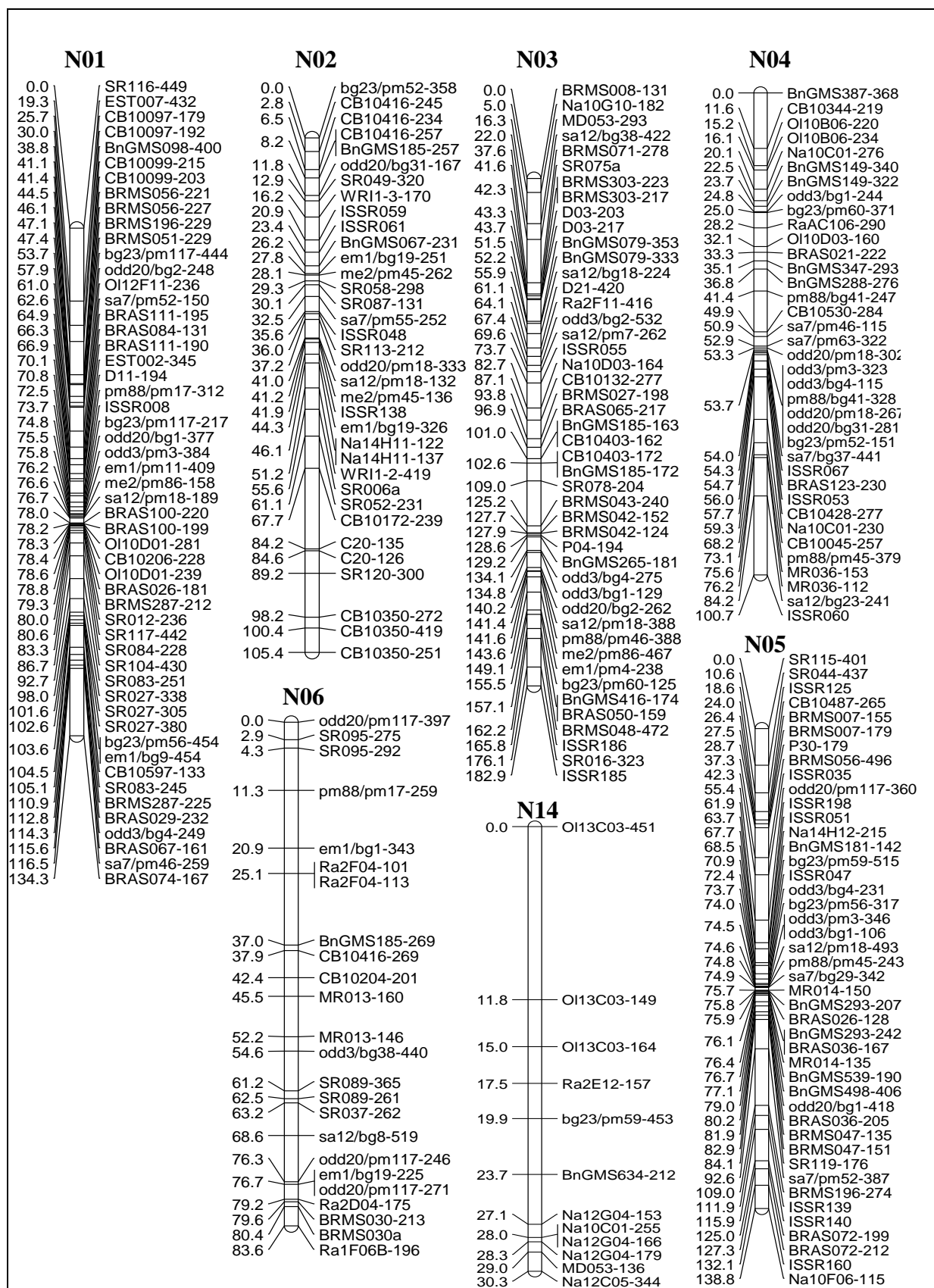
LGs	Total markers	SSR	ISSR	SCAR	Length (cM)	Marker density (cM/marker)
N01	43	43	-	-	115.4	2.7
N02	9	9	-	-	71.3	7.9
N03	19	17	2	-	103.1	5.4
N04	36	30	6	-	103.8	2.9
N05	23	17	6	-	132.0	5.7
N06	13	12	1	-	47.0	3.6
N07	20	19	1	-	95.6	4.8
N08	11	11	-	-	43.4	3.9
N09	17	13	1	3	95.1	5.6
N10	10	10	-	-	52.8	5.3
N11	21	19	2	-	108.3	5.2
N12	19	15	2	2	112.9	5.9
N13	25	22	2	1	107.8	4.3
N14	15	14	1	-	97.1	6.5
N15	17	16	1	-	53.2	3.1
N16	18	16	2	-	54.2	3.0
N17	12	12	-	-	112.6	9.4
N18	13	13	-	-	82.0	6.3
N19	8	8	-	-	62.6	7.8
Total	349	316	27	6	1650.2	4.7
Average	18.4	16.6	1.4	0.3	86.9	4.7

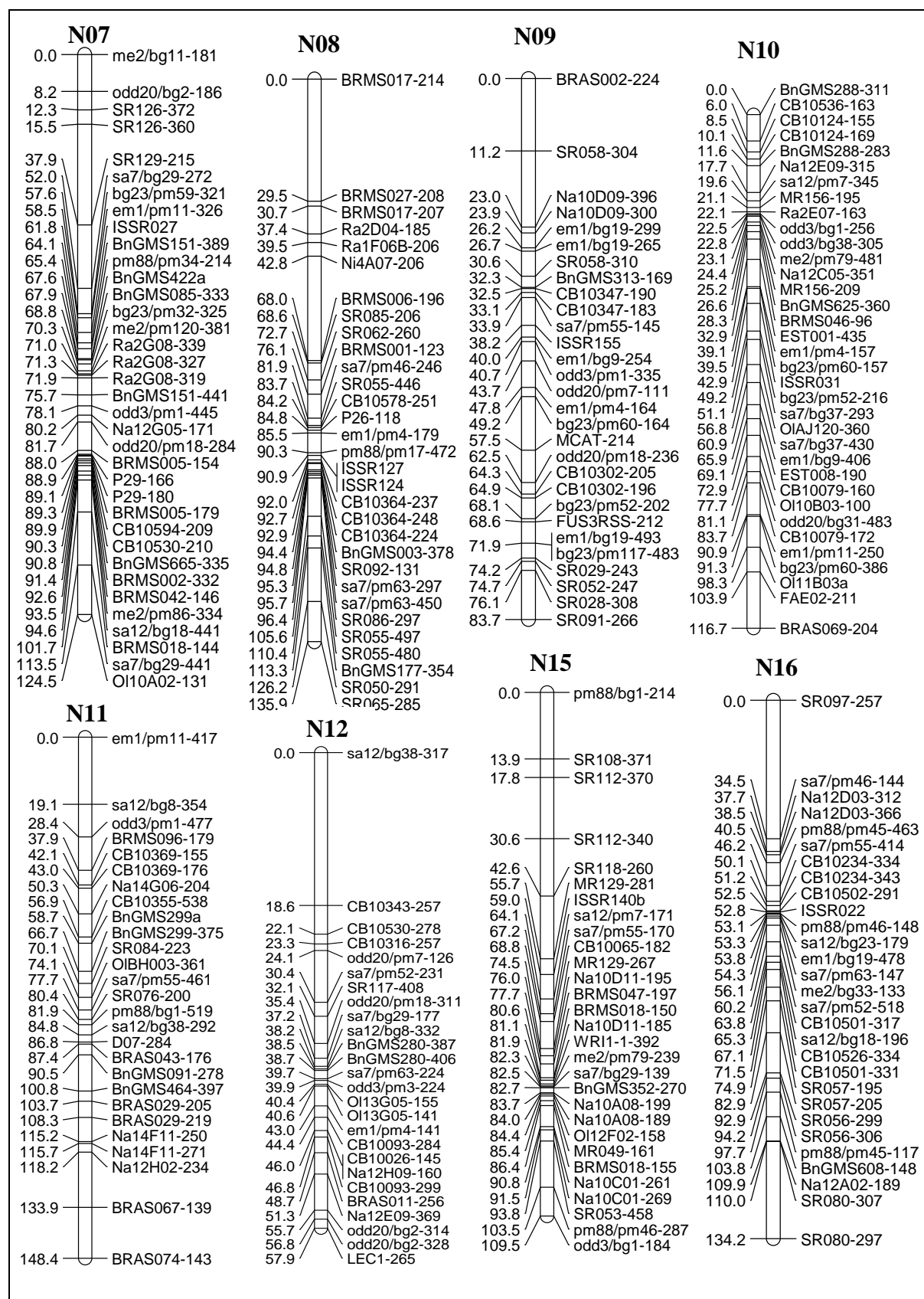
SSR: Simple Sequence Repeat markers, ISSR: Inter Simple Sequence Repeat markers, SCAR: Simple Characterized Amplified Region, cM: Centimorgan

Table 3.3 Main characteristics of integrated genetic map for *Brassica napus*

LGs	Total markers	Common markers*	SSR	ISSR	SRAP	SCAR	Length (cM)	Density (cM/marker)
N01	73	23	58	1	14	-	137.0	1.9
N02	40	4	25	4	9	2	129.1	3.2
N03	54	11	39	3	12	-	184.7	3.4
N04	55	18	34	6	15	-	104.1	1.9
N05	54	13	32	11	11	-	153.9	2.9
N06	32	5	23	1	8	-	84.0	2.6
N07	46	10	32	1	13	-	134.6	2.9
N08	45	7	36	2	7	-	130.6	2.9
N09	38	8	21	2	12	3	101.9	2.7
N10	38	7	23	1	13	1	117.4	3.1
N11	40	8	32	2	6	-	146.0	3.7
N12	35	10	20	2	11	2	94.0	2.7
N13	64	12	34	3	26	1	209.4	3.3
N14	18	9	16	1	1	-	83.7	4.7
N15	32	14	22	1	7	1	104.6	3.3
N16	37	10	24	2	11	-	108.9	2.9
N17	35	7	23	1	11	-	163.3	4.7
N18	25	5	19	-	5	1	121.9	4.9
N19	35	2	26	-	1	8	155.8	4.5
Total	796	183	539	44	193	19	2464.9	3.1
Average	41.9	18.1	54.1	4.6	19.3	4.1	129.7	3.1

Common markers represent the markers which are common to DH and RIL maps. SSR: Simple Sequence Repeat markers, ISSR: Inter Simple Sequence Repeat markers, SRAP: Sequence Related Amplified Region, SCAR: Simple Characterized Amplified Region, cM: Centimorgan.





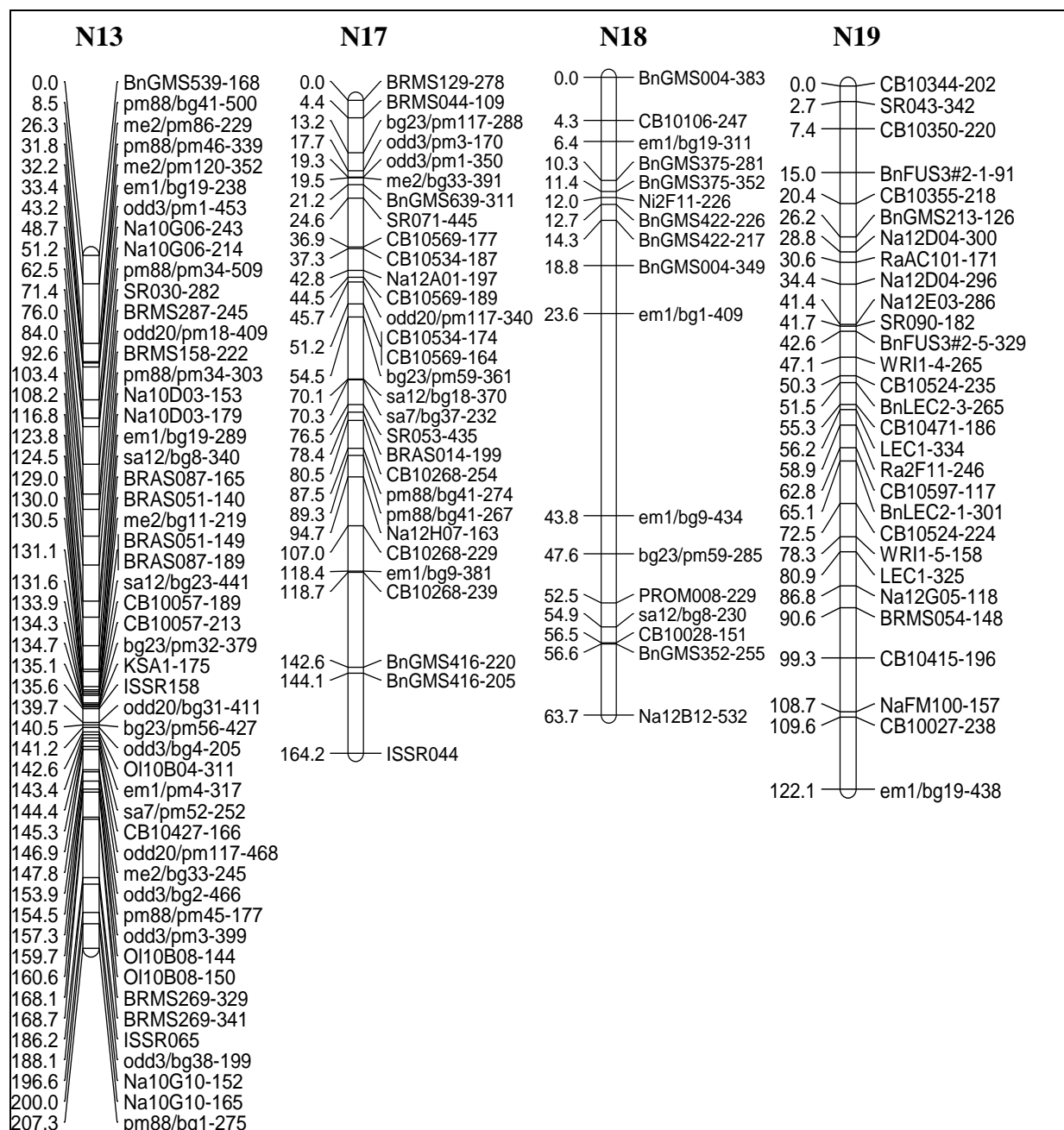
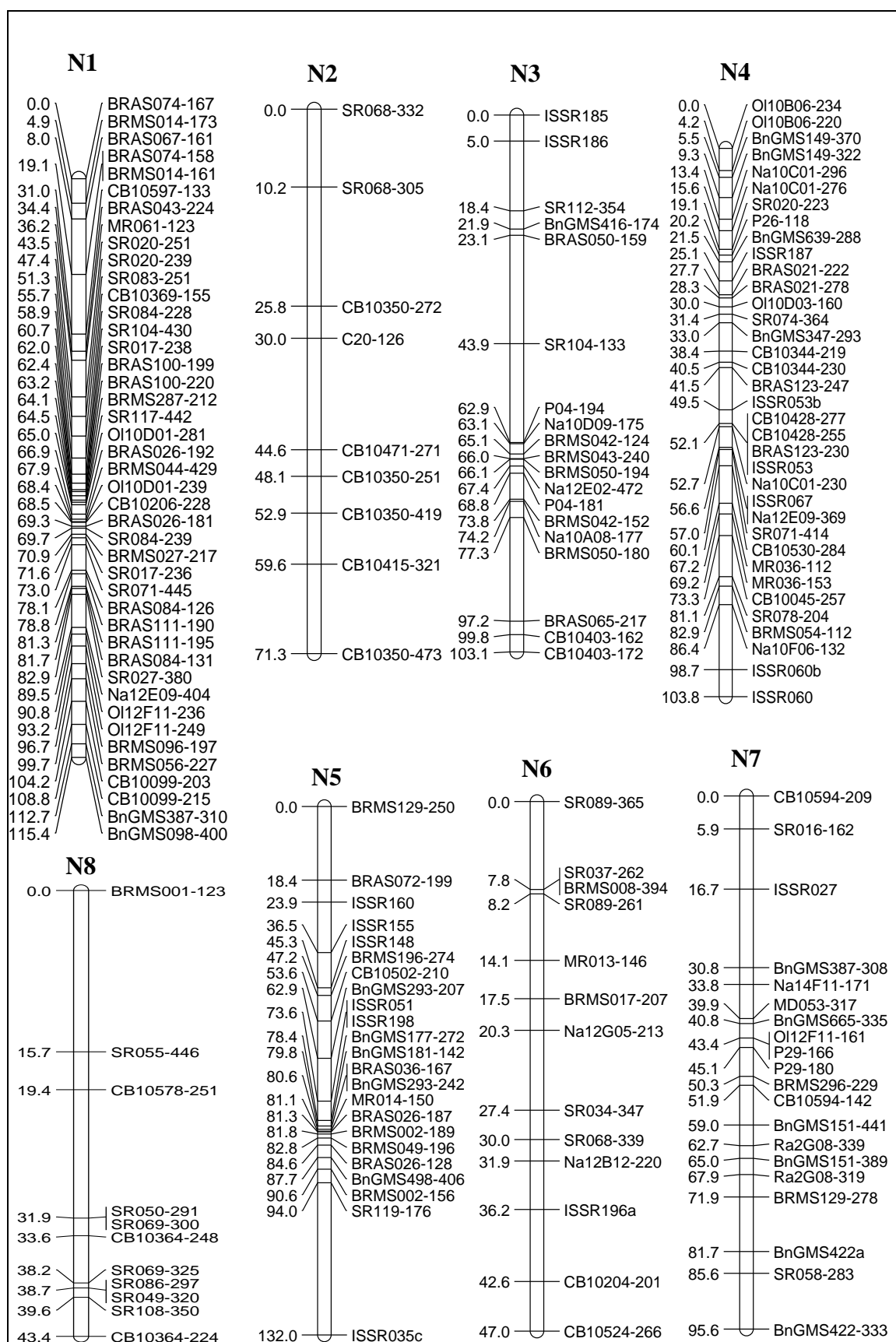
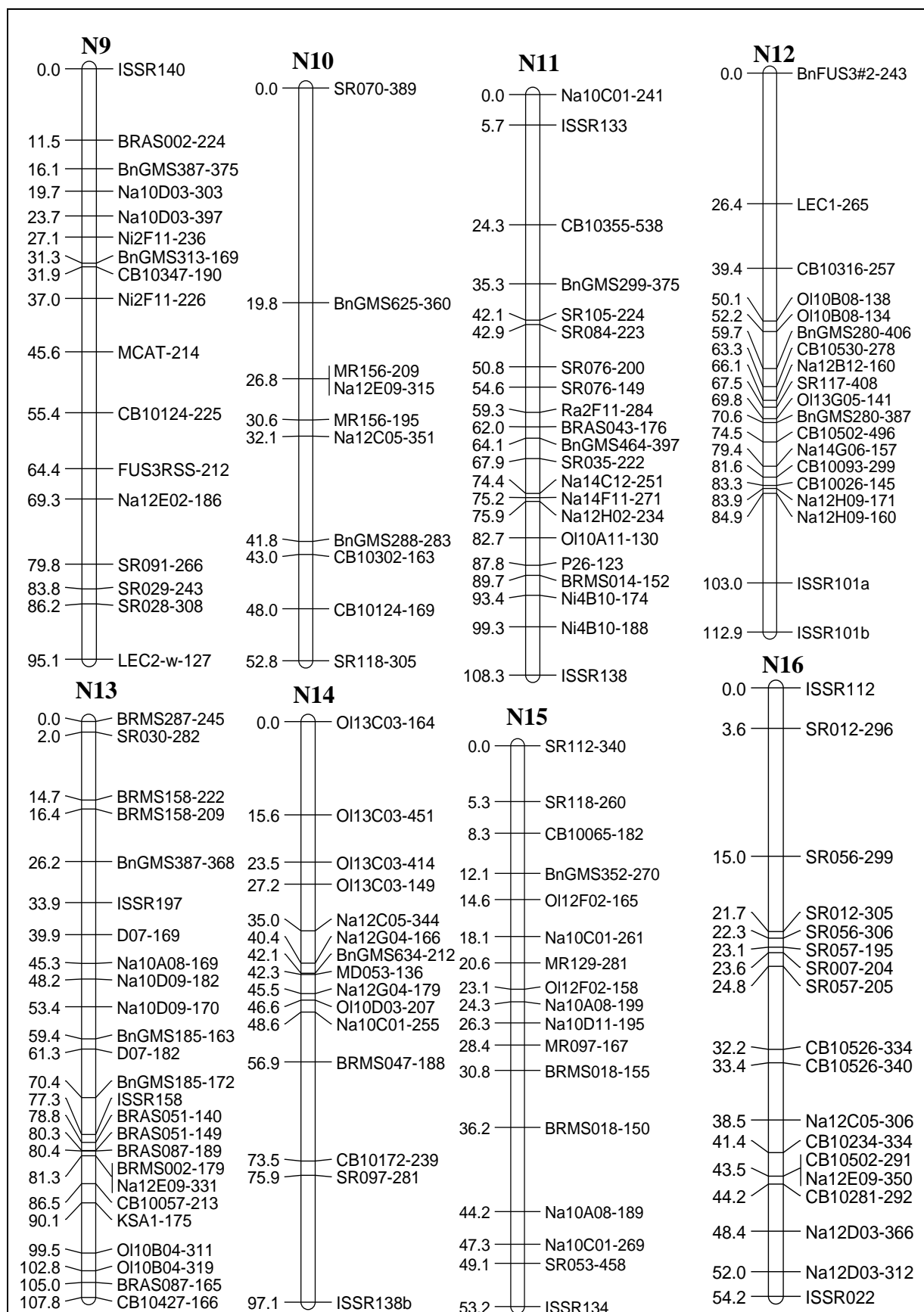


Fig. 3.1 A doubled haploid-based genetic map of *Brassica napus* population developed from the cross 'cv. Polo x cv. Topas'. The linkage groups were constructed from 190 doubled haploid lines using SSR, ISSR, SRAP and SCAR markers.





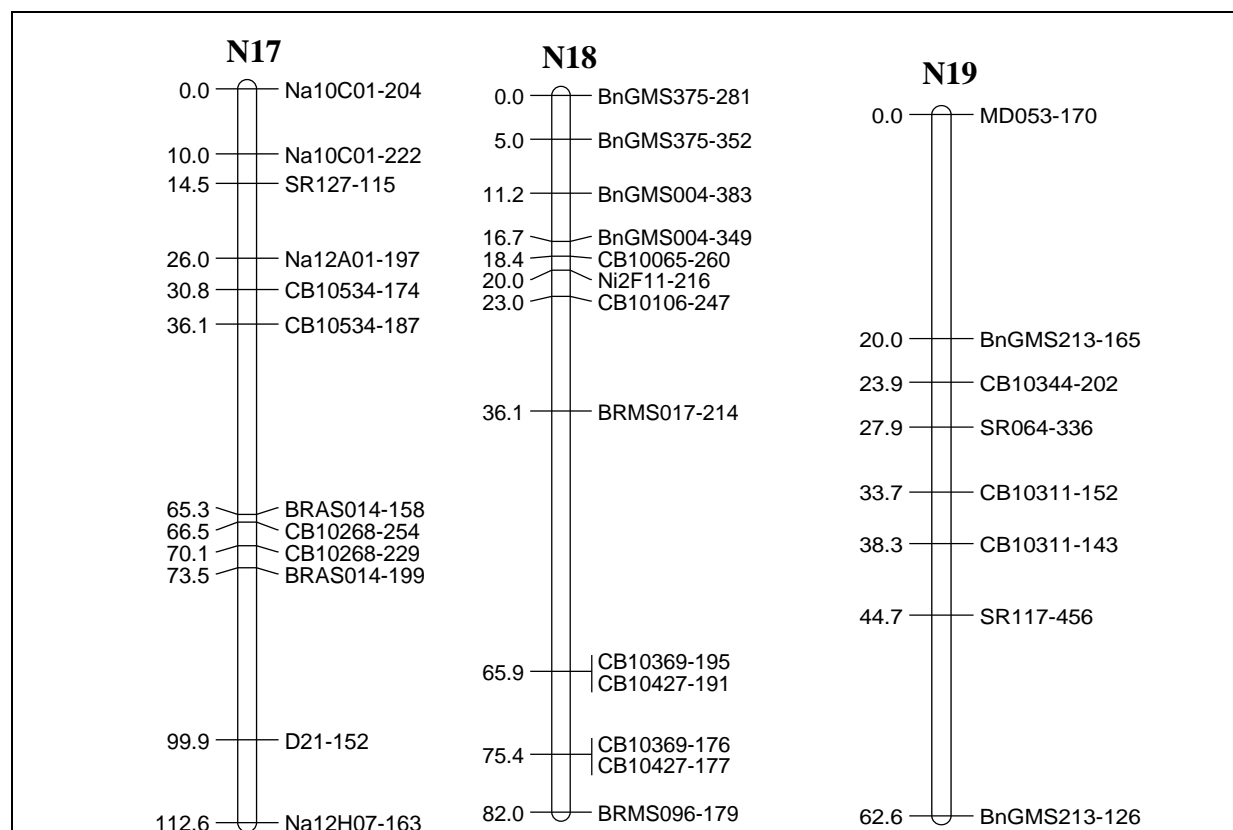
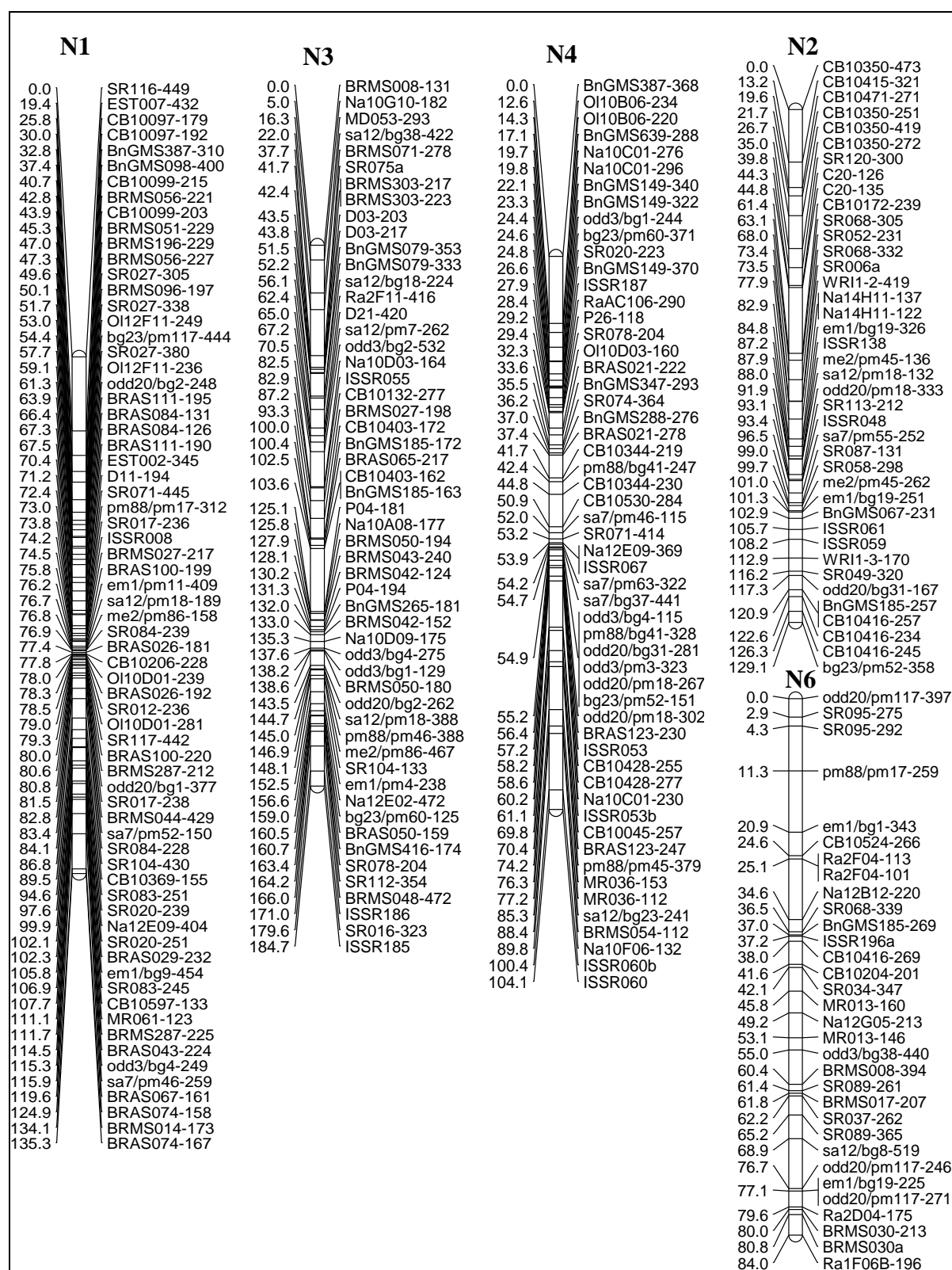
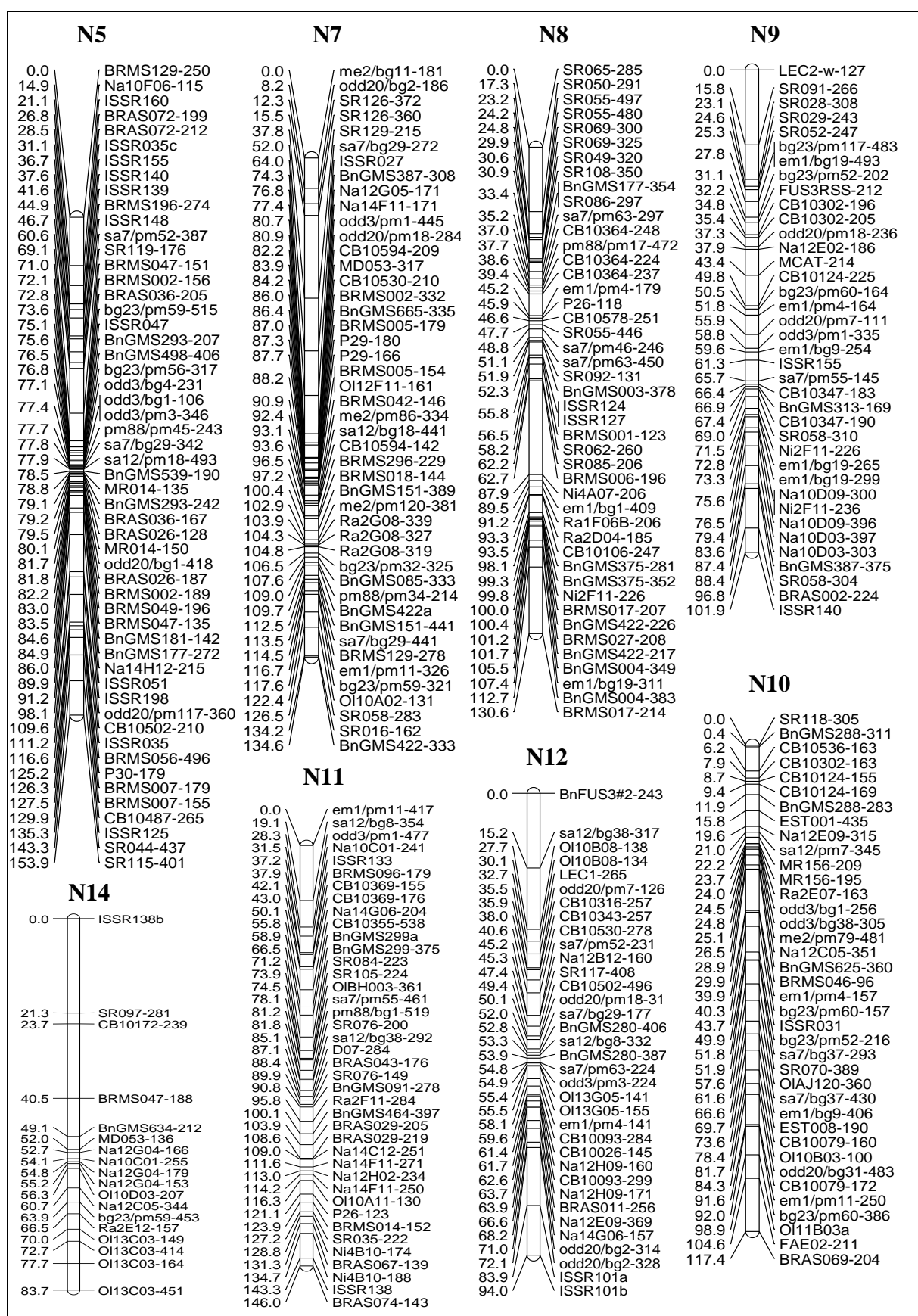


Fig. 3.2 A recombinant inbred line-based genetic map of *Brassica napus* population developed from the cross 'cv. Topas x 'cv. Polo'. The linkage groups were constructed from 94 recombinant inbred lines using SSR, ISSR, and SCAR markers.





