Broadening of genetic diversity in spring canola (*Brassica napus* L.) by use of the C-genome of *Brassica oleracea* var. *alboglabra* and *B. oleracea* var. *botrytis*.

by

Rameez Iftikhar

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

Master of Science

in

Plant Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

.

Abstract

Spring oilseed *Brassica napus* L. (AACC, 2*n* = 38) canola is one of the most important crop in Canada, widely grown in the Prairie Provinces Alberta, Manitoba and Saskatchewan. Presence of genetic diversity in breeding material is pre-requisite for developing new cultivars with desirable traits as well as for progress in breeding. The narrow genetic diversity in spring *B. napus* canola can be broadened by enriching its C-genome with the C-genome of progenitor species *Brassica oleracea* L. The present research was undertaken to study the feasibility of introgressing allelic diversity from *B. oleracea* var. *alboglabra* and *B. oleracea* var. *botrytis* into Canadian spring *B. napus* canola for the improvement of this crop. For this, *Brassica napus* × *B. oleracea* interspecific crosses were made and the F_1's were either self-pollinated for F_2 or backcrossed to the *B. napus* parent for BC_1 seeds. The F_2- and BC_1-derived populations were subjected to self-pollination with selection in each generation for different agronomic and seed quality traits including erucic acid and glucosinolate contents from where F_8 and BC_1F_7 families were developed. The interspecific cross derived plants were analysed by a flow cytometer to estimate their approximate chromosome number; while the extent of genetic diversity introgressed from *B. oleracea* into these plants was assessed by the use of simple sequence repeat (SSR) markers.

Plant fertility was low in early generation populations. However, inbreeding with selection for fertile plants resulted in *B. napus* plants in advanced generation populations. Silique size and number of seeds per silique in many of the advanced generation plants was comparable to the *B. napus* parent.

Segregation for erucic acid and glucosinolate contents in the populations derived from this interspecific cross involved only the C-genome alleles; this enabled efficient selection of canola quality plants from both F_2- and BC_1-derived populations. Molecular marker analysis showed that the plants derived from both F_2 and BC_1 are genetically distinct from the *B. napus* parent; this demonstrated the feasibility of introgressing allelic diversity from *B. oleracea* var. *alboglabra* and *B. oleracea* var. *botrytis* into spring *B. napus* canola.

Acknowledgements

All the praises and thanks to Allah Tallah who bestowed me with the potential and ability to contribute a little towards the existing scientific knowledge.

I am heartedly thankful to Dr. Habibur Rahman for giving me opportunity to work with the Canola Breeding Program of University of Alberta under his supervision. He has been a great supervisor as his continuous guidance helped me in polishing my plant breeding skills that led me in the completion of research project. I am also thankful to Dr. Rong-Cai Yang, my committee member, for his valuable suggestions and technical assistance during the course of research project.

I would like to say thanks and acknowledgement to dear Berisso Kebede for his continuous technical help as well as An Vo, Neil Hobson, Rudolph Fredua-Agyeman, Md. Zahidur Rahman, J. Salvador Lopez, and other colleagues of Canola Breeding Program for their support.

In the end, I want to acknowledge my beloved parents and siblings for their prayers and continuous support during my MSc. degree.

Table of Contents

List of Tables

List of Figures

List of Symbols and Abbreviations

±	Plus/minus
µl	Microliter
µmol/g seed	Micromoles per gram per seed
ng μL^{-1}	Nanogram per microliter
χ^2	Chi-square test statistics
2*n*	Diploid number of chromosomes
n	Number of observation
A^e	Zero erucic acid allele from *B. napus* A-genome
A^E	High erucic acid allele from *B. napus* A-genome
AAFC	Agriculture and Agri-Food Canada
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
BC_1	First backcross generation
BC_1F_1	First filial generation after first backcross
C^e	Zero erucic acid allele from *B. napus* C-genome
C^E	High erucic acid allele from *B. oleracea* C-genome
°C	Degrees Celsius
cm	Centimeter
cv.	Cultivar
df	Degree of freedom
DH	Doubled haploid
DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleotide triphosphate
F_1	First filial generation
FAO	Food and Agriculture Organization of the United Nations
Fig.	Figure
g	Gram
GSL	Glucosinolate

m	Meter
mM	Millimole
mm	Millimeter
min	Minute
MS	Mean squares
n	Haploid number of chromosomes
NIRS	Near infra-red spectroscopy
P-value	Probability value
PCR	Polymerase chain reaction
PCoA	Principal coordinate analysis
QTL	Quantitative trait loci
RFLP	Restriction fragment length polymorphism
SD	Standard Deviation
SSR	Simple sequence repeat
SS	Sum of squares
t-test	Test statistic for t-test
Taq polymerase	*Thermus aquaticus* polymerase
USDA	United States Department of Agriculture
var.	Variety

Chapter 1

Literature Review

1.1 Introduction

Brassica species of primary importance include three amphidiploids, *B. carinata* A. Br., *B. juncea* (L.) Czern. & Cross. and *B. napus* L., and three diploids, *B. nigra* (L.) Koch, *B. oleracea* L. and *B. rapa* L. (Rich 1991, Dixon 2007). *Brassica napus*, *B. juncea* and *B. rapa* are grown as oilseed crop. Other uses of different *Brassica* species include as vegetable, condiment, biodiesel, industrial lubricants and animal forage; thus, making the species of Brassicaceae family as one of the top ten most economically important plant families (Rich 1991 cited by Kasem et al. 2011). Among the different *Brassica* oilseed crop species, *B. napus* is the most extensively cultivated one. Two major forms of *B. napus* exists, the spring and winter types. The spring type is primarily grown in Australia, Northern Europe and Canada, while the winter type, which requires vernalization for flowering, is mainly grown in central Europe, Ukraine and Russia. Another is semi-winter type, which requires short duration of vernalization, primarily grown in China.

In Canada, *B. napus* is the predominant *Brassica* oilseed crop; while other species such as *B. rapa* and *B. juncea* grown on a small scale. These *Brassica* oilseed crops are grown mainly in the Prairie Provinces Alberta, Manitoba and Saskatchewan and limited acreage in British Columbia and Ontario. *Brassica* oilseed crop was first introduced in Canada as an industrial oil crop in early 1940's. Such oil of traditional *Brassica* oilseed crop contains more than 40% long-chain erucic fatty acid (C22:1). Later, intensive breeding efforts on this crop led to the development of edible oil types (zero or low erucic acid in oil) in 1960's and the first 'double low' or 'canola type' *B. napus* cultivar Tower, was released for commercial cultivation in 1974

(http://www.canolainfo.org). The term canola, abbreviation of Canadian Oil Low Acid, was coined and trademarked to describe the cultivars of *B. napus, B. juncea* and *B. rapa* which seed oil contain less than 2% erucic acid and the seed meal, remaining after oil extraction, contain less than 30 μmol of total aliphatic glucosinolates per gram dry matter (http://www.canolacouncil.org). The term canola distinguishes this improved type from the conventional non-canola type or rapeseed cultivars, as canola oil is very different from high erucic acid rapeseed oil in chemical, physical and nutritional properties. All edible oils are composed of primarily lipid compound triglycerides, which are the ester of one molecule of glycerol and three molecules of fatty acids. In canola oil, triglycerides constitute about 94.4 to 99.1% of the total lipid (Przybylski and Mag 2002).

Soon after the development of canola quality cultivars, this oilseed crop gained importance worldwide and now has become one of the major sources of edible oil after soybean and palm oil at global level (Fig.1.1). Today, canola oil is regarded as one of the healthiest vegetable oil in the world due to its balanced fatty acid composition (2:1 ratio of monounsaturated to polyunsaturated fatty acids). Canola oil contains, on an average, about 60% oleic acid (C18:1), 20% linoleic acid (C18:2) and 10% α-linolenic acid (C18:3) and has the lowest content of saturated fatty acid when compared with other commercially available dietary fats. Canola meal (remainder of canola seed after oil extraction) is second largest protein source after soybean meal for feeding animals, primarily for cattle and pigs (USDA report 2012).

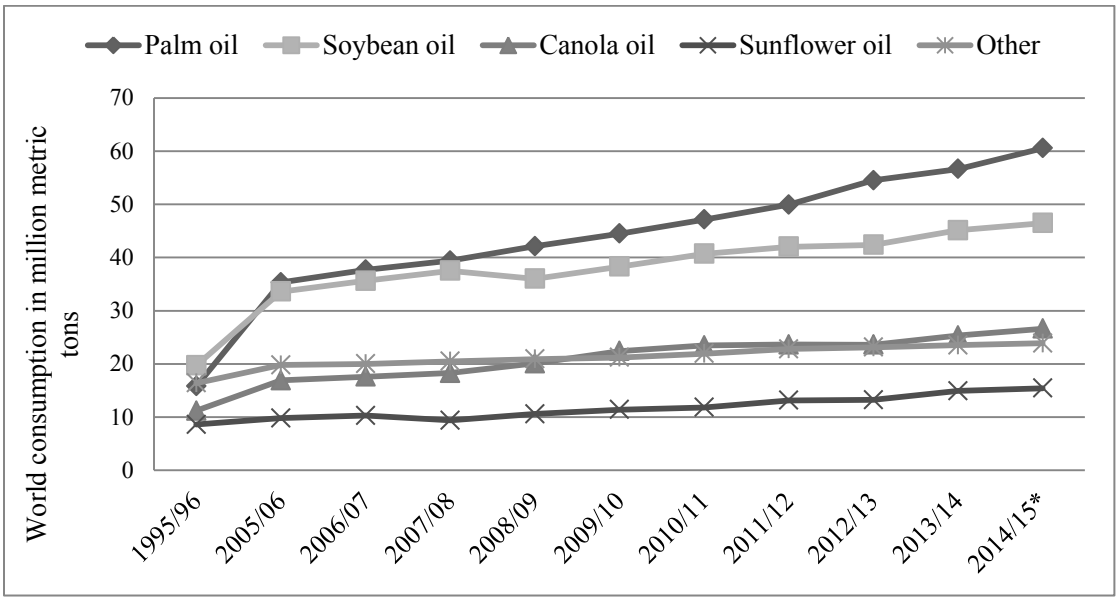


Fig. 1.1 Major vegetable oil production worldwide (USDA, March 2014). *Estimate as of November 2014.

Due to its premium quality oil, a continuous growing trend in cultivation of *Brassica* oilseed crops is evident in different parts of the world, such as Canada, China, India, Germany and Australia, during the past few decades (Fig. 1.2). In 2011-12, canola production in the world approached to 61.4 million tons where Canada was the top producer with a production of 14.6

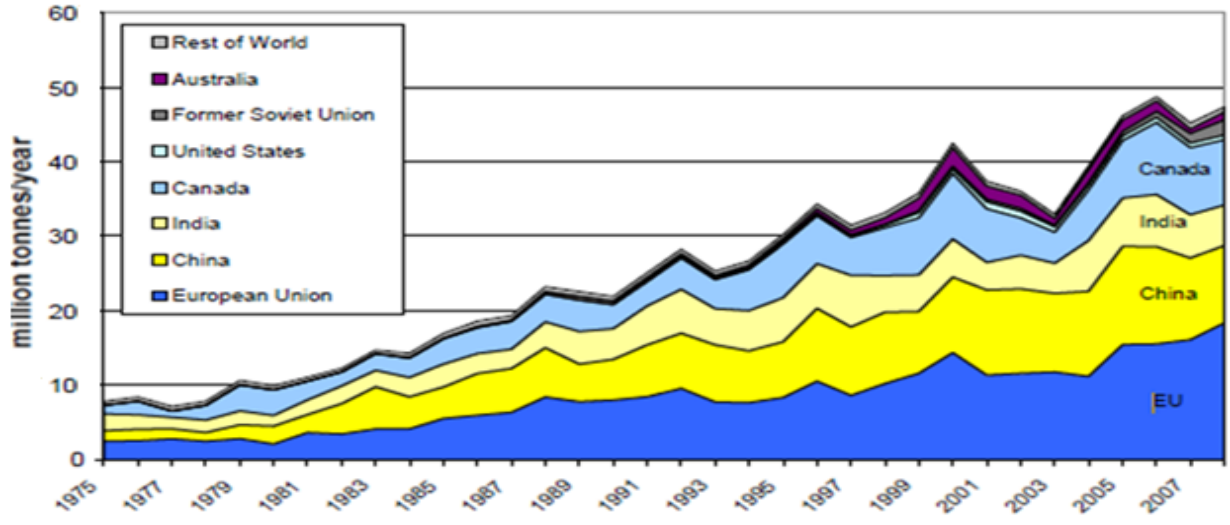


Fig. 1.2 World production of canola/rapeseed in 1975 to 2007 (Rosillo-Calle et al. 2009).

million tons followed by China 13.4 and India 6.4 million tons (FAO, 2013).

In Canada, spring canola is the second most economically important food crop after wheat. In 2011-12, canola contributed over $21 billion to the Canadian economy when its direct benefits, and indirect benefits and impacts such as crushing, port activities, transportation, jobs and wages are taken into account (Fig. 1.3).

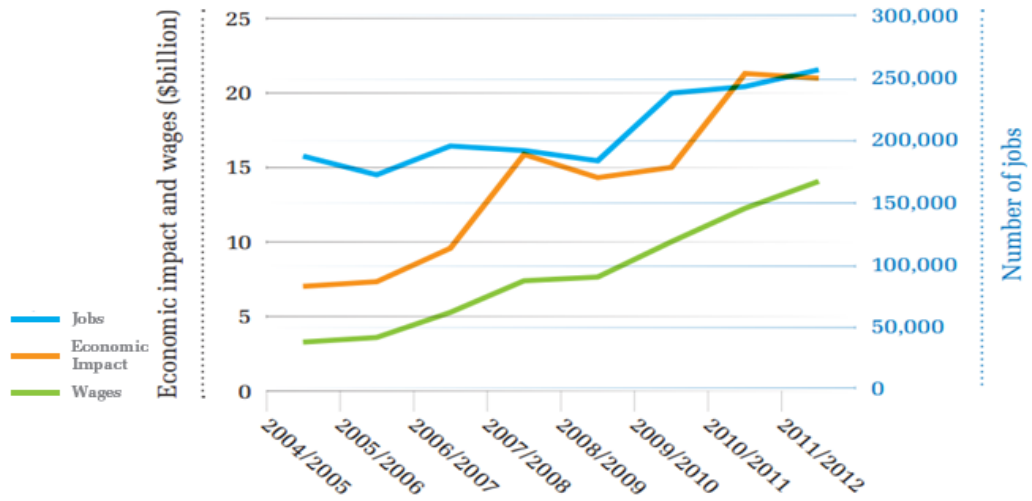


Fig. 1.3 Contribution of canola to Canadian economy (Canola Council of Canada, retrived on 10th Jan 2014).

In ancient times, *Brassica* oil crops have been cultivated in Asia for use its oil in cooking. Cultivation of this oil crop in Canada started during World War II and the first registered high erucic acid, high glucosinolate cultivar, 'Golden' was released in 1954 (http://www.canolainfo.org). However, this crop was not accepted in the market due to high content of erucic acid in oil. High level of erucic acid in oil is considered unhealthy for human consumption as its cause heart and skeletal muscle diseases; and high content of glucosinolates in seed meal is considered detrimental to animals causing retarded growth and liver abnormalities (EFSA report 2008). These two non-canola quality traits in early cultivars were the major constrain of cultivation of this crop and that remain until 1970's. Extensive breeding

research in 1950-1960's at different Canadian institutes led the development of canola quality cultivars. The gene for low erucic acid was found in *B. napus* forage cultivar 'Liho' in late 1950's (cited by Cao et al. 2010) and was transferred into *B. napus* cultivar 'Oro' in 1968 through backcross breeding (http://www.canolacouncil.org). However, high content of glucosinolates in meal remain the major hurdle for utilization of seed meal that was overcome when the Polish fodder rape cultivar 'Bronowski' was found to carry genes for low-glucosinolate contents in 1970's (Kondra and Steffonson 1970, Finlayson et al. 1973). By using 'Bronowski' as source of genes for low glucosinolate content, two plant breeders, Baldur Stefansson at the University of Manitoba and Keith Downey at the Canada Agriculture Research Station in Saskatoon changed the Canadian agriculture by developing the first low erucic acid, low glucosinolate *B. napus* cultivar 'Tower' in 1974 and *B. rapa* cultivar 'Candle' in 1977 respectively (Stefansson 1983) placing Canada at the forefront position in *Brassica* oilseed crop breeding worldwide; and the development of canola is described as a Canadian success story (http://www.canolainfo.org).

1.2 Erucic acid

Rapeseed oil containing high level of erucic acid (*cis*-1,3-docosenoic acid, C22:1) is considered to exert adverse effects on animals. High content of this fatty acid in diet may cause cardiac lesions by disrupting oxidation of other fatty acids (Charlton et al. 1975, Christophersen and Bremer 1972). Therefore, reduction in the level of this fatty acid in edible oil is desired. For this, better understanding of fatty acid biosynthesis and its genetic control is important. According to Harvey and Downey (1964), erucic acid biosynthesis in rapeseed (*B. napus*) is governed by two genes loci with additive effect of the genes. This two gene model of erucic acid biosynthesis has been confirmed by various researchers (Chen and Heneen 1989, Siebel and

Pauls 1989, Ecke et al. 1995, Coonrod et al. 2008, Pandey et al. 2013). Thorman et al. (1996) mapped these two loci on two linkage groups of their *B. napus* linkage map. Zhang et al. (2008) constructed a genetic linkage map covering all 19 linkage groups of *B. napus* by using a BC_1F_1 generation and identified two erucic acid loci on the A8 and C3 chromosomes. Similarly, Mahmood et al. (2003) reported two loci in *B. juncea* controlling erucic acid concentration in seed oil.

1.3 Glucosinolates

Glucosinolates are sulphur-rich secondary plant metabolites that occur naturally in economically important members of the family Brassicaceae (reviewed in Sønderby et al. 2010, Rahman et al. 2014). Through the use of the model plant *Arabidopsis,* researchers were able to understand the biosynthesis of glucosinolates (reviewed in Halkier and Du 1997) and genetic control of this trait in *Brassica* (Hasan et al. 2008, Bisht et al. 2009, Feng et al. 2012, Rahman et al. 2014). Based on origin of amino acid side chain, glucosinolates are grouped into three types: aliphatic having methionine-derived chain, aromatic with phenylalanine-derived chain and indole glucosinolates having chain from tryptophan. According to Velasco et al. (2008), aliphatic glucosinolates are predominant type of glucosinolates both in seed and leaf of *B. napus* accounting more than 90% of the total glucosinolates. Feng et al. (2012) reported 105 metabolite quantitative trait loci (mQTL) that influence glucosinolate production in either or both seed and leaf. More than 60% of these mQTL and epistatic loci were assigned to the A genome linkage groups. Rahnman et al. (2014) detected three QTL at the linkage groups A2, A7 and A9 controlling total seed glucosinolate content in *B. rapa*.

Biosynthesis of glucosinolates occurs through three stages: side chain elongation of precursor amino acids, formation of the core glucosinolates structure, and modifications of the amino acid side chain that is responsible for different glucosinolates in seed and leaf (reviewed in Halkier and Du 1997, Velasco et al. 2008, Feng et al. 2012). During crushing of seeds, glucosinolates may produce different type of products such as nitriles, thiocyanates, and isothiocyanates through hydrolysis by the enzyme myrosinase (reviewed in Sønderby et al. 2010, Feng et al. 2012). These breakdown products of glucosinolates are anti-nutritional to animals.

1.4 *Brassica* species and genome relationship

Over the past few decades, extensive molecular marker and genomic studies have been conducted by different researchers to understand the evolution of different species of the family Brassicaceae and their phylogenic relationship. However, most of the work has been centred on *B. napus* due to its enormous economic importance as oilseed crop in the world. *Brassica napus* is an amphidiploid species, contains the complete set of chromosomes of its diploid progenitor species *B. rapa* and *B. oleracea* (U 1935). The genome of these two diploid species show high chromosomal homoeology (Parkin et al. 1995, Cheung et al. 2009). This suggests that the A-genome of *B. rapa* and the C-genome of *B. oleracea* might have evolved from a common hexaploid ancestor, similar to *Arabidopsis thaliana* (Lagercrantz et al. 1996, Truco et al. 1996, Lysak et al. 2005, Parkin et al. 2005), through chromosomal fission, fusions and rearrangements to give rise the chromosome number variation of n = 8, 9 and 10 that we observe today in *B. nigra*, *B. oleracea* and *B. rapa*, respectively (Fig. 1.4). These genomes (A, B and C genomes) diversified from each other (Lysak et al. 2005, Cheng et al. 2013) over the period of time and different phylogenetic studies suggested that this divergence led to two distinct evolutionary lineages: *Nigra* lineage and *Rapa/Oleracea* lineage (Warwick and Black, 1991, Navabi et al.

2013). According to Warwick and Black (1991), *B. nigra* (*n* = 8) and its close relatives such as *Sinapsis alba* (*n* = 12) and *Sinapasis arvensis* (*n* = 9) are included in *Nigra* lineage, while *B. rapa* (*n* = 10) and *B. oleracea* (*n* = 9) and their close relatives including *B. oleracea* complex such as *B-rupestris-villosa* complex (*n* = 9) included in the *Rapa/Oleracea* lineage.

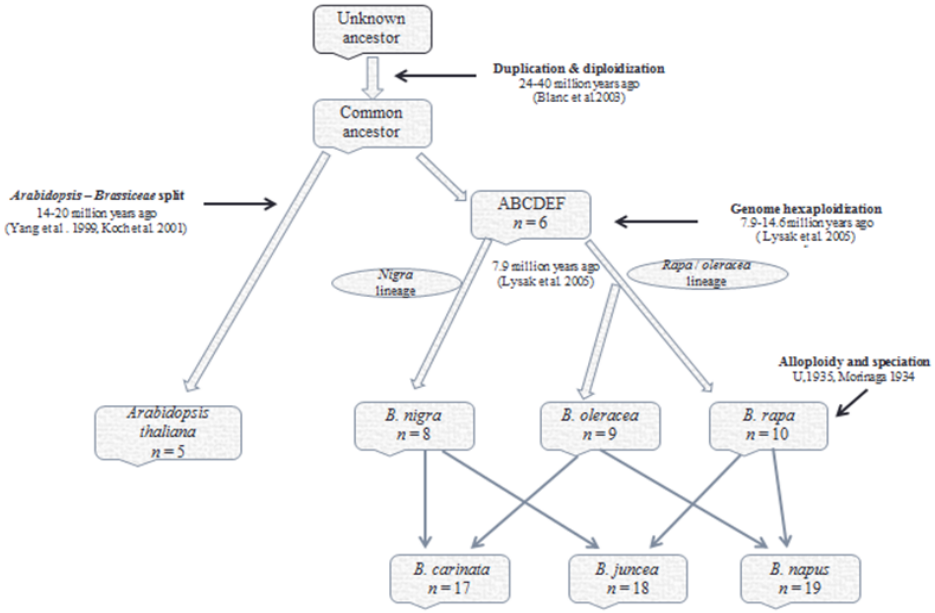


Fig. 1.4 Schematic representation of evolution of *Arabidopsis* and different *Brassica* genomes from a common ancestor over the period of time (Prakash et al. 2012).

U (1935) described the relationship between the six *Brassica* species in the form of a triangle (Fig. 1.5) based on their cross ability and chromosome pairing or fertility in the interspecific hybrids. Based on cytogenetic studies, Morinaga (1934) and U (1935) designated three diploid genomes as "A" for *B. rapa* (2*n* = 20), "B" for *B. nigra* (2*n* = 16) and "C" for *B. oleracea* (2*n* = 18) and suggested that the amphidiploid species *B. napus* (AACC, 2*n* = 38) resulted from cross between *B. rapa* (2*n* = 20) and *B. oleracea* (2*n* = 18), *B. juncea* (AABB, 2*n* = 36) from *B. rapa* (2*n* = 20) and *B. nigra* (2*n* =16) while, *B. carinata* (BBCC, 2*n* = 34) evolved from *B. nigra* (2*n* = 16) and *B. oleracea* (2*n* = 18) cross. Later, various genetic studies, such as,

resynthesis of the amphidiploids from the diploid species (Olsson 1960, Rahman 2001, Li et al. 2004), traditional and molecular cytogenetic analysis of the diploid and amphidiploid species (Palmer et al. 1983, Snowdon et al. 2002, Mason et al. 2010), and molecular marker analysis (Song and Osborn 1992, Parkin et al. 2005, Navabi et al. 2013) have supported this hypothesis as well as extended our knowledge of the relationships between different *Brassica* genomes.

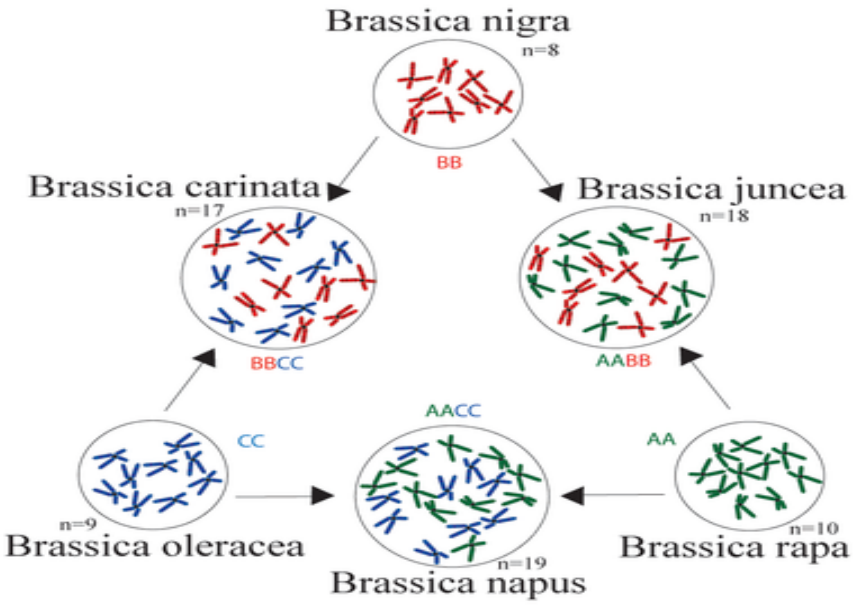


Fig .1.5 Genome relationships between different *Brassica* species, as described by Nagaharu U in 1935 (http://en.wikipedia.org/wiki/Triangle_of_U).

1.5 *Brassica napus* and *Arabidopsis thaliana*

Arabidopsis shares common ancestry with *B. napus* and both belongs to the same family Brassicaceae. Due to its small genome and efficient transformation system, *Arabidopsis* serves as model plant for geneticists to understand the genomic evolutionary pathway (Arabidopsis Genome Initiative 2000) that gave rise to different *Brassica* species as well as chromosomal homeology that exists among them. Marker based phylogenetic and comparative genomic studies unveiled relationship between *Brassica* species and *A. thaliana* to large extent suggesting that the

three genomes (A, B and C) of *Brassica* evolved from a common progenitor species similar to *Arabidopsis* through extensive duplication and reordering of the ancestral genome (Truco et al. 1996, Lan et al. 2000, Babula et al. 2003, Lysak et al. 2005, Li et al. 2003, Lukens et al. 2003). Parkin et al. (2005) mapped more than 1000 genetically linked loci in *B. napus* which are homologous to *Arabidopsis* genome by using RFLP markers and identified 21 conserved blocks in *Arabidopsis* genome, which may rearrange and/or duplicate to construct the *B. napus* genome. The *Brassica* and *Arabidopsis* genomes diverged from each other about 14 to 20 million years ago (Yang et al. 1999, Koch et al. 2001) and since then, a minimum of 74 gross rearrangements, 38 in the A genome and 36 in the C genome, has occurred (Parkin et al. 2005). From *Arbidopsis*-*Brassica* comparative genomic studies, researchers comprehend the phenomenon of gene function, genome divergence along with speciation as a result of polyploidization during evolution over the period of time.

