University of Alberta

Broadening genetic diversity in canola (*Brassica napus*) germplasm using the *B. oleracea* var. *alboglabra* C-genome

by

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Dedication

To the late Dr. Mohan Thiagarajah, whose passion for teaching, love of plant science, and kind human nature has influenced many. His influence lives on through all who had the privilege of knowing him.

Abstract

Genetic diversity in spring canola (*Brassica napus* L., AACC genome, *2n*=38) cultivars is narrow. Limited effort has been made to utilize genetic diversity from the diploid species *B. oleracea* (CC genome, *2n*=18), apparently due to the difficulty of producing *B. napus* × *B. oleracea* hybrids as well as lack of canola quality traits in seeds of this species. This Ph.D. research investigates the potential of genetic diversity of the C-genome of *B. oleracea* for the improvement of spring oilseed *B. napus*. Inbred lines were developed from F_1 and BC_1 plants of canola *B. napus* × *B. oleracea* var. *alboglabra* interspecific crosses through pedigree breeding. These populations were assessed for seed quality, effectiveness of selection based on morphological traits, genetic diversity using simple sequence repeat (SSR) markers, and ploidy levels using flow cytometry and cytological analysis of meiotic chromosomes. Heterotic potential of the F_8 lines were evaluated, and compared with two populations derived from winter × spring and spring × spring *B. napus* crosses. In addition, an early flowering F_5 line was used to generate a doubled haploid population for phenotypic and genetic analysis of the early flowering allele(s) of *B. oleracea* introgressed into *B. napus*.

Erucic acid content in the *B. napus* × *B. oleracea* interspecific plants depended on genotype at the C-genome locus (C^+C^+, C^+C^0, C^0C^0) as well as dosage effect of the zero-erucic allele from *B. napus* A-genome; and F_2 segregation deviated significantly from the 3:1 ratio based on disomic segregation of C-genome erucic acid alleles. Low glucosinolate plants were achieved from a relatively small segregating population, due to simpler segregation of high and

low glucosinolate alleles in C-genome only. Surprisingly, all inbred lines resulting from self-pollination of F_2 and BC_1 plants stabilized to *B. napus* type – none were found to have *B. oleracea* ploidy. On a population basis, hybrid yield of the inbred lines of *B. napus* × *B. oleracea* and winter × spring crosses did not deviate significantly from the tester parent Hi-Q; however, a number of lines displayed significant mid- and high-parent heterosis. No correlation was found between genetic distance of the inbred lines from Hi-Q and mid- or high-parent heterosis for seed yield. Two publicly available SSR markers, sR10417 and Ol13-G05, previously mapped to the *B. napus* chromosome N12, were found to be associated with the early flowering allele of *B. oleracea* introgressed into *B. napus*. In addition, two SSR markers, SSR-617 and SSR-129, were strongly associated with a QTL for high glucosinolate content originating from the C-genome of *B. oleracea*, presumably residing on the *B. napus* chromosome N19.

The findings from this Ph.D. research project suggested that it is feasible to introgress genetic diversity from the C-genome of *B. oleracea* into a canola quality and euploid *B. napus* background; and this diversity has great potential for improving yields of hybrid canola, as well as introducing alleles for improvement of specific traits such as earliness of flowering.

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Symbols and Abbreviations

χ^2	Chi-square test statistic
µE	Microeinstein
µmol g^{-1}	Micromoles per gram
A.U.	Arbitrary units
AFLP	Amplified Fragment Length Polymorphism
BC_1	First backcross generation
BC_1S_n	n^{th} self-pollinated generation after backcross
BnFRI	*Brassica napus FRIGIDA* gene
C^+	High erucic acid allele from *B. oleracea* C-genome
C^0	Recessive erucic acid allele from *B. napus* C-genome
cM	Centimorgans
CQ	Canola quality
C^w	Dominant white petal colour allele from *B. oleracea* C-genome
C^y	Recessive yellow petal colour allele from *B. napus* C-genome
DH	Doubled haploid
DTF	Days to flower
DTM	Days to maturity
F_1	First filial generation
F_n	n^{th} filial generation
GC	Gas chromatography / Growth chamber
GH	Greenhouse
GLS	Glucosinolate
HPH	High-parent heterosis
IN	Inbred lines derived from *Brassica napus* × *B. oleracea* interspecific cross
MPH	Mid-parent heterosis
N1 to N10	*B. napus* chromosomes corresponding to *B. rapa* A-genome
N11 to N19	*B. napus* chromosomes corresponding to *B. oleracea* C-genome
NIRS	Near-infrared spectroscopy
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
PMC	Pollen mother cell

QTL	Quantitative Trait Loci
RIL	Recombinant inbred line
RS	Resynthesized (rapeseed)
SS	Inbred lines derived from spring × spring cross
SSR	Simple sequence repeat (microsatellite)
t	Test statistic for t-test
UPGMA	Unweighted pair-group method with arithmetic mean
v/v	Volume to volume ratio
w/v	Weight to volume ratio
WS	Inbred lines derived from winter × spring cross

Chapter 1

Literature review

1.1 Introduction

Canola is a Canadian success story – it is a "Made in Canada" crop and is an achievement of the Canadian research community (Canola Council of Canada 2011). Rapeseed was first introduced to Canadian growers to fill a critical need for the oil as a lubricant for marine engines during World War II (Canola Council of Canada 2003). Beginning in the early 1970s, plant breeders developed rapeseed cultivars well adapted to Canadian growing conditions with anti-nutritional components erucic acid in oil and glucosinolate in seed meal largely removed. The public research sector, viz. Agriculture and Agri-Food Canada and the University of Manitoba, led the development and release of these early successful 'Argentine' (*Brassica napus* L.) and 'Polish' (*B. rapa* L.) cultivars. For example, the first erucic acid-free strain of *B. napus* was isolated in Winnipeg by Stefansson et al. (1961) from the German *B. napus* cultivar 'Liho'; and the first erucic acid-free strain of *B. rapa* was isolated in Saskatoon by Downey (1964). Finlayson et al. (1973), also in Saskatoon, reported the first low glucosinolate (total glucosinolate 10 μmol g^{-1} seed) genetic variation found in the *B. napus* Polish fodder cultivar 'Bronowski'. Alleles from this cultivar have been introduced into oilseed *Brassica*, and virtually all double-low cultivars used today carry the 'Bronowski' alleles. Canola breeding research was initiated at the University of Alberta in 1969. Since then, it has played a crucial role in the success of the canola industry in Alberta. To date, it has contributed an estimated $276 million to Alberta's economy (*Brassica*Corp Ltd. 2005). Due to its nature as a public research institution, it is able to devote greater attention to high risk and long term research than is possible in the private sector, for the benefit of the canola industry in Western Canada. Such initiatives include efforts such as broadening the genetic base of spring *B. napus* through interspecific hybridization with allied species (*Brassica*Corp Ltd. 2005).

In context of the foregoing introduction, this Ph.D. research project addresses a basic and pressing need to broaden genetic diversity in *B. napus*. Specifically, it explores the relatively unexploited C-genome of *B. oleracea* L. as a potential source of genetic diversity for long term improvement of spring canola. Alleles from the C-genome species with a positive effect on early maturity, disease resistance, high levels of heterosis, etc. in *B. napus* could potentially be introduced through interspecific hybridization. The following sections outline the importance of canola (rapeseed) worldwide and in Canada, as well as the status of genetic diversity in rapeseed crops both locally and abroad. They also provide a context for the research by giving an outline of genome relationships among the economically important *Brassica* crops and the challenges and opportunities associated with interspecific hybridization. Finally, the enormous benefits to the canola industry that could result from this research – including heterotic potential in hybrid breeding and genetic improvements for early flowering cultivars – are discussed.

1.2 Importance of canola (rapeseed)

1.2.1 Oilseed rape worldwide

Rapeseed (*B. napus* and *B. rapa*) is one of the most important oilseed crops in the world. It is second only to soybean in terms of overall world production (Fig. 1-1a), where about 58.4 million t were produced worldwide in 2010 (American Soybean Association 2011). It is of particular importance in temperate regions of the world such as Northern Europe, China, and Canada. In 2010, Canada produced approximately 11.8 million t of rapeseed, around 20% of the world total – only less than EU member states (36%) and China (22%) (USDA 2010). Canola oil is widely used for human consumption worldwide; while the residual meal is generally used as a high protein livestock feed supplement. In addition, rapeseed oil has a number of industrial applications. For example, High Erucic Acid Rapeseed (HEAR) oil is used in the production of erucamide, an additive used to prevent film adhesion in manufactured plastics (McVetty and Scarth 2002).

1.2.2 Canola production in Canada

Canola (rapeseed) is the most important oilseed crop in Canada, where 7.1 million ha were seeded in 2010 (Statistics Canada 2011; Fig. 1-1b). Canola annually generates an estimated $4.3 billion in direct economic benefit, and $13.8 billion when indirect benefits are included (Mark Goodwin Consulting Ltd. 2008). The term “canola” is derived from “Canadian oil”, and refers to rapeseed with specific oil and meal quality characteristics. The official definition of canola is rapeseed having less than 2% erucic acid in the oil and less than 30 µmol glucosinolates per gram of the solid component of the seed (Canola Council of Canada 2003). Canada is the single largest canola producer in the world (Canola Council of Canada 2011). The canola species *B. napus* accounts for almost all canola grown in Canada. For example, over 99% insured acres of canola in Alberta in 2010 were *B. napus* cultivars (Agricultural Financial Services Corporation 2010).

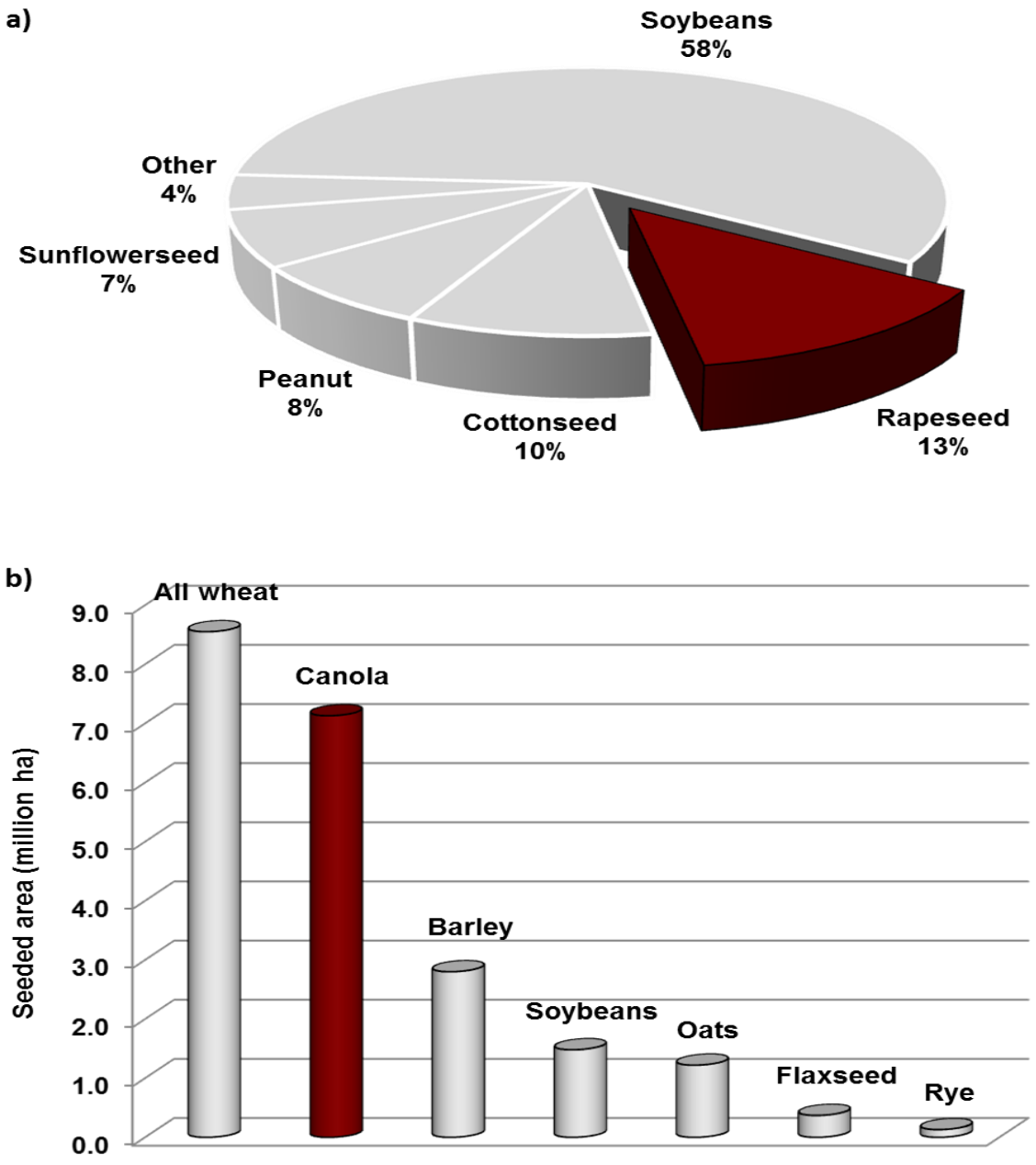

Figure 1-1. a) World oilseed production, 2010. Adapted from Soy Stats (American Soybean Association 2011); b) Relative importance of canola in Canada among agricultural field crops in 2010 (Statistics Canada 2011).

1.3 *Brassica* genome relationships

1.3.1 Origin of *Brassica* species

The species relationships in the genus *Brassica* were first postulated based on cytological studies conducted by Morinaga (1934) and U (1935). These researchers demonstrated the interrelationships between the diploid and tetraploid *Brassica* species (Fig. 1-2). The genomes of the diploid species were designated as 'A' for *B. rapa* (*n*=10), 'B' for *B. nigra* W. D. J. Koch (*n*=8), and 'C' for *B. oleracea* (*n*=9). The amphidiploid species *B. napus* (AC genome, *n*=19), *B. juncea* (L.) Czern. (AB genome, *n*=18), and *B. carinata* A. Braun (BC genome, *n*=17) are hypothesized to be the result of natural crossing between these diploid species. This has been verified through experimental synthesis of the amphidiploids from interspecific crosses between the diploid species followed by chromosome doubling by Frandsen (1943, 1947) and Olsson (1960). Frandsen (1943, 1947) also found that the synthetic amphidiploids are able to produce fertile offspring in crosses with natural amphidiploids of the same species.

While the exact origin of the cultivated diploid *Brassica* species is debated, theories exist based on geographical, historical, ecological, and genetic evidence. *Brassica oleracea* var. *sylvestris* is believed to be the most primitive form of *B. oleracea*, and it inhabits the coastal rocky cliffs from the Mediterranean to England (Prakash and Hinata 1980, Babula et al. 2007, Fig. 1-3). It is believed that domestication and adaptation have resulted in the various 'cole crop' varieties of this species, viz. cabbage, cauliflower, kale, broccoli, kohlrabi, and Brussels sprouts. The native habitat of *B. rapa* forms is a wide distribution, ranging from the Near East and Central Asia to Western Russia and throughout Europe (Prakash and Hinata 1980, Quijada et al. 2007, Fig. 1-3). Leafy forms of *B. rapa* seem to have been domesticated in China, Japan, and Korea (Hirai and Matsumoto 2007), while oleiferous forms originated from Europe and the area of northwest India (Quijada et al. 2007). Very little information is available on the origin of *B. nigra*; it is assumed to have come from the areas where it is

distributed in nature, viz. the Ethiopian plateau, Europe, and the Mediterranean (Prakash and Hinata 1980, Fig. 1-3).

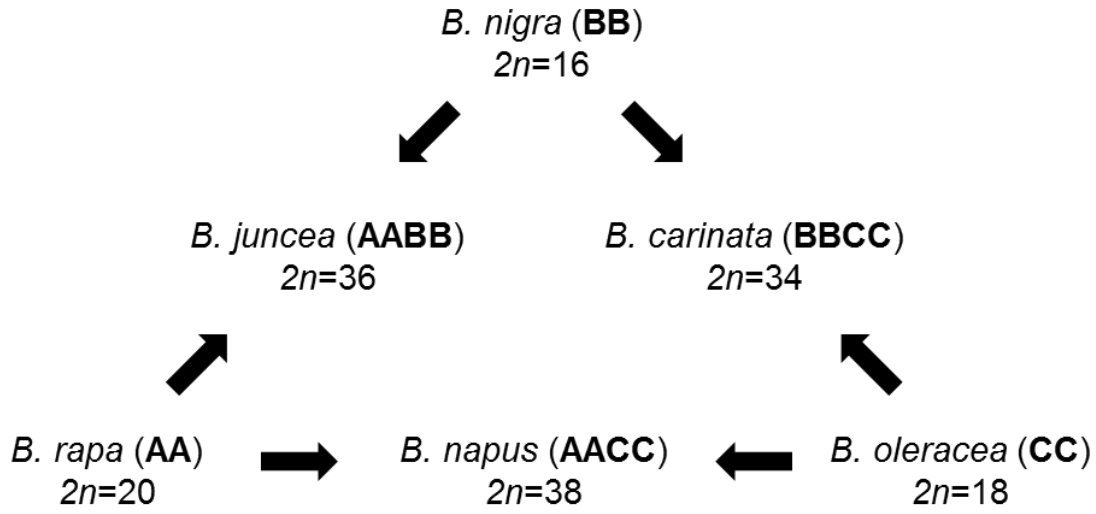

Figure 1-2. Interrelationships between diploid and tetraploid *Brassica* species, as set forth by U (1935), commonly known as the 'Triangle of U'.

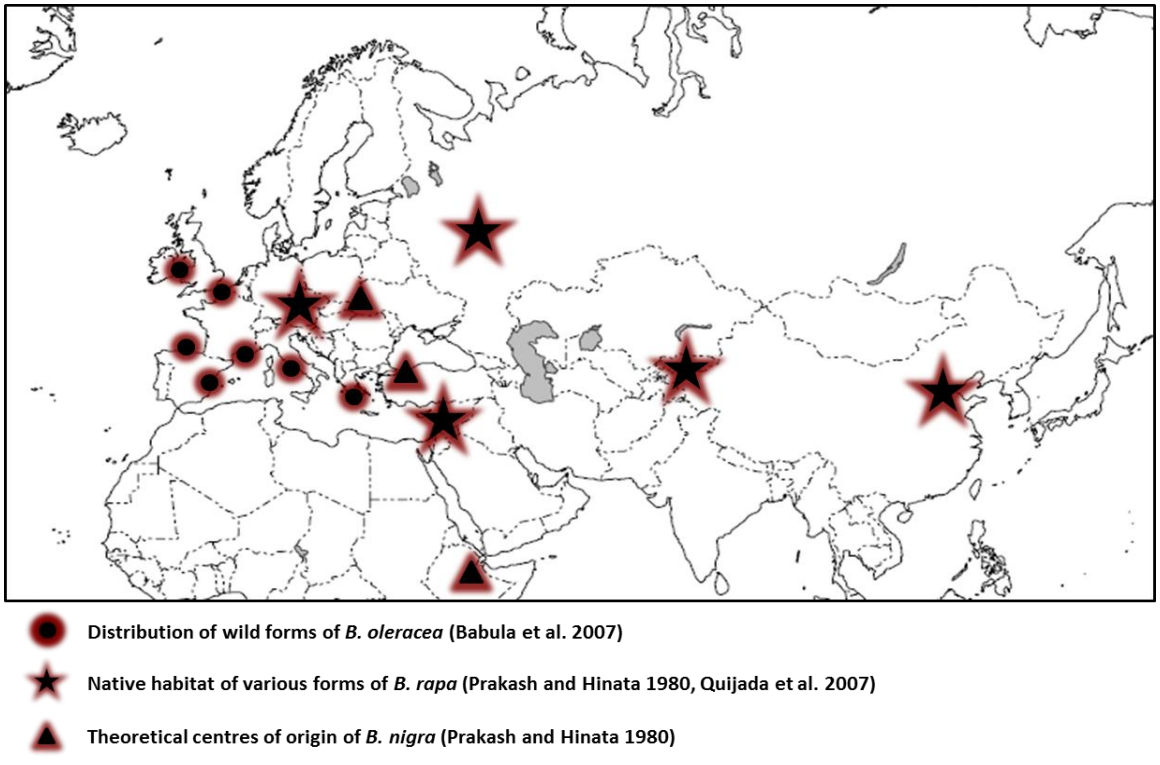

Figure 1-3. Hypothesized geographic origins of the diploid species *Brassica oleracea*, *B. rapa*, and *B. nigra*.

1.3.2 Diploid *Brassica* genomes

The cultivated *Brassica* species are the closest crop relatives to the model dicot plant *Arabidopsis thaliana*, both of which are members of the Brassicaceae (mustard) family. The phylogenetic distance of *Arabidopsis* and *Brassica* has been inferred using nucleotide sequence variation of two nuclear and chloroplast genes, and suggested that the two diverged approximately 20 million years ago (Mya) (Koch et al. 2001). This apparently was followed by the separation of the *nigra* (B genome) and the *rapa/oleracea* (A and C genome) lineages (Warwick and Black 1991), about 7.9 Mya (Lysak et al. 2005). Analysis of meiotic chromosomes in AC, AB, and BC amphihaploids showed high amount of pairing among A and C genome chromosomes, while only limited pairing occurred in those involving the B genome (Attia and Röbbelen 1986). This study suggests a closer relationship between the A- and C- genomes than either has with the B-genome.

The genomes within each of the three diploid *Brassica* species, viz. *B. oleracea*, *B. rapa*, and *B. nigra*, are known to be highly replicated; that is, multiple homologous regions occur within the genome of each species. Duplication of genes, whether individually, as chromosomal segments, or at the whole genome level (polyploidy), is thought to be a major contributor to both genetic and phenotypic diversity in plant species (Schranz et al. 2002). Although it is apparent that the diploid *Brassica* genomes have evolved from an ancient progenitor, the exact mechanism is still controversial. This includes basic chromosome number of a common progenitor, number and extent of genomic rearrangements that have occurred before or after species differentiation, and the extent to which orthologous genes have retained the same function.

Based on chromosome pairing in haploid *B. rapa*, *B. oleracea*, and *B. nigra* it has been proposed that the diploid species arose from a progenitor species of basic chromosome number of x=6 (Catcheside 1934, Röbbelen 1960, Armstrong and Keller 1981, 1982). However, not all researchers were as definitive about the

hypothesized basic chromosome complement of *x*=6, such as Truco et al. (1996) who deduced an ancestral genome of *x*=5 to *x*=7 based on comparative maps of each of the diploid species.

Lagercrantz and Lydiate (1996) performed a comparative analysis on the diploid species genomes using a common set of RFLPs, and found evidence that the diploid *Brassica* species result from genome-wide triplication of a common ancestor because many loci are present in three copies in these species. These researchers also suggested that the different chromosome numbers (8, 9, and 10) in the diploid species are likely the result of chromosome fission and fusion events, rather than addition/deletion of entire chromosomes. Based on key flowering time genes *CO* and *FLC*, Axelsson et al. (2001) found evidence to support the hypothesis of triplication of a common ancestral genome, as three homoeologous regions in each of the diploid *Brassica* species was found, corresponding to a single copy in *Arabidopsis*. However, although Lukens et al. (2004) found strong evidence of genome replication and rearrangement; these researchers found little evidence for genome triplication.

1.3.3 Amphidiploid *Brassica* species

Using guard cell size to estimate cellular DNA content of fossilized woody angiosperms, Masterson (1994) estimated that ~70% of angiosperms (flowering plants) have resulted from ancient polyploid events. At very least, this study confirms that polyploidy has been an important mechanism for the development of many of the angiosperm genomes we study today. Evidence suggests that the three diploid species *B. nigra*, *B. oleracea*, and *B. rapa* all derive from polyploidization events, and are secondary polyploids (Catcheside 1934, Röbbelen 1960). The formation of the amphidiploid species *B. napus*, *B. carinata*, and *B. juncea* through fusion of the diploid genomes is believed to be much more recent (Olsson 1960). Song et al. (1988) used RFLPs to study the phylogenetic origins of these six species, and confirmed that the three amphidiploid species were indeed the result of hybridizations between the three diploid species. They

suggested that the amphidiploid species, particularly *B. napus* and *B. juncea*, are of polyphyletic origin derived from different morphotypes of their diploid progenitors. The exact identity of the diploid parents involved in the formation of the amphidiploid *Brassica* species is unknown.

It is uncertain as to the level of change in genome structure that has occurred after the formation of each of the amphidiploid species *B. carinata*, *B. juncea* and *B. napus*. A study of genome organization of the A and B-genomes of *B. juncea*, as revealed by two RFLP maps, indicated that the marker order was highly conserved among the amphidiploid and diploid species (Axelsson et al. 2000). Likewise, using a set of 44 common markers between the *B. napus* and *B. rapa* A-genome, Suwabe et al. (2008) also found a high level of colinearity between corresponding genomes in these species. Based on the inheritance pattern of RFLP alleles from a cross between resynthesized (from *B. rapa* × *B. oleracea* cross) and natural *B. napus*, Parkin et al. (1995) suggested that very little change has occurred in the A and C genome of this amphidiploid since the initial fusion of the two diploid genomes.

On the other hand, Song et al. (1995) generated homozygous synthetic polyploids of *B. juncea* and *B. napus*, and found a wide range of changes in nuclear genomes, based on RFLP fragment patterns during the self-pollinated generations. These researchers hypothesized that such rapid changes may be due to instabilities created by interactions between diverse genomes. They also found twice as many changes in the AB as compared to the AC polyploid; and since the A and C-genome are more closely related, suggested that higher frequencies of genome change may be associated with divergence of the involved genomes. Most studies on genotypic and phenotypic effects of polyploidy in the amphidiploid *Brassica* species involved the generation of synthetic allopolyploids, followed by self-pollination or crosses with the corresponding natural form. In a review of these studies, Lukens et al. (2004) suggested that the rate of homoeologous recombination in resynthesized polyploids is typically much higher than is found in natural forms, which would partially explain the patterns observed by Song et al. (1995).

1.3.4 Practical implications

Understanding the level of homology for various chromosomes among related species can provide information on the likelihood of success of homoeologous recombination and successful gene transfer. Interspecies gene transfer is an important tool for *Brassica* breeders, as the Brassicaceae family includes species that are adapted to a very diverse number of habitats and possess many unique traits. Further, genomic tools from the model dicot species *Arabidopsis thaliana*, a member of the Brassicaceae family, can and have been exploited for the benefit of *Brassica* research (Parkin et al. 2005). Comparative genome analyses have also allowed researchers to estimate the number and degree of chromosomal rearrangements that have occurred among species, and this information is valuable for inferring the evolutionary history and assigning taxonomic characterization to these species.

1.4 Genetic diversity

1.4.1 Value of genetic diversity in crop species

Genetic diversity in breeding materials is essential for crop improvement – not only to enable continued yield improvement but also for adaptation of our crop species to factors such as local climate, disease pressure, etc. There is a need for broadening genetic diversity in a number of crop species, such as among United States rice (*Oryza sativa* L.) (Xu et al. 2004) cultivars and common bean (*Phaseolus* spp.) cultivars (Singh 2001). Broadening the genetic base of a crop species also helps to prevent dangers imposed due to genetic vulnerability – the large-scale susceptibility of genetically similar varieties to major losses imposed by biotic or abiotic stresses (Acquaah 2007). For these reasons, maintaining a broad genetic base in breeding materials is an important objective of plant breeders and plant geneticists (Kang 1997).

Many crops of economic importance have a narrow genetic base, particularly in the industrialized agricultural systems of developed countries,

where a large proportion of these crops are genetically similar lines or F_1 hybrid varieties (Cooper et al. 2001). Today, plant breeders are often under pressure to develop improved varieties in the short term. For this, breeders often use a set of elite material in crossing programs, and broadening the genetic base of breeding materials becomes a low priority (Cooper et al. 2001). For example, the large-scale replacement of open-pollinated and 'farmer' cultivars of maize with a few elite hybrid varieties greatly decreased the genetic base of this crop in the United States (Tallury and Goodman 2001). Recognizing the value of broadening the germplasm base of this crop in the US, efforts have been made to incorporate exotic germplasm for development of genetically diverse inbred lines (Nelson and Goodman 2008).

1.4.2 Assessment of diversity in *Brassica*

To allow breeders and organizations to have a necessary understanding of the genetic similarity and diversity that exists among various collections of *Brassica* spp. from around the world, numerous studies have been done to estimate genetic relatedness through a survey of selected genotypes (Diers and Osborn 1994, Hasan et al. 2006, Zhou et al. 2006). While the number of genotypes that can reasonably be included in a single study (generally <100) is limited, however, these studies provide useful information and identify general patterns and genotype groups.

A variety of methods are available to estimate diversity among genotypes. Before the modern tools of protein and molecular analysis came into wide use, morphological or phenotypic traits were generally used to estimate genetic relatedness, and these methods are still used (Yu et al. 2005, Hu et al. 2007). Measures of diversity such as Euclidean distance (ED) can be calculated based on phenotypic trait data (Teklewold and Becker 2006). The use of pedigrees, where available, also allows for estimates of the level of genetic relatedness among different cultivars – as was done for contemporary Australian canola cultivars (Cowling 2007). Several studies confirmed that genetic groupings obtained based

on phenotypic or molecular data correlate reasonably well with the known pedigree information (Diers and Osborn 1994, Becker et al. 1995, Hu et al. 2007).

Enzyme markers such as allozymes and isozymes are proteins that are the products of expressed genes, and differences among these proteins reflect differences in alleles of that specific gene. These have also been used as markers to perform genetic diversity studies (McGrath and Quiros 1992, Becker et al. 1995, Yu et al. 2005). However, with the availability of DNA-based molecular markers, the use of enzyme markers to evaluate genetic diversity has been limited due to the small number of marker loci available and low levels of polymorphism (Melchinger 1999).

DNA molecular markers are the most commonly used method to assess genetic diversity in *Brassica*. These markers are based on polymorphic segments of plant DNA that provide useful information on the genetic relationship between different individuals. Restriction fragment length polymorphism (RFLP) marker is a non-PCR (polymerase chain reaction) based DNA marker. This was among the first DNA-based markers to be used in genetic diversity studies in *Brassica* (McGrath and Quiros 1992, Diers and Osborn 1994, Becker et al. 1995). The invention and subsequent development of (PCR) techniques (Mullis and Faloona 1987) played an important role in facilitating the widespread use of molecular markers in research and breeding programs. Three PCR-based markers that have been used in more recent studies to survey genetic diversity among *Brassica* populations include random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) or microsatellite markers. AFLP markers are considered to be extremely useful for genetic diversity studies due to the large amounts of information that can be obtained in a short time and low cost (Seyis et al. 2003). SSR markers are advantageous in that they are highly polymorphic with regards to repeat numbers, have co-dominant inheritance, and are stably inherited (Plieske and Struss 2001).

Comparisons of genetic diversity among various *B. napus* populations revealed some common patterns. Spring and winter types generally fall into two distinct groups (Diers and Osborn 1994, Becker et al. 1995). Asian genotypes

surveyed (India, China, Japan) tended to be more genetically diverse than those from Australia, Europe, or Canada (Zhou et al. 2006, Chen et al. 2008). This may be due to introgressions of allelic diversity from the related *Brassica* species traditionally grown in these areas: *B. rapa* (China, e.g. Chinese cabbage) and *B. juncea* (India, e.g. Indian mustard). Resynthesized rapeseed types were clearly distinguishable from natural *B. napus* (Becker et al. 1995, Seyis et al. 2003).

1.4.3 Broadening genetic diversity in *B. napus*

Surveys of genetic diversity among the primary gene pool of *B. napus* taken from worldwide collections and including spring and winter, fodder, and leafy types disclosed that certain genotypes were somewhat genetically divergent from other members of the same type, which generally grouped closely together (Diers and Osborn 1994, Hasan et al. 2006). Thus, a limited amount of genetic variability can sometimes be identified within genotypes from the primary gene pool of the same type. However, diversity within these groups is limited, and does not provide the amount of variation needed for long-term breeding progress in *B. napus.*

Rapeseed varieties of different ecotypes, still within the primary gene pool, can also be used to broaden genetic diversity in spring type canola. One approach is to utilize the diversity of winter oilseed types in the breeding of spring oilseed types (Kebede et al. 2010, Rahman 2011). This represents a short to mid-term approach to broadening genetic diversity in spring type *B. napus*. This approach has the advantage of avoiding problems associated with crossability, fertility, and seed quality that would otherwise be encountered when more exotic germplasm is used.

Brassica napus (AACC, *2n*=38) is an amphidiploid species resulting from spontaneous hybridizations in nature between its diploid progenitors *B. rapa* (AA, *2n*=20) and *B. oleracea* (CC, *2n*=18) (U 1935). Phylogenetic studies based on molecular markers suggest that the A- and C-genomes of *B. napus* are genetically distinct from the corresponding genomes of its parental species (Song et al. 1988). Both of these diploid species harbour a vast amount of morphological and physiological diversity (Babula et al. 2007, Quijada et al. 2007). Therefore, they

serve as an excellent reservoir of genetic diversity for improving *B. napus* in the long term. Introgression of genetic diversity from these diploid species into *B. napus* has been done by direct crossing of these species followed by selection for *B. napus* types, particularly with *B. rapa* due to better crossability (Downey et al. 1980, Hansen et al. 2001) and availability of canola quality types. Qian et al. (2006) compared genetic diversity in *B. napus* x *B. rapa* interspecific cross derived *B. napus* lines with commonly available spring, winter, and semi-winter *B. napus* lines, and reported that the interspecific cross derived lines had the highest genetic diversity, and could be used to diversify the *B. napus* germplasm.

The resynthesis of *B. napus* by crossing various forms of *B. rapa* and *B. oleracea*, followed by chromosome doubling of the F_1 hybrid, is the other approach to utilizing genetic diversity from these diploid species. A major limitation to this approach is the introduction into resynthesized (RS) rapeseed lines of undesirable seed quality traits, viz. high erucic acid and glucosinolate content. This is particularly a problem in *B. oleracea*, where no double-low quality accession is available. In this regard, the development of *B. oleracea* accessions with double-low seed quality traits would greatly facilitate the usefulness of this method by enabling the generation of genetically diverse RS lines of appropriate seed quality. A low erucic acid mutant of *B. oleracea* var. *capitata* (white cabbage) has been used to generate RS *B. napus*, but in the zero-erucic RS lines glucosinolate content was still high (77 to 111 µmol mg^{-1}) (Rygulla et al. 2007). In addition, agronomic characteristics of RS *B. napus* lines *per se* are generally poor (Girke et al. 1999). However, evaluation of yield potential in test hybrids by including diverse RS lines either by direct crossing with adapted types (Kräling 1987, Girke et al. 1999), or as one of the parents to generate recombinant inbred lines and crossed with a tester (Gehringer et al. 2007), has shown very good potential.

Doubled haploid (DH) technology has facilitated the ability of plant breeders to create novel combinations of alleles in a much shorter breeding cycle than would be possible through other breeding methods. Basunanda et al. (2007) recovered double-low (zero erucic, <30 µmol glucosinolate g^{-1} seed) DH lines

from a cross between double-low and high erucic, high glucosinolate *B. napus* parents, and estimated that four beneficial QTL for seed quality were simultaneously introduced into these lines. In addition, some of these double-low lines still had a high number of alleles (50 to 55%) originating from the diverse '+ +' parent. Thus, canola lines with acceptable seed quality and high levels of introgressed diversity from a non-adapted parent can be quickly generated using DH technology.

1.4.4 Implications for canola breeding

There are both opportunities and challenges with regards to broadening the genetic base for the benefit of canola breeding. Some challenges are associated with selection of suitable genotypes due to the introduction of non-adapted material, such as genotypes having poor seed quality (Gehringer et al. 2007), vernalization requirement in the case of spring rapeseed breeding (Kebede et al. 2010), or low levels of fertility resulting in poor seed set resulting from wide crosses (Zou et al. 2010). However, the broadening of genetic diversity in canola can also pay great dividends in the long term by introducing disease resistance (Rygulla et al. 2007), early flowering (Rahman et al. 2011), and other specific traits where beneficial alleles are not normally found in local adapted varieties and breeding lines; as well as potential yield gains in new open-pollinated (Kebede et al. 2010) and hybrid (Quijada et al. 2004) varieties.

One area of particular interest with regards to genetic diversity in canola breeding is its application in the production of hybrid cultivars. In Alberta in 2009, an estimated 80% of the canola crop was seeded to hybrid cultivars (AFSC Crop Insurance Records 2010). The success of these cultivars depends on the level of heterosis, or hybrid vigour that can be achieved from the inbred parent lines. Heterosis is most often measured in terms of mid-parent heterosis (MPH) or high-parent heterosis (HPH), where the value of the F_1 hybrid is compared with either the parent mean or higher performing parent. Test hybrids have often been used to evaluate seed yield heterosis in crosses between elite breeding lines and genetically diverse material, and such hybrids have shown good potential in

spring (Qian et al. 2007, Quijada et al. 2004) and winter (Basunanda et al. 2007, Gehringer et al. 2007) material.

One of the most resource consuming aspects of any hybrid breeding program is the identification of suitable parents with high levels of heterosis. With the widespread availability of molecular markers in plant breeding programs, there has been an interest in utilizing marker-based estimates of genetic diversity among parental lines as a predictor of hybrid performance. Results have widely varied among studies in different crops from trait to trait and data set to data set. Melchinger (1999) proposed that correlation between parental genetic diversity and heterosis is strongest when related lines are used; but this relationship weakens as more distant material is involved in the hybrids. In rapeseed, Riaz et al. (2001) and Diers et al. (1996) reported a moderate, yet significant, correlation between genetic distance based on molecular markers and heterosis for seed yield in crosses between spring type cultivars. However, more work needs to be done in canola to evaluate this relationship when crosses between different ecotypes or genetically wider parental lines are involved (Qian et al. 2007).

1.5 Interspecific hybridization in *Brassica*

The selection of parental genotypes is of utmost importance in all plant breeding programs, because the fundamental goal is to develop cultivars with desirable combinations of genes. Crosses usually involve well adapted cultivars or breeding lines of the same species (Hadley and Openshaw 1980). However, depending upon the goal(s) of the breeding initiative and the genetic resources available, breeders may opt to use different species or genera in crosses with an adapted cultivar. The use of wide crosses is often very challenging, but can be extremely rewarding in the long-term (Hadley and Openshaw 1980).