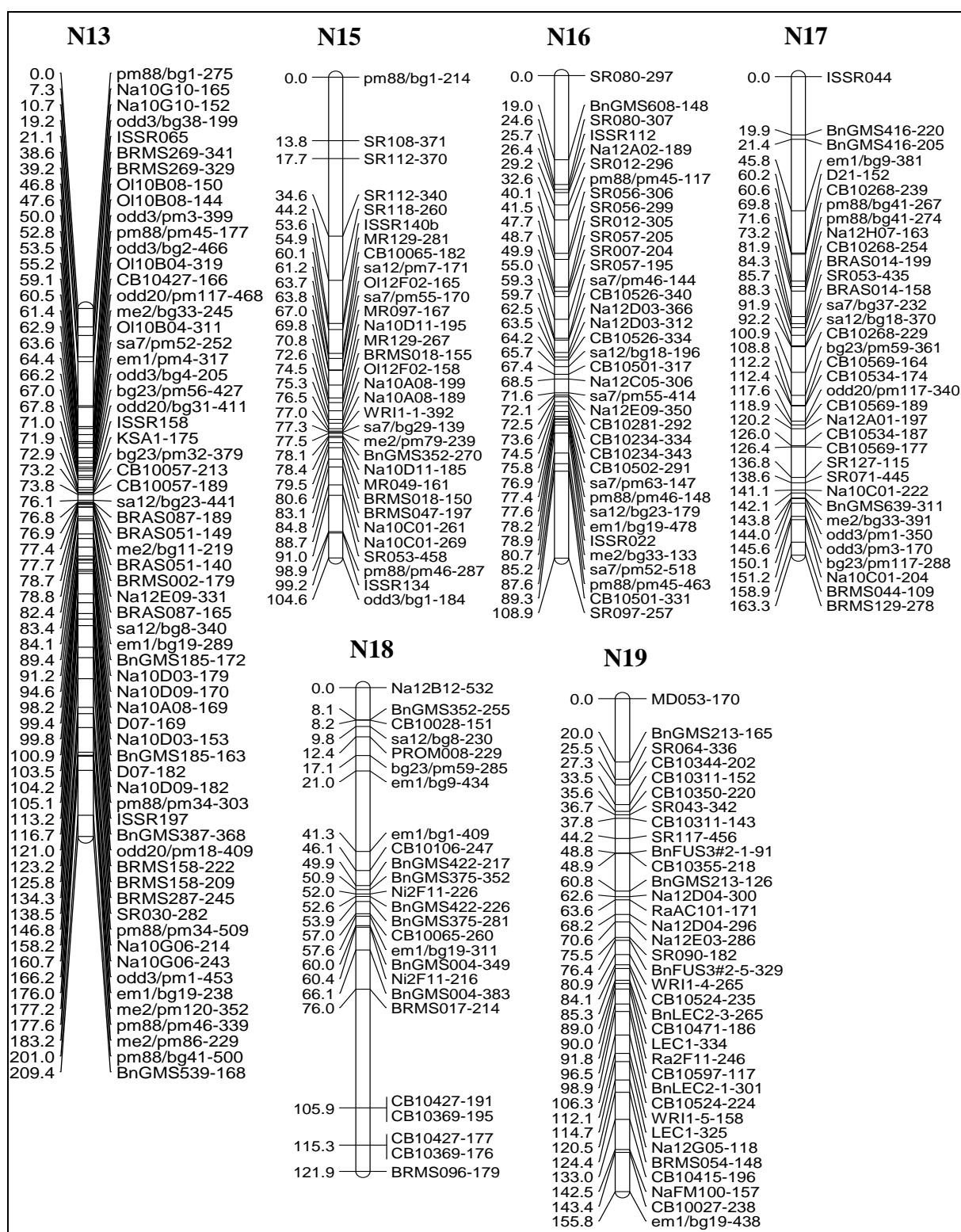


Fig. 3.3 An integrated genetic map of *Brassica napus* based on 194 doubled haploid lines population and a 94 recombinant inbred-lines population from the cross 'cv. Polo and 'cv. Topas

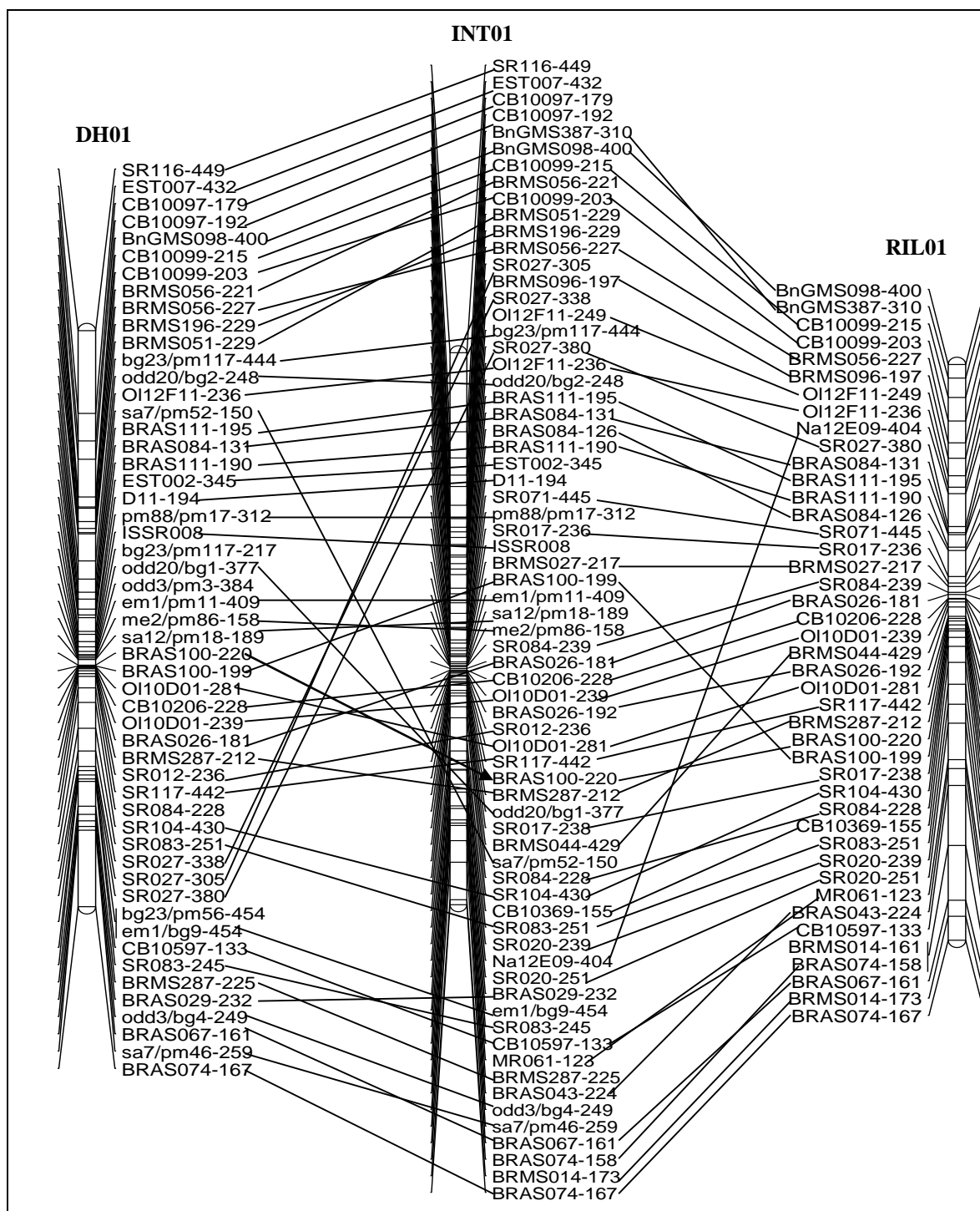


Fig. 3.4 Comparison of chromosome 1 between doubled haploid-based map, the recombinant inbred line-based map and the integrated genetic map. DH01 represents LG01 in the DH-based map, INT01 represents LG01 in the integrated map, and RIL01 represents LG01 in the RIL-based map.

4. IDENTIFICATION OF BRASSICA GENOTYPES AND MOLECULAR MARKERS FOR INCREASED SEED OIL CONTENT IN CANOLA

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4.1 Abstract

Carbon dioxide emissions by the transportation sector are major contributors to global climate change. Lower CO₂ emissions by the transportation sector are linked to the use of renewable fuels including biodiesel. Canola has high seed oil content, adaptation to temperate climates and favourable fatty acid composition which make it a preferred feedstock for biodiesel production. DH line, RI line and consensus genetics maps for mapping populations derived from Polo x Topas were developed. The DH line-based genetic map was then used for the identification and tagging of quantitative trait loci (QTL) controlling seed oil biosynthesis. This genetic map consisted of 620 loci identified using several different types of molecular markers and covered a map distance of 2241.1 cM with average marker distance of 3.7 cM. The phenotypic data on the mapping population for seed oil content and component fatty acids was collected from four-environment replicated (twice) field trials. One hundred and thirty-one QTL for various fatty acids in canola oil and 14 QTL for oil content were identified. These QTL, combined with marker-assisted selection, may assist breeders in their attempts to develop canola lines with improved oil quality, oil content and oil production per hectare for biodiesel production.

Key words: molecular markers, oil content, *B. napus*

Short title: molecular markers for oil quality and content in canola

4.2 Introduction

Among other greenhouse gases, the emission of carbon dioxide (CO₂) is a driver of climate change with the largest contributions to the Earth's energy budget (IPCC 2013). The concentration of atmospheric CO₂ has increased from 280 ppm from the Pre-Industrial era to a current concentration of 400 ppm mainly due to anthropogenic use of fossil fuels as an energy source (IPCC 2013; Rubino et al. 2013). Seventy-nine percent of the world's energy requirements are covered by fossil fuels (World Energy Council 2013). The transportation sector, exclusively fueled by fossil fuels, which currently accounts for over 50 percent of global oil consumption will increase its share in the next 25 years as the number of cars on the road doubles while the use of diesel fuel by trucks increases even faster than for cars over this time period (IEA 2012). Lower carbon emissions in the future are linked to adaptation of renewable energy sources of which biofuels are an important alternative (Soetaert and Vandamme 2009; World Energy Council 2013). Biodiesel and conventional diesel molecules have almost similar structural properties and they can be blended in any proportion for fueling diesel engines with minimal, if any, engine modifications (Shahabuddin et al. 2012). To reduce anthropogenic emission of greenhouse gases contributed by the transportation industry, to reduce dependence on fossil fuels, to revitalise rural economies and enhance energy security, National Governments in over 50 jurisdictions have mandated the production and sale of biofuels (primarily ethanol and biodiesel). These jurisdictions have mandated biodiesel in fossil diesel blends up to B20 to reduce air, water and soil pollution and greenhouse gas emissions. During the last decade, global biodiesel production has increased from 19.6×10^3 barrels per day to 294×10^3 barrels per day, paralleling the trend in consumption (United States Energy Information

Administration 2013). All Provinces in Canada have mandated B2 (a blend of 2% biodiesel with fossil diesel fuel), in ground transportation fuels currently or to come into effect on January 1, 2014. Canola is a major and preferred vegetable oil feedstock to produce biodiesel because of its high oil content, low saturated fatty acid content and low polyunsaturated fatty acid content (Anderson 2008). The Canadian B2 mandate creates an additional domestic demand of one million tonnes of canola seed annually (Canadian Canola Growers Association 2011). In order to meet the growing demand for canola oil, development of high oil content canola cultivars with improved agronomic performance remains a prime focus of many *Brassica* breeding programs globally (Abbadì and Leckband 2011; Delourme et al. 2006).

Different approaches can be employed to increase oil content and oil production per hectare in canola (Kole 2007; Weselake et al. 2008). For example, using reverse transgenic approaches, genes encoding proteins for critical processes in the fatty acid biosynthesis pathway have been manipulated to enhance oil content in canola under both controlled and field conditions (Roesler et al. 1997; Taylor et al. 2009; Vigeolas et al. 2007). Despite the varying level of success achieved using this approach and the appropriate plant biosafety provisions under the Plant Protection Act, no commercial transgenic canola cultivar with improved oil content has yet been released (Durrett et al. 2008; Somerville et al. 2000). An alternate approach is to employ conventional plant breeding techniques which have been instrumental in enhancing both oil content and seed yield in canola to date (Weselake et al. 2009). To make conventional plant breeding for increased oil content more efficient, the identification of Quantitative Trait Loci (QTL) for oil content and component fatty acids combined with the introgression of these identified QTL via marker-assisted selection into

elite germplasm is a promising approach (Chen et al. 2010; Collard et al. 2005; Delourme et al. 2006; Wurschum et al. 2012).

The effective utilization of marker assisted selection for QTL depends on the reliability of each QTL position and degree of impact each QTL has on the expression of the trait (Charmet et al. 1999; Dudley 1994). To obtain this kind of information, it is important that the effect of each QTL is verified through multi-year and multi-location validation experiments. In addition, it is also critical to develop high density genetic maps for reliably locating QTL's for selected traits and their associated markers to their specific chromosomes. In this paper, we report on the development of mostly simple sequence repeat (SSR) genetic maps based on a doubled haploid (DH) line population; on a Random Inbred (RI) line population and a consensus map as well as the use of the DH line based map to identify QTL for oil content and for component fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and arachidic acid) in *B. napus* canola. The results of these studies will assist Brassica breeders in their attempts to increase oil content and oil quality in canola. Increased oil content combined with increased seed yield in canola would enhance Canadian canola oil production to help meet increasing domestic and export market demand for canola oil for edible and biodiesel end uses.

4.3 Materials and Methods

The *B. napus* genetic mapping study utilized mainly simple sequence repeat (SSR) markers as well as sequence related amplified polymorphism (SRAP), inter-simple sequence repeats (ISSR), and sequence-characterized amplified region (SCAR) markers. SSR markers are PCR-based markers that are abundant in eukaryotic genomes and highly variable with respect to repeat numbers, moreover they are co-dominant, amenable to

automation and reproducible across labs. Three genetic maps were developed, a DH line population map based on 190 DH lines, an RI line population map based on 94 random inbred lines and a consensus genetic map derived from the combination of maps from a DH line population and from a RI line population. The DH line and RI line populations were developed by hybridizing two spring type canola cultivars that were previously registered in western and eastern Canada, the cv. Polo with approximately 48 % oil content and the cv. Topas with approximately 42 % oil content, respectively. The DH line population map was used to identify QTL in this study. Microspores were harvested from F₁ plants of the cross and DH progenies were developed using the procedure described by Coventry et al. (1988).

The DH line population was grown at two locations in 2009 and 2010 (four environments) in replicated nursery row trials. Seed from each DH line entry was harvested and evaluated for oil content by near infrared reflectance and fatty acid composition by gas chromatography. Phenotypic data on 136 DH lines for oil content and fatty acid composition of the oil results were analysed through ANOVA using the “Proc mixed” procedure of SAS version 9.1 (SAS Institute 2013). Individual fatty acid content was correlated between fatty acids and between each fatty acid and oil content using the “Proc corr” procedure of SAS.

4.4 General Findings and Relationship to Other Published Works

Genetic mapping: A detailed genetic map is essential to elucidate genome organization and to devise plant breeding strategies which efficiently develop new lines with enhanced traits. A genetic map is required to identify QTL controlling economic traits in crops which then can be used in marker-assisted selection as well as for cloning of the targeted QTL/gene. Several genetic maps have been developed for *B. napus*. Ferreira (1994) developed a genetic map consisting of 132 RFLP loci that covered 1016 cM of Brassica genome using a DH line population of *B. napus*. Uzunova et al. (1995) developed a slightly more saturated map to identify QTL controlling seed glucosinolate content using 204 RFLP loci, arranged in 19 linkage groups that covered 1441 cM in a *B. napus* DH line population. Delourme et al. (2006) constructed two genetic maps based on two DH line *B. napus* populations with distinct genetic backgrounds using SSR markers. The first map based on the “DY” population was derived from 305 loci distributed over 19 linkage groups which covered a genome distance of 2690 cM. The second map based on the “RNSL” population consisted of 259 SSR loci distributed over 19 linkage groups which covered a genomic distance of 2116 cM. Recently, Chen et al. (2010) developed a genetic map derived from a DH line *B. napus* population using 353 SRAP and 34 SSR markers which covered 1868 cM of the Brassica genome. Since the average map distance of *B. napus* is considered to be 2127 cM to 2480 cM (Lombard and Delourme 2001), the previously developed genetic maps provide fair coverage of the *B. napus* genome. However, several studies (Chen et al. 2010; Delourme et al. 2006; Wurschum et al. 2012) suggest that each genetic background harbors unique positive alleles specific to genetic backgrounds which can be used to further enhance oil content in *B. napus*.

Two independent genetic maps were previously developed at the University of Manitoba using 190 DH lines and 94 recombinant inbred (RI) lines from the cv. Polo x cv. Topas cross (Geng et al. 2012). The DH line population derived genetic map consisted of 620 polymorphic loci, with mostly SSR markers, and a few ISSR, SRAP and SCAR markers which were grouped into 19 linkage groups and then assigned to *B. napus* chromosomes using common SSR markers that were previously published. The map covered the genome distance of 2241.1 cM with an average marker density of 3.7 cM. The genetic map based on the RI line population consisted of 349 loci which included mostly SSR, ISSR and SCAR markers which were grouped into 19 linkage groups and which covered 1649.2 cM distance with an average marker density of 4.7 cM. A consensus map based on these two individual genetic maps was constructed using 2 to 23 common SSR markers. It was comprised of 796 markers, including 539 SSR, 193 SRAP, 45 ISSR and 19 SCAR markers which were found to form 19 linkage groups. The integrated map covered a genomic distance of 2464.9 cM with a marker density of 3.1 cM. The individual maps complimented each other with small only translocations noted. The consensus map was in agreement with the individual maps as well. These maps are saturated with the most SSR markers of any available genetic map in *B. napus* and they will be excellent maps to use to identify QTL for oil content and component fatty acids in canola.

Quantitative trait loci: Using a linkage mapping approach numerous QTL controlling oil content have been discovered in segregating populations of major oilseeds (Collard et al. 2005; Dudley 1994). Ecke et al. (1995) investigated the relationship between erucic acid content and oil content in a *B. napus* DH line population and identified three independent QTL controlling oil content. Two of these QTL were located close to the erucic acid genes.

These three QTL together explained 51% of the variation in oil content with additive effects. Delourme et al. (2006) identified 10 and 14 genomic regions of which two to five regions were found to be in common in two DH line populations with additive effects contributing to variation in oil content. Zhao et al. (2005a) identified 18 QTL controlling oil content with mainly additive effects and attributed the large number of QTL identified in their study to increased population size and enhanced genetic diversity since the population was developed from parents belonging to diverse European and Chinese gene pools. Chen et al. (2010), using the SRAP marker system in a DH line population, identified 27 QTL controlling oil content. Zhao et al. (2012) used a DH line population that was previously mapped with SSR markers and phenotyped it using seven additional locations to identify nine QTL which explained 57.79% variation in oil content.

Analysis of variance (ANOVA) of the phenotypic data collected over four environments indicated highly significant ($P < 0.0001$) differences among DH lines, and for the interaction of DH lines with both locations and years. Therefore, QTL identification was performed separately for each environment. A histogram of oil contents for the DH lines (Fig. 4.1) indicated continuous variation in the trait which is characteristic of quantitatively inherited traits. Transgressive segregates in the DH lines from the cv. Polo x cv. Topas cross with oil contents of up to 55% were observed. These transgressive segregate DH line oil contents are much higher than observed for either of the parents (42% to 48%); indicating the presence of substantial allelic variation in the parents.

The phenotypic correlation results for fatty acids were similar to those reported in previous studies. Oleic acid was found to be positively correlated with oil content ($r = 0.32$, $P < 0.0001$) as was stearic acid ($r = 0.25$, $P < 0.0001$). Since oleic acid comprises over 50% of

all the fatty acids in canola oil, this positive correlation was expected. The strongest negative correlations were observed between oleic acid and linoleic acid ($r = -0.77$, $P < .0001$) and between oleic acid and linolenic acid ($r = -0.71$, $P < .0001$). Oleic acid is the precursor fatty acid for linoleic and linolenic acid so a negative correlation among the fatty acids in this desaturation pathway was also expected.

The results of this study indicate that oil content is influenced by 14 QTL which individually explained circa 5 to 27% of the phenotypic variation for oil content. Chromosome N10 harbored two common QTL, which appeared consistently in three environments and which explained up to 11% and 8% of the total variation in oil content, respectively. A separate genomic region on N10 controlled oil content in all four environments studied. An independent QTL designated as QOIL-A10A, flanked by two SSR markers on N10, explained approximately 27% of the variation in oil content. Overall, 131 QTL controlled palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and arachidic acid content in all four environments. We found that the statistical correlation of fatty acids is related to the additive variances of two colocalized QTL on LG13 which consistently appeared in four environments. These QTL were associated with all fatty acids studied in all environments except for palmitic acid where they appeared only once.

In addition to the three genetic maps developed in this Green Crop Network-sponsored research and the substantial QTL information on oil content and component fatty acids generated in this study, several high oil content ($> 54\%$) lines were developed in this study. These arose as DH line transgressive segregates from the cv. Polo x cv. Topas cross. There were also two very high oil content lines ($> 55\%$) arising in the progeny of interspecific crosses of selected *B. juncea* and *B. napus* lines. These are in the patenting process

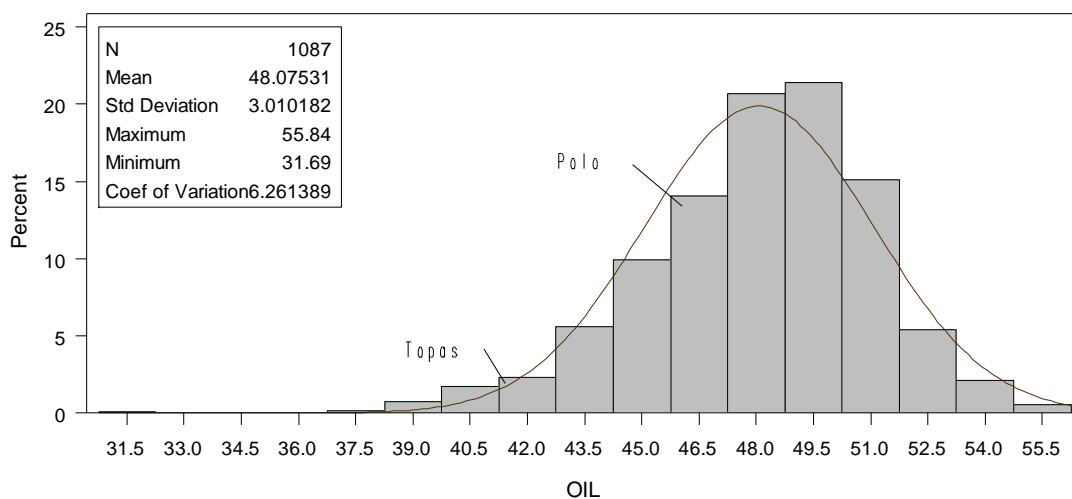
currently and will be made available to Canadian canola breeding organizations in the near future. These high oil content lines should be useful in attempts to increase the current average oil content ($< 45\%$) in canola.

4.5 Conclusions Regarding Relevance of Findings to Short- and Long- Term Greenhouse Gas Management

The genetic maps developed as part of the Green Crop Network sponsored research at the University of Manitoba (Geng et al. 2012) are saturated with the most SSR markers of any available genetic maps in *B. napus* and will be useful to identify QTL for oil content and component fatty acids in canola in the short-term. They will also provide a solid foundation for the development of genetic maps saturated with even more SSR markers in the long-term. The identification of QTL for oil content and for component fatty acids provides the basis for the development of molecular markers for oil content improvement in canola using marker assisted selection in both the short-term and the long-term. The development of several canola lines with oil contents $> 54\%$ suggests that the current oil content of canola can be significantly increased from the current value of $< 45\%$. A combination of increases in oil content and seed yield in Canadian produced canola will help satisfy the increasing market demand for canola oil for edible and biodiesel markets. Life Cycle Assessment (LCA) indicates that canola for biodiesel has a positive LCA balance (Anderson 2008; Requena et al. 2011). The area of greatest environmental impact identified by LCA is seed production (Requena et al. 2011). The use of hybrid canola cultivars grown under zero till conditions can potentially maximize seed yield and oil content while minimizing the environmental effects of canola production for biodiesel. Further, canola produced under these conditions can reduce CO₂ in the atmosphere since

the amount of carbon returned to the soil exceeds the amount of CO₂ produced by biodiesel (Anderson 2008). It therefore appears that canola-oil-based biodiesel used in the Canadian transportation sector has the potential to mitigate greenhouse gas emissions as well as provide several other benefits to the Canadian climate and economy.

Fig. 4.1 Oil content variation for cv. Polo x cv. Topas DH lines grown in four environments^a in western Canada, 2009-2010



^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

5. IDENTIFICATION OF QTL INFLUENCING SEED OIL CONTENT, FATTY ACID PROFILE AND DAYS TO FLOWERING IN *Brassica napus* L.

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In this research paper, Nasir Javed developed the mapping population, performed all the phenotyping, QTL analysis and wrote the manuscript.

5.1 Abstract

Quantitative trait loci analyses for seed oil content, fatty acid profile (palmitic acid content, stearic acid content, oleic acid content, linoleic acid content, linolenic acid content, arachidic acid content) and flowering time were performed using a composite interval mapping method in a doubled haploid (DH) *Brassica napus* population. This population was developed by hybridizing two spring type cultivars Polo and Topas. The population consisted of 156 lines and it was evaluated in the field for the above mentioned traits for two years at two locations (four environments). The available genetic map used for QTL identification was comprised of 620 marker loci, mostly SSR (383), ISSR (29), SRAP (191) and SCAR (17) marker loci. These markers were assembled into 19 linkage groups (LG), and anchored on 19 chromosomes. This linkage map covered a genetic distance of 2244.1 cM with an average marker interval of 3.7 cM.