1.6 Importance of genetic diversity

Presence of genetic diversity in breeding materials is vital for developing new cultivars that can meet the challenges of growth conditions including resistance to biotic and abiotic stresses and capable of producing high yield and improved quality. Crop improvement over the period of time occur mainly through selection of favourable type of cultivars carried out by farmers on field in ancient time, that gave rise to distinct landraces, and nowadays by plant breeders. This practice of selection brought about significant genetic gain (increase in performance by selection) in many crops like wheat, rice and maize. Land races that were evolved as a result of domestication and selection by the local farmers, provide important basic breeding-material needed to develop new cultivars by plant breeders. Unfortunately, a vast amount of genetic diversity that existed in the form of land race/native type cultivars have been

lost in many field crops due to their replacement with high yielding and genetically more uniform cultivars by farmers worldwide, especially after the advent of green revolution (McCouch 2004, van de Wouw et al. 2010). Moreover, the general trend of plant breeding during the last few decades has been the development of new cultivars in short period of time primarily based on elite lines and/or cultivars. These breeding materials contain only a fraction of the total genetic variation available in its gene pool (for review see Rahman 2013). Breeders usually avoid to use genetic variability from wide sources like progenitor and/or wild species in breeding programs as they carry undesirable alleles along with desirable ones, and use of these in cultivar development require repeated cycles of breeding. To avoid these difficulties, plant breeders often prefer to use elite materials that comprise limited genetic variation. All these activities gradually and unknowingly led to narrow down genetic diversity among the modern cultivars. Therefore, there is a need of increasing genetic diversity in many crops of economic importance, such as barley (Koebner et al. 2003), maize (Tallury and Goodman 2001), rice (Xu et al. 2004) and spring canola (Diers et al. 1996, Chen et al. 2010, Bennett et al. 2012, Rahman 2013). By the use of available genetic variability in spring canola germplasm, several researchers have demonstrated the feasibility of developing hybrid canola cultivars with relatively higher yield than open-pollinated cultivars, and emphasized the need of increasing genetic diversity in hybrid parental lines (Diers et al. 1996, Starmer et al. 1998, Riaz et al. 2001, Girke et al. 2012). Recent study on genetic diversity in Canadian spring canola by Fu and Gugel (2010) showed a clear trend of decreasing genetic variability in rapeseed cultivars over the period of time. This narrow genetic base in spring canola apparently due to intensive use of limited gene pool of elite inbred lines in breeding programs. Furthermore, genetic diversity among spring canola cultivars may have also been reduced by breeding emphasis on seed oil and meal quality traits, in particular

zero erucic acid (C22:1) in seed oil and low glucosinolate contents in seed meal (Hasan et al. 2008).

1.6.1 Increasing genetic diversity through the use of primary gene pool

Two main approaches that can be adapted to improve genetic diversity in spring canola (*B. napus*) includes the use of primary gene pool and the secondary gene pool. Rutabaga, winter and semi-winter types *B. napus*, which belongs to primary gene pool, are known to be genetically distinct from spring *B. napus* and harbour many alleles for favourable traits (Diers and Osborn 1994, Butruille et al. 1999, Hasan et al. 2006, Quijada et al. 2006, Chen et al. 2008, Basunanda et al. 2010, Kebede et al. 2010, Bus et al. 2011, Rahman and Kebede 2012). Use of these germplasm enables to utilize the amount of genetic variability of the A and C genomes captured in *B. napus* during evolution of this amphidiploid species in nature. Several studies have demonstrated the prospect of using winter and semi-winter gene pool to introgress allelic diversity into spring canola and to develop high yielding hybrid or open-pollinated cultivars. Butruille et al. (1999) evaluated the worth of introgressing alleles from winter *B. napus* into spring *B. napus* canola by developing doubled haploid (DH) lines from F_1 of cross between winter and spring types and evaluating the test-cross progeny of these DH lines. Some of these test-cross hybrids showed higher seed yield over commercial cultivars, inbreeds and hybrids from spring × spring type cross. Similarly, Kebede et al. (2010) reported DH lines from spring × winter crosses show significantly higher seed yield than spring check cultivar. According to Chen et al. (2008), the Chinese semi-winter germplasm is genetically distinct from spring canola, and thus can be used to broaden the gene pool of spring canola. Rahman et al. (2011b) introgressed clubroot resistance from winter canola cultivar Mendel into spring canola. All these studies demonstrated that the primary gene pool like winter germplasm is a valuable source to

broaden the genetic base of spring cultivars for specific traits as well as to boost seed yield in spring canola hybrids. However, spring type lines derived from crossing of winter × spring type may possess some undesirable traits like late flowering and maturity (Butruille et al. 1999, Rahman and Kebede 2012) and this would require a second cycle of breeding (Rahman 2013). The advantages of using canola quality winter and semi-winter *B. napus* in breeding is that crossability barrier and hybrid sterility as well as introduction of non-canola quality traits are not involved. On the other hand, use of non-canola quality *B. napus*, such as rutabaga (*B. napus* var. *napobrassica*), complicates the breeding as intensive selection for canola quality traits is required to develop canola quality spring type lines.

1.6.2 Increasing genetic diversity through the use of secondary gene pool

Enormous genetic diversity exits in the family Brassicaceae which contain over 3,700 species divided into 338 genera (Warwick et al. 2009). Among the cultivated *Brassica* species, enormous diversity exists in *B. rapa* and *B. oleracea* simply on the basis of morphological characteristics (Prakash and Hinata 1980 cited by Song et al 1988). For instance, *B. oleracea* can be grouped into different types (review by Prakash et al. 2012); kales (var. *acephala*) having strong main stem with edible foliage, branching bush kales (var. *fruticosa*) also edible foliage, Chinese kale (var. *alboglabra*) with edible flower and leaves, kohlrabi (var. *gongylodes*) having thick stem, inflorescence kale such as var. *botrytis* in which inflorescence forms a compact, whitish head used as vegetable and cabbage (var. *capitata)* with dense-leaved heads.

Similarly, *B. rapa* is grouped into leafy type (var. *chinensis*) used as vegetable, oleiferous type grown as oil crop, and thickened root type (var. *rapifera*) used as vegetable and fodder. Thus, these different forms of diploid progenitor species can serve as an excellent reservoir of genetic diversity for broadening the genetic base of *B. napus* as well as for

introgression of alleles for desired traits into this species (Song et al. 1988, Li et al. 2004, Qian et al. 2006, Chen et al. 2010, Rahman et al. 2011a, Bennett et al. 2012). Indeed, several researchers (Rahman 2001, Rahman 2005, Qian et al. 2006, Rahman et al. 2011a, 2011b, Bennett et al. 2012) has introgressed genetic diversity as well as specific traits from the diploid progenitor species into *B. napus* either by crossing with *B. napus* followed by selection for *B. napus* type plants or by resynthesizing *B. napus* from the diploid species *(B. rapa* and *B. oleracea*) followed by crossing of resynthesized *B. napus* to natural *B. napus.* For example, Muangprom et al. (2005) reported that the dwarf gene *Brrga1-d* from mutant *B. rapa* can be transferred into *B. napus* through hybridization to reduce lodging in *B. napus*. Similarly, Rahman et al. (2011a) demonstrated the prospects of developing reconstituted *B. napus* lines with earliness of flowering introgressed from *B. oleracea* through *B. napus* × *B. oleracea* interspecific cross. Thus, diploid progenitors as well as allied species can be explored to introgress favorable genes into *B. napus* germplasm (see Table 1.1). Bennett et al. (2012) developed genetically diverse elite *B. napus* lines from *B. napus* × *B. oleracea* crosses, and found that some of the test hybrids of these lines surpass in seed yield over the *B. napus* parent (Bennett 2012). By using resynthesized *B. napus* lines in hybrid breeding, Girke et al. (2012) found heterosis for seed yield and oil content. Similarly, Qian et al. (2005) reported that the *B. napus* lines, derived from *B. napus* × *B. rapa* crosses, containing the genome component of *B. rapa* exhibit heterosis when combined with cultivated *B. napus*.

Table 1.1 Introgression of alleles for favorable traits into *Brassica napus* through interspecific hybridization.

From	To	Traits	Reference
B. rapa (AA)	*B. napus*	Clubroot disease resistance	Lammerink (1970)
B. juncea (AABB)	*B. napus*	Blackleg disease resistance	Roy (1984), Sacristán and Gerdemann (1986)
B. juncea (AABB)	*B. napus*	Silique shattering	Prakash and Chopra (1990)
B. rapa (AA)	*B. napus*	Yellow seed color	Rahman (2001)
B. rapa (AA) and *B. oleracea* (CC)	*B. napus*	Self-incompatibilty	Rahman (2005)
B. rapa (AA)	*B. napus*	Black leg disease resistance	Leflon et al. (2007)
B. oleracea (CC)	*B. napus*	Early flowering	Rahman et al. (2011a)

Use of allied and progenitor species in breeding of spring *B. napus* canola not only introduces sterility in the interspecific hybrid progenies but also introduces unwanted alleles as linkage drag in the *B. napus* lines derived from interspecific crosses. These problems need to be resolved by plant breeders before the interspecific cross derived lines can be used for the development of commercial cultivars. Even, in the case of resynthesized *B. napus* which theoretically carry complete genomes of the diploid parental species, high sterility in resynthesized *B. napus* plants can occur over several generations due to meiotic irregularities (Pikaard 2001) and this make interspecific breeding a complex and laborious task. According to Szadkowski et al. (2010), the very first meiosis in resynthesized *B. napus* acts as genome blender leading to meiotic-driven genetic changes in the subsequent generations, and extent of such meiotic irregularities depend on cytoplasmic interactions (Cui et al. 2012). Selecting appropriate female parents in interspecific crosses can overcome to this problem to some extent. Despite

these challenges interspecific hybridization can be rewarding for the improvement of the genetic base of canola cultivars on long-term perspective to cope the changing agro-climatic conditions and to feed the growing human population.

1.7 Research objective

The long-term objective of this research is to broaden genetic diversity in *B. napus* through exploitation of the C-genome of the diploid progenitor species *B. oleracea.* Very little efforts have been made so far to introgress allelic diversity from *B. oleracea* into *B. napus* despite vast diversity present in this species. The proposed research is designed to understand the allelic diversity in two types of *B. oleracea* viz. *B. oleracea* var. *alboglabra* (CC, $2n = 18$) and *B. oleracea* var. *botrytis* (CC, $2n = 18$) for the improvement of Canadian spring *B. napus* canola. *Bassica oleracea* var. *alboglabra*, commonly called Chinese kale, is self-compatible and does not require vernalization for flowering, while *B. oleracea* var. *botrytis*, commonly called cauliflower, is self-incompatible. In short term, this MSc research project will study the following aspects:

i) Investigate the feasibility of developing canola quality *B. napus* ($2n = 38$) lines from F_2 and backcross derived populations of *B. napus* × *B. oleracea* interspecific crosses.

(ii) Study the inheritance of erucic acid and glucosinolate contents as well as response to selection for these two seed quality traits in different generations.

(iii) Estimate genetic diversity in the interspecific cross derived families by use of simple sequence repeat (SSR) markers.

1.7.1 Research hypothesis

The following hypothesis will be tested in this Master's thesis research project:

(1) Genetically diverse canola quality *B. napus* (2*n* = 38) lines can be developed from the progeny of *B. napus* × *B. oleracea* interspecific crosses through reconstitution of the C-genome of *B. napus* with the C-genome of *B. oleracea* with selection for the canola quality traits.

(2) The C-genomes of *B. oleracea* var. *botrytis* and *B. oleracea* var. *alboglabra* are genetically distinct from the C-genome of Canadian spring *B. napus* canola, and this diploid species would add new alleles into *B. napus* canola.

1.8 References

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

Babula, D., M. Kaczmarek, A. Barakat, M. Delseny, C. F. Quiros, and J. Sadowski, 2003: Chromosomal mapping of *Brassica oleracea* based on ESTs from *Arabidopsis thaliana*: complexity of the comparative map. Mol. Genet. Genomics 268: 656-665.

Basunanda, P., M. Radoev, W. Ecke, W. Friedt, H. C. Becker, and R. J. Snowdon, 2010: Comparative mapping of quantitative trait loci involved in heterosis for seedling and yield traits in oilseed rape (*Brassica napus* L.). Theor. Appl. Genet. 120: 271-281.

Bennett, R. A., 2012: Broadening genetic diversity in canola (*Brassica napus*) germplasm using the *B. oleracea* var. *alboglabra* C genome. Ph.D. thesis. University of Alberta, Edmonton, Canada.

Bennett, R. A., G. Séguin-Swartz, and H. Rahman, 2012: Broadening genetic diversity in canola (*Brassica napus* L.) using the C-genome species *B. oleracea*. Crop Sci. 52: 2030-2039.

Bisht, N. C., V. Gupta, N. Ramchiary, Y. S. Sodhi, A. Mukhopadhyay, N. Arumugam, D. Patel, and A. K. Pradhan, 2009: Fine mapping of loci involved with glucosinolate biosynthesis in oilseed mustard (*Brassica juncea*) using genomic information from allied species. Theor. Appl. Genet. 118: 413-421.

Blanc, G., H. Karsten, and K. H. Wolfe, 2003: A recent polyploidy superimposed on older large scale duplications in the *Arabidopsi*s genome. Genome Res. 13: 137-144.

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

Butruille, D.V., R. P. Guries, and T. C. Osborn, 1999: Increasing yield of spring oilseed rape hybrids through introgression of winter germplasm. Crop Sci. 39: 1491-1496.

Canola Council of Canada. http://www.canolacouncil.org. (Retrieved on date 10th Jan 2014)

Cao, Z., F. Tian, N. Wang, C. Jiang, B. Lin, W. Xia, J. Shi, Y. Long, C. Zhang and J. Meng, 2010: Analysis of QTLs for erucic acid and oil content in seeds on A8 chromosome and the linkage drag between the alleles for the two traits in *Brassica napus*. J. Genet. Genomics 37: 231-240.

Charlton, K. M., A. H. Corner, K. Davey, J. K. Kramer, S. Mahadevan, and F. D. Sauer, 1975: Cardiac lesions in rats fed rapeseed oils. Can. J. Comp. Med. 39: 261-269.

Chen, B. Y., and W. K. Heneen, 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, S., M. N. Nelson, K. Ghamkhar, T. Fu, and W. A. Cowling, 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.

Chen, S., J. Zou, W. A. Cowling , and J. Meng, 2010: Allelic diversity in a novel gene pool of canola-quality *Brassica napus* enriched with alleles from *B. rapa* and *B. carinata*. Crop Pasture Sci. 61: 483-492.

Cheng, F., T. Mandáková, J. Wu, Q. Xie, M. A. Lysak, and X. Wanga, 2013: Deciphering the diploid ancestral genome of the mesohexaploid *Brassica rapa*. Plant Cell 25: 1541-1554.

Cheung, F., M. Trick, N. Drou, Y. P. Lim, J. Y. Park, S. J. Kwonet J. A. Kim, R. Scott, J.C. Pires, A. H. Paterson, C. Town, and I. Bancroft 2009: Comparative analysis between homoeologous genome segments of Brassica napus and its progenitor species reveals extensive sequence-level divergence. Plant Cell 21: 1912-1928.

Christophersen, B. O., and J. Bremer, 1972: Erucic acid - an inhibitor of fatty acid oxidation in the heart. Biochim. Biophys. Acta 280: 506-514.

Coonrod, D., M. A. Brick, P. F. Byrne, L. DeBonte, and Z. Chen, 2008: Inheritance of long chain fatty acid content in rapeseed (*Brassica napus* L.). Euphytica 164: 583-592.

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

Cui, C., X. Ge, M. Gautam, L. Kang, and Z. Li, 2012: Cytoplasmic and genomic effects on meiotic pairing in *Brassica* hybrids and allotetraploids from pair crosses of three cultivated diploids. Genetics 191: 725-738.

Diers, B. W., T. C. Osborn, 1994: Genetic diversity of oilseed *Brassica napus* germ plasm based on restriction fragment length polymorphisms. Theor. Appl. Genet. 88: 662-668.

Diers, B. W., P. B. E. McVetty, and T. C. Osborn, 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.

Dixon, G.R., 2007: Vegetable Brassicas and related crucifers. CABI, Wallingford, UK; Cambridge, Massachusetts, USA.

EFSA report, 2008: Opinion of the scientific panel on contaminants in the food chain on a request from the European commission on glucosinolates as undesirable substances in animal feed. EFSA Journal 590: 1-76.

Ecke, W., M. Uzunova, and K. Weissleder, 1995: Mapping the genome of rapeseed (*Brassica napus* L.). II. Localization of genes controlling erucic acid synthesis and seed oil content. Theor. Appl. Genet. 91: 972-977.

Feng, J., Y. Long, L. Shi, J. Shi, G. Barker, and J. Meng, 2012: Characterization of metabolite quantitative trait loci and metabolic networks that control glucosinolate concentration in the seeds and leaves of *Brassica napus*. New Phytol.193: 96-108.

Finlayson, A. J., J. Krzymanski, and R. K. Downey, 1973: Comparison of chemical and agronomic characteristics of two *Brassica napus* L. cultivars, Bronowski and Target. J. Am. Oil Chem. Soc. 50: 407-410.

Food and Agriculture Organization, Food outlook report available online at http://www.fao.org/docrep/018/al999e/al999e.pdf retrieved on 14th Dec. 2013.

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

Girke, A., A. Schierholt, and H. C. Becker, 2012: Extending the rapeseed gene pool with resynthesized *Brassica napus* II: Heterosis. Theor. Appl. Genet. 124: 1017-1026.

Halkier, B. A., and L. Du, 1997: The biosynthesis of glucosinolates. Trends Plant Sci. 2:425-431.

Harvey, B. L., and R. K. Downey, 1964: The inheritance of erucic acid content in rapeseed (*Brassica napus*). Can. J. Plant Sci. 44: 104-111.

Hasan, M., F. Seyis, A. G. Badani, J. P. Kuhnemann , W. Lühs, W. Friedt, and R. J. Snowdon, 2006: Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet. Resour. Crop Evol. 53: 793-802.

Hasan, M., W. Friedt, J. P. Kühnemann, N. M. Freitag, K. Link, and R. J. Snowdon, 2008: Association of gene-linked SSR markers to seed glucosinolate content in oilseed rape (*Brassica napus* ssp. *napus*). Theor. Appl. Genet. 116: 1035-1049.

Kasem, W. T., A. Ghareeb, and E. Marwa, 2011: Seed morphology and seed coat sculpturing of 32 taxa of family Brassicaceae. Am. J. Sci. 7: 166-178.

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

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

Koebner, R. M. D., P. Donini, J. C. Reeves, R. J. Cooke, and J. R. Law, 2003: Temporal flux in the morphological and molecular diversity of UK barley. Theor. Appl. Genet. 106: 550-558.

Kondra, Z. P., and B. R. Stefansson, 1970: Inheritance of the major glucosinolates of rapeseed (*Brassica napus*) meal. Can. J. Plant Sci. 50: 643-647.

Lagercrantz, U., and D. J. Lydiate, 1996: Comparative genome mapping in *Brassica*. Genetics 144: 1903-1910.

Lammerink, J., 1970. Inter-specific transfer of clubroot resistance from *Brassica campestris* L. to *B. napus* L. New Zealand J. Agric. Res. 13: 105-110.

Lan, T.H., T. A. DelMonte, K. P. Reischmann, J. Hyman, S. P. Kowalski, J. McFerson, S. Kresovich, and A. H. Paterson, 2000: An EST-enriched comparative map of *Brassica oleracea* and *Arabidopsis thaliana*. Genome Res. 10: 776-788.

Leflon, M., H. Brun, F. Eber, R. Delourme, M. Lucas, P. Vallée, M. Ermel, M. Balesdent, and A. Chèvre, 2007: Detection, introgression and localization of genes conferring specific resistance to *Leptosphaeria maculans* from *Brassica rapa* into *B. napus*. Theor. Appl. Genet. 115: 897-906.

Li, G., M. Gao, B. Yang, and C. F. Quiros, 2003: Gene for gene alignment between the *Brassica* and *Arabidopsis* genomes by direct transcriptome mapping. Theor. Appl. Genet. 107: 168-180.

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

Lukens, L., F. Zou, D. Lydiate, I. Parkin, and T. Osborn, 2003: Comparison of a *Brassica oleracea* genetic map with the genome of *Arabidopsis thaliana*. Genetics 164: 359-372.

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

Mahmood, T., U. Ekuere, F. Yeh, A. G. Good, and G. R. Stringam, 2003: RFLP linkage analysis and mapping genes controlling the fatty acid profile of *Brassica juncea* using reciprocal DH populations. Theor. Appl. Genet. 107: 283-290.

Mason, A. S., V. Huteau, F. Eber, O. Coriton, G. Yan, M. N. Nelson, W. A. Cowling, and A. M. Chevre, 2010: Genome structure affects the rate of autosyndesis and allosyndesis in AABC, BBAC and CCAB *Brassica* interspecific hybrids. Chromosome Res. 18: 655-666.

McCouch, S., 2004: Diversifying selection in plant breeding. PLoS Biology 2: e347

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

Muangprom, A., S. G. Thomas, T. Sun and T. C. Osborn, 2005: A novel dwarfing mutation in a green revolution gene from *Brassica rapa*. Plant Physiol. 137: 931-938.

Navabi, Z. K., T. Huebert, A. G. Sharpe, C. M. O. Neill, I. Bancroft, and I. A. P. Parkin, 2013: Conserved microstructure of the *Brassica* B Genome of *Brassica nigra* in relation to homologous regions of *Arabidopsis thaliana*, *B. rapa* and *B. oleracea*. Genomics 14: 1-15.

Olin-Fatih, M., 1994: A new method for differential staining of *Brassica* metaphase chromosomes, and karyotypes of *B. campestris*, *B. oleracea*, and *B. napus*. Hereditas 120: 253-259.

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

Palmer, J. D., C. R. Shields, D. B. Cohen, and T. J. Orton, 1983: Chloroplast DNA evolution and the origin of amphidiploid *Brassica* species. Theor. Appl. Genet. 65: 181-189.

Pandey, Sharad, Manoj Kabdal, and M. K. Tripathi, 2013: Study of inheritance of erucic acid in Indian mustard (*Brassica juncea* L. Czern & Coss). Octa J. Biosci. 1: 77-84.

Parkin, I. A. P., A. G. Sharpe, D. J. Keith, and D. J. Lydiate, 1995: Identification of the A and C genomes of the amphidiploid *Brassica napus* (oilseed rape). Genome 38: 1122-1131.

Parkin, I. A. P., S. M. Gulden, A. G. Sharpe, L. Lukens, M. Trick, T. C. Osborn, and D. J. Lydiate, 2005: Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. Genetics 171: 765-781.

Pikaard, C. S., 2001: Genomic change and gene silencing in polyploids. Trends Genet. 17: 675-677.

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

Prakash, S., and L. V. Chopra, 1990: Reconstruction of alloploid *Brassicas* through non-homologous recombination: introgression of resistance to pod shatter in *Brassica napus*. Genet. Res. Camb. 56: 1-2.

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

Przybylski, R., and T. Mag, 2002: Canola/rapeseed oil. Vegetable oils in food technology: Composition, properties and uses: Blackwell publishing, CRC Press, USA/ Canada, pp. 98-127.

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

Qian, W., J. Meng, M. Li, M. Frauen, O. Sass, J. Noack, and C. Jung, 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.

Quijada, P. A., J. A. Udall, B. Lambert, and T. C. Osborn, 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.

Rahman, M. H., 2001: Production of yellow seeded *Brassica napus* through interspecific crosses. Plant Breed. 120: 463-472.

Rahman, M. H., 2005: Resynthesis of *Brassica napus* for self-incompatibility: Self-incompatibility reaction, inheritance and breeding potential. Plant Breed. 124: 13-19.

Rahman, M. H., R. A. Bennett, R-C Yang, B. Kebede, and M. R. Thiagarajah, 2011a: 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, M. H., A. Shakir, and M. J. Hasan, 2011b: Breeding for clubroot resistant spring canola (*Brassica napus* L.) for the Canadian prairies: Can the European winter canola cv. Mendel be used as a source of resistance? Can. J. Plant Sci. 91: 447-458.

Rahman, H., and B. Kebede, 2012: Improvement of spring canola *Brassica napus* (L.) by use of winter canola. J. Oilseed Brassica 3: 1-17.

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

Rahman, H., B. Kebede, C. Zimmerli, and R. C. Yang, 2014: Genetic study and QTL mapping of seed glucosinolate content *Brassica rapa* L. Crop Sci. 54: 1-7.

Riaz, A., G. Li, Z. Quresh, M. S. Swati, and C. F. Quiros, 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.

Rich, T. C. G., 1991: Crucifers of Great Britain and Ireland. Botanical Society of the British Isles, London, pp. 1–336.

Rosillo-Calle, F., Pelkmans L., and A. Walter, 2009: A global overview of vegetable oils, with reference to biodiesel. A Report for the IEA bioenergy task 40, IEA bioenergy.

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

Sacristán, M. D., and M. Gerdemann, 1986: Different behavior of *B. juncea* and *B. carinata* as sources of *Phoma lingam* resistance in experiments of interspecific transfer to *B. napus*. Plant Breed. 97: 304-314.

Siebel, J., and K. P. Pauls, 1989: A comparison of anther and microspore culture as a breeding tool in *Brassica napus*. Theor. Appl. Genet.78: 473-479.

Snowdon, R. J., T. Friedrich, W. Friedt, and W. Köhler, 2002: Identifying the chromosomes of the A and C genome diploid *Brassica* species *B. rapa* and *B. oleracea* in their amphidiploid *B. napus*. Theor. Appl. Genet. 104: 533-538.

Song, K. M., T. C. Osborn, and P. H. Williams, 1988: *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theor. Appl. Genet. 75: 784-794.

Song, K. and T. C. Osborn, 1992: Polyphyletic origins of *Brassica napus*: new evidence based on organelle and nuclear RFLP analyses. Genome 35: 992-1001.

Sønderby, I. E., F. Geu-Flores, and B. A. Halkier, 2010: Biosynthesis of glucosinolates-gene discovery and beyond. Trends Plant Sci. 15: 283-290.

Starmer, K. P., J. Brown, and J. B. Davis, 1998: Heterosis in spring canola hybrids grown in Northern Idaho. Crop Sci. 38: 376-380.

Stefansson, B. R., 1983: The development of improved rapeseed cultivars. In: Kramer JKG, Sauer FD and Pigden EJ (eds) High and low erucic acid rapeseed oils: Production, Usage, Chemistry, and Toxicological Evaluation. Academic Press, New York, pp 143-159.

Szadkowski, E., F. Eber, V. Huteau, M. Lode, C. Huneau, H. Belcram, O. Coriton, M. J. Manzanares- Dauleux, R. Delourme, G. J. King, B. Chalhoub, E. Jenczewski, and A. M. Chèvre, 2010: The first meiosis of resynthesized *Brassica napus*, a genome blender. New Phytol. 186: 102-112.

Tallury, S. P., and M. M. Goodman, 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.

Thormann, C. E., J. Romero, J. Mantet, and T. C. Osborn, 1996: Mapping loci controlling the concentrations of erucic and linolenic acids in seed oil of *Brassica napus* L. Theor. Appl. Genet. 93: 282-286.