Approaches to incorporating valuable genetic variation into *B. napus* from distant sources, which are cross-incompatible with *B. napus*, include application of asymmetric protoplast fusion (Jourdan et al. 1989, Wang et al. 2004) and transgenic (Himanen et al. 2008) approaches. Some *Brassica* species can more

easily cross with each other and readily form viable seeds upon cross-pollination, such as *B. napus* × *B. rapa* (Downey et al. 1980). However, obtaining viable seeds from other *Brassica* interspecific crosses is extremely difficult, as in the case of *B. napus* × *B. oleracea* (U 1935, Chiang et al. 1977, Bennett et al. 2008). The identity of the maternal parent also affects the success rate of hybridization between species (Downey et al. 1980). Thus, the approach and level of intervention required in an interspecific breeding program will depend upon the species involved and the direction of the cross.

The successful use of wide crosses for the improvement of *Brassica* crop species can be challenging. An initial problem is the difficulty of predicting the rate at which true hybrids will successfully be produced. In addition to the identity of the parental species and direction of the cross, hybrid production rate has also been found to vary depending on the health of parental plants and growth temperature (Chiang et al. 1977, Bennett et al. 2008), method of embryo rescue (Takeshita et al. 1980), age of cross-pollinated siliques (Rahman 2004), composition of culture media (Zhang et al. 2003), and genotypes involved (Chen et al. 1988). Thus, despite the intensive efforts required to generate hybrid plants, a number of factors influence hybrid production rate, which can result in populations of insufficient size to be of practical use in a breeding program (Ayotte et al. 1987).

Once true hybrids are obtained, another issue is low fertility among self-pollinated or backcrossed interspecific progeny due to aneuploidy (Quazi 1988, Inomata 2002). Thus, potentially useful breeding lines often require cytological or genetic investigation to determine ploidy. For example, a stable, low glucosinolate BC_1F_3 *B. juncea* line, developed from a *B. juncea* × *B. rapa* interspecific cross, was found to be nullisomic by cytological investigation (Cheng et al. 2001). Variations in ploidy levels of interspecific plants may also result from unreduced gametes, which have been found to be involved in the production of offspring resulting from wide *Brassica* crosses (Inomata 2002, Nelson et al. 2009). The introgression of useful alleles from an unadapted parent into an elite breeding line is often associated with the introduction of undesirable traits from the donor

parent (linkage drag). Additional efforts are therefore required to remove these unwelcome alleles from the elite breeding line.

Despite the challenges, our ability to utilize valuable genetic resources through wide crosses for the improvement of *Brassica* crops is increasing. This is facilitated by the improvement of embryo rescue techniques. In *Brassica* hybrids, several researchers (Quazi 1988, Chen et al. 1988, Ayotte et al. 1987) noted that early embryo abortion, which may be caused by abnormal endosperm development, is a predominant barrier to successful hybridization. Thus, the use of embryo rescue techniques is a good strategy for increasing success rate of hybrid production (Inomata 1993). A number of recent studies have focused on improving the embryo rescue success rate in *Brassica* interspecific crosses (Zhang et al. 2003, Rahman 2004, Bennett et al. 2008).

A number of beneficial traits have successfully been transferred into *B. napus* from related *Brassica* species (Table 1-1). Backcrossing hybrid progeny to a well-adapted recurrent parent is often done to introgress a specific trait into a desirable genetic background. A good example of this is the transfer of disease resistance genes from one species to another. Rahman et al. (2007) successfully transferred cotyledon and adult plant resistance to blackleg (*Leptosphaeria maculans*) pathotype PG2 from the B-genome of *B. carinata* (BBCC, *2n*=34) into *B. napus* cultivar 'Westar' background. This was accomplished through interspecific crosses between these two species, followed by backcrossing to 'Westar' and the subsequent production of DH lines.

Brassica oleracea var. *alboglabra* (L. H. Bailey) Musil (Chinese kale) has been a common choice as a C-genome parent in interspecific crossing efforts (Chen et al. 1988, Zaman 1988, Rahman 2001, Bennett et al. 2008, Rahman et al. 2011). This is due to its self-compatible nature, large flower bud that makes it amenable to cross-pollination, and its relatively short life cycle (no vernalization requirement) compared to other *B. oleracea* types.

Table 1-1. Selected traits introgressed into *Brassica napus* by interspecific crosses with related species

Trait improved	Donor	Reference
Early flowering	*B. rapa, B. oleracea*	Akbar (1989)
	B. oleracea	Rahman et al. (2011)
Yellow seed colour	*B. carinata, B. juncea*	Rashid et al. (1994)
	B. rapa	Rahman (2001)
Blackleg resistance	*B. juncea*	Roy (1984)
	B. carinata, B. juncea, B. nigra	Sjödin and Glimelius (1989)
Self-incompatibility (S-alleles)	*B. oleracea*	Ripley and Beversdorf (2003)
	B. rapa, B. oleracea	Rahman (2005)
Clubroot resistance	*B. rapa*	Gowers (1982)

1.6 Genetics of flowering time in *Brassica napus*

1.6.1 Agronomic importance

Flowering marks the transition from a vegetative to a reproductive state in crop species. The timing of flowering in a growing season is an important agronomic trait. For *Brassica* crops cultivated as leafy vegetables, such as *B. rapa* ssp. *pekinensis* (Chinese cabbage), delayed transition to flowering stage is preferable, as this increases the yield of vegetative parts and the quality of the product (Nishioka et al. 2005, Kim et al. 2007). For oilseed *Brassica* crops, where the economic value is in the seed, timing of flowering is critical to optimal seed production and seed quality. For *B. napus* canola cultivars, earliness of flowering is often a desirable trait, as this trait is a good indicator of maturity in *Brassica* oilseed crops (Miller 2001, Mahmood et al. 2007). In regions with a short growing season such as Northern Europe and Canada, earliness of flowering can circumvent reduced seed yield and oil quality due to frost damage. In low rainfall areas in Australia, earliness of flowering allows for seed maturity to be completed

before the onset of high temperatures and drought stress that reduce seed yield and oil accumulation (Robertson et al. 2002). The three major environmental factors affecting timing of flowering in *Brassica* are photoperiod, temperature, and vernalization (King and Kondra 1986, Robertson et al. 2002).

1.6.2 *Arabidopsis thaliana*

The dissection of the genetic control of flowering in *Brassica* has not been easy. Flowering is a complex trait, involving many gene loci with major or minor effect, interactions between genes, as well as interactions between the plant and environmental cues such as photoperiod and temperature. A large number of QTL, QTL x environment interactions and interacting loci make the study of genetic mechanisms underlying flowering time in *Brassica* species quite complex (Long et al. 2007, Uptmoor et al. 2008). Fortunately, the Brassicaceae family includes the model dicotyledon species *Arabidopsis thaliana*, whose complete genome sequence (The Arabidopsis Genome Initiative 2000) has allowed for the intensive study of the genetic basis of flowering time in this species. Due to its close relationship with crop *Brassica* species, it is expected that genetic and physiological processes involved in flowering time in *Arabidopsis* will be similar in *Brassica*. Indeed, the coding sequences and mode of action of key flowering time genes such as *FLOWERING LOCUS T* (*FT*) (Wang et al. 2009), *CONSTANS* (*CO*) (Robert et al. 1998), and *FLOWERING LOCUS C* (*FLC*) (Tadege et al. 2001) of *Arabidopsis* have been shown to be very similar in *B. napus*. Several of the key flowering time genes identified in *B. napus* and *B. rapa* correspond with well-characterized regions at the top of chromosome 4 (*FRI* and *LD*) and 5 (*FLC, CO,* and *FY*) in *Arabidopsis* (Osborn et al. 1997).

1.6.3 *Brassica rapa* and *B. oleracea*

The diploid progenitor species of *B. napus*, *B. oleracea* and *B. rapa*, include a diverse array of morphological types as well as variation for days required for flowering. Among all of these types, two flowering habits can be broadly distinguished – those that will flower in a single growing season (annual), and

those that require a period of vernalization (biennial) (Babula et al. 2007, Hirai and Matsumoto 2007). Comparative studies of flowering time loci between *B. rapa* and *B. napus* suggested that the genes in these two species having a large effect on flowering time are likely to be homoeologous (Osborn et al. 1997). For example, Osborn et al. (1997) hypothesized that the vernalization requirement for biennial *B. napus* types likely derived from biennial forms of *B. rapa*, as homologous chromosomal regions of N2 and N10 of these two species show large phenotypic effect on this trait.

However, it can be inferred that much of the allelic variation for flowering time present in diverse forms of *B. rapa* and *B. oleracea* has not been exploited for improvement of this trait in *B. napus*. Kubik et al. (1999) and Miller (2001) developed DH *B. napus* lines from a *B. napus* × *B. rapa* interspecific cross with significantly reduced number of days required to flower under Western Canadian conditions. Rahman et al. (2011) demonstrated that introgression of alleles from *B. oleracea* var. *alboglabra* into *B. napus* could also be used to improve earliness in this species, though *B. alboglabra* flowers 2-3 weeks later than *B. napus*. However, very little information is currently available on the early flowering gene(s) of the *B. oleracea* C-genome, their mode of inheritance and interactions with the flowering time genes of *B. napus*.

1.6.4 *Brassica napus* QTL studies

Since flowering time is a quantitative trait, quantitative trait loci (QTL) studies have greatly assisted in dissecting the genetic control of this trait in *B. napus*. A summary of the major loci identified by QTL studies is given in Table 1-2. In general, while these studies detected many QTL for flowering time, relatively few had large phenotypic effect and/or were stable across environments. Thus, it is possible that variation for flowering time in *B. napus* may be due to a relatively small number of genes with major effect, and many genes with minor effect. Quantitative traits, where a large number of genes with small effect are involved, are known to be highly influenced by environmental factors. Indeed, large numbers of QTL × environment interactions could be detected in *B. napus*,

indicating complexities involved in phenotypic variation for flowering time (Long et al. 2007, Cai et al. 2008). However, phenotypic variation explained by the main effect QTLs was much larger as compared to QTL × environment interactions (Cai et al. 2008).

In *Arabidopsis*, the loci involved in the control of flowering time are not randomly dispersed throughout the genome, but are grouped together in several clusters (Brachi et al. 2010). In comparative mapping between *Arabidopsis* and *B. napus*, Long et al. (2007) observed that many of the genomic regions of *Arabidopsis* involved in the control of flowering time were syntenic to the flowering time QTL regions of *B. napus*. Axelsson et al. (2001) reported three homoeologous regions in each of the diploid *Brassica* species (*B. rapa, B. oleracea, B. nigra*) and six in the amphidiploid *B. juncea*, and these regions corresponded to the top of *Arabidopsis* chromosome 5. Wang et al. (2009) reported six homologues of the *FT* gene in *B. napus* corresponding to a chromosomal block of *Arabidopsis* chromosome 1. These studies provide evidence that multiple QTL could be the result of polyploidization of the same ancestral gene(s), and confirm that *Brassica* species likely possess many homologous flowering time genes that have a similar mode of action.

Quantitative trait loci studies in *B. napus* have also disclosed co-localization of genes controlling days to flowering and other floral transition traits such as photoperiod sensitivity, budding, and bolting time (Long et al. 2007, Cai et al. 2008). In addition, flowering time QTL have been found to co-localize with other agronomically important traits such as seed yield, oil content, and plant height (Mei et al. 2009, Chen et al. 2010). It is not certain whether these observations are due to tight linkage of genes controlling the measured traits, or if there is a pleiotropic effect of the involved genes. Depending on the positive or negative correlation between the flowering time and linked QTL for other traits, it may be possible to simultaneously breed for early flowering along with other beneficial traits.

Despite the advantages of using QTL mapping to elucidating the genetics of flowering time in *B. napus*, researchers have reported several inherent challenges.

In the case of flowering time studies, the assumption of normal distribution of a quantitative trait is often violated (Mei et al. 2009, Chen et al. 2010). Further, non-Mendelian segregation of markers can occur in DH populations (Mei et al. 2009, Chen et al. 2010). These factors make statistical analysis and interpretation of results more difficult. A number of factors influence the detection of QTL, including population type (F_2, DH, Backcross, etc.), cross type (winter × winter, winter × spring, etc.), population size, extent of test environments, and statistical thresholds used (Mei et al. 2009). Thus, it is beneficial to use molecular approaches in conjunction with QTL studies, to uncover the genetic control of flowering time in rapeseed.

Molecular studies on specific key flowering time genes have been conducted to further understand the genetic basis of flowering in *B. napus*. These genes had previously been intensively studied in *Arabidopsis*, and their mode of action in this model diploid species is fairly well understood. For example, these have included complementation studies, where *B. napus* homologues of *CO* were introduced into a loss-of-function *Arabidopsis* mutant of the same gene, and the *B. napus* alleles were able to complement the recessive mutation (Robert et al. 1998). Genetic transformation of an early flowering ecotype of *Arabidopsis* with a *B. napus* homologue of the *FLC* gene delayed flowering significantly (Tadege et al. 2001). Thus, both studies indicated that the flowering time homologue from *B. napus* had a similar mode of action to the corresponding gene in *Arabidopsis*. Sequencing of these *B. napus* homologues revealed high levels of sequence homology at the nucleotide level (ca. 82% in *CO*, ca. 85-87% in *FT*) (Robert et al. 1998, Wang et al. 2009).

Table 1-2. Major loci affecting flowering time in *Brassica napus* as detected by quantitative trait loci (QTL) studies

Trait	Cross type	Population type and size	Markers used	Map size (cM)	Main reported QTL	Linkage group (No. of environ.)	Phenotypic variation explained (%)	Total no. QTL detected	Reference
Days to flowering	Spring × Spring?	150 DH lines	353 SRAP 34 SSR	1,868		LG14 (3) LG17 (1)	4.4 – 12.7 48.3	22	Chen et al. (2010)
Days to flowering	Spring × Spring	~150 DH lines	94 SRAP 82 SSR 72 AFLP	1,634	*dtf5* *dtf11* *dtf18*	N5 (2) N11 (2) N18 (2)	29.9 (combined) 28.4	16 (Main effect, Epistatic, QTL × E)	Cai et al. (2008)
Photoperiod sensitivity					*ps3* *ps10* *ps14* *ps18*	N3 (2) N10 (2) N14 (2) N18 (2)	53.3 (combined)		Cai et al. (2008)
Days to flowering	Winter × Spring	89 DH lines	203 RFLP 5 isozyme 2 disease res. 2 erucic acid 268 AFLP	1,016 (Ferriera et al. 1995)	*VFN1* *VFN2* *VFN3* *FN1*	N2 (1) N10 (1) LG16 (1) N2 (1)	46.9 8.1 6.4 29.1		Osborn et al. (1997); Ferriera et al. (1995)
Days to flowering	Winter × Semi-winter	202 DH lines; 404 Reconstructed F_2 lines	621 markers: TN mapping pop'n (Qiu et al. 2006) + 344 SSR, RFLP, SNP, MS-AFLP	2,060	*qFT10-4* *qFT16-3* *qFT10-5* *qFT3-2*	N10 (2) N16 (11) N10 (13) N3 (6)	52.4 17.9 15.6 12.5	42	Long et al. (2007)
Bolting/ budding time					*qBO10-2* *qBO3-1* *qBU10-2* *qBU10-1*	N10 (2) N3 (2) N10 (2) N10 (2)	24.3 14.9 25.9 15.4	16 (bolting) 15 (budding)	Long et al. (2007)
Days to flowering	Winter × Winter	145 $F_{2:3}$ lines	199 AFLP 42 SSR	2,094	*ft13* *ft17*	N3 (2) N12 (2)	29.5 – 30.4 13.1 – 14.6	6	Mei et al. (2009)

1.7 Research objectives

This Ph.D. research evolved from an MSc thesis project investigating the effects of silique age and growth condition on the efficiency of embryo rescue for production of *B. napus* × *B. oleracea* interspecific hybrids, and the inheritance of erucic acid content in self-pollinated BC_1 plants of (*B. napus* × *B. oleracea*) × *B. oleracea* (Bennett 2007). This Ph.D. research ulitizes the germplasm developed from the MSc study and investigates the effects of the genetic diversity of *B. oleracea* in the spring oilseed crop *B. napus*. Two populations, both resulting from interspecific hybridizations between *B. napus* and *B. oleracea* var. *alboglabra*, were generated. The first population was generated from the F_1 hybrid plants of *B. napus* × *B. oleracea* through self-pollination for several generations; while the second population was generated by backcrossing of the *B. napus* × *B. oleracea* F_1 hybrid plants to the *B. oleracea* parent followed by several generations of self-pollination. These populations were studied with the following objectives:

1.7.1 Long-term objectives

The long-term goal of the proposed research is to contribute to the knowledge of the value of *B. oleracea* for enriching diversity in the C-genome in spring type *B. napus*, as well as the discovery of alleles for specific traits that can further enrich *B. napus* germplasm.

1.7.2 Short-term objectives

Four primary research and development objectives are being pursued in the short-term in my Ph.D. program:

i) To study the feasibility of introgressing *B. oleracea* (C-genome) alleles into euploid *B. napus* (*2n*=38) recombinant inbred lines (RILs), while maintaining the canola quality traits (less than 2% erucic acid content in the oil and less than 30 µmol glucosinolate per g meal);

ii) To study the feasibility at which the canola quality traits can be introgressed into an euploid *B. oleracea* (*2n*=18) background;

iii) To investigate the value, as measured by mid- and high-parent heterosis of test hybrids, of genetic diversity of the C-genome of *B. alboglabra* introgressed into a spring type *B. napus*, and to compare these types with *B. napus* lines diversified using European winter and spring type canola for heterotic potential in hybrid spring canola;

iv) Uncover the genetic basis of the early flowering trait and glucosinolate content resulting from the introgression of *B. oleracea* C-genome alleles into a *B. napus* background.

1.7.3 Research hypotheses

The following five hypotheses are being tested in this Ph.D. research program:

i) Allelic diversity from the *B. oleracea* C-genome can be introgressed into *B. napus* (*2n*=38) through pedigree selection in the progeny of *B. napus* × *B. oleracea*;

ii) Canola quality traits from the C-genome of *B. napus* can be introgressed into a *B. oleracea* (*2n*=18) type background by pedigree selection in the progeny of (*B. napus* × *B. oleracea*) × *B. oleracea*;

iii) Mid- and high-parent heterosis for specific inbred *B. napus* lines, diversified using the *B. oleracea* C-genome or winter type *B. napus* alleles, will exceed the heterosis observed in test hybrids derived from spring × spring *B. napus* lines;

iv) Increased genetic distance among the inbred parental lines, as estimated by molecular markers, will have a significant and positive correlation with increased mid- and high-parent heterosis in test hybrids;

v) The early flowering allele(s) of the C-genome of *B. oleracea* var. *alboglabra* are different from the C-genome alleles of *B. napus*; and these alleles can be used for improvement of earliness of flowering and maturity in spring *B. napus*.

1.8 References

Acquaah G (2007) Principles of plant genetics and breeding. Blackwell Publishing, Malden, MA, USA

Agricultural Financial Services Corporation (2010) Acres Insured by Crop Variety – Alberta. Available at http://www.afsc.ca/doc.aspx?id=4182 (verified 3 Dec. 2010). AFSC, Lacombe, Alberta

Akbar A (1989) Resynthesis of *Brassica napus* aiming for improved earliness and carried out by different approach. Hereditas 111:239-246

American Soybean Association (2011) Soy Stats 2011. Available at http://www.soystats.com/2011/page_29.htm (verified 4 Nov. 2011)

Armstrong KC, Keller WA (1981) Chromosome pairing in haploids of *Brassica campestris*. Theor Appl Genet 59:49-52

Armstrong KC, Keller WA (1982) Chromosome pairing in haploids of *Brassica oleracea*. Can J Genet Cytol 24:735-739

Attia T, Röbbelen G (1986) Cytogenetic relationship within cultivated *Brassica* analyzed in amphihaploids from the three diploid ancestors. Can J Genet Cytol 28:323-329

Axelsson T, Bowman CM, Sharpe AG, Lydiate DJ, Lagercrantz U (2000) Amphidiploid *Brassica juncea* contains conserved progenitor genomes. Genome 43:679-688

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

Ayotte R, Harney PM, Souza Machado V (1987) The transfer of triazine resistance from *Brassica napus* L. to *B. oleracea* L. I. Production of F_1 hybrids through embryo rescue. Euphytica 36:615-624

Babula D, Kaczmarek M, Ziółkowski PA, Sadowski J (2007) *Brassica oleracea*. In: Kole C (ed) Genome Mapping and Molecular Breeding in Plants, volume 5. Springer-Verlag, Berlin/Heidelberg, Germany, pp 227-285

Basunanda P, Spiller TH, Hasan M, Gehringer A, Schondelmaier J, Lühs W, Friedt W, Snowdon RJ (2007) Marker-assisted increase of genetic diversity in a double-low seed quality winter oilseed rape genetic background. Plant Breed 126:581—587

Becker HC, Engqvist GM, Karlsson B (1995) Comparison of rapeseed cultivars and resynthesized lines based on allozyme and RFLP markers. Theor Appl Genet 91:62-67

Bennett RA (2007) Interspecific cross of *Brassica napus* x *B. oleracea* var. *alboglabra*: the effect of growth condition and silique age on hybrid production, and the inheritance of erucic acid in the self-pollinated backcross generation. MSc Thesis, University of Alberta, Edmonton

Bennett RA, Thiagarajah MR, King JR, Rahman MH (2008) Interspecific cross of *Brassica oleracea* var. *alboglabra* and *B. napus*: Effects of growth condition and silique age on the efficiency of hybrid production, and inheritance of erucic acid in the self-pollinated backcross generation. Euphytica 164:593-601

Brachi B, Faure N, Horton M, Flahauw E, Vazquez A, Nordborg M, Bergelson J, Cuguen J, Roux F (2010) Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. PLoS Genet 6(5):e1000940

*Brassica*Corp Ltd, SJ Campbell Investments Ltd (2005) A review and assessment of canola breeding at the University of Alberta. Lacombe, AB

Cai CC, Tu JX, Fu TD, Chen BY (2008) The genetic basis of flowering time and photoperiod sensitivity in rapeseed *Brassica napus* L. Russian J Gen 44:326-333

Canola Council of Canada (2003) Canola Growers Manual

Canola Council of Canada (2011) Canadian canola industry. Available at http://www.canolacouncil.org/ind_overview.aspx (verified 17 Nov. 2011). Winnipeg, MB

Catcheside DG (1934) The chromosomal relationships in the swede and turnip groups of *Brassica*. Ann Bot 48:601-633

Chen BY, Heneen WK, Jönsson R (1988) Resynthesis of *Brassica napus* L. through interspecific hybridization between *B. alboglabra* Bailey and *B. campestris* L. with special emphasis on seed colour. Plant Breed 101:52-59

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

Chen S, Nelson MN, Ghamkhar K, Fu T, Cowling WA (2008) Divergent patterns of allelic diversity from similar origins: the case of oilseed rape (*Brassica napus* L.) in China and Australia. Genome 51:1-10

Cheng BF, Séguin-Swartz G, Somers DJ, Rakow G (2001) Low glucosinolate *Brassica juncea* breeding line revealed to be nullisomic. Genome 44:738-741

Chiang MS, Chiang BY, Grant WF (1977) Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* var. *capitata*). I. Interspecific hybridization between *B. napus* and *B. oleracea* var. *capitata.* Euphytica 26:319-326

Cooper HD, Spillane C, Hodgkin T (2001) Broadening the genetic base of crops: an overview. In: Cooper HD, Spillane C, Hodgkin T (eds) Broadening the genetic base of crop production. IPGRI and FAO, CABI publishing, Wallingford, Oxon, UK, pp 1-23

Cowling WA (2007) Genetic diversity in Australian canola and implications for crop breeding for changing future environments. Field Crop Res 104:103-111

Diers BW, McVetty PBE, Osborn TC (1996) Relationship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (*Brassica napus* L.). Crop Sci 36:79-83

Diers BW, Osborn TC (1994) Genetic diversity of oilseed *Brassica napus* germ plasm based on restriction fragment length polymorphisms. Theor Appl Genet 88:662-668

Downey RK (1964) A selection of *Brassica campestris* L. containing no erucic acid in its seed oil. Can J Plant Sci 44:295

Downey RK, Klassen AJ, Stringam GR (1980) Rapeseed and mustard. pp 495-509 *in* Fehr WR, Hadley HH (eds) Hybridization of crop plants. ASA/CSSA, Madison, WI

Ferreira ME, Satagopan J, Yandell BS, Williams RH, Osborn TC (1995) Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theor Appl Genet 90:727-732

Finlayson AJ, Krzymanski J, Downey RK (1973) Comparison of chemical and agronomic characteristics of two *Brassica napus* L. cultivars, 'Bronowski' and 'Target'. J Am Oil Chem Soc 50:407-410

Frandsen KJ (1943) The experimental formation of *Brassica juncea* Czern. et Coss. Dansk Bot Arkiv 11:1-17

Frandsen KJ (1947) The experimental formation of *Brassica napus* L. var. *oleifera* D.C. and *Brassica carinata* Braun. Dansk Bot Arkiv 12:1-16

Gehringer A, Snowdon R, Tobias S, Basunanda P, Friedt W (2007) New oilseed rape (*Brassica napus*) hybrids with high levels of heterosis for seed yield under nutrient-poor conditions. Breed Sci 57:315-320

Girke A, Becker HC, Engqvist G (1999) Resynthesized rapeseed as a new genpool for hybrid breeding. Proc 10th Int Rapeseed Congress, v.4, Canberra, Australia

Gowers S (1982) The transfer of characters from *Brassica campestris* L. to *Brassica napus* L.: Production of clubroot-resistant oil-seed rape (*B. napus* ssp *oleifera*). Euphytica 31:971-976

Hadley HH, Openshaw SJ (1980) Interspecific and intergeneric hybridization. In: Fehr WR, Hadley HH (eds) Hybridization of crop plants. ASA/CSSA, Madison, Wisconsin, pp 133-159

Hansen LB, Siegismund HR, Jørgensen RB (2001) Introgression between oilseed rape (*Brassica napus* L.) and its weedy relative *B. rapa* L. in a natural population. Genet Resour Crop Evol 48:621-627

Hasan M, Seyis F, Badani AG, Pons-Kühnemann J, Friedt W, Lühs W, Snowdon RJ (2006) Surveying genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet Resour Crop Evol 53:793-802

Himanen SJ, Nissinen A, Auriola S, Poppy GM, Stewart NC Jr, Holopainen JK, Nerg AM (2008) Constitutive and herbivore-inducible glucosinolate concentrations in oilseed rape (*Brassica napus*) leaves are not affected by *Bt Cry1Ac* insertion but change under elevated atmospheric CO_2 and O_3. Planta 227:427-437

Hirai M, Matsumoto S (2007) *Brassica rapa*. In: Kole C (ed) Genome Mapping and Molecular Breeding in Plants, volume 5. Springer-Verlag, Berlin/Heidelberg, Germany, pp 185-190

Hu SW, Yu CY, Zhao HX, Sun GL, Zhao SL, Vyvadilova M, Kucera V (2007) Genetic diversity of *Brassica napus* L. Germplasm from China and Europe assessed by some agronomically important characters. Euphytica 154:9-16

Inomata N (2002) A cytogenetic study of the progenies of hybrids between *Brassica napus* and *Brassica oleracea*, *Brassica bourgeaui*, *Brassica cretica*, and *Brassica montana*. Plant Breed 121:174-176

Inomata N (1993) Embryo Rescue Techniques for Wide Hybridization. In: Labana KS, Banga SS, Banga SK (eds) Breeding Oilseed *Brassicas*. Narosa Publ House, New Delhi, pp 94-107

Jourdan PS, Earle ED, Mutschler MA (1989) Synthesis of male sterile, triazine-resistant *Brassica napus* by somatic hybridization between cytoplasmic male sterile *B. oleracea* and atrazine-resistant *B. campestris*. Theor Appl Genet 78:445-455

Kang MS (1997) Using genotype-by-environment interaction for crop cultivar development. Adv Agr 62:199-252

Kebede B, Thiagarajah MR, Zimmerli C, Rahman MH (2010) Improvement of open-pollinated spring rapeseed (*Brassica napus* L.) through introgression of genetic diversity from winter rapeseed. Crop Sci 50:1236-1243

Kim SY, Park BS, Kwon SJ, Kim J, Lim MH, Park YD, Kim DY, Suh SC, Jin YM, Ahn JH (2007) Delayed flowering time in *Arabidopsis* and *Brassica rapa* by the overexpression of *FLOWERING LOCUS C* (*FLC*) homologs isolated from Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Plant Cell Rep 26:327-336

King JR, Kondra ZP (1986) Photoperiod response of spring oilseed rape (*Brassica napus* L. and *B. campestris* L.) Field Crops Res 13:367-373

Koch M, Haubold B, Mitchell-Olds T (2001) Molecular systematics of the Brassicaceae: evidence from coding plastidic *matK* and nuclear *Chs* sequences. Am J Bot 88:534-544

Kräling K (1987) Utilization of genetic variability of resynthesized rapeseed. Plant Breed 99:209-217

Kubik TJ, Hawkins GP, Stringam GR (1999) Cytological stability of doubled haploid lines derived from interspecific crosses between *Brassica napus* L. and *B. rapa* L. Proc 10th Int Rapeseed Congr, Canberra, Australia, v.4

Lagercrantz U, Lydiate DJ (1996) Comparative genome mapping in *Brassica*. Genetics 144:1903-1910

Long Y, Shi J, Qiu D, Li R, Zhang C, Wang J, Hou J, Zhao J, Shi L, Park BS, Choi SR, Lim YP, Meng J (2007) Flowering time quantitative trait loci analysis of oilseed Brassica in multiple environments and genomewide alignment with Arabidopsis. Genetics 177:2433-2444

Lukens LN, Quijada PA, Udall J, Pires JC, Schranz ME, Osborn TC (2004) Genome redundancy and plasticity within ancient and recent *Brassica* crop species. Biol J Linnean Soc 82:665-674

Lysak MA, Koch MA, Pecinka A, Schubert I (2005) Chromosome triplication found across the tribe *Brassiceae*. Genome Res 15:516-525

Mahmood T, Rahman MH, Stringam GR, Yeh F, Good AG (2007) Quantitative trait loci for early maturity and their potential in breeding for earliness in *Brassica juncea*. Euphytica 154:101-111

Mark Goodwin Consulting Ltd (2008) Canola Socio-Economic Value Report.

Masterson J (1994) Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science 421-424

McGrath JM, Quiros CF (1992) Genetic diversity at isozyme and RFLP loci in *Brassica campestris* as related to crop type and geographical origin. Theor Appl Genet 83:783-790

McVetty PBE, Scarth R (2002) Breeding for improved oil quality in *Brassica* oilseed species. J Crop Prod 5:345-369

Mei DS, Wang HZ, Hu Q, Li YD, Xu YS, Li YC (2009) QTL analysis on plant height and flowering time in *Brassica napus*. Plant Breed 128:458-465

Melchinger AE (1999) Genetic diversity and heterosis. In: Coors JG, Pandey S (eds) Genetics and Exploitation of Heterosis in Crops. ASA, CSSA, Madison, WI, pp 99-118

Miller TA (2001) Agronomic and quality performance of three doubled haploid lines derived from a *Brassica napus/Brassica rapa* interspecific cross. MSc Thesis, University of Alberta, Edmonton

Morinaga T (1934) Interspecific hybridization in Brassica. VI. The cytology of F_1 hybrids of *B. juncea* and *B. nigra*. Cytologia 6: 62-67

Mullis K, Faloona F (1987) Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Meth Enzym 44:224-249

Nelson MN, Mason AS, Castello M-C, Thomson L, Yan G, Cowling WA (2009) Microspore culture preferentially selects unreduced (2n) gametes from an interspecific hybrid of *Brassica napus* L. × *Brassica carinata* Braun. Theor Appl Genet 119:497-505

Nelson PT, Goodman MM (2008) Evaluation of elite exotic maize inbreds for use in temperate breeding. Crop Sci 48:85-92

Nishioka M, Tamura K, Hayashi M, Fujimori Y, Ohkawa Y, Kuginuki Y, Harada K (2005) Mapping QTLs for bolting time in *Brassica rapa* (syn. *campestris*) under different environmental conditions. Breed Sci 55:127-133

Olsson G (1960) Species crosses within the genus *Brassica napus* L. II. Artificial *Brassica napus* L. Hereditas 46:351-396

Osborn TC, Kole C, Parkin IAP, Sharpe AG, Kuiper M, Lydiate DJ, Trick M (1997) Comparison of flowering time genes in *Brassica rapa*, *B. napus*, and *Arabidopsis thaliana*. Genetics 146:1123-1129

Parkin IAP, Gulden SM, Sharpe AG, Lukens L, Trick M, Osborn TC, Lydiate DJ (2005) Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. Genetics 171:765-781

Parkin IAP, Sharpe AG, Keith DJ, Lydiate DJ (1995) Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). Genome 38:1122-1131

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

Prakash S, Hinata K (1980) Taxonomy, cytogenetics and origin of crop Brassicas, a review. Opera Bot 55:1-57

Qian W, Meng J, Li M, Frauen M, Sass O, Noack J, Jung C (2006) Introgression of genomic components from Chinese *Brassica rapa* contributes to widening the genetic diversity in rapeseed (*B. napus* L.), with emphasis on the evolution of Chinese rapeseed. Theor Appl Genet 113:49-54

Qian W, Sass O, Meng J, Li M, Frauen M, Jung C (2007) Heterotic patterns in rapeseed (*Brassica napus* L.): I. Crosses between spring and Chinese semi-winter lines. Theor Appl Genet 115:27-34

Qiu D, Morgan C, Shi J, Long Y, Liu J, Li R, Zhuang X, Wang Y, Tan X, Dietrich E, Weihmann T, Everett C, Vanstraelen S, Beckett P, Fraser F, Trick M, Barnes S, Wilmer J, Schmidt R, Li J, Li D, Meng J, Bancroft I (2006) A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. Theor Appl Genet 114:67-80

Quazi MH (1988) Interspecific hybrids between *Brassica napus* L. and *B. oleracea* L. developed by embryo culture. Theor Appl Genet 75:309-318

Quijada PA, Udall JA, Polewicz H, Vogelzang R, Osborn TC (2004) Phenotypic effects of introgressing French winter germplasm into hybrid spring canola. Crop Sci 44:1982-1989

Quijada PJ, Cao J, Wang X, Hirai M, Kole C (2007) *Brassica rapa*. In: Kole C (ed) Genome Mapping and Molecular Breeding in Plants, vol 2. Springer-Verlag, Berlin/Heidelberg, Germany, pp 211-263

Rahman H (2011) Use of European winter canola *Brassica napus* L. for the improvement of Canadian spring canola: a practical breeding example. Proc 13th International Rapeseed Congr, Prague, pp. 875-878

Rahman MH (2005) Resynthesis of *Brassica napus* L. for self-incompatibility: self-incompatibility reaction, inheritance and breeding potential. Plant Breed 124:13-19

Rahman MH (2004) Optimum age of siliques for rescue of hybrid embryos from crosses between *Brassica oleracea, B. rapa* and *B. carinata*. Can J Plant Sci 84:965-969

Rahman MH (2001) Production of yellow-seeded *Brassica napus* through interspecific crosses. Plant Breed 120:463-472

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

Rahman MH, Hawkins G, Avery M, Thiagarajah MR, Sharpe AG, Lange R, Bansal V, Stringam GR (2007) Introgression of blackleg (*Leptosphaeria maculans*) resistance into *Brassica napus* from *B. carinata* and identification of microsatellite (SSR) markers. Proc of the 12th Int Rapeseed Congr 4:47-50

Rashid A, Rakow G, Downey RK (1994) Development of yellow-seeded *Brassica napus* L. through interspecific crosses. Plant Breed 112:127-134

Riaz A, Li G, Quresh Z, Swati MS, Quiros CF (2001) Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. Plant Breed 120:411-415

Ripley VL, Beversdorf WD (2003) Development of self-incompatible *Brassica napus*: (I) introgression of S-alleles from *Brassica oleracea* through interspecific hybridization. Plant Breed 122:1-5

Röbbelen G (1960) Beiträge zur analyse des *Brassica*-Genoms. Chromosoma 11:205-228

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

Robertson MJ, Watkinson AR, Kirkegaard JA, Holland JF, Potter TD, Burton W, Walton GH, Moot DJ, Wratten N, Farre I, Asseng S (2002) Environmental and genotypic control of time to flowering in canola and Indian mustard. Aust J Agric Res 53:793-809

Roy NN (1984) Interspecific transfer of *Brassica juncea*-type high blackleg resistance to *Brassica napus*. Euphytica 33:295-303

Rygulla W, Friedt W, Seyis F, Lühs W, Eynck C, von Tiedemann A, Snowdon RJ (2007) Combination of resistance to *Verticillium longisporum* from zero erucic acid *Brassica oleracea* and oilseed *Brassica rapa* genotypes in resynthesized rapeseed (*Brassica napus*) lines. Plant Breed 126:596-602

Schranz ME, Quijada P, Sung S-B, Lukens L, Amasino R, Osborn TC (2002) Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*. Genetics 162:1457-1468

Seyis F, Snowdon RJ, Lühs W, Friedt W (2003) Molecular characterization of novel resynthesized rapeseed (*Brassica napus*) lines and analysis of their genetic diversity in comparison with spring rapeseed cultivars. Plant Breed 122:473-478

Singh SP (2001) Broadening the genetic base of common bean cultivars: a review. Crop Sci 41:1659-1675

Sjödin C, Glimelius K (1989) Transfer of resistance against *Phoma lingam* to *Brassica napus* by asymmetric somatic hybridization combined with toxin selection. Theor Appl Genet 78:513-520

Song K, Lu P, Tang K, Osborn TC (1995) Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc Natl Acad Sci USA 92:7719-7723

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theor Appl Genet 75:784-794

Statistics Canada (2011) Field and special crops (Seeded area). Available at: http://www40.statcan.ca/l01/cst01/Prim11a-eng.htm (verified 24 Oct. 2011). Minister of Industry, Ottawa, Canada.