The genotype (line) and its interaction with the location and the year of evaluation of the DH lines had a significant effect ($P < 0.001$) on all the traits except palmitic acid content. The Spearman correlation for seed oil content with all other traits was significant ($P < 0.05$) except for palmitic acid. The seed oil content in this population of DH lines ranged from 36.5% to 55.84%. Both high and low in oil content parents (Polo and Topas) contributed positive as well as negative alleles for seed oil content QTL.

Fourteen QTL for oil content, 33 QTL for palmitic acid content, 18 QTL for stearic acid content, 21 QTL for oleic acid content, 20 QTL for linoleic acid content, 23 for linolenic acid, 16 QTL for arachidic acid content and 14 QTL for flowering time were identified in this study. A10 contained four oil content QTL of which one consistently appeared at 11.64 cM in all four environments. One major oil content QTL with a 3.81

LOD value and which explained 26.99% phenotypic variation in oil content was also identified on A10.

Clustering of some QTL was observed on C3 for all six fatty acids studied. Two map positions, 147.83 cM and 154.55 cM on C3 were found to be associated with QTL for the six fatty acids in all environments studied. The QTL appearing at these two map positions carried opposite additive effects for any two fatty acids. This formed a pattern that conforms with known correlations as well as to correlations based on the phenotypic data for the six fatty acids studied in this research. We propose this genomic region on C3 modulates correlations between fatty acids and thus further investigation of this region could provide insight into genes determining total seed oil content in *B. napus*.

5.2 Introduction

Oil content and quality are the most valuable components of *Brassica napus* L (*B. napus*) seed and important criteria for future Brassica breeding endeavours (Abbadi and Leckband 2011; Gupta and Pratap 2007). Based on 1716 harvest survey samples collected from the western provinces of Canada in 2013, the top grade *B. napus* seed samples contained on average 43.5% oil (Canadian Grain Commission 2013). This reflects a slight reduction from the 10-year average of 43.8% oil content. Interestingly, increases in seed oil content using the same seed yield per hectare will result in an increase of oil yield per hectare of land (Weselake et al. 2009). Actually, one percent increase in seed oil content is equal to an increase of 2.5% in seed yield (Wang 2004).

Variable number of QTL have been identified that control oil content in *Brassica napus*. Earlier studies (Cheung et al. 1998; Ecker et al. 1995) reported that a small number of QTL control oil content. In recent studies oil content has been reported to be controlled

by 14 to 63 QTL (Chen et al. 2010; Delourme et al. 2006; Wang et al. 2013; Zhao et al. 2005). The former two studies mostly report QTL that appear in one environment only. However, Chen et al. (2010) identified four oil content QTL which were common in two out of six environments. Whereas, Delourme et al. (2006) reported five QTL in different environments while only one QTL on N3 (A3) was potentially common between two populations studied. Consistent oil content QTL appearing across environments and genetic backgrounds are important targets to develop cultivars for improved oil production across range of growing conditions.

Different strategies, such as reverse genetics or traditional breeding can be employed to increase seed oil content in *B. napus*. Reverse genetic approaches are useful as genes regulating the enzymatic pathway of lipid biosynthesis are very well characterized (Li-Beisson et al. 2010). About 600 genes are involved in acyl lipid metabolism (Beisson et al. 2003). Using lipid metabolism gene information, the critical and rate limiting steps of lipid metabolism have been investigated. Taylor et al. (2009) developed transgenic *B. napus* by over-expressing *AtDGAT1* and *BnDGAT1*, which encode proteins that catalyze the final step of triacylglycerol (TAG) synthesis in the Kennedy pathway and reported an increase in oil content of 2.5% to 7%. Similarly, studies involving genes controlling critical processes of lipid synthesis have been undertaken. Conversion of acetyl-CoA into malonyl-CoA is catalyzed by plastidial heteromeric ACCase, which is a committed step in fatty acid synthesis that provides the substrate for enlarging the carbon molecule. ACCase activity is tightly regulated; therefore, Roesler et al. (1997) expressed the cytosolic homomeric ACCase of *Arabidopsis* (*ACCI*) in *B. napus* that resulted in an oil content increase of 5% in transgenic plants. Vigee et al. (2007) attempted to increase the supply of glycerol-3-

phosphate for enhanced TAG assembly by expressing the yeast cytosolic glycerol-3-phosphate dehydrogenase (*gpd1*) in *B. napus* and reported that oil content was 40% higher in transgenic plants. Despite increased genetic and molecular understanding of TAG synthesis, its enhancement in improved oilseed cultivars remains challenging (Durrett et al. 2008; Somerville et al. 2000).

Conventional breeding has been instrumental in increasing oil content in *B. napus* (Weselake et al. 2009). The efficiency of conventional breeding can now be further enhanced through the use of molecular tools. Before the advent of molecular markers, the effect of a single locus on a quantitative trait was difficult to determine (Dudley 1994). Moreover, the selection of quantitatively inherited phenotypes for further improvement is difficult because of the hindrance posed by genotype x environment interactions. The complexity associated with quantitative traits can be dissected through QTL mapping in which a genomic region's association is related to a marker on a molecular linkage map (Grandillo et al. 2013).

The oil profile that comprise of relative abundance of constituent fatty acids determines the end use of Brassica oil (Voelker and Kinney 2001). Several studies have suggested that the interaction among fatty acids limits the extent of oil accumulation in the seed (Barker et al. 2007; Voelker and Kinney 2001; Zhao et al. 2007). Zhao et al. (2007) identified several QTL that are likely affecting the correlations of different fatty acids. For example, a QTL on N6 (A6) had a negative effect on palmitic acid and a positive effect for stearic acid. However, few such loci with pleiotropic effects on several fatty acids for the regulation of total seed oil content have been reported in *B. napus*.

Seed yield and seed oil content determine total oil yield. Seed yield is affected by many traits of which, flowering is the most critical affecting seed oil content (Diepenbrock 2000). A non-significant but negative correlation of days to flowering with seed oil content in Brassica has been reported by Engqvist and Becker (1993) and Chen et al. (2010). However, Wurschum et al. 2012 speculated that flowering time had no relationship with oil accumulation/content and proposed that metabolic efficiencies of a plant are responsible. Simultaneous improvement of early maturity and increased seed oil content remain important canola breeding objectives.

The effective utilization of previously identified QTL depends upon the reliability of the QTL position and the degree of impact the QTL has on the expression of the trait (Charmet et al. 1999; Dudley 1994). To obtain this kind of information, it is critical that the effect of the QTL is verified by multiple year and multiple location experiments. Additionally, the development of high-density genetic maps for reliably locating the QTL with associated markers is critical. The current study focuses on identifying *B. napus* QTL that control total oil content, fatty acid profile (palmitic acid [C16:0], stearic acid [C18:0], oleic acid [C18:1], linoleic acid [C18:2], linolenic acid [C18:3] and arachidic acid [C20:0]) and flowering time.

5.3 Materials and Methods

Plant Material

Two spring canola varieties, Polo (maternal parent) and Topas, registered in Canada were selected to develop mapping populations for detection of QTL influencing seed oil content, fatty acid profile and flowering time. The variety Polo was registered by Mycogen Canada, Inc. (Dow AgroSciences) in 1994 for cultivation in western Canada. The variety

Topas was developed by Svalof A.B., Sweden and was registered in 1987 for cultivation in eastern Canada. According to the registration data of these varieties, Polo matures in 93 days and contains on average 46.2% seed oil content. The variety Topas matures in 101 days and contains seed oil content of 42%. Registered cultivars contain a suite of favorable alleles that are already selected for elite performance. To further build upon such genetics and differing oil content levels of cv Polo and cv Topas, the two parents were selected to develop a mapping population.

For the production of DH lines, the F₁ from Polo and Topas were grown in a growth chamber with the growing conditions of 5°C and a 15 hour day length. Before anthesis, the anthers of varying sizes were macerated and examined for uninucleate microspores. Anthers ranging from 3 to 4 mm had the highest frequency of uninucleate microspores and were selected for microspore embryogenesis. Haploid line plantlets were produced using standard microspore culture techniques as described by Coventry et al. (1988). The chromosome number of haploid line plants was doubled by keeping the plantlets slightly submerged in 0.02 % (w/v) colchicine solution for 5 hours. Plantlets were grown in greenhouse in pots, each containing a soil, sand and peat ratio of 2:2:1 and fertilized twice until flowering at the approximate rate of 3.3 ml/L (NPK: 20:20:20). One hundred fifty-six pollen producing DH plants were selected to produce DH lines for trait evaluation under field conditions.

Field Evaluation

The DH line population (Polo x Topas) was evaluated in the field for two years (2009 and 2010) at the University of Manitoba, Winnipeg (Win) and near Portage La Prairie (PlaP), approximately 100 km west of Winnipeg, Manitoba. The mapping population at

each location along with respective parents was evaluated in a randomized complete block design with two replications in single rows. Each DH line was seeded in a single row that was 3 meters long with 0.4 meter spacing between rows. Days to flowering was recorded when 50% plants in a row had flowered. Each DH line row was harvested separately at physiological maturity, dried in bundles in the field and threshed with a small plot combine (Wintersteiger, Salt Lake City, UT). A 30g seed sample from each DH line row was provided to oil quality laboratory, Department of Plant Science at the University of Manitoba for determining the oil content and fatty acid profile. The lab determined the seed oil content at zero percent seed moisture using American Oil Chemist's Society's approved methods using a FOSS 6500 near-infrared-reflectance-spectroscopy (Daun et al. 1994). The fatty acid profile of the oil including palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and arachidic acid (C20:0) was determined using gas chromatography (Christie 1989; Hougen and Bodo 1973) using the apparatus, Varian Model 3900, California, USA. The proportion of each fatty acid was expressed as a percentage of the total fatty acids identified.

Statistical analysis of field data

The analysis of variance (ANOVA; *PROC MIXED* of Statistical Analysis System, SAS ver. 9.1; SAS Institute, Cary, NC) partitioned source of variation into effects of lines, location, year, replicates, and error ($T_{ijkq} = \mu + g_i + l_j + y_k + ly_{jk} + rep_{jkq} + gl_{ij} + gy_{ik} + gly_{ijk} + e_{ijkq}$) where T_{ijkq} is the mean trait value of the i^{th} line of the k^{th} year at j^{th} location in q^{th} replication, and μ population mean, g_i line effect, l_j location effect, Y_k year effect, ly location x year effect, rep_{jkq} effect of replication, gl line x location effect, gy line x year effect, gly

line \times location \times year, and e_{ijkq} the residual. Phenotypic correlations between all pairs of traits were determined through the CORR procedure of SAS.

Molecular markers and genetic map

The genetic map used for QTL identification was developed by Geng et al. (2012). This saturated genetic map covers a map distance of 2241.1 cM of the *B. napus* genome with average marker-to-marker interval of 3.71 cM. This linkage map consists of 620 polymorphic loci, mostly SSR (383), ISSR (29), SRAP (191) and SCAR (17) molecular markers. These polymorphic loci form 19 linkage groups, which are anchored to chromosomes through common SSR markers (Cheng et al. 2009; Piquemal et al. 2005).

QTL mapping

The QTL analyses for the traits under study were performed using Windows QTL Cartographer version 2.5 with default settings (walking speed 1 cM, Model 6: Standard model) and the composite interval mapping method (Wang et al. 2011c). For each trait data set, a threshold-likelihood ratio (LR) was determined by selecting 1,000 fold permutations test ($\alpha = 0.05$) using ZMapQtl program built within the QTL software (Chen et al. 2010; Wang et al. 2011c). A QTL was considered significant if detected above the threshold LR or LOD value (logarithm of odds) (Churchill and Doerge 1994). The LOD peak (higher than the threshold-LOD value) corresponding to the genetic interval along the LG was taken as the likely position of a QTL (Liu 1998). The confidence interval of a QTL was determined from a LOD difference from either side of the QTL peak (Hackett 2002; Lander and Botstein 1989). If present, multiple QTL peaks exceeding one-half of the LOD difference were considered separate QTL (Chen et al. 2010; Flint-Garcia et al. 2003). A QTL was considered common if it appeared in more than one environment at the same map

position or within the same QTL interval on a LG. A QTL appearing in only one environment was assumed to be an independent QTL.

QTL were designated according to Raman et al. (2012b), where the ‘*q*’ indicates a QTL identified for a trait (abbreviated), followed by the related chromosome number (hyphenated). A suffix (a, b, c etc) to a chromosome number was added if multiple QTL were identified on a chromosome. For example, *qOIL-A10a* indicate detection of more than one QTL for oil content on chromosome A10.

5.4 Results

Trait Statistics

The analysis of variance (ANOVA) for all traits under study is given in Table 5.1. Based on ANOVA, seed oil content, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and flowering time in the mapping population of *B. napus* DH lines were affected by the year and location of evaluation. This is evident from highly significant ($P < 0.001$) interaction effects (line*Year*Loc) determined for all the traits studied with the exception of palmitic acid content (Table 5.1). The effects of genotypes as fixed effect for all the traits studied were found statistically significant in the study as well. Since, the genetic effects of DH lines (genotypes) were influenced by environment (location and year), the data set from each environment was analyzed separately for the identification of QTL controlling each trait.

Histograms of means for all individual DH lines in the mapping population for all traits under study are given in Fig. 5.1 to 5.8. The histograms for all traits display continuous variation, suggesting that all of these traits are quantitative traits. Additionally,

transgressive segregation for all traits was observed. This was evident for seed oil content (Fig. 5.1) because a maximum seed oil content of 55.84% was observed in one DH line which was 9.34% higher than the average oil content of the high oil parent Polo. This significant transgressive segregation for oil content in the segregating DH line mapping population is a manifestation of the presence of substantial allelic variation for seed oil content in the parents.

Correlation between traits

The Spearman's rank correlations based on means of DH lines for the respective traits revealed correlations of variable magnitude and direction (Table 5.2). The oil content was positively correlated with oleic acid content ($r = 0.32$, $P < 0.0001$) and flowering time ($r = 0.30$, $P < 0.0001$). The correlation of stearic acid content with arachidic acid content was positive ($r = 0.85$, $P < 0.0001$). Oleic acid content was negatively correlated with linoleic acid content ($r = -0.77$, $P < 0.0001$) and linolenic acid content ($r = -0.71$, $P < 0.0001$).

QTL identification

Summarized lists of QTL detected for the field trials combined over four environments are given in Tables 5.3 to 5.10. The linkage group locations of major QTL as well as of the collocating QTL for traits across the four environments of the study are given in Fig. 5.9 and appendix i. Graphical presentation of all the identified QTL in four environments for the traits studied is given in Appendix ii to Appendix ix.

QTL identification for seed oil content

Oil content QTL identified using composite interval mapping are summarized in Table 5.3. In total, 14 QTL were found to influence seed oil content in the four environments

used in this study. The oil content QTL were scattered on five linkage groups namely, A3, A10, C1, C5, and C6. QTL *qOIL-A10c* was the only common QTL identified in all four environments. Three QTL, *qOIL-A10b*, *qOIL-C5a* and *qOIL-C5b* were found to be common in three environments. Five QTL were detected in two environments, of which two (*qOIL-A3a* and *qOIL-A3b*) appeared on A3 and C1 (*qOIL-C1b* & *qOIL-C1c*) and one QTL, *qOIL-C6b* on C6. Five QTL were identified only in one environment (independent); two were present on A10 (*qOIL-A10a* and *qOIL-A10d*), and one each on A3 (*qOIL-A3c*), C1 (*qOIL-C1a*) and C6 (*qOIL-C6a*). The proportion of phenotypic variation in oil content explained by individual QTL ranged from 5.23% to 26.99%. The additive effect of oil content QTL ranged from -2.32% to 2.38%. The QTL *qOIL-A10b* had the strongest association with seed oil content as judged by its LOD value of 4.72.

Three major oil content QTL were located on the upper part of A10 (Table 5.3). The QTL *qOIL-A10c* was identified in all four environments and explained up to 12.65% phenotypic variation for oil content. The QTL *qOIL-A10b* was found in three environments, explained up to 11.45 % variation in oil content and carried negative additive effects for seed oil content. QTL *qOIL-A10a* had the largest effect on seed oil content and explained 26.99% variation in oil content in one environment. The allele for this QTL was inherited from the low oil content parent Topas as the associated marker of this QTL was absent in the other parent. Both parents contributed positive as well as negative alleles for oil content QTL. Topas contributed negative alleles for four QTL (*qOIL-A3a* and *qOIL-A3b*, *qOIL-A10b*, *qOIL-A10d*) whereas Polo contributed negative alleles for five QTL (*qOIL-C1a*, *qOIL-C1b*, *qOIL-C1c*, *qOIL-C5a* and *qOIL-C6b*) (Table 5.3). The low oil content parent Topas contributed positive alleles for oil content for two

QTL (*qOIL-A10a* and *qOIL-C5b*) whereas, the high oil content parent Polo contributed positive alleles for three oil content QTL (*qOIL-A3c*, *qOIL-A10c* and *qOIL-C6a*).

Among the QTL controlling oil content, six collocated at map positions for fatty acid profile QTL of which four (*qOIL-A10c*, *qOIL-A10d*, *qOIL.C5a*, *qOIL.C5b*) influenced palmitic acid content (Appendix i) and one (*qOIL-A10c*) simultaneously affected oil content, palmitic acid and linolenic acid content. The fifth QTL (*qOIL-C1a*) affected only stearic acid content and arachidic acid content. The sixth oil content QTL, *qOIL-A10b* affected linolenic acid content. Only the oil content QTL, *qOIL-C5b* was found to collocate with a QTL for flowering time.

QTL identification for palmitic acid content

The composite interval mapping analyses for the identification of palmitic acid content QTL are given in the Table 5.4. The variation in palmitic acid was controlled by 33 QTL across the four environments studied. The QTL were scattered on ten linkage groups namely A3, A4, A5, A8, A10, C2, C3, C5, C8 and C9. Seven QTL, one on A8 (*qC16:0-A8a*), two on C8 (*qC16:0-C8b* and *qC16:0-C8c*) and four on A10 (*qC16:0-A10a*, *qC16:0-A10b*, *qC16:0-A10c* and *qC16:0-A10d*) were discovered to be common QTL in two environments. A majority (26 QTL) of the palmitic acid content QTL appeared in only one environment. The phenotypic variation explained by individual palmitic acid QTL across four environments ranged from 5.21% to 32.41%. The palmitic acid content QTL identified in this study carried minor additive effects and their contribution towards the variation in the palmitic acid content ranged from -0.33% to 0.12%.

Averaged over four environments, 10 major palmitic acid content QTL were identified on linkage groups, A5, A8, A10, C2, C8 and C9 (Table 5.4). The maximum phenotypic variation (32.41%) in palmitic acid content was explained by a major QTL, *qC16:0-C8b*.

Alleles for QTL having negative or positive additive effects on palmitic acid content were contributed by both parents regardless of their oil content (Table 5.4). However, among the seven QTL appearing only in one environment on C2 (Win10), four were contributed by Polo and three by Topas. The effect of Polo alleles for these seven QTL was positive whereas Topas alleles were involved both in the increase and decrease of palmitic acid content. QTL *qC16:0-C2c* inherited the allele from Topas and explained 21.73% variation in palmitic acid content. This QTL had an additive effect of 0.12% for palmitic acid content and its LOD value was 6.19, twice the threshold for QTL linkage detection used in this study.

QTL identification for stearic acid content

The results of composite interval mapping for the identification of stearic acid QTL are summarized in Table 5.5. Eighteen QTL controlling the content of stearic acid in the mapping population of *B. napus* were distributed on six LG namely A1, A3, A8, C1, C3 and C8. Three common QTL on C3 were identified in all four environments. Two common QTL were found in two environments and both were identified on A1. The majority of stearic acid content QTL (13) were detected only in one environment; two QTL on A1, three QTL on A3, one QTL on A8, three QTL on C1, two QTL on C3 and C8. The individual stearic acid QTL explained a phenotypic variance ranging from 4.68% to 22.71% across four environments of the study. Seven QTL explained more than 10% of the phenotypic variation in stearic acid. The most significant linkage of marker with a

stearic acid phenotype was determined for the QTL *qC18:0-C3e*. This QTL had a LOD value of 11.27 and explained 22.71% of the phenotypic variation for stearic acid content.

Both parents contributed positive and negative alleles for stearic acid content variation in the study. However, high oil parent (Polo) alleles were found to be associated with the majority of the stearic acid content QTL detected (Table 5.5). A maximum additive effect of 0.17% in stearic acid content was observed with the QTL *qC18:0-A1c* and this positive allele was contributed by the parent Polo. QTL *qC18:0-C3d* had the most negative additive influence on stearic acid content. The negative allele contributed to this QTL was inherited from Polo as well.

QTL identification for oleic acid content

The results of the composite interval mapping (CIM) analysis for the identification of QTL controlling oleic acid content in the mapping population are summarized in Table 5.6. Twenty-one QTL determined the content of oleic acid in the mapping population grown in four environments. These QTL were located on five linkage groups A1, A2, A3, C3 and C8. Four common oleic acid content QTL, three on C3, and one on A3 were discovered in all four environments. Two oleic acid content QTL on A2 were found common in three environments. Three oleic acid content QTL, one on A1 and two on A2 were common in two environments. Twelve oleic acid content QTL, five on A1, three on A2 and A3 and one on C8 were detected in one environment. The phenotypic variance explained by identified oleic acid content QTL ranged from 4.57% to 28.47%. QTL *qC18:1-C3c* was the most significant QTL having the highest LOD value of 16.84 and it also explained the most phenotypic variation (28.47%) for oleic acid content across all environments of the study.

Ten major oleic acid content QTL individually explained more than 10% variation in oleic acid content in at least one environment. A major QTL (*qC18:1-C3c*) explained from 14.25% to 28.47% of the phenotypic variation in oleic acid content in all environments of the study. The low oil content parent Topas contributed the allele for QTL *qC18:1-C3c*. This allele increased oleic acid content by 1.05%. Another major QTL *qC18:1-A2a* that appeared in three environments explained a major portion of variation for oleic acid content and was associated with the SCAR marker (WRI1-3-170).

Both parents contributed positive and negative alleles for QTL controlling variation in oleic acid content (Table 5.6). In most instances, the high oil content parent Polo contributed the allele for the QTL identified for oleic acid content QTL with positive additive effect. However, the low oil content parent Topas contributed a positive allele for QTL *qC18:1-C3c* with the highest additive effect among oleic acid content QTL. This QTL increased the oleic acid content by 1.05%.

QTL identification for linoleic acid content

A complete list of linoleic acid content QTL identified in the mapping population is given in Table 5.7. A total of 20 QTL for linoleic acid content were identified on seven linkage groups, A1, A2, A3, A5, C1, C3 and C9. Eight QTL were common in three environments, two on A1, three on A2 and three on C3 were identified in the study. Three common linoleic acid content QTL, two on A2 and one on A3 were identified in two environments. Nine linoleic acid content QTL, one on A1, two on A3, one on A5, C1 and C3 and three on C9 were identified in only one environment of the study. Individually, linoleic acid content QTL explained phenotypic variance in the range of 4% to 22.09%. The most significant marker linkage with linoleic acid content was determined for QTL

qC18:2-C3c. This QTL, among the linoleic acid content QTL identified across three environments, had the highest LOD value of 13.21 and explained the most phenotypic variation (22.09%) for the linoleic acid content.

Among the linoleic acid content QTL, eight had major phenotypic effects. Individually, they explained more than 10% variation for linoleic acid content in at least one environment (Table 5.7). The major QTL *qC18:2-C3c* was flanked by two SRAP markers which had a strong linkage with linoleic acid content as indicated by the respective LOD value of 13.21. This QTL also explained the most phenotypic variance (22.09%) for linoleic acid content in the mapping population.

QTL identification for linolenic acid content

The results of the composite mapping analysis for the identification of linolenic acid content QTL are summarized in Table 5.8. Across four environments of the study, the linolenic acid content in the mapping population is influenced by 23 QTL. These QTL were scattered on eight linkage groups, A1, A2, A3, A5, A9, A10, C3 and C6. Seven linolenic acid content QTL were common in all four environments of the study. Out of these common linolenic acid content QTL, four were detected on A3 and three on C3. Only one linolenic acid content QTL on A10 was common in three environments. Five linolenic acid content QTL on A2 were common in two environments. Ten linolenic acid content QTL appeared only in one environment. Two of the independent linolenic acid content QTL were on A1, one QTL each on A3, A5 and C6, two QTL on A9 and three QTL on A10. The individual linolenic acid content QTL explained phenotypic variation varying from 4.82% to 35.21%. The most significant marker linkage with linolenic acid content was

determined for QTL *qC18:3-C3c*. This QTL had a LOD value of 18.06 and explained 35.21% of the phenotypic variation for linolenic acid content (Table 5.8).

Ten linolenic acid content QTL indicated major phenotypic effects in at least one environment. Three major QTL appeared on C3 (Table 5.8). Two of these major QTL namely *qC18:3-C3a* and *qC18:3-C3c* had negative additive effects and respectively explained 26.61% and 35.21% of the phenotypic variation in the linolenic acid content, respectively. The third QTL, *qC18:3-C3b* had a positive effect on the linolenic acid content and explained a phenotypic variance of 22.90%.

Both parents contributed positive and negative alleles for the QTL controlling variation in linolenic acid content in the mapping population (Table 5.8). However, the high oil parent Polo contributed the most alleles (eleven QTL) with negative effects. The low oil parent Topas, contributed a positive allele for three linolenic acid content QTL which carried minor phenotypic effects.

QTL identification for arachidic acid content

The results of the composite mapping analysis for the identification of arachidic acid content QTL are summarized in Table 5.9. Sixteen QTL related to arachidic acid content were identified on A1, A10, C1, C3, C5, C8 and C9. Among arachidic acid content QTL, three QTL on C3 were common in all four environments of the study. Three arachidic acid content QTL were common in two environments, two on A1 and one on C1. Ten arachidic acid content QTL were independent; two were identified on A1, C3, C8, and one QTL each on A10, C1, C5 and C9. The individual arachidic acid content QTL explained a phenotypic variance between 4.63 to 26.51% across four environments. The most significant marker linkage with a phenotype was determined for the QTL *qC20:0-C3d*. This QTL had a LOD

value of 11.85 and explained a phenotypic variation of 26.51% in arachidic acid content. The positive allele for this QTL was contributed by Topas.

Consistently both parents contributed both the positive and the negative alleles for the variation in arachidic acid content (Table 5.9). However, minor additive effects for arachidic acid content QTL were observed. The range of additive effects of arachidic acid content QTL varied from -0.03 % to 0.05%.

QTL identification for flowering time

The flowering time QTL identified using composite interval mapping in the mapping population are summarized in Table 5.10. In this study 14 QTL were found that were associated with flowering time. They were scattered on five linkage groups, A1, A2, A10, C3, and C5 across all four environments studied. Three common QTL, two on A2 and one on C3 were found in three environments of the study. Another three common QTL, two on A10 and one on A1 were found in two environments of the study. Eight QTL were detected only in one environment. Of the independent flowering time QTL two were present on A1, A10 and C3 and one each on A2 and C5. Individual, flowering time QTL explained a phenotypic variance varying from 3.59% to 43.22%. The most significant marker linkage with the early flowering phenotype was determined for the QTL *qFLR-A2c*. This QTL had a LOD value of 21.69 and explained 43.22% of the phenotypic variation. Moreover, the positive allele for this QTL promoted earliness in *B. napus* and was contributed by high oil parent Polo.

A minor QTL, *qFLR-A2a* (Table 5.10) was linked with a SCAR marker. The late flowering parent Topas contributed the allele for this QTL which carried a negative additive effect of -0.34 % and delayed the flowering time.

Alleles promoting earliness were only contributed by the early maturing and high oil maternal parent (Polo), whereas alleles for the late flowering QTL had negative additive effects and were inherited from both parents (Table 5.10).

Collocation of QTL

Thirty-four genomic regions were associated with QTL for more than one trait (Tables 5.3 to 5.10, Fig. 5.9 and appendix i). These co-localized genomic regions i.e. a locus related with QTL of more than one trait were distributed on nine linkage groups; nine were on A1, five on A2 and A10, four on A3 and C3, two on C5 and C8, and one each on A5, C1, and C9. Some QTL identified for flowering time, oil content and fatty acid profile were found to collocate on the same linkage group at the same mapping positions. Six genomic regions, three on A10, one on C1 and two on C5 were found to collocate for oil content, palmitic acid content, stearic acid content, linolenic acid content, arachidic acid content and flowering time QTL. However, no collocation of oil content QTL with any of the oleic acid content QTL or linoleic acid QTL was found. The QTL for the fatty acids were frequently found to collocate with each other. Oleic acid content QTL found at 19 genomic regions collocated with several different fatty acids and flowering time (Fig. 5.9 and appendix i). Each of three genomic regions on C3 (144.4 cM, 147.8 cM and 154 cM) were co-localized with the QTL of palmitic acid content, stearic acid content, oleic acid content, linoleic acid content, linolenic acid content and arachidic acid content. Five genomic regions on A1,

A2, A10 and C5 were involved in the collocation of flowering time QTL with combinations of different fatty acids.