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

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

United States Department of Agriculture report (USDA), 2012. Available at http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx#.UWbBYLUxDA4.

United States Department of Agriculture (USDA), publicated in March 2014. Available at http://www.statista.com/statistics/263937/vegetable-oils-global-consumption/

van de Wouw, M., C. Kik, T. van Hintum, R. van Treuren, and B. Visser, 2010: Genetic erosion in crops: concept, research results and challenges. Plant Genet. Resour. 8: 1-15.

Velasco, P., P. Soengas, M. Vilar, and M. E. Cartea, and M. del Rio, 2008: Comparison of glucosinolate profiles in leaf and seed tissues of different *Brassica napus* crops. JASHS 133: 551-558.

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

Warwick, S. I., A. Francis, and I. A. Al-Shehbaz, 2006: Brassicaceae: species checklist and database on CD-Rom. Pl. Syst. Evol. 259: 249-258.

Warwick, S. I., A. Francis, R. K. Gugel, 2009: Guide to wild germplasm of *Brassica* and allied crops (tribe *Brassiceae*, Brassicaceae) 3rd edn. Agriculture and Agri-Food Canada Techical Bulletin, Ottawa, Canada.

Wikipedia, retrived in Feb 2014. Available at http://en.wikipedia.org/wiki/Triangle-of-U

Wroblewski, T., S. Coulibaly, J. Sadowski, and C. F. Quiros, 2000: Variation and phylogenetic utility of the *Arabidopsis thaliana* Rps2 homolog in various species of the tribe *Brassiceae*. Mol. Phylog. Evol. 16: 440-448.

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

Yang, Y. W., K. N. Lai, P. Y. Tai, and W. H. Li, 1999: Rates of nucleotide substitution in angiosperm mitochondrial DNA sequence and dates of divergence between *Brassica* and other angiosperm lineages. J. Mol. Evol. 48: 597-604.

Zhang, J. F., Q. I. Cun-Kou, P. U. Hui-Ming, C. Song, C. Feng, G. A. O. Jian-Qin, C. Xin-Jun, G. U. Hui, and F. U. Shou-Zhong, 2008: QTL identification for fatty acid content in rapeseed (*Brassica napus* L.). Acta Agron. Sin. 34: 54-60.

Chapter 2

Development of interspecific recombinant inbred lines (RIL's) from *B. napus* × *B. oleracea* interspecific crosses

2.1 Introduction

Brassica napus L. (AACC, 2*n* = 38) is one of the most important species of the Brassicaceae family that evolved through hybridization between *Brassica rapa* L. (AA, 2*n* = 20) and *Brassica oleracea* L. (CC, 2*n* = 18) (U, 1935). Both these diploid progenitor (*B. rapa* and *B. oleracea*) species show high chromosomal homoeology suggesting their origin from a common *Brassica* ancestor similar to *Arabidopsis thaliana* (Lagercrantz et al. 1996, Truco et al. 1996, Babula et al. 2003, Li et al. 2003, Lysak et al. 2005, Ziolkowski et al. 2006).

In Canada, spring type *Brassica napus* L. (AACC, 2*n* = 38) is the predominant *Brassica* oilseed crop contributing over $19 billion to the Canadian economy each year (http://canolacouncil.org). This oilseed crop is grown mainly in the Prairie Provinces: Alberta, Manitoba and Saskatchewan. In Canada, cultivation of *B. napus* started in early 1940's for its seed oil as high quality lubricant. This oil was considered unhealthy for edible purposes due to high content of erucic acid (C22:1). Similarly, the value of its seed meal (remainder after oil extraction) could not be harvested due to the presence of high content of glucosinolates. Erucic acid and glucosinolate contents were reduced to less than 2% in oil and less than 30 μmol/g dry matter respectively, during 1970's through extensive breeding research in Canada (Stefansson 1983, for review see Gupta and Pratap 2007). Such seed quality improved *B. napus* and *B. rapa* cultivars are called "canola" and/or "double low" cultivars. Today, canola oil is considered as premium quality vegetable oil in the world due to its balanced fatty acid composition, and the

canola meal is the second largest source of protein supplement for livestock after soybean meal (http://www.ers.usda.gov).

Presence of genetic diversity in breeding materials is pre-requisite to develop new cultivars with desirable agronomic traits and high yield. However, genetic diversity in spring *B. napus* canola has decreased over period of time (Fu and Gugel 2010) and therefore, this needs to be increased (Hasan et al. 2006, Cowling 2007, for review see Rahman 2013). For this, *B. oleracea* (CC, 2*n* = 18) and *B. rapa*, the progenitor species of *B. napus*, as well as other allied *Brassica* species can be used. Often, it is difficult to obtain viable hybrids from crossing of *B. napus* with its allied species from secondary or tertiary gene pool due to compatibility barriers like sexual incompatibility and/or hybrid breakdown. Meiotic irregularities such as, formation of unreduced gametes and multivalent pairing (Parkin et al. 1995, Pikaard 2001, Inomata 2002, Szadkowski et al. 2010) often occurs in interspecific hybrids and in subsequent generation plants, and that result sterility in hybrid progenies. These are some of the difficulties for introgression of allelic diversity from allied species into *B. napus*. With the development of different cell and tissue culture techniques like embryo rescue, ovule culture, ovary culture, protoplast fusion and somatic hybridization, the chances of producing viable interspecific hybrids have been increased (Rahman 2004, Bennett et al. 2008) and favorable traits have been introgressed from allied species into *B. napus* (see Table 1.1 in chapter 1).

Very few interspecific cross has been made to introgress genetic diversity from the C-genome of *B. oleracea* into *B. napus*, perhaps due to the difficulty of producing interspecific *B. napus* × *B. oleracea* hybrids, as well as lack of canola quality of *B. oleracea* (Bennett et al. 2008). However, some studies like Bennett (2012), Li et al. (2014) and Rahman et al. (2015)

demonstrated the prospect of developing canola quality *B. napus* lines from *B. napus* × *B. oleracea* interspecific crosses.

The overall objective of this study was two-fold; to determine the feasibility of introgression of allelic diversity from the C genome of *B. oleracea* into *B. napus* for the improvement of this crop as well as to develop genetically distinct canola quality spring growth habit *B. napus* recombinant inbred lines. However, the present research was designed to extend our knowledge on this *B. napus* × *B. oleracea* interspecific cross through a comparative study by the use of two different morphotypes of *B. oleracea* (var. *alboglabra* and var. *botrytis*) and two breeding methods for the development of canola quality *B. napus* lines.

2.2 Materials and methods

2.2.1 Parental Germplasm

The parental germplasm used for this study were one canola quality (zero erucic acid and <15 μmol per g seed glucosinolate) spring type *B. napus* (AACC, 2*n* = 38) doubled haploid line A04-73NA developed by the Canola Program of the University of Alberta, and two *B. oleracea* lines/cultivars, viz. *B. oleracea* var. *alboglabra*-NRC (PBI) and *B. oleracea* var. *botrytis* (BARI Cauliflower-1), which hereafter will be referred to as *B. oleracea* var. *alboglabra* and *B. oleracea* var. *botrytis*, respectively. Both *B. oleracea* parents are of non-canola quality types.

B. oleracea var. *alboglabra*, commonly called Chinese kale, is self-compatible with spring growth habit and is characterized by having white flowers (von Bothmer et al. 1995). This plant is used for a variety of purposes including human food and animal feed. While, the other parent *B. oleracea* var. *botrytis*, commonly called cauliflower, is an important vegetable crop, and self-incompatible in nature (Sharma et al. 2005).

2.2.2 Development of F_1 and BC_1 hybrids

The following two interspecific crosses were made by the Canola Program using *B. napus* as female and *B. oleracea* as male to develop the breeding materials for this research.

(1) A04-73NA × *B. oleracea* var. *alboglabra* (cross ID 5CA1300)

(2) A04-73NA × *B. oleracea* var. *botrytis* (cross ID 5CA1343)

The F_1 plants were self-pollinated to produce F_2 seeds as well as backcrossed to the *B. napus* parent A04-73NA to develop backcross (BC_1) seeds:

(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA (cross ID 5CA1676)

(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA (cross ID 5CA1677)

Backcrossing of the interspecific F_1 hybrids to the *B. napus* parent A04-73NA was done to increase the chance of developing canola quality elite lines through self-pollination of the BC_1 plants, though overall genetic diversity in this population is, theoretically, expected to be lower than the population derived from F_2.

2.2.3 Development of F_2- and BC_1-derived inbred lines

Two types of populations were developed from the above mentioned interspecific crosses through self-pollination: population derived from F_2 and BC_1 (Fig. 2.1). For my thesis research, I got F_3 and BC_1F_2 populations along with different data from the previous generation populations from the Canola Program. The F_3 and BC_1F_2, F_4 and BC_1F_3, and F_6 and BC_1F_5 populations were grown in greenhouse (21°/18° ± 2°C day/night) during spring 2012, winter 2012-13 and 2013-14, respectively while, F_5 and BC_1F_4, and F_7 and BC_1F_6 population were grown in field spring season 2013 and 2014, respectively at the Edmonton Research Station of the University of Alberta. Plot size was single row of 2 m with 50 cm space between the rows. Self-pollinated seeds in each generation were obtained by bagging individual plants with transparent and micro-

perforated plastic bags that were seeded to grow next generation population. Selection in these generations primarily focused on plant fertility and seed quality traits.

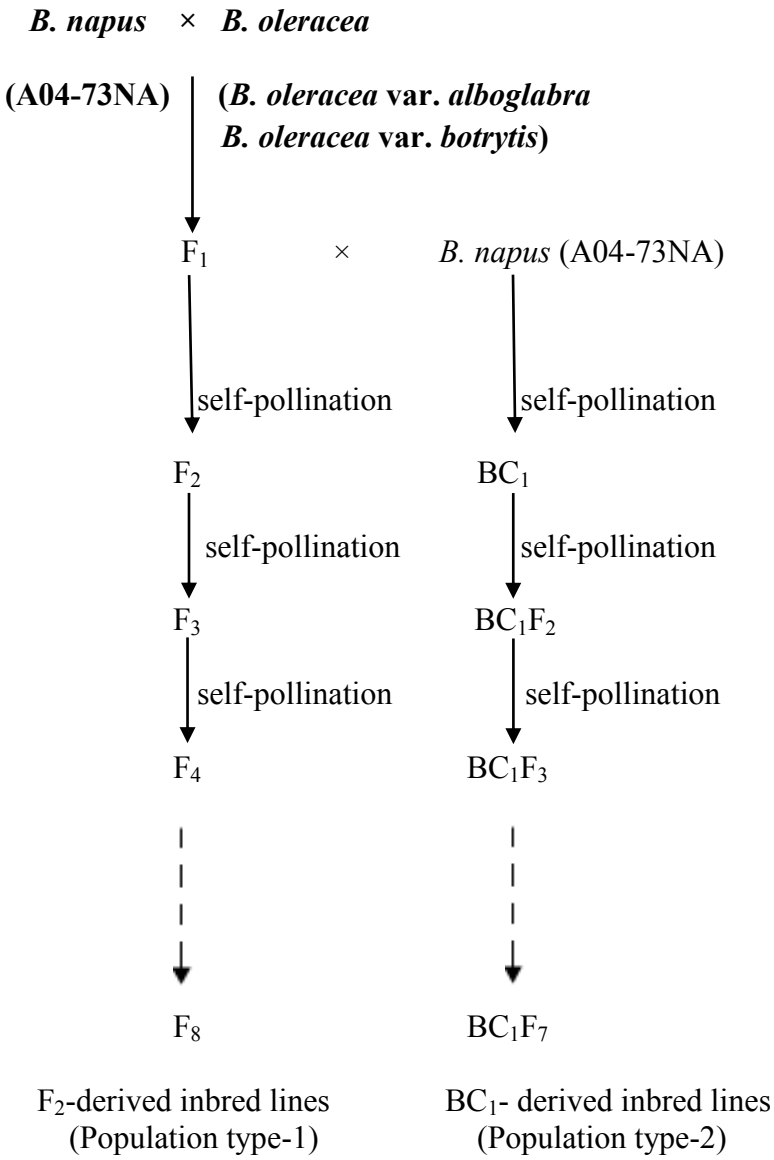


Fig. 2.1 A schematic diagram of the *B. napus* × *B. oleracea* interspecific cross for the development of genetically diverse *B. napus* lines.

2.2.4 Plant fertility

Plant fertility in F_2 and BC_1 generation population was estimated on the basis of seed yield per plant (g). On the other hand, plant fertility in the subsequent later generation

populations (F_3, BC_1F_2, F_4, BC_1F_3, F_5, BC_1F_4, F_6, BC_1F_5, F_7, BC_1F_6, F_8 and BC_1F_7) was estimated based on silique length (mm), number of seeds per silique, and seed yield per plant (g). For this, three to five siliques from the middle to upper half of the main raceme of the individual plants were selected randomly to estimate silique length (mm). Seeds from the same siliques were counted and average number of seeds per silique was calculated. Data of these traits were compared with the *B. napus* parent A04-73NA in each generation to estimate plant fertility.

2.2.5 Fatty acid analysis

Fatty acid profile of F_2 and F_2-derived populations (F_3, F_4, F_5 and F_6) as well as BC_1 and BC_1-derived populations (BC_1F_3, BC_1F_4, and BC_1F_5) were performed on self-pollinated seeds harvested from individual plants. For this, 0.10 to 0.25 g seed from each plant was used. Seeds were crushed in N-pentane in 50 ml conical tube, centrifuged at 1500 rpm for 15-20 min, and the supernatant was transferred to 10 × 75 mm glass tube. The N-pentane was evaporated, leaving behind the extracted oil. Extracted oil was methylated to produce fatty acid methyl esters that were analyzed by gas chromatographic technique (Ackman, 1966) to determine fatty acid profile by using a Hewlett-Packard chromatograph (model 6890 N) equipped with a flame ionization detector (for detail, see Bennett et al. 2008).

2.2.6 Glucosinolate analysis

Glucosinolate (GLS) content in F_2- and BC_1-derived generation populations was determined by using near-infrared spectroscopy (NIRS, FOSS NIRSystems model 6500). For this, 2.5 to 4 g self-pollinated seeds harvested from individual plants grown in greenhouse, or 5 to 8 g open-pollinated bulk seeds harvested from several plants grown in field plots was used. Glucosinolate content was calculated on 8.5% moisture basis and reported as μmol/ g seed.

2.2.7 Ploidy analysis

Flow cytometric analysis for relative nuclear DNA content (or Partec value) was done on F_6 and BC_1F_5 as well as on F_8 and BC_1F_7 generation plants to estimate their approximate chromosome number − whether the plants were close to the *B. napus* or *B. oleracea* parent. For this, approximately 0.5 cm^2 leaf tissue from each plant was collected and chopped with a razor blade in extraction buffer and incubated for 1 to 2 minute. After that, samples were filtered through Partec CellTrics Disposable Filter and 1.6 ml staining buffer was added to the samples. Ploidy level was analyzed by Partec ploidy analyzer (Partec GmbH, Münster, Germany).

2.2.8 Statistical analysis

Data recorded on different agronomic and seed quality traits were analyzed by using different statistic feature of MS excel and Statistical Analysis Software (SAS version 9.3). Comparison of different generation populations was made by using the following SAS statement;

model response variable = cross generation cross*generation;

lsmeans cross generation cross*generation/adjust = tukey;

repeated/group = cross*generation.

where, Tueky test was used due to its conservative nature to control type-I error while, the term repeated/group was used in the model statement to take into account the generation variance.

2.3 Results

2.3.1 Study of plant fertility

Plant fertility in F_2-derived populations of *B. napus* × *B. oleracea* var. *alboglabra* (cross ID 5CA1300) and *B. napus* × *B. oleracea* var. *botrytis* (cross ID 5CA1343) as well as in BC_1-derived populations of (*B. napus* × *B. oleracea* var. *alboglabra*) × *B. napus* (cross ID 5CA1676) and (*B. napus* × *B. oleracea* var. *botrytis*) × *B. napus* (cross ID 5CA1677) crosses was estimated on the basis of silique length and number of seeds per silique. A confidence limits for silique length and seeds number per silique was calculated for the *B. napus* parent A04-73NA grown along with these F_2- and BC_1-derived populations and used to compare with these interspecific cross derived generation populations. Data on silique length, number of seeds per silique and percent plant fertility in different generation populations are presented in Table 2.1, Table 2.2 and Table 2.3, respectively.

F_3 and BC_1F_2 population

Silique length in F_3 population of 5CA1300 varied between 11.2 to 54.6 mm with a mean 29.1 ± 8.23 mm SD; which was significantly (P-value < 0.01) longer than the BC_1F_2 population (21.3 ± 6.11 mm SD) of 5CA1676 (Table 2.1). Significant difference for number of seeds per silique was found between the F_3 and BC_1F_2 populations as well. Mean silique length and number of seeds per silique in F_3 population of 5CA1343 was 24.3 ± 5.70 mm SD (range 9.6 to 37.8 mm) and 2.6 ± 2.11 SD (range 0.2 to 10.6), respectively; while the BC_1F_2 population of 5CA1677 had mean silique length of 22.1 ± 6.00 mm SD (range 10.8 to 37.3 mm) with 0.8 ± 1.55 SD (range 0 to 6.0) seeds per silique. The difference between the F_3 and BC_1F_2 population for number seeds per silique was statistically significant (P-value < 0.01) in this case also (Table 2.2). Overall of the two crosses, mean values of silique length and number of seeds per silique

were 27.4 ± 7.75 mm SD and 3.8 ± 3.49 SD in F_3 while 21.7 ± 5.98 mm SD and 0.7 ± 1.33 SD in BC_1F_2 population (Table 2.3). About 71% and 43% plants, respectively of the F_3 and BC_1F_2 population produced viable seeds (Table 2.3).

F_4 and BC_1F_3 population

Mean silique length and number of seeds per silique of the F_4 and BC_1F_3 populations of both crosses were similar, however significantly different from the *B. napus* parent A04-73NA (P-value < 0.01) (Table 2.1, 2.2). However, 13% F_4 and 25% BC_1F_3 plants had silique size either similar or longer than the *B. napus* parent (Table 2.1). None of the F_4 or BC_1F_3 plants produced number seeds per silique comparable to A04-73NA (Table 2.2). Percent seed producing plants in F_4 and BC_1F_3 generation was 41% and 35%, respectively (Table 2.3).

F_5 and BC_1F_4 population

F_5 and BC_1F_3 populations were grown in field in 2013. In this case, silique length and number of seeds per silique data was collected from open pollinated branches of the same plants which were self-pollinated by bag isolation.

F_5 population of both crosses (5CA1300 and 5CA1343) had significantly longer silique (P-value < 0.05) as compared to their respective BC_1F_4 populations (Table 2.1). About, 19% F_5 plants and 20% BC_1F_4 plants had silique size similar to the *B. napus* parent A04-73NA or greater than this parent (Table 2.1). As compared to the *B. napus* parent, both F_5 and BC_1F_4 populations of the two crosses produced significantly (P-value < 0.05) fewer number of seeds per silique.

More than 90% of the F_5 and BC_1F_4 plants produced seeds under open-pollination which was significantly greater than the previous or the following generation plants grown in greenhouse. This apparently resulted from better pollination under open pollination condition in the field.

F_6 and BC_1F_5 population

The F_6 and BC_1F_5 populations of both crosses still produced significantly lower number of seeds per silique compared to the *B. napus* parent (Table 2.2). At this stage, more than 45% of both F_6 and BC_1F_5 plants had silique size similar to A04-73NA (Table 2.1) and more than 60% plants produced seeds under self-pollination (Table 2.3).

F_7 and BC_1F_6 population

The F_7 and BC_1F_6 populations were grown in the field during 2014 and data recorded on silique from open-pollinated branches of the plants that were self-pollinated by bag isolation. Silique length of both F_7 and BC_1F_6 populations from the two crosses was statistically similar; but, this was significantly shorter (P-value < 0.05) than the *B. napus* parent A04-73NA (Table 2.1). These populations also produced significantly fewer numbers of seeds per silique as compared to A04-73NA (Table 2.2); however, all (100%) of the plants produced seed under open-pollination in field (Table 2.3).

In summary, silique length in both F_2- and BC_1-derived populations of both crosses improved by about 2-fold, and number seeds per silique by about 10-fold through selection for fertile plants in each generation. However, on average, these populations still produced significantly shorter silique with less number of seeds per silique than the *B. napus* parent. The range of variation observed in these populations for these traits suggests that some of the advanced generation plants are suffering from aneuploidy. Overall, populations derived from F_2 or BC_1 were not significantly different for these two plant fertility traits.

Table 2.1 Silique length in F_2- and BC_1-derived populations of *Brassica napus* × *B. oleracea* var. *alboglabra* and *Brassica napus* × *B. oleracea* var. *botrytis* interspecific crosses.

			Whole population					Selected population		
Cross ID[1]	Gen.	Growth Cond.[2]	No. plants (families)	Range	Mean ± SD[3]	% *B. napus* type[†]	% better than *B. napus*[††]	No. plants (families)	Range	Mean ± SD
5CA1300	F_3	GH	137 (37)	11.2 - 54.6	29.1 ± 8.23b	0	4%	51 (29)	13.4 - 54.6	30.8 ± 7.45
5CA1676	BC_1F_2	GH	108 (43)	10.0 - 38.8	21.3 ± 6.11d	0	0	all to next generation		
5CA1343	F_3	GH	74 (35)	9.6 - 37.8	24.3 ± 5.70cd	0	0	39 (25)	15.2 - 35.2	25.5 ± 0.88
5CA1677	BC_1F_2	GH	111 (46)	10.8 - 37.3	22.1 ± 6.00c	0	0	all to next generation		
A04-73NA[4]		GH	4	42.4 - 44.0	43.8 ± 0.91a					
5CA1300	F_4	GH	78 (36)	18.3 - 51.0	31.5 ± 7.60b	3%	5%	all to next generation		
5CA1676	BC_1F_3	GH	117 (59)	7.0 - 60.0	31.2 ± 10.54b	6%	10%	all to next generation		
5CA1343	F_4	GH	86 (29)	11.8- 59.0	29.5 ± 9.17b	2%	3%	all to next generation		
5CA1677	BC_1F_3	GH	122 (59)	12.5 - 59.3	30.6 ± 8.35b	5%	4%	all to next generation		
A04-73NA[4]		GH	7	41.3 - 45.8	44.1 ± 1.44a					
5CA1300	F_5	Field	205 (72)	13.3 - 63.7	41.3 ± 11.22b	8%	6%	39 (22)	16.0 - 60.0	41.1 ± 11.19
5CA1676	BC_1F_4	Field	261 (92)	11.7 - 59.0	37.9 ± 11.28c	4%	1%	62 (37)	17.0 - 59.0	40.9 ± 9.92
5CA1343	F_5	Field	208 (72)	12.7 - 65.3	40.3 ± 10.08bc	3%	2%	42 (23)	27.0 - 65.3	44.2 ± 8.59
5CA1677	BC_1F_4	Field	246 (89)	11.7 - 60.7	34.8 ± 10.30d	15%	0.4%	45 (29)	17.3 - 58.0	40.4 ± 9.26
A04-73NA[4]		Field	45	40.7 - 64.0	57.6 ± 4.48a					
5CA1300	F_6	GH	88 (35)	13.7 - 59.0	35.3 ± 11.87c	13%	5%	70 (29)	13.7 - 59.0	35.7 ± 11.52
5CA1676	BC_1F_5	GH	95 (58)	15.0 - 59.3	38.3 ± 9.71bc	19%	5%	86 (55)	15.0 - 59.3	38.6 ± 9.46
5CA1343	F_6	GH	75 (36)	17.0 - 67.7	41.3 ± 11.21ab	14%	12%	61 (33)	17.0 - 67.7	40.8 ± 11.89
5CA1677	BC_1F_5	GH	90 (41)	19.0 - 62.7	40.8 ± 10.93ab	7%	14%	78 (39)	19.0 - 62.7	41.5 ± 11.07
A04-73NA[4]		GH	8	45.7 - 59.3	50.0 ± 4.50a					
5CA1300	F_7	Field	126 (61)	24.3 - 81.0	53.4 ± 9.57b	2%	13%	63 (37)	38.3 - 81.0	55.6 ± 9.10
5CA1676	BC_1F_6	Field	138 (72)	27.0 - 69.7	51.4 ± 7.43b	4%	5%	57 (35)	35.3 - 65.7	51.2 ± 6.34
5CA1343	F_7	Field	112 (55)	33.3 - 74.3	53.4 ± 8.62b	3%	14%	43 (20)	40.7 - 74.3	57.2 ± 8.48
5CA1677	BC_1F_6	Field	128 (64)	31.3 - 76.0	53.8 ± 10.13b	2%	19%	61 (37)	33.7 - 75.0	56.3 ± 9.73
A04-73NA[4]		Field	51	48.0 - 73.3	62.3 ± 5.72a					

[1]Cross ID; 5CA1300 = *B. napus* × *B. oleracea* var. *alboglabra*, 5CA1676 = (*B. napus* × *B. oleracea* var. *alboglabra*) × *B. napus*, 5CA1343 = *B. napus* × *B. oleracea* var. *botrytis*, and 5CA1677 = (*B. napus* × *B. oleracea* var. *botrytis*) × *B. napus.*

[2]Growing condition, GH = greenhouse.

[3]Sharing of same letter indicate no significant difference according to Tukey test (P-value > 0.05).

[4]Confidence limits of *B. napus* parent A04-73NA for silique length was 42.9-44.7, 43.0-45.2, 56.2-58.9, 46.9-53.1 and 60.7-63.9 for the plants grown along with F_3 & BC_1F_2, F_4 & BC_1F_3, F_5 & BC_1F_4, F_6 & BC_1F_5 and F_7 & BC_1F_6 generation population, respectively.

[†]% plants falling within confidence limits of the *B. napus* parent A04-73NA for silique size (mm); [††]% plants with silique size (mm) longer than the *B. napus* parent A04-73NA.

Table 2.2 Number of seeds per silique in F_2- and BC_1-derived populations of *Brassica napus* × *B. oleracea* var. *alboglabra* and *Brassica napus* × *B. oleracea* var. *botrytis* interspecific crosses.