Stefansson BR, Hougen FW, Downey RK (1961) Note on the isolation of rape plants with seed oil free from erucic acid. Can J Plant Sci 41:218-219

Suwabe K, Morgan C, Bancroft I (2008) Integration of *Brassica* A genome genetic linkage map between *Brassica napus* and *B. rapa*. Genome 51:169-176

Tadege M, Sheldon CC, Helliwell CA, Stoutjesdijk P, Dennis ES, Peacock WJ (2001) Control of flowering time by *FLC* orthologues in *Brassica napus*. The Plant J 28:545-553

Takeshita M, Kato M, Tokumasu S (1980) Application of ovule culture to the production of intergeneric or interspecific hybrids in *Brassica* and *Raphanus*. Jpn J Genet 55:373-387

Tallury SP, Goodman MM (2001) The state of the use of maize genetic diversity in the USA and sub-Saharan Africa. In: Cooper HD, Spillane C, Hodgkin T (eds) Broadening the genetic base of crop production. IPGRI and FAO, CABI publishing, Wallingford, Oxon, UK, pp 159-179

Teklewold A, Becker HC (2006) Comparison of phenotypic and molecular distances to predict heterosis and F_1 performance in Ethiopian mustard (*Brassica carinata* A. Braun) Theor Appl Genet 112:752-759

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796-815

Truco MJ, Hu J, Sadowski J, Quiros CF (1996) Inter- and intra-genomic homology of the *Brassica* genomes: implications for their origin and evolution. Theor Appl Genet 93:1225-1233

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

United States Department of Agriculture (2010) World Agricultural Production, Table 15 'Rapeseed Area, Yield, and Production'. Foreign Agricultural Service

Uptmoor R, Schrag T, Stützel H, Esch E (2008) Crop model based QTL analysis across environments and QTL based estimation of time to floral induction and flowering in *Brassica oleracea*. Mol Breeding 21:205-216

Wang J, Long Y, Wu B, Liu J, Jiang C, Shi L, Zhao J, King GJ, Meng J (2009) The evolution of *Brassica napus FLOWERING LOCUS T* paralogues in the context of inverted chromosomal duplication blocks. BMC Evol Biol 9:271

Wang YP, Snowdon RJ, Rudloff E, Wehling P, Friedt W, Sonntag K (2004) Cytogenetic characterization and *fae1* gene variation in progenies from asymmetric somatic hybrids between *Brassica napus* and *Crambe abyssinica*. Genome 47:1-8

Warwick SI, Black LD (1991) Molecular systematics of *Brassica* and allied genera (subtribe *Brassicinae*, *Brassiceae*) – chloroplast genome and cytodeme congruence. Theor Appl Genet 82:81-92

Xu Y, Beachell H, McCouch SR (2004) A marker-based approach to broadening the genetic base of rice in the USA. Crop Sci 44:1947-1959

Yu CY, Hu SW, Zhao HX, Guo AG, Sun GL (2005) Genetic distances revealed by morphological characters, isozymes, proteins and RAPD markers and their relationships with hybrid performance in oilseed rape (*Brassica napus* L.). Theor Appl Genet 110:511-518

Zaman MW (1988) Limitations for introgression of yellow seed coat colour in *Brassica napus.* J Swed Seed Assoc 98:157-161

Zhang GQ, Zhou WJ, Gu HH, Song WJ, Momoh EJJ (2003) Plant regeneration from the hybridization of *Brassica juncea* and *B. napus* through embryo culture. J Agronomy Crop Sci 189:347-350

Zhou WJ, Zhang GQ, Tuvesson S, Dayteg C, Gertsson B (2006) Genetic survey of Chinese and Swedish oilseed rape (*Brassica napus* L.) by simple sequence repeats (SSRs). Genet Res Crop Evol 53:443-447

Zou J, Zhu J, Huang S, Tian E, Xiao Y, Fu D, Tu J, Fu T, Meng J (2010) Broadening the avenue of intersubgenomic heterosis in oilseed *Brassica*. Theor Appl Genet 120:283-290

Chapter 2

Broadening genetic diversity in canola: development of double-low quality inbred lines from a *Brassica napus* x *B. oleracea* cross

2.1 Introduction

Brassica napus L. (*2n*=38, AACC genome) is an oilseed crop with importance economically among countries where it is cultivated. For example, it was recently estimated that this crop adds $13.8 billion in economic activity to the Canadian economy (Mark Goodwin Consulting Ltd. 2008). The narrow genetic base of *B. napus* has been of concern to many researchers, as diversity is critical for continued improvement of the crop, as well as for adaptation to changing environments and markets (Cowling 2007b). Cowling (2007a) reported loss of genetic diversity among contemporary Australian spring type cultivars in a relatively closed breeding population. Using simple sequence repeat (SSR) markers, Hasan et al. (2006) found that among 96 rapeseed accessions from European genebanks tested, spring oilseed types had the lowest number of unique alleles – fewer than winter and vegetable types. Low levels of genetic diversity among spring and winter accessions compared to interspecific cross-derived Chinese semi-winter types were detected by Qian et al. (2006) using amplified fragment length polymorphism (AFLP) markers. Thus, there is a need for broadening genetic diversity in spring type *B. napus* cultivars, which are most commonly grown in Northern Europe, Canada, and Australia.

Molecular marker based phylogenetic studies have confirmed that the amphidiploid species *B. napus* arose from diploid progenitor species *B. rapa* L. (*2n*=20, AA genome) and *B. oleracea* L. (*2n*=18, CC genome) (Song et al. 1988); and there is evidence to suggest that multiple hybridization events led to modern day cultivated oilseed rape *B. napus* (Song and Osborn 1992). However, it is very unlikely that all genetic variation of the diploid parental species was included during its evolution. Within each diploid species there is a vast amount of morphological diversity (Babula et al. 2007, Hirai and Matsumoto 2007), and the

diploid genomes are genetically distinct from the corresponding genomes in *B. napus* (Song et al. 1988). Therefore, these diploid species are viewed as an important reservoir for increasing genetic diversity in *B. napus*. *Brassica rapa* can easily hybridize with *B. napus* even under natural conditions (Jørgensen and Andersen 1994, Hansen et al. 2001); therefore, it has been more commonly used for crossing with *B. napus*. More than 50% of the Chinese *B. napus* cultivars are derived from *B. napus* × *B. rapa* interspecific cross (Liu 1985, cited by Chen et al. 2008). However, a major constraint of utilizing the genetic diversity of *B. oleracea* for the improvement of *B. napus* is the difficulty of producing hybrids of these two species (Quazi 1988, Ripley and Beversdorf 2003, Bennett et al. 2008). Furthermore, interspecific crossing of *B. napus* × *B. oleracea* introduces high content of erucic acid and glucosinolate (GLS) in *B. napus* due to the lack of canola quality *B. oleracea* germplasm. Therefore, not much effort has been made on utilizing the C-genome species for increasing genetic diversity in *B. napus*.

The inheritance of erucic acid in the diploid species *B. rapa* is controlled by one gene locus (Dorrell and Downey 1964, Rahman et al. 1994); while in the amphidiploid species, *B. napus*, *B. juncea* (L.) Czern. and *B. carinata* A. Braun, it is controlled by two gene loci – one corresponding to each genome (Harvey and Downey 1964, Kirk and Hurlstone 1983, Chen and Heneen 1989, Getinet et al. 1997). Erucic acid levels in seeds are determined by the embryonic genotype; and erucic acid alleles generally act in an additive fashion. Rahman et al. (2001) reported that at least four gene loci are involved in the control of total GLS content in seed in spring type *B. napus*; and low GLS is due to the presence of recessive alleles in the homozygous condition at all loci. Other studies based on quantitative trait loci (QTL) mapping (Toroser et al. 1995, Uzunova et al. 1995, Howell et al. 2003) and phenotypic analysis (Magrath et al. 1993, Rücker and Röbbelen 1994) in both spring and winter type *B. napus* drew similar conclusions.

Our objective is to broaden genetic diversity in *B. napus* through exploitation of the C-genome of *B. oleracea*. Seeds of *B. oleracea* generally contain ~40% erucic acid in oil and >100 μmol GLS per g seed. Hybridization of *B. napus* and *B. oleracea* introduces these undesirable traits in the interspecific

hybrid progenies. Therefore, an understanding on the inheritance of these traits in interspecific hybrid progenies is needed for the development of double-low quality *B. napus* from this wide cross. Thus, the objective of this research was to study the inheritance of erucic acid content in an F_2 population of *B. napus* × *B. oleracea*; as well as to investigate the response to selection for low GLS content and *B. napus* type plants in different generations in the development of interspecific *B. napus* (*2n*=38) inbred lines. Furthermore, the interspecific inbred lines were evaluated for genetic diversity by use of SSR markers. In this research, we used *B. oleracea* var. *alboglabra* as model due to its spring growth habit and self-compatible nature.

2.2 Materials and methods

2.2.1 Parent material

One high erucic (40% erucic acid), high GLS (>100 μmol g^{-1} seed) self-compatible inbred (F_7) *B. oleracea* var. *alboglabra* (L. H. Bailey) Musil (CC, *2n*=18) line, and two zero-erucic, low (10 to 15 μmol g^{-1} seed) GLS *B. napus* L. (AACC, *2n*=38) doubled haploid lines, Hi-Q and A01-104NA, were used as parents of the interspecific crosses. *Brassica oleracea* var. *alboglabra* (Chinese kale) was domesticated in China (Prakash and Hinata 1980) and is a form of *B. oleracea*. It will hereafter be referred to as *B. oleracea*.

2.2.2 Production of F_1 hybrid plants

The following two crosses were made using *B. napus* as female: Hi-Q × *B. oleracea* and A01-104NA × *B. oleracea*. The details of the *in vitro* ovule culture technique applied for production of F_1 plants is described by Bennett et al. (2008). F_1 plants were grown in a growth chamber set at 20°/15°C day/night temperatures and 16 h photoperiod with photosynthetic flux density of 450 μE-m^{-2} s^{-1} at plant level. Three F_1 plants were grown from each cross and manually self-pollinated for generation of F_2 seeds.

2.2.3 F_2 to F_8 inbred lines

The F_2, F_3, F_5, F_7 and F_8 generation populations were grown in a growth chamber or a greenhouse (21°/18° ± 2°C day/night); and the F_4 and F_6 generation populations were grown in field plots (1- to 2-row plots of one to six m long) at the Edmonton Research Station of the University of Alberta. During the course of inbred development, only self-pollinated seed from individual plants produced by covering with transparent, micro-perforated plastic bags were used for propagation of the next generation population. In total, 65 F_8 generation inbred lines from Hi-Q × *B. oleracea* cross and 4 F_7 inbred lines from A01-104NA × *B. oleracea* were developed.

2.2.4 Fatty acid analysis

A total of 93 F_2 seeds, 52 from A01-104NA × *B. oleracea* and 41 from Hi-Q × *B. oleracea*, were analyzed for fatty acid profile by the half-seed fatty acid analysis technique. A minimum of four seeds from each of the three parents (A01-104NA, Hi-Q, and *B. oleracea*) were also analyzed for comparison. The details of the half-seed fatty acid analysis is described elsewhere (Bennett et al. 2008). Fatty acid analysis in F_3 plants was done on bulk seed sample, for which 0.20 to 0.25 g seed harvested from individual plants was used. Seeds were ground in N-pentane in 50 ml conical tubes, centrifuged at 1500 rpm for 10 min, and the supernatant was transferred to 10 × 75 mm glass tubes. The N-pentane was evaporated, leaving behind extracted oil. Extracted oil was methylated to produce fatty acid methyl esters. Fatty acid profiles of the extracted oil were determined by gas chromatography (Thies 1980).

2.2.5 Glucosinolate analysis

Bulked seed harvested from individual field plots were analyzed for total GLS content using near-infrared spectroscopy (NIRS, FOSS NIRSystems model 6500). A sample of 4 to 5 g seed was used for analysis. A calibration equation developed in our laboratory (unpublished) using WinISI II (Infrasoft International, LLC.)

was used, which allowed quantification of GLS content. This laboratory is accredited by the Canadian Grain Commission for doing GLS and fatty acid analysis. GLS content was calculated on a whole seed dry basis in μmol g^{-1} seed and the content for each seed family is reported as an average value of two replications.

2.2.6 Plant fertility

Plant fertility in F_5 to F_6 generation was estimated based on silique length (mm), number seeds per silique and seed yield per plant (g); while in early generations (F_3 to F_4) this was estimated based on seed yield (g) per plant only. For silique length and seed set per silique, 10 siliques from the main raceme of individual plants were used. Silique length was measured following the description by the Canadian Food Inspection Agency (2008). Seeds from 10 siliques of each plant were counted and average number of seeds per silique was calculated. Data was compared with their *B. napus* parent Hi-Q and A01-104NA.

2.2.7 Flow cytometry

Ploidy level in the 65 F_8 inbred lines for Hi-Q × *B. oleracea* and 4 F_7 A01-104NA × *B. oleracea* cross was estimated by flow cytometric analysis of nuclear DNA content, in which the intensity of the fluorescence from stained nuclei is measured relative to the *B. napus* control. For this, pieces (~1 cm^2) of leaf tissue were excised and chopped with a razor blade in Partec buffer supplemented with the nuclear fluorochrome DAPI (4,6-diaminido-2-phenylindole, Sigma, product no. D-9542). Samples were filtered using Partec CellTrics(tm) fitted with nylon gauze (30 μm pore size) and run through a Partec Ploidy Analyzer (Partec GmbH, Münster, Germany).

2.2.8 Meiotic analysis

Meiotic chromosome preparations were made according to Cheng et al. (1994) with slight modification. Flower buds were fixed in Farmer's solution (3:1 absolute alcohol:glacial acetic acid, v/v) for 24 h, transferred to 70% ethanol, and

stored at 4ºC until use. Prior to staining, the flower buds were fixed in Farmer's solution amended with ferrous chloride as a mordant for two days. The flower buds were then stained in Snow's carmine in a water bath at 60ºC for 4 h. Squash preparations were made in Hoyer's medium (Cunningham 1972).

2.2.9 Marker analysis

The 65 F_8 inbred lines of Hi-Q × *B. oleracea* cross were genotyped by SSR markers. For this, young, healthy leaves from each line and the two parental genotypes, grown in a greenhouse, were used for extraction of genomic DNA using a SIGMA DNA extraction kit (Sigma-Aldrich, St. Louis, MO) and following the manufacturer's instructions. Genomic DNA was diluted to 5 ng μL^{-1}. The two parents (Hi-Q and *B. oleracea*) were screened for polymorphic marker alleles using 191 SSR primer pairs covering all 19 *B. napus* linkage groups. Markers were obtained from various sources: Agriculture and Agri-Food Canada (AAFC), Biotechnology and Biological Science Research Council (UK), Celera AgGen Brassica Consortium, Uzunova and Ecke (1999), Suwabe et al. (2002), Tamura et al. (2005), and Cheng et al. (2009). Based on this, 19 SSR markers producing distinct fragments and clear polymorphism were selected for genotyping the inbred lines. Polymerase chain reactions (PCR) and labelling of PCR products were done following Kebede et al. (2010). A capillary ABI sequencer No. 3730 (Applied Biosystems, Foster City, CA) was used for detection of amplification products.

2.3 Results

2.3.1 Erucic acid segregation in F_2

Seeds of the two *B. napus* parents, Hi-Q and A01-104NA, contained 0.4% erucic acid in seed oil, while seeds of *B. oleracea* contained approximately 40% of this fatty acid. The distribution of the F_2 seeds for erucic acid content is presented in Fig. 2-1. The average erucic acid content in the F_2 population was 15.4%, which was significantly lower than the mean of the two parental species ($t = -4.76$, $P <$

0.01). A distinct zero-erucic acid group was observed (Fig. 2-1) and this included nine seeds. Erucic acid content in 75 seeds ranged between 4 and 27% with two peaks – one at about 8 to 10% erucic acid content and the second peak at 18% of this fatty acid content (Fig. 2-1). Nine F_2 seeds contained >30% erucic acid (Fig. 2-1). The F_2 distribution 84:9 for presence and absence of zero-erucic deviated significantly from the expected 3:1 segregation for the C-genome alleles (χ^2 = 11.65, $P < 0.01$).

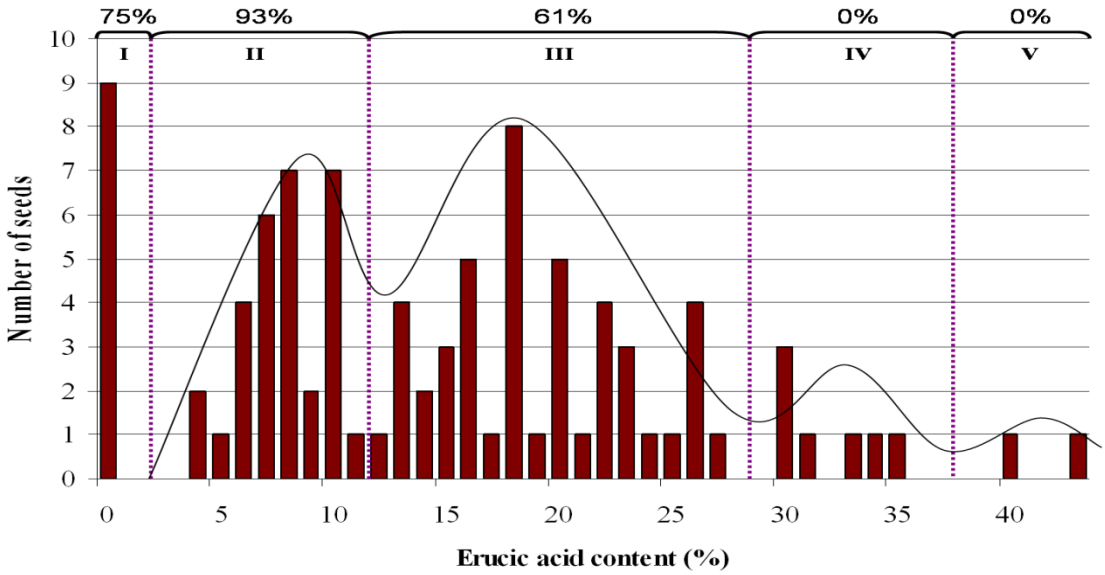

Figure 2-1. Distribution of the F_2 seeds of *B. napus* × *B. oleracea* for erucic acid content (n = 93). Seeds fall into five rough groups based on phenotype: I) zero-erucic acid, II) low erucic acid, III) intermediate erucic acid, IV) moderate-high erucic acid, and V) high erucic acid. Percentages along the top of each group indicate proportion of the total F_2 seeds that produced fertile F_2 plants yielding F_3 seeds.

The 93 F_2 seeds resulted in 72 F_2 plants; however, 20 were sterile and produced no viable seed. Of the 8 and 28 plants of the zero and low erucic acid groups, 75 and 93% plants respectively were fertile and produced viable seeds on self-pollination (Fig. 2-1). On the other hand, of the 33 and 3 plants of the intermediate and moderate-high erucic acid groups, 61 and 0% plants respectively produced seed (Fig. 2-1). The average erucic acid content of the F_2 seeds resulting fertile or semi-fertile F_2 plants was 11.2% (range 0 to 24%).

2.3.2 Petal colour in F_2

The petal colour of all F_2 plants was recorded and compared with the presence '+' or absence '-' of erucic acid. F_2 plants with white or creamy white petal colour carried the dominant white petal colour allele from *B. oleracea* (C^w); while the plants with yellow petal colour carried the recessive alleles (C^y) from *B. napus*. Likewise, '+' erucic plants had at least one high erucic acid allele (C^+) from *B. oleracea*; while '-' plants had only the zero-erucic acid (C^0) alleles from *B. napus*. Of the 72 F_2 plants observed, seven plants, i.e. 9.7% of the total number of plants, had recombinant phenotypes for these two traits (Table 2-1).

Table 2-1. Segregation for petal colour and presence/absence of erucic acid in F_2 population of *B. napus* × *B. oleracea* interspecific cross

Parental type		**Recombinant type**	
White '+'[z]	Yellow '-'	White '-'	Yellow '+'
60	5	3	4
65		**7**	

[z]'+' erucic acid present in seed; '-' erucic acid absent in seed; White & Yellow = Petal colour

2.3.3 Erucic acid in F_3 plants

Twenty-one F_3 seed families, derived from self-pollination of 6 zero-erucic acid and 15 low (<10%) erucic acid plants, were selected from where 222 F_3 plants were grown. In the F_3 generation, 70 plants from 18 families produced >0.2 g seeds. Seeds of 37 F_3 plants, descendant of five zero-erucic F_3 seed families, were all zero-erucic acid type. Erucic acid content in the seeds of the remaining 33 plants from 13 low erucic acid F_3 seed families ranged between zero and 15% with a mean of 5.6%; of which 10 plants were zero-erucic acid type.

2.3.4 Seed glucosinolate content

GLS content of the *B. napus* genotypes, A01-104NA and Hi-Q, grown together with different segregating populations ranged between 6.7 and 14.3 μmol g^{-1} seed, with a mean of 10.6 ± 1.6. In the F_4 generation, GLS content among the 21 families ranged from 14.6 to 67. 5 μmol g^{-1} seed with a mean of 38.0 ± 13.3. For

this population, seven families (33%) had GLS content ≤30 μmol g^{-1} seed and one (5%) family had a content similar (≤15 μmol g^{-1} seed) to the *B. napus* parents. In case of F_6, 25 families had a GLS content ranging from 10.6 to 50.7 μmol g^{-1} seed with a mean of 24.7 ± 10.7. For this population, 18 families (72%) had ≤30 μmol g^{-1} seed, and seven (28%) were similar to the *B. napus* parents. A weak but significant parent/progeny correlation was observed between the F_4 and F_6 generation (r = 0.26, $P < 0.05$).

2.3.5 Plant fertility

Data on silique length and seed set for F_3 to F_6 generation families is presented in Table 2-2. In each generation, the proportion of families with seed set not significantly lower than their *B. napus* parent increased; and in F_6, none of the families had significantly lower seed set ($P < 0.05$). Similarly, only one F_6 family was found to have significantly shorter silique length than its *B. napus* parent (Table 2-2). Silique length was positively correlated with number seeds per silique under both greenhouse (r = 0.65) and field (r = 0.36) conditions.

Table 2-2. Seed set and silique length (mm) in F_5 and F_6 generation families of *B. napus* × *B. oleracea* interspecific cross

Generation	No. of families[z]	Silique length (mm)[y]	No. of families not sig. shorter[x]	No. seeds/silique[y]	No. of families not sig. less[x]
F_5	14 (70)	40.4 - 60.9 51.0 ± 1.1 (51.8 ± 1.5)	12 (86%)	10.4 - 19.8; 15.7 ± 0.6 (16.4 ± 1.2)	12 (86%)
F_6	25 (50)	52.8 - 71.3 63.4 ± 0.7 (60.0 ± 0.8)	24 (96%)	22.3 - 35.3; 28.1 ± 0.5 (27.0 ± 1.9)	25 (100%)

[z] Total number plants measured given in brackets
[y] Data presented as range and mean ± SE; in brackets, *B. napus* parent mean ± SE
[x] For seed set and silique length, number families (in brackets percent families) not significantly less than *B. napus* parent at $P < 0.05$

2.3.6 Ploidy

The *B. napus* parents had Partec values of 195.8 ± 0.7 (A01-104NA) and 195.7 ± 3.8 (Hi-Q), while the *B. oleracea* parent had a value of 117.4 ± 1.3. Partec values

in the four inbred lines derived from A01-104NA × *B. oleracea* cross ranged from 191.1 to 222.7, with an average value of 208.0 ± 15.7. This was not significantly different from the A01-104NA parent (t = 1.54, $P = 0.22$). The 65 inbred lines derived from Hi-Q × *B. oleracea* cross had a range of 175.0 to 208.0 with a mean of 196.3 ± 4.9. The difference between this population and the Hi-Q parent was non-significant (t = 1.02, $P = 0.31$). However, both inbred line populations were significantly different from the *B. oleracea* parent for Partec values ($P < 0.01$).

Six F_8 inbred lines of Hi-Q × *B. oleracea* cross, three with lowest and three with highest Partec values, were examined for seed set – a measure to estimate the fertility of the inbred lines for both self-pollination and reciprocal crosses with their *B. napus* parent (Table 2-3). Seed set was generally good with the exception of family 9-285 (Table 2-3). Furthermore, the meiotic chromosome number in 13 pollen mother cells (PMC's) at diakinesis revealed 18 to 19 bivalents in family 9-296 (extreme low Partec value); suggesting that the chromosome number of these plants was close to *B. napus* (Fig. 2-3).

Table 2-3. Seed set in selected six F_8 lines of *B. napus* × *B. oleracea* cross and their crossability with their *B. napus* parent Hi-Q. Partec values of these lines are also given as an estimate of chromosome number

		No. seeds per silique		
F_8 line	**Partec value**	**Self- pollination**	**Hi-Q × F_8**	**F_8 x Hi-Q**
9-296	175.0	11.0	15.0	14.8
9-261	185.1	16.5	7.9	13.3
9-267	188.4	12.5	17.0	12.9
9-289	202.8	10.8	13.0	5.5
9-251	203.5	13.9	12.3	14.0
9-285	208.0	0.0	7.7	4.2
Hi-Q (check)	195.7	17.1	-	-

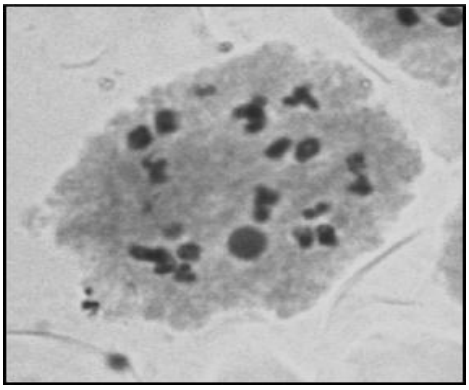

Figure 2-2. Pollen mother cell (PMC) from the F_8 line '9-296' of *B. napus* × *B. oleracea* with Partec value 175.0 showing 18 to 19 bivalents at diakinesis.

2.3.7 Marker analysis

The 19 polymorphic SSR markers from the nine C-genome linkage groups were tested on the 65 F_8 inbred lines of Hi-Q × *B. oleracea* cross. These markers detected a total of 44 alleles. The occurrence of *B. oleracea* alleles among the 65 inbred lines ranged from 0.0 to 72.7% with a mean of 25.9%. The F_8 lines (n=30) originating from the F_6 families with GLS content similar to Hi-Q (≤15 μmol g^{-1} seed) had an average of 16.3% (range 0.0 to 26.7%) alleles from *B. oleracea*. The 22 F_6 families with GLS content 16 to 30 μmol g^{-1} seed averaged 30.7% (range 0.0 to 66.7%), while the 13 lines >30 μmol g^{-1} seed averaged 39.9% (range 15.8 to 72.7%) for the *B. oleracea* alleles.

Genetic similarity among 61 F_8 inbred lines, estimated based on UPGMA method is presented in Fig. 2-3. The dendrogram depicted two main groups (I and II) at a genetic similarity coefficient of 0.58. Group I, consisting of 46 inbred lines, showed greater similarity with Hi-Q and included most of the low GLS lines. However, in Group I two subgroups are evident with genetic similarity coefficient of about 0.88. On the other hand, Group II, which consisted of 15 inbred lines, had genetic similarity coefficient of only 0.58 with Hi-Q. About 50% of these inbred lines had GLS content of ≤30 μmol g^{-1} seed, suggesting the possibility of extracting low GLS lines with greater genetic diversity.

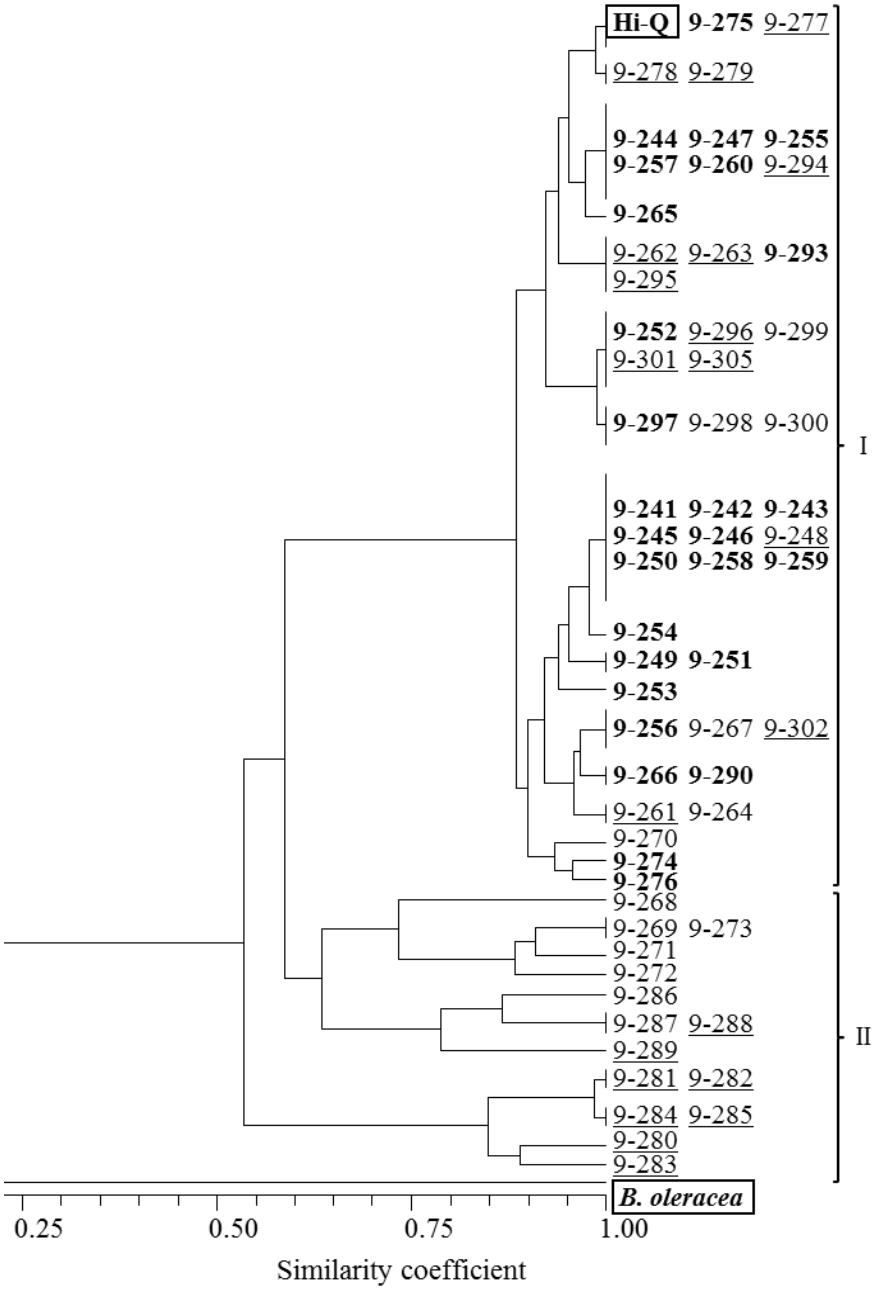

Figure 2-3. Dendrogram showing genetic similarity for 61 F_8 inbred lines derived from a *Brassica napus* Hi-Q × *B. oleracea* interspecific cross using unweighted pair-group method with arithmetic mean (UPGMA) clustering based on genetic fingerprint of polymorphic simple sequence repeat (SSR) markers. The inbred lines with GLS content similar to Hi-Q (≤15 µmol g^{-1}) are in bold font, those containing 16 to 30 µmol g^{-1} are underlined, and >30 µmol g^{-1} lines are in regular font.

2.4 Discussion

Introgression of genetic diversity into *B. napus* from its parental species *B. oleracea* imposes a greater challenge compared to introgression of genetic diversity from *B. rapa* primarily due to high erucic acid and high GLS contents in the seed of this donor species. In this study, we examined the inheritance of erucic acid content in F_2 populations of *B. napus* × *B. oleracea* interspecific crosses. The genomic composition of the digenomic triploid (ACC) F_1 plants in respect to erucic acid alleles can be designated as $A^0C^+C^0$, where A^0C^0 are the zero-erucic alleles of the *B. napus* parent, and C^+ is the high erucic acid allele of *B. oleracea*. Assuming no allosyndetic pairing of chomosomes in meiosis of the F_1 plants, the C-genome chromosomes are expected to undergo normal disomic segregation, while the haploid set of the A-genome chromosomes would be randomly included in the gametes. If no differential viability of the gametes occurs due to inclusion of different number of A-genome chromosomes, the following types of gametes in F_1 plants are expected to be formed in equal frequency in respect to erucic acid alleles: C^+, C^0, A^0C^+, and A^0C^0. The expected F_2 genotypic frequencies based on random fertilization of the gametes as well as their phenotype based on additive contribution of the erucic acid alleles (Harvey and Downey 1964) is presented in Table 2-4. Chi-square test showed that the number of F_2 seeds falling in these phenotypic groups deviated significantly ($\chi^2 = 45.78$, $P < 0.01$) from the expected numbers. The zero-erucic acid (group I), moderate-high erucic acid (group IV), and high erucic acid groups (group V) (Fig. 2-1) had significantly lower number of seeds than expected; while the low erucic acid group (group II) had significantly greater number of seeds than expected. The observed number of seeds falling into the intermediate erucic acid group (group III) did not deviate significantly from expected.

The presence or absence of erucic acid in F_2 seeds depended on segregation of the erucic acid alleles at one gene locus on the C-genome, and the F_2 seeds containing erucic acid would carry at least one C^+ allele. Thus, a 3:1 segregation for presence:absence of this fatty acid was expected among the F_2 seeds. However, we observed an almost 9:1 segregation for presence:absence of erucic

acid in this population – i.e., 90% of F_2 seeds inherited at least one C^+ allele. This observed segregation significantly deviated from the expected F_2 segregation (χ^2 = 11.65, $P < 0.01$). Thus, the pattern of segregation for erucic acid observed in this F_2 population strongly indicates that (i) segregation distortion occurred in favour of the high erucic acid (C^+) allele originating from *B. oleracea*; and (ii) aneuploid gametes from F_1 hybrid plants carrying greater numbers of A-genome chromosomes were more viable and were more likely to form fertile zygotes. Higher viability of gametes that have a greater number of chromosomes has been implicated in several aneuploid populations generated from interspecific hybrids involving *B. napus*, e.g. in *B. carinata* × *B. napus* (Fernandez-Escobar et al. 1988), *B. napus* × *B. oleracea* (Bennett et al. 2008), and *B. napus* × *B. carinata* (Stead 2008). This might be the reason that the inbred lines in this study stabilized to a ploidy level similar to *B. napus* and predominantly had *B. napus* type morphology, even as early as in F_3 generation. In addition, 93% of F_2 plants from the low erucic acid category (group II, Fig. 2-1), predicted to have both copies of the A^0 allele, were fertile and able to produce F_3 seed.

Table 2-4. Expected F_2 seed genotypes frequency and their phenotypes based on random segregation of erucic acid alleles in digenomic triploid ($A^0C^+C^0$) F_1 plants of *B. napus* × *B. oleracea* interspecific cross

Possible F_2 genotypes[z]	Expected frequency[y]	Expected phenotypes[x]	Group in Fig. 2-1
C^0C^0	1/16 (6.25)	0	I
$A^0C^0C^0$	2/16 (12.5)	0	I
$A^0A^0C^0C^0$	1/16 (6.25)	0	I
C^+C^0	2/16 (12.5)	20	III
$A^0C^+C^0$	4/16 (25.0)	~15	III
$A^0A^0C^+C^0$	2/16 (12.5)	10	II
C^+C^+	1/16 (6.25)	40	V
$A^0C^+C^+$	2/16 (12.5)	~30	IV
$A^0A^0C^+C^+$	1/16 (6.25)	20	III

[z] C^0 = zero-erucic allele from the C-genome of *B. napus* parent; C^+ = erucic acid allele from *B. oleracea* parent; A^0 = zero-erucic allele from the A-genome of *B. napus* parent
[y] Expected percent given in brackets
[x] F_2 seed erucic acid content (%), based on an additive contribution of each allele

According to Chen et al. (1988), the white petal colour allele (C^w) of *B. oleracea* var. *alboglabra* is dominant over the yellow petal colour allele (C^y) of *B. napus*. Therefore, a 3:1 segregation for white:yellow petal colour would be expected in F_2. However, a 7:1 segregation for white:yellow was observed among these plants, which deviated significantly from expected ($\chi^2 = 6.00$, $P < 0.05$), as was observed in the case of erucic acid content. The similar pattern of segregation of erucic acid and petal colour is due to linkage between these two traits (Chen et al. 1992). Woods and Séguin-Swartz (1997) reported that the C-genome petal colour locus is located at a distance of approximately 3.2 cM from the erucic acid locus. In previous studies, we also found the erucic acid allele of the C-genome to be linked to the petal colour allele at a distance of approximately 5 to 8 cM (Rahman et al. 2001, Bennett et al. 2008). Data presented from this study substantiate earlier reports of linkage between the white petal colour and high erucic acid alleles in the C-genome. The erucic acid locus (*Bn-FAE1.2*) of the C-genome is mapped on the linkage group N13 (Basunanda et al. 2007, Rahman et al. 2008); and based on this, it can be anticipated that the petal colour gene of the C-genome is also located on N13.

Approximately two to three major loci, along with several loci having minor effect, are involved in the control of GLS in the C-genome of *B. napus* (Basunanda et al. 2007, Howell et al. 2003). A more or less continuous variation was observed for this trait among F_4 and F_6 plant families. This is not unexpected taking into consideration the influence of minor genes and environmental effect on phenotype of this polygenic trait. The relatively high proportion of low GLS genotypes that could be obtained among F_6 plant families is likely due to relatively simpler segregation for this trait in this interspecific population (only C-genome involved) compared to segregation for this trait in a cross between high and low *B. napus* genotypes (both A and C genomes involved).

In *Brassica* interspecific crosses between the amphidiploid and diploid species, the digenomic triploids often stabilizes into amphidiploid type after several generations of inbreeding (Zaman 1988, Rahman 2001). In the present study, we also obtained stable *B. napus* type plants in the F_3 to F_4 generation.

Ploidy in these lines was estimated using the Partec flow cytometry system. For these populations, an A01-104NA plant was selected as the control and set at value of 196.3. The mean Partec value of the 65 Hi-Q × *B. oleracea* evaluated in this study was similar to the *B. napus* check. In this population, the line 9-296 had the greatest deviation from the check (Partec measurement = 175.0) and was found to have 18 to 19 bivalents in 13 PMCs examined. In addition, five of six lines with greatest deviation from the *B. napus* check showed good seed set on self-pollination as well as crossing with *B. napus*. These observations provide strong evidence that the majority of inbred lines developed in this study have a euploid or near-euploid *B. napus* (*2n*=38) genomic constitution.