5.5 Discussion

Oil Content

A majority of the oil content QTL identified were detected in more than one environment (Table 5.3). The oil content QTL detected consistently in different environments where the environmental effects are significant on phenotypes, indicates the existence of genes that are insensitive to environmental conditions (Li et al. 2011). One such QTL in this study was *qOIL-A10c* that flanked by SSR markers and detected in all four environments. Li et al. (2011) studied differentially expressed QTL for oil content in two *B. napus* populations grown at higher and lower altitudes. Significant correlation existed between oil content and the environments. Based on altitude, the variation in oil content ranged from 0.00 to 18.66%. This difference in oil contents was attributed to the presence of five oil content QTL. Li et al. (2011) considered these QTL insensitive to environmental conditions. Fluctuation in environmental conditions may regulate the onset of expression of genes that are responsive to either optimal or stressful conditions (Li et al. 2011). The environmentally induced expression of genes may lead to a varying number of QTL or entirely unique QTL being identified in different studies for oil content (Chen et al. 2010; Delourme et al. 2006; Li et al. 2011; Lionneton et al. 2002; Yan et al. 2011; Zhao et al. 2005).

The alleles increasing seed oil content as identified by the positive additive effect of a QTL were inherited from both parents (Table 5.3). The low oil content parent, Topas, contributed an oil increasing allele for the QTL *qOIL-A10a* that explained 26.99% of the

variation for seed oil content. This phenotypic variation in oil content is higher than found in previous studies (Delourme et al. 2006; Sun et al. 2012; Wang et al. 2013; Yan et al. 2009). However, the magnitude of effect of a QTL across studies is dependent on the respective population size (Wang et al. 2013). In smaller populations (92 DH lines), such as used by Sun et al. (2012), the effect of a QTL may be overestimated (Wang et al. 2013).

The increased oil content (Fig. 5.1) in some DH lines observed in the current study can be attributed to inheritance of positive and negative alleles being contributed by both parents. Delourme et al. (2006) mapped seed oil content in a *B. napus* population designated as RNSL. The parents of the RNSL population contained seed oil content of 47.7% and 41.7% respectively. The alleles increasing oil content in the RNSL population were mostly inherited from the high oil parent. Consequently less transgressive segregation for seed oil content was observed for the RNSL population in comparison to our current study.

The upper region of A10 contained major QTL for seed oil content in this study. This segment of A10 is collinear with chromosome 5 of *Arabidopsis* and reported to contain flowering time genes (*FLC*, *FY* and *CO*) (Osborn et al. 1997), and erucic acid QTL (Qiu et al. 2006). Presence of genes on a chromosome controlling the same or different traits may be due to genetic linkage or pleiotropy of genes (Ramchiary et al. 2007; Tanksley et al. 1996). The upper region of A10 likely contains structurally important genes controlling different pathways (flowering and lipid synthesis) that may be interesting targets for the exploration of genes that are expressed in wide range of environments and backgrounds.

The mapping population used in this study was devoid of erucic acid content; however, the synthesis of arachidic acid content would require the activity of FAE for which one

QTL was identified in this study on A10. In rapeseed, erucic acid is a major component of oil content (Burns et al. 2003; Ecke et al. 1995). In canola cultivars, the influence of erucic acid on seed oil content has been reduced. Interestingly, the region between 4.01 cM to 17.70 cM on A10 is associated with the oil content QTL that explained the greatest phenotypic variation in the study. This region on A10 has also been reported to contain a linoleic acid QTL (Smooker et al. 2011), erucic acid and oil content QTL (Qiu et al. 2006). The study by Delourme et al. (2006) reported an oil content QTL in multiple environments on A10 that was located less than 1 cM from a SSR marker, MR156 in a *DY* designated population. The marker MR 156 in our study is located approximately 10 cM from the confidence intervals of the three oil content QTL identified on A10. Thus, A10 is an interesting target to increase seed oil content in a wide range of environmental conditions.

Identification of the oil content QTL on LG, A3, C1, C5 and C6 in the current study is consistent with earlier reports. The two oil content QTL reported by Wang et al. (2013) on A3 are likely to be similar to our study as in both studies these QTL exist close to a common SSR marker (BRAS050). This marker is positioned 25.30 cM away from A3 QTL in the study by Wang et al. (2013) and only at 7.99 cM from the A3 QTL (*qOIL-A3c*) in the current study. The novelty of the additional QTL identified in our study from the QTL reported by Wang et al. (2013) is difficult to ascertain as these QTL are not flanked by common markers. Similarly, another QTL of our study on C5, *qOIL.C5a* was located 17 cM distance from a SSR marker, Na10D11-195. Delourme et al. (2006) has also identified a QTL for oil content on C5 (LG *DY*-N15) at a distance of 23.40 cM from Na10D11.

Delourme et al. (2006) developed two genetic maps (*DY* and *RNSL*) using the same set of molecular markers. The LG-*DY*-N11 (C1) was marked by a common SSR marker

(CB 10369) that was associated a QTL, *qOIL-C1a* in the current study. No QTL however on DY-N11 (C1) linkage group was reported by Delourme et al. (2006). In contrast, RNSL-N11 (C1) lacked the integration of CB 10369 marker in the RNSL-LG N11 (C1) but upper part of C1 harbored an oil content QTL with a confidence interval overlapping with our study's QTL, *qOIL-C1a*. The RNSL population segregated for oil content which has more similarity to Polo x Topas population compared to DY designated population that harbors a dwarfism gene and winter type pedigree (Delourme et al. 2006). Additionally, we identified two QTL, *qOIL.C5b* and *qOIL.C6a*, that were found on somewhat similar locations compared with QTL reported by Sun et al. (2012) (*OW-C5*) and Zhao et al. (2012) (*OilC6*). However, no common markers were used in these multiple studies so confirmation of the similarity of these QTL is tenuous.

Palmitic acid content

The detection of seven QTL for palmitic acid in the current study on C2 may be linked to the *B. napus* response to increased environmental temperatures. These QTL were identified in one environment (Win10) when the growing conditions at the seed filling stage experienced environmental temperatures peaking around 30 °C for 12 days in the month of August, 2010 (weather data not shown). Increased temperature during seed maturation has been reported to reduce oil content in *B. napus*. Zhu et al. (2012) explained the effect of increased temperature on oil content at a molecular level. They developed two near isogenic lines (*B. napus*), NIL-9 and NIL-1 that differed for a QTL, *qOC.C2.2* and further analyzed the developing seeds of the isogenic lines for variations in transcriptomes under different growing temperatures. This QTL region was only detected at high temperatures (30°C) and enhanced the oil content. This temperature sensitive oil content

QTL, *qOC.C2.2* was flanked by SSR markers, O|13G05 and CB 10530 that were mapped in our study with multiple C16:0 QTL. The former marker was linked with a major QTL, *qC16:0-C2c* that explained 21.73% variation in the trait.

In plants, pathways of fatty acid synthesis are reported to be largely regulated by transcriptional factors (Ohlrogge and Jaworski 1997). The transcriptional factor *FUSCA3* (*FUS3*) works downstream of *LEC1* and *LEC2* and are all major transcriptional factors (TFs) influencing oil content. *FUS3* redundantly controls the expression of genes related to seed storage proteins (Baud et al. 2002). We have found a *FUS3* SCAR marker (BnFUS3#2-1-91) that is linked to a QTL (*qC16:0-C9a*) with negative additive effects on palmitic acid content and explains 12.43% of the phenotypic variance (Table 5.4). The negative effect on the content of palmitic acid by the QTL, *qC16:0-C9a* suggests that it may be regulating the synthesis long chain fatty acids.

Stearic acid content

Wang et al. (2013) combined QTL information from eight populations of different genetic background or gene pools to perform meta QTL analysis to identify consensus oil content QTL in *B. napus*. Seven consensus QTL on the consensus LG, A1 were projected in a map distance of 9.20 cM between common SSR markers, BRAS111 and BRAS026/CB10206. The QTL peaks of three stearic acid content QTL on A1 (*qC18:0-A1a* to *qC18:0-A1c*) in our study spanned a region of 13.25 cM and were located between the previously mentioned common SSR markers. Therefore, the three stearic acid content QTL on A1 in our study represent the consensus oil content QTL projected on A1 by Wang et al. (2013).

In the current study, the stearic acid content QTL on A3 were detected on different map positions than the oil content QTL, suggesting these QTL may have involvement either in the desaturation or elongation pathways. The QTL peaks of all the three stearic acid content QTL on A3 of our study fall within the QTL span of oleic acid and linoleic acid content identified by Smooker et al. (2011) on N3 (A3). Both studies detected these QTL detected in close proximity of a common marker, Na10D03-164. These stearic acid content QTL are most likely involved in substrate supply for the elongation and desaturation activities.

In our study, the stearic acid content QTL on C1, *qC18:0-C1a* peaked at the same position as an oil content QTL (*qOIL-C1a*). Both of these QTL are in coupling phase, one reducing oil content and the other increasing stearic acid. This pleiotropy has been reported previously for an erucic acid content (*BnA8.FAE1*) and oil content QTL (Cao et al. 2010).

Based on QTL mapping results of the previous studies, C3 consistently harbors oil content QTL across different gene pools of *B. napus* (Wang et al. 2013; Zhao et al. 2012). Examining the five stearic acid content QTL on C3 in the current study, two QTL, *qC18:0-C3b* and *qC18:0-C3c* were flanked by common SSR markers (CB 10057 and CB10427). The genomic region between these markers reported to contain QTL intervals of two consensus oil content QTL (*DY-qOC-3* and *GS/12-qOC-3*) on C3 (Wang et al. 2013). In our study, *qC18:0-C3b* showed environmental sensitivity as it was detected only in one environment and was linked to a SCAR marker (KSAI-175). Whereas, the latter QTL, in agreement with Wang et al. (2013) was detected in all four environments of our study and also explained major phenotypic variation for stearic acid.

Oleic acid content

Mono unsaturated fatty acid (C18:1) is produced through the activity of the *Fatty Acid Biosynthesis 2 (FAB2)* (Kachroo et al. 2007) that encodes a stearyl-ACP desaturase (SAD). Using locus specific markers, Smooker et al. (2011) mapped a *FAB2* QTL on A3; 10 cM away from a SSR marker (Na10D03-155). In our study, *qC18:1-A3d* and *qC18:0-A3c* were identified 15.30 cM away from the same marker (Na10D03-164). The collocation of the QTL, *qC18:1-A3d* and *qC18:0-A3c* also indicate that they are involved in the desaturation pathway.

With respect to the current information available on the location of *FAD2* genes in *B. napus*, we identified six oleic acid content QTL on A1 in this study. The location of QTL *qC18:1-A1d* in this study is in close proximity to a *FAD2* locus that was mapped by Scheffler et al. (1997) with a RFLP marker, pO12e. The QTL *qC18:1-A1d* is not mapped with a common marker but since RFLP and SSR markers have a strong correlation in estimating the genetic distance, it is possible that *qC18:1-A1d* represents a *FAD2* locus. Similarly, Hu et al. (2006) using independent markers (SSR and RFLP) identified two oleic acid content QTL, one with a larger LOD (3.5) and the other with a lower LOD value (2.5) on the top and bottom of LG N1 (A1). Identification of stronger oleic acid content QTL (*qC18:1-A1c* & *qC18:1-A1f*) with larger LOD value is in agreement with Wang et al. (2013) who reports that A1 could contain oil content QTL in most genetic backgrounds of *B. napus*. No oleic acid content QTL on A5, C1 or C5 were detected in the current study which is in contradiction to other known locations of *FAD2* genes. This could be due to lack of segregation for these *FAD2* loci in our mapping population (Zhao et al. 2008).

The current study has mapped on A2; seven oleic acid QTL and five of these explained major phenotypic variation and were flanked by SSR markers. The QTL, *qC18:1A2a* was linked with a transcription factor marker (WRI1-3-170) and explained 14% variation in oleic acid content. A marker Na14H11 has been mapped (Smooker et al. 2011; Yan et al. 2011) in proximity of a QTL for fatty acid profile on C2. Whereas, this marker was mapped on A2 in close proximity of *qC18:1-A2f* QTL in our study. This might be due to the fact that most mapped genetic loci of A genome are known to have homeologous loci in C genome.

Burns et al. (2003) reported that fatty acid composition QTL can affect total seed oil content in *B. napus*. The interplay of mechanisms controlling fatty acid composition affects the rate of fatty acid biosynthesis (Barker et al. 2007) and ultimately oil content. Genetically correlated traits (such as fatty acid composition and total fatty acid content) exhibit QTL co-localization on certain linkage groups (Lionneton et al. 2004). This could be due to genetic linkage and pleiotropic effects of genes controlling correlated traits (Ramchiary et al. 2007). In our study, the oleic acid QTL, *qC18:1A2a* influence multiple traits (C18:2, C18:3 and flowering time; Fig 5.9). A similar effect is expected from the oleic acid QTL appearing on A3, C3 and C8 that may be influencing multiple fatty acids due to collocation.

Linoleic acid content

Seed coat color and oil content are correlated traits and efficient selection for variation in seed coat color is an important objective in the breeding of *B. napus*. The LG A5 in the current study contained a QTL for linoleic acid content and linolenic acid content that was linked with the SSR marker BRAS072-199. This marker was also mapped 9.10 cM away from the QTL, *qC16:0-A5d* where it positively affected the content of palmitic acid and

decreased both contents of linoleic acid and linolenic acid in our study. The QTL, *qC18:2-A5* and *qC18:3-A5* are in repulsion which suggest an interaction that might be affecting another phenotype. This assumption is supported with the association of the marker, BRAS072 that has been reported to be linked to a QTL of seed color variation, *qSCBB05A-7-2* (Fu et al. 2007).

The most likely candidate of a *FAD2* locus in our study is located on two map positions, 104.51 cM and 112.78 cM on A1. The opposite additive effects of oleic acid and linoleic acid content QTL appearing on these two map positions indicate an interaction. Addition of a double bond in a fatty acid requires electron donations that are provided by cytochrome b5 (*Cb5*) protein that is located in endoplasmic reticulum. *Brassica napus* has 6-8 gene copies of *Cb5* of which some are reported on A1 (Scheffler et al. 1997). The *FAD2* locus in our study has a similar map position as reported by Scheffler et al. (1997); however, lack of common markers between studies warrants further verification.

Linolenic acid content

The content of linolenic acid in our study was controlled by 23 QTL of which six QTL were independent and 17 QTL collocated with the QTL of oil content, saturated and unsaturated fatty acids content QTL and flowering time (appendix i). This redundancy of genetic control highlights the difficulty of reducing the content of linolenic acid to improve the shelf life of canola oil. However, the QTL, *qC18:3-C3b* explained 22.90% variation in the content of linolenic acid and positively affected the phenotype. Avoiding this QTL integration improves the possibility of reduction in linolenic acid in *B. napus*.

In addition to fatty acid profile QTL identified in the current study on A2 (20.16 cM to 33.54 cM), A3 (43.68 cM to 56.95 cM) and C3 (144.42 cM to 154.55 cM), the QTL that

may also contain *FAD3* loci are *qC18:3-A1b* and *qC18:3-A5*. Hu et al. (2006) reported a *FAD2* locus on LG N1 (A1) and N5 (A5). A plastidial desaturase (*FAD7*) which converts C18:2 to C18:3 has been reported on N5 (A5) and was flanked by RFLP markers, pN215aNP and pO123CNP (Scheffler et al. 1997). However, since the lack of common markers and map alignments with these two aforementioned studies, this remains to be verified whether the QTL, *qC18:3-A1b* and *qC18:3-A5* represent desaturase encoding loci.

Arachidic acid content

QTL of saturated fatty acids and unsaturated fatty acids have been reported to collocate to one map position of a chromosome (Burns et al. 2003; Lionneton et al. 2002). Stepwise elongation from C16 to C18 is required for the synthesis of very long chain fatty acids (VLCFA) (Baud et al. 2003). Oleic acid is a preferred substrate of the activity of FAE for the synthesis VLCFA such as erucic acid (Bao et al. 1998) or arachidic acid. The arachidic acid content, *qC20:0-A1b* and oleic acid content QTL, *qC18:1-A1d* collocated on the same position and likely represent a *FAE* locus in our study.

Flowering time

In the current study, 14 QTL have been identified for flowering time. The number of QTL identified for flowering time in previous studies varied (six QTL, nine QTL and 23 QTL) due to the genetics of the populations, the marker types used and the environments studied (Chen et al. 2010; Delourme et al. 2006; Wang et al. 2011b).

There are inconsistent reports on the effects of flowering time on oil content. In some reports, days to flowering was negatively correlated to oil content (Chen et al. 2010). In contrast, Delourme et al. (2006) reported two genomic regions where both early and late

flowering QTL collocated with high oil content. However, most of QTL for these two traits were independent of each other in the study. In our study, the phenotypic correlation between flowering time and seed oil content was positive and significantly correlated ($r=0.30$, $P<.0001$, Table 5.2). This relationship was further supported in our study by map position of a flowering time QTL, *qFLR-C5* that promoted lateness. An oil content QTL, *qOIL-C5b*, occupied the same map position on C5. Both of these QTL inherited the lateness and oil increasing allele from Topas, indicating that late flowering increases oil content.

With the exception of *qFLR-A1b*, all flowering time QTL that colocalized with other traits promoted late flowering. For example, flowering time QTL, *qFLR-A1c*, *qFLR-A2a*, *qFLR-A10b* and *qFLR-C5* with negative additive effects delayed flowering, and collocated to positions of QTL controlling other traits (appendix i). Delayed flowering due to the QTL, *qFLR-C5* had positive phenotypic effect on oil content. In total, six flowering time QTL independently promoted delayed flowering and three flowering time QTL promoted earliness. This collocation of flowering time QTL explains why flowering time ($r=0.30^{**}$ Table 5.2) was positively correlated with oil content in the current study.

The SCAR marker (*WRL1-3-170*) for a transcription factor (*WRINKLE 1*) affecting lipid biosynthesis was found to be linked to a late flowering QTL (*qFLR-A2a*). The genomic region of this flowering time QTL also had association with oleic acid, linoleic acid and linolenic acid QTL, which are major component of seed oil content in *B. napus*. This further explains why flowering time was significantly correlated with seed oil content in our study. Recently, the role of phosphatidylcholine (PC), a phospholipid produced in the endoplasmic reticulum during lipid synthesis has been studied on flowering initiation in *Arabidopsis* by Nakamura et al. (2014). In the study, FLOWERING LOCUS T (FT)

protein increasingly binds in shoot apex with phosphatidylcholine, which triggers flowering. Increased PC level promoted earliness and reduced delayed flowering.

Transition to flowering in *Arabidopsis* is regulated by five pathways: photoperiod, vernalization, gibberellic acid (GA), autonomous pathway, and thermal clock (Putterill et al. 2004). There are two growth types of *B. napus*, the annual (spring type) and biennial (winter types) types (Teutonico and Osborn 1995). The biennial types require vernalization to induce flowering. A region on LG 9 reported to contain a vernalization QTL (Ferreira et al. 1995) aligned with N2 (A02) of *B. napus* (Teutonico and Osborn 1995). This segment of A02 in our study also contains a major flowering time QTL *qFLR-A2c*. This QTL explained 43.22% of the variation in flowering time and the allele for earliness for this QTL was contributed by the early flowering parent (Polo). Presence of flowering time QTL in this study at the reported location of a vernalization QTL may be due to the fact that these are related traits. Additionally, vernalization in annual growth types of *B. napus* can promote earliness (Medham and Scott 1975).

Early flowering and early maturing varieties in *B. napus* have value in agro-climatic zones where the growing period is short. A flowering time QTL identified in this study on A2 can be used to develop early flowering and early maturing *B. napus* lines with increased oil content. The QTL *qFLR-A2c* explained 43.22% of the variation in flowering time and the high oil parent Polo contributed the earliness alleles. Another QTL, *qFLR-A2b* explained 15.17% variation in flowering. However, the late flowering allele was also contributed by the high oil and early flowering parent (Polo). This is consistent with the phenotypic correlation observed between days to flowering and oil content (Table 5.2). The phenotypic correlation often observed between two traits is dependent on the environment

and genetic background (Lynch and Walsh 1998). The QTL *qFLR-A2c* on A2 is most likely a candidate for a vernalization gene that has lost function in spring types of *B. napus*.

Collocation of QTL

Multiple collocation sites of QTL controlling variations in the unsaturated fatty acid contents were identified on LG A2 and A3. We found that the upper part of LG A2 (20.16 cM to 33.54 cM) contained QTL for oleic acid content, linoleic acid content and linolenic acid content. The clustering of QTL on A2 can be explained with three pieces of evidence. First, the QTL of unsaturated fatty acids are linked with a transcription factor (marker WRI1-3-170) that affects multiple pathways. Secondly, the newly developed and integrated SSR marker on A2 in the current study, SR058-299 corresponds to a *B. rapa* gene, Bra008247 (data not shown) that encode a protein for pollen development. Since linolenic acid content of pollen has strong correlation ($r=87$) with seed linolenic acid content (Jourdain et al. 1996) this collocation of QTL is expected. Thirdly, independent studies have reported oil content QTL on this part of A2 (Delourme et al. 2006; Wang et al. 2013) in particular by Zhao et al. (2012) who has mapped QTL *OilC2* that represent a *DGAT/PDAT* locus (*At5g136040* and *At5g10160*) and functions in fatty acid biosynthesis (Li-Beisson et al. 2010). The clustering of QTL for different fatty acids on the upper region of A2 is therefore likely related to DGAT activity, which has broad specificity for fatty acids biosynthesis (Topfer et al. 1995).

Collocation and clustering of multiple traits was observed on A1, A2, A3, A10, C1, C3, C5, C8 and C9 (Fig. 5.9). An oleic acid content and linoleic acid content QTL collocated to the same map position of 104.51 cM on A1. Both the alleles, increasing oleic acid content and decreasing linolenic acid content were contributed by Polo (Table 5.6).

Considering the opposing effects of QTL of two traits where the two traits are both contributed by the same parent highlights the difficulties of introgressing multiple alleles from one parent (Ramchiary et al. 2007).

We report two genomic regions (QTL) on C3 that are likely controlling the mutual correlations of fatty acids in *B. napus*. QTL associated with all fatty acids studied in this research clustered at two genomic regions, 147.83 cM and 154.55 cM of C3 referred as Correlation QTL (*rQTL*). The *rQTL* explain a high proportion of the phenotypic variance. The additive effects of *rQTL* either positive or negative affect the trait of influence. The presumptive interaction of additive effects of *rQTL* for fatty acids studied, results in a pattern (Table 5.11) that is indicative of the direction of correlation between fatty acids. This deduced correlation of *rQTL* also coincides with the phenotypic correlations observed between fatty acids (Table 5.2). These findings are consistent with the study by Zhao et al. (2007) who found opposing additive effects for oleic acid content (negative) and erucic acid content QTL (positive) which indicate their genetic correlation. The *rQTL* had higher LOD values highlighting the possibility that they contain pleiotropic loci affecting multiple traits. Zhao et al. (2012) mapped *Arabidopsis* lipid orthologous genes in *B. napus* and reported genes at the bottom of C3 that are related to plastidial fatty acid synthesis, fatty acid elongation, and wax and cutin metabolism. Further fine mapping and comparative genomic studies at these two genomic positions of C3 may provide insight into genes controlling seed oil content, which is influenced in part by the correlations between fatty acids (Sanyal and Linder 2012).

This study has presented QTL identification results that were obtained using a saturated linkage map derived mainly of SSR markers. The relatively large population size and

reduced marker interval of 3.77 cM for this linkage map allowed detection of QTL with minor effects. Common/stable QTL for oil content, fatty acid profile and flowering time have been identified. Some of the identified QTL have been compared with published results where common markers between studies permitted such comparison. Certain fatty acid QTL reported here are associated with transcription factors for lipid biosynthesis. QTL explaining major phenotypic variations in oil content (26.99%), 35.21% in linolenic acid content, and 43.22% in flowering time have been reported in this study. These stable QTL can possibly be introgressed into elite germplasm of *B. napus* using marker-assisted selection.

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Table 5.1 Analysis of variance for days to flowering, oil content, palmitic acid content, stearic acid content, oleic acid content, linoleic acid content, linolenic acid content and arachidic acid content for doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

Source of variation	DF ^b	OIL ^c	C16:0 ^d	C18:0 ^e	C18:1 ^f	C18:2 ^g	C18:3 ^h	C20:0 ⁱ	FLR ^j
Line	136	24.65**	0.31**	0.42**	21.79**	11.30**	4.34**	0.03**	19.21**
Year	1	1094.01 ^{NS}	9.02 ^{NS}	4.42 ^{NS}	97.76 ^{NS}	10.60 ^{NS}	88.53 ^{NS}	0.60 ^{NS}	6681.75 ^{NS}
Location (loc)	1	1400.45 ^{NS}	15.24 ^{NS}	0.06 ^{NS}	64.82 ^{NS}	22.14 ^{NS}	162.12 ^{NS}	0.00 ^{NS}	120.86 ^{NS}
Rep(Year*Loc)	4	5.76 ^{NS}	0.35**	0.65	2.95 ^{NS}	0.94 ^{NS}	0.44 ^{NS}	0.02**	1.94 ^{NS}
Line*Loc	136	3.63 ^{NS}	0.01 ^{NS}	0.29**	1.79 ^{NS}	0.47 ^{NS}	0.18 ^{NS}	0.02 ^{NS}	2.21 ^{NS}
Year*Loc	1	1050.60**	3.05 ^{NS}	0.18 ^{NS}	237.84 ^{NS}	3.14 ^{NS}	45.88**	0.37 ^{NS}	6736.47**
Line*Year	136	7.69**	0.04**	0.06**	3.68**	1.02**	0.42**	0.00**	2.53 ^{NS}
Line*Year*Loc	136	3.45**	0.01 ^{NS}	0.02**	1.78**	0.47**	0.22**	0.00**	2.01**
Residual	539	1.86	0.01	0.01	0.81	0.29	0.12	0.00	1.30

**significant, P<0.00

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b Degrees of freedom, ^c OIL: Seed oil content (%), ^d C16:0 palmitic acid, ^e C18:0 stearic acid, ^f C18:1 oleic acid, ^g C18:2 linoleic acid, ^h C18:3 linolenic acid, ⁱ C20:0 arachidic acid and ^j FLR: Days to flowering (DF: Residual 541, DF for OIL: Line*Year*Loc 135 & Residual 536).

Table 5.2 Phenotypic correlations between trait means of a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

	FLR ^b	OIL ^c	C16:0 ^d	C18:0 ^e	C18:1 ^f	C18:2 ^g	C18:3 ^h	C20:0 ⁱ
FLR		0.30**	0.03 ^{NS}	-0.18**	-0.01 ^{NS}	0.05 ^{NS}	0.13**	-0.36**
OIL	0.30**		0.02 ^{NS}	-0.29**	0.32**	-0.10 ^{NS}	-0.28**	-0.42**
C16:0	0.03 ^{NS}	0.02 ^{NS}		-0.16**	-0.23**	0.25**	0.04 ^{NS}	-0.08 ^{NS}
C18:0	-0.18**	-0.29**	-0.16**		0.25**	-0.34**	-0.35**	0.85**
C18:1	-0.01 ^{NS}	0.32**	-0.23**	0.25**		-0.77**	-0.71**	0.20**
C18:2	0.05 ^{NS}	-0.10 ^{NS}	0.25**	-0.34**	-0.77**		0.41**	-0.36**
C18:3	0.13**	-0.28**	0.04 ^{NS}	-0.35**	-0.71**	0.41**		-0.35**
C20:0	-0.36**	-0.42**	-0.08 ^{NS}	0.85**	0.20**	-0.36**	-0.35**	

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b FLR: days to flowering, ^c OIL: Seed oil content (%), ^d C16:0 palmitic acid, ^e C18:0 stearic acid, ^f C18:1 oleic acid, ^g C18:2 linoleic acid, ^h C18:3 linolenic acid and ⁱ C20:0 arachidic acid.

Table 5.3 Summarized list of QTL detected for oil content using composite interval mapping in a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

QTL ^b	Location	LG ^c	Position	LOD ^d	R ² *	ADD ^e	Allele ^f	Flanked markers
<i>qOIL-A3a</i>	Win10, PlaP09	A3	129.23	2.85	5.87	-0.48	Topas	BnGMS265-181/odd3/bg4-275
<i>qOIL-A3b</i>	Win10, PlaP09	A3	141.43	3.23	6.55	-0.51	Topas	sa12/pm18-388/pm88/pm46-388
<i>qOIL-A3c</i>	PlaP09	A3	149.11	2.54	5.23	0.47	Polo	em1/pm4-238/bg23/pm60-125
<i>qOIL-A10a</i>	Win09	A10	4.01	3.81	26.99	0.99	Topas	BnGMS288-311/CB10536-163
<i>qOIL-A10b</i>	Win10, PlaP10, PlaP09	A10	10.12	4.72	11.45	-2.32	Topas	CB10124-169/BnGMS288-283
<i>qOIL-A10c</i>	Win10, PlaP10, Win09, PlaP09	A10	11.64	3.61	12.65	2.38	Polo	BnGMS288-283/Na12E09-315
<i>qOIL-A10d</i>	PlaP09	A10	17.70	2.50	5.46	-0.47	Topas	Na12E09-315/sa12/pm7-345
<i>qOIL-C1a</i>	Win09	C1	42.09	2.73	6.17	-0.53	Polo	CB10369-155/CB10369-176
<i>qOIL-C1b</i>	Win10, PlaP09	C1	68.69	2.92	6.54	-0.48	Polo	BnGMS299-375/SR084-223
<i>qOIL-C1c</i>	Win10, PlaP09	C1	77.73	2.86	5.84	-0.49	Polo	sa7/pm55-461/SR076-200
<i>qOIL-C5a</i>	Win10, Win09, PlaP09	C5	62.98	2.67	6.34	-0.51	Polo	ISSR140b/sa12/pm7-171
<i>qOIL-C5b</i>	Win10, Win09, PlaP09	C5	67.12	4.15	9.26	0.62	Topas	sa12/pm7-171/sa7/pm55-170
<i>qOIL-C6a</i>	Win10	C6	45.54	3.87	12.69	0.71	Polo	pm88/pm45-463/sa7/pm55-414
<i>qOIL-C6b</i>	Win10, PlaP09	C6	53.76	3.97	8.14	-0.57	Polo	em1/bg19-478/sa7/pm63-147

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b: QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (*OIL*, seed oil content), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome, ^c: LG: Linkage groups, ^d: LOD: Estimation of LOD score at the QTL peak, * % of phenotypic variation explained by the QTL, ^e:ADD: Additive effect of QTL, ^f:Allele: The source of allele inheritance for the respective QTL.