			Whole population					Selected population		
Cross ID[1]	Gen.	Growth Cond.[2]	No. plants (families)	Range	Mean ± SD[3]	% *B. napus* type[†]	% better than *B. napus*[††]	No. plants (families)	Range	Mean ± SD
5CA1300	F_3	GH	137 (37)	0.2 - 23	3.9 ± 3.96b	0.8%	0	51 (29)	0 - 23	5.2 ± 4.09
5CA1676	BC_1F_2	GH	108 (43)	0 - 7	0.6 ± 1.22d	—	—	all to next generation		
5CA1343	F_3	GH	74 (35)	0.2 - 10.6	2.6 ± 2.11c	0	0	39 (25)	0-10.6	3.0 ± 2.20
5CA1677	BC_1F_2	GH	111 (46)	0 - 6	0.8 ± 1.55d	—	—	all to next generation		
A04-73NA[4]		GH	4	23.2 - 25.0	24.0 ± 1.09a					
5CA1300	F_4	GH	78 (36)	0 - 12.8	3.0 ± 3.11b	0	0	all to next generation		
5CA1676	BC_1F_3	GH	117 (59)	0 - 13.0	2.4 ± 2.69b	0	0	all to next generation		
5CA1343	F_4	GH	86 (29)	0 - 10.8	2.5 ± 2.45b	0	0	all to next generation		
5CA1677	BC_1F_3	GH	122 (59)	0 - 10.5	2.6 ± 2.42b	0	0	all to next generation		
A04-73NA[4]		GH	7	20.0 - 23.5	22.2 ± 0.92a					
5CA1300	F_5	Field	205 (72)	0 - 34.7	19.7± 7.93b	0.5%	0	39 (22)	8 - 34.7	20.4 ± 6.93
5CA1676	BC_1F_4	Field	261 (92)	0 - 35.0	19.3 ± 8.65b	13%	0	62 (37)	5.3 - 34.7	21.8 ± 7.72
5CA1343	F_5	Field	208 (72)	0 - 34.0	20.5 ± 7.50b	0.5%	0	42 (23)	11.3 - 34.0	23.9 ± 5.59
5CA1677	BC_1F_4	Field	246 (89)	0 - 39.3	15.6 ± 7.11c	0.8%	0.4%	45 (29)	0 - 39.3	18.4 ± 7.27
A04-73NA[4]		Field	45	30.7 - 37.7	34.3 ± 1.37a					
5CA1300	F_6	GH	88 (35)	0 - 25.3	7.3 ± 6.00c	1%	1%	70 (29)	0 - 25.3	7.3 ± 5.93
5CA1676	BC_1F_5	GH	95 (58)	0.3 - 22.0	8.4 ± 5.59bc	2%	0	86 (55)	0.3 - 22.0	8.6 ± 5.59
5CA1343	F_6	GH	75 (36)	0.7 - 24.7	9.7 ± 5.79b	1%	1%	61 (33)	0.7 - 24.7	9.5 ± 6.10
5CA1677	BC_1F_5	GH	90 (41)	0.7 - 24.3	8.8 ± 5.67bc	0.5%	3%	78 (39)	0.7 - 24.3	8.9 ± 5.83
A04-73NA[4]		GH	8	20.0 - 24.3	22.3 ± 1.44a					
5CA1300	F_7	Field	126 (61)	2.7 - 41.3	26.0± 8.37bc	8.8	10.9	63 (37)	13.3 - 41.3	27.9 ± 6.13
5CA1676	BC_1F_6	Field	138 (72)	1.3 - 38.0	24.2 ± 7.50bc	5%	5%	57 (35)	5.3 - 35.3	24.9 ± 5.62
5CA1343	F_7	Field	112 (55)	2.4 - 37.3	26.2 ± 8.20b	11%	14%	43 (20)	10.7 - 37.3	29.5 ± 5.40
5CA1677	BC_1F_6	Field	128 (64)	1.2 - 36.6	22.8 ± 8.04c	3.3%	5%	61 (37)	9.3 - 34.7	24.7 ± 6.64
A04-73NA[4]		Field	51	29.0 - 38.3	33.5 ± 2.43a					

[1]Cross ID; 5CA1300 = *B. napus* × *B. oleracea* var. *alboglabra*, 5CA1676 = (*B. napus* × *B. oleracea* var. *alboglabra*) × *B. napus*, 5CA1343 = *B. napus* × *B. oleracea* var. *botrytis*, and 5CA1677 = (*B. napus* × B.*oleracea* var. *botrytis*) × *B. napus.*

[2]Growth condition, GH = greenhouse.

[3]Sharing of same letter indicate no significant difference according to Tukey test (P-value > 0.05).

[4]Confidence limits of *B. napus* parent A04-73NA for number of seeds per silique was 22.9-25.1, 21.5-22.9, 33.9-34.7, 21.3-23.3 and 32.8 - 34.2 while grown with F_3 & BC_1F_2, F_4 & BC_1F_3, F_5 & BC_1F_4, F_6 & BC_1F_5 and F_7 & BC_1F_6 generation, respectively.

[†]% plants falling within confidence limits of the *B. napus* parent A04-73NA for number of seeds per silique

[††]% plants with number of seeds per silique greater than the *B. napus* parent A04-73NA.

Table 2.3 Silique length and number of seeds per silique in F_2- and BC_1-derived populations of *B. napus* × *B. oleracea* interspecific crosses.

Generation[x]	Growth cond.	No. plants[y]	Silique length (mm)		Seeds per silique		Percent fertile plants[z]
			Range	Mean ± SD	Range	Mean ± SD	
F_3	GH	296 (211)	9.6-54.6	27.4 ± 7.75	0.2-23.0	3.8 ± 3.49	71%
BC_1F_2	GH	505 (219)	10.0-38.8	21.7 ± 5.98	0-7.0	0.7 ± 1.33	43%
F_4	GH	399 (164)	11.8-59.0	30.5 ± 8.49	0-12.8	2.7 ± 2.78	41%
BC_1F_3	GH	680 (239)	7.0-60.0	30.9 ± 9.47	0-13.0	2.5 ± 2.55	35%
F_5	Field	432 (413)	12.7-65.3	40.8 ± 10.66	0-34.7	20.3 ± 7.71	96%
BC_1F_4	Field	546 (507)	11.7-60.7	36.4 ± 10.91	0-39.3	17.5 ± 8.15	93%
F_6	GH	257 (163)	13.7-67.7	38.0 ± 11.92	0-25.3	8.4 ± 6.02	63%
BC_1F_5	GH	272 (185)	15.0-62.7	39.5 ± 10.4	0.3-24.3	8.6 ± 5.62	68%
F_7	Field	238 (238)	24.3 - 81.0	53.4 ± 9.11	2.4 - 41.3	25.4 ± 8.25	100%
BC_1F_6	Field	266 (266)	27.0 - 76.0	52.6 ± 8.88	1.2 - 38.0	23.6 ± 7.78	100%

[x]Pooled data of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* in case of F_2-derived populations while, pooled data of *B. napus* × (*B. napus* × *B. oleracea* var. *alboglabra*) and *B. napus* × (*B. napus* × *B. oleracea* var. *botrytis*) crosses in case of BC_1- derived populations.

[y]Within brackets, number plants producing silique with seeds.

[z]Calculated based on number plants producing seeds divided by total number of observed plants and multiplied by hundred.

2.3.2 Flow cytometric analysis

Flow cytometric analysis was done to estimate the relative nuclear DNA content (partec value) in F_6 and BC_1F_5 as well as in F_8 and BC_1F_7 generation plants by the use of a Partec flow cytometer.

F_6 and BC_1F_5 population

The partec value of A04-73NA ranged from 354 to 399 with a mean of 381 ± 15.95 SD. In case of the *B. oleracea* parents' var. *alboglabra* and var. *botrytis*, partec values ranged from 202 to 241 and 201 to 248 with means of 222 ± 13.49 SD and 218 ± 16.17 SD, respectively. Confidence limits of the *B. napus* and *B. oleracea* parents for partec value was calculated, which was 375 to 387 for *B. napus* and 213 to 227 for *B. oleracea*. Based on this, the F_6 and BC_1F_5 plants were classified into five different groups. The plants with partec value falling within the confidence limits of the *B. napus* parent were considered as "*B. napus* type" while those falling within the confidence limits of *B. oleracea* were considered as "*B. oleracea* type". The plants having partec value in between *B. oleracea* and *B. napus* were considered as "intermediate type". Plants with partec value less than *B. oleracea* or greater than *B. napus* were grouped into two different groups. Plant fertility, estimated based on silique length and number seed per silique, as well as seed quality traits of the plants falling in these groups is presented in Table 2.4.

Of the 226 F_6 plants, four plants had partec value less than *B. oleracea* with mean of 192 ± 3.75 SD, while only one of the 248 BC_1F_5 plants had partec value less than *B. oleracea* (Group-1). However, one of the four F_6 plants produced silique of 25.4 mm size with 3.5 seeds per silique while the other three plants were sterile. On the other hand, the single BC_1F_5 plant belonging to Group-1 produced 39.7 mm long silique with 5.3 seeds per silique. This BC_1F_5

plant contained 0.03% erucic acid in seed oil and had 24.1 µmol/g seed glucosinolate. None of the other F_6 and BC_1F_5 plants had partec value similar to *B. oleracea* (Table 2.4).

Forty two F_6 and 72 BC_1F_5 plants fell within the confidence limits of *B. napus* (Group-4). The mean partec values in this group of plants were 380 ± 4.11 SD and 381 ± 4.23 SD for F_6 and BC_1F_5, respectively. Mean silique length and number of seeds per silique in this group of plants was 42.5 ± 12.14 mm SD and 9.9 ± 5.01 SD in case of F_6 while, 40.7.8 ± 0.48 mm SD and 9.8 ± 6.55 SD in case of the BC_1F_5 plants, respectively. Plant fertility, estimated based on number plants producing viable seed was 88% and 83 % in F_6 and BC_1F_5 population, respectively. This group of F_6 plants had erucic acid and GSL contents of 0.17 ± 0.02% SD and 22.06 ± 10.60 µmol/g seed SD, respectively, while erucic acid and GSL contents in BC_1F_5 plants was 0.22 ± 0.23% SD and 18.08 ± 8.28 µmol/g seed SD , respectively (Table 2.4).

Sixty nine F_6 and 43 BC_1F_5 plants that had partec value in between the confidence limits of *B. oleracea* and *B. napus* were placed in Group-3 and were considered "intermediate type". Mean partec value of the F_6 plants belonging to this group was 355 ± 13.47 SD (range 302 to 372) while for BC_1F_5 plants it was 358 ± 15.77 SD (range 292 to 372). These F_6 plants had mean silique length of 37.6 ± 11.08 mm SD with 8.5 ± 6.88 SD seeds per silique, while, the BC_1F_5 plants had mean silique length of 41.1 ± 9.94 mm SD with 10.0 ± 6.00 SD seeds per silique. This group of F_6 and BC_1F_5 plants had erucic acid content of 0.24 ± 0.12% SD and 0.19 ± 0.21% SD and GSL contents of 21.26 ± 9.55 SD and 19.89 ± 8.16 µmol/g seed SD, respectively.

One hundred eleven F_6 plants with partec values of 438 ± 55.33 SD (range 388 to 623) and 132 BC_1F_5 plants with partec value of 420 ± 15.35 SD (range 387 to 502) fall beyond the confidence limits of the *B. napus* parent A04-73NA (Group-5). The F_6 plants falling in this group showed mean silique length of 34.6 ± 11.29 mm SD and 6.7 ± 4.86 SD seeds per silique. On the

other hand, the BC_1F_5 plants falling in this group had mean silique length of 37.9 ± 8.93 SD mm with 7.1 ± 4.09 SD seeds per silique. About 53% F_6 and 62% BC_1F_5 plants of this group produced silique with seeds (Table 2.4). The F_6 and BC_1F_5 plants had 1.39 ± 3.95 SD and 0.53 ± 1.33 SD % erucic acid in seed oil, and 22.7 ± 10.07 SD and 24.36 ± 10.34 SD µmol GSL/g seed, respectively (Table 2.4).

Silique length and number of seeds per silique was highest in the plants belonging to Group-3 and Group-4. Results during this study revealed that relative nuclear DNA content in most of the F_6 and BC_1F_5 plants reached close to the *B. napus* parent A04-73NA ($2n = 38$), while only few plants had relative nuclear DNA content similar to *B. oleracea* ($2n = 18$).

Table 2.4 Estimation of relative nuclear DNA content (partec value) in F_6 and BC_1F_5 generation populations of *B. napus* × *B. oleracea* interspecific crosses.

Group[a]	Gen.[b]	Total[c]	Partec value[d]	Silique length (mm)	Seeds per silique	Percent fertile plants[e]	Erucic acid %	Glucosinolate (µmol/g seed)
Group-1 (<*B. oleracea*)	F_6	4 (1)	**192 ± 3.75** (188 - 197)	25.4 ± 0	3.5 ± 0	25%	---	---
	BC_1F_5	1 (1)	**199 ± 0**	39.7 ± 0	5.3 ± 0	100%	0.03 ± 0	24.06 ± 0
Group-2 (*B. oleracea*)	F_6	---	---	---	---	---	---	---
	BC_1F_5	---	---	---	---	---	---	---
Group-3 (Intermediate)	F_6	69 (51)	**355 ± 13.47** (302 - 372)	37.6 ± 11.08	8.5 ± 6.88	74%	0.24 ± 0.12	21.26 ± 9.55
	BC_1F_5	43 (39)	**358 ± 15.77** (292 - 372)	41.1 ± 9.94	10.0 ± 6.00	91%	0.19 ± 0.21	19.89 ± 8.16
Group-4 (*B. napus* type)	F_6	42 (37)	**380 ± 4.11** (373 - 387)	42.5 ± 12.14	9.9 ± 5.01	88%	0.17 ± 0.02	22.06 ± 10.60
	BC_1F_5	72 (60)	**381 ± 4.23** (373 - 387)	40.7 ± 10.48	9.8 ± 6.55	83%	0.22 ± 0.23	18.08 ± 8.28
Group-5 (>*B. napus*)	F_6	111 (59)	**438 ± 55.33** (388 - 623)	34.6 ± 11.29	6.70 ± 4.86	53%	1.39 ± 3.95	22.7 ± 10.07
	BC_1F_5	132 (82)	**420 ± 15.35** (387 - 502)	37.9 ± 8.93	7.1 ± 4.09	62%	0.53 ± 1.33	24.36 ± 10.34
A04-73NA (*B. napus* check)		24	**381 ± 15.95** (358 - 400)	52.2 ± 5.92	22.3 ± 10.39	---	0.14 ± 0.05	11.68 ± 3.56
B. oleracea var. *alboglabra*		9	**222 ± 13.49** (202 - 244)	---	---	---	---	---
B. oleracea var. *botrytis*		9	**218 ± 16.17** (201 - 248)	---	---	---	---	---

[a]Groups based on confidence limits of *B. napus* A04-73NA (375-387) and *B. oleracea* parents' var. *alboglabra* and var. *botrytis* (213-227).

[b]F_6 plants of A04-73NA × *B. oleracea* var. *alboglabra* and A04-73NA × *B. oleracea* var. *botrytis*, and BC_1F_5 plants of A04-73NA × (A04-73NA × *B. oleracea* var. *alboglabra*) and A04-73NA × (A04-73NA× *B. oleracea* var. *botrytis*) crosses.

[c]Total number of plants, within brackets number plants producing silique with seeds.

[d]Partec value or relative nuclear DNA content presented as Mean ± SD in bold font; inside brackets range of partec values given.

[e]Plant fertility estimated based on number plants producing silique with seeds divided by total number of plants and multiplied by hundred.

F_8 and BC_1F_7 population

In the case of F_8 and BC_1F_7 populations, two samples per plant were analysed by flow cytometer and mean values were used for statistical analysis. The mean values of relative nuclear DNA content (partec value) of the F_8 and BC_1F_7 populations were not significantly (P-value > 0.05) different from *B. napus* parent A04-73NA; however, these values were significantly (P-value < 0.05) different from *B. oleracea* (Table 2.5). This suggested that F_8 and BC_1F_7 plants derived from *B. napus* × *B. oleracea* and (*B. napus* × *B. oleracea*)× *B. napus* crosses had chrosome number similar to *B. napus* ($2n = 38$).

Table 2.5 Relative nuclear DNA content in F_8 and BC_1F_7 generation population of *B. napus* × *B. oleracea* interspecific cross.

Generation	Observation	Range	Partec value (Mean ± SD)[†]
F_8[x]	105	341 – 396	369 ± 9.51a
BC_1F_7[y]	96	351 – 406	368 ± 10.30a
B. oleracea[z]	6	187 – 206	198 ± 7.06b
A04-73NA (*B. napus*)	51	341 – 384	365 ± 8.76a

[x]F_8 plants of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis*.
[y]BC_1F_7 plants of (*B. napus* × *B. oleracea* var. *alboglabra*) × *B. napus* and (*B. napus* × *B. oleracea* var. *botrytis*) × *B. napus*.
[z]*B. oleracea* parents' var. *alboglabra* and var. *botrytis*.
[†]Sharing of same letter indicate no significant difference according to Tukey test (P-value > 0.05).

2.3.3 Erucic acid

Erucic acid content in F_2 and subsequent generations of the crosses A04-73NA × *B. oleracea* var. *alboglabra* (cross ID 5CA1300) and A04-73NA × *B. oleracea* var. *botrytis* (cross ID 5CA1343) as well as in BC_1 of crosses (A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA (cross ID 5CA1676) and (A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA (cross ID 5CA1677) are presented in Table 2.6 and pooled data of these crosses are summarized in Table 2.8

F_2 and BC_1 generation

Erucic acid content in F_2 plants of 5CA1300 and 5CA1343 ranged between 0 to 18.7 % and 0.4 to 20.1 % with mean of 12.52 % ± 4.65 SD and 8.35 % ± 6.78 SD, respectively. The difference between the means of the two F_2 populations was not statistically significant (P-value > 0.05). Frequency distribution of the F_2 and BC_1 population for erucic acid content is presented in Fig. 2.4 and 2.5, respectively. The mean erucic acid content in BC_1 population of the two backcrosses 5CA1676 and 5CA1677 was 7.19 % ± 5.29 SD and 7.96 % ± 5.78 SD, respectively (Table 2.6).

F_3 generation

A total of 150 F_3 plants from two crosses were analysed. Mean erucic acid content in this population was 11.15 % ± 7.60 SD and varied between 0.0 and 28.0 % (Table 2.8). The F_3 plants of the two crosses showed similar variation for this fatty acid, and the mean values were not significantly different (11.83 % ± 7.37 SD for 5CA1300 and 9.85 % ± 7.95 SD for 5CA1343). The mean of the selected population of the two crosses was significantly (P-value < 0.05) lower than the whole F_3 population (Table 2.8).

Erucic acid analysis could not be performed on the seeds harvested from BC_1F_2 plants due to lack of the amount of seeds needed (0.2 g) for this analysis on bulk sample basis.

F_4 and BC_1F_3 generation

The mean erucic acid content in F_4 population of 5CA1300 and 5CA1343 was 6.55 % ± 7.48 SD and 3.33 % ± 5.41 SD, respectively; this difference between the two crosses was statistically significant (P-value < 0.05). The mean erucic acid content in BC_1F_3 population of 5CA1676 and 5CA1677 was 4.39 % ± 6.34 SD and 6.70 % ± 8.19 SD, respectively (Table 2.6). About 61% F_4 and 57% BC_1F_3 plants had eruci content less than 1% (Fig. 2.2, 2.3).

F_5 and BC_1F_4 generation

In F_5 population, erucic acid ranged from 0.0 to 24.0 % with mean 4.53 ± 7.29 SD in case of the cross 5CA1300 and 0.02 to 23.3 % with a mean of 1.48 ± 4.20 SD in 5CA1343. The BC_1F_4 population of the two crosses, 5CA1676 and 5CA1677, were similar (P-value > 0.05) for erucic acid content with mean values of 2.91± 5.66 SD and 3.62 ± 6.61 SD, respectively.

F_6 and BC_1F_5 generation

Most of the F_6 and BC_1F_5 generation plants were zero or low erucic acid type as evident from their mean values (Table 2.8). Thus, these results demonstrated that zero erucic acid families can be achieved in the populations derived from both F_2 and BC_1 of both *B. napus* × *B. oleracea* crosses through repeated selection for low erucic acid content.

Table 2.6 Erucic acid content (% of total fatty acids) in F_2- and BC_1-derived populations of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* interspecific crosses.

			Whole population			Selected population		
Cross ID[1]	Gen.	Growth Cond.[2]	No. plants (families)	Range	Mean ± SD[3]	No. plants (families)	Range	Mean ± SD
5CA1300	F_2	GH	21 (1)	0.2 - 18.7	12.52 ± 4.65b	all to next generation		
5CA1676	BC_1	GH	36 (1)	0.1 - 19.2	7.19 ± 5.29c	all to next generation		
5CA1343	F_2	GH	15 (1)	0.4 - 20.1	8.35 ± 6.78bc	all to next generation		
5CA1677	BC_1	GH	28 (1)	0.2 - 19.8	7.96 ± 5.78c	all to next generation		
A04-73NA[4]		GH	5	0.0 - 0.2	0.10 ± 0.05a			
5CA1300	F_3	GH	99 (35)	0.0 - 27.5	11.83 ± 7.37b	47 (28)	0.0 - 17.3	7.26 ± 5.66
5CA1343	F_3	GH	51 (24)	0.1 - 28.0	9.85 ± 7.95b	31 (19)	0.1 - 23.1	6.13 ± 5.44
A04-73NA[4]		GH	4	0.1 - 0.2	0.13 ± 0.03a			
5CA1300	F_4	GH	65 (31)	0.0 - 20.2	6.55 ± 7.48c	all to next generation		
5CA1676	BC_1F_3	GH	100 (53)	0.1 - 29.7	4.39 ± 6.34bc	all to next generation		
5CA1343	F_4	GH	61 (23)	0.1 - 19.5	3.33 ± 5.41b	all to next generation		
5CA1677	BC_1F_3	GH	90 (39)	0.1 - 29.6	6.70 ± 8.19c	all to next generation		
A04-73NA[4]		GH	4	0.0 - 0.2	0.13 ± 0.09a			
5CA1300	F_5	Field	116 (48)	0.0 - 24.0	4.53 ± 7.29ac	39 (22)	0.0 - 11.4	1.47 ± 3.18
5CA1676	BC_1F_4	Field	180 (75)	0.0 - 22.0	2.91 ± 5.66abc	62 (36)	0.0 - 1.4	0.21 ± 0.24
5CA1343	F_5	Field	116 (47)	0.0 - 23.3	1.48 ± 4.20ab	42 (23)	0.0 - 0.6	0.17 ± 0.16
5CA1677	BC_1F_4	Field	139 (64)	0.0 - 34.6	3.62 ± 6.61ac	45 (28)	0.0 - 5.4	0.41 ± 0.79
A04-73NA[4]		Field	7	0.1 - 0.16	0.13 ± 0.02a			
5CA1300	F_6	GH	88 (35)	0.03 - 17.6	1.13 ± 3.34ac	70 (29)	0.0 - 0.7	0.28 ± 0.20
5CA1676	BC_1F_5	GH	95 (58)	0.0 - 0.7	0.10 ± 0.17ab	86 (55)	0.0 - 0.7	0.09 ± 0.16
5CA1343	F_6	GH	75 (36)	0.0 - 0.6	0.15 ± 0.14ab	61 (33)	0.0 - 0.6	0.16 ± 0.15
5CA1677	BC_1F_5	GH	90 (41)	0.0 - 6.6	0.39 ± 0.91ac	78 (39)	0.0 - 0.9	0.15 ± 0.27
A04-73NA[4]		GH	4	0.1 - 0.2	0.15 ± 0.02a			

[1]Cross ID; 5CA1300 = *B. napus* × *B. oleracea* var. *alboglabra*, 5CA1676 = (*B. napus* × *B. oleracea* var. *alboglabra)* × *B. napus*, 5CA1343 = *B. napus* × *B. oleracea* var. *botrytis*, and 5CA1677 = (*B. napus* × *B. oleracea* var. *botrytis*) × *B. napus*.

[2]Growth condition, GH = greenhouse.

[3]Sharing of same letter indicate no significant difference according to Tukey test (P-value > 0.05).

[4]Confidence limits of the *B. napus* parent A04-73NA for erucic acid content was 0.06 - 0.14, 0.10 - 0.16, 0.05 - 0.21, 0.11 - 0.15 and 0.13 - 0.17 for the plants grown along with F_2 & BC_1, F_3 & BC_1F_2, F_4 &BC_1F_3, F_5 & BC_1F_4, and F_6 & BC_1F_5 generation population, respectively.

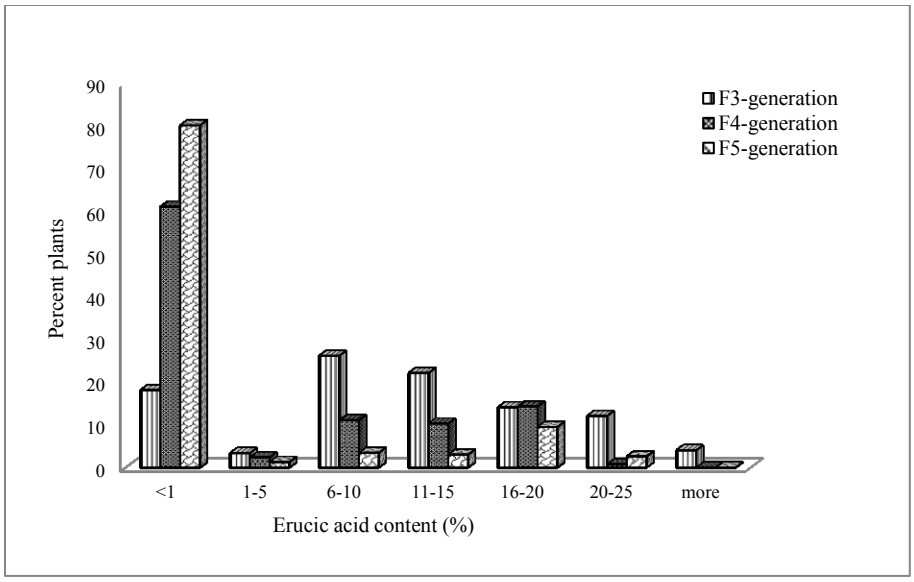


Fig. 2.2. Frequency distribution of the proportion of different generation population of *B. napus* × *B. oleracea* interspecific cross for erucic acid content. Pooled data of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* interspecific crosses presented.

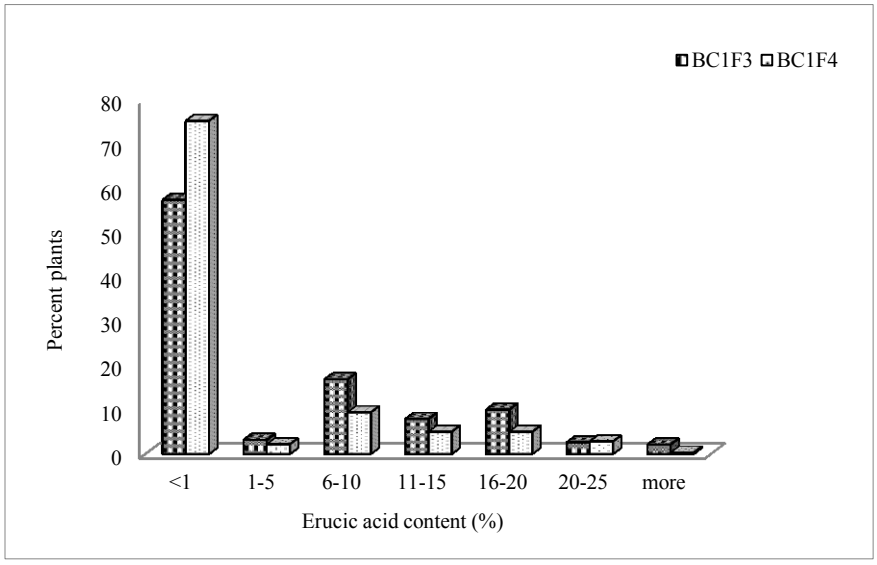


Fig. 2.3 Frequency distribution of the proportion of different generation population of (*B. napus* × *B. oleracea*) × *B. napus* interspecific cross for erucic acid content. Pooled data of (*B. napus* × *B. oleracea* var. *alboglabra*) × *B. napus* and (*B. napus* × *B. oleracea* var. *botrytis*) × *B. napus* interspecific crosses presented.