The objective of this study was to broaden genetic diversity in canola quality *B. napus* with a reasonable proportion of the C-genome originating from *B. oleracea*. While all the 65 Hi-Q × *B. oleracea* inbred lines had zero-erucic acid content, these lines varied for levels of GLS. Genotyping of these inbred lines with SSR markers revealed that the lines with lower GLS content in seeds generally had lower number of *B. oleracea*-specific alleles. However, of the very genetically distinct lines depicted in Group II of the dendrogram, about 50% had GLS content of $\leq$30 μmol g^{-1} seed. In addition, of the mildly diverse subgroup depicted in Group I with genetic similarity of about 0.88 to Hi-Q, approximately 75% had $\leq$15 μmol g^{-1} seed GLS. These lines can be used in breeding programs for developing improved genetically diverse, canola quality open-pollinated and hybrid spring type cultivars.

In conclusion, segregation distortion for erucic acid alleles was found in F_2 where the proportion of zero-erucic genotype was significantly less than the expected numbers. This needs to be taken into account while designing a breeding program for the introgression of genetic diversity from *B. oleracea* into double-low quality *B. napus*. The tight linkage between the white petal colour allele and high erucic acid allele residing on the *B. oleracea* C-genome serves as a convenient morphological marker for selecting zero-erucic types in crosses involving white flowered C-genome species. As expected, an intensive effort for generating a *B. napus* × *B. oleracea* interspecific population was needed.

However, double-low quality, fully fertile and genetically diverse *B. napus* genotypes can be obtained from a relatively small segregating population. Knowledge on the utility of these lines can be further extended by producing test hybrids with adapted breeding lines and evaluating the hybrids for heterosis for seed yield and other traits.

2.5 References

Babula D, Kaczmarek M, Ziółkowski PA, Sadowski J (2007) *Brassica oleracea*. In: Kole C (ed) Genome Mapping and Molecular Breeding in Plants, volume 5. Springer-Verlag, Berlin/Heidelberg, Germany, pp 227-285

Basunanda P, Spiller TH, Hasan M, Gehringer A, Schondelmaier J, Lühs W, Friedt W, Snowdon RJ (2007) Marker-assisted increase of genetic diversity in a double-low seed quality winter oilseed rape genetic background. Plant Breed 126:581-587

Bennett RA, Thiagarajah MR, King JR, Rahman MH (2008) Interspecific cross of *Brassica oleracea* var. *alboglabra* and *B. napus*: Effects of growth condition and silique age on the efficiency of hybrid production, and inheritance of erucic acid in the self-pollinated backcross generation. Euphytica 164:593-601

Canadian Food Inspection Agency (2008) Cruciferous crop inspection procedures: Appendix VII – Plant descriptions and illustrations of mustard and canola. Available at: http://www.inspection.gc.ca/english/plaveg/seesem/man/swi-crue.shtml#a22.

Chen BY, Heneen WK (1989) Fatty acid composition of resynthesized *Brassica napus* L., *B. campestris* L., and *B. alboglabra* Bailey with special reference to the inheritance of erucic acid content. Heredity 63:309-314

Chen BY, Heneen WK, Jönsson R (1988) Resynthesis of *Brassica napus* L. through interspecific hybridization between *B. alboglabra* Bailey and *B. campestris* L. with special emphasis on seed colour. Plant Breed 101:52-59

Chen BY, Simonsen V, Lannér-Herrera C, Heneen WK (1992) A *Brassica campestris-alboglabra* addition line and its use for gene mapping, intergenomic gene transfer and generation of trisomics. Theor Appl Genet 84:592-599

Chen S, Nelson MN, Ghamkhar K, Fu T, Cowling WA (2008) Divergent patterns of allelic diversity from similar origins: the case of oilseed rape (*Brassica napus* L.) in China and Australia. Genome 51:1-10

Cheng BF, Heneen WK, Chen BY (1994) Meiotic studies on a *Brassica campestris-alboglabra* monosomic addition line and derived *B. campestris* primary trisomics. Genome 37:584-589

Cheng X, Xu J, Xia S, Gu J, Yang Y, Fu J, Qian X, Zhang S, Wu J, Liu K (2009) Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor Appl Genet 118:1121-1131

Cowling WA (2007a) Genetic diversity in Australian canola and implications for crop breeding for changing future environments. Field Crop Res. 104:103-111

Cowling WA (2007b) Genetic diversity in canola for changing environments. Proc 12th Int Rapeseed Congr 1:243-246

Cunningham JL (1972) A miracle mounting fluid for permanent whole-mounts of microfungi. Mycologia 64:906-911

Dorrell DG, Downey RK (1964) The inheritance of erucic acid in rapeseed (*Brassica campestris*). Can J Plant Sci 44:499-504

Fernandez-Escobar J, Dominguez J, Martin A, Fernandez-Martinez JM (1988) Genetics of the erucic acid content in interspecific hybrids of Ethiopian mustard (*Brassica carinata* Braun) and rapeseed (*B. napus* L.). Plant Breed 100:310-315

Getinet A, Rakow G, Raney JP, Downey RK (1997) The inheritance of erucic acid content in Ethiopian mustard. Can J Plant Sci 77:33-41

Hansen LB, Siegismund HR, Jørgensen RB (2001) Introgression between oilseed rape (*Brassica napus* L.) and its weedy relative *B. rapa* L. in a natural population. Genet Resour Crop Evol 48:621-627

Harvey BL, Downey RK (1964) The inheritance of erucic acid content in rapeseed (*Brassica napus*). Can J Plant Sci 44:104-111

Hasan M, Seyis F, Badani AG, Pons-Kühnemann J, Friedt W, Lühs W, Snowdon RJ (2006) Surveying genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet Resour Crop Evol 53:793-802

Hirai M, Matsumoto S (2007) *Brassica rapa*. In: Kole C (ed) Genome Mapping and Molecular Breeding in Plants, volume 5. Springer-Verlag, Berlin/Heidelberg, Germany, pp 185-190

Howell PM, Sharpe AG, Lydiate DJ (2003) Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). Genome 46:454-460

Jørgensen RB, Andersen B (1994) Spontaneous hybridization between oilseed rape (*Brassica napus*) and weedy *B. campestris* (Brassicaceae): A risk of growing genetically modified oilseed rape. Am J Bot 81:1620-1626

Kebede B, Thiagarajah MR, Zimmerli C, Rahman MH (2010) Improvement of open-pollinated spring rapeseed (*Brassica napus* L.) through introgression of genetic diversity from winter rapeseed. Crop Sci 50:1236-1243

Kirk JTO, Hurlstone CJ (1983) Variation and inheritance of erucic acid in *Brassica juncea*. Z Pflanzenzüchtg 90:331-338

Liu H (1985) Rapeseed genetics and breeding. Shanghai Science and Technology Press, Shanghai.

Magrath R, Herron C, Giamoustaris A, Mithen R (1993) The inheritance of aliphatic glucosinolates in *Brassica napus*. Plant Breed 111:55-72

Mark Goodwin Consulting Ltd (2008) Canola Socio-Economic Value Report.

Prakash S, Hinata K (1980) Taxonomy, cytogenetics and origin of crop Brassicas, a review. Opera Bot 55:1-57

Qian W, Meng J, Li M, Frauen M, Sass O, Noack J, Jung C (2006) Introgression of genomic components from Chinese *Brassica rapa* contributes to widening the genetic diversity in rapeseed (*B. napus* L.), with emphasis on the evolution of Chinese rapeseed. Theor Appl Genet 113:49-54

Quazi MH (1988) Interspecific hybrids between *Brassica napus* L. and *B. oleracea* L. developed by embryo culture. Theor Appl Genet 75:309-318

Rahman M, Sun Z, McVetty PBE, Li G (2008) High throughput genome-specific and gene-specific molecular markers for erucic acid genes in *Brassica napus* (L.) for marker-assisted selection in plant breeding. Theor Appl Genet 117:895-904

Rahman MH (2001) Production of yellow-seeded *Brassica napus* through interspecific crosses. Plant Breed 120:463-472

Rahman MH, Rahman L, Stølen O, Sørensen H (1994) Inheritance of erucic acid content in yellow- and white-flowered yellow sarson x Canadian *Brassica campestris* L. Acta Agric Scand 44:94-97

Rahman MH, Joersbo M, Poulsen MH (2001) Development of yellow-seeded *Brassica napus* of double low quality. Plant Breed 120:473-478

Ripley VL, Beversdorf WD (2003) Development of self-incompatible *Brassica napus*: (I) introgression of S-alleles from *Brassica oleracea* through interspecific hybridization. Plant Breed 122:1-5

Rücker B, Röbbelen G (1994) Inheritance of total and individual glucosinolate contents in seeds of winter oilseed rape (*Brassica napus* L.). Plant Breed 113:206-216

Song KM, Osborn TC (1992) Polyphyletic origins of *Brassica napus*: new evidence based on organelle and nuclear RFLP analyses. Genome 35:992-1001

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theor Appl Genet 75:784-794

Stead KE (2008) Mapping the introgression of the *Brassica carinata* C and B genomes into *Brassica napus*. Ph.D. Thesis, University of Alberta, Edmonton

Suwabe K, Iketani H, Nunome T, Kage T, Hirai M (2002) Isolation and characterization of microsatellites in *Brassica rapa* L. Theor Appl Genet 2002:1092-1098

Tamura K, Nishioka M, Hayashi M, Zhang Z, Lian C, Hougetsu T, Harada K (2005) Development of microsatellite markers by ISSR-suppression-PCR method in *Brassica rapa*. Breed Sci 55:247-252

Thies W (1980) Method of the Canadian Grain Commission. In: J. Daun and D. I. McGregor (eds), Analytical chemistry of rapeseed. The Canola Council of Canada, Winnipeg

Toroser D, Thormann CE, Osborn TC, Mithen R (1995) RFLP mapping of quantitative trait loci controlling seed aliphatic-glucosinolate content in oilseed rape (*Brassica napus* L.). Theor Appl Genet 91:802-808

Uzunova M, Ecke W (1999) Abundance, polymorphism and genetic mapping of microsatellites in oilseed rape. Plant Breed 118:323-326

Uzunova M, Ecke W, Weissleder K, Röbbelen G (1995) Mapping the genome of rapeseed (*Brassica napus* L.) I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theor Appl Genet 90:194-204

Woods DL, Séguin-Swartz G (1997) An investigation on linkage between white flower colour and erucic acid in summer rape. Can J Plant Sci 78:109-111

Zaman MW (1988) Limitations for introgression of yellow seed coat colour in *Brassica napus.* J Swed Seed Assoc 98:157-161

Chapter 3

Broadening genetic diversity in canola (*Brassica napus* L.) using the C-genome species *B. oleracea*[1]

3.1 Introduction

Canola is an important crop in Canada, with approximately 7.5 million ha seeded in 2011, second in seeded area to wheat (8.8 million ha) (Statistics Canada 2011). Of the total canola acreage, *Brassica napus* L. accounted for 99.6% of insured canola grown in Alberta in 2010 (AFSC 2010), representative of the pre-eminence of this species in Canada. The rate of improvement of this crop for seed yield and agronomic traits as well as for the ability to adapt to changing environments may be severely limited due to its narrow genetic base. Introgression of new allelic diversity will therefore be required, particularly among populations of spring type cultivars commonly grown in Australia (Cowling 2007), Europe (Hasan et al. 2006), and Canada (Fu and Gugel 2010), for continued improvement in this crop.

The diploid progenitor species of *Brassica napus* (AACC genome, *2n*=38), viz. *B. rapa* L. (AA genome, *2n*=20) and *B. oleracea* L. (CC genome, *2n*=18), harbour a vast amount of morphological and genetic diversity (Babula et al. 2007, Hirai and Matsumoto 2007, Song et al. 1988) and thus represent a valuable source of alleles for broadening genetic diversity in *B. napus* (Seyis et al. 2003). To exploit this, two approaches can be taken: direct crossing of *B. napus* to one of its diploid progenitor species or resynthesis of *B. napus* by interspecific crossing between various forms of its diploid progenitor species, followed by chromosome doubling. In resynthesized *B. napus*, high levels of genome changes in early generations have been reported (Song et al. 1995); which is another potential source of new genetic variation. *Brassica rapa* has been used to some extent in the breeding of *B. napus*, particularly in China (Qian et al. 2006) – however, very little work has been done for the improvement of *B. napus* by using *B. oleracea*.

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A major obstacle in the use of resynthesized *B. napus* in a practical breeding program is that of introducing non-canola quality traits in the breeding materials, which require extra efforts for removal (Schelfhout et al. 2008). Canola quality characteristics include zero-erucic acid content in the seed oil and low aliphatic glucosinolate content (<30 µmol g^{-1}) in seed meal. Erucic acid content in *B. napus* is controlled by two gene loci, one locus on each of the A- and C-genomes (Chen and Heneen 1989, Dorrell and Downey 1964). At least four gene loci are involved in the control of total seed glucosinolate content in spring type *B. napus*; and low glucosinolate is due to the presence of recessive alleles in the homozygous condition at all loci (Rahman et al. 2001). Although canola quality *B. rapa* genotypes are available, no canola quality *B. oleracea* exists, even though naturally occurring low erucic acid mutants have been identified in this species (Lühs et al. 2000; cited in Seyis et al. 2003). Thus, resynthesized *B. napus* are generally non-canola quality type which severely limits their usefulness in a practical breeding program. The development of a canola quality form of *B. oleracea*, which could be crossed with canola quality *B. rapa*, would therefore be a valuable tool for resynthesis of canola quality *B. napus* for efficient introgression of genetic diversity from these parental species into canola *B. napus*.

Interspecific crossing of *B. napus* and *B. oleracea* results in hybrids of digenomic triploid genome composition ACC. Previous studies with *B. carinata* A. Braun × *B. oleracea* interspecific crosses have demonstrated that digenomic triploids (BCC) often stabilize into amphidiploid types and backcrossing of the BCC hybrids to the diploid parent leads to diploid type progeny (Rahman 2001, Zaman 1988). Therefore, it is hypothesized that backcrossing of the ACC plants of *B. napus* × *B. oleracea* to *B. oleracea* would result in a greater probability of stabilizing into *B. oleracea* types in self-pollinated generations. With respect to the alleles for erucic acid and seed glucosinolate content of the C-genome of *B. napus*, these canola quality alleles would be randomly segregating in the self-pollinated progeny of ACC × *B. oleracea*, resulting in euploid (*2n*=18) *B. oleracea* of canola quality type. However, the hybrid progenies of ACC × *B. oleracea* are theoretically expected to be composed of euploid to aneuploid (CC +

0A to CC + 10A) type plants. Therefore, self-pollinated progenies of F_1 × *B. oleracea* backcross hybrids would also be expected to result in *B. napus* types with greater amount of genetic diversity from *B. oleracea* and retaining canola quality traits of the C-genome of *B. napus*.

Thus, the objective of the current research is two-fold: i) to assess the feasibility of introgressing canola quality traits from *B. napus* into *B. oleracea* for the purpose of developing a canola quality *B. oleracea*; and ii) to develop *B. napus* with greater genetic diversity from *B. oleracea* while retaining canola quality traits. The development of canola quality *B. oleracea* is for the longer-term objective of resynthesizing genetically diverse *B. napus* from its diploid progenitor species *B. oleracea* and *B. rapa* without the loss of canola quality traits; and thus to make use of resynthesized *B. napus* lines directly in practical breeding for cultivar development. In this study, *B. oleracea* var. *alboglabra* was used as a model *B. oleracea* genotype for testing these hypotheses.

3.2 Materials and methods

3.2.1 Parent Material

Two spring-type *B. napus* (AACC genome, *2n*=38) parents were used in this study: Hi-Q, a cultivar registered in Canada in 1999 (Stringam et al. 2000) and A01-104NA, a breeding line maintained at the University of Alberta. Both *B. napus* parents are canola quality (zero-erucic acid in seed oil, 10 to 15 μmol g^{-1} glucosinolate in seed meal) doubled haploid (DH) lines. The *B. oleracea* var. *alboglabra* (L. H. Bailey) Musil (Chinese kale), considered to be a vegetable form of *B. oleracea* (Babula et al. 2007), was selected as a model *B. oleracea* due to its self-compatible nature and lack of vernalization requirement for flowering. The line used in this study was highly inbred (F_7), and high in erucic acid content in seed oil (40%) and glucosinolate content in seed meal (>80 μmol g $seed^{-1}$). For simplicity, it will hereafter be referred to as *B. oleracea*.

3.2.2 Population Development

Hi-Q and A01-104NA were used as female parents in interspecific crosses between *B. napus* and *B. oleracea.* F_1 hybrids were produced through application of ovule culture technique. The detail of this technique is described by Bennett et al. (2008). Three F_1 hybrid plants from each cross (Hi-Q × *B. oleracea* and A01-104NA × *B. oleracea*) were pollinated with *B. oleracea*, and the ovule culture technique was again applied to generate backcross (BC_1) hybrids. Subsequent generations were obtained through self-pollination of individual plants under bag isolation (BC_1S_1 to BC_1S_7). Due to extremely low fertility in BC_1 to BC_1S_3 generation plants, manual self-pollination was also applied to maximize number of seeds for the next generation. The two populations resulting from these crosses will be referred to as Population I (resulting from A01-104NA parent) and Population II (from Hi-Q parent).

Plant populations up to BC_1S_7 were managed in growth chamber and greenhouse facilities at the University of Alberta. Growth chamber (Conviron, Winnipeg, Canada) conditions were as follows: 16 h photoperiod, 20-15°C/15-10°C day/night temperature, photosynthetic flux density of approximately 450 $\mu E\text{-}m^{-2}s^{-1}$ at plant level. Greenhouse conditions were as follows: 21°/18° ± 3°C day/night temperature and 16 h artificial light supplement using T5 high efficiency bulbs (450 $\mu E\text{-}m^{-2}\ s^{-1}$ output). In both greenhouse and growth chamber conditions, plants were grown in five or six-inch pots in a soilless mixture (SunGro® Sunshine Mix 4) consisting of sphagnum peat moss, perlite, limestone and gypsum. Plants were watered daily and fertilized weekly with 15-30-15 N-P-K fertilizer (10 g fertilizer/10 l H_2O). In addition to controlled environments, BC_1S_5 families were grown in field plots with two replications at the Edmonton Research Station of the University of Alberta. Plots were single-row, 1 m long with 0.3 m space between plots.

3.2.3 Fatty Acid Analysis

The half-seed technique of gas chromatographic fatty acid analysis was applied to determine the fatty acid profile of individual BC_1S_1 to BC_1S_4 seeds, along with

their parents. Details of this technique are described elsewhere (Bennett et al. 2008).

3.2.4 Glucosinolate Analysis

Bulked seed harvested from BC_1S_5 families grown in field plots (two replications), and self-pollinated seed of individual BC_1S_5 plants grown under greenhouse or growth chamber environments, were analyzed for total glucosinolate content, i.e. alkenyl and indole, using near-infrared spectroscopy (NIRS, FOSS NIRSystems model 6500). A sample size of 4 to 5 g seed was used for analysis. A calibration equation developed in our laboratory (unpublished) using WinISI II (Infrasoft International, LLC.) was used, which allows quantification of glucosinolate. In cases where less than 4 g seed was available, ring inserts and a calibration equation specifically designed for samples of 0.5 g seed were used (Raney and Serblowski 2007). Glucosinolate content was calculated in μmol g^{-1} on a whole seed dry basis.

3.2.5 Plant Morphology and Fertility

Individual plants of the BC_1S_2 to BC_1S_5 generations were evaluated for morphological characteristics. Leaf morphology including lobe development and shape of leaf margin, as well as silique characteristics were assessed based on the description of canola by the Canadian Food Inspection Agency (2008). In addition, leaf clasping, stem thickness, and inflorescence morphology were subjectively evaluated. For each of these individual traits, the following scale was used: 0 (*B. oleracea* type), 1 (intermediate type), or 2 (*B. napus* type). The total phenotypic score of the individual plants formed the basis for grouping the next generation population. Other traits recorded were petal color, days to flower, silique length (mm), number of seeds per silique, and seed yield per plant (g).

Plant fertility was estimated in two ways: first, individual plants were visually classified as either fertile (anthers well-developed; abundant pollen), semi-fertile (anthers poorly developed; small amount of pollen), or sterile (no pollen production). Second, pollen viability counts were done on an individual

plant basis. For this, one unopened bud (ca. one day prior to anthesis) from the plants was used, where the anthers were crushed on a glass slide containing 1 to 2 drops of acetocarmine. The debris was removed and the slide was covered with a cover slip. Counts of pollen grains were made under a microscope at 40x or 100x magnification from four to six non-overlapping fields. Pollen grains were classified as either (i) viable, characterized by well-formed pollen grains and stained red, or (ii) non-viable, characterized by unstained and shrunken appearance. Pollen fertility was expressed as the percentage of viable pollen to the total number of pollen grains counted.

3.2.6 Marker analysis

Young, healthy leaves were collected from all individual BC_1S_5 plants and their parents and stored at -80°C. The 30 individual plants (14 *B. oleracea*, 14 intermediate, and 2 *B. napus* type) from Population I with the highest level of fertility as determined by number of seeds per silique were subjected to microsatellite (SSR) marker analysis. In addition, a Population I BC_1S_5 plant family categorized as having predominantly *B. napus* type morphology and consisting of six individuals (two intermediate and four *B. napus* type), was also genotyped. Genomic DNA was extracted using the SIGMA extraction method. Genomic DNA was diluted to 5 ng μL^{-1} and the two parents (A01-104NA and *B. oleracea*) were screened for polymorphic marker alleles using 191 publicly available SSR primer pairs covering all 19 *B. napus* linkage groups. Source of markers was as reported in section 2.2.9. From these, 26 SSR markers producing distinct bands and clear polymorphism were used for genotyping BC_1S_5 plants. Polymerase chain reactions (PCR) and labelling of PCR products were done as described by Kebede et al. (2010). A capillary ABI sequencer No. 3730 (Applied Biosystems, Foster City, CA) was used for detection of amplification products.

3.2.7 Flow cytometry

Ploidy level of the BC_1S_6 plants was estimated by flow cytometry. For this, leaf tissues of ~1 cm^2 size were excised and chopped with a razor blade in Partec

buffer supplemented with nuclear fluorochrome DAPI (4,6-diaminido-2-phenylindole, Sigma, product no. D-9542). Samples were filtered using Partec CellTrics® fitted with nylon gauze (30 µm pore size) and run through a Partec Ploidy Analyzer (Partec GmbH, Münster, Germany). Partec values are relative to nuclear DNA content of *B. napus* (A01-104NA) which was set at value of 200 as the control, and are reported as arbitrary units (A.U.). Values for the 84 BC_1S_6 families (Population I = 79; Population II = 5), were obtained by averaging the A.U. from three plants per family. For each parent A01-104NA, Hi-Q, and *B. oleracea* six plants were used.

3.2.8 Meiotic chromosome analysis

Meiotic chromosome preparations were made according to Cheng et al. (1994) with the following modifications. Flower buds were fixed in Farmer's solution (3:1 ethanol:glacial acetic acid, v/v) for 24 h, transferred to 70% ethanol, and stored at 4ºC. Prior to staining, the flower buds were fixed in Farmer's solution amended with ferrous chloride as mordant for two days. The flower buds were then stained in Snow's carmine in a water bath at 60ºC for 4 h. Squash preparations were made in Hoyer's medium (Cunningham 1972). Pollen mother cells (PMCs) were examined using a Zeiss Primo Star light microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) under oil immersion at 1000x magnification.

3.3 Results

3.3.1 Erucic acid segregation

Mean erucic acid content of the parental genotypes, grown along with the BC_1S_1 to BC_1S_4 generation, was 0.2 ± 0.2, 0.2 ± 0.2, and 45.4 ± 4.4%, respectively for A01-104NA, Hi-Q, and *B. oleracea*. Segregation for erucic acid content into the three groups described below was similar among the families from the two crosses of *B. napus* × *B. oleracea* (BC_1S_1 $\chi^2 = 2.25$, $P = 0.32$; BC_1S_2 $\chi^2 = 2.83$, $P =$

0.24); therefore, data from these two crosses were pooled and presented together. Plants resulting from the seeds with zero-erucic acid content in oil were considered to be fixed for this trait; therefore, no further analysis was done on the offspring of these plants.

A total of 109 BC_1S_1 seeds were analyzed, where 19 seeds were zero-erucic type (0-2% erucic acid), three seeds were high erucic type (≥31% erucic acid), and 87 seeds had an intermediate level of erucic acid (7 to 30% erucic acid). The proportion of seeds falling into these three groups, resulting from self-pollination of BC_1S_1, BC_1S_2, and BC_1S_3 plants of intermediate levels of erucic acid, is given in Fig. 3-1. Self-pollination of 20 BC_1S_1 plants resulted in 6/138 (4.3%) zero-erucic seeds; while 124/138 (89.9%) seeds were obtained with intermediate levels of erucic acid. In the case of BC_1S_3 seeds obtained from self-pollination of 20 BC_1S_2 plants, 8/145 (5.5%) zero-erucic seeds were obtained, while 135/145 (93.1%) had intermediate levels of this fatty acid.

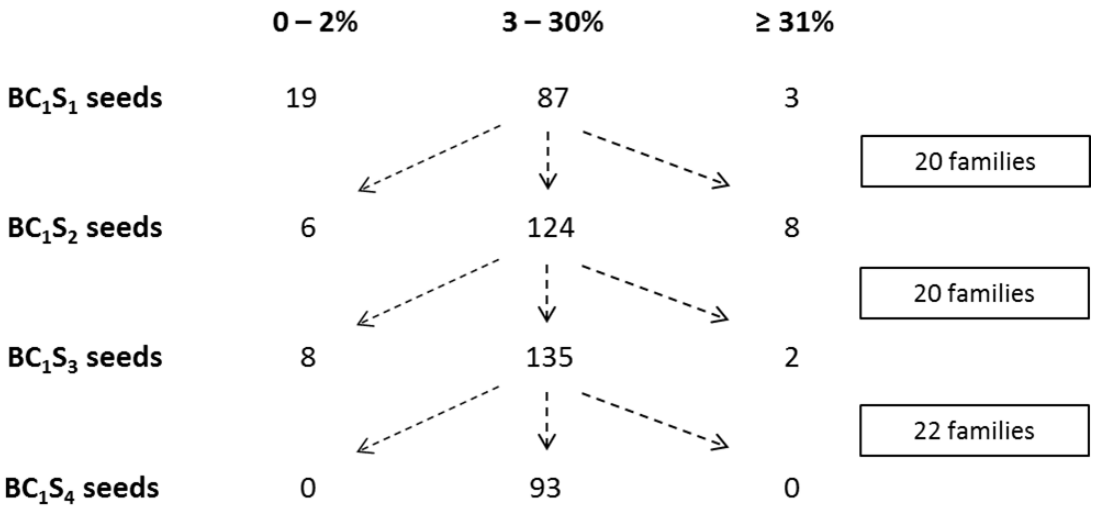

Figure 3-1. Flowchart of segregation for erucic acid in seed oil in the progeny of BC_1S_1, BC_1S_2, and BC_1S_3 plants of (*B. napus* × *B. oleracea*) × *B. oleracea* interspecific cross containing intermediate levels (3-30%) of this fatty acid, as determined by half-seed fatty acid analysis. Progeny of the zero-erucic (0-2%) and high erucic (≥31%) BC_1S_1, BC_1S_2, and BC_1S_3 seeds are not included in fatty acid analysis.

Ninety-three BC_1S_4 seeds obtained from 22 BC_1S_3 plants having 11.1 to 23.7% (average 17.4%) erucic acid content were analyzed for erucic acid content; however, none of these BC_1S_4 seeds was zero-erucic acid type. Erucic acid content in the 93 seeds ranged from 11.7 to 22.0% with a mean of 18.8%.

The proportion of plants fixed for the zero-erucic acid trait gradually increased with the progression of generations, as follows: 16/92 (17.4%) in BC_1S_1, 15/58 (25.9%) in BC_1S_2, and 62/199 (31.2%) in BC_1S_3 plants. In the BC_1S_4 and BC_1S_5 plant populations, more than 90% of the plants were zero-erucic acid type: 428/468 (91.5%) in BC_1S_4 and 225/243 (92.6%) in BC_1S_5. All BC_1S_6 plants grown for flow cytometry analysis were free from this fatty acid.

3.3.2 Glucosinolates

Evaluation for seed glucosinolate content was done in the BC_1S_5 generation of morphologically *B. napus* and *B. oleracea* type families of Population I and II grown under field and growth chamber/greenhouse environments (Table 3-1). The canola quality *B. napus* parents had glucosinolate content of 11.6 ± 1.6 μmol (A01-104NA) and 12.0 ± 2.5 μmol (Hi-Q) g^{-1} seed. Glucosinolate content in the zero-erucic acid *B. napus* type families ranged from 28.1 to 50.9 μmol g^{-1} seed (Table 3-1), where two families had <30 μmol g^{-1} glucosinolate. In the case of zero-erucic acid *B. oleracea* type plants, of the 42 plants with sufficient seed set for NIRS analysis, glucosinolate content ranged from 10.3 to 60.2 μmol g^{-1} seed where 18 had seed glucosinolate content of ≤ 30 μmol.

Table 3-1. NIRS estimate of glucosinolate content (μmol g^{-1}) in seeds obtained from four groups of BC_1S_5 plants of (*B. napus* × *B. oleracea*) × *B. oleracea* interspecific crosses

Morphological type[z]	No.	Average (± SD)	Range
Field[y]			
B. napus '-'	5	39.6 (± 10.8)	28.1 – 50.9
B. napus '+'	41	40.0 (± 12.3)	24.7 – 63.7
A01-104NA/Hi-Q	12	11.2 (± 1.1)	8.8 – 12.7
Greenhouse/ Growth chamber[x]			
B. oleracea '-'	42	32.4 (± 12.9)	10.3 – 60.2
B. oleracea '+'	8	45.9 (± 5.4)	38.0 – 54.1
A01-104NA/Hi-Q	3	10.5 (± 2.6)	7.6 – 12.6

[z] '+' indicates presence of erucic acid; '-' indicates free from erucic acid.
[y] Two replication trial, open-pollinated seeds used for glucosinolate analysis.
[x] Self-pollinated seeds used for glucosinolate analysis.

3.3.3 Backcross population morphology

A total of 38 BC_1 plants were grown, of which 27 were completely sterile, and the remaining 11 produced visible pollen. Ten of these plants were analyzed for pollen viability, where percent viable pollen ranged from 10.3 to 91.0. The number of fertile plants in the two subsequent population generations was 58/92 (63.0%) in BC_1S_1 and 58/72 (80.6%) in BC_1S_2. Pollen viability of the morphologically *B. napus*, *B. oleracea*, and intermediate type BC_1S_3 and BC_1S_4 plants and the three parents is summarized in Table A-1. Pollen viability was higher in these generations compared to BC_1.

Due to significant differences in flowering time and maturity between *B. oleracea* and *B. napus* type plants under greenhouse conditions, to promote synchrony of flowering time/maturity among plant groups, *B. oleracea* type plants were raised in a growth chamber, while the other type plants were grown in a greenhouse. A summary of the effect of selection for the three morphological types among different generations is given in Table 3-2. In general, morphological characteristics of the plants, whether *B. napus*, *B. oleracea*, or intermediate type, were not reflected in the progeny generation.

Table 3-2. Overall morphology of the self-pollinated populations of (*B. napus* × *B. oleracea*) × *B. oleracea* interspecific crosses

		Previous generation		Current generation morphology		
Pop'n[z]	**Generation**	**No. plants**	**Type**[y]	***B. napus***	**Intermediate**	***B. oleracea***
I	BC_1S_3	8	Nap	3	3	2
		8	Int	6	0	2
		4	Ole	0	2	2
	BC_1S_4	23	Nap	6	12	5
		8	Int	1	5	2
		18	Ole	7	6	5
	BC_1S_5	3	Nap	1	2	0
		4	Int	0	3	1
		19	Ole	0	10	9
II	BC_1S_3	0	Nap	0	0	0
		2	Int	0	0	2
		3	Ole	0	1	2
	BC_1S_4	0	Nap	0	0	0
		0	Int	0	0	0
		5	Ole	2	2	1
	BC_1S_5	1	Nap	0	1	0
		0	Int	0	0	0
		2	Ole	0	1	1

[z] Population I: (A01-104NA × *B. oleracea*) × *B. oleracea*; Population II: (Hi-Q × *B. oleracea*) × *B. oleracea*.
[y] Ole = *B. oleracea* type, Int = intermediate type, Nap = *B. napus* type.

Fertility for the three morphological types of BC_1S_3 to BC_1S_5 generation plants was estimated based on seed yield per plant and number of seeds per silique (Table A-2). On average, seed yield in these plants was poor compared to the *B. napus* parents, particularly in Population II. However, some individual plants in Population I had high pollen fertility and good seed set; plants with up to 19.7, 20.3, and 23.0 seeds per silique were obtained in the BC_1S_3, BC_1S_4, and BC_1S_5 populations. A wide variability for different morphological traits was also noted among the plants in both populations. For example, of the 199 BC_1S_5 plants of Population I, days to flower ranged from 33 to 88 (average 47.5), plant height from 30 to 111 cm (average 78.3), and silique length from 9.7 to 58.6 mm (average 30.0).

3.3.4 Molecular marker analysis

The number of seeds per silique in the 30 BC_1S_5 plants, selected for molecular marker analysis based on high fertility, averaged 12.7 (range 10.4 – 26.1); while

A01-104NA averaged 16.1 and *B. oleracea* averaged 11.9 seeds per silique. The *B. napus* type family averaged 2.9 (range 0.7 – 8.5) seeds per silique. Six of the 14 *B. oleracea*-type plants were '+' erucic, while all other 30 plants were free from this fatty acid. Two morphologically *B. napus* and one intermediate type plant had yellow petals and the remaining 33 had white petals.

Of 191 SSR markers screened on A01-104NA and *B. oleracea*, 60 (31.4%) were found to be polymorphic and amplified 140 alleles. Of all markers screened, 109 were previously mapped to the C-genome, of which 37 (33.9%) were polymorphic, amplifying 89 alleles. The frequency of *B. oleracea* specific alleles among the three morphological groups was as follows: *B. napus* type ranged from 0.0 to 36.4 (average 22.5%); intermediate type ranged from 23.1 to 58.3 (average 35.8%); and *B. oleracea* type ranged from 0.0 to 57.1 (average 41.6%). A higher proportion of *B. oleracea*-specific alleles were found among the six '+' erucic plants (52.1%) than in the 30 '-' erucic plants (32.6%) ($P < 0.05$). In addition, of 624 marker loci, 211 (33.8%) were heterozygous. This is significantly higher than the expected proportion of 10 (1.6%) in the BC_1S_5 generation ($\chi^2 = 4113.0$, $P < 0.001$). Using this marker data, the first and second principal coordinates of a PCoA explained 11.1 and 8.5% of the variation, respectively (Fig. 3-2). Based on this, two plants grouped closely to the *B. napus* parent and a group of nine plants were near to the *B. oleracea* parent. The remaining 25 plants were somewhat intermediate of the two parents.

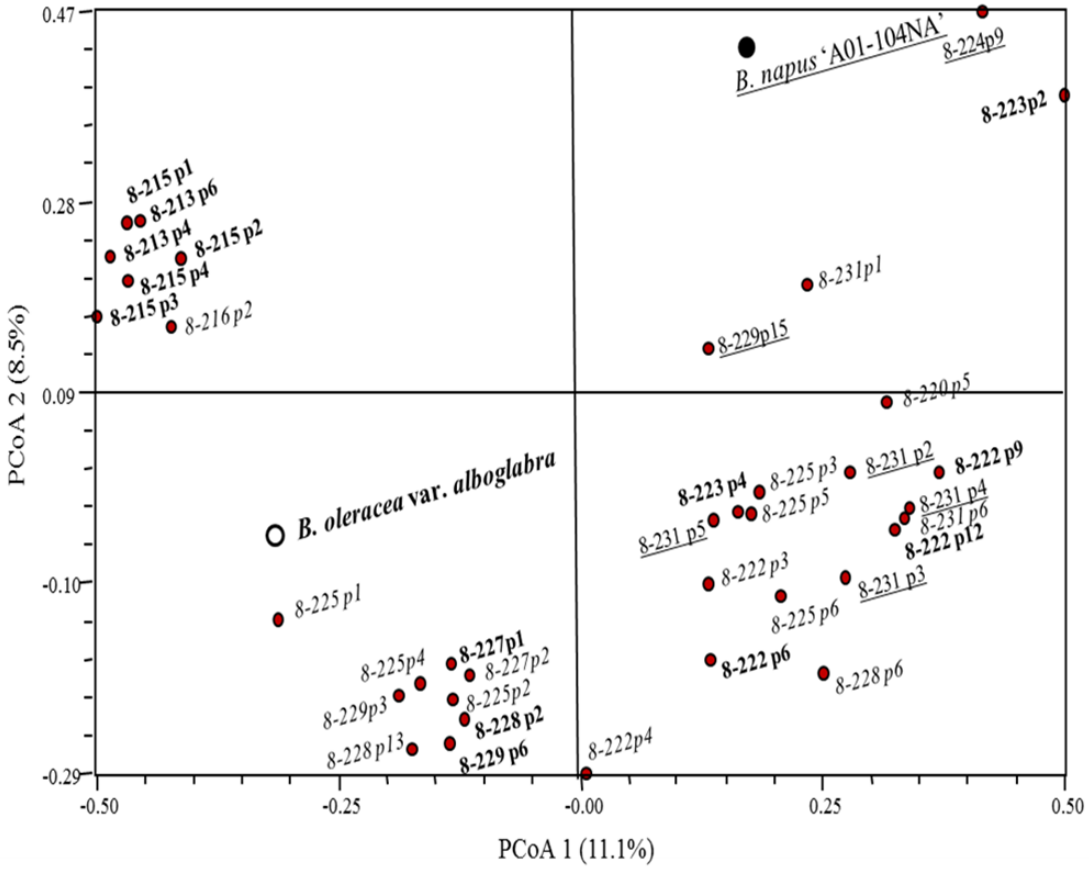

Figure 3-2. Plot of the first and second principal coordinates for 36 BC_1S_5 lines derived from a (*Brassica napus* × *B. oleracea*) × *B. oleracea* interspecific cross based on polymorphic bands derived from simple sequence repeat (SSR) markers. Morphology of individual plants is represented by **bold type face** = *B. oleracea* type, *italicized* = intermediate type, and <u>underlined</u> = *B. napus* type.