Table 5.4 Summarized list of QTL detected for palmitic acid content using composite interval mapping in a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

QTL ^b	Location	LG ^c	Position	LOD ^d	R ² *	ADD ^e	Allele ^f	Flanked markers
<i>qC16:0-A3</i>	Win10	A3	73.69	3.30	6.25	0.06	Topas	ISSR055/Na10D03-164
<i>qC16:0-A4</i>	PlaP09	A4	19.14	2.81	5.21	0.06	Polo	O110B06-234/Na10C01-276
<i>qC16:0-A5a</i>	Win10	A5	1.01	2.94	5.45	-0.06	Polo	SR115-401/SR044-437
<i>qC16:0-A5b</i>	Win09	A5	82.89	3.01	7.11	-0.20	Topas	BRMS047-151/SR119-176
<i>qC16:0-A5c</i>	PlaP09	A5	100.62	3.01	6.16	0.07	Polo	sa7/pm52-387/BRMS196-274
<i>qC16:0-A5d</i>	Win10	A5	113.94	5.02	11.12	0.08	Topas	ISSR139/ISSR140
<i>qC16:0-A8a</i>	Win09, PlaP10	A8	90.96	2.87	5.92	-0.05	Polo	ISSR124/CB10364-237
<i>qC16:0-A8b</i>	PlaP10	A8	94.43	3.06	6.91	0.06	Topas	BnGMS003-378/SR092-131
<i>qC16:0-A8c</i>	Win10	A8	119.35	3.60	14.52	-0.10	Polo	BnGMS177-354/SR050-291
<i>qC16:0-A10a</i>	PlaP10, PlaP09	A10	11.64	5.50	10.33	-0.09	Polo	BnGMS288-283/Na12E09-315
<i>qC16:0-A10b</i>	PlaP10, PlaP09	A10	17.70	3.89	7.56	0.08	Topas	Na12E09-315/sa12/pm7-345
<i>qC16:0-A10c</i>	PlaP10, Win10	A10	22.85	4.52	10.91	-0.08	Polo	odd3/bg38-305/me2/pm79-481
<i>qC16:0-A10d</i>	PlaP10, PlaP09	A10	26.19	4.88	10.62	0.08	Topas	MR156-209/BnGMS625-360
<i>qC16:0-A10e</i>	PlaP09	A10	32.94	4.60	8.75	-0.09	Polo	EST001-435/em1/pm4-157
<i>qC16:0-C2a</i>	Win10	C2	35.36	2.96	6.24	0.06	Polo	SR117-408/odd20/pm18-311
<i>qC16:0-C2b</i>	Win10	C2	38.16	3.89	9.68	0.08	Polo	odd20/pm18-311/sa7/bg29-177
<i>qC16:0-C2c</i>	Win10	C2	42.65	6.19	21.73	0.12	Topas	O113G05-141/em1/pm4-141
<i>qC16:0-C2d</i>	Win10	C2	45.41	3.59	8.26	-0.07	Topas	CB10093-284/CB10026-145
<i>qC16:0-C2e</i>	Win10	C2	46.86	3.90	8.11	0.07	Polo	CB10093-299/BRAS011-256
<i>qC16:0-C2f</i>	Win10	C2	48.72	2.58	5.43	-0.06	Topas	BRAS011-256/Na12E09-369
<i>qC16:0-C2g</i>	Win10	C2	51.33	3.36	6.97	0.07	Polo	Na12E09-369/odd20/bg2-314
<i>qC16:0-C3a</i>	PlaP09	C3	144.42	4.01	7.04	0.07	Polo	sa7/pm52-252/CB10427-166
<i>qC16:0-C3b</i>	PlaP09	C3	147.83	3.41	5.91	0.07	Polo	me2/bg33-245/odd3/bg2-466
<i>qC16:0-C3c</i>	PlaP09	C3	154.55	4.66	7.98	-0.07	Topas	pm88/pm45-177/odd3/pm3-399
<i>qC16:0-C3d</i>	PlaP09	C3	160.56	4.83	8.16	-0.08	Polo	O110B08-150/BRMS269-329
<i>qC16:0-C3e</i>	Win10	C3	188.15	3.96	7.54	0.07	Polo	odd3/bg38-199/Na10G10-152
<i>qC16:0-C5a</i>	PlaP09	C5	62.98	2.76	6.13	0.07	Topas	ISSR140b/sa12/pm7-171

Table 5.4 continued

QTL ^b	Location	LG ^c	Position	LOD ^d	R ² *	ADD ^e	Allele ^f	Flanked markers
<i>qC16:0-C5b</i>	PlaP09	C5	67.20	4.37	7.38	-0.07	Topas	sa12/pm7-171/sa7/pm55-170
<i>qC16:0-C8a</i>	PlaP09	C8	43.78	3.29	5.94	0.07	Polo	em1/bg9-434/bg23/pm59-285
<i>qC16:0-C8b</i>	PlaP09, PlaP10	C8	46.78	4.55	32.41	-0.12	Topas	em1/bg9-434/bg23/pm59-285
<i>qC16:0-C8c</i>	PlaP09, PlaP10	C8	54.90	4.98	10.43	0.07	Topas	sa12/bg8-230/CB10028-151
<i>qC16:0-C9a</i>	Win09	C9	19.05	3.68	12.43	-0.33	Topas	BnFUS3#2-1-91/CB10355-218
<i>qC16:0-C9b</i>	Win09	C9	87.77	2.87	9.79	-0.26	Polo	Na12G05-118/BRMS054-148

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b: QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C16:0, palmitic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome, ^c: LG: Linkage groups, ^d: LOD: Estimation of LOD score at the QTL peak, * % of phenotypic variation explained by the QTL, ^e:ADD: Additive effect of QTL, ^f:Allele: The source of allele inheritance for the respective QTL.

Table 5.5 Summarized list of QTL detected for stearic acid content using composite interval mapping in a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

QTL ^b	Location	LG ^c	Position	LOD ^d	R ² *	ADD ^e	Allele ^f	Flanked markers
<i>qC18:0-A1a</i>	Win10	A1	62.58	3.89	8.02	-0.11	Polo	sa7/pm52-150/BRAS111-195
<i>qC18:0-A1b</i>	Win09	A1	70.83	2.84	5.51	0.06	Polo	D11-194/pm88/pm17-312
<i>qC18:0-A1c</i>	Win10, PlaP09	A1	75.83	5.37	15.04	0.17	Polo	odd3/pm3-384/em1/pm11-409
<i>qC18:0-A1d</i>	Win10, PlaP09	A1	80.01	5.94	14.77	0.15	Polo	SR012-236/SR117-442
<i>qC18:0-A3a</i>	PlaP10	A3	60.95	2.82	6.18	0.07	Polo	sa12/bg18-224/D21-420
<i>qC18:0-A3b</i>	PlaP09	A3	64.12	2.67	5.76	-0.07	Topas	D21-420/Ra2F11-416
<i>qC18:0-A3c</i>	PlaP09	A3	67.14	4.69	14.62	0.10	Polo	Ra2F11-416/odd3/bg2-532
<i>qC18:0-A8</i>	Win10	A8	84.24	3.82	7.51	0.09	Polo	CB10578-251/P26-118
<i>qC18:0-C1a</i>	PlaP09	C1	42.09	3.54	6.47	0.07	Polo	CB10369-155/CB10369-176
<i>qC18:0-C1b</i>	Win09	C1	50.29	2.64	4.68	0.07	Polo	Na14G06-204/CB10355-538
<i>qC18:0-C1c</i>	PlaP09	C1	56.93	2.58	5.27	-0.06	Polo	CB10355-538/BnGMS299a
<i>qC18:0-C3a</i>	PlaP10	C3	126.47	2.54	5.80	0.08	Polo	sa12/bg8-340/BRAS087-165
<i>qC18:0-C3b</i>	PlaP10	C3	135.16	3.63	7.38	0.09	Polo	KSA1-175/ISSR158
<i>qC18:0-C3c</i>	Win10, PlaP10, Win09, PlaP09	C3	145.28	6.20	14.86	0.13	Topas	CB10427-166/odd20/pm117-468
<i>qC18:0-C3d</i>	Win10, PlaP10, Win09, PlaP09	C3	147.83	7.58	16.90	-0.13	Polo	me2/bg33-245/odd3/bg2-466
<i>qC18:0-C3e</i>	Win10, PlaP10, Win09, PlaP09	C3	154.55	11.27	22.71	0.12	Topas	pm88/pm45-177/odd3/pm3-399
<i>qC18:0-C8a</i>	PlaP10	C8	0.01	4.87	10.07	0.09	Topas	BnGMS004-383/CB10106-247
<i>qC18:0-C8b</i>	PlaP10	C8	7.42	2.66	6.11	-0.0	Polo	em1/bg19-311/BnGMS375-281

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b: QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C18:0, stearic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome, ^c: LG: Linkage groups, ^d: LOD: Estimation of LOD score at the QTL peak, * % of phenotypic variation explained by the QTL, ^e:ADD: Additive effect of QTL, ^f:Allele: The source of allele inheritance for the respective QTL.

Table 5.6 Summarized list of QTL detected for oleic acid content using composite interval mapping in a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

QTL ^b	Location	LG ^c	Position	LOD ^d	R ² *	ADD ^e	Allele ^f	Flanked markers
<i>qC18:1-A1a</i>	PlaP10	A1	44.40	2.52	4.92	-0.43	Topas	CB10099-203/BRMS056-221
<i>qC18:1-A1b</i>	PlaP10	A1	46.13	2.70	5.21	0.45	Polo	BRMS056-227/BRMS196-229
<i>qC18:1-A1c</i>	Win09	A1	66.32	4.14	5.96	-0.44	Topas	BRAS084-131/BRAS111-190
<i>qC18:1-A1d</i>	Win09	A1	73.48	3.21	10.37	0.58	Topas	pm88/pm17-312/ISSR008
<i>qC18:1-A1e</i>	Win10, PlaP09	A1	104.51	3.28	5.13	0.56	Polo	CB10597-133/SR083-245
<i>qC18:1-A1f</i>	PlaP09	A1	112.78	3.02	4.57	-0.45	Topas	BRAS029-232/odd3/bg4-249
<i>qC18:1-A2a</i>	Win10, PlaP09, Win09	A2	20.16	7.20	14.10	-0.73	Topas	WRI1-3-170/ISSR059
<i>qC18:1-A2b</i>	Win09, PlaP09	A2	23.41	6.70	10.73	0.61	Polo	ISSR061/BnGMS067-231
<i>qC18:1-A2c</i>	Win10	A2	26.18	5.21	8.89	-0.58	Topas	BnGMS067-231/em1/bg19-251
<i>qC18:1-A2d</i>	Win09, PlaP09, Win10	A2	29.06	7.90	13.41	0.68	Polo	me2/pm45-262/SR058-298
<i>qC18:1-A2e</i>	Win09, PlaP09	A2	33.54	7.10	12.29	0.65	Polo	sa7/pm55-252/ISSR048
<i>qC18:1-A2f</i>	PlaP09	A2	40.21	2.50	6.18	0.46	Topas	odd20/pm18-333/sa12/pm18-132
<i>qC18:1-A2g</i>	PlaP10	A2	71.73	2.61	12.89	0.70	Polo	CB10172-239/C20-135
<i>qC18:1-A3a</i>	Win10	A3	43.68	5.88	9.34	-0.56	Topas	D03-217/BnGMS079-353
<i>qC18:1-A3b</i>	Win09	A3	51.53	5.16	8.71	0.56	Polo	BnGMS079-353/BnGMS079-333
<i>qC18:1-A3c</i>	Win10, PlaP10, Win09, PlaP09	A3	55.95	10.04	14.99	0.70	Polo	sa12/bg18-224/D21-420
<i>qC18:1-A3d</i>	Win09	A3	67.14	3.56	7.55	-0.50	Polo	Ra2F11-416/odd3/bg2-532
<i>qC18:1-C3a</i>	Win10, PlaP10, Win09, PlaP09	C3	144.42	9.09	19.40	-0.87	Polo	sa7/pm52-252/CB10427-166
<i>qC18:1-C3b</i>	Win10, PlaP10, Win09, PlaP09	C3	147.83	8.51	17.66	-0.83	Polo	me2/bg33-245/odd3/bg2-466
<i>qC18:1-C3c</i>	Win10, PlaP10, Win09, PlaP09	C3	154.55	16.84	28.47	1.05	Topas	pm88/pm45-177/odd3/pm3-399
<i>qC18:1-C8</i>	Win10	C8	54.47	2.70	6.26	-0.49	Polo	PROM008-229/sa12/bg8-230

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b: QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C18:1, oleic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome, ^c: LG: Linkage groups, ^d: LOD: Estimation of LOD score at the QTL peak, * % of phenotypic variation explained by the QTL, ^e:ADD: Additive effect of QTL, ^f:Allele: The source of allele inheritance for the respective QTL.

Table 5.7 Summarized list of QTL detected for linoleic acid content using composite interval mapping in a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

QTL ^b	Location	LG ^c	Position	LOD ^d	R ^{2*}	ADD ^e	Allele ^f	Flanked markers
<i>qC18:2-A1a</i>	PlaP10	A1	92.73	3.15	7.51	0.56	Topas	SR083-251/SR027-338
<i>qC18:2-A1b</i>	Win10, PlaP10, Win09	A1	104.51	6.04	10.32	-0.52	Polo	CB10597-133/SR083-245
<i>qC18:2-A1c</i>	Win10, PlaP10, Win09	A1	112.78	4.32	10.17	0.70	Topas	BRAS029-232/odd3/bg4-249
<i>qC18:2-A2a</i>	Win10, Win09, PlaP09	A2	20.16	6.22	12.10	0.47	Topas	WRI1-3-170/ISSR059
<i>qC18:2-A2b</i>	Win10, PlaP09	A2	23.41	5.46	9.84	-0.42	Polo	ISSR061/BnGMS067-231
<i>qC18:2-A2c</i>	Win10, PlaP09	A2	26.18	5.55	10.06	0.42	Topas	BnGMS067-231/em1/bg19-251
<i>qC18:2-A2d</i>	Win10, Win09, PlaP09	A2	29.06	7.59	14.07	-0.50	Polo	me2/pm45-262/SR058-298
<i>qC18:2-A2e</i>	Win10, Win09, PlaP09	A2	33.54	6.05	11.56	-0.46	Polo	sa7/pm55-252/ISSR048
<i>qC18:2-A3a</i>	Win09	A3	43.68	3.17	4.49	0.29	Topas	D03-217/BnGMS079-353
<i>qC18:2-A3b</i>	Win09	A3	52.24	2.80	4.00	0.27	<u>Polo</u>	BnGMS079-333/sa12/bg18-224
<i>qC18:2-A3c</i>	Win10, Win09	A3	55.24	3.06	9.30	-0.41	<u>Polo</u>	BnGMS079-333/sa12/bg18-224
<i>qC18:2-A5</i>	Win10	A5	125.01	3.08	5.00	-0.30	Topas	BRAS072-199/BRAS072-212
<i>qC18:2-C1</i>	PlaP10	C1	133.91	3.78	8.16	-0.59	<u>Topas</u>	BRAS067-139/BRAS074-143
<i>qC18:2-C3a</i>	Win10, Win09, PlaP09	C3	144.42	5.03	9.76	0.42	Polo	sa7/pm52-252/CB10427-166
<i>qC18:2-C3b</i>	Win10, Win09, PlaP09	C3	147.83	3.32	6.39	0.34	Polo	me2/bg33-245/odd3/bg2-466
<i>qC18:2-C3c</i>	Win10, Win09, PlaP09	C3	154.55	13.21	22.09	-0.64	Topas	pm88/pm45-177/odd3/pm3-399
<i>qC18:2-C3d</i>	PlaP09	C3	157.36	5.70	12.56	0.72	Polo	odd3/pm3-399/O110B08-144
<i>qC18:2-C9a</i>	PlaP09	C9	0.01	3.56	7.60	-0.90	Topas	CB10344-202/SR043-342
<i>qC18:2-C9b</i>	PlaP09	C9	8.40	2.92	7.15	-0.82	Topas	CB10350-220/BnFUS3#2-1-91
<i>qC18:2-C9c</i>	Win09	C9	41.75	2.59	3.61	-0.25	Topas	SR090-182/BnFUS3#2-5-329

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b: QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C18:2 linoleic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome, ^c: LG: Linkage groups, ^d: LOD: Estimation of LOD score at the QTL peak, * % of phenotypic variation explained by the QTL, ^e:ADD: Additive effect of QTL, ^f:Allele: The source of allele inheritance for the respective QTL.

Table 5.8 Summarized list of QTL detected for linolenic acid content using composite interval mapping in a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

QTL ^b	Location	LG ^c	Position	LOD ^d	R ² *	ADD ^e	Allele ^f	Flanked markers
<i>qC18:3-A1a</i>	PlaP10	A1	44.40	3.45	6.23	0.22	Topas	CB10099-203/BRMS056-221
<i>qC18:3-A1b</i>	PlaP10	A1	46.13	3.97	7.04	-0.24	Polo	BRMS056-227/BRMS196-229
<i>qC18:3-A2a</i>	Win10, PlaP09	A2	19.16	4.07	7.60	0.22	Topas	WRI1-3-170/ISSR059
<i>qC18:3-A2b</i>	Win10, PlaP09	A2	23.41	3.68	4.22	-0.19	Polo	ISSR061/BnGMS067-231
<i>qC18:3-A2c</i>	Win10, PlaP09	A2	25.41	3.87	17.81	0.33	Polo	ISSR061/BnGMS067-231
<i>qC18:3-A2d</i>	Win10, PlaP09	A2	29.06	5.07	8.39	-0.23	Polo	me2/pm45-262/SR058-298
<i>qC18:3-A2e</i>	Win10, PlaP09	A2	33.54	4.27	7.51	-0.22	Polo	sa7/pm55-252/ISSR048
<i>qC18:3-A3a</i>	PlaP10	A3	22.00	4.03	8.00	0.26	Topas	sa12/bg38-422/BRMS071-278
<i>qC18:3-A3b</i>	Win10, PlaP10, Win09, PlaP09	A3	37.58	5.91	11.48	-0.30	Polo	BRMS071-278/SR075a
<i>qC18:3-A3c</i>	Win10, PlaP10, Win09, PlaP09	A3	42.29	7.82	14.69	-0.34	Polo	BRMS303-217/D03-203
<i>qC18:3-A3d</i>	Win10, PlaP10, Win09, PlaP09	A3	51.53	5.83	11.85	-0.31	Polo	BnGMS079-353/BnGMS079-333
<i>qC18:3-A3e</i>	Win10, PlaP10, Win09, PlaP09	A3	56.95	6.57	18.81	-0.34	Polo	sa12/bg18-224/D21-420
<i>qC18:3-A5</i>	Win10	A5	130.32	2.78	15.23	-0.31	Topas	BRAS072-212/ISSR160
<i>qC18:3-A9a</i>	PlaP10	A9	23.90	3.61	6.43	-0.23	Topas	Na10D09-300/em1/bg19-299
<i>qC18:3-A9b</i>	PlaP10	A9	32.27	2.69	4.82	0.20	Polo	BnGMS313-169/CB10347-190
<i>qC18:3-A10a</i>	Win10, Win09, PlaP09	A10	8.50	4.36	6.92	-0.21	Polo	CB10124-155/CB10124-169
<i>qC18:3-A10b</i>	Win10	A10	11.64	4.67	7.26	-0.22	Polo	BnGMS288-283/Na12E09-315
<i>qC18:3-A10c</i>	Win10	A10	19.59	3.32	5.24	-0.20	Polo	sa12/pm7-345/MR156-195
<i>qC18:3-A10d</i>	Win10	A10	47.91	2.52	5.25	-0.20	Topas	ISSR031/bg23/pm52-216
<i>qC18:3-C3a</i>	Win10, PlaP10, Win09, PlaP09	C3	145.28	12.59	26.61	-0.42	Topas	CB10427-166/odd20/pm117-468
<i>qC18:3-C3b</i>	Win10, PlaP10, Win09, PlaP09	C3	147.83	10.61	22.90	0.38	Polo	me2/bg33-245/odd3/bg2-466
<i>qC18:3-C3c</i>	Win10, PlaP10, Win09, PlaP09	C3	154.55	18.06	35.21	-0.47	Topas	pm88/pm45-177/odd3/pm3-399
<i>qC18:3-C6</i>	PlaP10	C6	59.12	2.69	13.32	0.33	Polo	me2/bg33-133/sa7/pm52-518

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b: QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C18:3 linolenic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome, ^c: LG: Linkage groups, ^d: LOD: Estimation of LOD score at the QTL peak, * % of phenotypic variation explained by the QTL, ^e:ADD: Additive effect of QTL, ^f:Allele: The source of allele inheritance for the respective QTL.

Table 5.9 Summarized list of QTL detected for arachidic acid content using composite interval mapping in a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

QTL ^b	Location	LG ^c	Position	LOD ^d	R ² *	ADD ^e	Allele ^f	Flanked markers
<i>qC20:0-A1a</i>	Win10	A1	61.97	4.89	10.21	-0.02	Polo	Ol12F11-236/sa7/pm52-150
<i>qC20:0-A1b</i>	Win09	A1	72.48	2.81	5.00	-0.01	Topas	pm88/pm17-312/ISSR008
<i>qC20:0-A1c</i>	Win09, Win10	A1	76.19	5.62	11.33	-0.03	Topas	em1/pm11-409/me2/pm86-158
<i>qC20:0-A1d</i>	Win10, Win09	A1	80.01	7.21	13.21	0.03	Polo	SR012-236/SR117-442
<i>qC20:0-A10</i>	PlaP10	A10	24.08	2.80	15.43	-0.03	Topas	me2/pm79-481/Na12C05-351
<i>qC20:0-C1a</i>	Win10, Win09	C1	40.91	2.97	5.05	0.01	Polo	BRMS096-179/CB10369-155
<i>qC20:0-C1b</i>	Win10	C1	147.91	2.88	4.63	0.01	Topas	BRAS067-139/BRAS074-143
<i>qC20:0-C3a</i>	PlaP10	C3	115.20	3.21	13.02	-0.03	Polo	Na10D03-153/Na10D03-179
<i>qC20:0-C3b</i>	Win10, PlaP10, Win09, PlaP09	C3	145.28	4.76	16.79	0.05	Topas	CB10427-166/odd20/pm117-468
<i>qC20:0-C3c</i>	Win10, PlaP10, Win09, PlaP09	C3	147.83	5.57	12.87	-0.03	Polo	me2/bg33-245/odd3/bg2-466
<i>qC20:0-C3d</i>	Win10, PlaP10, Win09, PlaP09	C3	154.55	11.85	26.51	0.04	Topas	pm88/pm45-177/odd3/pm3-399
<i>qC20:0-C3e</i>	PlaP09	C3	157.36	4.77	11.72	-0.03	Polo	odd3/pm3-399/Ol10B08-144
<i>qC20:0-C5</i>	Win10	C5	23.81	2.52	12.79	-0.02	Topas	SR112-370/SR112-340
<i>qC20:0-C8a</i>	PlaP10	C8	0.01	3.47	6.63	0.02	Topas	BnGMS004-383/CB10106-247
<i>qC20:0-C8b</i>	PlaP09	C8	18.78	2.50	5.52	-0.02	Polo	BnGMS004-349/em1/bg1-409
<i>qC20:0-C9</i>	PlaP09	C9	17.40	3.87	8.73	-0.03	Topas	BnFUS3#2-1-91/CB10355-218

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b: QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C20:0, arachidic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome, ^c: LG: Linkage groups, ^d: LOD: Estimation of LOD score at the QTL peak, * % of phenotypic variation explained by the QTL, ^e:ADD: Additive effect of QTL, ^f:Allele: The source of allele inheritance for the respective QTL.

Table 5.10 Summarized list of QTL detected for flowering time using composite interval mapping in a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

QTL ^b	Location	LG ^c	Position	LOD ^d	R ² *	ADD ^e	Allele ^f	Flanked markers
<i>qFLR-A1a</i>	Win10, Win09	A1	56.75	2.80	11.14	-0.57	Polo	bg23/pm117-444/odd20/bg2-248
<i>qFLR-A1b</i>	Win10	A1	60.97	2.51	3.59	0.34	Polo	Ol12F11-236/sa7/pm52-150
<i>qFLR-A1c</i>	Win10	A1	66.32	2.65	4.01	-0.36	Topas	BRAS084-131/BRAS111-190
<i>qFLR-A2a</i>	Win10	A2	17.16	2.70	4.05	-0.34	Topas	WRI1-3-170/ISSR059
<i>qFLR-A2b</i>	Win10, Win09, PlaP09	A2	67.73	6.41	15.17	-0.70	Polo	CB10172-239/C20-135
<i>qFLR-A2c</i>	Win10, Win09, PlaP09	A2	83.73	21.69	43.22	1.13	Polo	CB10172-239/C20-135
<i>qFLR-A10a</i>	Win09	A10	42.53	2.49	4.50	-0.39	Topas	bg23/pm60-157/ISSR031
<i>qFLR-A10b</i>	Win10, PlaP09	A10	47.91	5.49	9.99	-0.58	Topas	ISSR031/bg23/pm52-216
<i>qFLR-A10c</i>	Win09, Win10	A10	53.06	3.51	7.14	0.48	Polo	sa7/bg37-293/OlAJ120-360
<i>qFLR-A10d</i>	Win10	A10	60.85	2.78	4.32	-0.36	Polo	OlAJ120-360/sa7/bg37-430
<i>qFLR-C3a</i>	PlaP10	C3	168.71	3.05	8.33	1.77	Polo	BRMS269-341/ISSR065
<i>qFLR-C3b</i>	PlaP10	C3	185.71	2.97	8.31	-1.18	Polo	BRMS269-341/ISSR065
<i>qFLR-C3c</i>	Win10, Win09, PlaP09	C3	206.02	5.28	9.84	-0.54	Topas	Na10G10-165/pm88/bg1-275
<i>qFLR-C5</i>	Win10	C5	66.12	2.82	23.64	-0.83	Topas	sa12/pm7-171/sa7/pm55-170

^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010, ^b: QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (FLR, flowering time), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome, ^c: LG: Linkage groups, ^d: LOD: Estimation of LOD score at the QTL peak, * % of phenotypic variation explained by the QTL, ^e:ADD: Additive effect of QTL, ^f:Allele: The source of allele inheritance for the respective QTL.

Table 5.11 C3 genomic regions of a doubled haploid line population—Polo X Topas harboring two QTL (*rQTL*) for fatty acids which are modulating correlation between different fatty acids through their respective additive effects.

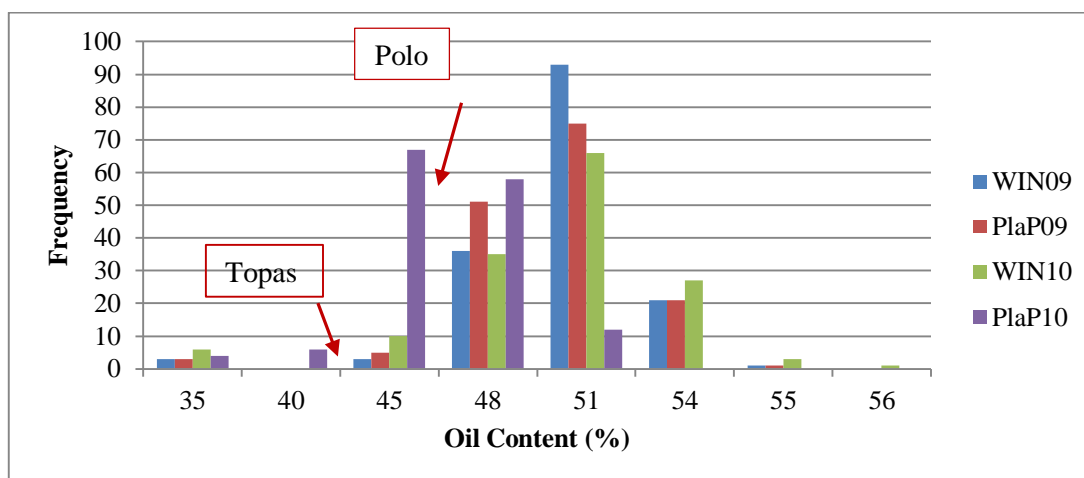
Map position (cM)	C16:0 ^a	C18:0 ^b	C18:1 ^c	C18:2 ^d	C18:3 ^e	C20:0 ^f
147.83	+	-	-	+	+	-
154.55	-	+	+	-	-	+
		*				
			**			

Note: At any of these map positions of C3, the appearing QTL if have alternate direction of polarity of additive effects between two fatty acids, it will result in a negative correlation between those fatty acids otherwise positive.

*: negative correlation, **: positive correlation.