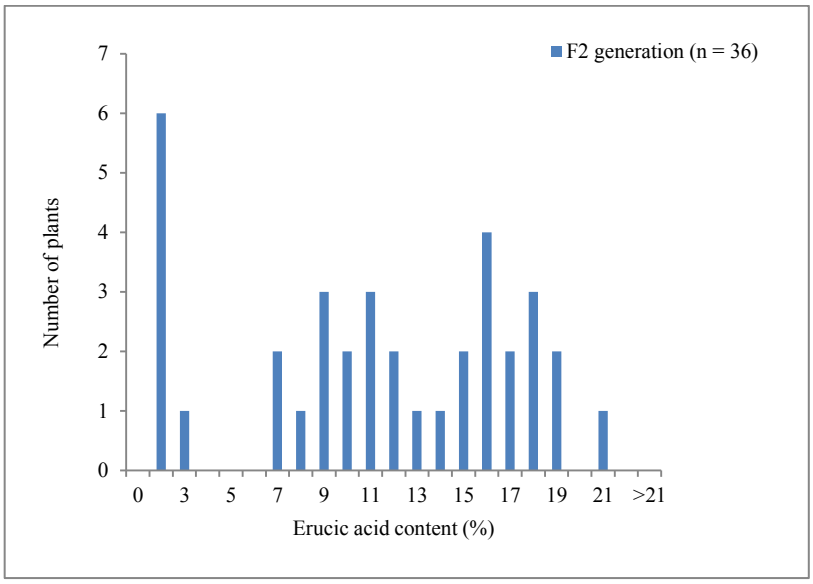


Fig. 2.4 Frequency distribution of the F_2 (n = 36) plants of *B. napus* × *B. oleracea* interspecific cross for erucic acid content. Pooled data of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* interspecific crosses included.

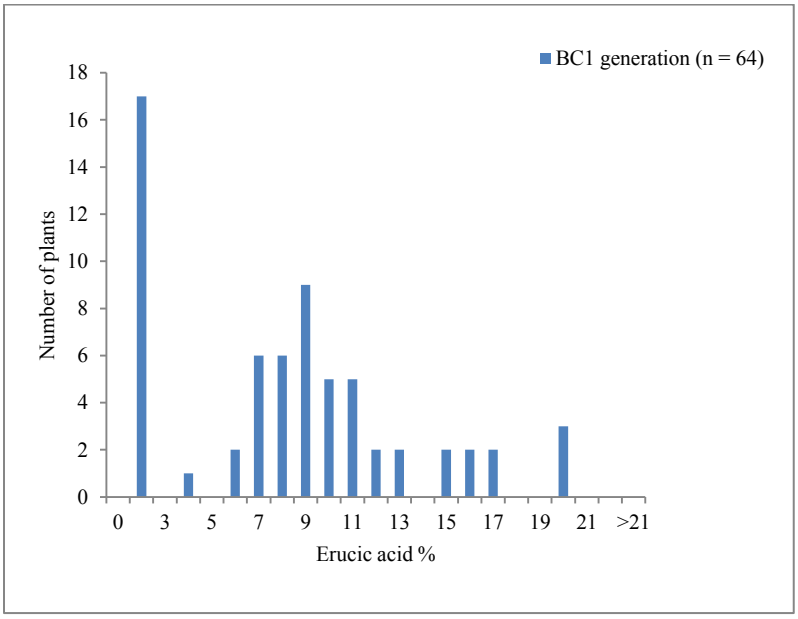
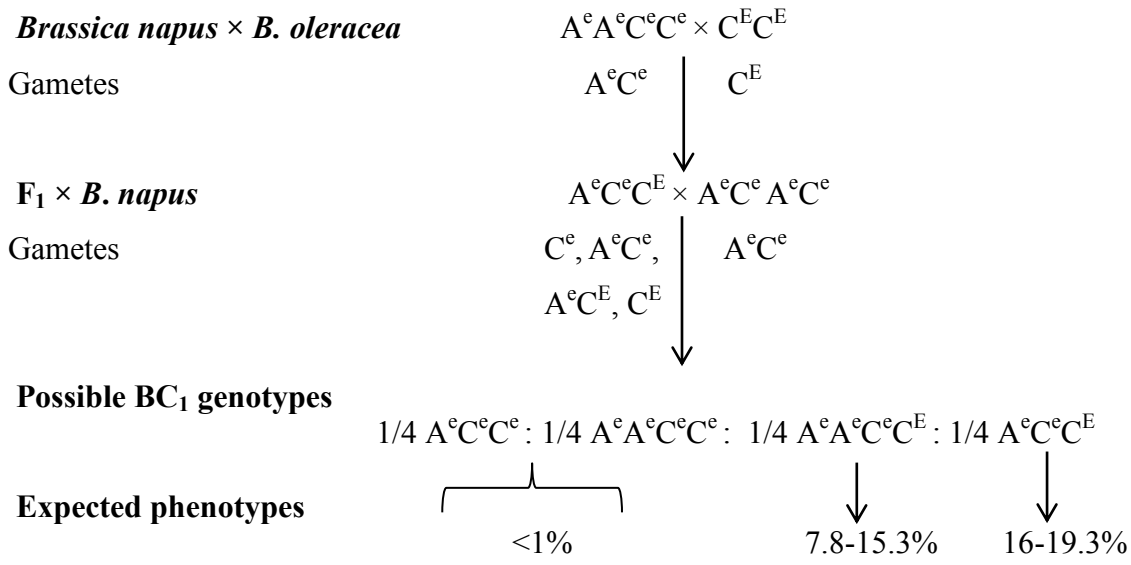


Fig. 2.5 Frequency distribution of the BC_1 (n = 64) plants of (*B. napus* × *B. oleracea*) × *B. napus* interspecific cross for erucic acid content. Pooled data of (*B. napus* × *B. oleracea* var. *alboglabra*) × *B. napus* and (*B. napus* × *B. oleracea* var. *botrytis*) × *B. napus* interspecific crosses included.



where;

A^e = zero erucic acid allele from the *B.napus* A genome

C^e = zero erucic acid allele from the *B.napus* C genome

C^E = zero erucic acid allele from the *B. oleracea* C genome

Fig. 2.6 Expected genotypic frequency of BC_1 plants and their phenotype based on segregation of C-genome erucic acid alleles.

2.3.4 Glucosinolate (GSL) content

Glucosinolate (GSL) content in different generation populations derived from F_2 of A04-73NA × *B. oleracea* var. *alboglabra* (cross ID 5CA1300) and A04-73NA × *B. oleracea* var. *botrytis* (cross ID 5CA1343) crosses, and BC_1 of (A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA (cross ID 5CA1676) and (A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA (cross ID 5CA1677) crosses are presented in Table 2.7; and pooled data of these crosses are summarized in Table 2.7. Confidence limits for the *B. napus* parent A04-73NA, grown along with these populations, was calculated and used to compare with each of these populations. Due to poor seed in F_2, BC_1 and BC_1F_2 generation, analysis for GSL content was started in F_3 and BC_1F_3 generation.

F_3 generation

Mean GSL content in seeds harvested from F_3 plants of the two crosses ranged from 7.5 to 60.8 µmol/g seed with mean of 34.13 ± 12.37 SD (Table 2.7). Variation for GSL content, and the mean values of the two crosses, 5CA1300 and 5CA1343, was not significantly (P-value > 0.05) different (Table 2.7). About 9% F_3 plants of the two crosses had GSL content similar or lower than the *B. napus* parent A04-73NA. Emphasis in this generation was given on selection of plants with high fertility (*B. napus* type); therefore, mean GSL content in the population selected for growing the next generation population was not significantly different from the whole population mean (Table 2.7).

F_4 and BC_1F_3 generation

Glucosinolate content in F_4 plants of the cross 5CA1300 varied from 11.9 to 54.0 µmol/g seed with mean 30.56 ± 9.81 SD, and in cross 5CA1343 it ranged from 7.1 to 62.3 µmol/g seed with mean 34.49 ± 13.94 SD. These two F_4 means were not significantly different (P-value >

0.05) from their F_3 population means (32.2 ± 13.94 SD and 33.0 ± 10.85 SD) (Table 2.7). In case of the two backcross populations 5CA1676 and 5CA1677, GSL content varied from 8.6 to 52.8 µmol/g seed and 8.6 to 61.0 µmol/g seed with mean of 29.01 ± 10.36 SD and 31.29 ± 12.77 SD, respectively (Table 2.7). About 4% of the F_4 and BC_1F_3 plants fell within the confidence limits of A04-73NA or had GSL content lower than this parent (Table 2.7). No selection for plant fertility or GSL content was done in F_4 and BC_1F_3 generation populations.

F_5 and BC_1F_4 generation

Glucosinolate content in F_5 generation of 5CA1300 and 5CA1343 ranged from 11.0 to 65.7 and 11.7 to 59.3 µmol/g seed with mean of 30.78 ± 12.54 SD and 32.94 ± 11.39 SD, respectively. In BC_1F_4 generation of 5CA1676 and 5CA1677, GSL varied from 8.1 to 54.2 and 8.6 to 57.2 µmol/g seed with mean of 25.48 ± 10.12 SD and 26.69 ± 11.29 SD, respectively (Table 2.7). Both F_5 and BC_1F_4 populations had significantly (P-value < 0.05) higher GSL content than the *B. napus* parent A04-73NA; however, about 34 % of the F_5 and 48% of the BC_1F_4 plants had GSL content similar or lower than the check A04-73NA (Table 2.7). Selection for low GSL content was done in these populations. This reflected in the F_5 and BC_1F_4 plants selected for growing the next generation population, which had significantly lower (P-value < 0.05) content of GSL as compared to the respective whole population.

F_6 and BC_1F_5 generation

F_6 populations of the cross 5CA1300 had significantly (P-value < 0.05) lower GSL content than that of the cross 5CA1343; however, GSL content in the two BC_1F_5 populations of the crosses 5CA1676 and 5CA1677 were similar (Table 2.7). About 22% of the F_6 and 31% of the BC_1F_5 plants had GSL content similar to the *B. napus* parent A04-73NA (Table 2.7).

F_7 and BC_1F_6 generation

GSL content in F_7 and BC_1F_6 populations, estimated on seeds harvested from field, varied from about 12 to more than 45 µmol/g seed. However, seeds of majority of the F_7 and BC_1F_6 plants had GSL content similar to the *B. napus* parent as evident from no significant (P-value > 0.05) difference between these two populations (except F_7 of 5CA1343) and A04-73NA. A total of more than 80% of the F_7 and BC_1F_6 plants had GSL content comparable to the *B. napus* check A04-73NA (Table 2.7).

Overall, plants with GSL content comparable to the *B. napus* parent A04-74NA were achieved from both F_2- and BC_1-derived populations of the two interspecific crosses. Compared to F_2-derived populations, greater proportion of plants with low GSL content was obtained in BC_1-derived populations (Table 2.7). In general, there was a gradual increase in the proportion of low GSL plants with the progression of generations from F_2 to F_7 and BC_1 to BC_1F_6. For instance, the proportion of plants that had GSL content comparable to the *B. napus* parent A04-73NA was 3% in F_3, and that increased to 5% in F_4 and 39% in F_7 generation population (Fig. 2.7). Similarly, the proportion of low GSL plants in BC_1F_3 population was 9% and that increase to 39% in BC_1F_6 population (Fig. 2.8).

Table 2.7 Glucosinolate content (µmol/g seed) in populations derived from F_2 and BC_1 of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* interspecific crosses.

			Whole population					Selected population		
Cross ID[1]	Gen.	Growth Cond.[2]	No. plants (families)	Range	Mean ± SD[3]	% *B. napus* type[†]	% better than *B. napus*[††]	No. plants (families)	Range	Mean ± SD
5CA1300	F_3	GH	71 (29)	7.5 - 60.8	34.64 ± 12.70b	0.0	1.4	40 (24)	7.5 - 60.8	32.24 ± 13.94
5CA1343	F_3	GH	27 (17)	7.2 - 52.8	32.8 0 ± 11.55b	3.7	3.7	16 (13)	7.3 - 44.3	32.97 ±10.85
A04-73NA[4]			4	8.6 - 12.5	10.29 ± 1.61a					
5CA1300	F_4	GH	54 (28)	11.9 - 54.0	30.56 ± 9.81b	0.0	0.0	all to next generation		
5CA1676	BC_1F_3	GH	77 (45)	8.6 - 52.8	29.01 ± 10.36b	1.3	0.0	all to next generation		
5CA1343	F_4	GH	45 (22)	7.1 - 62.3	34.49 ± 13.94b	0.0	4.4	all to next generation		
5CA1677	BC_1F_3	GH	70 (43)	8.6 - 61.0	31.29 ± 12.77b	2.9	0.0	all to next generation		
A04-73NA[4]			7	8.4 - 10.9	9.61 ± 0.87a					
5CA1300	F_5	Field	67 (67)	11.0 - 65.7	30.78 ± 12.54cd	3.0	16.4	22 (22)	8.1 - 26.6	18.04 ± 5.01
5CA1676	BC_1F_4	Field	92 (92)	8.1 - 54.2	25.48 ± 10.12b	5.4	17.4	37 (37)	8.1 - 26.6	18.04 ± 5.01
5CA1343	F_5	Field	61 (61)	11.7 - 59.3	32.94 ± 11.39d	1.6	13.1	22 (22)	11.7-30.7	21.81 ± 6.37
5CA1677	BC_1F_4	Field	88 (88)	8.6 - 57.2	26.69 ± 11.29bc	5.7	19.3	29 (29)	8.9 - 26.8	18.15 ± 5.54
A04-73NA[4]			13	14.6 - 20.0	17.36 ± 1.60a					
5CA1300	F_6	GH	55 (25)	8.5 - 45.0	19.18 ± 7.97b	5.5	5.5	31 (22)	8.6 - 29.6	16.65 ± 15.38
5CA1676	BC_1F_5	GH	69 (48)	6.6 - 46.6	20.70 ± 8.93bc	7.2	11.6	65 (47)	6.6 - 46.6	20.09 ± 8.79
5CA1343	F_6	GH	56 (29)	7.2 - 46.5	25.09 ± 11.04c	3.6	7.1	32 (25)	7.2- 39.7	22.99 ± 8.64
5CA1677	BC_1F_5	GH	74 (39)	6.3 - 42.8	19.99 ± 9.05b	4.1	8.1	64 (36)	6.3 - 42.6	18.15 ± 7.58
A04-73NA[4]			7	8.4 - 11.7	10.03 ± 1.13a					
5CA1300	F_7	Field	63 (63)	12.6 - 45.5	23.35 ± 8.97 ab	1.6	49.2	36 (36)	12.6 - 24.3	17.15 ± 3.18
5CA1676	BC_1F_6	Field	84 (84)	12.7 - 54.8	26.23 ± 10.29 abc	3.6	33.3	37 (37)	12.7 - 24.5	17.80 ± 3.05
5CA1343	F_7	Field	59 (59)	13.2 - 54.4	28.23 ± 10.99 c	5.1	28.8	22 (22)	13.2 - 24.4	17.30 ± 3.05
5CA1677	BC_1F_6	Field	77 (77)	12.1 - 54.4	22.45 ± 10.72ab	2.6	58.4	39 (39)	12.1 - 19.6	14.85 ± 1.20
A04-73NA[4]			28	16.0 - 24.5	20.22 ± 2.34 a					

[1]5CA1300 = *B. napus* × *B. oleracea* var. *alboglabra*, 5CA1676 = (*B. napus* × *B. oleracea* var. *alboglabra)* × *B. napus*, 5CA1343 = *B. napus* × *B. oleracea* var. *botrytis*, and 5CA1677 = (*B. napus* × *B. oleracea* var. *botrytis*) × *B. napus*.

[2]GH = greenhouse

[3]Sharing of same letter indicate no significant difference according to Tukey test (P-value > 0.05).

[4]Confidence limits of *B. napus* parent A04-73NA for glucosinolate content (µmol/g seed) was 8.7-11.9, 9.0-10.3, 16.5-18.3, 9.2-10.9 and 19.4-21.1 while grown with F_3, F_4 & BC_1F_3, F_5 & BC_1F_4, F_6 & BC_1F_5 and F_7 & BC_1F_6 generation, respectively.

[†]% plants falling within the confidence limits of the *B. napus* parent A04-73NA for glucosinolate content (µmol/g seed).

[††]% plants with glucosinolate content (µmol/g seed) lower than the *B. napus* parent A04-73NA.

Table 2.8 Summary of erucic acid (%) and glucosinolate content (µmol/g seed) in F_2- and BC_1-derived populations of *B. napus* × *B. oleracea* interspecific crosses.

		Erucic acid (%)			Glucosinolate content (µmol/g seed)			
Generation[1]	Growth cond.	No. plants	Range	Mean ± SD	No. plants	Range	Mean ± SD	Percent plants "00" type[3]
F_3	GH[2]	149	0 - 28.0	11.15 ± 7.60	98	7.3 - 60.8	34.13 ± 12.37	2.0%
F_4	GH	126	0 - 20.2	4.99 ± 6.73	99	7.1 - 62.3	32.34 ± 11.96	6.0%
BC_1F_3	GH	190	0.1 - 29.7	5.49 ± 7.35	147	8.6 - 61.0	30.09 ± 11.59	8.2%
F_5	Field	232	0 - 24.0	3.01 ± 6.13	128	11.0 - 65.7	31.81 ± 12.01	14.8%
BC_1F_4	Field	319	0 - 34.6	3.22 ± 6.09	180	8.1 - 57.2	26.07 ± 10.69	20.0%
F_6	GH	163	0.02 - 17.6	0.68 ± 2.49	111	7.2 - 46.5	22.2 ± 10.05	30.6%
BC_1F_5	GH	185	0 - 6.6	0.29 ± 0.66	143	6.3 - 46.6	20.3 ± 8.97	30.1%

[1] F_2-derived populations of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* and BC_1-derived populations of *B. napus* × (*B. napus* × *B. oleracea* var. *alboglabra*) and *B. napus* × (*B. napus* × *B. oleracea* var. *botrytis*).
[2]GH = greenhouse.
[3]Double low (or 00) type plants with less than 1 % erucic acid in seed oil and less than 15 µmol/g glucosinolate content per gram seed.

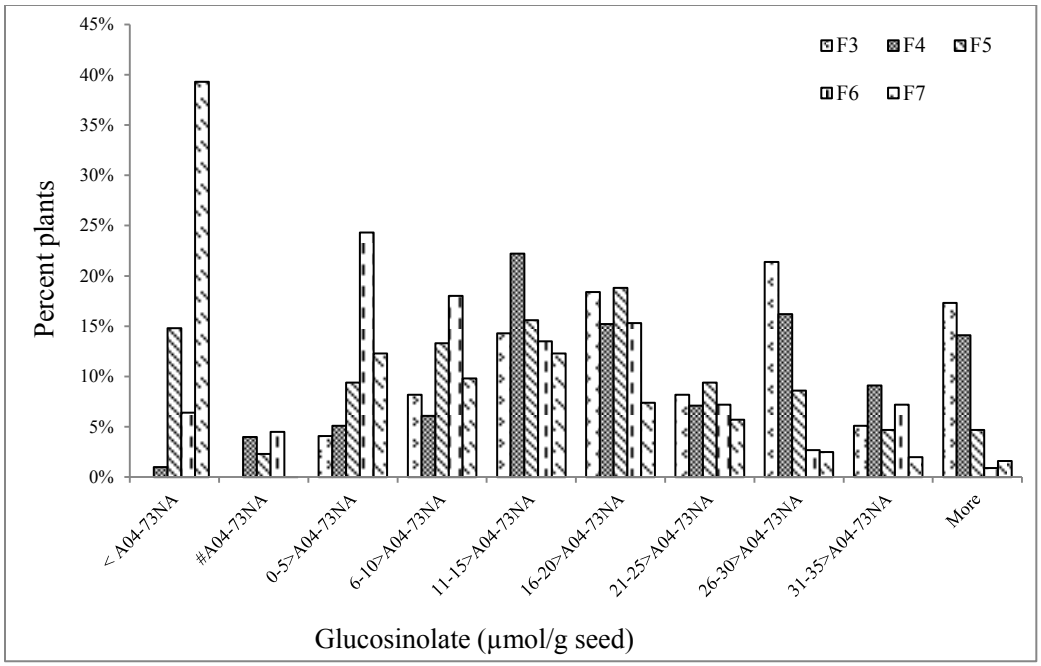


Fig. 2.7 Frequency distribution for seed glucosinolate content (µmol/g seed) in different generation populations derived from F_2 of *B. napus* × *B. oleracea* interspecific cross. Pooled data of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* interspecific crosses included.

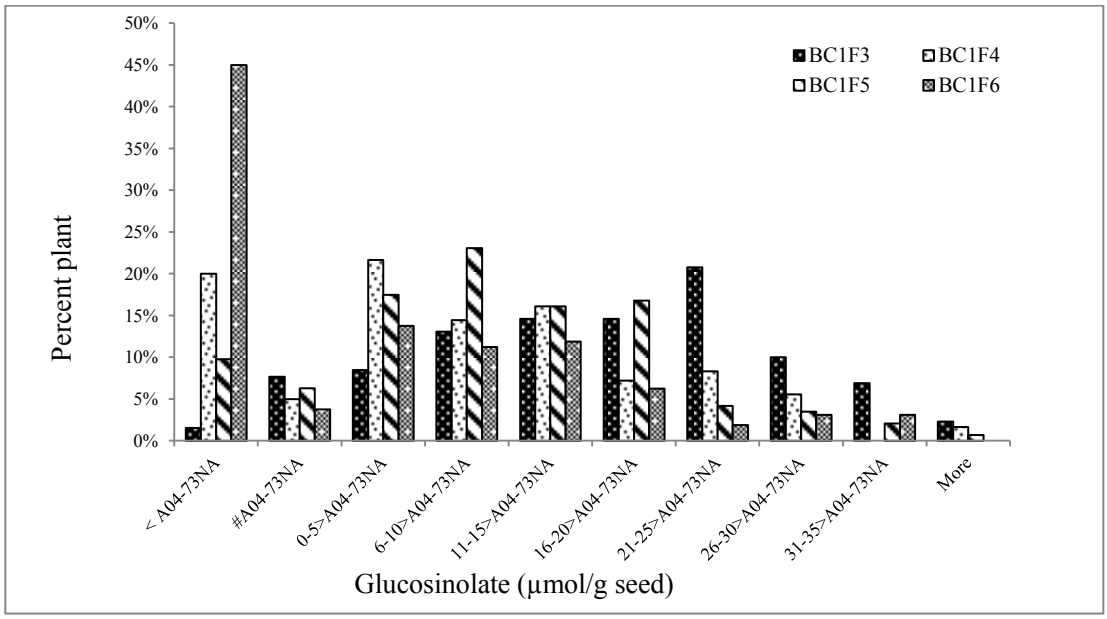


Fig. 2.8 Frequency distribution for seed glucosinolate content (µmol/g seed) in different generation populations derived from BC_1 of (*B. napus* × *B. oleracea*) × *B. napus* interspecific cross. Pooled data of (*B. napus* × *B. oleracea* var. *alboglabra*) × *B. napus* and (*B. napus* × *B. oleracea* var. *botrytis*) × *B. napus* interspecific crosses included.

Correlation between GSL content of parent vs. offspring

Correlation between the parent generation and their offspring generation was calculated for seed GSL content. As, the different generation populations were grown under different growth conditions (greenhouse or field), GSL content data was therefore adjusted based on GSL content of the *B. napus* parent A04-73NA grown along with these populations. For this, GSL content of A04-73NA was subtracted from GSL content of the F_2- or BC_1-derived plants grown under same growth condition along with A04-73NA. This resulted either positive (GSL content higher than confidence limits of A04-73NA) or negative (GSL content lower than confidence limits of A04-73NA) or zero (falling within confidence limits of A04-73NA) values for GSL content of the F_2- or BC_1-derived plants. Based on this data scatter diagrams of parent vs. offspring generations are presented in Fig. 2.9 for the F_2-derived populations and in Fig. 2.10 for the BC_1-derived populations.

Significant correlation (P-value < 0.05) for GSL content was found between F_3 and F_4 and F_5 and F_6 for both 5C1300 and 5CA1343 crosses. In case of the other populations, significant correlation was found between F_4 and F_5 of the cross 5CA1300, and F_6 and F_7 (P-value < 0.01) of the cross 5CA1343. Thus, no consistent correlation between the parent and offspring generation population could be found in case of the populations derived from F_2. Similarly, no specific trend of correlation between the parents and offspring generations could be found in case of populations derived from BC_1.

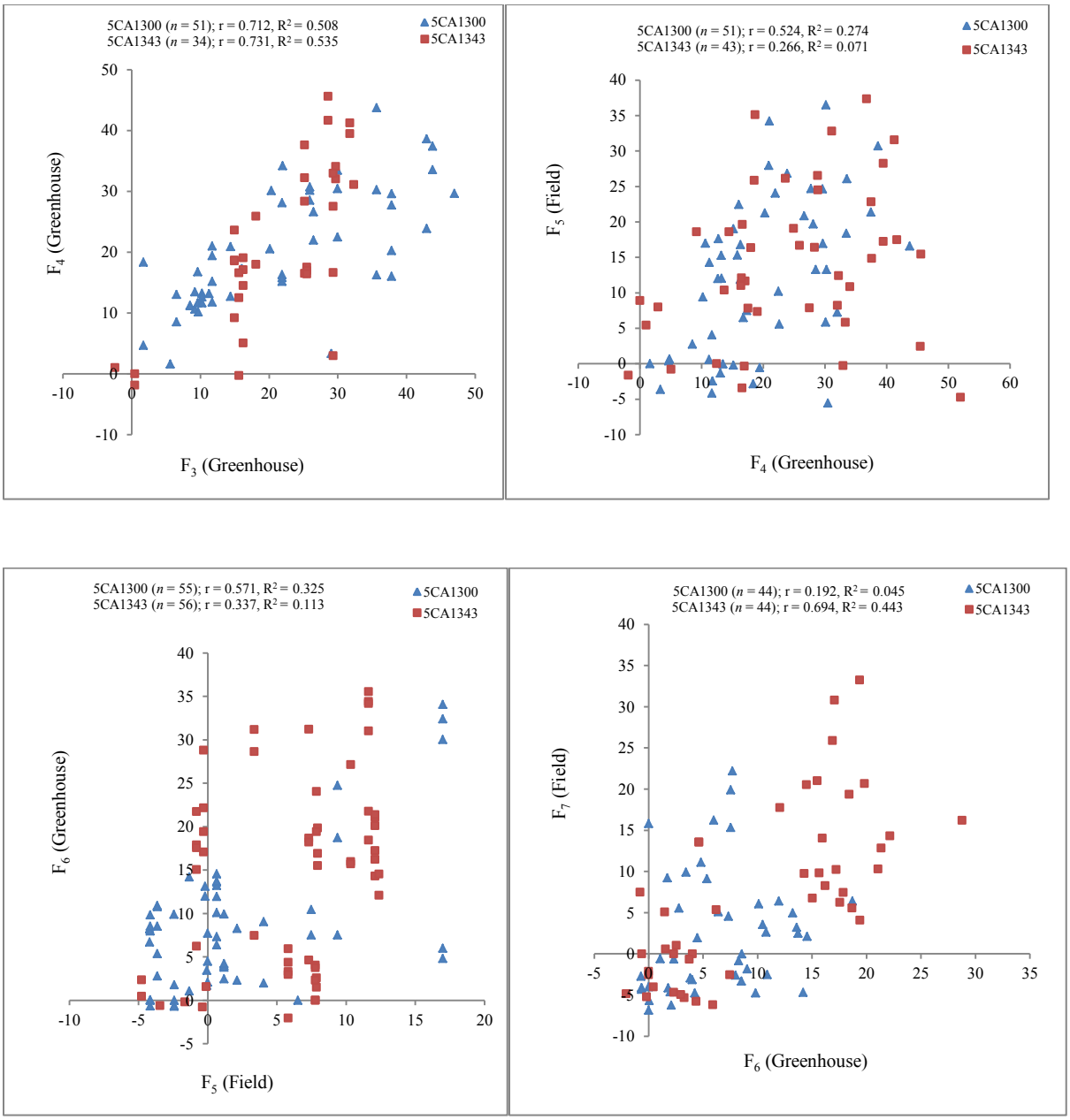


Fig. 2.9 Scatter diagram of parent vs. offspring generation populations derived from F_2 of *Brassica napus* × *B. oleracea* var. *alboglabra* (cross ID 5CA1300) and *Brassica napus* × *B. oleracea* var. *botrytis* (cross ID 5CA1343) interspecific crosses for seed glucosinolate content (µmol/g seed) in comparison to the *B. napus* parent. In brackets, plant growth condition indicated.