3.3.5 Flow cytometry

The *B. oleracea* parent gave a value of 114.7 ± 3.1 A.U. (range 108.9 to 117.3); while the values for A01-104NA and Hi-Q were 196.8 ± 7.8 (range 179.9 to 203.8) and 198.0 ± 2.1 (range 194.9 to 200.5), respectively. The A.U. value of *B. oleracea* was significantly different from the values of the two *B. napus* parents ($t = 32.7$, $P < 0.01$), while the two *B. napus* parents were statistically similar ($t = -0.40$, $P = 0.70$). A total of 84 BC_1S_6 families were evaluated for chromosome number/ploidy level. Based on morphological characteristics of their

corresponding BC_1S_5 plants, 66 BC_1S_6 families were considered to be *B. oleracea* type, 16 intermediate type, and 2 *B. napus* type. Flow cytometry data of the three BC_1S_6 groupings along with parents is presented in Fig. 3-3. The *B. oleracea* type BC_1S_6 families had a mean A.U. value of 209.7 ± 6.9 (range 196.8 to 228.3); while it was 206.2 ± 5.5 (range 198.3 to 221.8) for the intermediate families and 210.9 ± 4.9 (range 207.4 to 214.4) for the *B. napus* type families – indicating that chromosome number in the BC_1S_6 plants reached to *B. napus* despite rigorous selection for morphologically *B. oleracea* type plants performed in each generation.

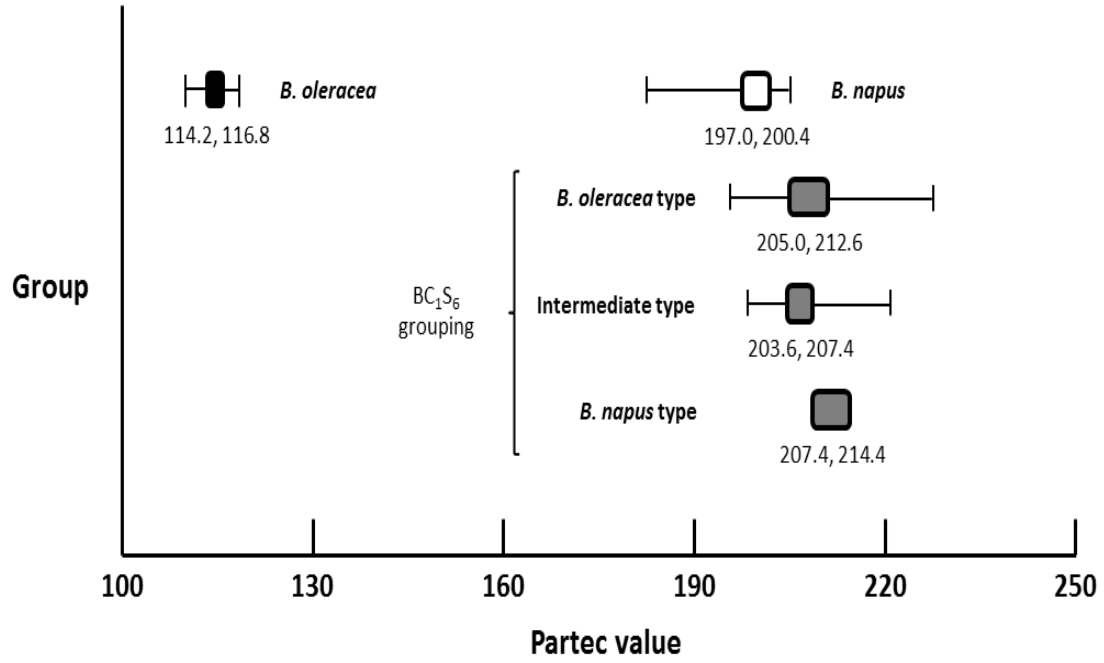

Figure 3-3. Nuclear DNA content, as revealed by flow cytometry analysis, in 84 self-pollinated offspring (BC_1S_6) derived from three types of BC_1S_5 plants, classified based on morphological characteristics: *B. oleracea* type (n=66 BC_1S_6 families), intermediate type (n=16 BC_1S_6 families), and *B. napus* type (n=2 BC_1S_6 families). Data of the two *B. napus* parents as well as BC_1S_6 plants derived from two (*B. napus* × *B. oleracea*) × *B. oleracea* interspecific crosses are pooled together. Filled bars and numerical values below the bars show the 25th to 75th percentile and extended bars show the range of A.U. values.

3.3.6 Meiotic chromosome analysis

Six BC_1S_6 plant families with extreme A.U. values, the three lowest (198.3 to 198.9) and three highest (221.9 to 227.4), were further examined cytologically through meiotic analysis of chromosomes. Families with the lowest A.U. values appeared to have from about 16 to 19 bivalents in diakinesis stage of prophase I (Fig. 3-4a); and similar counts were also made for this group in PMCs in anaphase I stage (Fig. 3-4b). The extreme high samples appeared to have a few more chromosomes; however, it was not possible to count the exact number. Fragments, bridges (Fig. 3-4c) and abnormal spindle formation (Fig. 3-4d) were observed among PMCs from the families with highest A.U. values.

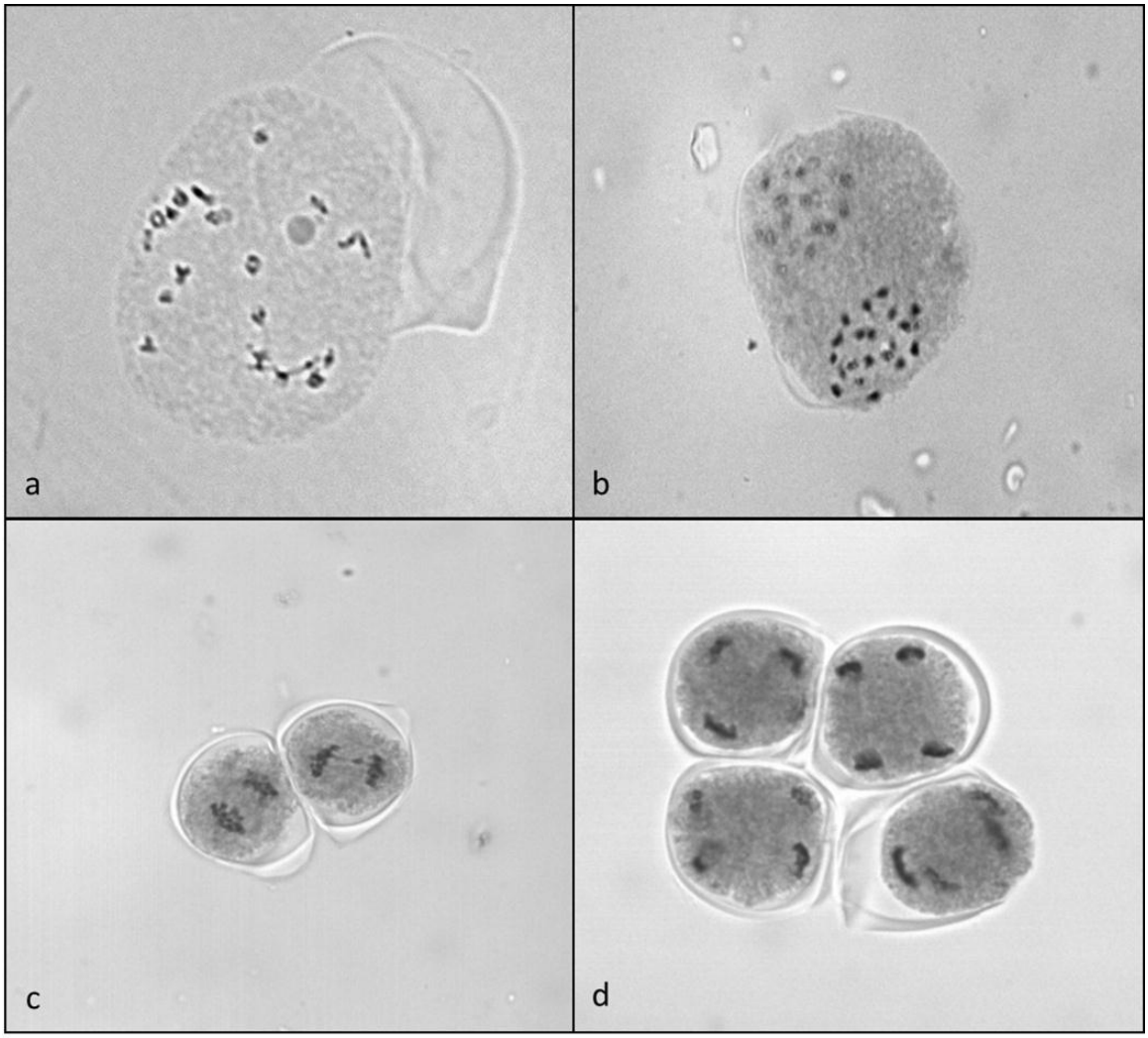

Figure 3-4. Meiotic analysis of chromosomes in BC_1S_6 plants of (*B. napus* × *B. oleracea*) × *B. oleracea* interspecific cross. Pollen mother cells from (a) family 8-303 (A.U. value = 198.9 ± 2.7) in diakinesis stage of prophase I with about 19 bivalents; (b) family 8-289 (A.U. value = 198.8 ± 19.1) in anaphase I, which appears to have about 19 chromosomes at each pole; (c) family 8-249 (A.U. value = 221.9 ± 9.1) showing two cells in anaphase I, with bridge in one cell (right); and (d) family 8-249 showing four cells in anaphase II, with parallel spindles in one cell (top right) and normal spindle configuration in other cells.

3.4 Discussion

One of the objectives of this (*B. napus* × *B. oleracea*) × *B. oleracea* interspecific cross was that a portion of the inbred F_1 × *B. oleracea* backcross families would stabilize into euploid (*2n*=18) *B. oleracea* type plants. Surprisingly, all 84 BC_1S_6 families had a nuclear DNA content similar to that of *B. napus*, indicating that all

backcross lines stabilized to *B. napus* or near *B. napus* ploidy level (*2n*=38). This precluded us from the possibility of obtaining a canola quality *B. oleracea* type line through self-pollination of the backcross hybrids with selection based on only morphological traits. These results suggest that while selection based on morphological traits was effective in retaining the *B. oleracea* alleles of the C-genome in the interspecific progeny, it was not effective in selecting for types with similar ploidy levels to *B. oleracea*. The results obtained in the present study are in contrast to the results reported from Chen et al. (1988), who reported a partially yellow seeded *B. alboglabra* developed from interspecific hybridization between *B. alboglabra* and *B. carinata*; and used this interspecific cross derived *B. alboglabra* for resynthesis of *B. napus* by crossing with *B. rapa*. This indicates that euploid (*2n*=18) *B. oleracea* can be obtained from *B. carinata* × *B. oleracea* cross. Rahman (2001) and Zaman (1988) also obtained *B. oleracea* type plants abundantly from (*B. carinata* × *B. oleracea*) × *B. oleracea* interspecific backcross populations. Unfortunately, no information is available on chromosome number in these phenotypically *B. oleracea* type plants.

In this study, morphological traits were used in the early generations (BC_1S_2 to BC_1S_5) to obtain *B. oleracea* and *B. napus* plants from this interspecific cross. It was assumed that selecting a representation of plants from each morphological group among all generations would result in inbred backcross lines with *2n*=18 and *2n*=38 chromosomes. However, both flow cytometry and cytological analysis suggested that ploidy levels of these families were similar to the *B. napus* parents. Thus, we found that selection for *2n*=18 lines from (*B. napus* × *B. oleracea*) × *B. oleracea* was not effective using the phenotypic characters. To achieve this, cytological analysis of the early generation plants for *2n*≈18 chromosomes would be needed. However, the other objective of developing genetically diverse lines with *2n*≈38 chromosomes (*B. napus*) was achieved, and the frequency of this type of plants was much higher than anticipated.

Chiang et al. (1979) obtained four BC_1 plants from *B. napus* × *B. oleracea* hybrid (ACC) backcrossed to *B. oleracea*. Chromosome number in these plants varied from *2n*=18 to *2n*=38 with pollen fertility 6.78% in the *2n*=18 chromosome

plant and 20.5% in the *2n*=38 plant. The 38 chromosome plant apparently generated from union of reduced and unreduced gametes; and this type of plant would carry more than two sets of C-genome chromosomes. In the present study, wide variation for fertility among the BC_1 plants was also found; where, of the 38 plants, 27 were sterile and the remaining 11 plants showed variable fertility. Therefore, it is likely that some of the BC_1 plants with higher fertility resulted from unreduced gametes. Inomata (2002) reported *2n*=37 (ACCC) chromosome F_1 plants of *B. napus* × *B. oleracea* interspecific cross – resulting from the union of reduced *B. napus* gamete and unreduced *B. oleracea* gamete. Fertility of the 37 chromosome plants was higher than *2n*=28 chromosome F_1 plants. Backcrossing of these 37 chromosome plants to *B. napus* resulted progenies with *2n*=29 to *2n*=56 chromosomes, where the 56 chromosome plant apparently resulted from the union of unreduced female (*n*=37) and reduced male (*n*=19) gametes. Nelson et al. (2009) also reported unreduced male gametes in *B. napus* × *B. carinata* interspecific hybrids, and these unreduced gametes were preferentially included in microspore culture. Similarly, Chèvre et al. (1998) reported unreduced female gametes in *B. napus* × *Raphanus raphanistrum* (*2n*=18) hybrid plants. Thus, there is evidence that unreduced male and female gametes can frequently occur in *Brassica* interspecific crosses. In the present study, flow cytometry and cytology analysis suggested that most of the inbred backcross lines possessed nuclear DNA content similar to or greater than that of the *B. napus* parent; and one BC_1S_6 family, derived from a BC_1S_5 plant, had two plants with ploidy level similar to *B. napus* while the third plant had 1.5x DNA content compared to *B. napus* (A.U. value 312 vs. 217). This provides evidence that unreduced gametes were produced in the present interspecific crosses and that these resulted in plants with *2n*≈38 or higher chromosome numbers in self-pollinated generations of (*B. napus* × *B. oleracea*) × *B. oleracea* cross. This is further substantiated from the occurrence of *B. oleracea* type plants with chromosome number 2n≈38 or higher – apparently derived from the plants generated from unreduced gametes and carrying more than two sets of C-genome chromosomes.

Although the inheritance of erucic acid in *Brassica* has been well studied based on intraspecific crosses between types containing high and low-erucic acid alleles (Chen et al. 1988, Rahman et al. 1994), few reports exist for the inheritance of this fatty acid in interspecific populations. While definite predictions of genotypic composition of erucic acid alleles are difficult to make based solely on phenotype of individual plants, some general observations can be made. It was previously predicted that based solely on disomic segregation of C-genome erucic acid alleles, the expected ratio of genotypes in the BC_1S_1 generation would be 5 C^+C^+: 2 C^+C^0 : 1 C^0C^0 (Bennett et al. 2008). Based on this, the theoretical number of zero-erucic acid genotypes among the 109 BC_1S_1 seeds in this study would be 14 – very close to the actual number observed in this study (n=19). In addition, one-fourth of these BC_1S_1 seeds would be expected to have the heterozygous genotype of C^+C^0. Of the 20 self-pollinated BC_1S_1 plants of intermediate erucic acid content (Fig. 3-1), five segregated for zero-erucic seeds, as expected. Chen et al. (1988) previously demonstrated the additive mode of inheritance of erucic acid content based on C-genome alleles of *B. oleracea* var. *alboglabra*. Based on this, plants with C^+C^0 genotype in this study would likely have erucic acid content of 20 ± 5%. However, the five apparently heterozygous (C^+C^0) BC_1S_1 plants had erucic acid content ranging from 9.5 to 14.7%, demonstrating that dosage effect from the zero-erucic acid alleles from A-genome (A^0) is an important factor in overall production of this fatty acid in these interspecific plants. Due to this dosage effect of A^0 alleles, it is difficult to distinguish C^+C^0 and C^+C^+ genotypes based on phenotype of individual plants. The 22 self-pollinated BC_1S_3 plants (Fig. 3-1), ranging from 11.1% to 23.7% erucic acid content, all appear to have genotype of C^+C^+ based on the fact that 0/93 BC_1S_4 seeds segregated for zero-erucic trait. Thus, the genetic control of levels of erucic acid in segregating populations in this study appears to be strongly influenced both by the alleles at the erucic acid locus on the C-genome (C^+C^+, C^+C^0, C^0C^0) as well as the dosage effect of the A-genome alleles (0 to 2 doses theoretically possible).

Glucosinolates had a more complex inheritance pattern, and as expected, a continuous variation was observed for this trait among the BC_1S_5 plants. Our study on selection for seed quality traits among the relatively small segregating population demonstrated that plants with glucosinolate levels similar to that of the *B. napus* parent, as well as zero-erucic acid content, are achievable from (*B. napus* × *B. oleracea*) × *B. oleracea* interspecific crosses. Given the genetic control of low glucosinolate content in *B. napus* of about four gene loci (Rahman et al. 2001) and erucic acid of two gene loci (Chen and Heneen 1989, Dorrell and Downey 1964), the achievement of low glucosinolate, zero-erucic type plants is apparently due to the involvement of only C-genome alleles for these two seed quality traits as well as simpler segregation of the alleles.

Despite the difficulty in recovering plant families of *B. oleracea* ploidy level, all 84 BC_1S_6 families appeared to have a chromosome complement similar to *B. napus*. These families were free from erucic acid and a total of 20/47 zero-erucic plants/families analyzed in BC_1S_5 had <30 μmol g^{-1} glucosinolate content. The morphological diversity among these families reveals that segregation for allelic diversity from the *B. oleracea* parent must have occurred and been included in generous proportions among later generations of plants. This observation was further confirmed by marker analysis where an average of 35.8% *B. oleracea*-specific alleles were detected among 36 BC_1S_5 plants. This was also demonstrated in the PCoA where the majority (34 of 36) of plants clustered near to *B. oleracea* or fell intermediate of the two parents.

The findings from this study demonstrate that there is a good possibility to introduce genetic diversity into *B. napus* recombinant inbred lines derived from backcross of the F_1 of *B. napus* × *B. oleracea* to *B. oleracea*, without the loss of canola quality traits. These genetically diverse, *2n*=38 lines can be used in the breeding program for enhancement of specific traits and may have great potential for developing improved genetically diverse, canola quality open-pollinated and hybrid spring type varieties.

3.5 References

Agricultural Financial Services Corporation (2010) Acres Insured by Crop Variety – Alberta. Available at http://www.afsc.ca/doc.aspx?id=4182 (verified 24 Oct. 2011). AFSC, Lacombe, Alberta

Babula D, Kaczmarek M, Ziółkowski PA, Sadowski J (2007) *Brassica oleracea*. In: Kole C (ed) Genome Mapping and Molecular Breeding in Plants, volume 5. Springer-Verlag, Berlin/Heidelberg, Germany, pp 227-285

Bennett RA, Thiagarajah MR, King JR, Rahman MH (2008) Interspecific cross of *Brassica oleracea* var. *alboglabra* and *B. napus*: Effects of growth condition and silique age on the efficiency of hybrid production, and inheritance of erucic acid in the self-pollinated backcross generation. Euphytica 164:593-601

Canadian Food Inspection Agency (2008) Cruciferous crop inspection procedures: Appendix VII – Plant descriptions and illustrations of mustard and canola. Available at: http://www.inspection.gc.ca/english/plaveg/seesem/man/swi-crue.shtml#a22

Chen BY, Heneen WK (1989) Fatty acid composition of resynthesized *Brassica napus* L., *B. campestris* L., and *B. alboglabra* Bailey with special reference to the inheritance of erucic acid content. Heredity 63:309-314

Chen BY, Heneen WK, Jönsson R (1988) Resynthesis of *Brassica napus* L. through interspecific hybridization between *B. alboglabra* Bailey and *B. campestris* L. with special emphasis on seed colour. Plant Breed 101:52-59

Cheng BF, Heneen WK, Chen BY (1994) Meiotic studies on a *Brassica campestris-alboglabra* monosomic addition line and derived *B. campestris* primary trisomics. Genome 37:584-589

Chèvre AM, Eber F, Baranger A, Hureau G, Barret P, Picault H, Renard M (1998) Characterization of backcross generations obtained under field conditions from oilseed rape-wild radish F_1 interspecific hybrids: an assessment of transgene dispersal. Theor Appl Genet 97:90-98

Chiang MS, Chiang BY, Grant WF (1979) Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* ssp. *capitata*). III. First backcross and F_2 progenies from interspecific hybrids between *B. napus* and *B. oleracea* ssp. *capitata*. Euphytica 28:257-266

Cowling WA (2007) Genetic diversity in Australian canola and implications for crop breeding for changing future environments. Field Crop Res. 104:103-111

Cunningham JL (1972) A miracle mounting fluid for permanent whole-mounts of microfungi. Mycologia 64:906-911

Dorrell DG, Downey RK (1964) The inheritance of erucic acid in rapeseed (*Brassica campestris*). Can J Plant Sci 44:499-504

Fu YB, Gugel RK (2010) Genetic diversity of Canadian elite summer rape (*Brassica napus* L.) cultivars from the pre- to post-canola era. Can J Plant Sci 90:23-33

Hasan M, Seyis F, Badani AG, Pons-Kühnemann J, Friedt W, Lühs W, Snowdon RJ (2006) Surveying genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet Resour Crop Evol 53:793-802

Hirai M, Matsumoto S (2007) *Brassica rapa*. In: Kole C (ed) Genome Mapping and Molecular Breeding in Plants, volume 5. Springer-Verlag, Berlin/Heidelberg, Germany, pp 185-190

Inomata N (2002) A cytogenetic study of the progenies of hybrids between *Brassica napus* and *Brassica oleracea*, *Brassica bourgeaui*, *Brassica cretica*, and *Brassica montana*. Plant Breed 121:174-176

Kebede B, Thiagarajah MR, Zimmerli C, Rahman MH (2010) Improvement of open-pollinated spring rapeseed (*Brassica napus* L.) through introgression of genetic diversity from winter rapeseed. Crop Sci 50:1236-1243

Lühs W, Seyis F, Voss A, Friedt W (2000) Genetics of erucic acid content in *Brassica oleracea* seed oil. Czech J Genet Plant Breed 36:116-120

Nelson MN, Mason AS, Castello M-C, Thomson L, Yan G, Cowling WA (2009) Microspore culture preferentially selects unreduced (2n) gametes from an

interspecific hybrid of *Brassica napus* L. × *Brassica carinata* Braun. Theor Appl Genet 119:497-505

Qian W, Meng J, Li M, Frauen M, Sass O, Noack J, Jung C (2006) Introgression of genomic components from Chinese *Brassica rapa* contributes to widening the genetic diversity in rapeseed (*B. napus* L.), with emphasis on the evolution of Chinese rapeseed. Theor Appl Genet 113:49-54

Rahman MH (2001) Production of yellow-seeded *Brassica napus* through interspecific crosses. Plant Breed 120:463-472

Rahman MH, Rahman L, Stølen O, Sørensen H (1994) Inheritance of erucic acid content in yellow- and white-flowered yellow sarson x Canadian *Brassica campestris* L. Acta Agric Scand 44:94-97

Rahman MH, Joersbo M, Poulsen MH (2001) Development of yellow-seeded *Brassica napus* of double low quality. Plant Breed 120:473-478

Raney JP, Serblowski G (2007) Inserts for FOSS NIRS 6500 spinning ring cups. Proc. 12th Inter. Rapeseed Congr. 5:39-42

Schelfhout CJ, Wroth JM, Yan G, Cowling WA (2008) Enhancement of genetic diversity in canola-quality *Brassica napus* and *B. juncea* by interspecific hybridisation. Austr J Agric Res 59:918-925

Seyis F, Snowdon RJ, Lühs W, Friedt W (2003) Molecular characterization of novel resynthesized rapeseed (*Brassica napus*) lines and analysis of their genetic diversity in comparison with spring rapeseed cultivars. Plant Breed 122:473-478

Song K, Lu P, Tang K, Osborn TC (1995) Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc Natl Acad Sci USA 92:7719-7723

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theor Appl Genet 75:784-794

Statistics Canada (2011) Field and special crops (Seeded area). Available at: http://www40.statcan.ca/l01/cst01/Prim11a-eng.htm (verified 24 Oct. 2011). Minister of Industry, Ottawa, Canada

Stringam GR, Degenhardt DF, Thiagarajah MR, Bansal VK (2000) Hi-Q summer rape. Can J Plant Sci 80:835–836

Zaman MW (1988) Limitations for introgression of yellow seed coat colour in *Brassica napus.* J Swed Seed Assoc 98:157-161

Chapter 4

Broadening genetic diversity in spring canola (*Brassica napus* L.): patterns of heterosis in three distinct inbred populations

4.1 Introduction

Canola (rapeseed) is the most important oilseed crop in Canada, where about 7.5 million ha were seeded in 2011 (Statistics Canada 2011). Most cultivars grown by producers are *Brassica napus* L. species of spring growth habit. Presence of adequate genetic variability is critical to the continued improvement of the crop for seed yield and agronomic traits as well as adaptability to changing climate (Barnes et al. 2010). However, the narrow genetic diversity among *B. napus* cultivars, particularly in spring type cultivars based on unique SSR alleles compared to winter fodder and vegetable genotypes (Hasan et al. 2006), as well as compared to Chinese semi-winter and interspecific-derived types estimated by AFLP markers (Qian et al. 2006), strongly indicate the need for introduction of allelic diversity in breeding germplasm.

Increasing genetic diversity in canola is of particular interest with regards to the breeding of hybrid cultivars. Genetically diverse inbred lines are considered essential in developing new hybrid cultivars with increased levels of heterosis, particularly for seed yield (Qian et al. 2009). Winter type rapeseed has been shown to be genetically distinct from spring types (Diers and Osborn 1994), and alleles from winter type introgressed into a spring type background have been shown to have great potential for increased seed yield in test hybrids (Butruille et al. 1999, Quijada et al. 2004) as well as in open-pollinated lines (Kebede et al. 2010, Rahman 2011). Quijada et al. (2006) identified QTL alleles of winter cultivars that increased seed yield in spring type DH lines and their test hybrids.

Some recent studies have also demonstrated the potential value of utilizing allelic diversity of the allied *Brassica* species for increased heterosis in *B. napus* (Basunanda et al. 2007, Zou et al. 2010). The A- (A^n) and C- (C^n) genomes of *B. napus* are genetically distinct from the A-genome of *B. rapa* L. (A^r) and *B. juncea*

(L.) Czern (A^j), and the C-genome of *B. oleracea* L. (C^o) and *B. carinata* A. Braun (C^c) (Li et al. 2004). Qian et al. (2007, 2009) suggested that some of the alleles in Chinese semi-winter type rapeseed, likely introgressed from *B. rapa*, may contribute to high levels of heterosis for seed yield in both spring and winter rapeseed hybrids. Zou et al. (2010) observed high heterosis in test hybrids while using germplasm diversified with the A-genome of *B. rapa* (A^r) and the C-genome of *B. carinata* (C^c). A number of European researchers have also studied diversity of resynthesized (RS) rapeseed (*B. oleracea* × *B. rapa*) for the improvement of winter rapeseed open-pollinated or hybrid cultivars (Basunanda et al. 2007, Gehringer et al. 2007, Rygulla et al. 2007, Radoev et al. 2008). Udall et al. (2006) detected two QTL in a spring *B. napus* test cross population where the allele for increased seed yield came from a RS rapeseed parent. Thus, *B. napus* diversified using its allied species has shown excellent potential for increasing levels of heterosis in this crop.

The major limitation of using genetic diversity of the C-genome of *B. oleracea* (C^o) for the improvement of canola quality spring *B. napus* is that *B. oleracea* often requires vernalization for flowering and typically contains very high levels of glucosinolate and erucic acid (Rygulla et al. 2007). Very few studies have been conducted so far to evaluate heterotic potential of *B. napus* lines derived from *B. napus* × *B. oleracea* interspecific crosses. Riaz et al. (2001) reported a high level of high parent heterosis for seed yield in hybrids involving a line developed by Quazi (1988) from *B. napus* × *B. oleracea* cross. Several researchers have also reported heterosis for seed yield in hybrids developed based on different cultivar/lines of spring *B. napus* (Diers et al. 1996, Riaz et al. 2001, Cuthbert et al. 2009) as well as spring type lines derived from winter × spring *B. napus* crosses, crossed with spring type cultivars/lines (Butruille et al. 1999, Quijada et al. 2004). However, no report exists on comparative evaluation of the allelic diversity of *B. oleracea* and winter *B. napus* on seed yield heterosis in spring rapeseed, *B. napus*.

Assessment of genetic diversity between parental lines based on molecular markers has been suggested as a method to predict heterosis and F_1 performance.

In spring rapeseed, Diers et al. (1996) and Riaz et al. (2001) reported moderate associations between parental genetic diversity and heterosis. Based on research results from maize, rice, rapeseed, soybean, and wheat, Melchinger (1999) proposed that correlation between parental genetic diversity and heterosis is strongest when genetically related parental lines are involved in hybrids; but this relationship weakens as more distant parents are involved. In the case of canola, more work needs to be done to explain this relationship, especially when crosses between different ecotypes or widely diverse parental material are involved (Qian et al. 2007). Diers et al. (1996) found that general combining ability (GCA) and genetic diversity estimates combined in a multiple regression model improved their ability to predict hybrid seed yield.

The purpose of this study was two-fold: (i) to compare the level of heterosis among three genetically distinct populations derived from spring × spring and winter × spring *B. napus*, and *B. napus* × *B. oleracea* crosses; and (ii) to investigate the relationship between genetic distances as estimated by SSR markers and seed yield heterosis in these populations. For this purpose, a *B. napus* spring-type cultivar was used as a common parent in crosses with a spring breeding line, a winter cultivar, and a *B. oleracea* line to generate three sets of inbred lines with allelic diversity from these three distinct sources. Test hybrids of these inbred lines were produced by crossing with the *B. napus* parent involved in the original three crosses. Using this experimental design, we hypothesized that heterosis observed among the test hybrids would be due to differences in allelic diversity among the inbred lines.

4.2 Materials and methods

4.2.1 Population development

The *B. napus* (AACC genome, *2n*=38) doubled haploid (DH) spring cultivar Hi-Q (Stringam et al. 2000) was used as a common parent in three crosses to generate the three populations of inbred lines used in this study. Cross #1 was made with a spring type, self-compatible *B. oleracea* var. *alboglabra* (L. H. Bailey) Musil (CC

genome, *2n*=18) line, and the resultant inbred lines were termed as the 'IN' (interspecific-derived) population. Cross #2 was made with a winter type *B. napus* cultivar 'Aviso', obtained from Danisco Seed, Holeby, Denmark, and the inbred lines from this cross were termed as the 'WS' (winter × spring derived) population. Cross #3 was made with a spring type breeding line A03-14NI developed at the University of Alberta, and the inbred lines from this cross were termed as the 'SS' (spring × spring derived) population. Hi-Q was used as female parent in crosses with *B. oleracea*; while it was used as male in crosses with Aviso and A03-14NI.

Due to poor seed quality traits (40% erucic acid content in oil; >80 µmol glucosinolate g^{-1} seed) of the *B. oleracea* parent and poor fertility in early generations of the *B. napus* × *B. oleracea* interspecific hybrids, selection in the segregating generations was performed for the two seed quality traits (zero-erucic acid and low glucosinolate content) and for euploid *B. napus* plants. In the case of winter × spring cross, plants with spring growth habit and earliness of flowering were selected to allow for evaluation under field conditions in Canada. Aside from this, no selection for other traits was performed during the development of these three populations. Single plants were self-pollinated by bag isolation in a growth chamber or greenhouse, and in this way 65 inbred lines were developed from each cross.

4.2.2 SSR genotyping

F_8 lines from the IN population and F_5 lines from the WS and SS population were grown in a greenhouse with their four parents. Young leaves from all genotypes were collected and used for genomic DNA extraction, using a SIGMA DNA extraction kit (Sigma-Aldrich, St. Louis, MO) and following manufacturer's instructions. Quantification of total genomic DNA was done for individual samples using 1.5 µl samples on a NanoDrop® Spectrophotometer (model 'ND-1000') and each was diluted to 5 ng μl^{-1}. The four parental genotypes Hi-Q, *B. oleracea* var. *alboglabra*, Aviso, and A03-14NI were screened using 194 publicly available SSR primer pairs from the 19 *B. napus* linkage groups. Source of

markers was as reported in section 2.2.9. A total of 32 polymorphic SSR markers having clear bands and good reproducibility were used for genotyping of the IN population; while 36 were used for genotyping of the WS population and 14 were used for genotyping the SS population. The lower number of markers in the SS population was primarily due to difficulty of finding polymorphic SSRs between the parents. Polymerase Chain Reactions (PCR), labelling of PCR products, and detection of amplification products for genotyping of each population was done as described by Kebede et al. (2010).

4.2.3 Test hybrid production

Using genotypic data obtained from SSR analysis of the three inbred populations, 35 lines from each population were selected based on an estimate of genetic distance from Hi-Q, where an attempt was made to select inbred lines with a wide range of genetic distances from Hi-Q. This estimate was expressed as a percentage and was calculated based on the number of non-Hi-Q alleles in each genotype divided by the total number of alleles detected and multiplied by 100. Test hybrid seeds were produced using the 80 inbred lines as pollen donor and Hi-Q as the common tester. For this, unopened flower buds of Hi-Q were emasculated and pollinated with fresh pollen from the inbred lines and seeds were harvested at maturity.

4.2.4 Field plot design

Field plots were grown in two replications at the University of Alberta South Campus Research Farm, Edmonton in 2010 and 2011 (referred to as SC2010 and SC2011); and at the University of Alberta St. Albert farm, located approximately 25 km north of the research farm, in 2011 (referred to as SA2011). Plot size was 0.92 m width × 2 m length = 1.84 m^2; with 1 g seed used in four rows per experimental plot. Field plots were laid out in a nested split-plot design, where main plots were the three populations and subplots were inbred lines and their respective test hybrid. Inbred lines were always grown side-by-side with their respective test hybrids. Randomization of lines within blocks and order of

subplots was done using Statistical Analysis System (SAS 9.1; SAS Institute, Cary, NC).

4.2.5 Agronomic traits

The Canola/Rapeseed Objective Description traits, described by the Plant Breeders' Rights Office (CFIA 2009), were used for phenotypic characterization. All inbred lines and their test hybrids were evaluated for seven agronomic and seed quality traits on a whole-plot basis at all locations: Days to flowering, plant height (cm), days to maturity, seed yield (kg ha^{-1}), and seed oil (%), protein (%) and total glucosinolate (µmol g^{-1} seed) contents. In addition, a subset of eight inbred lines and their respective hybrids from each population were evaluated for five agronomic traits at the South Campus site over both years: Leaf length and width (cm), silique length (mm), number seeds per silique, and 1000-seed weight (g). Days to flowering was recorded as the number of days from seeding to approximately 50% plants having at least one open flower. For leaf measurements, basal leaves of randomly selected 10 plants per plot were used. Likewise, 10 siliques from the middle to upper-half of the main raceme from at least three plants were used to estimate silique length, number seeds per silique, and 1000-seed weight. Seed oil, protein, and glucosinolate contents were estimated using near-infrared spectroscopy (NIRS, FOSS NIRSystems model 6500) on a whole seed basis at 8.5% moisture. NIRS analysis was done in the Analytical Laboratory of the Canola Program of the University of Alberta, which is accredited by the Canadian Grain Commission for this analysis.

4.2.6 Data analysis

Analysis of variance (ANOVA) was done using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Least squares means were obtained for all traits using Proc MIXED, where replication and block nested in replication were random effects. Main plot (three population types) and sub-plot (inbred or test hybrid) effects were compared using 'pdiff' command. Proc CORR was used to calculate Pearson correlation coefficients. Two checks were included in each block of every

trial: Hi-Q (common tester parent), and 95-53, a registered hybrid canola cultivar in Canada. Seed yield was expressed as a percentage of each check, calculated as: ((Test hybrid - check)/check) × 100. Due to significant genotype × environment interactions for many traits, each environment was analyzed separately.

Calculation of coefficients of similarity among the inbred lines was performed separately for each of the three populations using Multi-Variate Statistical Package software (Kovach 2007). For this, SSR data was transformed into a binary matrix and Nei and Li's (1979) genetic distance was used. To obtain coefficients for genetic distance from Hi-Q, where greater diversity from Hi-Q and higher heterosis values would result in positive correlation, one minus similarity coefficient was used. For estimates of heterosis for seed yield, mid-parent heterosis (MPH) was calculated as ((Test hybrid - mid-parent value)/mid-parent value) × 100; and high parent heterosis (HPH) was calculated as ((Test hybrid – high parent)/high parent) × 100.

Single marker analysis was performed using MapDisto version 1.7.5.1 (Lorieux 2007), which computes F-tests for a trait by grouping individuals based on marker genotype at each loci. In this study, genotypic (marker) data from inbred lines and phenotypic data from the respective test hybrid were used.

4.3 Results

4.3.1 Agronomic performance

4.3.1.1 Seed yield

Analysis of variance of seed yield for the three population types (IN, WS, and SS) and two cross types (inbred and test hybrid) in each of three environments is presented in Table 4-1. In each environment there were significant differences for seed yield among both the population and cross types (Table 4-1). The average yield of the Hi-Q check among the three environments was 4.60 ± 0.67 t ha^{-1}; while it was 5.30 ± 0.81 t ha^{-1} for the hybrid check 95-53 (Table 4-2). As a whole, seed yield of the inbred IN population was significantly lower than Hi-Q in all three environments (t = -4.23 to -4.76, $P < 0.001$); while the SS inbred population

was significantly lower in SC2010 and SC2011 (t = -2.77 to -3.52, $P < 0.01$). The inbred WS population was not significantly different from Hi-Q under any environment. While comparing inbred lines and the corresponding test hybrids at a population level (IN, WS, and SS), the test hybrid populations in all cases had significantly greater yield under all three environments at $P < 0.01$. The highest mean yield over all three environments among the inbred lines was observed in the WS population, and this was significantly higher than IN ($P < 0.001$) and SS ($P < 0.05$) inbred lines. Comparing test hybrids of the three populations, the highest average yield was observed for the IN population at SC2010 and SC2011, although neither of these average test hybrid yields were statistically higher from others under these two environments. The WS population had the highest average yield of test hybrids under the SA2011 environment.