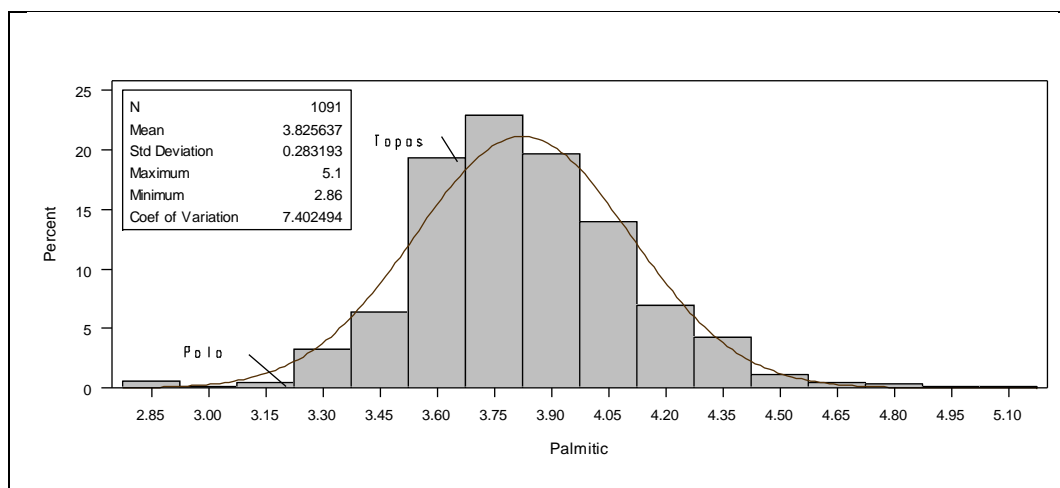
^a C16:0: palmitic acid, ^b C18:0: stearic acid, ^c C18:1: oleic acid, ^d C18:2: linoleic acid, ^e C18:3: linolenic acid and ^f C20:0: arachidic acid.

Fig. 5.1 Histogram for mean seed oil content of a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10



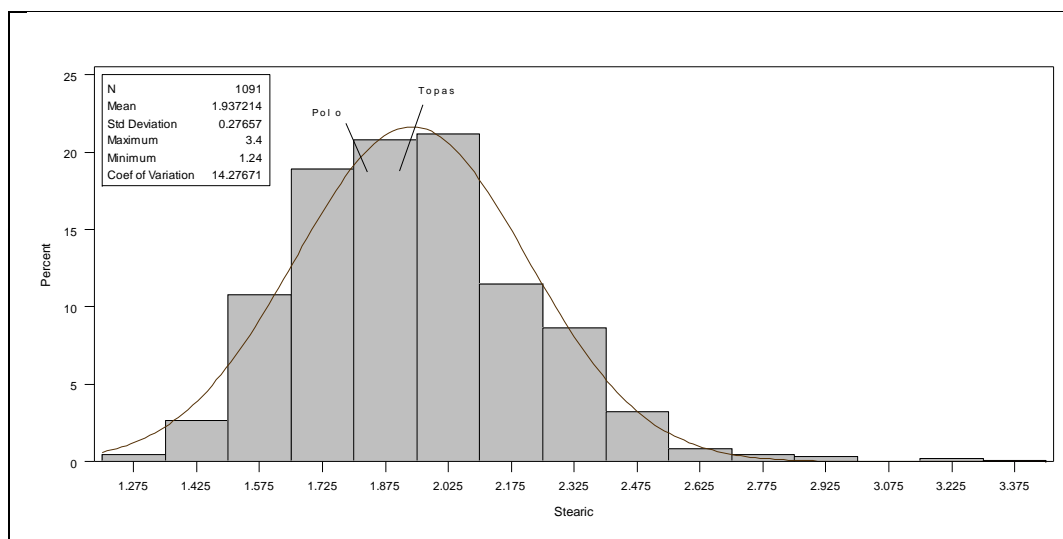
^a: Winnipeg 2009-WIN09, Portage la Prairie 2009-PlaP09, Winnipeg 2010-WIN10, and Portage la Prairie 2010-PlaP10.

Fig. 5.2 Histogram for mean content of palmitic acid of a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10



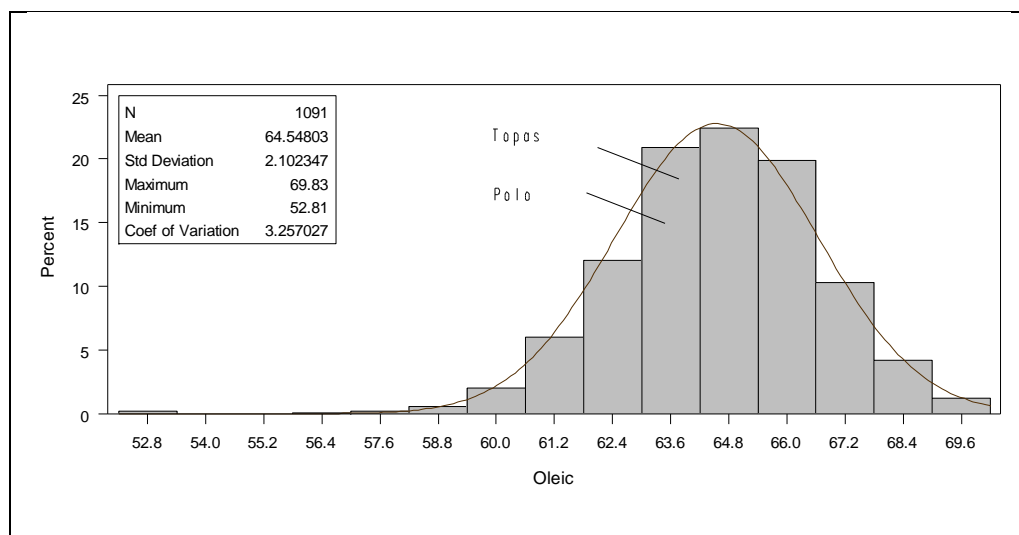
^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

Fig. 5.3 Histogram for mean content of stearic acid of a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10



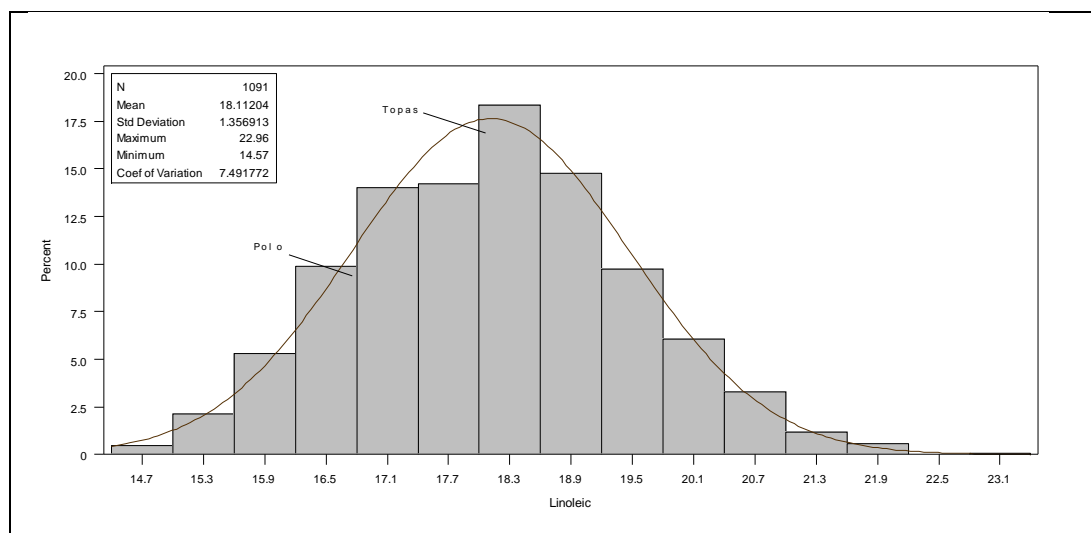
^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

Fig. 5.4 Histogram for mean content of oleic acid of a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10



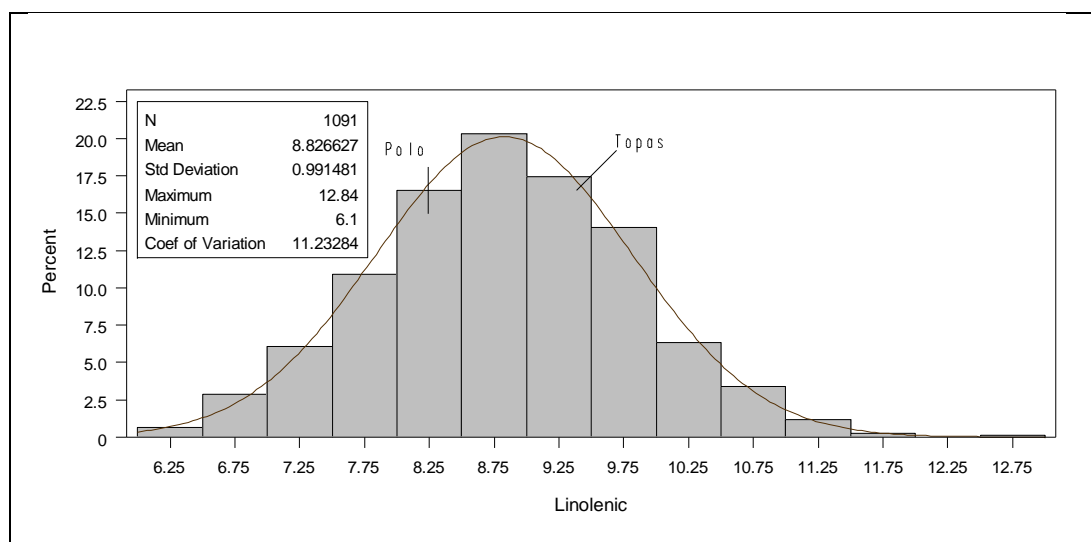
^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

Fig. 5.5 Histogram for mean content of linoleic acid of a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10



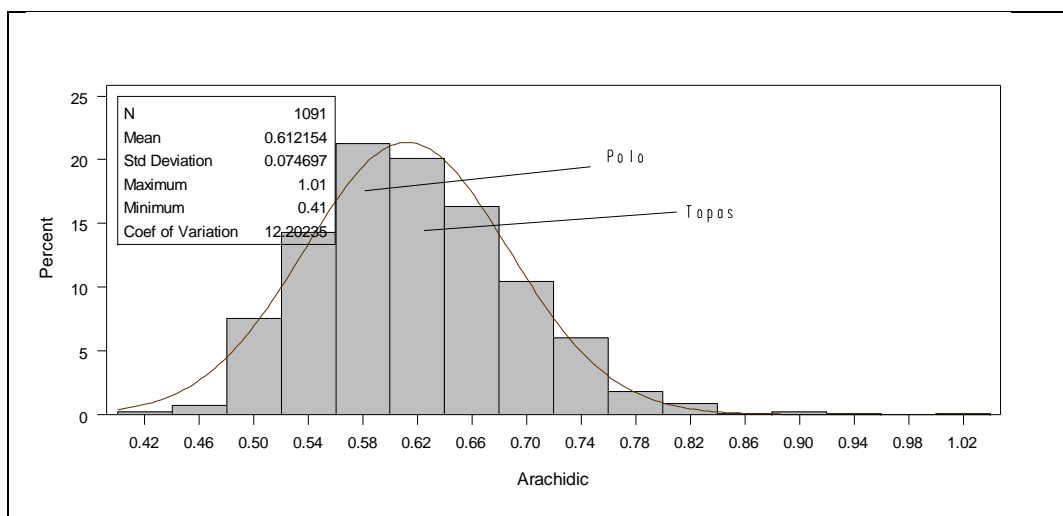
^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

Fig. 5.6 Histogram for mean content of linolenic acid of a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10



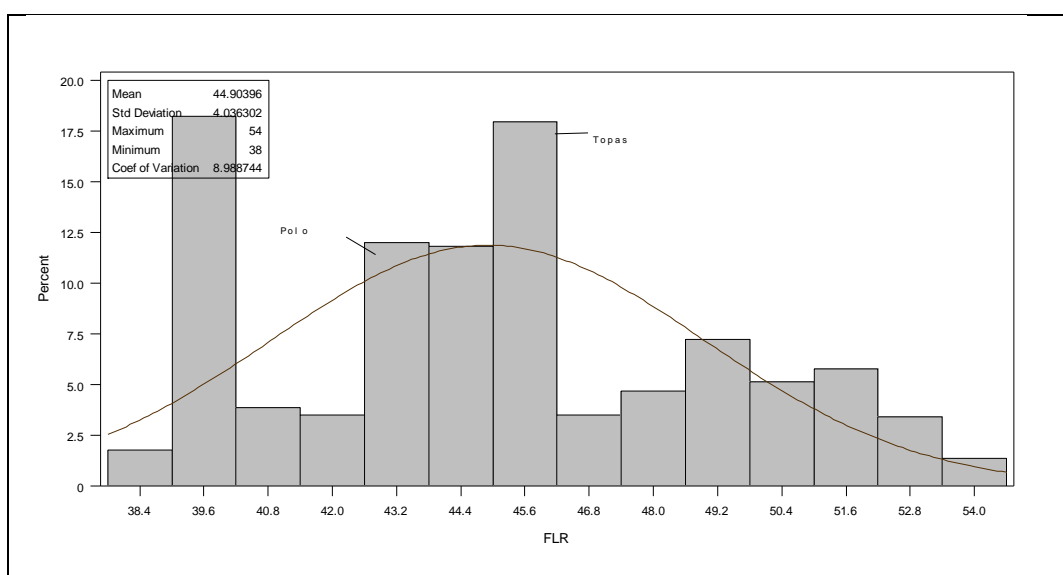
^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

Fig. 5.7 Histogram for mean content of arachidic acid of a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10

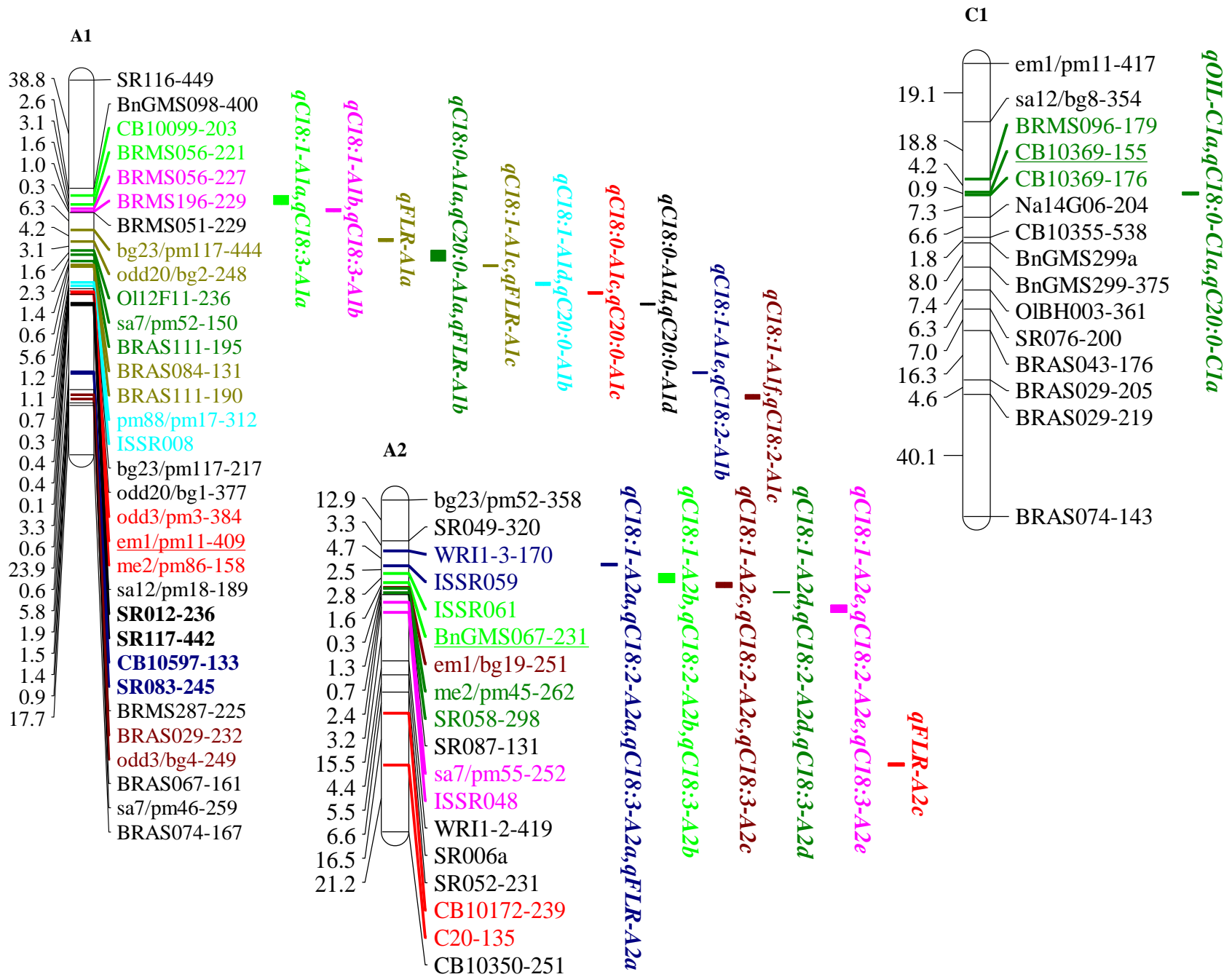


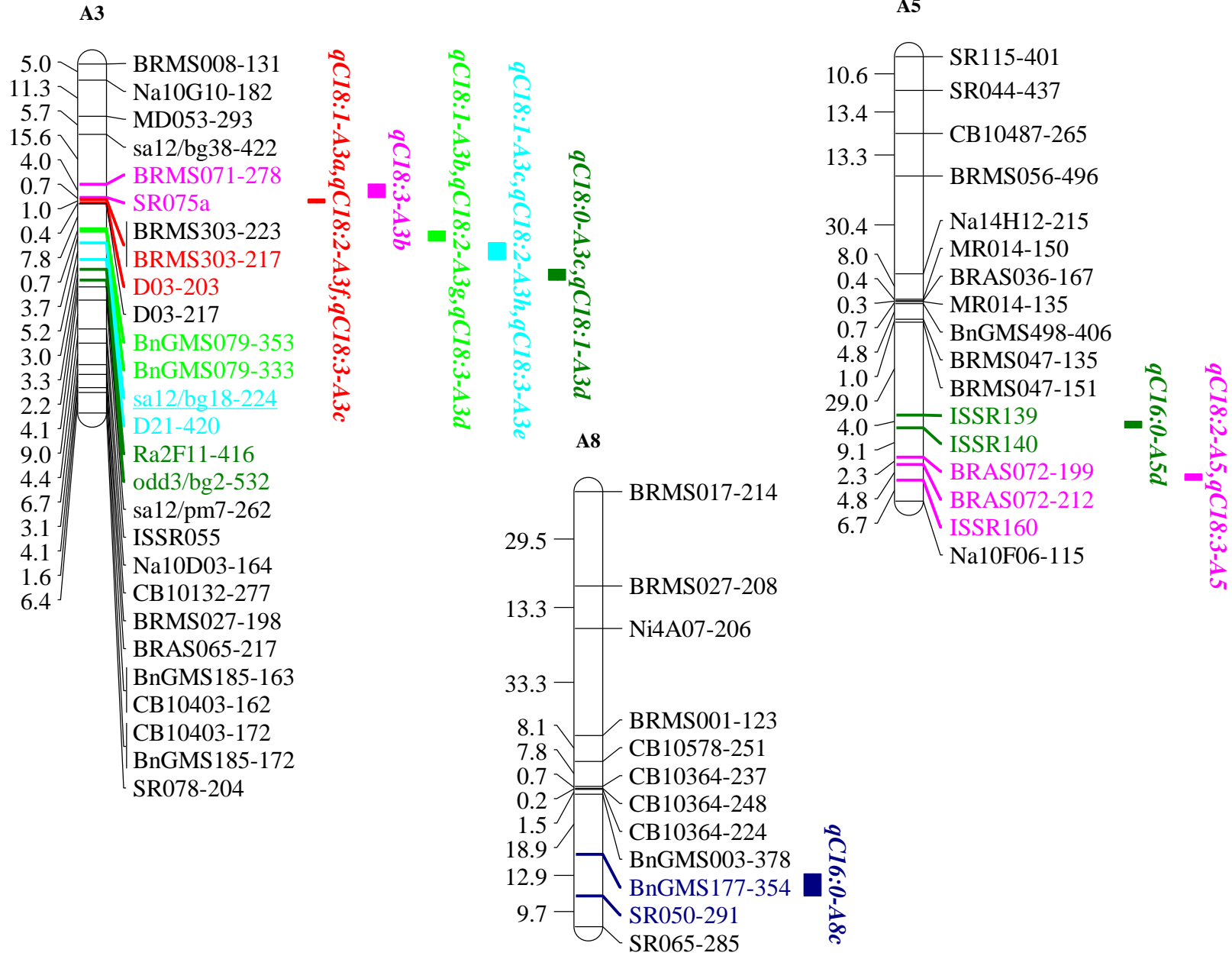
^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

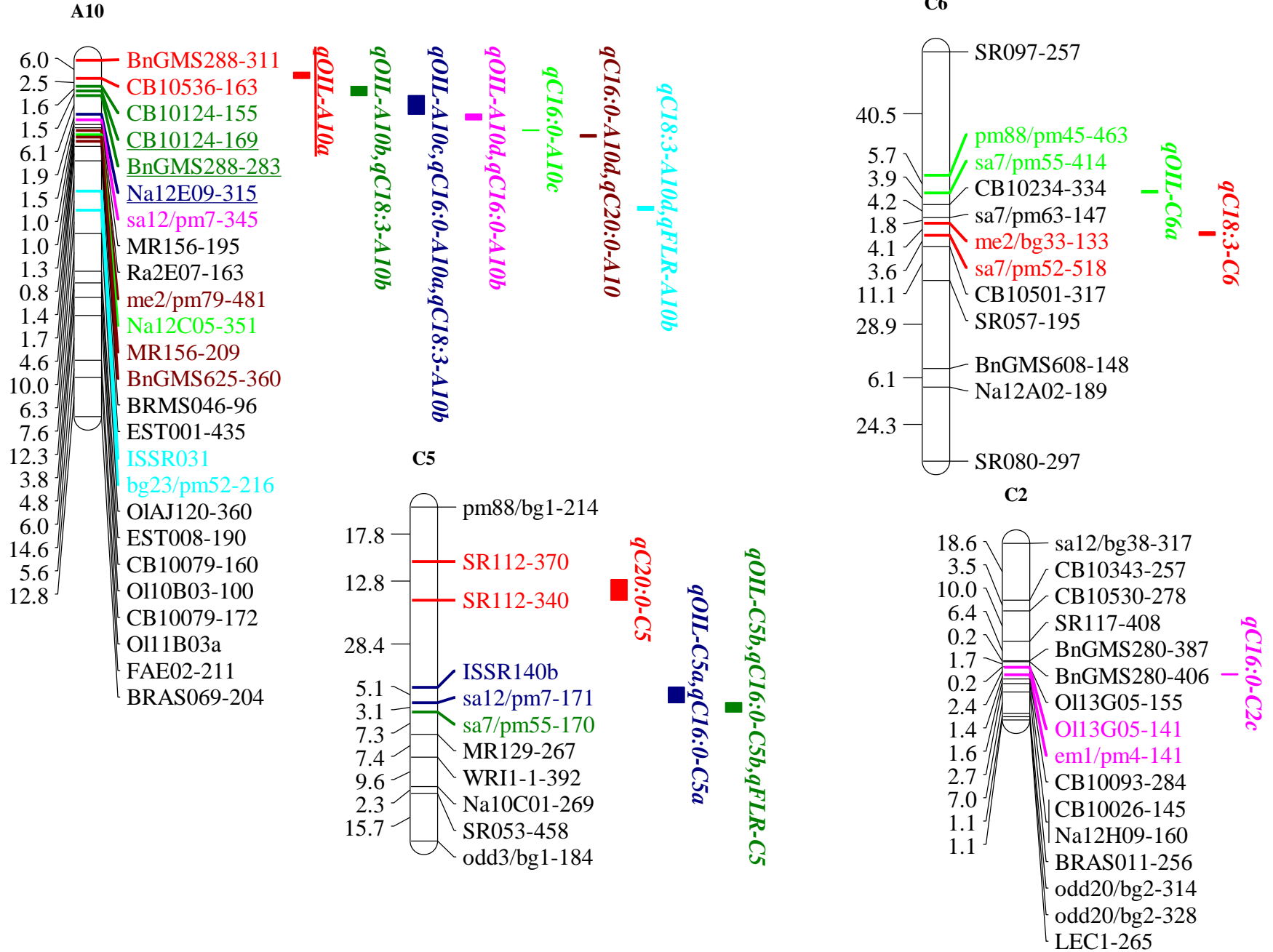
Fig. 5.8 Histogram for mean days to flowering of a doubled haploid line population—Polo X Topas grown in four environments^a during 2009-10



^a: Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010







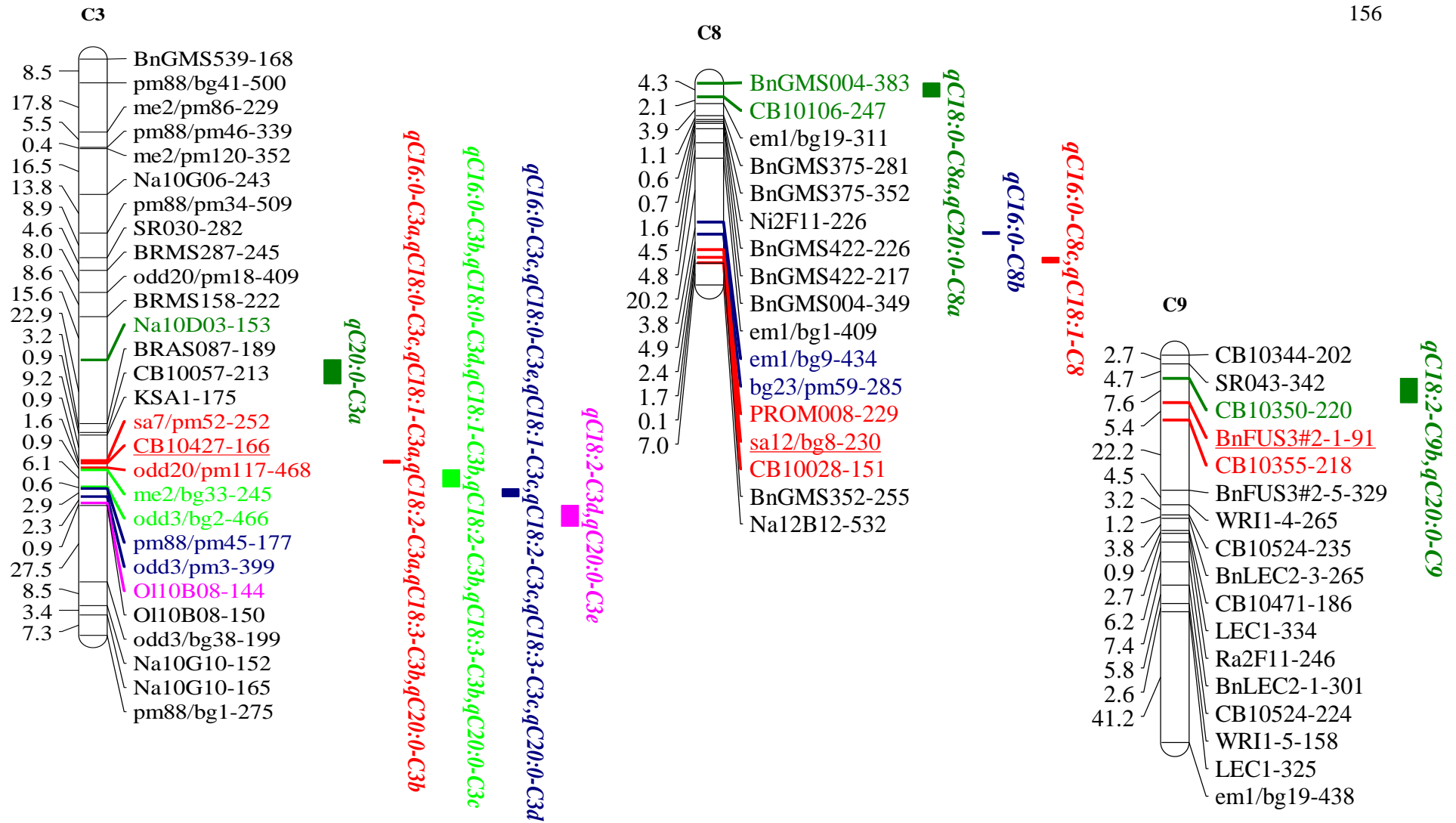


Fig. 5.9 Illustration of collocating and major QTL identified in the doubled haploid line population—Polo X Topas. The flanking markers (the underlined marker locus; flanks with the neighboring loci) and the respective QTL have matching color. Genetic distance between markers is given on the left side of the LG.

6. GENERAL DISCUSSION

In this research, a doubled haploid line population and a recombinant inbred line population were developed from a F₁ hybrid derived from a cross between cv. Polo and cv. Topas. Two individual genetic maps from both of these populations were developed. These individual genetic maps were further combined to develop an integrated genetic map. The genetic map derived from the DH line population was used to identify quantitative trait loci (QTL) associated with seed oil content, fatty acid profile (palmitic acid content, stearic acid content, oleic acid content, linoleic acid content, linolenic acid content and arachidic acid content) and flowering time.