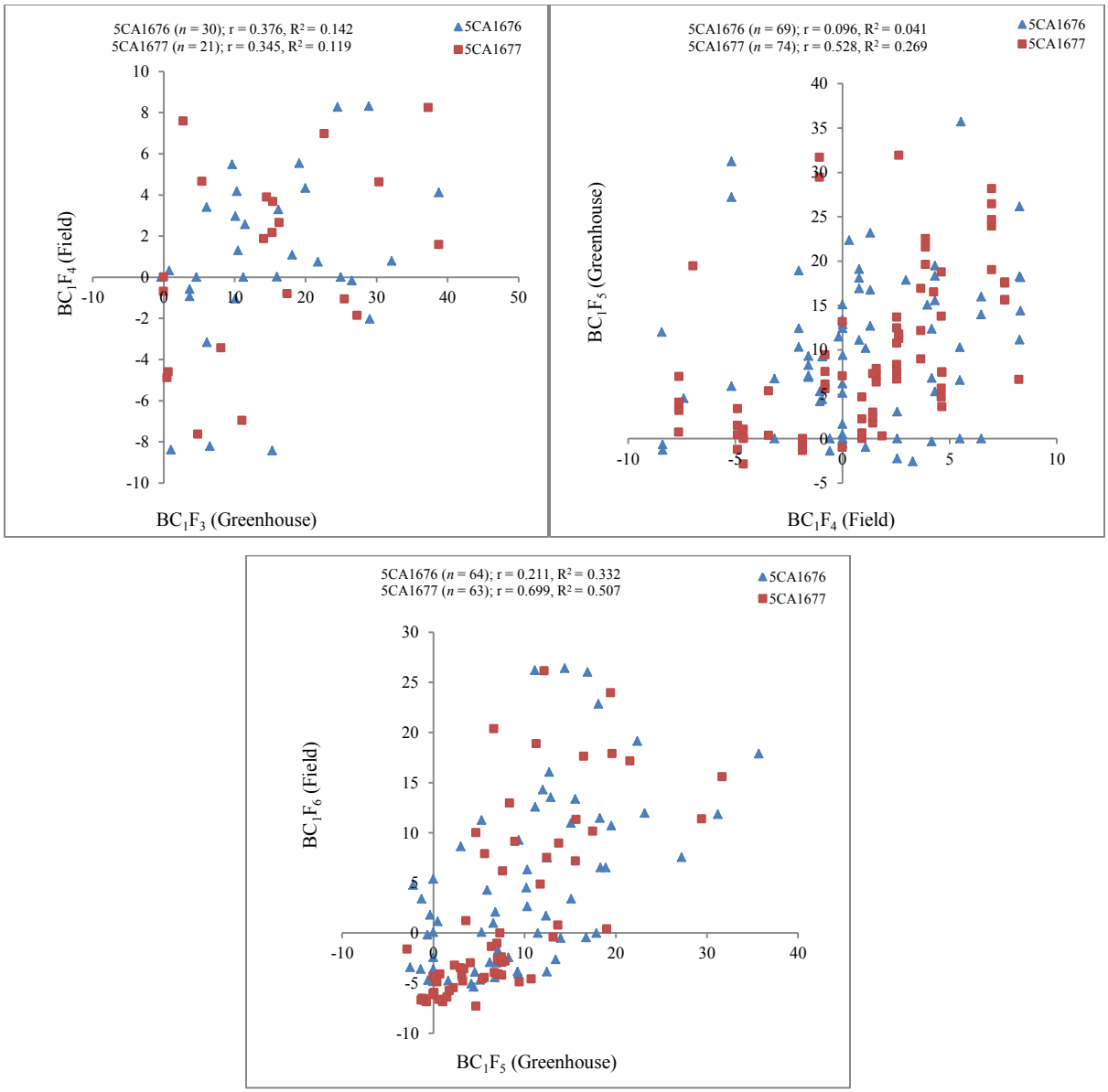


Fig. 2.10 Scatter diagram of parent vs. offspring generation populations derived from BC_1 of (*Brassica napus* × *B. oleracea* var. *alboglabra*) × *Brassica napus* (cross ID 5CA1676) and (*Brassica napus* × *B. oleracea* var. *botrytis*) × *Brassica napus* (cross ID 5CA1677) interspecific crosses for seed glucosinolate content (µmol/g seed) in comparison to the *B. napus* parent. In brackets, plant growth condition indicated.

2.4 Discussion

Cytological studies can provide better insight on chromosome number of the interspecific cross derived plants and their behaviour in meiosis; however this is difficult to apply in a large breeding population derived from interspecific crosses for the advancement of generations. In this study, selection primarily for plant fertility and zero erucic acid and low GLS content resulted canola quality plants. These plants had $2n \approx 38$ chromosomes as evident from flow cytogenetic analysis. Plant fertility was low in early generation populations − apaprantly resulted from the production of aneuploid gametes in high proportion due to meiotic abnormalities; however this improved with the advancement of generation. Rahman et al. (2015) found similar results in F_8 generation plants derived from *B. napus* × *B. oleracea* var. *alboglabr* interspecific cross. Bennett et al. (2012) found $2n \approx 38$ chromosomes in BC_1F_5 plants despite the trigenomic F_1 (ACC) plants of *B. napus* × *B. oleracea* was backcrossed to *B. oleracea* parent. Seed set and plant fertility was higher in open-pollinated plants as compared to self-pollinated plants. Similarly, Li et al. (2013) observed higher seed set on open-pollination in hexaploid plants derived from *B. rapa* × (*B. napus* × *B. oleracea*) cross as compared to self-pollinated plants.

In the present study, inheritance of erucic acid in different generation populations was investigated (Table 2.6). It is well established that erucic acid biosynthesis in *B. napus* is controlled by two major gene loci, one on each genome (A and C) (Harvey and Downey 1964). By using microspore derived *B. napus* plants, Chen and Beversdorf (1990) confirmed that each locus carries two alleles which show additive effect. The *B. napus* parent used in the present study carry zero erucic acid allele in both A and C genomes, while the *B. oleracea* parents carry only high erucic acid allele. Therefore, segregation for erucic acid observed in F_2 and BC_1 populations reflects primarily the segregation of the erucic acid alleles of the C genome. In this

study, erucic acid content in seed oil of F_2 plants ranged from 0 to 20% where none had the content of this fatty acid close to the *B. oleracea* parent (>40%). This is in contrast to Rahman et al. (2015) who reported a variation of <1 (≈ zero) to 43% erucic acid in F_2 population derived from *B. napus* × *B. oleracea* var. *alboglabra* interspecific cross.The possible reason of this difference could be small number of plants (n = 36) analysed in the present study. Segregation for erucic acid in the F_2 population (n = 36) deviated significantly (χ^2 = 10.13, P-value < 0.01) from a 3:1 ratio for presence vs. absence of this fatty acid. This agrees with the results reported by Rahman et al. (2015). Backcrossing of the F_1 ($A^eC^eC^E$ where, e = zero erucic acid allele, E = high erucic acid allele) plants to the *B. napus* parent ($A^eA^eC^eC^e$) expected to produce $A^0C^0C^0$, $A^0A^0C^0C^0$, $A^0A^0C^0C^+$ and $A^0C^0C^+$ genotypes for erucic acid alleles (Fig. 2.6). The genotypes $A^0C^0C^0$ and $A^0A^0C^0C^0$ are expected to be virtually free (<1%) from erucic acid, while the plants with $A^0A^0C^0C^+$ and $A^0C^0C^+$ genotype expected to produce about 10 % (7.8 – 15.3%) and 15% (16 – 19.3%) erucic acid in seed oil (Bennett et al. 2008), respectively. The observed distribution of the BC_1 plants (Fig.2.5) fall roughly into three classes <1%, 3-13% and 15-20% erucic acid. This distribution deviated significantly (χ^2 = 40.34, P-value < 0.01) from expected phenotypic segregation ratio of 2:1:1. However, plants producing low content of erucic acid were obtained in F_2 and BC_1 populations, as well as from self-pollinated progeny of the intermediate erucic acid plants. This study demonstrated that interspecific inbred lines with zero erucic acid in seed oil are achievable from *B. napus* × *B. oleracea* interspecific crosses.

Genetic control of seed glucosinolate (GSL) content is more complex in *B. napus* than the genetic control of erucic acid due to involvement of multiple gene loci (Kondra and Stefansson 1970) and environmental influence on this trait (Röcker and Röbbelen 1994). Different studies (Toroser et al. 1995, Rahman et al. 2001, Howell et al. 2003, Hasan et al. 2008)

have shown that at least three to five loci are involved in the control of seed GSL content in *B. napus*. In case of the A genome of *B. rapa*, which shares one of the two genomes of *B. napus*, three QTL were found for seed GSL content (Rahman et al. 2014). Among the three major types of GSL (aliphatic, aromatic, and indole), the aliphatic GSLs determines the total seed GSL content (Velasco et al. 2008). Predominance of additive gene control of this trait (Rücker and Röbbelen 1994, Rahman et al. 2014) suggests that selection for low GSL content would be effective. In the present study, a continuous variation for GSL content in F_3 to F_7 and BC_1F_3 to BC_1F_6 generation was observed from where repeated selection led to the development of low GSL lines (Table 2.7). This is apparently due to simpler segregation for this trait in the interspecific hybrid progeny where high GSL alleles only from the C genome were involved.

This research study demonstrated that it is feasible to develop canola quality inbred lines with $2n = 38$ chromosomes from *B. napus* × *B. oleracea* interspecific crosses by using *B. oleracea* var. *alboglabra* and var. *botrytis* variants in the crosses through application of pedigree or limited backcross breeding methods. Li et al. (2014) reported significant correlation between genetic diversity of *B. oleracea* introgressed into *B. napus* and heterosis for seed yield in *B. napus*. Therefore, the germplasm derived from this study expected to be valuable in hybrid breeding programs.

2.5 References

Ackman, R. G., 1966: Analysis of the monoethylenic fatty acids of rapeseed oil by open tubular gas chromatography. J. Am. Oil Chem. Soc. 43: 483-486.

Babula, D., M. Kaczmarek, A. Barakat, M. Delseny, C. F. Quiros, and J. Sadowski, 2003: Chromosomal mapping of *Brassica oleracea* based on ESTs from *Arabidopsis thaliana*: complexity of the comparative map. Mol. Genet. Genomics 268: 656-665.

Bennett, R. A., M. R. Thiagarajah, J. R. King, and M. H. Rahman, 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: 593-60.

Bennett, R. A., 2012: Broadening genetic diversity in canola (*Brassica napus*) germplasm using the *B. oleracea* var. *alboglabra* C genome. Ph.D. thesis. University of Alberta, Edmonton, Canada.

Bennett, R. A., G. Séguin-Swartz, and H. Rahman, 2012: Broadening genetic diversity in canola (*Brassica napus* L.) using the C-genome species *B. oleracea*. Crop Sci. 52: 2030-2039.

Canola Council of Canada. http://www.canolacouncil.org. (Retrieved on 10th Jan 2014).

Chen, J. L., and W. D. Beversdorf, 1990: Fatty acid inheritance in microspore-derived populations of spring rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 80: 465-469.

Chen, W., Y. Zhang, X. Liu, B. Chen, J. Tu, and F. Tingdong, 2007: Detection of QTL for six yield-related traits in oilseed rape (*Brassica napus*) using DH and immortalized F_2 populations. Theor. Appl. Genet. 115: 849-858.

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

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

Gaeta, R. T., and J. C. Pires, 2010: Homoeologous recombination in allopolyploids: the polyploid ratchet. New Phytol. 186: 18-28.

Gupta, S. K., and A. Pratap, 2007: History, origin, and evolution. In: Gupta, S.K. (Ed.), Advances in botanical research: Incorporating advances in plant pathology, vol. 45. Academic Press/Elsevier, San Diego, pp. 1-20.

Harvey, B. L., and R. K. Downey, 1964: The inheritance of erucic acid content in rapeseed (*Brassica napus*). Can. J. Plant Sci. 44: 104-111.

Hasan, M., F. Seyis, A. G. Badani, J. P. Kuhnemann , W. Lühs, W. Friedt, and R. J. Snowdon, 2006: Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet. Resour. Crop Evol. 53: 793-802.

Hasan, M., W. Friedt, J. P. Kühnemann, N. M. Freitag, K. Link, and R. J. Snowdon, 2008: Association of gene-linked SSR markers to seed glucosinolate content in oilseed rape (*Brassica napus* ssp. *napus*). Theor. Appl. Genet. 116: 1035-1049.

Howell, P. M., A. G. Sharpe, and D. J. Lydiate, 2003: Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). Genome 46: 454-460.

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., and H. Rahman, 2014: Quantitative trait loci (QTL) mapping of silique length and petal colour in *Brassica rapa*. Plant Breed 133: 609-614.

Kondra, Z. P., and B. R. Stefansson, 1970: Inheritance of the major glucosinolates of rapeseed (*Brassica napus*) meal. Can. J. Plant Sci. 50: 643-647.

Lagercrantz, U., and D. J. Lydiate, 1996: Comparative genome mapping in *Brassica*. Genetics 144: 1903-910.

Li, G., M. Gao, B. Yang, and C. F. Quiros, 2003: Gene for gene alignment between the *Brassica* and *Arabidopsis* genomes by direct transcriptome mapping. Theor. Appl. Genet. 107: 168-180.

Li, Q. F., J. Q. Mei, Y. J. Zhang, J. N. Li, X. H. Ge, Z. Y. Li, and W. Qian, 2013: A large-scale introgression of genomic components of *Brassica rapa* into *B. napus* by the bridge of hexaploid derived from hybridization between *B. napus* and *B. oleracea*. Theor. Appl. Genet. 126: 2073-2080.

Li, Q., Q. Zhou, J. Mei, Y. Zhang, J. Li, Z. Li, X. Ge, Z. Xiong, Y. Huang and W. Qian, 2014: Improvement of *Brassica napus* via interspecific hybridization between *B. napus* and *B. oleracea*. Mol. Breeding 1-9.

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

Parkin, I. A. P., A. G. Sharpe, D. J. Keith, and D. J. Lydiate, 1995: Identification of the A and C genomes of the amphidiploid *Brassica napus* (oilseed rape). Genome 38: 1122-1131.

Parkin, I., and D. J. Lydiate, 1997: Conserved patterns of chromosome pairing and recombination in *Brassica napus* crosses. Genome 40: 496-504.

Pikaard, C. S., 2001: Genomic change and gene silencing in polyploids. Trends in Genetics 17: 675-677.

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

Rahman, M. H., 2002: Fatty acid composition of resynthesized *Brassica napus* and trigenomic *Brassica* void of genes for erucic acid in their A genomes. Plant Breed. 121: 357-359

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

Rahman, M. H., R. A. Bennett, R. C. Yang, B. Kebede, and M. R. Thiagarajah, 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, M. H., 2013: Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic germplasm. Can. J. Plant Sci. 10: 24-29.

Rahman, H., B. Kebede, C. Zimmerli, and R. C. Yang, 2014: Genetic study and QTL mapping of seed glucosinolate content *Brassica rapa* L. Crop Sci. 54: 1-7.

Rahman, H., R. A. Bennett, and G. Séguin-Swartz, 2015: Broadening genetic diversity in *Brassica napus* canola: Development of canola-quality spring *B. napus* from *B. napus* × *B. oleracea* var. a*lboglabra* interspecific crosses. Can. J. Plant Sci. 95: 1-13.

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

Seyis, F., W. Friedt, and W. Lühs, 2006: Yield of *Brassica napus* L. hybrids developed using resynthesized rapeseed material sown at different locations. Field Crops Res. 96: 176-180.

Sharma, S. R., P. K. Singh, V. Chable, and S. K. Tripathi, 2005: A review of hybrid cauliflower development. J. New Seeds 6: 151-193.

Stefansson, B. R., 1983: The development of improved rapeseed cultivars. In: Kramer JKG, Sauer FD and Pigden EJ (eds) High and low erucic acid rapeseed oils: Production, Usage, Chemistry, and Toxicological Evaluation. Academic Press, New York, pp 143-159.

Szadkowski, E., F. Eber, V. Huteau, M. Lode, C. Huneau, H. Belcram, O. Coriton, M. J. Manzanares- Dauleux, R. Delourme, G. J. King, B. Chalhoub, E. Jenczewski, and A. M. Chèvre, 2010: The first meiosis of resynthesized *Brassica napus*, a genome blender. New Phytol. 186:102-112.

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

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

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

Udall, J. A., P. A. Quijada, H. Polewicz, R. Vogelzang, and T. C. Osborn, 2004: Phenotypic effects of introgressing Chinese winter and resynthesized *Brassica napus* L. germplasm into hybrid spring canola. Crop Sci. 44: 1990-1996.

United State Department of Agricultuere, economic research service, http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx

Velasco, P., P. Soengas, M. Vilar, and M. E. Cartea, and M. del Rio, 2008: Comparison of glucosinolate profiles in leaf and seed tissues of different *Brassica napus* crops. J. Am. Soc. Hort. Sci. 133: 551-558.

von Bothmer, R., M. Gustafsson, and S. Snogerup, 1995: *Brassica* sect. *Brassica* (Brassicaceae). II. Inter- and intraspecific crosses with cultivars of *B. oleracea*. Genet. Resour. Crop Evol. 42: 165-178.

Ziolkowski, P. A., M. Kaczmarek, D. Babula, and J. Sadowski, 2006: Genome evolution in *Arabidopsis*/*Brassica*: conservation and divergence of ancient rearranged segments and their breakpoints. Plant J. 47: 63-74.

Chapter 3

Estimation of allelic diversity introgressed from *B. oleracea* into *B. napus* by the use of SSR markers

3.1 Introduction

The *Brassicacea* family is comprised of many crop species. Some of which are important sources of food in the form of edible plant parts such as roots, leaves, stems, flowers and seeds (Branca and Cartea 2011). Among these, *Brassica napus* L. became one the most important crop species due to the use of its seed oil for edible purposes and meal for animal since the 1970's, following the release of canola cultivars (<2% erucic fatty acid in oil and glucosinolate < 20 µmol/g seed meal) in the market (reviewed by Abbadi and Leckband 2011). In the last two decades, *B. napus* has also gained attention to produce different raw materials such as lauric and erucic acids for industrial purposes by modifying its seed oil fatty acid composition (Friedt and Lühs 1998).

Amphidiploid *B. napus* originated approximately, 7500 years ago in the Mediterranean region of Southwest Europe, and has a relatively short history of domestication as compared to its diploid progenitor species *B. rapa* and *B. oleracea* (reviewed by Prakash et al. 2012). Genetic diversity in *B. napus* germplasm has decreased due to this short history of domestication (Becker et al. 1995) along with breeding bottlenecks, such as the selection for low erucic acid and glucosinolate content (Hasan et al. 2008, Friedt and Snowdon 2010, Bus et al. 2011), as well as growing of a specific form, such as spring, semi-winter and winter type, in a specific region and keeping the gene pool of these types isolated from each other (Diers and Osborn 1994, Butruille et al. 1999, Plieske and Struss 2001, Qian et al. 2006, Kebede et al. 2010, Bonneuil et al. 2012).

Becker et al. (1995) compared genetic diversity in resynthesized *B. napus*, created from the progenitor species *B. rapa* and *B. oleracea*, with commercial *B. napus* cultivars from different geographical regions and found that resynthesized *B. napus* can be a potential resource for broadening the genetic base of *B. napus* cultivars. This has been confirmed by other researchers (Girke et al. 1999, Seyis et al. 2003, Udall et al. 2004). Thus, resynthesized *B. napus* offers a novel source of gene pool for the improvement of spring *B. napus* canola. Seyis et al. (2006) reported that these resynthesized *B. napus* lines have the potential to develop high yielding canola hybrid cultivars. Thus, the progenitor species *B. rapa* and *B. oleracea* are a valuable reservoir of genetic variability (von Bothmer et al. 1995, Branca and Cartea 2011, Prakash et al. 2012) to use in spring *B. napus* canola breeding. According to Qian et al. (2006), Chinese oilseed *B. napus* is genetically distinct from oilseed *B. napus* of other parts of the world. Chinese breeders often used *B. napus* × *B. rapa* interspecific crosses to develop *B. napus* cultivars; this resulted frequent introgression of *B. rapa* alleles into *B. napus* cultivars (Liu 1985 cited by Chen et al. 2008). Mei et al. (2011) found that the narrow genetic base of cultivated oilseed *B. napus* can be broadened by the C subgenome; this has been supported by Rahman et al. (2015) by using the use of *B. oleracea* in the breeding of *B. napus*.

The use of molecular markers in plant breeding has provided opportunities to estimate genetic variation at the DNA level, making plant breeding more efficient and cost effective. Among the PCR based molecular markers, simple sequence repeats (SSRs) or microsatellites are found to be suitable for different studies, such as population genetics, genetic diversity, and mapping of genes in different crop species (Hearne et al. 1992, Powell et al. 1996, Cieslarová et al. 2011) due to their co-dominant inheritance and relatively high abundance throughout the genome. Through the screening of a *B. napus* genomic library of 15,000 recombinant clones,

Kresovich et al. (1995) found GA- simple sequence repeat is more abundant than other repeats (CA and GATA-), and also found that SSR markers are suitable for analysis of the genetic resources. By using SSR markers, Plieske and Struss (2001) were able to clearly differentiate winter type *B. napus* from spring type. Similarly, Hasan et al. (2006) classified the primary gene pool of *B. napus* into distinct groups by using SSR markers.

The overall hypothesis of this breeding research was that genetic diversity in spring *B. napus* canola can be broadened by enriching its C genome with genetic variation from the C genome of *B. oleracea*. The specific objective of this study was to assess the extent of introgression of allelic diversity from *B. oleracea* into spring *B. napus* using SSR markers.

3.2 Materials and Methods

3.2.1 Plant material

A total of 93 interspecific cross derived plants were selected from F_4 and BC_1F_3 generations to quantify the allelic diversity introgressed from *B. oleracea* var. *alboglabra* and *B. oleracea* var. *botrytis* into spring *B. napus* canola. This population consisted of 16 F_4 derived from A04-73NA × *B. oleracea* var. *alboglabra*-NRC(PBI), 12 F_4 from A04-73NA × *B. oleracea* var. *botrytis* (BARI Cauliflower-1), 36 BC_1F_3 from (A04-73NA × *B. oleracea* var. *alboglabra*-NRC(PBI)) × A04-73NA, and 29 BC_1F_3 derived from [A04-73NA × *B. oleracea* var. *botrytis* (BARI Cauliflower-1)] × A04-73NA crosses. Pedigree information of the F_4 and BC_1F_3 plants is presented in supplementary Table S3.1.

3.2.2 Screening for parental polymorphism

The three parents, A04-73NA, *B. oleracea* var. *alboglabra* – NRC (PBI) and *B. oleracea* var. *botrytis* (BARI Cauliflower-1) were screened for polymorphism with 414 SSR markers. These SSR markers were obtained from Agriculture and Agri-Food Canada (AAFC). Twenty six polymorphic SSR markers, spanning the nine C genome linkage groups C11 to C19 were selected based on clear and reproducible bands (Table 3.1).

3.2.3 Genotyping of F_4 and BC_1F_3 plants

Leaf samples were harvested from F_4 and BC_1F_3 plants at the age of 3-4 weeks after seeding grown in greenhouse. Genomic DNA was extracted from samples with SIGMA DNA extraction Kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer's instructions. DNA was diluted to a concentration of 15 ± 5 ng μL^{-1} for polymerase chain reactions (PCR). PCR were performed with 15 ng of template DNA, 1 pmol of each forward and reverse primers, 0.2 mM dNTPs mix, 2.5 mM MgCl2, 1x PCR reaction buffer, and 0.25 unit of Taq DNA polymerase in a final volume of 15 µL. Amplifications were performed in a GeneAmp PCR System 9700 thermal cycler. PCR products were initially verified by agarose gel electrophoresis. Later, a capillary ABI sequencer No. 3730 (Applied Biosystems, Foster City, CA) was used to measure the amplicon sizes.

3.2.4 Data analysis

Marker amplicons were given a score of 1 when present in a sample and a score of 0 when absent. A binary data matrix based on these scores for different SSR markers was produced. Only clear bands with sharp peaks in ABI sequencer were considered, while ambiguous or weak bands were not included in the analysis.

Dice genetic similarity coefficients (Nei and Li, 1979) were calculated from the data matrix using the software Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.2; Rohlf, 2000). These similarity coefficients were used for an unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis in order to develop dendrogram.

Genetic relationships among the interspecific F_4 and BC_1F_3 plants were also calculated by principal coordinate analysis (PCoA) using NTSYSpc (Rohlf, 2000). Analysis of molecular variance (AMOVA) for F_4 and BC_1F_3 was done by using GenAlEx6 software (Peakall and Smouse, 2006)

Table 3.1 List of SSR markers used to genotype F_4 and BC_1F_3 plants of *B. napus* × *B. oleracea* interspecific crosses.

Primer Name	Primer #	Linkage group	No. of amplified bands
sS1876	2276	11	10
sN2087	2278	11	6
sN3734	2279	11	8
sN0691	2286	11	4
sNRG67	160	12	5
sN11913	90	13	5
sN2316	110	13	3
sN2429	112	13	4
sNRA88	159	13	2
sN11819 (b)	2091	13	9
sN3508F (a)	2109	14	10
sNRC89 (cNM)	2105	14	8
sORH46gNP	604	11, 15, 18, 19	2
sN1711aNP	610	11, 14, 15, 17, 19	3
sN0761a	721	5, 15	7
sR1211	2449	15	4
sN12056	2360	16	7
sN11746	2361	16	3
sR0293	2368	16	5
sORC76 (cNM)	2119	17	12
sN12508I	2389	17	5
sORF37	2393	17	11
sN1708	2395	17	5
sN12822	2424	17	5
sN9070B	153	19	6
sN7271a? (x)	834	19	5

3.3 Results

3.3.1 Genetic diversity

The 26 SSR markers from the *Brassica* C genome (Table 3.1) amplified a total 156 genomic regions among the 93 F_4 and BC_1F_3 plants of *B. napus* × *B. oleracea* interspecific crosses. Within the whole population of the two crosses and their backcrosses, genetic variability accounted for 98% of the total variation as shown by AMOVA (Table 3.2). However, variation between the F_4 and BC_1F_3 populations from the two interspecific crosses contributed only 2% to the total genetic variation.

Table 3.2 Results of AMOVA performed with 26 SSR loci in F_4 and BC_1F_3 population of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* interspecific crosses.