Several inbred IN lines consistently displayed good specific combining ability with Hi-Q, as measured by high-parent heterosis (HPH). Under SC2010, SC2011, and SA2011 environments, IN276 had 21.4, 40.8, and 16.3% HPH; while it was 21.9, 7.9, and 37.5% for IN279 and 11.3, 16.3, and 39.5% for IN262. Other IN inbred lines gave large HPH under one or two of the environments, such as IN257, which had 32.1% HPH in SC2010 and 40.1% HPH in SA2011; and IN275 gave 48.0% HPH in SC2010.

Table 4-1. Analysis of variance of the inbred lines derived from *Brassica napus* × *B. oleracea* interspecific cross (IN), and winter × spring (WS) and spring × spring (SS) crosses of *B. napus* and their test hybrids in three environments

Source	DF	F value	*P* > F
SC2010			
Population[z]	2	5.8	0.003
Cross type[y]	1	113.4	<0.001
Pop*Cross type	2	7.2	0.001
SC2011			
Population	2	5.2	0.006
Cross type	1	85.1	<0.001
Pop*Cross type	2	8.9	<0.001
SA2011			
Population	2	8.9	<0.001
Cross type	1	86.8	<0.001
Pop*Cross type	2	5.3	0.006

[z] Population: IN, WS, and SS

[y] Cross type: Inbred and test hybrid

Table 4-2. Seed yield (t ha^{-1}) of the inbred lines derived from *Brassica napus* × *B. oleracea* interspecific cross (IN), and winter × spring (WS) and spring × spring (SS) crosses of *B. napus* and their test hybrids in three environments

Population	Type	Location	No. lines	Range	Average yield ± SD	% Hi-Q[z]	% 95-53[y]
IN	Inbred	SC2010	33	2.27 – 5.64	3.67 ± 0.81	- 22.3	- 29.8
		SC2011	23	1.61 – 5.72	3.58 ± 1.19	- 31.2	- 41.7
		SA2011	27	0.67 – 4.83	2.20 ± 1.15	- 43.5	- 51.4
				0.67 – 5.72	**3.16**	**- 31.2**	**- 40.2**
	Test hybrid	SC2010	34	4.20 – 6.99	5.10 ± 0.63	+ 8.1	- 2.5
		SC2011	33	3.32 – 7.97	5.39 ± 0.94	+ 3.7	- 12.1
		SA2011	30	2.98 – 6.65	4.20 ± 0.84	+ 8.1	- 7.1
				2.98 – 7.97	**4.92**	**+ 6.9**	**- 7.1**
WS	Inbred	SC2010	21	3.17 – 5.16	4.37 ± 0.55	- 7.3	- 16.3
		SC2011	21	2.75 – 6.67	4.77 ± 1.05	- 8.3	- 22.3
		SA2011	21	1.45 – 5.55	3.69 ± 1.10	- 5.0	- 18.3
				1.45 – 6.67	**4.28**	**- 7.0**	**- 19.2**
	Test hybrid	SC2010	21	3.33 – 6.70	5.03 ± 0.73	+ 6.5	- 3.8
		SC2011	20	4.58 – 6.24	5.39 ± 0.46	+ 3.6	- 12.2
		SA2011	14	3.28 – 5.77	4.48 ± 0.70	+ 15.2	- 1.0
				3.28 – 6.70	**5.02**	**+ 9.0**	**- 5.2**
SS	Inbred	SC2010	25	2.73 – 5.07	4.03 ± 0.57	- 14.7	- 23.0
		SC2011	25	2.31 – 6.86	3.83 ± 0.97	- 26.4	- 37.6
		SA2011	22	1.85 – 5.01	3.32 ± 0.84	- 14.6	- 26.6
				1.85 – 6.86	**3.74**	**- 18.7**	**- 29.4**
	Test hybrid	SC2010	25	4.06 – 5.66	4.89 ± 0.44	+ 3.6	- 6.5
		SC2011	25	3.99 – 6.04	4.89 ± 0.56	- 6.0	- 20.3
		SA2011	20	2.74 – 5.62	4.46 ± 0.72	+ 14.8	- 1.4
				2.74 – 6.04	**4.77**	**+ 3.6**	**- 10.0**
Checks	Hi-Q	SC2010			4.72 ± 0.89		
		SC2011			5.20 ± 1.26		
		SA2011			3.89 ± 1.22		
					4.60		
	95-53	SC2010			5.23 ± 0.82		
		SC2011			6.14 ± 1.22		
		SA2011			4.52 ± 1.31		
					5.30		

[z] Based on comparison with mean value of the parent check Hi-Q in each environment

[y] Based on comparison with mean value of the commercial hybrid check 95-53 in each environment

4.3.1.2 Yield related traits

For each of the yield contributing traits, silique length, number of seeds per silique, and seed weight, significant differences were detected among the genotypes ($P < 0.05$) in both environments. For silique length and number of

seeds per silique, the mean and standard deviation of the three inbred line populations, their test hybrids and the tester Hi-Q are presented in Fig. 4-1. Hi-Q averaged 64.7 (SC2010) and 69.0 (SC2011) mm silique length, and 30.4 (SC2010) and 31.5 (SC2011) seeds per silique. The average silique length and number of seeds per silique was higher for the test hybrids compared to the inbred lines in each population under both environments (Fig. 4-1). While comparing the three populations, mean silique length and number of seeds per silique were greatest in the IN population, followed by WS and SS under both environments for both inbred lines and their test hybrids (Fig. 4-1); however, these differences in most cases were marginal and not statistically significant. These two traits had a significant positive correlation in both years – SC2010 ($r = 0.599$, $P < 0.001$) and SC2011 ($r = 0.550$, $P < 0.001$). Seed yield had a weak positive correlation with number of seeds per silique in SC2010 ($r = 0.213$, $P < 0.05$), and in SC2011 with silique length ($r = 0.354$, $P < 0.01$) and seed weight ($r = 0.259$, $P < 0.05$).

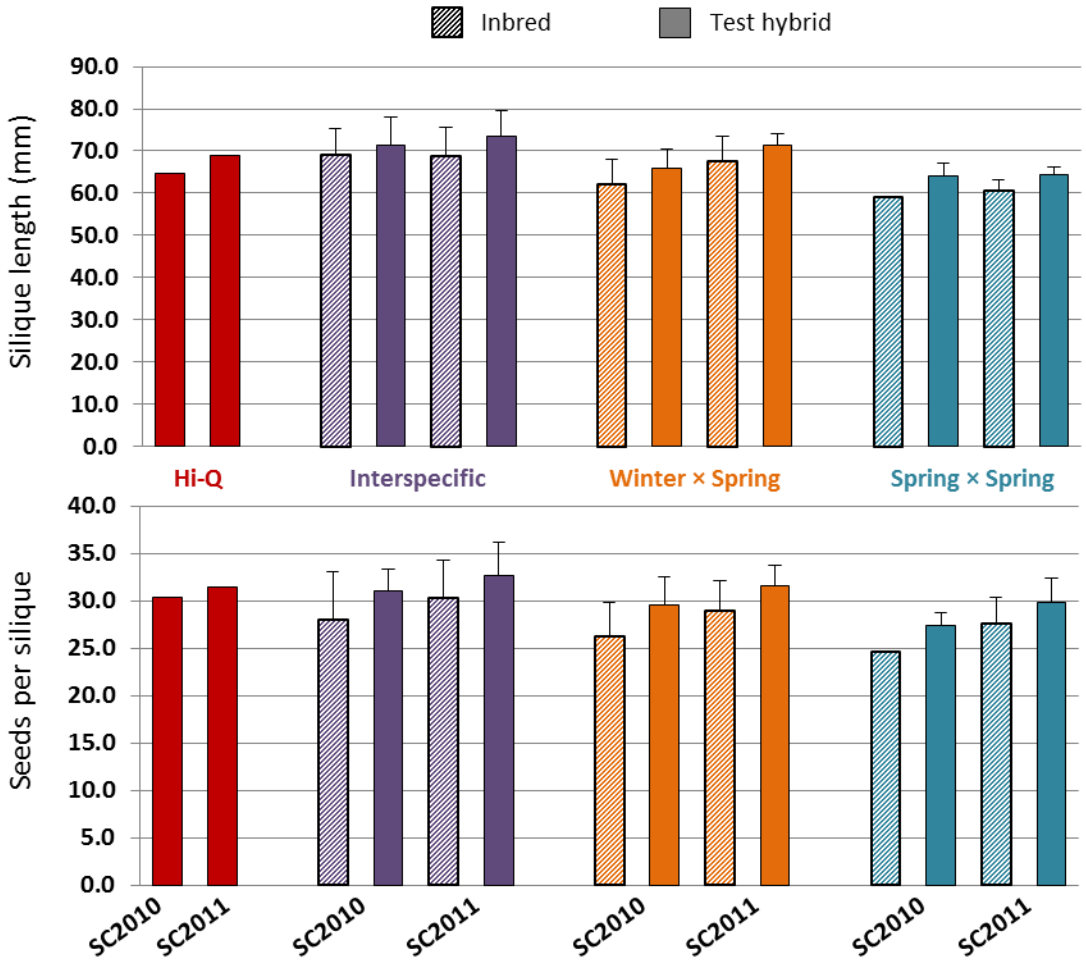

Figure 4-1. Silique length and number of seeds per silique of the inbred lines derived from *Brassica napus* × *B. oleracea* interspecific cross, and winter × spring and spring × spring crosses of *B. napus* and their test hybrids in two environments.

4.3.1.3 Agronomic traits

All inbred lines and test hybrids of the three populations, as well as the Hi-Q check, flowered and matured earlier in 2010 than in 2011. In general, IN inbred lines flowered and matured slightly earlier than their test hybrids; and these two populations were earlier than the Hi-Q parent. However, the WS test hybrids generally flowered and matured slightly earlier than their respective inbred lines; and the WS test hybrid means were very similar to the Hi-Q parent (e.g. SC2011: 57.0 vs. 57.1 for days to flowering and 120.3 vs. 120.0 for days to maturity). Almost no difference was observed between the inbred lines and their test hybrids

of the SS population for these two traits, or between these two populations and the Hi-Q parent.

Leaf length, leaf width, and plant height for the inbred lines and their test hybrids of the three populations under the SC2011 environment are displayed in Fig. 4-2. In this environment, Hi-Q had a mean leaf length of 30.2 cm and width of 13.2 cm, and plant height of 121.8 cm. Figure 4-2 shows that for each of the three populations, test hybrids on average had larger size leaf and were taller than the inbred lines. This trend was also observed in the other two environments. Comparing the three populations, the WS population had the largest leaves and tallest plants; while the IN population had smallest leaf size and shortest plants. The SS population was intermediate for these traits.

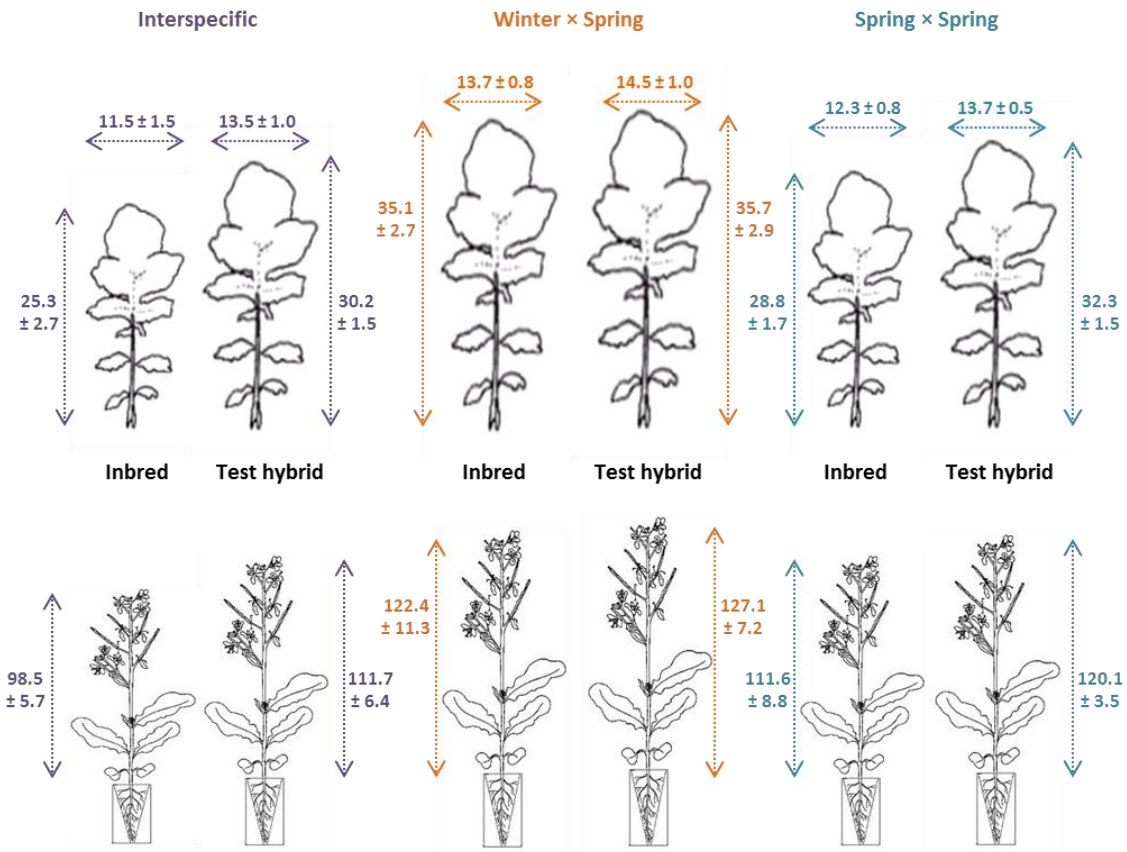

Figure 4-2. Diagrammatic representation of mean ± SD of leaf length and width (cm) and plant height (cm) of the inbred lines derived from *Brassica napus* × *B. oleracea* interspecific cross, and winter × spring and spring × spring crosses of *B. napus* and their test hybrids under SC2011 field environment.

4.3.1.4 Seed quality traits

Three seed quality traits were evaluated for harvested seed at each location: oil, protein, and glucosinolate content. A summary of the mean, standard deviation, and range of each of these traits in SC2010 trial for the three inbred populations and their test hybrids as compared to Hi-Q is presented in Table 4-3. Compared to Hi-Q, inbred lines from the IN population had significantly lower oil content and higher seed glucosinolate content (Table 4-3). However, no significant differences between Hi-Q and IN test hybrids were observed for each of the three traits. The WS inbred lines and their test hybrids had significantly lower protein content than Hi-Q (Table 4-3). Likewise, both inbred lines and test hybrids from the SS population had significantly lower protein content, however, higher oil content compared to Hi-Q (Table 4-3). While comparing the various populations, 4 – 7% variability was observed among the genotypes for oil content, while 3 – 8% variability occurred among the genotypes for protein content.

Table 4-3. Oil, protein and glucosinolate contents (mean ± SD and range) in the inbred lines derived from *Brassica napus* × *B. oleracea* interspecific cross (IN), and winter × spring (WS) and spring × spring (SS) crosses of *B. napus* and their test hybrids under SC2010 field environment

Population	Type	No. lines	Oil content (%)	Protein content (%)	Seed glucosinolate content (μmol g^{-1})
IN	Inbred	33	**44.6** ± 2.0*, 41.7 – 48.5	**27.4** ± 1.7, 22.3 – 30.6	**20.5** ± 10.7***, 10.2 – 51.8
	Test hybrid	34	**46.0** ± 1.6, 41.5 – 48.4	**26.6** ± 0.7, 25.5 – 28.4	**16.3** ± 7.7, 9.0 – 37.2
WS	Inbred	21	**46.0** ± 1.6, 43.8 – 49.6	**26.1** ± 1.3**, 22.1 – 27.9	**14.2** ± 2.4, 10.0 – 18.5
	Test hybrid	21	**46.4** ± 1.2, 44.8 – 50.0	**26.1** ± 1.0**, 22.9 – 27.3	**12.0** ± 1.1, 10.3 – 14.0
SS	Inbred	25	**48.0** ± 1.4***, 44.9 – 50.9	**24.8** ± 1.1***, 22.6 – 26.8	**10.4** ± 1.0, 8.4 – 12.4
	Test hybrid	25	**47.8** ± 1.0***, 45.9 – 49.9	**25.5** ± 0.8***, 23.6 – 26.7	**10.3** ± 0.6, 9.4 – 11.5
Check	Hi-Q		**45.7**	**27.2**	**13.2**

Significantly different from Hi-Q at * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$

4.3.2 Genotypic data

4.3.2.1 Correlation: Genetic diversity vs. heterosis

No correlation was found between genetic distance of the inbred lines from Hi-Q, as estimated by SSR markers, and either mid-parent (MPH) or high-parent heterosis (HPH) for seed yield. Under field trial SC2010, correlation coefficients were close to zero in all three populations (IN population: HPH = 0.002 and MPH = -0.048; WS population: HPH = -0.051 and MPH = -0.067; and SS population: HPH = -0.028 and MPH = 0.144). These values were also low under the SC2011 and SA2011 environments.

For the IN population, genetic distance of the inbred lines from Hi-Q ranged from 0.241 (most similar) to 0.760 (least similar), where the inbred lines with genetic distance of 0.358 to 0.435 resulted greatest HPH while the lines with distance of 0.279 to 0.447 resulted highest MPH (Fig. 4-3). WS inbred lines resulting greatest HPH were slightly more diverse from Hi-Q, where highest HPH and MPH was found for the inbred lines with genetic distance of 0.517 to 0.700. Interestingly, the WS inbred resulting test hybrid with lowest HPH and MPH also fell within this range of genetic distance (0.649). In contrast to the IN and WS populations, no SS test hybrid with extreme levels of heterosis was observed; and this was particularly evident for HPH (Fig. 4-3).

Although no correlation was observed for the IN population, the line of best fit for MPH and HPH regressed on genetic distance from Hi-Q was non-linear. For this set of data, use of a 2^{nd} degree polynomial greatly increased the R-squared value over simple linear regression, where highest levels of seed yield heterosis would be found intermediate of the two extremes for genetic distance from Hi-Q (Fig. 4-3). For the WS and SS population, there was little difference in terms of R-squared value between simple linear regression and non-linear, therefore simple linear regression was used.

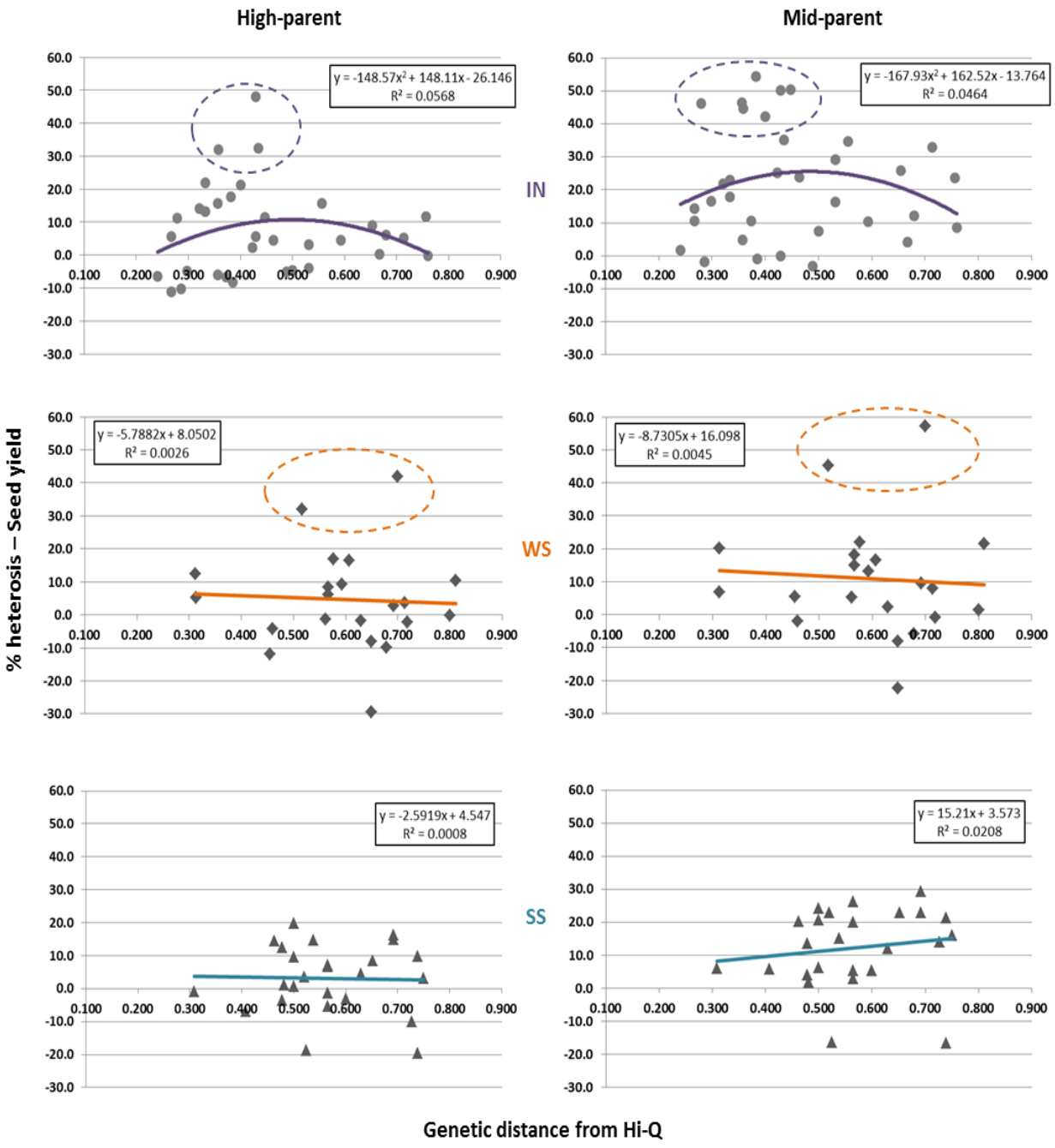

Figure 4-3. Simple linear regression of high- and mid-parent heterosis for seed yield (kg ha^{-1}) in SC2010 on genetic distance from Hi-Q in two test hybrid populations (winter × spring and spring × spring), and non-linear regression (2^{nd} order polynomial) on *B. napus* × *B. oleracea* interspecific-derived population. Equation and R-squared values are given in top corner; dotted oval highlight lines showing the hybrids with highest levels of heterosis.

4.3.2.2 Single marker analysis

Each marker was tested individually among all three environments for all traits, to determine whether the presence of specific markers in the inbred lines affected the phenotype of the resulting test hybrids. Due to limited population size, the probability associated with each F-test was considered significant at $P < 0.001$. Using this criterion, no significant association was found among individual markers and traits in the WS population. In the SS population, a single marker of N8 was associated with differences in seed oil and protein content in the SC2010 environment (Table 4-4).

The greatest number of significant single marker associations were observed for the IN population. These markers were all previously mapped to the C-genome of *B. napus* (Table 4-4), which would be expected considering that the inbred lines in this population resulted from a *B. napus* × *B. oleracea* interspecific cross. Four markers were found to be strongly associated with oil content in test hybrids under all three environmental conditions, and a fifth marker under SC2010 and SC2011 conditions (Table 4-4). Of these, three were previously mapped to N15, while the other two markers were mapped to N13 and N18. In all cases, the *B. oleracea* allele was responsible for a decrease in seed oil content.

The IN inbred lines also had two markers which were observed to affect days to flowering among test hybrid lines. The marker sR10417, previously mapped to N12 by Agriculture and Agri-Food Canada, was found to be significantly associated with days to flowering where the *B. oleracea* allele resulted in earliness of flowering; while it was the opposite for the marker CB10211 from N16. CB10211, from Celera AgGen Brassica Consortium, also was significantly associated with an increase in plant height when *B. oleracea* allele was present (Table 4-4).

Table 4-4. Single marker analysis for SSR marker loci of inbred lines of *Brassica napus* × *B. oleracea* interspecific cross (IN), and winter × spring (WS) and spring × spring (SS) crosses of *B. napus* and their effect on phenotype of test hybrids under three environments

Pop'n	Trait	Environment	Marker	*B. napus* map location	Level of significance[z]	Effect of non-Hi-Q allele[y]
IN	Days to flowering	SC2010	sR10417	N2, N12	***	-
		SC2011	CB10211	N16	***	+
	Plant height	SC2010	CB10211	N16	***	+
	Oil content	SC2010	SSR-54	N15	****	-
			sS2129	N15	*****	-
			Ol10-D02	N15	***	-
			Ol12-D05	N18	*****	-
			CB10057	N13	*****	-
		SC2011	SSR-54	N15	****	-
			sS2129	N15	****	-
			Ol10-D02	N15	***	-
			Ol12-D05	N18	****	-
			CB10057	N13	****	-
		SA2011	SSR-54	N15	***	-
			sS2129	N15	***	-
			Ol12-D05	N18	***	-
			CB10057	N13	***	-
SS	Oil content	SC2010	sS1702	N8	***	+
	Protein content	SC2010	sS1702	N8	***	-

[z] F-test level of significance where ***, ****, and ***** = $P < 0.001$, $P < 0.0001$, and $P < 1.0 \times 10^{-5}$, respectively
[y] '+' signifies greater value; '-' signifies lesser value

4.4 Discussion

The experimental design of this study was such that Hi-Q was selected as both a common parent of all three inbred (IN, WS, and SS) populations, as well as the female tester to which all selected inbred lines were crossed. Thus, any differentiated heterotic effects observed among the three test hybrid populations is

expected to be due to allelic variation from inbred lines originating from the non-Hi-Q parent. While seed yield for IN and WS test hybrids were not significantly different from Hi-Q as a population, however, certain lines showed significant heterosis. Most of the studies which evaluated test hybrids of rapeseed diversified using the *B. oleracea* C-genome have been by way of resynthesized (RS) rapeseed from various genotypes of *B. rapa*, crossed with selected *B. oleracea* types followed by chromosome doubling (Kräling 1987, Girke et al. 1999, Gehringer et al. 2007, Radoev et al. 2008). These RS lines were either crossed directly with a tester, or crossed with a natural *B. napus* and DH lines were generated, which were then used to generate test hybrids. These reports have shown great potential of the diploid parental species for increasing heterosis in *B. napus*. To the best of our knowledge, very few studies have investigated heterotic potential of inbred lines derived directly from *B. napus* × *B. oleracea* cross. Our results demonstrate that very good heterotic potential can be achieved using inbred *B. napus* lines directly diversified with the *B. oleracea* C-genome.

In this study, IN inbred lines had, on average, lower yields at all three locations compared with the WS and SS inbred lines. However, the average yield of the IN test hybrids was the highest at the South Campus site in both 2010 and 2011; and specific test hybrids with the greatest yield potential came from the IN population under all three environments (Table 4-2). Riaz et al. (2001) evaluated 12 hybrids involving parents from different genetic diversity groups, and reported the highest MPH and HPH for the hybrids that involved a parental line derived from a *B. napus* × *B. oleracea* cross which was developed by Quazi (1988). Girke et al. (1999) reported that the RS rapeseed lines used in their study showed poor performance for seed yield; however good potential was observed for seed yield in test hybrids, particularly for higher yielding RS lines. In the present study, a weak to moderate positive correlation was found between IN inbred line seed yield and yield of the respective test hybrids, ranging from r = 0.261 to 0.509 among environments. It can be anticipated that several unadapted alleles were introduced into the IN inbred lines from the *B. oleracea* parent resulting in poor performance of these lines on a population basis. However, many of these lines

showed very good heterotic potential in the hybrids – particularly for those that were somewhat intermediate in genetic distance from Hi-Q. This may be due to the fact that while ample diversity exists in these lines, the proportion of unadapted alleles giving negative effect may have been too high for many of these inbred lines.

This study also confirmed the good potential of introgression of alleles from winter *B. napus* for improving spring *B. napus*. Use of the double-low winter cultivar to broaden genetic diversity in spring *B. napus* is advantageous from the perspective that no selection is needed for canola seed quality traits. Also, many positive alleles characteristic of an elite canola cultivar are present in this winter type parent. The primary challenge for use of this germplasm was the high proportion of inbred lines screened out due to vernalization requirement or too late flowering and maturity. Among WS test hybrid lines, >30% high-parent heterosis was observed, confirming the good potential of spring type diversified with winter type canola as a hybrid parent, as suggested by Butruille et al. (1999) and Quijada et al. (2004).

An increase in seed yield, plant height, leaf size, silique length, and seeds per silique was apparent among the test hybrids compared to the inbred lines of all three populations. The field design was such that inbred plots and their respective test hybrid plots were laid out side-by-side, allowing for ease of comparison between the two types. The test hybrid plots generally showed increased early season vigour as well as vegetative growth at the time of bolting/first flowering compared to their respective inbred lines. This observation was similar to reports of vigorous growth of European × Canadian spring hybrids compared to their parents noted by Cuthbert et al. (2009). However, there was no apparent difference among means between inbred lines and test hybrids for seed quality traits, with the exception of glucosinolate content for the IN population. This difference exists due to the high glucosinolate alleles introduced into IN inbred lines from the *B. oleracea* parent, where crossing with Hi-Q invariably increased proportion of low glucosinolate alleles in the test hybrid population. Butruille et al. (1999) also noted no change in oil content in test hybrids of winter × spring

cross derived DH lines crossed with two spring cultivars. The fact that in this study, all IN inbred lines were free from erucic acid, as well as the fact that the mean glucosinolate content among test hybrid lines from this population was not significantly different than Hi-Q, demonstrates that these *B. napus* × *B. oleracea* inbred lines can be used in a practical hybrid breeding program. This is further illustrated by the fact that of the four inbred lines that gave HPH of >20% averaged over all three environments, these had GLS content of 11.8 ± 0.6, 12.3 ± 1.3, 15.3 ± 0.8, and 23.0 (one environment). Gehringer (2007) noted the challenge of obtaining double-low quality DH lines from a double-low cultivar crossed with a RS high erucic, high glucosinolate rapeseed line.

No correlation was observed between genetic distance from Hi-Q and heterosis for seed yield. This was contrary to the finding of Diers et al. (2006) and Riaz et al. (2001), who found a positive correlation between genetic distance among parental lines and seed yield of hybrids. The findings of these authors were based on diversity among spring type *B. napus* lines or cultivars and hybrids generated from intercrossing of these lines. Despite these findings, Diers et al. (2006) stated that genetic distance estimates cannot be reliably used alone to estimate high yielding combinations, and Riaz et al. (2001) hypothesized that significant linkage between some of the markers used in their study and QTL for seed yield might be a reason they found significant positive correlation between genetic distance and seed yield. Yu et al. (2005) did not find significant correlation between genetic distance among parental lines and F_1 yield in winter type CMS × restorer crosses, and postulated that a measure of genetic diversity in the parents may be a better predictor of heterosis if the markers involved were known to be associated with coding regions or have some significant linkage to agronomic traits. Thus, inclusion of molecular markers known to be associated with QTL for seed yield and other agronomic traits, or coding regions, may improve the ability to predict the performance of hybrids based on genetic distance of the parents.

The C-genome of *B. oleracea* var. *alboglabra* carries allele(s) for early flowering which can be used for the improvement of earliness in *B. napus*

(Rahman et al. 2011). This is described more fully in the DH study reported in Chapter 5 of this thesis. In Chapter 5 an SSR marker, Ol13-G05, was observed to be significantly associated with earliness of flowering in *B. napus* DH lines under multiple environments at $P < 0.01$, when the lines carry the *B. oleracea* allele. This marker had been previously mapped to 20 to 30 cM from the top of the *B. napus* chromosome N12 (Piquemal et al. 2005, Qiu et al. 2006). In the present study, an additional SSR marker sR10417 for days to flowering was identified based on test hybrid data; and in this case also the allele from *B. oleracea* resulted earlier flowering in test hybrids. This marker was also previously mapped to the middle of *B. napus* chromosome N12 by Agriculture and Agri-Food Canada (AAFC). This provides further evidence that an allele for early flowering from *B. oleracea* is present on N12; and this allele apparently exerted dominance effect in the hybrids, as IN inbred lines carrying the N12 allele of *B. oleracea* were earlier (3 to 4 days) in flowering ($t = -2.65$ to -3.14, $P < 0.01$) as well as produced early flowering hybrids (2 to 3 days) ($t = -2.25$ to -4.12, $P < 0.05$). Other researchers also found significant QTL affecting days to flowering on this chromosome, termed *ft17* (Mei et al. 2009) and *qFT12-1* (Long et al. 2007). These QTL were positioned at the mid to lower half of the *B. napus* N12 chromosome. It is unclear whether the early flowering allele of *B. oleracea* corresponds to a similar region on N12 as these previously identified QTLs.

Delourme et al. (2006) identified a QTL for oil content on N15 in a DH population of 442 lines derived from Darmor-*bzh* (winter) × Yudal (Korean spring) cross. This was one of five QTL detected in their study across all three locations, and the N15 QTL explained the largest amount of phenotypic variation at each location compared to the QTL detected on other chromosomes. Thus, it is apparent that a genomic region, residing on C-genome chromosome N15, affects seed oil content in *B. napus*. In addition, an SSR QTL marker (CB10057) from N13 affecting oil content was also detected in the present study; however, this marker mapped about 34 cM away from a QTL region on N13 detected by Delourme et al. (2006). The *B. oleracea* alleles in our study obviously impart lower seed oil content. In a QTL mapping study of RILs developed from a cross

between yellow and black-seeded lines from China, Yan et al. (2009) found co-localization of seed oil content, seed hull content, and seed coat colour QTL on N8 of the *B. napus* A-genome. Apparently this chromosomal region has genes which affect multiple seed characteristics. We detected strong association of one N8 marker (sS1702) in the SS population which was associated with an increase in oil content and a decrease in protein content when the spring parent A03-14NI allele was present. This marker may be associated with the same QTL region for oil content, *qOC06W-8*, identified by Yan et al. (2009).

Plot size and number of replications in experiments evaluating test hybrids are often limited by availability of seed, which is influenced by the method of hybrid seed production (e.g. hand crosses vs. CMS) and breeding materials used. In this study, seed availability of the test hybrids was limited due to the fact that no CMS Hi-Q line was available, which necessitated the generation of hybrid seed by emasculation and hand pollination of all hybrid combinations in the greenhouse during the winter preceding each growing season. If seed availability were not a limitation, intra-hybrid variance for seed yield could have been reduced by increasing plot size and number of replications.

In conclusion, there was no correlation between high- and mid-parent heterosis for seed yield and genetic distance of the inbred lines based on SSR marker dataset. However, the genetic diversity introduced into a spring *B. napus* background from *B. oleracea* var. *alboglabra* and from the winter cultivar Aviso both showed very good heterotic potential. The *B. napus* × *B. oleracea* derived inbred lines are particularly interesting as certain genotypes produced test hybrids with greatest yields under all three environmental conditions. Furthermore, despite the non-canola quality traits of seeds of the *B. oleracea* parent, the inbred and particularly test hybrid population displayed seed quality characteristics closely approaching that of spring canola. The heterotic effect of these diversified lines could possibly be even greater should alternative tester lines, unrelated to Hi-Q, be used to generate test hybrids. Thus, the continued improvement of spring cultivars of *B. napus*, and in particular hybrid varieties, will likely include the

utilization of greater genetic diversity from winter types and the allied species including the C-genome of *B. oleracea*.

4.5 References

Barnes AP, Wreford A, Butterworth MH, Semenov MA, Moran D, Evans N (2010) Adaptation to increasing severity of phoma stem canker on winter oilseed rape in the UK under climate change. J Agr Sci 148:683-694

Basunanda P, Spiller TH, Hasan M, Gehringer A, Schondelmaier J, Lühs W, Friedt W, Snowdon RJ (2007) Marker-assisted increase of genetic diversity in a double-low seed quality winter oilseed rape genetic background. Plant Breed 126:581-587

Butruille DV, Guries RP, Osborn TC (1999) Linkage analysis of molecular markers and quantitative trait loci in populations of inbred backcross lines of *Brassica napus* L. Genetics 153:949-964

Canadian Food Inspection Agency (2009) Canola/rapeseed objective description. Plant Breeders' Rights Office, Ottawa, Canada

Cuthbert RD, Crow G, McVetty PBE (2009) Assessment of agronomic performance and heterosis for agronomic traits in hybrid high erucic acid rapeseed (HEAR). Can J Plant Sci 89:227-237

Delourme R, Falentin C, Huteau V, Clouet V, Horvais R, Gandon B, Specel S, Hanneton L, Dheu JE, Deschamps M, Margale E, Vincourt P, Renard M (2006) Genetic control of oil content in oilseed rape (*Brassica napus* L.) Theor Appl Genet 113:1331-1345

Diers BW, McVetty PBE, Osborn TC (1996) Relationship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (*Brassica napus* L.). Crop Sci 36:79-83

Diers BW, Osborn TC (1994) Genetic diversity of oilseed *Brassica napus* germ plasm based on restriction fragment length polymorphisms. Theor Appl Genet 88:662-668

Gehringer A, Snowdon R, Spiller T, Basunanda P, Friedt W (2007) New oilseed rape (*Brassica napus*) hybrids with high levels of heterosis for seed yield under nutrient-poor conditions. Breed Sci 57:315-320

Girke A, Becker HC, Engqvist G (1999) Resynthesized rapeseed as a new genpool for hybrid breeding. Proc 10th Int Rapeseed Congress, v.4, Canberra, Australia

Hasan MF, Seyis F, Badani AG, Pons-Kühnemann J, Friedt W, Lühs W, Snowdon RJ (2006) Surveying genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet Resour Crop Evol 53:793-802

Kebede B, Thiagarajah MR, Zimmerli C, Rahman MH (2010) Improvement of open-pollinated spring rapeseed (*Brassica napus* L.) through introgression of genetic diversity from winter rapeseed. Crop Sci 50:1236-1243

Kovach WL (2007) MVSP – A multivariate statistical package for windows, ver. 3.1. Kovach Computing Services, Pentraeth, Wales, UK

Kräling K (1987) Utilization of genetic variability of resynthesized rapeseed. Plant Breed 99:209-217

Li M, Qian W, Meng J, Li Z (2004) Construction of novel *Brassica napus* genotypes through chromosomal substitution and elimination using interploid species hybridization. Chromosome Res 12:417-426

Long Y, Shi J, Qiu D, Li R, Zhang C, Wang J, Hou J, Zhao J, Shi L, Park BS, Choi SR, Lim YP, Meng J (2007) Flowering time quantitative trait loci analysis of oilseed Brassica in multiple environments and genomewide alignment with Arabidopsis. Genetics 177:2433-2444

Lorieux M (2007) MapDisto, a free user-friendly program for computing genetic maps. Computer demonstration (P958) given at the Plant and Animal Genome XV Conference, Jan 13-17 2007, San Diego, CA. URL: http://mapdisto.free.fr/

Mei DS, Wang HZ, Hu Q, Li YD, Xu YS, Li YC (2009) QTL analysis on plant height and flowering time in *Brassica napus*. Plant Breed 128:458-465

Melchinger AE (1999) Genetic diversity and heterosis. *In* Coors JG, Pandey S (eds.) Genetics and Exploitation of Heterosis in Crops. ASA, CSSA, Madison, WI.