Polymorphisms between nucleotides at a locus constitute a genetic marker and several molecular marker detection techniques such as SSR, ISSR, SRAP and SCAR are routinely used for genetic mapping (Meksem and Kahl 2005). The choice of molecular markers for mapping studies depends on simplicity in genotyping procedure, cost and transferability of results across different research groups (Meksem and Kahl 2005).

Linkage maps provide insight into a genome by linearly ordering molecular markers based on their recombination frequencies. Linkage maps are crucial in determining genes that are involved in controlling qualitative or quantitative traits. Among several genetic maps available in *B. napus*, the SRAP based genetic map developed by Sun et al. (2007) had coverage of 1604.8 cM with marker saturation of 0.12 cM. Piquemal et al. (2005) developed a linkage map based on SSR markers by using six F₂ populations that covered a map distance of 1692 cM with marker interval of 7.2 cM. The coverage of a genetic map could be improved by increasing the population size and molecular markers or by

employing different types of molecular markers (Voorrips et al. 1997). For this purpose, two genetic maps, and an integrated genetic map derived from multiple marker systems and multiple population types were constructed. The first genetic map derived from a DH line population consisted of 620 polymorphic loci comprised of 387 SSR, 29 ISSR, 191 SRAP and 17 SCAR markers. These loci were assembled into 19 linkage groups and anchored to respective chromosomes by published SSR markers (Cheng et al. 2009; Piquemal et al. 2005). Considering the genome length of *B. napus* that ranges between 2,127–2,480 cM (Lombard and Delourme 2001), the map length of 2244.1 cM attained in this research is close to complete coverage of the *B. napus* genome. A marker interval of 3.7 cM was attained in the linkage map that offers utility in QTL mapping and efficient marker-assisted selection of traits. The second genetic map derived from the recombinant inbred line population was accomplished with 349 loci comprised of 316 SSR, 27 ISSR and 6 SCAR markers and covered a genome distance of 1650.2 cM with marker saturation of 4.7 cM. A minimum of two and up to 23 common markers of the same linkage group of the DH line population and the RI population maps were combined to develop an integrated genetic map. The integrated map covered a genomic distance of 2464.9 cM with a marker interval of 3.1 cM.

Comparing common markers of homologous LG groups of the two populations allowed the comparison of marker order in the integrated genetic map. Between the DH line population and the RIL population 194 markers were common, of which 183 (94.3%) were assembled into an integrated genetic map. Of the common markers, 170 markers assembled into the same LG group in both the DH line population and the RIL population.

In *B. napus*, publically available SSR markers are not as abundant compared to other crops such as rice and soybean (Suwabe et al. 2006). In this study, we used online tools to develop SSR markers pertaining to different lengths of di-repeats (e.g. [AT]_n, [CT]_n, and [GA]_n). A total of 146 SSR markers are new, among them 92 SSR markers were integrated on the A genome and 45 were integrated on to the C genome. Nine SSR markers were scattered in both genomes.

The genetic maps developed as part of this Ph.D. research, sponsored by Green Crop Network at the University of Manitoba (Geng et al. 2012) are saturated genetic maps. These genetic maps, can serve as a breeding tool to identify QTL for oil content and component fatty acids in *B. napus*. In the long term, these maps can further be saturated with different or even more molecular markers. The flanking markers of the identified QTL for seed oil content and fatty acid profile can be used in marker-assisted introgression of the respective traits.

In recent studies, the number of QTL controlling oil content in *B. napus* has varied from seven to thirty-six QTL (Delourme et al. 2006; Qiu et al. 2006; Wang et al. 2013). In the current study 14 QTL for seed oil content were identified. The number of QTL identified across different studies are due to difference in population size, range of difference in the trait phenotype of the mapping population and map resolution (Wang et al. 2013).

Of the 14 oil content QTL, four QTL were detected on A10 where two were discovered in one environment and two other individual QTL were found in three and four environments. This confirms previous research where oil content QTL were reported on A10 of *B. napus* (Delourme et al. 2006; Qiu et al. 2006; Wang et al. 2013). The oil content

QTL of our study on A10 were identified close to the common marker MR156-195 (Delourme et al. 2006) and BnGMS625-360 (Wang et al. 2013). Synthesis of erucic acid requires the activity of enzyme encoded by *FAEI* gene that utilizes C18:1 as substrate. Both erucic acid and oleic acid are major component of Brassica oil. Erucic acid QTL have been detected on the upper region of A10 along with oil content QTL by Qiu et al. (2006). It is likely due to presence of *FAEI* on A10, which led to the detection of multiple and stable/common QTL for seed oil content on A10 across different environments of the study.

One major finding of this research is the identification of seed oil content QTL (*qOIL-A10a*; Table 5.3) which explained 26.99% variation in oil content and the positive allele of this QTL was inherited from the low oil, pollen parent Topas. For the remaining oil content QTL, both parents contributed positive as well as negative alleles. This led to transgressive segregation for oil content and values up to 55.5% were observed. Introgression of high oil content alleles in canola cultivars (current average of 44.8% oil content) would result in more seed oil per unit area.

Seed oil quality and total oil content is dependent upon component fatty acids, ultimately affecting its utilization as edible or industrial oil (Abadi and Leckband 2011; Sanyal and Linder 2012). In this study, across four environments, we have identified 33 QTL for palmitic acid content, 18 for stearic acid content, 21 for oleic acid content, 20 for linoleic acid content, 23 QTL for linolenic acid content and 16 QTL for arachidic acid content.

The phenotypic variation explained by palmitic acid content QTL ranged from 5.21% to 32.41%. In *B. napus* Yan et al. (2011) mapped two palmitic acid content QTL N8 (A8) close to a SSR marker (CB10364-237) which controlled major trait variation (34.5%).

Similarly, in our study, the common marker CB10364-237 was linked with QTL, *qC16:0-A8a* (Table 5.4). Furthermore, seven QTL for palmitic acid were identified only in one environment on C2, mostly with positive additive effects. Individually, one QTL explained variation of 21.73% in palmitic acid content. These QTL correspond (common marker O|13G05) to a QTL region (*qOC.C2.2*) as reported by Zhu et al. (2012) on C2 that is detected in hot weather (25 °C to 30 °C) and enhances oil content. Incorporation of these QTL into cultivars could compensate for oil content during growing seasons with above average heat accumulation. However, validation of these QTL is suggested before embarking on any venture.

A marker (JICB0676) of the fatty acid elongase1 (*FAEI*) gene involved in the synthesis of erucic acid is reported to be located at 128 cM on C3 (Smooker et al. 2011). BRMS-287-254 is located 57cM away from JICB0676 (Smooker et al. 2011) , and in our study, it had similar location with respect to a SCAR marker, KSA1-175. The QTL of our study, *qC18:0-C3b* is associated with KSA1-175 and therefore most likely represent is a *FAE* locus. The gene marked by the SCAR marker, KSA1-175 encodes a β -ketoacyl-ACP synthases (KAS) enzyme that has three isoforms which are required for the production of 18 carbon fatty acids in plastids (Somerville et al. 2000).

Scheffler et al. (1997) reported a 30 cM genomic region of chromosome 3 of *A. thaliana* containing *fad2*, *fad5*, and *fad7* genes that is collinear with six regions of *B. napus* present on A1, C1, A3, C3, A5, and C5. All of the oleic acid content QTL identified in the current study are located on these chromosomes with the exception of *qC18:1-C8*. Yan et al. (2011) has reported oleic acid QTL on A8 and C3 (56 cM-78.8 cM) as well. Oleic acid content is a major component of seed oil content in canola (Pavlista et al. 2011). The QTL

qC18:1-C3c (Table 5.6) has a large LOD value up to 16.84, providing evidence of the significance of the QTL. It explained 28.47% of the phenotypic variation in oleic acid content. This QTL could be a promising target to develop custom-tailored cultivars high in oleic acid content.

Yang et al. (2012) recently reported four copies of *FAD2* in *B. napus* on A1, C1, A5, and C5. In agreement with Yang et al. (2012), we found linoleic acid content QTL on A1 A5 and C1 (Table 5.7). A SSR marker CB10403 on A3 was mapped (Yang et al. 2012) in the QTL interval of *OLEA3*. Another linoleic acid QTL, *LAA3* was also reported in close proximity of the common marker (CB10403). In our study, the three linoleic acid QTL on A3 have different positions and were located at about 44.76 cM from the common marker (CB10403). The linoleic acid QTL in our study could potentially be novel based on their map positions.

Yang et al. (2012) reported six copies of *FAD3* gene present equally on A and C genome of *B. napus*. These loci are present on A3, C3, A4, C4, and A5. Likewise, we have identified five and three linolenic acid content QTL, respectively on A3 and C3, and one QTL on A5 (Table 5.8). Chromosome A5 of our map aligns with Yang et al. (2012) where two markers, CB10487 and BRMS007 have similar marker order and were located close to oleic acid and linoleic acid QTL. The Yang et al. (2012) study also has a linolenic acid QTL on A5 at different position compared to our study where *qC18:3-A5* was identified near the bottom of A5. The *qC18:3-A5* explains major phenotypic variation and reduces the content of linolenic acid. This QTL could be an interesting target to reduce the linolenic acid content in canola oil with the goal of improving shelf life. The SCAR marker, WRI1-3-170 was also associated with linolenic acid content, linoleic acid and oleic acid content

QTL. It increased the phenotypic effects for the former two fatty acids while it decreased the content of oleic acid. Therefore, this QTL could be involved in substrate supply for the elongation pathway. The map position related to these unsaturated fatty acids on A2 corresponds to C2 that is reported to contain an oil content QTL by Zhao et al. (2012).

Sixteen arachidic acid content QTL were observed on map positions and LGs known to contain erucic acid content QTL (Qiu et al. 2006; Smooker et al. 2011). The mapping population studied was of canola quality, thus, our population had very low levels of erucic acid. However, the condensation reaction to convert C18 to C22:1 is catalyzed by a protein encoded by *FAEI* (Somerville et al. 2000). Considering the stepwise elongation of carbon chain to form arachidic acid, this collocation between arachidic acid content QTL and erucic acid content QTL is logical. Smooker et al. (2011) identified an erucic acid content QTL (independent marker) on C9 at 7.7 cM that was 10 cM away from our study QTL, *qC20:0-C9* (Table 5.9) which was associated with a SCAR marker (BnFUS3#2-1-9) for arachidic acid.

This research is the first to report two QTL (*rQTL*) on C3 at 147.83 cM and 154.55 cM, which may be affecting the correlation between fatty acids. These *rQTL* are major loci explaining a high proportion of the phenotypic variance for the respective traits. The additive effects (positive or negative) of either of the *rQTL* affect the respective trait's phenotype which forms a pattern that is indicative of the correlation of the fatty acids (Table 5.11). The correlation between fatty acids based on *rQTL* interaction coincides with the phenotypic correlations determined for different fatty acids (Table 5.2). The assumption that these QTL are modulating the correlations between fatty acids is in agreement with Zhao et al. (2007) who observed opposing additive effects for oleic acid content QTL

(negative) and erucic acid content QTL (positive). In our study, these two fatty acids have negative correlation (Zhao et al. 2007).

In the current study, a QTL (*qFLR-C5*) with negative effect on flowering time and a positive effect on oil content (*qOIL-C5b*) collocated on C5. Another, flowering time QTL was associated with the SCAR marker, WRI1-3-170 for *WRINKLED1* that is known to influence on oil content. The contributed allele for both of these QTL was inherited from the late flowering parent (Topas). Together, this suggests a positive correlation between flowering time and seed oil content and this is demonstrated in our results (Table 5.2). The QTL of correlated traits often collocate on the same linkage groups (Ramchiary et al. 2007; Yan et al. 2009). This is in agreement with our results as we detected QTL for flowering time and oil content on A1 or C1, A10, A3 or C3, and C5. In our study, a flowering time QTL, *qFLR-A2c* was identified on A2 in three environments that explained a maximum phenotypic variation of 43.22%. This QTL promoted early flowering and the contributing allele was inherited from the high oil parent Polo, indicating prospects of developing early flowering *B. napus* with improved oil content.

Chromosomal locations marked by flanking markers that affect economically important traits of Brassica such as oil content, fatty acid profile and flowering time have been identified. The identified QTL explaining major variation in traits of their influence such as *qOIL-A10a*, *qC16:0-C2c*, *qC16:0-C8b*, *qC18:0-A1c*, *qC18:1-A3c*, *qC18:2-A2d*, *qC18:3-A3e*, *qC20:0-C3b*, *qFLR-C5*, in addition to *rQTL* appearing on C3 may accelerate the development of canola cultivars with improved oil content and desirable oil profiles with optimal flowering time.

7. SUMMARY/CONCLUSION

The canola crop has an annual economic impact of \$19.3 in Canada (Canola Council of Canada 2014a). The diversified uses of canola and rapeseed oil encourage their increased demand. Therefore, increasing the seed oil content for increased production of oil per unit area remains a focus of many Brassica breeding programs.

A prerequisite of QTL mapping is availability of a genetic linkage map. In the current study, two separate genetic maps were developed from a DH line and the second from a RI line mapping population. These maps were then combined into an integrated genetic map (consensus map). Comparison of the consensus map to the individual maps allowed insight into the genetic structure of both types of populations. This comparison also confirmed that the two maps are equally suited for QTL mapping studies as the marker positions and linkage of 94% of the markers remained unchanged in the consensus map. It was the first attempt to develop an integrated genetic map derived from two types of populations having the same pedigree. The DH line based genetic map provided near complete coverage of the genome, covering 2244.1cM distance with an average marker interval of 3.7 cM. All mapped loci were placed into 19 linkage groups that were anchored to chromosomes for comparison to different published maps and identified QTL. Considering the combination of different molecular marker types used, the genome coverage achieved and number of molecular markers scored, the consensus map developed in this study is therefore, a saturated map, which could serve as a valuable tool for improvement of economically important traits in *B. napus*.

In this research, we found 14 QTL controlling oil content (Table 5.3). Five of these QTL were detected in one environment, one QTL (*qOIL-A10c*) was identified in all four

environments of the study and the remaining oil content QTL appeared in multiple environments (2-3). Environmentally insensitive QTL that are detected in a range of environments are important to breed *B. napus* cultivars with stable oil content (Li et al. 2011). A QTL of this nature identified in our study was *qOIL-A10c*, which controlled a range of phenotypic variation in oil content across different environments. This study further reports three major QTL for oil content. Of these major oil content QTL, the maximum variation of 26.99% in oil content was explained by QTL, *qOIL-A10a*, followed by QTL, *qOIL-C6a* and *qOIL-A10b* that respectively explained 12.65% and 11.45% variation in specific environments. The associated markers of these QTL can be employed to target genomic regions to manipulate oil content in *B. napus*.

In total, 33 QTL were identified for palmitic acid content and two QTL are of particular interest. First, *qC16:0-C9a* explained 12.43% phenotypic variation was linked with a transcription factor *FUS3* (SCAR marker: BnFUS3#2-1-91) that is a major regulator of the seed maturation process. The second, *qC16:0-C2c* increased and explained 21.73% variation in palmitic acid content. The region on C2 containing this QTL is known to contain a oil content QTL (*qOC.C2.2*) that has been characterized and detected only in hot environments and increases oil content in *B. napus* (Zhu et al. 2012). The flanking markers of this QTL on C2 could be valuable to breed cultivars which can perform consistently better under diverse climatic conditions without negative fluctuation in oil content.

We identified 21 oleic acid content QTL, 20 linoleic acid content QTL, and 23 linolenic acid content QTL that colocalized on A1, A2, A3 and C3. Higher than 65% oleic acid content and reduced contents of linoleic acid and linolenic acid are desirable traits in canola cultivars as they improve shelf life and reduced oil rancidity. Findings of this research

related to colocalization of QTL of polyunsaturated fatty acids may be useful in breeding of canola cultivars with specific oil profile. In this context QTL, *qC18:1-A1a&b* and *qC18:3-A1a&b* (likely *FAD3* loci), *qC18:2-A1b* (*FAD2* locus), *qC18:0-A3c* (*FAB2* locus), *qC18:2-A5* (*FAD3* locus) identified in the current study can be employed to develop canola cultivars with desirable oil profile.

For arachidic acid, 16 QTL were identified that contributed to phenotypic variation. Most positions of C20:0 QTL collocated with QTL for other fatty acids studied. One SCAR marker (*BnFUS3#2*) was associated with QTL *qC20:0-C9*. The synthesis of very long chain fatty acids, such as C20:0 and C22:1 is catalyzed by the 3-ketoacyl-CoA synthase enzyme which is encoded by the *FAE1* gene (Puyaubert et al. 2005). The transcription factors FUS3 and ABI3 positively regulate each other and the former affect plastidial fatty acid synthesis and the latter influence fatty acid elongation reaction (Weselake et al. 2009). This SCAR marker, *BnFUS3#2*, can be used to target the *FAE1* gene to increase very long fatty acids during the cultivar development process.

This is the first report of two QTL (*rQTL*) on C3 at 147.83 cM and 154.55 cM that are consistently involved in the detection of QTL for all the studied fatty acids in single or multiple environments. The *rQTL* through their respective additive effects either increase or decrease a target phenotype. The effect of *rQTL* is consistent for all the fatty acids studied. These *rQTL* had higher LOD values and generally explained major phenotypic variation for the respective traits. Further validation and manipulation of these two loci may enable breeders to manipulate the relative contribution of different fatty acids for total seed oil content, thereby altering the oil composition and also the extent of oil content in *B. napus* seed.

Flowering time was affected by 14 QTL across all environments and one QTL, *qFLR-A2c* explained up to 43.22% of the variation for flowering time. The identification of this flowering time QTL on A2 is consistent with earlier studies that report a vernalization QTL on A2 of *B. napus* (Ferreira et al. 1995; Raman et al. 2013). The flowering time QTL of the current study can be utilized for the development of early and later maturing *B. napus* cultivars.

The LGs harboring flowering time QTL in the current study on A1, A10 and C3 have been shown to contain oil content and fatty acid composition QTL in literature. The interaction of flowering time pathways with lipid synthesis can be deduced from the association of the *WRINKLE1* transcription factor (SCAR marker, WRI1-3-170) with a flowering time QTL, *qFLR-A2a*. Another QTL that explains the relationship of flowering time with oil content is *qFLR-C5*. This QTL explained 23.64% variation in flowering time and also affected seed oil content.

The oil content ranged from 31.5% to 55.5% in the progeny of the mapping population with the extremes being significantly lower or higher than the parents. This suggests that the parents harbor allelic variation for oil content and new genetic recombination from the cross (Polo x Topas) unmasked this genetic potential. The oil content of selected lines from the mapping population (55.5%) exceeded the oil content (44%) generally found in canola grown in western Canada (Canadian Grain Commission 2013). This Polo x Topas-derived mapping population could help breeders understand the genetic control of seed oil content in *B. napus* and also introgress the desired variation for oil content into elite canola breeding lines.

The results obtained in this study provide a better understanding of the genetic control of oil content, fatty acid profile and flowering time in *B. napus*. Genomic regions have been identified that are independent or harbor collocalizing QTL for oil content, fatty acid profile and flowering time. Since QTL collocalization suggests genetic interaction (Lionneton et al. 2004), the associated genetic markers for these QTL can serve as important resources for *B. napus* geneticists, physiologists and breeders. The identified independent QTL are also vital for specific trait enhancements. The identified QTL and associated markers offer excellent potential for pyramiding these desired traits into elite *B. napus* breeding lines.

8. RECOMMENDATIONS FOR FURTHER STUDY

Plant species produce 120 different fatty acids. Many of the genes encoding the enzymes required for the function of lipid metabolism have been studied in *Arabidopsis*. Nevertheless, how plants determine total seed oil content is still unknown (Somerville et al. 2000). Critical steps of lipid synthesis and triacylglycerol synthesis in the ER have been manipulated through reverse transgenic studies but no commercial cultivar with enhanced oil content has been released to date. It is assumed that the entire metabolic pathway(s) needs to be regulated to produce consistent oil content enhancement.

Alternative to a biparental segregating mapping population as used in the current study, future QTL studies on oil content can employ association mapping. Association mapping can resolve a QTL position with high resolution that can then be utilized in a breeding program. Genotyping in prospective association mapping studies can make use of recent *B. napus* genome sequence information that annotates 2229 lipid biosynthesis genes in both subgenomes (Chalhoub et al. 2014). This genotyping can shed light on lipid synthesis genes that are more responsive to selection pressure. Additionally, future association mapping studies should be phenotype in a large number of environments (up to eight or more). This may enable accurate QTL analysis and reduce environmental noise, consequently increasing precision in the estimation of a QTL effect and QTL x environment interaction (Xu 2010).

Further research that is required involves the validation of the identified QTL in different genetic backgrounds. Validation of major trait QTL can also be performed by developing Near Isogenic Lines (NILs) from the DH population-Polo x Topas. These NILs may differ only in the QTL region of interest and subsequent phenotyping of such NILs

can help characterize the molecular nature of the QTL. Additionally, the *rQTL* can be fine mapped and characterized to confirm their function. Development of a fine mapping population from the DH population-Polo x Topas and saturation of its genetic map on specific linkage groups (A2, A10 and C3) can be performed using SNP markers. As, recent sequencing of *B. napus* genome has revealed that the single nucleotide differences account for major (86%) allelic differences in *B. napus* from its progenitors (Chalhoub et al. 2014).

Furthermore, the extreme oil content genotypes developed from the DH population-Polo x Topas can be further studied for the differential gene expression during seed development. Many biochemical pathways are involved in the regulation of total seed oil content. How these pathways interact with each other to determine the extent of oil content in various plant ssp. remains relatively unknown. Therefore, a genome wide differential gene expression study between extreme oil content phenotypes could elucidate the regulatory networks of total seed oil content.

Multiple pathways flowering time and lipid biosynthesis exert influence that affect the extent of seed oil content. This influence in the current study is evident through the association of transcription factors with flowering time (SCAR marker, WRI1-3-170) and the QTL, *qFLR-C5* that simultaneously affected flowering time and oil content. The newly available *B. napus* genome sequence can be utilized to further dissect flowering time. This might help to identify the functional genes that are causing such correlations between these two pathways.

Finally, high oil content progeny lines can be crossed with agronomically superior breeding lines to enhance seed yield, seed oil content, desired fatty acid profile and improved adaptability of *B. napus* cultivars.

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10. APPENDICES

Appendix i

Collocation of QTL with their additive effects as marked by the direction of arrows on the respective phenotypes in the doubled haploid population-Polo x Topas of *B. napus*.

LG/Map position	Phenotype							
A1	OC	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	FLR
44.4				↓	↔ <i>FAD3</i> ↔	↑		
46.13				↑		↓		
56.75								↓
60.97			↓		↔ Putative <i>FAE</i> ↔		↓	↑
66.32				↓				↓
70.83			↑		↔ <i>FAE</i> ↔			
73.48				↑			↓	
75.83			↑				↓	
80.01			↑				↑	
92.73					↑			
104.51				↑	↔ <i>FAD2</i> ↔			
112.78				↓				
A2								
20.16				↓	↑	↑		↓
23.41				↑	↓	↓		
26.18				↓	↑	↑		
29.06				↑	↓	↓		
33.54				↑	↓	↓		
40.21				↑				
67.73								↓
71.73				↑				
83.73								↑
A3								
22.00						↑		
37.58						↓		
43.68				↓	↑	↓		
51.53				↑	↑	↓		
55.95				↑	↓	↓		
60.95			↑					
64.12			↓					
67.14			↑	↔ <i>FAB2</i> ↔	↓			
73.69		↑						
129.23	↓							
141.43	↓							
149.11	↑							

Appendix i Continued:

LG/Map position		Phenotype						
A4	OC	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	FLR
	19.14	↑						
A5								
	1.01	↓						
	82.89	↓						
	100.62	↑						
	113.94	↑						
	125.01				↓	↓		
					FAD3			
A8								
	84.24		↑					
	90.96	↓						
	94.43	↑						
	119.35	↓						
A9								
	23.9					↓		
	32.27					↑		
A10								
	4.01	↑						
	10.12	↓				↓		
	11.64	↑	↓			↓		
	17.7	↓	↑					
	19.59					↓		
	22.85	↓						
	26.19	↑						
	32.94	↓						
	42.53							↓
	47.91					↓		↓
	53.06							↑
	60.85							↓

Appendix i Continued:

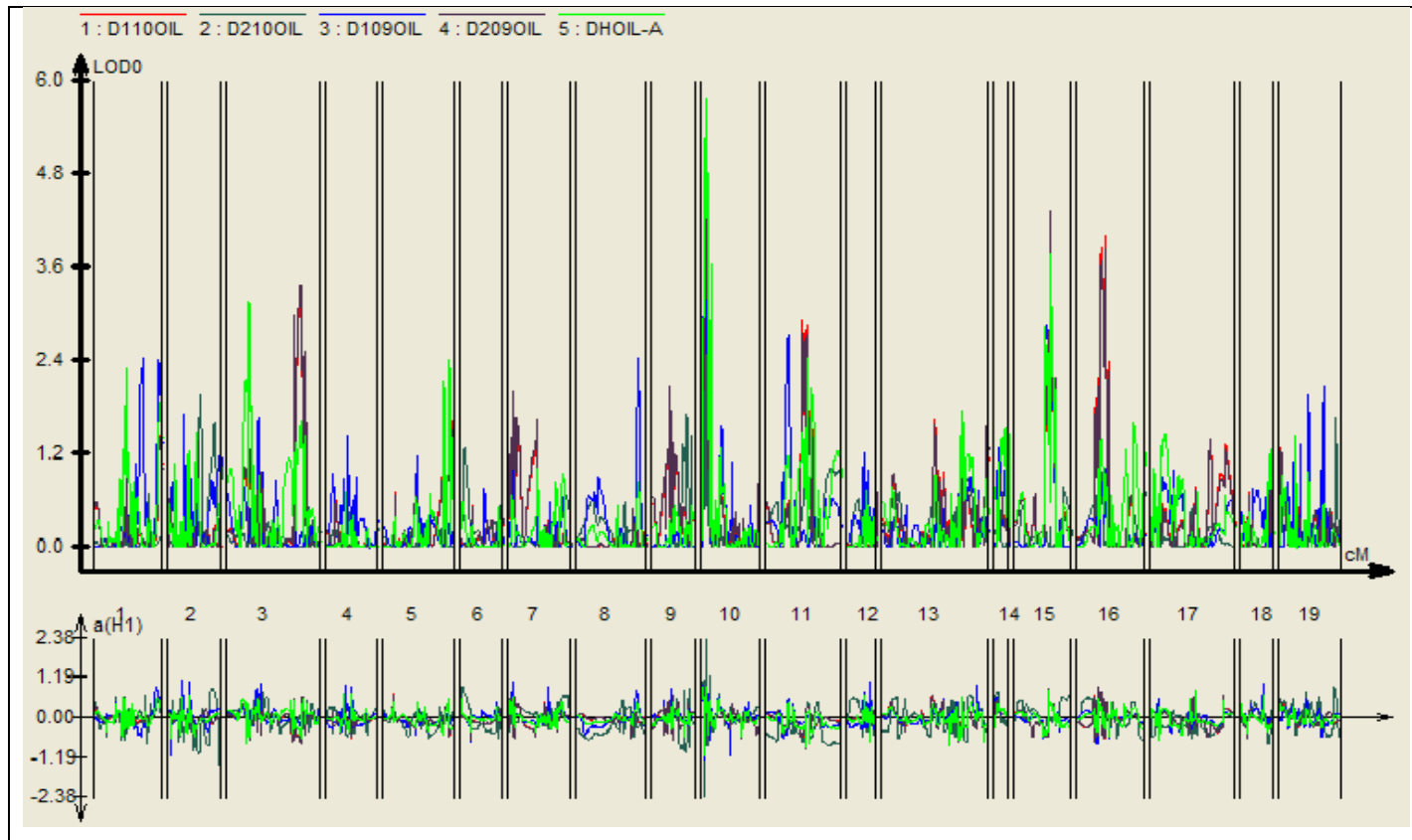
LG/Map position		Phenotype						
C1	OC	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	FLR
42.09	↓		↑				↑	
50.29			↑					
56.29			↓					
68.69	↓							
77.73	↓							
133.91					↓			
147.91							↑	
C2								
35.36		↑						
38.16		↑						
42.65		↑						
45.41		↓						
46.86		↑						
48.72		↓						
51.33		↑						
C3								
115.2							↓	
126.47			↑					
135.16			↑					
144.83		↑	↑	↓	↑	↓	↑	
147.83		↑	↓	↓	↑	↑	↓	
154.55		↓	↑	↑	↓	↓	↑	
157.7					↑		↓	
160.56		↓						
168.71								↑
185.71								↓
188.15		↑						
206.02								↓

Appendix i continued:

LG/Map position		Phenotype							
C5		OC	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	FLR
	23.81							↓	
	62.98	↓	↑						
	67.12	↑	↓						↓
C6									
	45.54	↑							
	53.76	↓							
	59.12						↑		
C8									
	0.01			↑				↑	
	7.42			↓					
	18.78							↓	
	43.78	↑							
	46.78	↓							
	54.98	↑	FAB2			↓			
C9									
	0.01					↓	FAE		
	8.4					↓	↓		↓
	19.05	↓							
	41.75					↓			
	87.77	↓							

Appendix ii

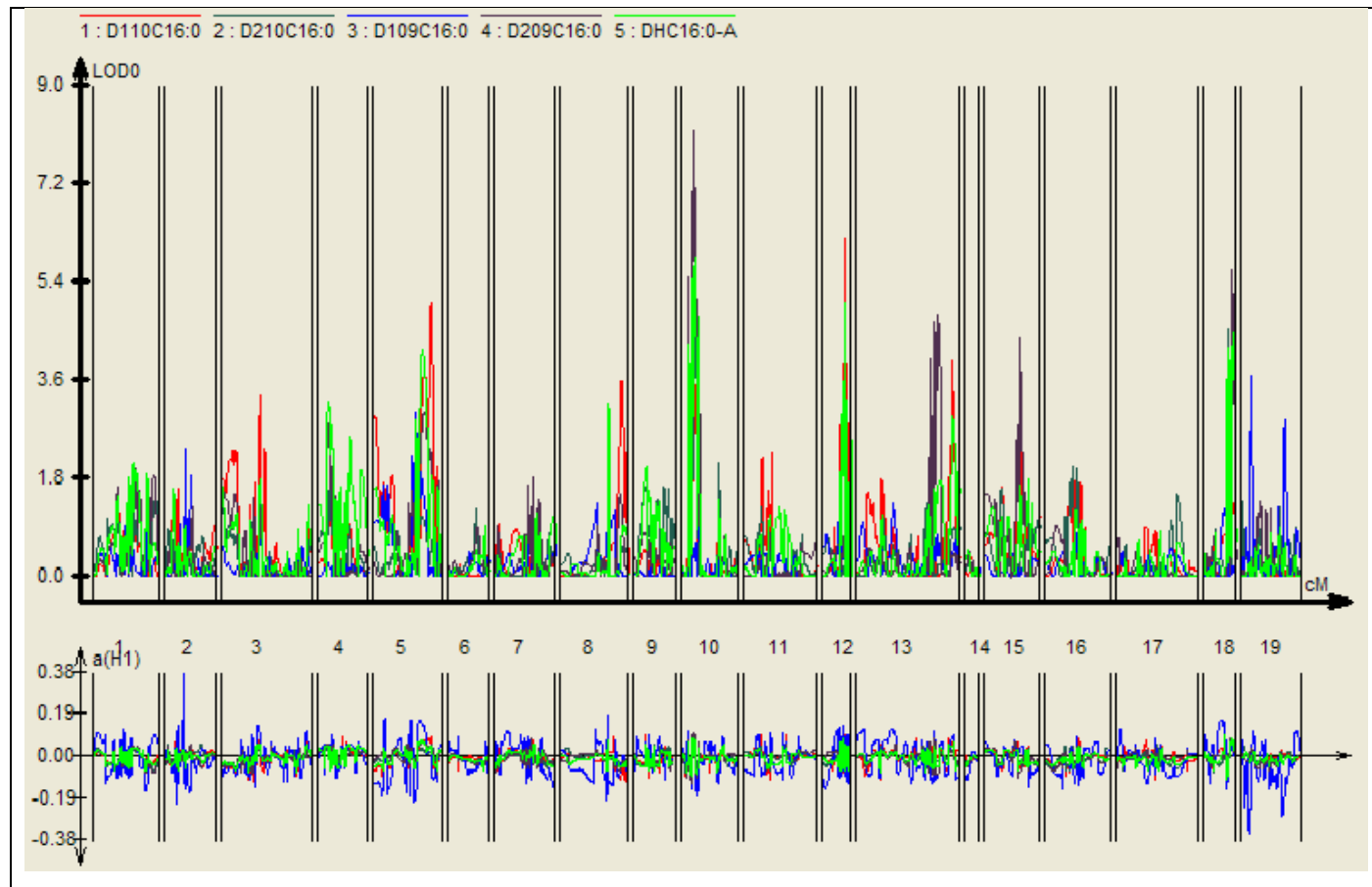
QTL detection graphs for oil content. The graphs were generated through Window QTL Cartographer version 2.5 using the composite interval mapping method for four locations and average trait value. Y axis: LOD value (threshold of QTL detection 2.5), X axis: Linkage groups.