Source	df	SS	MS	Estimated Variance	% Variation	F_{st}	P-value
Among population	3	84.99	28.33	0.40	2%	0.01	0.06
Within population	92	1739.96	19.55	19.55	98%		
Total	95	1824.95		19.95	100%		

3.3.2 Percent *B. oleracea* introgression

A total of 23 *B. oleracea* var. *alboglabra* and 35 *B. oleracea* var. *botrytis* specific alleles were amplified by 26 SSR markers in their respective F_4 and BC_1F_3 plants. Frequency distribution of the F_4 and BC_1F_3 plants of the two crosses for the percentage of *B. oleracea* allele introgression is shown in Fig. 3.1. The percentage of alleles specific to *B. oleracea* var.

alboglabra in the F_4 and BC_1F_3 plants of *B. napus* × *B. oleracea* var. *alboglabra* ranged from 8.69 to 43.5% and 13.0 to 52.2%, with mean of 27.4 ± 2.50 SE and 28.9 ± 1.65 SE, respectively (Table 3.3). This difference between the F_4 and BC_1F_3 plants was not significantly different (t = 0.52, P-value > 0.05). In case of the F_4 and BC_1F_3 plants of *B. napus* × *B. oleracea* var. *botrytis* cross, the percentage of alleles specific to *B. oleracea* var. *botrytis* ranged from 0 to 28.6% and 11.4 to 40.0% with mean of 13.8 ± 2.22 SE and 23.6 ± 1.37 SE, respectively (Table 3.3). This difference was statistically significantly (t = 3.83, P-value < 0.05) indicating that the BC_1F_3 plants carry greater frequency of *B. oleracea* alleles as compared to F_4 plants**.** Overall of the two crosses, 21.6% and 26.6 % *B. oleracea* specific alleles were detected in F_4 and BC_1F_3 population, respectively.

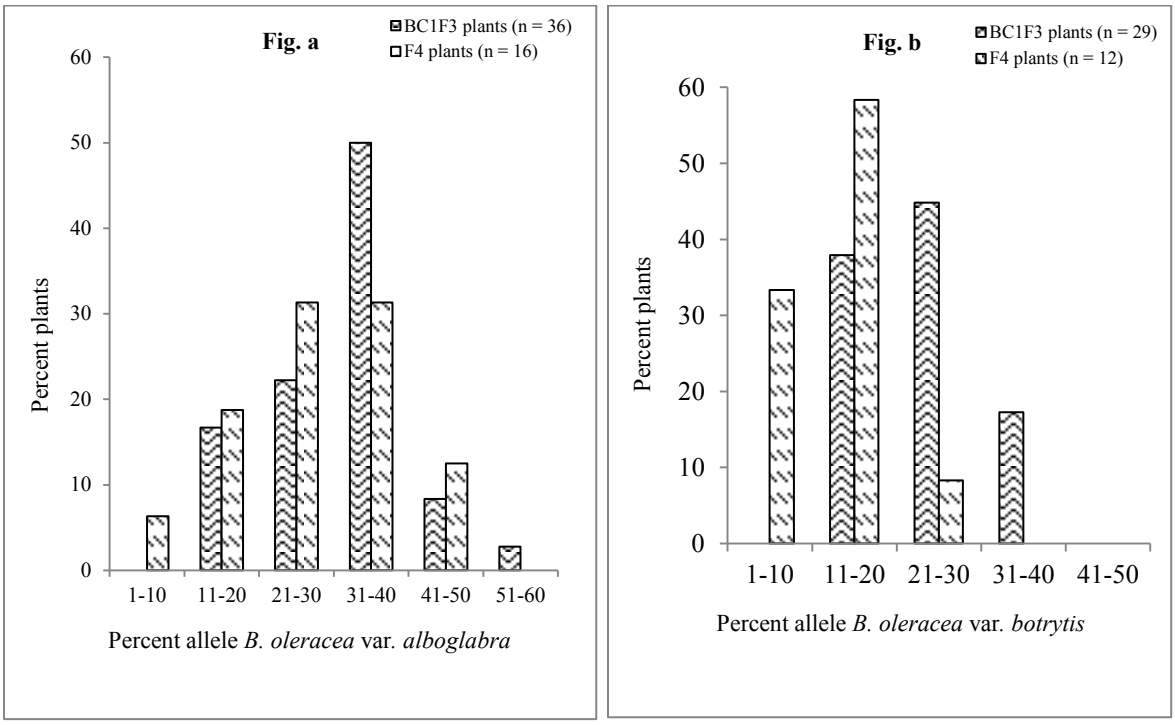


Fig. 3.1 Frequency distribution of F_4 ( ) and BC_1F_3 ( ) plants based on *B. oleracea* var. *alboglabra* (Fig. a) and *B. oleracea* var. *botrytis* (Fig. b) alleles introgressed into these plants.

Table 3.3 Occurrence of SSR alleles (percent of the total alleles detected) from *B. oleracea* var. *alboglabra* and *B. oleracea* var. *botrytis* in F_4 and BC_1F_3 plants of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis*.

	B. oleracea var. *alboglabra*[1]			*B. oleracea* var. *botrytis*[2]		
Generation	No. plants (n)	Mean ± SE	Range	No. plants (n)	Mean ± SE	Range
F_4	16	27.4 ± 2.50	8.7 - 43.5	12	13.8 ± 2.22	0 - 28.6
BC_1F_3	36	28.9 ± 1.65	13.0 - 52.2	29	23.6 ± 1.37	11.4 - 40.0

[1]Estimated based on 23 *B. oleracea* var. *alboglabra* alleles amplified by 26 SSR markers.
[2]Estimated based on 35 *B. oleracea* var. *botrytis* alleles amplified by 26 SSR markers.

3.3.3 Genetic relationship

UPGMA cluster analyses indicated no major groups between F_4 and BC_1F_3 plants derived from *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* (Fig. 3.2 and 3.3) crosses. However, four BC_1F_3 plants, Albo-BC-23, Albo-BC-37, Albo-BC-32 and Albo-BC-6, and two F_4 plants Albo-F-72 and Albo-F-76, formed a small group close to *B. oleracea* var. *alboglabra* parent as shown in Fig 3.2. Overall, genetic variability was larger in the plants derived from *B. napus* × *B. oleracea* var. *botrytis* than *B. napus* × *B. oleracea* var. *alboglabra* cross.

The principal coordinate analysis was employed to further examine the genetic diversity. The first and second principal coordinates respectively explained 9.9 % and 6.0 % of the variation in 93 plants (Fig. 3.4). Most of the plants fall in between the *B. oleracea* and *B. napus* parents with the majority being clustered in the *B. napus* quadrant. A few BC_1F_5 plants fall in the quadrant of the *B. oleracea* var. *botrytis* parent.

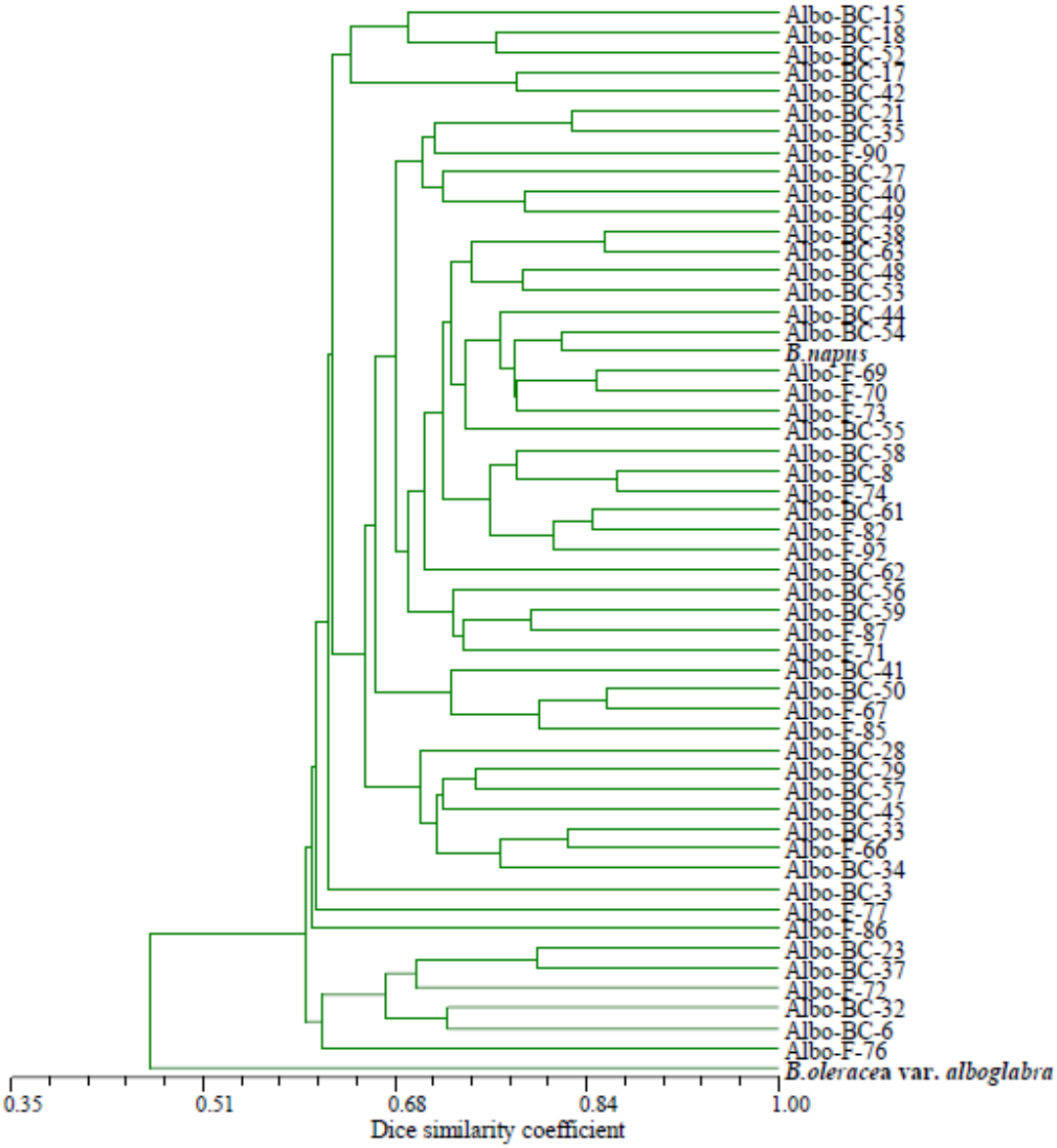


Fig. 3.2 Dendrogram showing genetic similarity for 16 F_4 and 36 BC_1F_3 plants derived from *Brassica napus* × *B. oleracea* var. *alboglabra* interspecific cross using unweighted pair-group method with arithmetic mean (UPGMA) clustering based on genetic fingerprint by use of simple sequence repeat (SSR) markers.

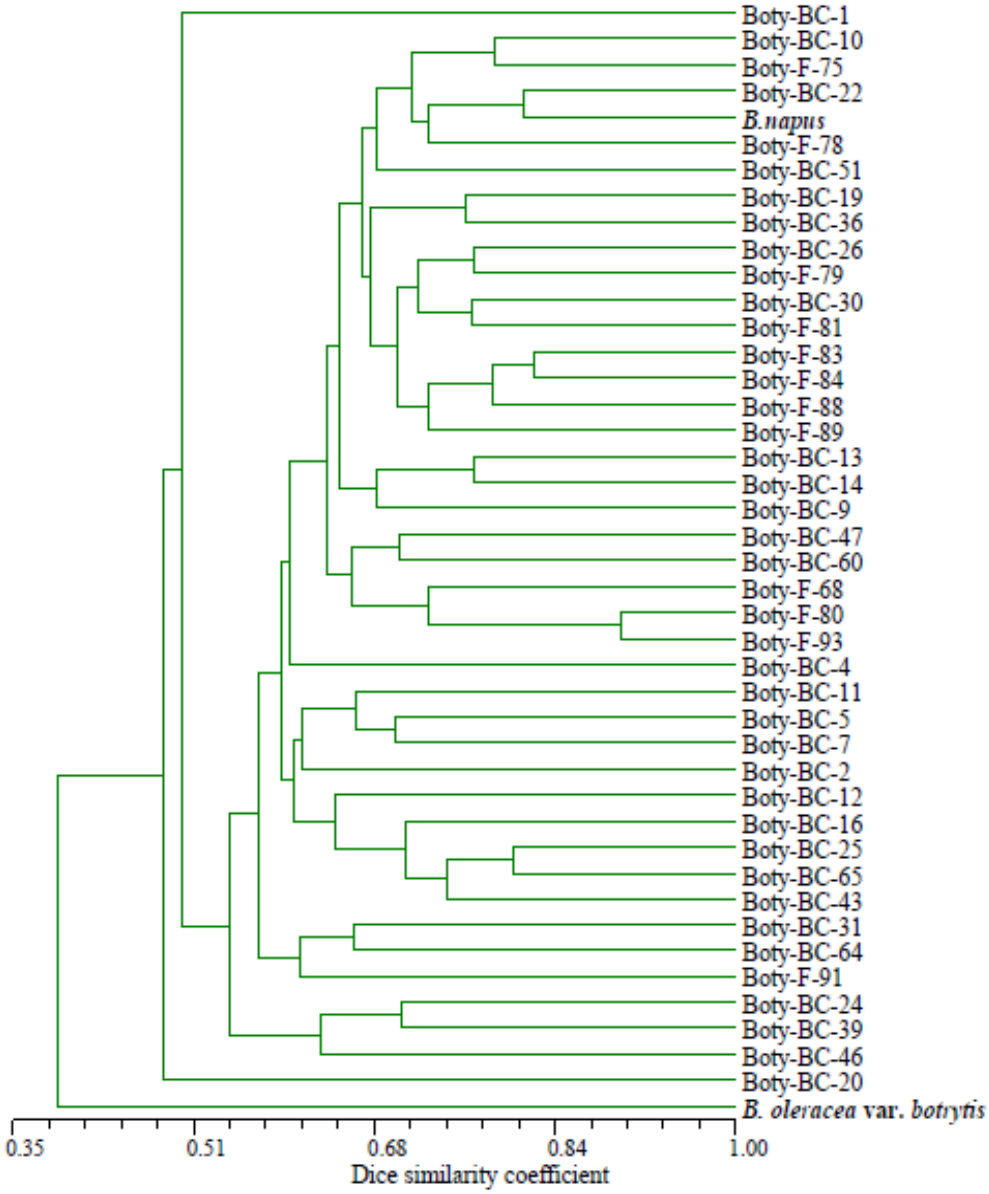


Fig. 3.3 Dendrogram showing genetic similarity for 12 F_4 and 29 BC_1F_3 plants derived from *Brassica napus* × *B. oleracea* var. *botrytis* interspecific cross using unweighted pair-group method with arithmetic mean (UPGMA) clustering based on genetic fingerprinting by use of simple sequence repeat (SSR) markers.

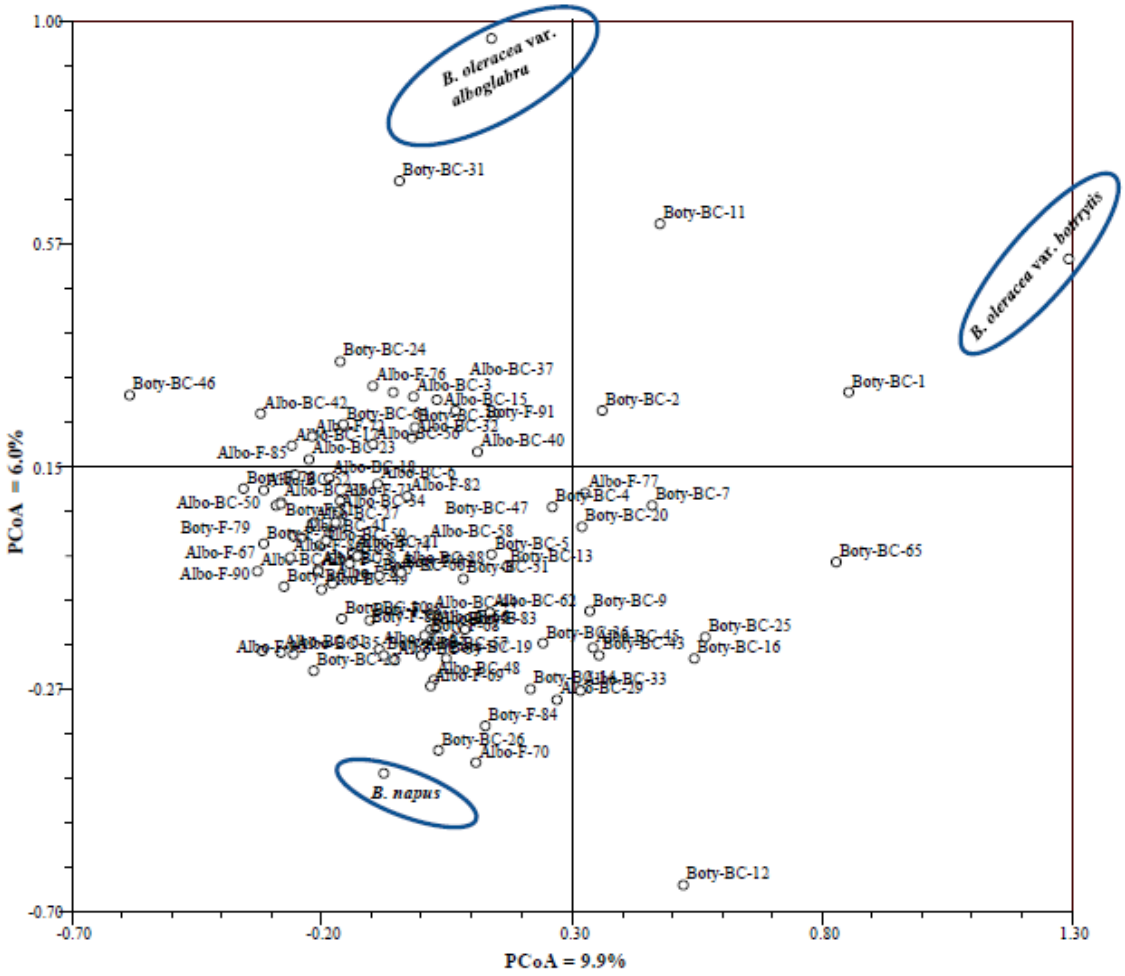


Fig. 3.4 Plot of the first and second principal coordinates for 28 F_4 and 65 BC_1F_3 plants derived from *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* interspecific crosses based on simple sequence repeat (SSR) marker analysis.

3.4 Discussion

Genetic diversity in cultivated crops represents only a fraction of the total variation present in their gene pool (reviewed by Fernie et al. 2006). It is therefore important to explore exotic germplasm, such as land races, progenitor and/or allied species for favorable alleles (Xiao et al. 1996, McCouch 2004, Reif et al. 2005, Rahman 2013) which may got lost in recently cultivated crops during breeding, or may not have been included during the course of domestication (van de Wouw et al. 2009). In last few decades, alleles introgression from the

progenitor species *B. rapa* resulted substantial increase in genetic diversity in *B. napus* in its A genome (Qian et al. 2006, Chen et al. 2008). However, during recent time researchers like Bennett et al. (2012), Li et al. (2014), and Rahman et al. (2015) have emphasized the need to diversify *B. napus* in its C genome by using the progenitor species *B. oleracea*. A recent study of Wang et al. (2014) revealed that the *Brassica* C genome carries more favorable alleles to confer elite traits than the A genome. In this research, analysis of molecular variance (Table 3.2) revealed non-significant difference of genetic variation between the populations derived from *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* crosses. The molecular variance results were supported by UPGMA analysis (Fig. 3.2, 3.3, 3.4) in which the F_2 and BC_1 derived plants could not be demarcated into separate major groups. Theoretically, the F_2 derived plants expected to possess greater allelic diversity than the BC_1 derived plants. However, in the present case, BC_1F_3 population possessed significantly ($t = 2.24$, $P < 0.05$) higher *B. oleracea* specific alleles as compared to F_4 population which might have resulted from strong selection for *B. napus* phenotype and canola quality traits during the development of the F_4 population as compared to selection pressure imposed during the development of BC_1F_3 population. Apparently, selection in F_2 derived population improved canola quality and other plant fertility traits, however, also reduced the amount of *B. oleracea* introgression in these plants. These results agree with the earlier reports (Hasan et al. 2008, Bus et al. 2011) that selection for canola quality traits was one of the major factors for reducing genetic diversity in *B. napus*. Some of the interspecific cross derived plants sharing the same pedigree (Table S3.1) were positioned at different levels of genetic similarity in the dendogram. This might be due to limited number of marker allele used in this study where each marker allele apparently contributed large effect on genetic diversity. Other possible reasons for this divergence might be

due to homoelogous recombination between the A and C genome chromosomes (Udall et al. 2005, Gaeta et al. 2007, Gaeta and Pires 2010) as well as increased frequency of sequence elimination (Shaked et al. 2001) in interspecific hybrid progenies. According to Zou et al. (2011), genomic alterations occurred in interspecific hybrids had played significant role in evolution and crop improvement.

On the other hand, many plants also dispersed between the *B. napus* and *B. oleracea* parents, as expected. However, most of the plants clustered in the quadrant of the *B. napus* parent (Fig. 3.4). In general, the plants derived from *B. napus* × *B. oleracea* var. *botrytis* were found to be more genetically diverse as compared to the plants derived from *B. napus* × *B. oleracea* var. *alboglabra* cross.

There is growing interest among researchers to develop genetically diverse *B. napus* germplasm for developing hybrid cultivars as many studies showed encouraging results from the use of such lines in hybrid breeding. For instance, Qian et al. (2005) evaluated heterotic potential of the inbred lines developed by reconstituting the A genome of *B. napus* with the A genome of *B. rapa*. They found about 90% of the hybrid combinations exceeded seed yield of the tester and more than 25% of the hybrids surpassed the elite Chinese cultivars. Similarly, Seyis et al. (2006) evaluated the performance of 18 test cross hybrids developed by using resynthesized *B. napus* lines over three locations and found that two of them had the highest average seed yield. According to Zou et al. (2010), heterosis in *B. napus* can be enhanced through introgression of genomic regions from allied *Brassica* species into *B. napus* hybrid parent lines. Bennett (2012) developed *B. napus* lines from *B. napus* × *B. oleracea* var. *alboglabra* cross and evaluated these lines in hybrid trials by using the *B. napus* parent as tester and observed mid to high parent heterosis in some of the test cross hybrid combinations. A recent study by Li et al. (2014)

showed that introgression from the C genome of *B. oleracea* into *B. napus* can have significant effect on heterosis for seed yield. Thus, it would be important to evaluate heterotic potential of the *B. napus* lines developed in this research to produce commercial hybrid canola cultivars.

In conclusion, this MSc thesis research project demonstrated the feasibility of introgression of genetic diversity from *B. oleracea* var. *alboglabra* and var. *botrytis* into spring *B. napus*. Introgression of allelic diversity from the C genome of *B. oleracea* would expand genetic diversity in the Canadian spring *B. napus* canola gene pool.

3.5 References

Abbadi, A., and G. Leckband, 2011: Rapeseed breeding for oil content, quality, and sustainability. Eur. J. Lipid Sci. Technol. 113: 1198-1206.

Becker, H. C., G. M. Engqvist, and B. Karlsson, 1995: Comparison of rapeseed cultivars and resynthesized lines based on allozyme and RFLP markers. Theor. Appl. Genet. 91: 62-67.

Bennett, R. A., 2012: Broadening genetic diversity in canola (*Brassica napus*) germplasm using the *B. oleracea* var. *alboglabra* C genome. Ph.D. thesis. University of Alberta, Edmonton, Canada.

Bennett, R. A., G. Séguin-Swartz, and H. Rahman, 2012: Broadening genetic diversity in canola (*Brassica napus* L.) using the C-genome species *B. oleracea*. Crop Sci. 52: 2030-2039.

Bonneuil, C., R. Goffaux, I. Bonnin, P. Montalent, C. Hamon, F. Balfourier, and I. Goldringer, 2012: A new integrative indicator to assess crop genetic diversity. Ecol. Indic. 23: 280 289.

Branca, F., and E. Cartea, 2011: *Brassica*. In wild crop relatives: genomic and breeding resources. Springer Berlin/Heidelberg pp. 17-36.

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

Butruille, D.V., R. P. Guries, and T. C. Osborn, 1999: Increasing yield of spring oilseed rape hybrids through introgression of winter germplasm. Crop Sci. 39:1491-1496.

Chen, S., M. N. Nelson, K. Ghamkhar, T. Fu, and W. A. Cowling, 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.

Cieslarová, J., P. Hanáček, E. Fialová, M. Hýbl, and P. Smýkal, 2011: Estimation of pea (*Pisum sativum* L.) microsatellite mutation rate based on pedigree and single-seed descent analyses. J. Appl. Genet. 52: 391-401.

Diers, B. W., and T. C. Osborn, 1994: Genetic diversity of oilseed *Brassica napus* germ plasm based on restriction fragment length polymorphisms. Theor. Appl. Genet. 88: 662-668.

Fernie, A. R., Y. Tadmor, and D. Zamir, 2006: Natural genetic variation for improving crop quality. Curr. Opin. Plant Biol. 9: 196-202.

Friedt, W., and W. Lühs, 1998: Recent developments and perspectives of industrial rapeseed breeding. Lipid 100: 219-226.

Friedt, W., and R. Snowdon, 2010: "Oilseed rape" Oil crops. Springer New York 91-126.

Gaeta, R. T., J. C. Pires, F. Iniguez-Luy, E. Leon, and T. C. Osborn, 2007: Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. Plant Cell 19: 3403-3417.

Gaeta, R. T., and J. C. Pires, 2010: Homoeologous recombination in allopolyploids: the polyploid ratchet. New Phytol. 186: 18-28.

Girke, A., H. C. Becker, and G. Engqvist, 1999: Resynthesized rapeseed as a new gene pool for hybrid breeding. Proc. 10th International Rapeseed Congress, Canberra, Australia.

Hasan, M., F. Seyis, A. G. Badani, J. P. Kuhnemann , W. Lühs, W. Friedt, and R. J. Snowdon, 2006: Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet. Resour. Crop Evol. 53: 793-802.

Hasan, M., W. Friedt, J. P. Kühnemann, N. M. Freitag, K. Link, and R. J. Snowdon, 2008: Association of gene-linked SSR markers to seed glucosinolate content in oilseed rape (*Brassica napus* ssp. *napus*). Theor. Appl. Genet. 116: 1035-1049.

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

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

Kresovich, S., A. K. Szewc-McFadden, S. M. Bliek, and J. R. McFerson, 1995: Abundance and characterization of simple-sequence repeats (SSRs) isolated from a size-fractionated genomic library of *Brassica napus* L. (rapeseed). Theor. Appl. Genet. 91: 206-211.

Li, Q., Q. Zhou, J. Mei, Y. Zhang, J. Li, Z. Li, X. Ge, Z. Xiong, Y. Huang and W. Qian, 2014: Improvement of *Brassica napus* via interspecific hybridization between *B. napus* and *B. oleracea*. Mol. Breeding 1-9.

Liu, H., 1985: Rapeseed genetics and breeding. Shanghai Science and Technology Press, Shanghai pp 556-559.

McCouch, S., 2004: Diversifying selection in plant breeding. PLoS Biology 2: e347.

Mei, J., Q. Li, L. Qian, Y. Fu, J. Li, M. Frauen, and W. Qian, 2011: Genetic investigation of the origination of allopolyploid with virtually synthesized lines: application to the C subgenome of *Brassica napus*. Heredity 106: 955-961.

Nei, M., and W.H. Li, 1979: Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269-5273.

Peakall, R., and P. E. Smouse, 2006: GENALEX 6: Genetic analysis in Excel-Population genetic software for teaching and research. Mol. Ecol. Notes 6: 288-295.

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

Powell, W., G. C. Machray, and J. Provan, 1996: Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 1: 215-222.

Prakash, S., X. M. Wu, and S. R. Bhat, 2012: History, evolution, and domestication of *Brassica* crops. Plant Breed. Rev. 35: 19-84.