Nei M, Li W (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5256-5273

Piquemal J, Cinquin E, Couton F, Rondeau C, Seignoret E, Doucet I, Perret D, Villeger M-J, Vincourt P, Blanchard P (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor Appl Genet 111:1514-1523

Qian W, Li Q, Noack J, Sass O, Meng J, Frauen M, Jung C (2009) Heterotic patterns in rapeseed (*Brassica napus* L.): II. Crosses between European winter and Chinese semi-winter lines. Plant Breed 128:466-470

Qian W, Meng J, Li M, Frauen M, Sass O, Noack J, Jung C (2006) Introgression of genomic components from Chinese *Brassica rapa* contributes to widening the genetic diversity in rapeseed (*B. napus* L.), with emphasis on the evolution of Chinese rapeseed. Theor Appl Genet 113:49-54

Qian W, Sass O, Meng J, Li M, Frauen M, Jung C (2007) Heterotic patterns in rapeseed (*Brassica napus* L.): I. Crosses between spring and Chinese semi-winter lines. Theor Appl Genet 115:27-34

Qiu D, Morgan C, Shi J, Long Y, Liu J, Li R, Zhuang X, Wang Y, Tan X, Dietrich E, Weihmann T, Everett C, Vanstraelen S, Beckett P, Fraser F, Trick M, Barnes S, Wilmer J, Schmidt R, Li J, Li D, Meng J, Bancroft I (2006) A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. Theor Appl Genet 114:67-80

Quazi MH (1988) Interspecific hybrids between *Brassica napus* L. and *B. oleracea* L. developed by embryo culture. Theor Appl Genet 75:309-318

Quijada PA, Udall JA, Lambert B, Osborn TC (2006) Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (*Brassica napus* L.): 1. Identification of genomic regions from winter germplasm. Theor Appl Genet 113:549-561

Quijada PA, Udall JA, Polewicz H, Vogelzang R, Osborn TC (2004) Phenotypic effects of introgressing French winter germplasm into hybrid spring canola. Crop Sci 44: 1982-1989

Radoev M, Becker HC, Ecke W (2008) Genetic analysis of heterosis for yield and yield components in rapeseed (*Brassica napus* L.) by quantitative trait locus mapping. Genetics 179:1547-1558

Rahman H (2011) Use of European winter canola *Brassica napus* L. for the improvement of Canadian spring canola: a practical breeding example. Proc 13th Inter Rapeseed Congr, Prague, p 875-878

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

Riaz A, Li G, Quresh Z, Swati MS, Quiros CF (2001) Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. Plant Breed 120:411-415

Rygulla W, Friedt W, Seyis F, Lühs W, Eynck C, von Tiedemann A, Snowdon RJ (2007) Combination of resistance to *Verticillium longisporum* from zero erucic acid *Brassica oleracea* and oilseed *Brassica rapa* genotypes in resynthesized rapeseed (*Brassica napus*) lines. Plant Breed 126:596-602

Statistics Canada. 2011. Field and special crops (Seeded area). Available at: http://www40.statcan.ca/l01/cst01/Prim11a-eng.htm (verified 24 Oct. 2011). Minister of Industry, Ottawa, Canada.

Stringam GR, Degenhardt DF, Thiagarajah MR, Bansal VK (2000) Hi-Q summer rape. Can J Plant Sci 80:835-836

Udall JA, Quijada PA, Lambert B, Osborn TC (2006) Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (*Brassica napus* L.): 2. Indentification of alleles from unadapted germplasm. Theor Appl Genet 113:597-609

Yan XY, Li JN, Fu FY, Jin MY, Chen L, Liu LZ (2009) Co-location of seed oil content, seed hull content and seed coat color QTL in three different environments in *Brassica napus* L. Euphytica 170:355-364

Yu CY, Hu SW, Zhao HX, Guo AG, Sun GL (2005) Genetic distances revealed by morphological characters, isozymes, proteins and RAPD markers and their relationships with hybrid performance in oilseed rape (*Brassica napus* L.). Theor Appl Genet 110:511-518

Zou J, Zhu J, Huang S, Tian E, Xiao Y, Fu D, Tu J, Fu T, Meng J (2010) Broadening the avenue of intersubgenomic heterosis in oilseed *Brassica*. Theor Appl Genet 120:283-290

Chapter 5

Uncovering the genetic basis of early flowering and glucosinolate content in *Brassica napus* introgressed from the C-genome of *Brassica oleracea* var. *alboglabra*

5.1 Introduction

Canola is the most important oilseed crop in Canada, where *Brassica napus* L. dominates the acreage due to its yield advantage over *B. rapa* L. The development of earlier maturing *B. napus* is an important breeding objective for cultivars targeted to regions with short growing seasons, such as northern Canada and Europe. Flowering marks the transition from a vegetative to a reproductive state, is an important agronomic trait for crop reproduction, and has been reported as a good indicator of maturity in *Brassica* oilseed crops (Miller 2001, Mahmood et al. 2007). Therefore, days to flowering (DTF) has been used by several breeding programs for the selection of early maturing *B. napus*.

Days to flowering is a complex (quantitative) trait that involves several gene loci with large or small effect, interactions between the genes, as well as interactions of the plant with environmental cues such as photoperiod and temperature. Due to its large genome size, amphidiploid nature, and high level of duplicated genomic regions both within and between the A- and C- genomes (Parkin et al. 2003), the genetic regulation of flowering time in *B. napus* is quite complex and remains largely unknown.

Quantitative trait loci (QTL) studies have been very instrumental in identifying genomic regions underlying the determination of flowering time in *B. napus* (Ferreira et al. 1995, Osborn et al. 1997, Long et al. 2007, Cai et al. 2008, Mei et al. 2009, Chen et al. 2010). In these studies, mapping populations have been derived from a variety of crosses, including spring × spring, winter × spring, winter × winter, and winter × semi-winter types. Doubled haploid (DH) populations derived from these crosses have most often been used for generation

of genotypic and phenotypic data, although $F_{2:3}$ and reconstructed F_2 populations have also been employed for this purpose.

Collectively, a large number of QTL affecting flowering time have been identified in *B. napus*. QTL × environment interactions have been found to play an important role in the genetic control of flowering time (Cai et al. 2008); thus, many of these loci are strongly influenced by environmental factors. However, QTLs with large phenotypic effect on flowering time are often stable across the environments. It has been suggested by Long et al. (2007) that the number of such QTL in *B. napus* is between four and nine. Sequence information from the closely related model dicot species *Arabidopsis thaliana*, in which the genes regulating flowering time are well characterized, has been used for identification of candidate genes underlying flowering time QTL in *B. napus*, such as *CONSTANS* (*BnCO*) (Robert et al. 1998) and *FLOWERING LOCUS T* (*BnFT*) (Wang et al. 2009).

High levels of glucosinolates (GLS) in seed meal, e.g. sinigrin and gluconapin, impart an adverse effect on the taste and smell of animal feed; while other GLS, e.g. progoitrin (goitrogenic), are antinutritional and decrease the feeding value of this high-protein meal (Bjerg et al. 1989). Therefore, reducing the levels of total and/or specific GLS increases the value of this seed meal. QTL studies have been performed to identify the chromosomal regions associated with the inheritance of seed GLS content. Uzunova et al. (1995), using a DH population derived from a cross between high and low GLS winter-type *B. napus* lines, identified four QTL for the total GLS content. These QTLs explained 61% of the phenotypic variation in this population. Based on spring-type DH lines, Toroser et al. (1995) identified five QTL for total GLS which behaved in an additive fashion and explained 71% of the phenotypic variation in this population. Howell et al. (2003) also identified four genomic regions associated with seed GLS content in backcross lines from intervarietal winter rapeseed crosses. Thus, total seed GLS content appears to be controlled by four to five gene loci and low GLS phenotype results from recessive alleles in all loci in homozygous condition.

Most often, introgression of early flowering alleles of the A-genome of *B. rapa* have been attempted for improvement of this trait in *B. napus*. We recently demonstrated that allele(s) from the C-genome of the late flowering species *B. oleracea* L. can also improve earliness in *B. napus* (Rahman et al. 2011), despite the fact that the most early flowering C-genome species flower at least 2-3 weeks later than *B. napus*. However, the chromosomal location and nature of the allele contributing to this improved earliness are unknown. From a *B. napus* × *B. oleracea* interspecific cross, we developed an inbred line which flowers earlier than the *B. napus* parent but possesses high GLS content in seed. Therefore, the objective of this study was to investigate the genetic basis of the early flowering and high GLS content in *B. napus* resulting from introgression of the C-genome alleles from *B. oleracea* into a *B. napus* background.

5.2 Materials and methods

5.2.1 Plant material

The parental material used in this study was a spring *B. napus* L. (AACC, *2n*=38) DH canola cultivar Hi-Q and an inbred (F_5) line RIL-144. The line RIL-144 was derived from an interspecific cross between Hi-Q and a highly inbred (F_7) *B. oleracea* var. *alboglabra* (L. H. Bailey) Musil (CC, *2n*=18) line. Hi-Q is a registered cultivar in Canada (Stringam et al. 2000). *Brassica alboglabra* is self-compatible, and does not require vernalization for flowering. It is one of the earliest flowering variants of the C-genome *Brassica* species; however, it flowers two to three weeks later than Canadian spring *B. napus*.

The RIL-144 was selected from an early flowering F_5 plant family 9-123 (n=5 plants). This family had a mean of 9.5 days earlier flowering than Hi-Q (n=12 plants) under greenhouse conditions. This family had *B. napus* morphology and was highly fertile, producing 16.1 ± 3.4 seeds per silique vs. 15.4 ± 4.7 of Hi-Q under greenhouse conditions. Flow cytometry analysis (Partec system) of self-pollinated offspring from this F_5 family revealed a nuclear DNA content almost identical to the *B. napus* parent: 196.3 vs. 195.7 (Hi-Q); while *B. alboglabra* had

Partec value of 117.4. These observations suggest that the chromosome number of this plant family was the same as *B. napus*. Seed GLS content of RIL-144 was 21.7 µmol g^{-1} seed under greenhouse condition (Hi-Q 14.4 µmol g^{-1} seed).

5.2.2 Doubled haploid (DH) population

Crosses between a single Hi-Q plant as female and a single plant of RIL-144 as male were made in a greenhouse. Three F_1 plants were grown in a growth chamber set at 10°/7°C day/night temperature with 16 h photoperiod. However, two plants were used as microspore donors for the development of DH lines. Both plants were confirmed true hybrids by five polymorphic SSR markers. A total of 110 DH lines, 90 from one F_1 plant and 20 from the other F_1 plant, were produced. For microspore isolation and culture, a procedure modified from Chuong and Beversdorf (1985), as described by Thiagarajah and Stringam (1993), was used. Young flower buds of petal approximately two-thirds length of anther, were picked from main raceme or lateral branches of F_1 plants and surface sterilized with a 7% (w/v) calcium hypochlorite solution. Isolated microspores were suspended in modified Nitsch and Nitsch medium (Lichter 1985) 'NN13' with 13% sucrose and 50 mg l^{-1} colchicine (Möllers et al. 1994) and incubated at 30°C for 24 h. Microspores were subsequently re-suspended in NN13 medium without colchicine and incubated at 30°C for approximately two weeks. For embryo germination and plantlet development, cotyledonary embryos were transferred to B5 medium containing gibberillic acid (0.1 mg L^{-1}) and solidified with 0.8% agar (Coventry et al. 1988). Developed plantlets were transplanted to a soil-free mix in the greenhouse, and the fertile, pollen-producing plants were self-pollinated and seeds harvested as DH lines.

5.2.3 Phenotypic data

Flowering data was acquired for 97 DH lines, their parents, and F_1 from replicated trials in growth chamber and field. A series of four trials in growth chambers were conducted. For each trial, plants were grown in two replications – either in one large or two small growth chamber(s). The mean value of two plants of each DH

line and four plants of each of the parents and F_1 in each replication were used for analysis. A 16 h photoperiod was used for all growth chamber trials. Of these four trials, three trials were set at 20°C constant temperature, while the fourth trial was set at 20/16°C day/night temperature. For these trials, DTF was recorded on individual plant basis as the number of days from seeding to opening of the first flower.

Field trials were seeded at the South Campus Research Station of the University of Alberta in 2010 and 2011; as well as at the St. Albert Research farm of the University of Alberta and at Langdon and Prosper sites, North Dakota in 2011 to study days to flowering as well as seed yield of the DH lines. For the trials on days to flowering, each trial consisted of two replications in a 10 block × 20 entries/block and the experimental design was alpha-lattice. Plot size in all locations was 2 m long, single row with seeding 0.6 m apart. Self-pollinated seeds were used for this study. For yield estimation, open-pollinated seed of the DH lines and the parents collected from the 2010 field trial were used. These trials consisted of four row plots of 6 m length and 1.2 m width, and the trials were grown in two replications at each of the three 2011 locations. A modified randomized complete block design, as described by Mahmood et al. (2007), was used due to large size of the trials. For this, the 97 DH lines, RIL-144, and F_1 hybrid (generated from additional Hi-Q × RIL-144 crosses) were nested into three sets and randomized within each set. For both days to flowering and yield trials (single-row plots and full plots), two checks were included in each block: Hi-Q and Peace. Peace is an early *B. napus* cultivar developed at the University of Alberta, and registered in 2001 in Canada. The nine environments are designated as GC20C = Growth chamber 20°C constant, GC20-16 = 20/16°C day/night temperature, SC10-SR = South campus 2010 single row (days to flowering trial), SC11-SR = South Campus 2011 single row, SA11-SR = St. Albert 2011 single row, ND11-SR = North Dakota 2011 single row, SC11-FP = South Campus 2011 full plot (yield trial), SA11-FP = St. Albert 2011 full plot, and ND11-FP = North Dakota 2011 full plot.

For all field trials, DTF was recorded on a plot basis as the number of days from seeding to approximately 50% of plants having at least one open flower. In addition to DTF, days to maturity (DTM) was estimated on a whole plot basis when loss of moisture and change in coloration was apparent in seeds of main raceme of at least 75% of plants. Seed oil (%), protein (%), and total GLS (µmol g^{-1} seed) contents were estimated using near-infrared spectroscopy (NIRS, FOSS NIRSystems model 6500) on a whole seed basis at 8.5% moisture. NIRS analysis was done in the Analytical Laboratory of the Canola Program of the University of Alberta, which is accredited by the Canadian Grain Commission for this analysis.

5.2.4 Genotypic data

Young leaves from the two parents, their F_1, and 93 DH lines, grown in a greenhouse, were used. Genomic DNA was extracted using SIGMA extraction method following the manufacturer's instructions. Quantification of total genomic DNA of each sample was done using 1.5 µl samples measured on a NanoDrop® Spectrophotometer (model 'ND-1000'), and each sample was diluted to 5 ng μl^{-1}. The two parents (Hi-Q and RIL-144) were used to screen for polymorphism using 613 simple sequence repeat (SSR or microsatellite) primer pairs covering all 19 *B. napus* linkage groups. Source of markers was as reported in section 2.2.9. A few markers with proprietary right to another research group have also been used, and these are designated with the prefix 'SSR-'. Polymerase chain reactions (PCR) and labelling of PCR products were done as described by Kebede et al. (2010). Amplified Fragment Length Polymorphism (AFLP) analysis was done following the protocol developed by Vos et al. (1995). For this, 250 ng genomic DNA extracted from the RILs and the parents was used for digestion with the restriction enzymes *Eco*RI and *Mse*I. Polymorphisms were detected using primer combinations, which involved four *Eco*RI and ten *Mse*I primers. The AFLP markers were named using the codes of each *Eco*RI and *Mse*I primer; for example E3453.300 means *Eco* primer number 34 and *Mse* primer number 53, and band size of 300 bp for the AFLP allele. *Eco*RI primers were labeled with four florescent dyes: FAM, VIC, NED and PET (Applied Biosystems, Foster City,

CA). Detection of the amplification products of AFLP and SSR primers were performed on a capillary ABI sequencer No. 3730 (Applied Biosystems, Foster City, CA).

An additional 40 primer pairs were designed based on 13 key flowering time genes selected from Ehrenreich et al. (2009) – Table S1, identified as important in the model dicot species *Arabidopsis thaliana*. For this, DNA sequence alignment information from *A. thaliana* (Ehrenreich et al. 2009 – File S1) was used to perform a BLAST search on The National Center for Biotechnology Information (NCBI) database, and primers were designed from publicly available homologous sequences in *B. napus*, *B. oleracea*, and *B. rapa*. Where no *Brassica* homologous sequences were found, sequence information from *A. thaliana* was used directly. In this case, exon regions were identified using GENSCAN software (Burge and Karlin 1997) and primers were designed within these regions. All primers were designed using Primer3 software (Rozen and Skaletsky 2000).

5.2.5 Data analysis

Analysis of variance (ANOVA) and correlation were done using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Data is presented as least squares means, calculated by Proc GLM in the case of growth chamber experiments and Proc MIXED in the case of field trials, where replication and block nested in replication were random effects. Proc CORR was used to calculate Pearson correlation coefficients among various datasets.

For marker data, normal Mendelian segregation of 1:1 in the DH population was tested using χ^2 analysis. All markers showing severe segregation distortion ($\chi^2 > 50$) were excluded from data analysis. Single marker analysis and construction of genetic linkage groups was done using MapDisto version 1.7.5.1 (Lorieux 2007). For linkage group construction, a LOD value of 5 was used. The ‘order sequence’ and ‘ripple order’ commands were used to find best marker order. The Kosambi function (Kosambi 1944) was used for calculating distance between the markers, and the marker distance was expressed in centimorgan

(cM). QTL analysis was done using Windows QTL Cartographer version 2.5.010 (Wang et al. 2011).

5.3 Results

5.3.1 Phenotypic data

Data for DTF of pedigree selections leading to the family 9-123, from which RIL-144 was selected, is presented in Table 5-1. RIL-144 had *B. napus* type appearance, including similar leaf and stem morphology, as well as yellow petal colour. Both parents, Hi-Q and RIL-144, had zero erucic acid content and similar levels of total saturated fatty acids in seed oil. A comparison of GLS and DTF, as well as the average silique length and number of seeds per silique under greenhouse conditions, of the two parental plants used in the cross for generating a DH population is given in Table 5-2.

Table 5-1. Days to flower for pedigree generations of plant family 9-123, derived from *B. napus* Hi-Q x *B. oleracea* var. *alboglabra* interspecific cross, as compared to Hi-Q

Generation	Plant family	No. of plants	DTF (Avg ± SD)	Range
F_2 (Growth chamber)*	9-006	26	68.8 ± 8.5	55 to 89
	Hi-Q	1	64.0	-
F_3 (Greenhouse)	9-015	30	35.6 ± 2.6	31 to 41
	Hi-Q	6	39.3 ± 1.5	38 to 42
F_4 (Field)	9-106	2 plots	44.5 ± 3.5	42 to 47
	Hi-Q	4 plots	47.8 ± 2.2	45 to 50
F_5 (Greenhouse)	9-123	5	35.0 ± 1.9	33 to 37
	Hi-Q	12	44.5 ± 3.1	42 to 51

*F_2 seeds germinated on petri dish for fatty acid analysis by half-seed technique. The germinated seedlings were transplanted in pots, and this apparently retarded normal growth and development, and thus delayed flowering

Table 5-2. Comparison of phenotypic traits, under greenhouse conditions, of the two parental plants used to generate a doubled haploid population

Parents of DH	Days to flowering	Glucosinolate content (μmol g^{-1})	Avg. silique length (mm)	No. seeds per silique
RIL-144	33	23.4	51.6	13.6
Hi-Q	43	15.5	55.3	17.8

The rate of success of obtaining DH plants from culture of microspores is given in Table 5-3. Of 516 embryos initially transferred from liquid media to solid media, 110 (21.3%) gave diploid plants, i.e. DH lines, in the greenhouse and were included in the present experiment.

Table 5-3. Success rate for obtaining doubled haploid (DH) lines of *Brassica napus* through microspore culture

Step	Total no.	% success from previous step
Transfer of embryos to solid media	516	
Transfer of plantlets to soilless media	364	70.5
Evaluation of mature plants	269	73.9
No. diploids, i.e. DH	110	40.9

Distribution of the DH lines for DTF showed moderate departures from normality and were generally skewed towards earliness. Combined analysis showed significant location × genotype interaction ($P < 0.001$); therefore, data were analyzed separately for each environment. In all environments, significant differences were found among the genotypes ($P < 0.01$). Under growth chamber conditions, the RIL-144 flowered 3.4 to 5.0 days earlier than Hi-Q (37.2 to 39.0 days to flower), and the DH lines required 31.0 to 43.5 days to flower (Fig. 5-1). Under Albertan field conditions, the RIL-144 flowered 3.3 to 7.5 days earlier than Hi-Q (53.6 to 57.8 days to flower), and the DH lines required 45.6 to 63.5 days to flower (Fig. 5-2). All experimental materials flowered earlier in the two North Dakota sites than in Alberta, and less variation was evident among the DH lines. In these two sites, the RIL-144 flowered only 1.5 to 1.9 days earlier than Hi-Q,

and DH lines ranged from 37.0 to 44.0 for DTF (Fig. 5-2). Under all environments, the F_1 was either intermediate of the two parents or very similar (0.0 to 1.3 days later) to the RIL-144 parent (Fig. 5-1, Fig. 5-2).

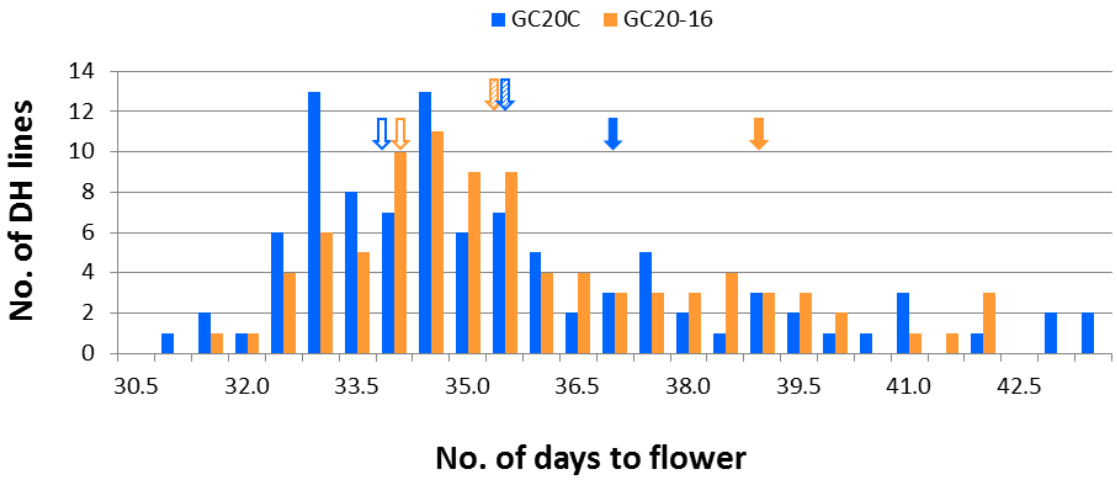

Figure 5-1. Distribution of the *Brassica napus* doubled haploid population for days to flowering in two growth chamber (20°C constant and 20/16°C day/night) environments. Parents of the DH population are represented by open arrow (RIL-144) and filled arrow (Hi-Q); F_1 is represented by hatched arrow.

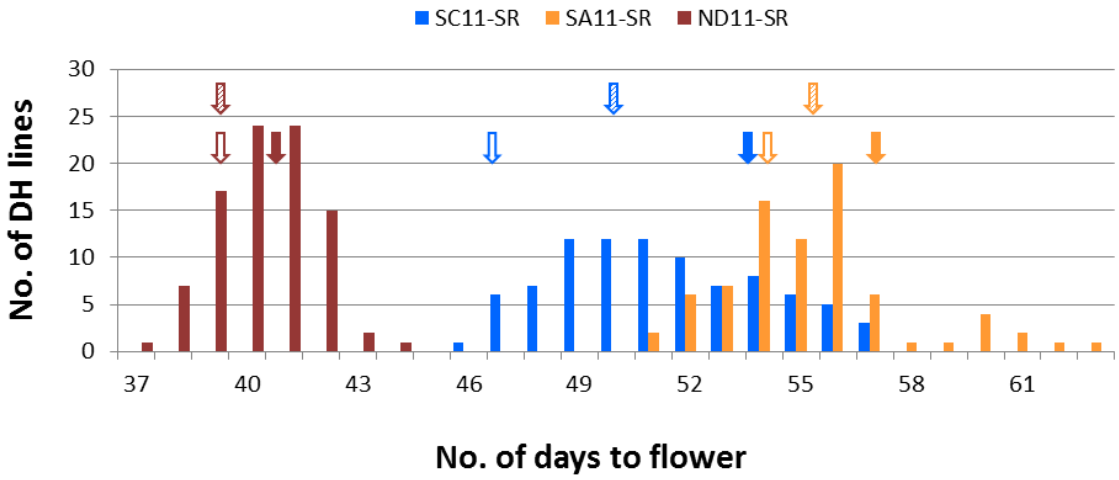

Figure 5-2. Distribution of the *Brassica napus* doubled haploid population for days to flowering in field trials at three locations (South Campus, St. Albert and North Dakota) in 2011. Parents of the DH population are represented by open arrow (RIL-144) and filled arrow (Hi-Q); F_1 is represented by hatched arrow.

A distinct bimodal distribution is evident among DH lines for GLS content at all four environments (Fig. 5-3); where the two peaks approximately correspond to the value of the two parents. Glucosinolate level in the low GLS parent Hi-Q remained very similar among years, locations, and type of trials (Fig. 5-3a, b); however, GLS level in the high parent RIL-144 was affected by plot size. Seeds harvested from single row plots were about 14 µmol g^{-1} seed higher GLS than seeds from full plots. Analysis of variance revealed significant difference between the DH lines ($P < 0.001$), and significant location × genotype ($P < 0.001$) interaction for the DH population.

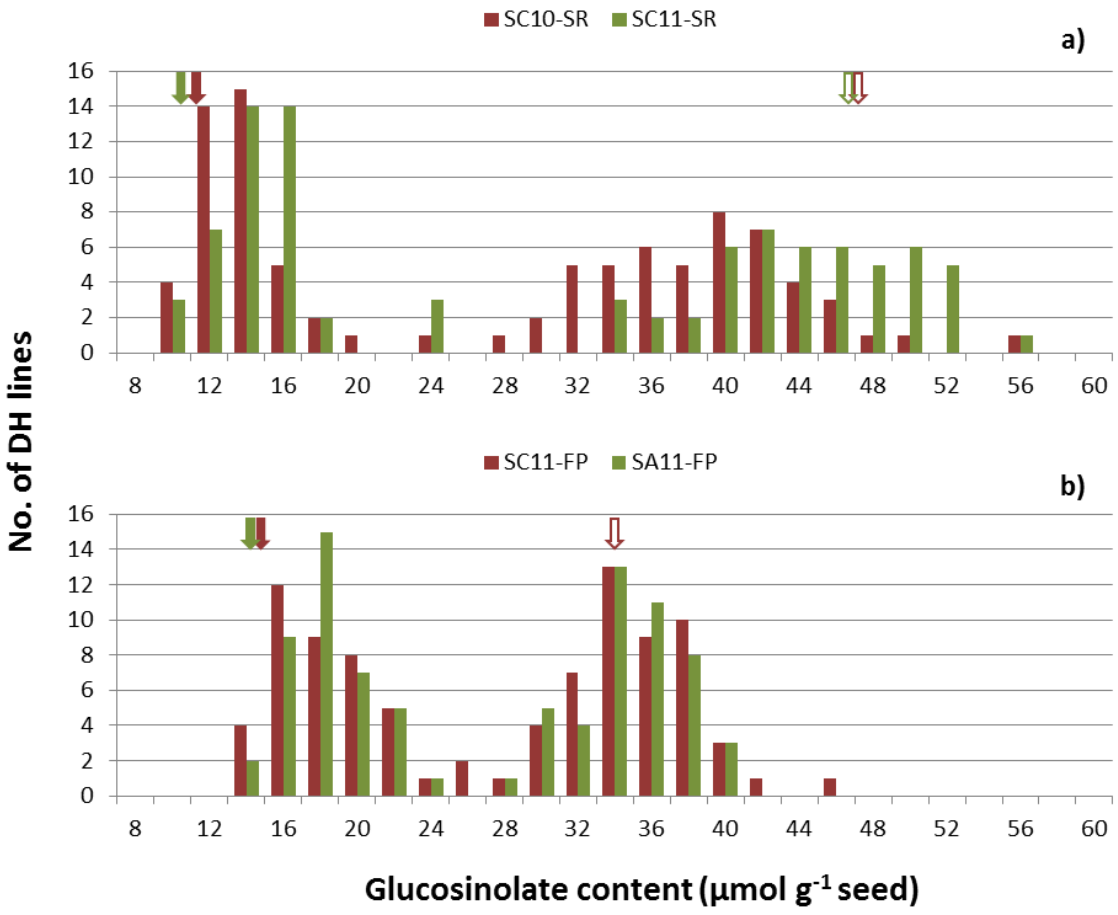

Figure 5-3. Distribution of the *Brassica napus* doubled haploid population for total glucosinolate content in field trials for a) single row plots grown at South Campus in 2010 and 2011, and b) full size plots grown at South Campus and St. Albert sites in 2011. Parents of the DH population are represented by open arrow (RIL-144) and filled arrow (Hi-Q). No data available for RIL-144 from St. Albert site.

The correlation coefficient between the two growth chamber conditions for DTF in the DH population was 0.779; while under the five Alberta trials it ranged from 0.471 to 0.711 (Table 5-4). There was moderate correlation for this trait among the North Dakota full plot trial and five Alberta trials (r = 0.422 to 0.509); however, none was observed with the single row North Dakota trial (r = -0.118 to 0.059). DTF was also found to have moderate positive correlation with days to maturity (DTM). Higher yields were weakly correlated with later DTF. DTF shows almost no correlation with seed oil and GLS content (Table 5-4).

Table 5-4. Correlation of days to flowering (DTF) between pairs of environments in the *Brassica napus* DH lines, and its correlation with days to maturity (DTM) in Alberta field trials

Trait(s) and locations	No. environments	Correlation coefficient	Significance
DTF (GC)	2	0.779	$P < 0.001$
DTF (Field: Alberta)	5	0.471 to 0.711	$P < 0.001$
DTF (Field: Alberta – ND)	5, 2	-0.118 to 0.509	$P < 0.001$ to $P = 0.78$
DTF – DTM (Field: Alberta)	5	0.503 to 0.664	$P < 0.001$

GC = Growth chamber

5.3.2 Genotypic data

Of the 613 SSR markers from 19 *B. napus* linkage groups tested on the two parents Hi-Q and RIL-144, only 22 (3.6%) were polymorphic. Of the 40 SSR primers designed based on flowering time gene sequence, PCR products of the parents were produced by 26 primers; however, only one showed polymorphism between the parents. This primer pair was developed from a *BnFRI* gene sequence. Sixteen AFLP primer pairs generated a total of 254 polymorphic loci – an average of 16 loci per primer pair. The proportion of markers significantly ($P < 0.01$) deviating from expected 1:1 segregation in the DH population was 79.5%

(202/254) for AFLP markers and 31.8% (7/22) for SSR markers. The marker designed based on *BnFRI* did not deviate significantly from the expected segregation ($\chi^2 = 2.53$, $P = 0.11$). Thus, levels of segregation distortion were very high for AFLP markers used in this study. Fifty-five AFLP markers with severe segregation distortion (χ^2 value greater than 50) were excluded from data analysis.

Single marker analysis was performed for DTF in each of the nine environments and for GLS content in the four environments. Six markers with significant association with DTF were found. One marker, Ol13-G05, showed a strong association with this trait across all GC and Alberta field trials, where the presence of the RIL-144 allele resulted in earliness of flowering. Of the remaining five markers, RIL-144 alleles were associated with earlier flowering in three cases; while in other two cases, the RIL-144 alleles were associated with a delay in flowering.

For seed GLS content, nine markers having significant association with the trait across all four environments were identified. Two of these markers, SSR-129 and SSR-617, showed strong association with seed GLS content in all four environments, where the presence of RIL-144 alleles was associated with higher level of GLS. Interestingly, the remaining seven markers had smaller but significant association with lower GLS content when the RIL-144 alleles were present – despite the fact that this parent had 20 – 30 µmol higher GLS content than the Hi-Q parent under field conditions. The markers with largest effect on the two traits, the effect of presence of alleles from RIL-144, and the level of significance in various environments is summarized in Table 5-5.

Table 5-5. Major marker loci associated with days to flowering (DTF) and seed glucosinolate (GLS) content in the *Brassica napus* doubled haploid population as revealed by single marker analysis under multiple environments

Marker	Effect of RIL-144 allele	Level of significance			
		***P* < 0.05**	***P* < 0.01**	***P* < 0.001**	***P* < 0.0001**
Ol13-G05	Earlier DTF	SC10-SR	SA11-FP	SC11-SR SA11-SR SC11-FP ND11-FP	GC20C GC20-16
BnGMS168	Earlier DTF	GC20C	GC20-16 SC10-SR SC11-SR SA11-SR SC11-FP SA11-FP		
SSR-129	High GLS				SC10-SR SC11-SR SC11-FP SA11-FP
SSR-617	High GLS				SC10-SR SC11-SR SC11-FP SA11-FP

The three SSR markers of RIL-144, and thus of *B. oleracea*, significantly associated with earliness of flowering in *B. napus* were previously mapped to N12 (Ol13-G05; Biotechnology and Biological Science Research Council (BBSRC), UK (http://www.brassica.bbsrc.ac.uk/BrassicaDB)), N13 (BnGMS168; Cheng et al. 2009), and N19 (BRAS050; Celera AgGen Brassica consortium). Two additional SSR markers, SSR-623 and sN3514f, were found to be linked to Ol13-G05. The marker sN3514f was previously mapped to N12 by Agriculture and Agri-Food Canada (AAFC) (Fig. 5-4). This provides evidence that a QTL for earliness of flowering, originating from *B. oleracea*, may be located on N12. The marker developed based on the flowering gene *BnFRI* did not show any association with DTF or seed GLS content.

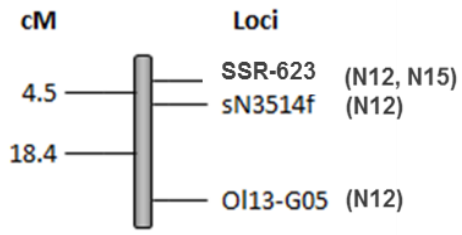

Figure 5-4. Schematic diagram of a genomic region showing the SSR marker Ol13-G05 from the *Brassica napus* chromosome N12 which showed linkage association with a QTL for earliness of flowering in a *Brassica napus* doubled haploid population and an additional SSR marker, which previously mapped to the same chromosome.

The two SSR markers, SSR-129 and SSR-617, showed strong association with GLS content; and these two markers were linked at a distance of 6.2 cM (Fig. 5-5). The marker SSR-129 was previously mapped to N19, while SSR-617 was mapped to N15. An additional marker, sNRG42, previously mapped to N19, was also linked with these two markers. Therefore, it is probable that both of these markers are associated with a QTL for GLS content, and this QTL is likely located on the N19 chromosome of *B. napus*.

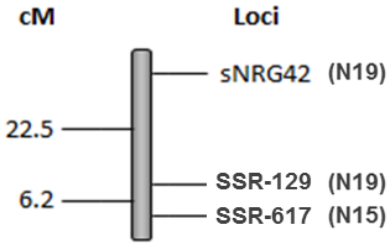

Figure 5-5. Schematic diagram of a genomic region showing the SSR markers SSR-129 and SSR-617 which showed linkage association with a QTL for glucosinolate content in a *Brassica napus* doubled haploid population under four environments, along with one additional SSR marker, which mapped to the chromosome N19.

5.4 Discussion

In this study, we have attempted to identify genomic regions, originating from the C-genome of *B. oleracea*, responsible for earliness of flowering and for high GLS content in *B. napus*. Marker Ol13-G05 is an SSR marker publicly available through the Biotechnology and Biological Sciences Research Council (BBSRC) microsatellite programme. It was developed from *B. oleracea*, and was previously mapped to N12 on *B. napus* (Piquemal et al. 2005). This marker formed a small linkage group with two additional SSR markers, sN3514f and SSR-623, used in this study. The marker sN3514f was previously mapped to the middle region of N12 by AAFC SSR marker consortium. Thus, the allele/QTL responsible for earliness of flowering in the *B. napus* RIL-144, introgressed from *B. oleracea*, seems to originate from N12.

Previous studies have identified significant QTL for DTF on N12. Mei et al. (2009) detected a significant QTL on this linkage group, termed *ft17*, in a winter × winter $F_{2:3}$ population under two environments. This QTL explained 13.1 to 14.6% of the phenotypic variation and was the largest QTL detected on the C-genome. Long et al. (2007) aligned an N12 flowering time QTL *qFT12-1* detected in their study with a region from *A. thaliana* chromosome 5 that encodes the *EARLY FLOWERING 5* (*ELF5*) gene. The QTL *qFT12-1* detected by Long et al. (2007) was the only one on N12 in an extensive study where 42 total flowering time QTLs on 11 *B. napus* linkage groups were detected. It may be that a gene homologous to *ELF5* in *B. oleracea* var. *alboglabra* underlies the variation for earliness of flowering detected in this DH population.

In this study, two SSR markers, SSR-617 and SSR-129, both strongly associated with a QTL for GLS content, were found to be linked at a distance of 6.2 cM. A third marker, sNRG42, was also mapped closely to these markers, and was previously mapped to the *B. napus* linkage group N19. Based on this data, it can be inferred that the GLS QTL is located on N19; and in this case, the present finding correlates well to the QTL mapping of GLS inheritance in *B. napus* done by Basunanda et al. (2007), who found two large-scale QTLs for this trait located on N17 and N19. Howell et al. (2003) also found three QTL for GLS content

common in two *B. napus* winter × winter backcross populations, two of which were located on the C-genome chromosomes N12 and N19.