1 : Winnipeg 2010, 2 : Portage La Prairie 2010, 3 : Winnipeg 2009, 4 : Portage La Prairie 2009, 5 : Trait average.

Appendix iii

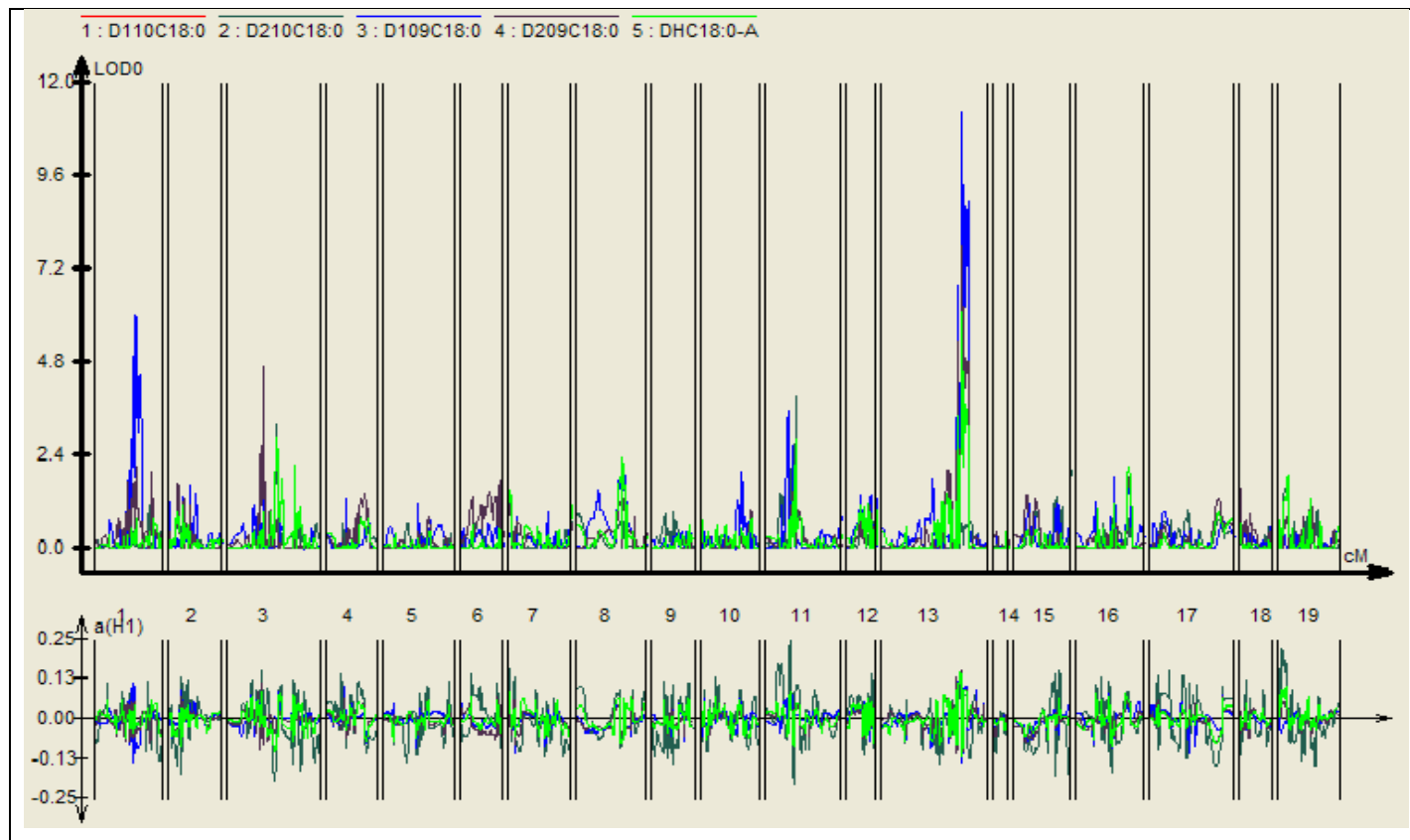
QTL detection graphs for palmitic acid content. The graphs were generated through Window QTL Cartographer version 2.5 using the composite interval mapping method for four locations and average trait value. Y axis: LOD value (threshold of QTL detection 2.5), X axis: Linkage groups.



1 : Winnipeg 2010, 2 : Portage La Prairie 2010, 3: Winnipeg 2009, 4 : Portage La Prairie 2009, 5 : Trait average.

Appendix iv

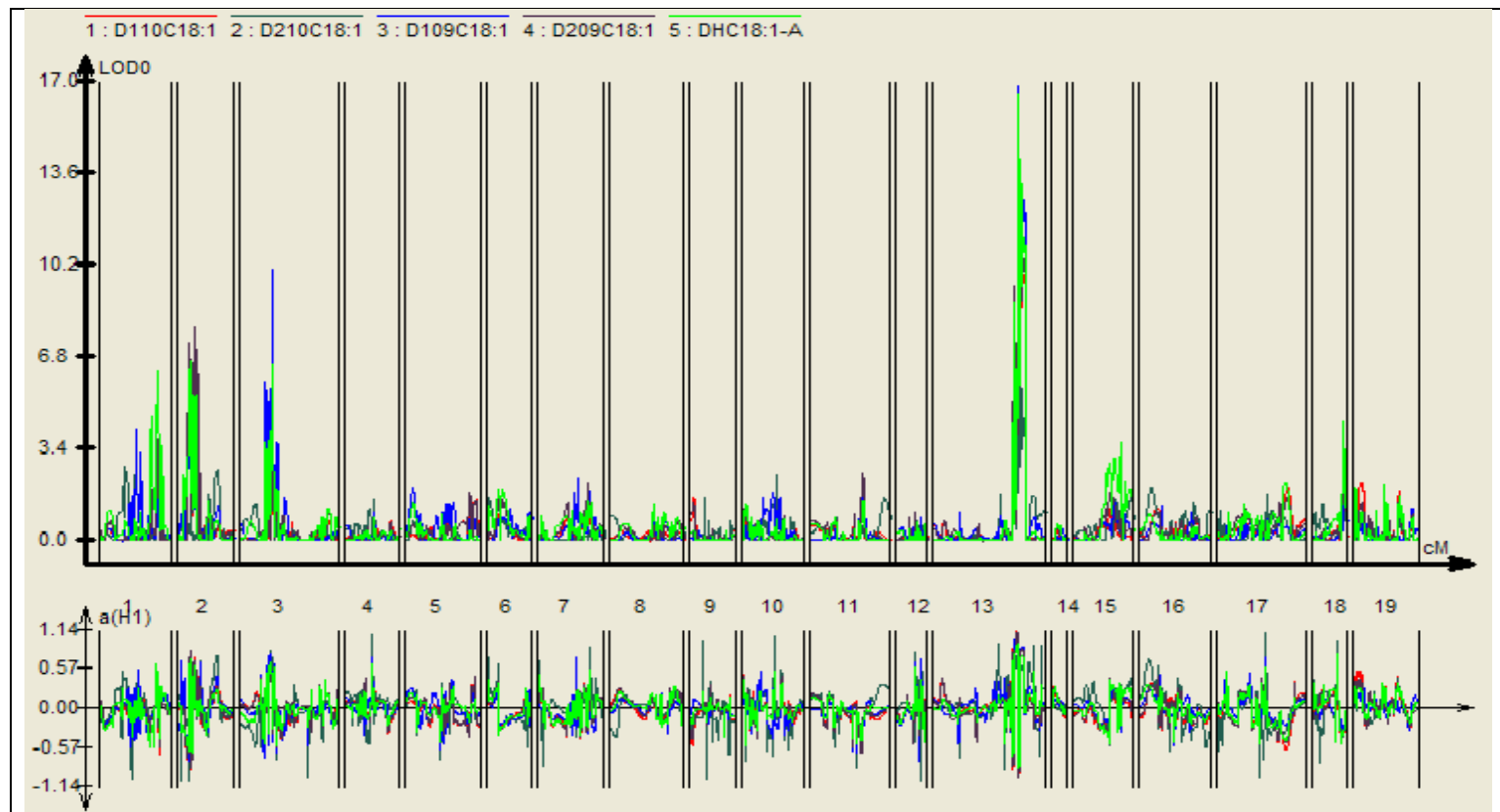
QTL detection graphs for content of stearic acid. The graphs were generated through Window QTL Cartographer version 2.5 using the composite interval mapping method for four locations and average trait value. Y axis: LOD value (threshold of QTL detection 2.5), X axis: Linkage groups



1 : Winnipeg 2010, 2 : Portage La Prairie 2010, 3: Winnipeg 2009, 4 : Portage La Prairie 2009, 5 : Trait average.

Appendix v

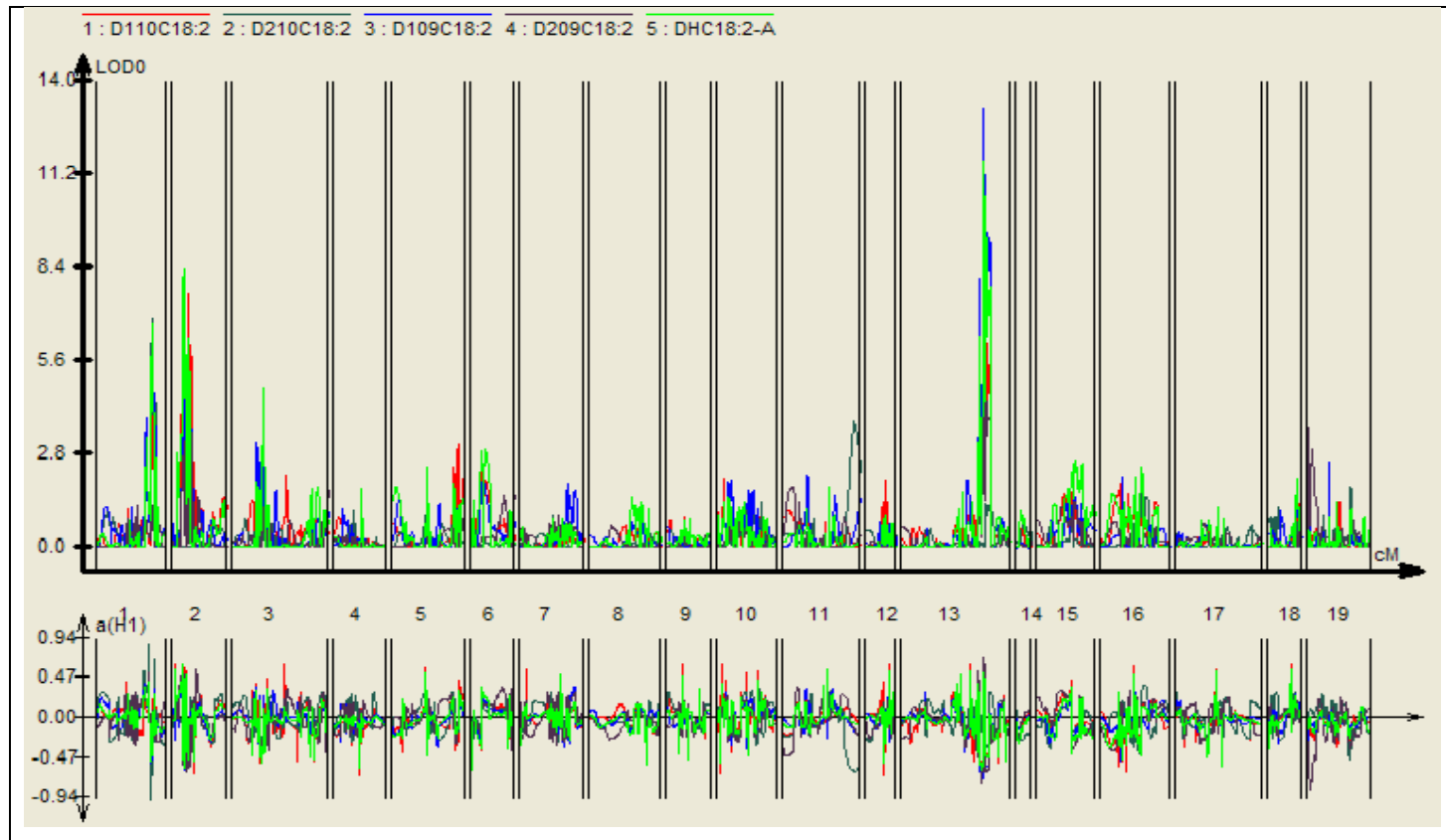
QTL detection graphs for content of oleic acid. The graphs were generated through Window QTL Cartographer version 2.5 using the composite interval mapping method for four locations and average trait value. Y axis: LOD value (threshold of QTL detection 2.5), X axis: Linkage groups.



1 : Winnipeg 2010, 2 : Portage La Prairie 2010, 3: Winnipeg 2009, 4 : Portage La Prairie 2009, 5 : Trait average.

Appendix vi

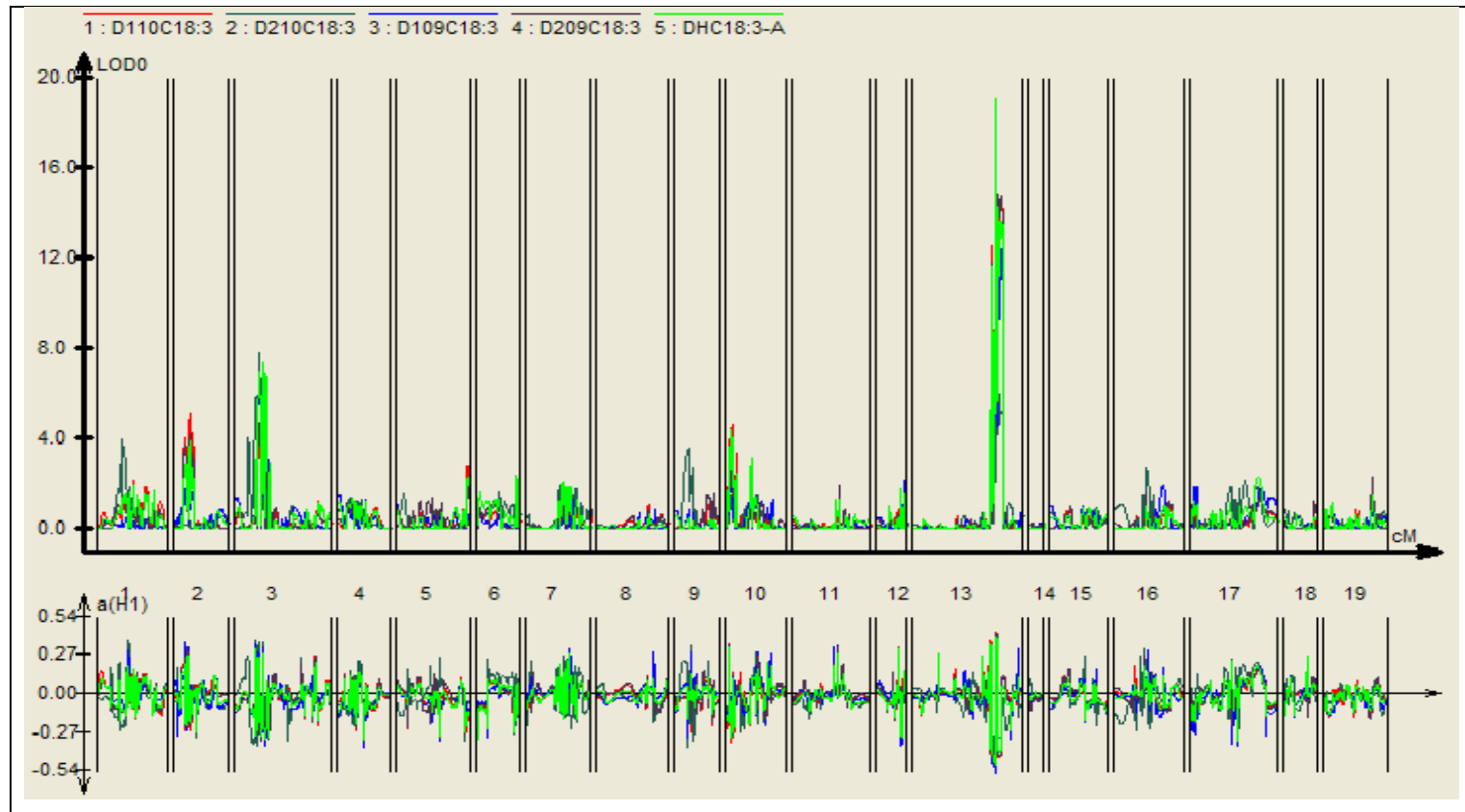
QTL detection graphs for content of linoleic acid. The graphs were generated through Window QTL Cartographer version 2.5 using the composite interval mapping method for four locations and average trait value. Y axis: LOD value (threshold of QTL detection 2.5), X axis: Linkage groups.



1 : Winnipeg 2010, 2 : Portage La Prairie 2010, 3: Winnipeg 2009, 4 : Portage La Prairie 2009, 5 : Trait average.

Appendix vii

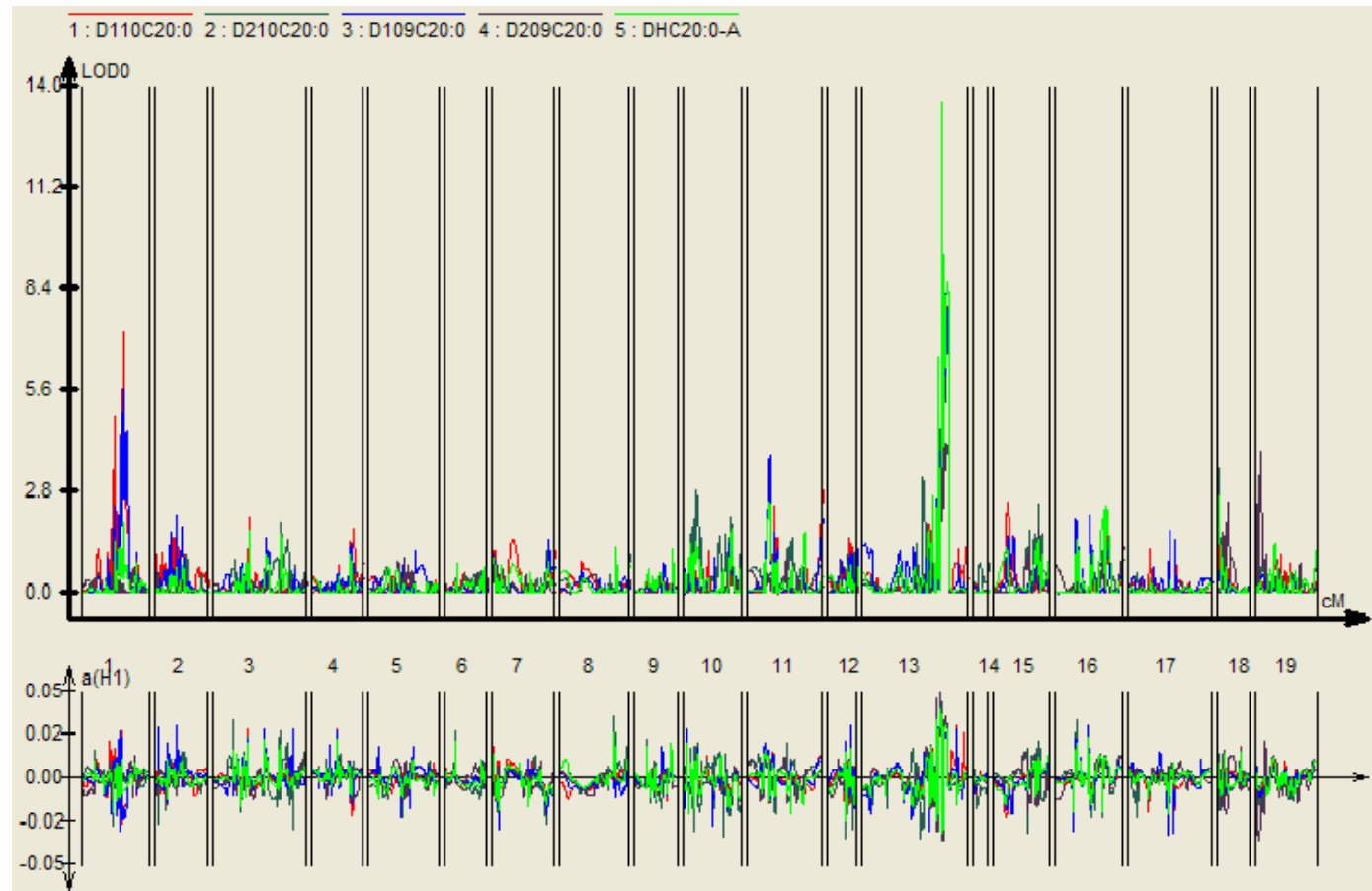
QTL detection graphs for content of linolenic acid. The graphs were generated through Window QTL Cartographer version 2.5 using the composite interval mapping method for four locations and average trait value. Y axis: LOD value (threshold of QTL detection 2.5), X axis: Linkage groups.



1 : Winnipeg 2010, 2 : Portage La Prairie 2010, 3: Winnipeg 2009, 4 : Portage La Prairie 2009, 5 : Trait average.

Appendix viii

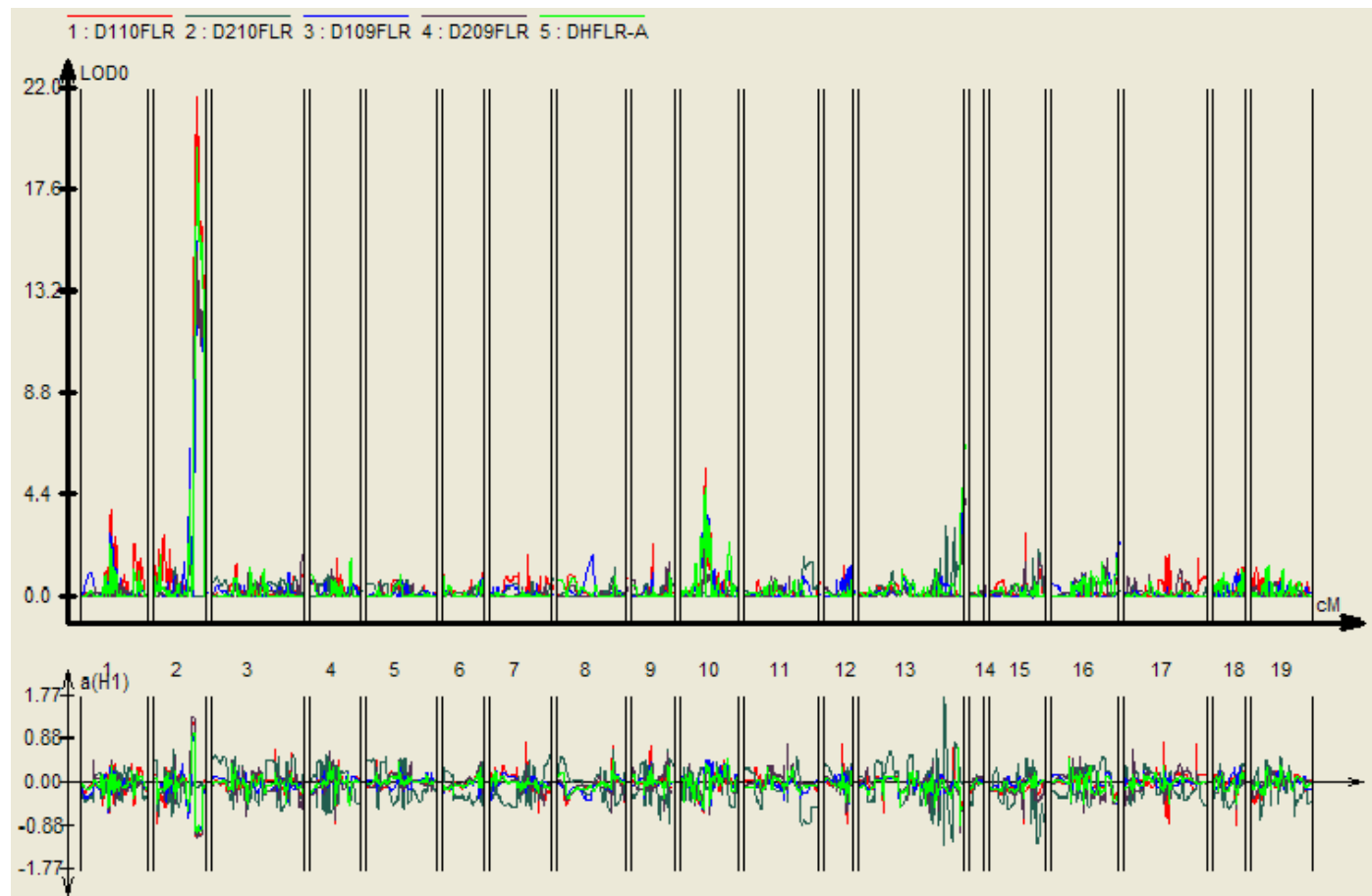
QTL detection graphs for content of arachidic acid. The graphs were generated through Window QTL Cartographer version 2.5 using the composite interval mapping method for four locations and average trait value. Y axis: LOD value (threshold of QTL detection 2.5), X axis: Linkage groups.



1 : Winnipeg 2010, 2 : Portage La Prairie 2010, 3: Winnipeg 2009, 4 : Portage La Prairie 2009, 5 : Trait average.

Appendix ix

QTL detection graphs for days to flowering. The graphs were generated through Window QTL Cartographer version 2.5 using the composite interval mapping method for four locations and average trait value. Y axis: LOD value (threshold of QTL detection 2.5), X axis: Linkage group.



1 : Winnipeg 2010, 2 : Portage La Prairie 2010, 3: Winnipeg 2009, 4 : Portage La Prairie 2009, 5 : Trait average.