For single row plots (Fig. 5-3a), two distinct GLS groups are formed – 10 to 26 µmol g^{-1} seed and 28 to 56 µmol g^{-1} seed, consisting of 85 and 98 lines, respectively. Each followed an approximately normal distribution. The number of lines in each group did not deviate significantly from a 1:1 segregation ratio (χ^2 = 0.92, P = 0.34). For full plots (Fig. 5-3b), the groups are likewise distinct – 14 to 26 µmol g^{-1} seed and 28 to 46 µmol g^{-1} seed, consisting of 80 and 94 lines, respectively. This observation also fits well with 1:1 segregation (χ^2 = 1.13, P = 0.29). Thus, it appears that RIL-144 carried one major locus for high GLS content from N19 chromosome of *B. oleracea*, and DH lines form two groups depending on the inheritance of the allele from Hi-Q or from RIL-144. Since GLS is a quantitative trait, minor loci and environmental factors would account for the relatively normal distribution of each group. Distribution of the high GLS group was relatively wider for 1-row plots compared to full plots (Fig. 5-3). It is apparently due to relatively greater environmental influence on the plants in 1-row plots. Strong environmental influence on seed GLS content in *Brassica* crops is well documented (Velasco et al. 2007, Cartea and Velasco 2008).

The level of segregation distortion for SSR marker alleles (31.8%) observed in this DH population of 93 lines was similar to that reported by Foisset et al. (1996) in a DH population of 152 lines from winter × spring *B. napus* cross – 30% for isozyme, 30% for RFLP, and 36.6% for RAPD markers (Foisset et al. 1996). Several researchers also used AFLP markers with relatively few primer combinations for genetic mapping studies in *Brassica* as this marker technique often generates greater amount of genotypic data. However, Lionneton et al. (2002) reported only 18.3% (74/405) AFLP markers showing distorted segregation in a *B. juncea* (L.) Czern. mapping population of 131 DH lines; and in another *B. juncea* population of 123 DH lines, Pradhan et al. (2003) found a similar rate (159/996, 16.0%) of segregation distortion. However, segregation distortion for AFLP marker alleles was much more severe in the present study (79.5%). In a *B. oleracea* mapping population of 107 DH lines, a much higher

rate of segregation distortion (64.1%) was observed for RFLP and AFLP markers by Voorrips et al. (1997), although only 92 markers in total were evaluated in their study. According to Geng et al. (2007), segregation distortion can affect the order of markers on the same linkage group as well as estimation of genetic distances.

The low rate of polymorphism as detected by SSR and key flowering gene markers may indicate that a relatively small proportion of the *B. oleracea* genome had been stably introgressed into the inbred parent RIL-144 used in this study. Also, RIL-144 carries the *B. napus* cytoplasm, as Hi-Q was originally used as female parent. Luan et al. (2009) suggested that the use of near-isogenic lines as parents in QTL studies may be advantageous in that the progeny segregate for small and defined genomic regions where much background noise is eliminated. Because of the high level of homology between the *B. napus* and *B. oleracea* C-genome (Attia and Röbbelen 1986), it is likely that these introgressed regions behave normally in meiosis with the homologous C-genome chromosomes of *B. napus*. Therefore, some of the advantages described by Luan et al. (2009) may have been realized in this study. However, the generation of a conventional genetic map with well-defined linkage groups containing many markers for QTL analysis is precluded by using genetically similar parents.

In their study on the genetic basis of flowering time in *A. thaliana*, Brachi et al. (2010) noted that variation for flowering time under field conditions did not closely correlate with the data obtained under greenhouse conditions. In this study, growth chamber data of the DH lines positively correlated ($r = 0.512$ to 0.660) with field trial data. However, while comparing flowering time data of the two parents from growth chamber, field, and greenhouse trials, the widest difference between the two parents was found under greenhouse conditions. On the other hand, difference between the two parents for seed GLS content was much more pronounced in seed collected from field trials as compared to greenhouse conditions (Table 5-2, Fig. 5-3).

The correlation coefficient for DTF among the two growth chamber and five Alberta field environments was high and significant – which was also

observed in a similar experiment using DH lines in field trials in Manitoba (Chen et al. 2010 supplementary data). However, lower correlation for DTF was found between Alberta and North Dakota field trials. Wet conditions and late seeding in North Dakota in 2011 may have contributed to this difference. Significant correlation between DTF and DTM has previously been reported (Miller 2001, Mahmood et al. 2007), as was also observed in all five environments in this study. Thus, for our material, DTF was a fairly reliable predictor of maturity. The relatively non-significant correlation between DTF and seed oil content observed in this study was also similar to that observed by Chen et al. (2010), except one of their trials, which showed significant correlation between these two traits.

More detailed genetic mapping of the early flowering gene of *B. oleracea* is currently underway. It is hoped that suitable molecular marker(s) can be identified that will allow marker assisted selection for the introgression of this gene(s) into other spring *B. napus* lines. The effect of this introgressed genomic region on plant physiology warrants more detailed study, as does its responsiveness to varying environmental conditions, viz. temperature and/or photoperiod. The findings from this study suggest that further improvement of earliness in spring *B. napus* using allelic variation from the late flowering species *B. oleracea* is possible. The study also provides evidence of the *B. oleracea* chromosomes carrying the early flowering and high glucosinolate alleles which were introgressed from this species into *B. napus*.

5.5 References

Attia T, Röbbelen G (1986) Cytogenetic relationship within cultivated *Brassica* analyzed in amphihaploids from the three diploid ancestors. Can J Genet Cytol 28:323-329

Basunanda P, Spiller TH, Hasan M, Gehringer A, Schondelmaier J, Lühs W, Friedt W, Snowdon RJ (2007) Marker-assisted increase of genetic diversity in a double-low seed quality winter oilseed rape genetic background. Plant Breed 126:581-587

Bjerg B, Eggum BO, Jacobsen I, Otte J, Sørenson H (1989) Antinutritional and toxic effects in rats of individual glucosinolates (± myrosinases) added to a standard diet (2). Zeitschr Tierphysiol, Tierernährung u Futtermittel-kd 61:227-244

Brachi B, Faure N, Horton M, Flahauw E, Vazquez A, Nordborg M, Bergelson J, Cuguen J, Roux F (2010) Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. PLoS Genet 6(5):e1000940

Burge C, Karlin S (1997) Prediction of complete gene structures in human genomic DNA. J Mol Biol 268:78-94

Cai CC, Tu JX, Fu TD, Chen BY (2008) The genetic basis of flowering time and photoperiod sensitivity in rapeseed *Brassica napus* L. Russian J Gen 44:326-333

Cartea ME, Velasco P (2008) Glucosinolates in *Brassica* foods: bioavailability in food and significance for human health. Phytochem Rev 7:213-229

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

Cheng X, Xu J, Xia S, Gu J, Yang Y, Fu J, Qian X, Zhang S, Wu J, Liu K (2009) Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor Appl Genet 118:1121-1131

Chuong PV, Beversdorf WD (1985) High frequency embryogenesis through isolated microspore culture in *Brassica napus* L. and *Brassica carinata* Braun. Plant Sci 39:219-226

Coventry J, Kott L, Beversdorf WD (1988) Manual for microspore culture technique for *Brassica napus*. OAC Publication 0489, University of Guelph, Canada

Ehrenreich IM, Hanzawa Y, Chou L, Roe JL, Kover PX, Purugganan MD (2009) Candidate gene association mapping of Arabidopsis flowering time. Genetics 183:325–335

Ferreira ME, Satagopan J, Yandell BS, Williams RH, Osborn TC (1995) Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theor Appl Genet 90:727-732

Foisset N, Delourme R, Barret P, Hubert N, Landry BS, Renard M (1996) Molecular-mapping analysis in *Brassica napus* using isozyme, RAPD and RFLP markers on a doubled-haploid progeny. Theor Appl Genet 93:1017-1025

Geng JF, Zhu CS, Zhang XW, Cheng Y, Zhang YM, Hou XL (2007) A genetic linkage map of nonheading Chinese cabbage. J Amer Soc Hort Sci 132:816-823

Howell PM, Sharpe AG, Lydiate DJ (2003) Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). Genome 46:454-460

Kebede B, Thiagarajah MR, Zimmerli C, Rahman MH (2010) Improvement of open-pollinated spring rapeseed (*Brassica napus* L.) through introgression of genetic diversity from winter rapeseed. Crop Sci 50:1236-1243

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172-175

Lichter R (1985) From microspores to rape plants: A tentative way to low glucosinolate strains. In: Sorenson H (ed) Advances in the production and utilization of cruciferous crops. Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands, pp 268-277

Lionneton E, Ravera S, Sanchez L, Aubert G, Delourme R, Ochatt S (2002) Development of an AFLP-based linkage map and localization of QTLs for seed fatty acid content in condiment mustard (*Brassica juncea*). Genome 45:1203-1215

Long Y, Shi J, Qiu D, Li R, Zhang C, Wang J, Hou J, Zhao J, Shi L, Park BS, Choi SR, Lim YP, Meng J (2007) Flowering time quantitative trait loci analysis of oilseed Brassica in multiple environments and genomewide alignment with Arabidopsis. Genetics 177:2433-2444

Lorieux M (2007) MapDisto, a free user-friendly program for computing genetic maps. Computer demonstration (P958) given at the Plant and Animal Genome XV Conference, Jan 13-17 2007, San Diego, CA. URL: http://mapdisto.free.fr/

Mahmood T, Rahman MH, Stringam GR, Yeh F, Good AG (2007) Quantitative trait loci for early maturity and their potential in breeding for earliness in *Brassica juncea*. Euphytica 154:101-111

Mei DS, Wang HZ, Hu Q, Li YD, Xu YS, Li YC (2009) QTL analysis on plant height and flowering time in *Brassica napus*. Plant Breed 128:458-465

Miller TA (2001) Agronomic and quality performance of three doubled haploid lines derived from a *Brassica napus/Brassica rapa* interspecific cross. MSc Thesis, University of Alberta, Edmonton

Möllers C, Iqbal MCM, Röbbelen (1994) Efficient production of doubled haploid *Brassica napus* plants by colchicine treatment of microspores. Euphytica 75:95-104

Osborn TC, Kole C, Parkin IAP, Sharpe AG, Kuiper M, Lydiate DJ, Trick M (1997) Comparison of flowering time genes in *Brassica rapa*, *B. napus*, and *Arabidopsis thaliana*. Genetics 146:1123-1129

Parkin IAP, Sharpe AG, Lydiate DJ (2003) Patterns of genome duplication within the *Brassica napus* genome. Genome 46:291-303

Piquemal J, Cinquin E, Couton F, Rondeau C, Seignoret E, Doucet I, Perret D, Villeger M-J, Vincourt P, Blanchard P (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor Appl Genet 111:1514–1523

Pradhan AK, Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS, Pental D (2003) A high-density linkage map in *Brassica juncea* (Indian mustard) using AFLP and RFLP markers. Theor Appl Genet 106:607-614

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

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

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, pp 365-386

Stringam GR, Degenhardt DF, Thiagarajah MR, Bansal VK (2000) Hi-Q summer rape. Can J Plant Sci 80:835–836

Thiagarajah MR, Stringam GR (1993) A comparison of genetic segregation in traditional and microspore-derived populations of *Brassica juncea* L. Czern and Coss. Plant Breed 111:330-334

Toroser D, Thormann CE, Osborn TC, Mithen R (1995) RFLP mapping of quantitative trait loci controlling seed aliphatic-glucosinolate content in oilseed rape (*Brassica napus* L.). Theor Appl Genet 91:802-808

Uzunova M, Ecke W, Weissleder K, Röbbelen G (1995) Mapping the genome of rapeseed (*Brassica napus* L.) I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theor Appl Genet 90:194-204

Velasco P, Cartea ME, González C, Vilar M, Ordás A (2007) Factors affecting the glucosinolate content of kale (*Brassica oleracea* acephala group). J Agric Food Chem 55:955-962

Voorrips RE, Jongerius MC, Kanne HJ (1997) Mapping of two genes for resistance to clubroot (*Plasmodiophora brassicae*) in a population of doubled haploid lines of *Brassica oleracea* by means of RFLP and AFLP markers. Theor Appl Genet 94:75-82

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Homes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucl Acids Res 23:4407-4414

Wang J, Long Y, Wu B, Liu J, Jiang C, Shi L, Zhao J, King GJ, Meng J (2009) The evolution of *Brassica napus FLOWERING LOCUS T* paralogues in the context of inverted chromosomal duplication blocks. BMC Evol Biol 9:271

Wang S, Basten CJ, Zeng Z-B (2011) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Chapter 6

Summary and General Discussion

6.1 Overview

This Ph.D. research investigated the value of *B. oleracea* L. (CC genome; *2n*=18) for enriching diversity in the C-genome in spring type *B. napus* L. (AACC genome; *2n*=38), as well as discovery of alleles for specific traits that can further enrich *B. napus* germplasm. An overview of the populations developed from these initial crosses is represented schematically in Fig. 6-1. These populations were used to study four primary objectives, corresponding to Roman numerals in Fig. 6-1: **i)** study of efficiency at which *B. oleracea* (C-genome) alleles can be introgressed into euploid *B. napus* recombinant inbred lines (RILs), while maintaining the canola quality traits; **ii)** study the feasibility at which the canola quality traits can be introgressed into an euploid *B. oleracea* background; **iii)** investigation of the value of genetic diversity of the C-genome of *B. oleracea* introgressed into a spring type *B. napus* as compared to genetic diversity introgressed from European winter and different spring type canola for heterotic potential in hybrid spring canola; and **iv)** uncovering the genetic basis of early flowering and high glucosinolate content resulting from the introgression of *B. oleracea* C-genome alleles into a *B. napus* background.

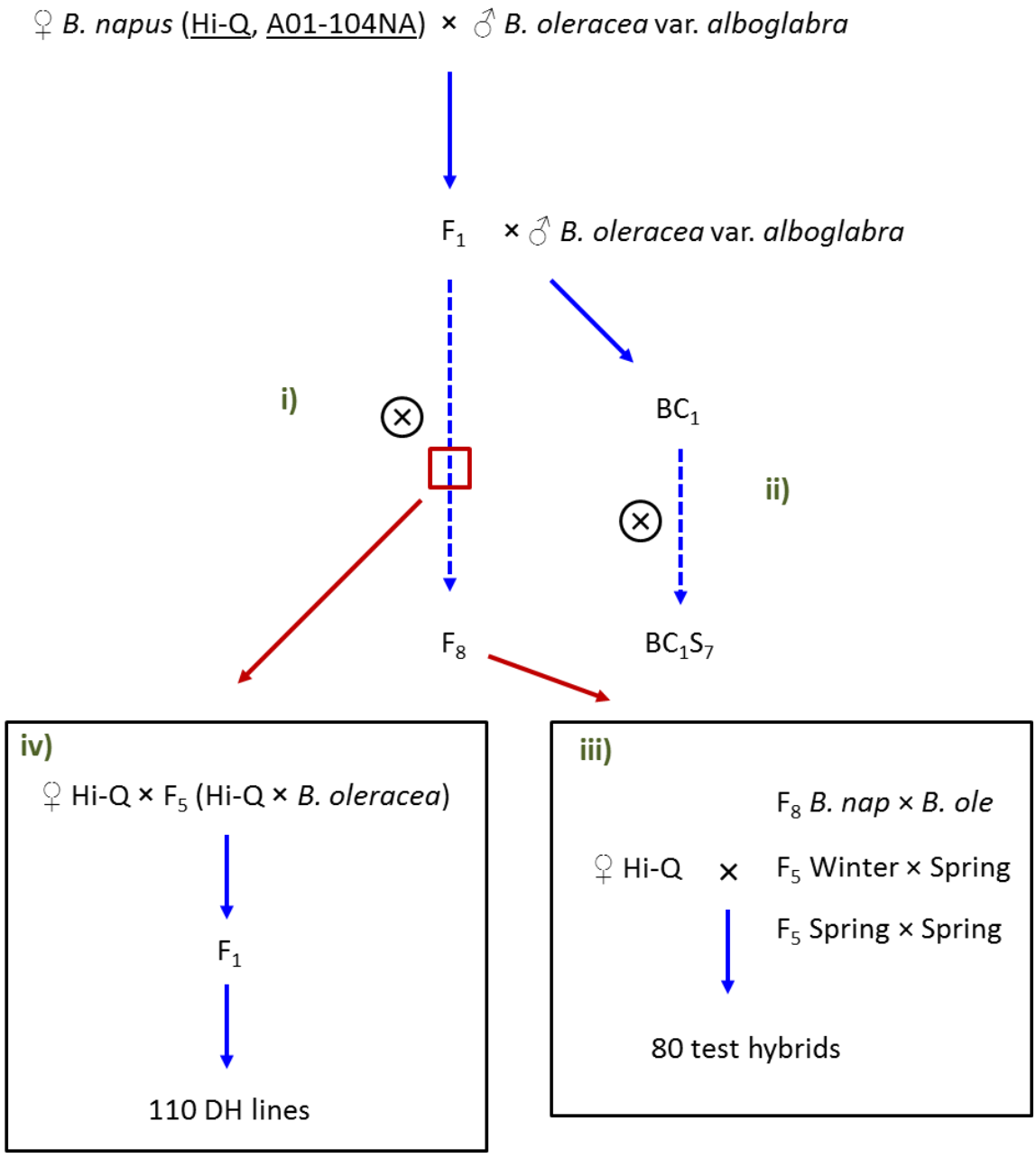

Figure 6-1. Schematic overview of crossing scheme and population development in this Ph.D. thesis, corresponding to study of four primary objectives (represented by Roman numerals).

6.2 Key findings

6.2.1 Recombinant inbred lines

- Zero-erucic, ≤30 μmol g^{-1} glucosinolate genetically diverse lines, with genetic similarity coefficient <0.60 to *B. napus* parent, were achieved in a relatively small segregating population. A mildly diverse sub-group (similarity coefficient ~0.88) consisted of about 75% of lines having seed quality similar to the *B. napus* parent (zero-erucic, ≤15 μmol g^{-1} glucosinolate).

- An approximately 9:1 presence:absence of erucic acid ratio was observed in F_2 *B. napus* × *B. oleracea* seeds, rather than the expected 3:1 based on normal disomic segregation of erucic acid in AC^0C^+ F_1 hybrid plants.

- Aneuploid gametes involved in the formation of F_2 seeds, resulting from meiosis in digenomic triploid ACC F_1 plants, seemed to be more viable and likely to be involved in seed formation when carrying a greater number of A-genome chromosomes.

- Earlier reports of the linkage between petal colour locus and erucic acid locus (Chen et al. 1992, Woods and Séguin-Swartz 1997, Rahman et al. 2001, Bennett 2007), at a distance of approximately 3 – 8 cM, have been substantiated.

- Inbred lines, resulting from several generations of self-pollination of F_2 plants, stabilized to the amphidiploid type. The majority of these lines had comparable fertility to the *B. napus* parents.

6.2.2 Backcross inbred lines

- Selection based on phenotypic traits of interspecific-derived backcross plants was not an effective means of selecting for ploidy level; nor was it a good predictor of family morphology in the subsequent generation.

- No inbred backcross lines stabilized into *B. oleracea* (*2n*=18) types, as was predicted; however, all lines stabilized to *B. napus* (*2n*=38) or near *B. napus* ploidy level.

- Molecular marker analysis and the resulting Principal Coordinates Analysis revealed that the majority of inbred backcross lines had a generous (avg. 35.8%) proportion of *B. oleracea* alleles.

- Unreduced gametes appeared to have played a role in the formation of fertile or semi-fertile (*B. napus* × *B. oleracea*) × *B. oleracea* BC_1 plants; as well as in self-pollinated generations. This agrees with previous reports of the role of unreduced gametes in *Brassica* interspecific crosses (Chiang et al. 1979, Chèvre et al. 1998, Inomata 2002, Nelson et al. 2009).

- Erucic acid content in these interspecific lines seems to be dependent on genotype at the C-genome locus (C^+C^+, C^+C^0, C^0C^0) as well as dosage effect of the zero-erucic allele from *B. napus* A-genome (0 to 2 doses theoretically possible).

- Although glucosinolates exhibited a more complex inheritance than erucic acid, low glucosinolate genotypes were achieved. Given the current evidence that seed glucosinolate in *B. napus* is controlled by 4 to 5 major loci (Rahman et al. 2001), the low glucosinolate types were easily achieved due to simpler segregation of high and low glucosinolate alleles in C-genome only.

6.2.3 Heterotic potential of diverse lines

- Although on a population basis the IN and WS test hybrids did not yield significantly different than the Hi-Q tester parent, a number of individual lines displayed significant mid- and high-parent heterosis.

- A comparison of inbred lines and test hybrids among the IN, WS, and SS populations showed that seed yield, plant height, leaf size, silique length, and seeds per silique were all on average greater in the test hybrid population. Visually, increased early season vigour and vegetative growth at bolting and flowering stage of test hybrid lines compared to their respective inbred parent was also evident.

- Seed quality of the IN test hybrids, on average, was not significantly different than the canola quality tester cultivar Hi-Q.

- No correlation was found between genetic distance of the inbred lines from Hi-Q, based on a set of polymorphic SSR markers, and mid- or high-parent heterosis for seed yield.

- Inbred lines of the IN population resulting in greatest high-parent heterosis had genetic distances of 0.358 to 0.435 from the Hi-Q tester; and in the case of WS population, greatest high-parent heterosis was achieved with a slightly greater genetic distance (0.517 to 0.700).

- An SSR marker, sR10417, previously mapped to the *B. napus* C-genome linkage group N12, was found to be significantly associated with early flowering among the IN test hybrids, when the allele originating from *B. oleracea* was present.

- Single marker analysis identified several markers for seed oil content, where the alleles from *B. oleracea* resulted in a decrease in oil content in IN test hybrids. Three of these markers are from N15; while the other two are from N13 and N18.

- An SSR marker allele of A03-14NI in the SS population was associated with an increase in oil and decrease in protein content. This marker, sS1702, was previously mapped to the *B. napus* A-genome chromosome N8.

6.2.4 Early flowering and glucosinolate content

- An SSR marker, Ol13-G05, previously mapped to the *B. napus* chromosome N12, was found to be most closely associated with the early flowering allele/QTL originating from the C-genome of *B. oleracea*.

- The distribution of the DH lines for total glucosinolate content was bimodal and fitted well to 1:1 segregation. This suggested that RIL-144 carried a major locus for high glucosinolate from *B. oleracea*, and the resultant DH lines fell into one of two groups depending on the inheritance of this allele from RIL-144 or Hi-Q.

- Two SSR markers, SSR-617 and SSR-129, were strongly associated with a QTL for high glucosinolate content originating from the C-genome of *B. oleracea*. Based on linkage association of these markers with a marker from N19, it is presumed that this QTL for high glucosinolate content resides on the *B. napus* chromosome N19.

- A significant and positive correlation for flowering time ($r = 0.512$ to 0.660) was observed between growth chamber and field trial data.

- Seed glucosinolate content was most pronounced among the two parents under field conditions, while widest difference for days to flower was observed in the greenhouse.

- The correlation between days to flower and days to maturity was significant and positive; indicating days to flower was a fairly reliable predictor of maturity.

6.3 General discussion

As outlined in section 1.4.1 of this thesis, there is real value in broadening the genetic base of crop species, including the spring canola cultivars grown on the Canadian prairies. The allied species *B. oleracea* represents a vast reservoir of morphologic and genetic diversity (Babula et al. 2007), but the major obstacle of using this species in the breeding of spring canola *B. napus* has been the undesirable seed quality traits (high erucic acid and glucosinolate content) carried by this species, as well as the difficulty of producing hybrids from *B. napus* × *B. oleracea* interspecific cross. Recently, Bennett et al. (2008) reported that efficient production of this interspecific hybrid can be achieved through adjustment in growth conditions (temperature) of the parental plants and stage of application of embryo rescue technique. Thus, study of the inheritance of the two canola quality traits in several generations of pedigree breeding of this interspecific cross; and assessment of chromosome number and genetic diversity in the resultant lines, as well as heterosis of these lines in test hybrids were all important considerations in this Ph.D. research.

Many reports are available on the inheritance of the two important seed quality traits, erucic acid and glucosinolate content, in several intraspecific as well as some interspecific crosses (Dorrell and Downey 1964, Harvey and Downey 1964, Chen et al. 1988, Fernandez-Escobar 1988, Rahman et al. 1994). This thesis investigated the inheritance of these traits in two populations derived from *B. napus* × *B. oleracea* crosses. Interestingly, while zero-erucic acid seeds were

obtained in self-pollinated generations of BC_1 plants at a proportion consistent with normal disomic segregation of C-genome erucic acid alleles (Bennett et al. 2008), however, the proportion was significantly lower than expected in F_2 seeds in the present study. The knowledge of the additive gene action of erucic acid alleles of the A and C-genomes, coupled with the fact that each genome harbours one erucic acid locus (Harvey and Downey 1964), theoretically would allow inferences to be made regarding transmission of A and C-genome chromosomes carrying the erucic acid allele in early generations. However, in the case of interspecific hybridization where aneuploid gametes are formed at high frequency, and unreduced gametes also contribute significantly to the formation of self-pollinated seed, frequency of genotypes for erucic acid alleles does not occur as expected in the progeny of interspecific hybrids. Contribution of unreduced gametes in *Brassica* interspecific hybrids has previously been reported by other researchers (Inomata 2002, Nelson et al. 2009). It is also evident from the present study, based on segregation of erucic acid alleles, that gametes with higher chromosome number, where a greater number of A-genome chromosomes are included, are more viable in early generations and more likely to produce fertile plants.

Seed GLS content was most reliably assessed for seed obtained from F_4, F_6, and BC_1S_5 field trials. The results obtained from analysis of these generations of inbred lines was further substantiated in the test hybrid study, where the 34 selected F_8 lines gave an average GLS content of 20.5 ± 10.7 µmol g seed^{-1} (mean ± SD). The presence or absence of a high GLS allele in the C-genome was also clearly demonstrated in the present study by developing a DH population from a purposely chosen high GLS, early flowering IN line crossed with the low GLS *B. napus* parent Hi-Q. This allowed for identification of the genomic region carrying a QTL for GLS content in the C-genome of *B. oleracea*. The knowledge gained from the analysis of seed quality traits in the advanced inbred, test hybrid, and DH populations will be valuable for future research and breeding efforts attempting to utilize diversity from *B. oleracea* for improvement of *B. napus*.

These studies also demonstrate that molecular markers may be used in breeding efforts in a variety of useful ways. For example, single marker analysis of the DH population was able to simultaneously elucidate genomic regions from *B. oleracea* containing allele(s) for early flowering (positive trait) and high GLS (negative trait). Likewise, the early flowering allele identified in the DH population was also identified in the test hybrid population. Genotyping of the F_8 population for markers, polymorphic between the *B. napus* and *B. oleracea* parents, was used not only for estimation of the level of diversity included in these lines, but also to identify genetic distance from *B. napus* of those lines that displayed high levels of heterosis. The value of using molecular markers in genetic diversity studies was underscored by the fact that phenotypic selection did not always reflect genotypic content, as described in Chapter 3.

Taken together, these studies give greater insight into both the challenges and opportunities encountered by using the *B. oleracea* C-genome to improve spring *B. napus*. The stabilization of both inbred populations into amphidiploid type, including the first-backcross derived population, indicated that intensive efforts in early generations are required in order to develop canola quality *B. oleracea* type via interspecific cross. Seed yield of inbred lines per se were generally low, but some individual lines showed good potential, and certainly in test crosses with Hi-Q where many test hybrids displayed both good yield potential and seed quality characteristics. Therefore, these diversified *B. napus* types can be used for further improvement of existing spring *B. napus* cultivars. It is feasible to be able to combine the identified early flowering allele of *B. oleracea* with seed quality alleles from *B. napus*, in a line that will give good heterotic potential.

6.4 Future research

One of the important objectives of this research was to generate canola quality *B. oleracea* type (*2n*=18) plants for the resynthesis of genetically diverse and canola quality *B. napus* lines, and the evaluation of the utility of these lines for

improving spring type *B. napus* cultivars grown in Canada. To achieve this, it seems necessary that cytological evaluation is done on earlier generation (e.g. BC_1, BC_1S_1) plants to obtain self-pollinated seeds from the plants having ~9 bivalents in diakinesis stage of prophase I or ~18 chromosomes in mitotic cell (root tip) preparations. It would also be interesting to evaluate the frequency of dyad vs. tetrad formation from anther preparations in the interspecific progenies compared to the parents, as was done by Nelson et al. (2009). This would give indication as to the rate of unreduced gamete formation.

Due to greenhouse space and amount of time required for production of test hybrid seeds by hand emasculation and pollination, inclusion of additional tester parents in the test hybrid study was not feasible. However, it would be beneficial to evaluate heterotic potential of the interspecific cross-derived inbred lines with other *B. napus* testers, genetically distinct from Hi-Q, and evaluate combining ability. Constraints on amount of available test hybrid seed (all generated from manual cross-pollination) limited trials to half sized plots, and only two replications at two sites in a year were possible. However, it would be beneficial to evaluate this material in full plot trials, with at least three replications at each site, to determine whether the heterotic potential would remain similar to that observed in these experiments. Further, molecular mapping of heterotic QTL by construction of a linkage map and performing composite interval mapping analysis would give valuable information on *B. oleracea* genomic regions and alleles involved in heterotic response of test hybrids. In studies involving multiple testers, the construction of a map would also be of benefit with regards to mapping loci involved in specific and general combining ability.

The construction of a molecular linkage map using the DH population developed in this study would be of value for mapping of flowering time QTL, as well as other QTL affecting the physiology of flowering. This would allow more precision in determining the position on chromosome N12 of the early flowering allele of *B. oleracea*, as well as the location on N19 of the *B. oleracea* high GLS allele. The identification of putative flowering time genes in the identified region can also more readily be done as the genome sequence of *Brassica* becomes

available in the next few years. The availability of this information would allow for a sequence based approach, where sequences from coding regions of key flowering time genes from *Arabidopsis* or other close relatives could be used to search for close matches in the QTL region of interest.

The economic importance of *Brassica napus* canola / oilseed rape worldwide, and particularly in Canada (Mark Goodwin Consulting Ltd. 2008), justifies the need for research efforts to provide tools and means for continued improvement of this crop. This Ph.D. research project suggested that it is feasible to introgress genetic diversity from the C-genome of *B. oleracea* into a canola quality and euploid *B. napus* background; and this diversity has great potential for improving yields of hybrid canola, as well as introducing alleles for improvement of specific traits such as earliness of flowering. It is hoped that both the findings and material developed from this Ph.D. research will be beneficial for future canola breeding efforts, and utilized for the good of the canola community both in Canada and worldwide.

6.5 References

Babula D, Kaczmarek M, Ziółkowski PA, Sadowski J (2007) *Brassica oleracea*. In: Kole C (ed) Genome Mapping and Molecular Breeding in Plants, volume 5. Springer-Verlag, Berlin/Heidelberg, Germany, pp 227-285

Bennett RA (2007) Interspecific cross of *Brassica napus* x *B. oleracea* var. *alboglabra*: the effect of growth condition and silique age on hybrid production, and the inheritance of erucic acid in the self-pollinated backcross generation. MSc Thesis, University of Alberta, Edmonton

Bennett RA, Thiagarajah MR, King JR, Rahman MH (2008) Interspecific cross of *Brassica oleracea* var. *alboglabra* and *B. napus*: Effects of growth condition and silique age on the efficiency of hybrid production, and inheritance of erucic acid in the self-pollinated backcross generation. Euphytica 164:593-601

Chen BY, Heneen WK, Jönsson R (1988) Resynthesis of *Brassica napus* L. through interspecific hybridization between *B. alboglabra* Bailey and *B. campestris* L. with special emphasis on seed colour. Plant Breed 101:52-59

Chen BY, Simonsen V, Lannér-Herrera C, Heneen WK (1992) A *Brassica campestris-alboglabra* addition line and its use for gene mapping, intergenomic gene transfer and generation of trisomics. Theor Appl Genet 84:592-599

Chèvre AM, Eber F, Baranger A, Hureau G, Barret P, Picault H, Renard M (1998) Characterization of backcross generations obtained under field conditions from oilseed rape-wild radish F_1 interspecific hybrids: an assessment of transgene dispersal. Theor Appl Genet 97:90-98

Chiang MS, Chiang BY, Grant WF (1979) Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* ssp. *capitata*). III. First backcross and F_2 progenies from interspecific hybrids between *B. napus* and *B. oleracea* ssp. *capitata*. Euphytica 28:257-266

Dorrell DG, Downey RK (1964) The inheritance of erucic acid in rapeseed (*Brassica campestris*). Can J Plant Sci 44:499-504

Fernandez-Escobar J, Dominguez J, Martin A, Fernandez-Martinez JM (1988) Genetics of the erucic acid content in interspecific hybrids of Ethiopian mustard (*Brassica carinata* Braun) and rapeseed (*B. napus* L.). Plant Breed 100:310-315

Mark Goodwin Consulting Ltd (2008) Canola Socio-Economic Value Report.

Nelson MN, Mason AS, Castello M-C, Thomson L, Yan G, Cowling WA (2009) Microspore culture preferentially selects unreduced (2n) gametes from an interspecific hybrid of *Brassica napus* L. × *Brassica carinata* Braun. Theor Appl Genet 119:497-505

Harvey BL, Downey RK (1964) The inheritance of erucic acid content in rapeseed (*Brassica napus*). Can J Plant Sci 44:104-111

Inomata N (2002) A cytogenetic study of the progenies of hybrids between *Brassica napus* and *Brassica oleracea*, *Brassica bourgeaui*, *Brassica cretica*, and *Brassica montana*. Plant Breed 121:174-176

Rahman MH, Joersbo M, Poulsen MH (2001) Development of yellow-seeded *Brassica napus* of double low quality. Plant Breed 120:473-478

Rahman MH, Rahman L, Stølen O, Sørensen H (1994) Inheritance of erucic acid content in yellow- and white-flowered yellow sarson x Canadian *Brassica campestris* L. Acta Agric Scand 44:94-97

Woods DL, and Séguin-Swartz G (1997) An investigation on linkage between white flower colour and erucic acid in summer rape. Can J Plant Sci 78:109-111

Appendix

Supplementary tables from Chapter 3

Table A-1. Percent viable pollen in 10 BC_1 plants of (*B. napus* × *B. oleracea*) × *B. oleracea* and their selected BC_1S_3 and BC_1S_4 generation populations

BC_1 plant[z]	Plant type[y]	Mean	BC_1S_3 family	Plant type	No. plants	Range, Mean	BC_1S_4 family	Type	No. plants	Range, Mean
8-001 p5	Int	46.5								
8-001 p6	Ole	38.7	8-092	Int	3	76 – 96, 89.3	8-138	Nap	3	74 – 84, 79.6
			8-093	Int	3	94 – 98, 96.5	8-141	Nap	3	80 – 87, 84.3
			8-097	Nap	3	83 – 98, 92.3	8-152	Nap	3	73 – 82, 78.0
			8-098	Nap	3	80 – 92, 86.9	8-153	Nap	3	78 – 86, 81.0
							8-107	Ole	3	32 – 45, 38.3
							8-118	Int	3	75 – 79, 77.0
8-001 p8	Int	47.7								
8-002 p3	Ole	10.3								
8-002 p4	Int	27.8								
8-002 p8	Int	46.4	8-100	Ole	3	42 – 84, 56.9				
8-002 p9	Nap	61.9								
8-002 p12	Int	91.0								
9-002 p6	Nap	38.7	9-085	Ole	3	3 – 70, 47.0				
9-003 p6	Nap	44.0								
A01-104NA	-					79 – 84, 81.7				
Hi-Q	-					97 – 97, 96.9				
B. oleracea	-					74 – 84, 77.0				

[z] 8-001 and 8-002: Population I: (A01-104NA × *B. oleracea*) × *B. oleracea*; 9-002 and 9-003: Population II: (Hi-Q × *B. oleracea*) × *B. oleracea*; descendants of these plants in same row

[y] Ole = *B. oleracea* type, Int = intermediate type, Nap = *B. napus* type

Table A-2. Seed set based on three morphological groupings in two (*B. napus* × *B. oleracea*) × *B. oleracea* populations

Pop'n[z]	Generation	No. plants	Seeds per plant (g)[y]			Seeds per silique		
			Nap[x]	Int	Ole	Nap	Int	Ole
I	BC_1S_3	128	1.53, 0.01 – 3.70	0.44, 0.00 – 1.60	0.72, 0.00 – 3.91	10.6, 0.1 – 18.1	4.6, 0.0 – 14.3	4.1, 0.0 – 19.7
	BC_1S_4	373	0.33, 0.00 – 1.45	0.34, 0.00 – 1.52	0.30, 0.00 – 1.84	4.7, 0.0 – 20.3	5.6, 0.0 – 16.2	5.0, 0.0 – 16.7
	BC_1S_5	199	0.10, 0.01 – 0.27	0.56, 0.00 – 9.40	0.66, 0.00 – 4.42	2.9, 0.7 – 8.5	4.6, 0.0 – 26.1	6.4, 0.0 – 23.0
	A01-104NA	33	1.95, 0.06 – 9.16	-	-	15.5, 1.8 – 27.6	-	-
	B. oleracea	27	-	-	1.21, 0.01 – 5.50	-	-	9.3, 0.0 – 24.3
II	BC_1S_3	41	-	0.03, 0.00 – 0.08	0.14, 0.00 – 1.08	-	0.3, 0.0 – 1.0	1.8, 0.0 – 10.4
	BC_1S_4	48	0.02, 0.00 – 0.13	0.21, 0.00 – 0.69	0.08, 0.00 – 0.31	0.4, 0.0 – 2.0	3.8, 0.0 – 12.1	1.3, 0.0 – 4.7
	BC_1S_5	23	-	0.09, 0.00 – 0.43	0.04, 0.01 – 0.14	-	0.7, 0.0 – 5.3	1.1, 0.1 – 2.1
	Hi-Q	32	1.99, 0.08 – 6.81	-	-	15.6, 2.8 – 24.1	-	-
	B. oleracea	27	-	-	1.21, 0.01 – 5.50	-	-	9.3, 0.0 – 24.3

[z] Population I: (A01-104NA × *B. oleracea*) × *B. oleracea*; Population II: (Hi-Q × *B. oleracea*) × *B. oleracea*

[y] Mean, Range

[x] Ole = *B. oleracea* type, Int = intermediate type, Nap = *B. napus* type