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

Qian, W., J. Meng, M. Li, M. Frauen, O. Sass, J. Noack, and C. Jung, 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, M. H., 2013: Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic germplasm. Can. J. Plant Sci. 10: 24-29.

Rahman, H., R. A. Bennett, and G. Séguin-Swartz, 2015: Broadening genetic diversity in *Brassica napus* canola: Development of canola-quality spring *B. napus* from *B. napus* × *B. oleracea* var. a*lboglabra* interspecific crosses. Can. J. Plant Sci. 95: 1-13.

Reif, J. C., P. Zhang, S. Dreisigacker, M. L. Warburton, M. van Ginkel, D. Hoisington, M. Bohn and A. E. Melchinger, 2005: Wheat genetic diversity trends during domestication and breeding. Theor. Appl. Genet. 110: 859-864.

Rohlf, F. J., 2000: NTSYS-pc numerical taxonomy and multivariate analysis system. Exeter Software, New York.

Seyis, F., R. J. Snowdon, W. Luhs, and W. Friedt, 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.

Seyis, F., W. Friedt, and W. Lühs, 2006: Yield of *Brassica napus* L. hybrids developed using resynthesized rapeseed material sown at different locations. Field Crops Res. 96: 176-180.

Shaked, H., K. Kashkush, H. Ozkan, M. Feldman, and A. A. Levy, 2001: Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell 13: 1749-1759.

Udall, J. A., P. A. Quijada, H. Polewicz, R. Vogelzang, and T. C. Osborn, 2004: Phenotypic effects of introgressing Chinese winter and resynthesized *Brassica napus* L. germplasm into hybrid spring canola. Crop Sci. 44: 1990-1996.

Udall, J. A., P.A. Quijada, and T. C. Osborn, 2005: Detection of chromosomal rearrangements derived from homeologous recombination in four mapping populations of *Brassica napus* L. Genetics 169: 967-979.

van de Wouw, M., C. Kik, T. van Hintum, R. van Treuren, and B. Visser, 2009: Genetic erosion in crops: concept, research results and challenges. Plant Genet. Resour. 8: 1-15.

von Bothmer, R., M. Gustafsson, and S. Snogerup, 1995: *Brassica* sect. *Brassica* (Brassicaceae). II. Inter- and intraspecific crosses with cultivars of *B. oleracea*. Genet. Resour. Crop Evol. 42: 165-178.

Wang, N., F. Li, B. Chen, K. Xu, G. Yan, J. Qiao, J. Li, G. Gao, I. Bancroft, J. Meng, G. J. King, and X. Wu, 2014: Genome-wide investigation of genetic changes during modern breeding of *Brassica napus*. Theor. Appl. Genet. 127: 1817-1829.

Xiao, J., S. Grandillo, S. N. Ahn, S. R. McCouch, S. D. Tanksley, J. Li, L. Yuan, L. Stenflo and M. Y. Yu, 1996: Genes from wild rice improve yield. Nature 384: 223-224.

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

Zou, J., D. Fu, H. Gong, W. Qian, W. Xia, J. C. Pires, R. Y. Li, Y. Long, A. S. Mason, T. J. Yang, Y. P. Lim, B. S. Park, and J. L. Meng, 2011: De novo genetic variation associated with retrotransposon activation, genomic rearrangements and trait variation in a recombinant inbred line population of *Brassica napus* derived from interspecific hybridization with *Brassica rapa*. Plant J. 68: 212-224.

Chapter 4

General Discussion and Conclusions

4.1 General discussion

B. napus L. (AACC genome, 2*n* = 38) canola is the third most important source of vegetable oil in the world. In Canada, the economic importance of spring *B. napus* canola is significant; contribution of this crop to the Canadian economy is about $19 billion per year. For developing improved cultivars with desirable traits, existence of genetic diversity in breeding material is important. A decline in genetic diversity in spring *B. napus* canola has been observed in the last few decades (Cowling 2007, Fu and Gugel 2010), which is at least partly due to intensive breeding for seed quality traits (Girke et al. 1999). This decline in genetic diversity has raised concerns among researchers for sustainable breeding progress in spring *B. napus* canola. Therefore, many breeding programs had put effort to broaden genetic diversity in spring *B. napus* canola either by crossing with winter type (Butruille et al. 1999, Kebede et al. 2010) or semi-winter type *B. napus* (Udall et al. 2004), or by utilizing its diploid progenitor species *B. rapa* and *B. oleracea*, such as through resynthesis of *B. napus* (Kräling 1987, Becker et al. 1995, Udall et al. 2006, Seyis et al. 2006) or crossing the diploid species with *B. napus* (Qian et al. 2006, Bennett et al. 2012, Li et al. 2014, Rahman et al. 2015). Rahman (2013) reviewed different strategies for broadening genetic diversity in spring *B. napus* canola. One of the ways to broaden genetic diversity is to reconstitute the A genome of *B. napus* with the A genome of *B. rapa*. This type of reconstituted *B. napus* has shown significant increase in seed yield (Liu et al. 2002, Qian et al. 2003, 2005). However, little information can be found in literature about the use of other progenitor species *B. oleracea*, despite this species carry vast untapped genetic variability. This

is perhaps due to the presence of high erucic acid (> 40 %) in oil and GSL content (>100 µmol/g seed) seed meal in *B. oleracea*, and its low crossing ability with *B. napus* (Bennett et al. 2008). The erucic acid content in *B. napus* is controlled by two gene loci (Harvey and Downey 1964), one on each genome as reported by different researchers (Rahman et al. 1994, Chen and Heneen 1989, Bennett et al. 2008, 2012, Zhang et al. 2008). On the other hand, GSL content is controlled by at least three gene loci (Rahman et al. 2001, Howell et al. 2003, Hasan et al. 2008, Feng et al. 2012), and therefore, it is relatively more difficult to develop a low GSL line from cross involving high and low GSL type parents. Thus, intensive breeding effort is needed to develop a canola quality line from *Brassica* interspecific crosses (Rahman et al. 2001).

Plant sterility is another major obstacle in *Brassica* interspecific hybrids, such as *B. napus* × *B. oleracea*, due to meiotic abnormalities (Pikaard 2001, Szadkowski et al. 2010) resulting from haploid set of chromosomes as well as frequent homoeologous chromosome pairing between the chromosomes (Song et al. 1995, Nicolas et al. 2009, Tian et al. 2010, reviewed by Chen et al. 2011). This makes the plants instable in early segregating generations. Despite these barriers, some researchers (Rahman et al. 2011, Bennett et al. 2012, Ding et al. 2013, Rahman et al. 2015) have shown the prospects of introgressing allelic diversity from *B. oleracea* into *B. napus*. Therefore, the present research was undertaken with primary objective to investigate the feasibility of developing genetically distinct canola quality *B. napus* ($2n = 38$) recombinant inbred lines of spring growth habit from *B. napus* × *B. oleracea* and (*B. napus* × *B. oleracea*) × *B. napus* interspecific crosses by using two morphotypes of *B. oleracea* var. *alboglabra* and var. *botrytis* with hypothesis that C genomes of these two *B. oleracea* types are genetically distinct from the C genome of *B. napus*, and would increase genetic diversity in spring *B. napus* canola.

4.2 Conclusions

The following conclusions were drawn from the present research:

- Genetically diverse canola quality (<1% erucic acid, <15 µmol/g seed GSL) *B. napus* lines can be achieved from the progenies of *B. napus* × *B. oleracea* var. *alboglabra* and *B. napus* × *B. oleracea* var. *botrytis* interspecific crosses through reconstitution of the C genome of *B. napus* with the C genome of *B. oleracea*.
- Presence or absence of erucic acid in F_2 and BC_1 derived population was dictated by one gene locus of the C genome. This simple genetic control enabled selection of zero erucic acid type from early segregating generations.
- A good number of families derived from *B. napus* × *B. oleracea* interspecific cross had low GSL content (< 20 µmol/g seed). This is due to segregation for GSL genes of the C genome only.
- Low plant fertility in early generation populations suggested occurrence of aneuploid plants. This is also evident from flow cytometric analysis of the F_6 and BC_1F_3 generation plants. However, inbred lines with good plant fertility comparable to the *B. napus* parent were achieved through repeated inbreeding with selection for *B. napus* type plants with high fertility.
- Results of molecular marker analysis depicted that the plants derived from both F_2 and BC_1 are genetically distinct from the *B. napus* parent, and showed high genetic variability within the population. This demonstrated that genetic base of the C genome of spring *B. napus* canola can be broadened substantially by using *B. oleracea* var. *alboglabra* and *B. oleracea* var. *botrytis*.

4.3 Future research

Cytological analysis during early generations of *B. napus* × *B. oleracea* cross may reduce the number of generations required to develop *B. napus* plants with $2n = 38$ chromosomes. However, this will require huge effort. A more fair assessment of comparative genetic diversity in the F_2 and BC_1 derived population as well as the prospect of developing canola quality lines from these two approaches can be made by increasing the population size. High sterility in the interspecific hybrid progenies, and limited greenhouse space and other resources has been the major hindrance for this.

These interspecific cross derived lines ($2n = 38$) need to be tested for heterosis for seed yield and other agronomic traits as well as for general and specific combining abilities. QTL mapping of the genomic regions involved in heterosis in *B. napus* and investigation of the impact of the alleles introgressed from *B. oleracea* will be needed for use in marker assisted breeding of hybrid cultivars.

4.4 References

Becker, H. C., G. M. Engqvist, and B. Karlsson, 1995: Comparison of rapeseed cultivars and resynthesized lines based on allozyme and RFLP markers. Theor. Appl. Genet. 9: 62-67.

Bennett, R. A., M. R. Thiagarajah, J. R. King, and M. H. Rahman, 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.

Bennett, R. A., 2012: Broadening genetic diversity in canola (*Brassica napus*) germplasm using the *B. oleracea* var. *alboglabra* C genome. Ph.D. thesis. University of Alberta, Edmonton, Canada.

Bennett, R. A., G. Séguin-Swartz, and H. Rahman, 2012: Broadening genetic diversity in canola (*Brassica napus* L.) using the C-genome species *B. oleracea*. Crop Sci. 52: 2030-2039.

Butruille, D. V., R. P. Guries, and T. C. Osborn, 1999: Increasing yield of spring oilseed rape hybrids through introgression of winter germplasm. Crop Sci. 39: 1491-1496.

Chen, B. Y., and W. K. Heneen, 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, S., M. N. Nelson, A. M. Chèvre, E. Jenczewski, Z. Li, A. S. Mason, J. Meng, J. A. Plummer, A. Pradhan, K. H. M. Siddique, R. J. Snowdon, G. Yan, W. Zhou and W.A. Cowling, 2011: Trigenomic bridges for *Brassica* improvement. Crit. Rev. Plant Sci. 30: 524-547.

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

Ding, Y., J. Mei, Q. Li, Y. Liu, H. Wan, L. Wang, H.C. Becker and W. Qian, 2013: Improvement of *Sclerotinia sclerotiorum* resistance in *Brassica napus* by using *B. oleracea*. Genet. Resour. Crop Evol. 60: 1615-1619.

Ecke, W., M.Uzunova, and K. Weissleder, 1995: Mapping the genome of rapeseed (*Brassica napus* L.). II. Localization of genes controlling erucic acid synthesis and seed oil content. Theor. Appl. Genet. 91(6-7): 972-977.

Feng, J., Y. Long, L. Shi, J. Shi, G. Barker, and J. Meng, 2012: Characterization of metabolite quantitative trait loci and metabolic networks that control glucosinolate concentration in the seeds and leaves of *Brassica napus*. New Phytol.New Phytol. 193: 96-108.

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

Girke, A., H. C. Becker, and G. Engqvist, 1999: Resynthesized rapeseed as a new gene pool for hybrid breeding. Proc 10th Int Rapeseed Congress, v.4, Canberra, Australia.

Harvey, B. L., and R. K. Downey, 1964: The inheritance of erucic acid content in rapeseed (*Brassica napus*). Can. J. Plant Sci. 44: 104-111.

Hasan, M., W. Friedt, J. P. Kühnemann, N. M. Freitag, K. Link, and R. J. Snowdon, 2008: Association of gene-linked SSR markers to seed glucosinolate content in oilseed rape (*Brassica napus* ssp. *napus*). Theor. Appl. Genet. 116: 1035-1049.

Howell, P. M., A. G. Sharpe, and D. J. Lydiate, 2003: Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). Genome 46:454-460.

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

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

Li, Q., Q. Zhou, J. Mei, Y. Zhang, J. Li, Z. Li, X. Ge, Z. Xiong, Y. Huang and W. Qian, 2014: Improvement of *Brassica napus* via interspecific hybridization between *B. napus* and *B. oleracea*. Mol. Breeding 1-9.

Liu, R., W. Qian, and J. Meng, 2002: Association of RFLP markers and biomass heterosis in trigenomic hybrids of oilseed rape (*Brassica napus* × *B. campestris*). Theor. Appl. Genet. 105: 1050-1057.

Nicolas, S. D., M. Leflon, H. Monod, F. Eber, O. Coriton, V. Huteau, and E. Jenczewski, 2009: Genetic regulation of meiotic cross-overs between related genomes in *Brassica napus* haploids and hybrids. Plant Cell 21: 373-385.

Pikaard, C. S., 2001: Genomic change and gene silencing in polyploids. Trends Genet. 17: 675-677.

Qian, W., R. Liu, and J. Meng, 2003: Genetic effects on biomass yield in interspecific hybrids between *Brassica napus* and *B. rapa*. Euphytica 134: 9-15.

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

Qian, W., J. Meng, M. Li, M. Frauen, O. Sass, J. Noack, and C. Jung, 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, M. H., L. Rahman, O. Stùlen, and H. Sorensen, 1994: Inheritance of erucic acid content in yellow- and white-flowered yellow sarson × Canadian *Brassica campestris* L. Acta Agric. Scand., Sect. B 44: 94 -97.

Rahman, M. H., M. Joersbo, and M. H. Poulsen, 2001: Development of yellow-seed *Brassica napus* of double low quality. Plant Breed.120: 473-478.

Rahman, M. H., 2002: Fatty acid composition of resynthesized *Brassica napus* and trigenomic *Brassica* void of genes for erucic acid in their A genomes. Plant Breed.121: 357-359.

Rahman, M. H., R. A. Bennett, R-C Yang, B. Kebede, and M. R. Thiagarajah, 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, M. H., 2013: Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic Germplasm. Can. J. Plant Sci. 10: 24-29.

Rahman, H., B. Kebede, C. Zimmerli, and R. C. Yang, 2014: Genetic study and QTL mapping of seed glucosinolate content *Brassica rapa* L. Crop Sci. 54: 1-7.

Rahman, H., R. A. Bennett, and G. Séguin-Swartz, 2015: Broadening genetic diversity in *Brassica napus* canola: Development of canola-quality spring *B. napus* from *B. napus* × *B. oleracea* var. a*lboglabra* interspecific crosses. Can. J. Plant Sci. 95: 1-13.

Seyis, F., W. Friedt, and W. Lühs, 2006: Yield of *Brassica napus* L. hybrids developed using resynthesized rapeseed material sown at different locations. Field Crops Res. 96: 176-180.

Song, K, P. Lu, K. Tang, and T. C. Osborn, 1995: Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proceedings of the National Academy of Sciences 92: 7719-7723.

Szadkowski, E., F. Eber, V. Huteau, M. Lode, C. Huneau, H. Belcram, O. Coriton, M. J. Manzanares- Dauleux, R. Delourme, G. J. King, B. Chalhoub, E. Jenczewski, and A. M. Chèvre, 2010: The first meiosis of resynthesized *Brassica napus*, a genome blender. New Phytol.186: 102-112.

Tian, E., Y. Jiang, L. Chen, J. Zou, F. Liu, and J. Meng, 2010: Synthesis of a *Brassica* trigenomic allohexaploid (*B. carinata* × *B. rapa*) de novo and its stability in subsequent generations. Theor. Appl. Genet. 121: 1431-1440.

Udall, J. A., P. A. Quijada, H. Polewicz, R. Vogelzang, and T. C. Osborn, 2004: Phenotypic effects of introgressing Chinese winter and resynthesized *Brassica napus* L. germplasm into hybrid spring canola. Crop Sci. 44: 1990-1996.

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

Zhang, J. F., Q. I. Cun-Kou, P. U. Hui-Ming, C. Song, C. Feng, G. A. O. Jian-Qin, C. Xin-Jun, G. U. Hui, and F. U. Shou-Zhong, 2008: QTL identification for fatty acid content in rapeseed (*Brassica napus* L.). Acta Agron. Sin. 34:54-60.

Thesis References

Abbadi, A., and G. Leckband, 2011: Rapeseed breeding for oil content, quality, and sustainability. Eur. J. Lipid Sci. Technol. 113: 1198-1206.

Ackman, R. G., 1966: Analysis of the monoethylenic fatty acids of rapeseed oil by open tubular gas chromatography. J. Am. Oil Chem. Soc. 43: 483-486.

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

Babula, D., M. Kaczmarek, A. Barakat, M. Delseny, C. F. Quiros, and J. Sadowski, 2003: Chromosomal mapping of *Brassica oleracea* based on ESTs from *Arabidopsis thaliana*: complexity of the comparative map. Mol. Genet. Genomics 268:656-665.

Basunanda, P., M. Radoev, W. Ecke, W. Friedt, H. C. Becker, and R. J. Snowdon, 2010: Comparative mapping of quantitative trait loci involved in heterosis for seedling and yield traits in oilseed rape (*Brassica napus* L.). Theor. Appl. Genet. 120: 271-281.

Becker, H. C., G. M. Engqvist, and B. Karlsson, 1995: Comparison of rapeseed cultivars and resynthesized lines based on allozyme and RFLP markers. Theor. Appl. Genet. 91: 62-67.

Bennett, R. A., M. R. Thiagarajah, J. R. King, and M. H. Rahman, 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: 593-60.

Bennett, R. A., 2012: Broadening genetic diversity in canola (*Brassica napus*) germplasm using the *B. oleracea* var. *alboglabra* C genome. Ph.D. thesis. University of Alberta, Edmonton, Canada.

Bennett, R. A., G. Séguin-Swartz, and H. Rahman, 2012: Broadening genetic diversity in canola (*Brassica napus* L.) using the C-genome species *B. oleracea*. Crop Sci. 52: 2030-2039.

Bisht, N. C., V. Gupta, N. Ramchiary, Y. S. Sodhi, A. Mukhopadhyay, N. Arumugam, D. Patel, and A. K. Pradhan, 2009: Fine mapping of loci involved with glucosinolate biosynthesis in oilseed mustard (*Brassica juncea*) using genomic information from allied species. Theor. Appl. Genet. 118: 413-421.

Blanc, G., H. Karsten, and K. H. Wolfe, 2003: A recent polyploidy superimposed on older large scale duplications in the *Arabidopsi*s genome. Genome Res. 13: 137-144.

Bonneuil, C., R. Goffaux, I. Bonnin, P. Montalent, C. Hamon, F. Balfourier, and I. Goldringer, 2012: A new integrative indicator to assess crop genetic diversity. Ecol. Indic. 23: 280 289.

Branca, F., and E. Cartea, 2011: *Brassica*. In wild crop relatives: genomic and breeding resources. Springer Berlin/Heidelberg pp. 17-36.

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

Butruille, D. V., R. P. Guries, and T. C. Osborn, 1999: Increasing yield of spring oilseed rape hybrids through introgression of winter germplasm. Crop Sci. 39: 1491-1496.

Canola Council of Canada. http://www.canolacouncil.org. (Retrieved on date 10th Jan 2014)

Cao, Z., F. Tian, N. Wang, C. Jiang, B. Lin, W. Xia, J. Shi, Y. Long, C. Zhang and J. Meng, 2010: Analysis of QTLs for erucic acid and oil content in seeds on A8 chromosome and the linkage drag between the alleles for the two traits in *Brassica napus*. J. Genet. Genomics 37: 231-240.

Charlton, K. M., A. H. Corner, K. Davey, J. K. Kramer, S. Mahadevan, and F. D. Sauer, 1975: Cardiac lesions in rats fed rapeseed oils. Can. J. Comp. Med. 39: 261-269.

Chen, B. Y., and W. K. Heneen, 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, J. L., and W. D. Beversdorf, 1990: Fatty acid inheritance in microspore-derived populations of spring rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 80: 465-469.

Chen, W., Y. Zhang, X. Liu, B. Chen, J. Tu, and F. Tingdong, 2007: Detection of QTL for six yield-related traits in oilseed rape (*Brassica napus*) using DH and immortalized F_2 populations. Theor. Appl. Genet. 115: 849-858.

Chen, S., M. N. Nelson, K. Ghamkhar, T. Fu, and W. A. Cowling, 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.

Chen, S., J. Zou, W. A. Cowling , and J. Meng, 2010: Allelic diversity in a novel gene pool of canola-quality *Brassica napus* enriched with alleles from *B. rapa* and *B. carinata*. Crop Pasture Sci. 61: 483-492.

Chen, S., M. N. Nelson, A. M. Chèvre, E. Jenczewski, Z. Li, A. S. Mason, J. Meng, J. A. Plummer, A. Pradhan, K. H. M. Siddique, R. J. Snowdon, G. Yan, W. Zhou and W.A. Cowling, 2011: Trigenomic bridges for *Brassica* improvement. Crit. Rev. Plant Sci. 30: 524-547.

Cheng, F., T. Mandáková, J. Wu, Q. Xie, M. A. Lysak, and X. Wanga, 2013: Deciphering the diploid ancestral genome of the mesohexaploid *Brassica rapa*. Plant Cell 1-14.

Cheung, F., M. Trick, N. Drou, Y. P. Lim, J. Y. Park, S. J. Kwonet J. A. Kim, R. Scott, J.C. Pires, A. H. Paterson, C. Town, and I. Bancroft 2009: Comparative analysis between homoeologous genome segments of *Brassica napus* and its progenitor species reveals extensive sequence-level divergence. Plant Cell 21:1912-1928.

Christophersen, B. O., and J. Bremer, 1972: Erucic acid - an inhibitor of fatty acid oxidation in the heart. Biochim. Biophys. Acta 280: 506-514.

Cieslarová, J., P. Hanáček, E. Fialová, M. Hýbl, and P. Smýkal, 2011: Estimation of pea (*Pisum sativum* L.) microsatellite mutation rate based on pedigree and single-seed descent analyses. J. Appl. Genet. 52: 391-401.

Coonrod, D., M. A. Brick, P. F. Byrne, L. DeBonte, and Z. Chen, 2008: Inheritance of long chain fatty acid content in rapeseed (*Brassica napus* L.). Euphytica 164: 583-592.

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

Cui, C., X. Ge, M. Gautam, L. Kang, and Z. Li, 2012: Cytoplasmic and genomic effects on meiotic pairing in *Brassica* hybrids and allotetraploids from pair crosses of three cultivated diploids. Genetics 191: 725-738.

Diers, B. W., and T. C. Osborn, 1994: Genetic diversity of oilseed *Brassica napus* germ plasm based on restriction fragment length polymorphisms. Theor. Appl. Genet. 88: 662-668.

Diers, B. W., P. B. E. McVetty, and T. C. Osborn, 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.

Ding, Y., J. Mei, Q. Li, Y. Liu, H. Wan, L. Wang, H.C. Becker and W. Qian, 2013: Improvement of *Sclerotinia sclerotiorum* resistance in *Brassica napus* by using *B. oleracea*. Genet. Resour. Crop Evol. 60: 1615-1619.

Dixon, G.R., 2007: Vegetable Brassicas and related crucifers. CABI, Wallingford, UK; Cambridge, Massachusetts, USA.

EFSA report, 2008: Opinion of the scientific panel on contaminants in the food chain on a request from the European commission on glucosinolates as undesirable substances in animal feed. EFSA Journal 590: 1-76.

Ecke, W., M. Uzunova, and K. Weissleder, 1995: Mapping the genome of rapeseed (*Brassica napus* L.). II. Localization of genes controlling erucic acid synthesis and seed oil content. Theor. Appl. Genet. 91: 972-977.

Feng, J., Y. Long, L. Shi, J. Shi, G. Barker, and J. Meng, 2012: Characterization of metabolite quantitative trait loci and metabolic networks that control glucosinolate concentration in the seeds and leaves of *Brassica napus*. New Phytol. 193: 96-108.

Fernie, A. R., Y. Tadmor, and D. Zamir, 2006: Natural genetic variation for improving crop quality. Curr. Opin. Plant Biol. 9: 196-202.

Finlayson, A. J., J. Krzymanski, and R. K. Downey, 1973: Comparison of chemical and agronomic characteristics of two *Brassica napus* L. cultivars, Bronowski and Target. J. Am. Oil Chem. Soc. 50: 407-410.

Food and Agriculture Organization, Food outlook report available online at http://www.fao.org/docrep/018/al999e/al999e.pdf retrieved on 14th Dec. 2013.

Friedt, W., and W. Lühs, 1998: Recent developments and perspectives of industrial rapeseed breeding. Lipid 100: 219-226.

Friedt, W., and R. Snowdon, 2010: "Oilseed rape" Oil crops. Springer New York 91-126.

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

Gaeta, R. T., J. C. Pires, F. Iniguez-Luy, E. Leon, and T. C. Osborn, 2007: Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. Plant Cell 19: 3403-3417.

Gaeta, R. T., and J. C. Pires, 2010: Homoeologous recombination in allopolyploids: the polyploid ratchet. New Phytol.186: 18-28.

Girke, A., H. C. Becker, and G. Engqvist, 1999: Resynthesized rapeseed as a new gene pool for hybrid breeding. Proc. 10th International Rapeseed Congress, Canberra, Australia.

Girke, A., A. Schierholt, and H. C. Becker, 2012: Extending the rapeseed gene pool with resynthesized *Brassica napus* II: Heterosis. Theor. Appl. Genet. 124: 1017-1026.

Gupta, S. K., and A. Pratap, 2007: History, origin, and evolution. In: Gupta, S.K. (Ed.), Advances in botanical research: Incorporating advances in plant pathology, vol. 45. Academic Press/Elsevier, San Diego, pp. 1-20.

Halkier, B. A., and L. Du, 1997: The biosynthesis of glucosinolates. Trends Plant Sci. 2: 425-431.

Harvey, B. L., and R. K. Downey, 1964: The inheritance of erucic acid content in rapeseed (*Brassica napus*). Can. J. Plant Sci. 44: 104-111.

Hasan, M., F. Seyis, A. G. Badani, J. P. Kuhnemann , W. Lühs, W. Friedt, and R. J. Snowdon, 2006: Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet. Resour. Crop Evol. 53: 793-802.

Hasan, M., W. Friedt, J. P. Kühnemann, N. M. Freitag, K. Link, and R. J. Snowdon, 2008: Association of gene-linked SSR markers to seed glucosinolate content in oilseed rape (*Brassica napus* ssp. *napus*). Theor. Appl. Genet. 116: 1035-1049.

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

Howell, P. M., A. G. Sharpe, and D. J. Lydiate, 2003: Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). Genome 46: 454-460.

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.

Kasem, W. T., A. Ghareeb, and E. Marwa, 2011: Seed morphology and seed coat sculpturing of 32 taxa of family Brassicaceae. Am. J. Sci. 7: 166-178.

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

Kebede, B., and H. Rahman, 2014: Quantitative trait loci (QTL) mapping of silique length and petal colour in *Brassica rapa*. Plant Breed. 133: 609-614.

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

Koebner, R. M. D., P. Donini, J. C. Reeves, R. J. Cooke, and J. R. Law, 2003: Temporal flux in the morphological and molecular diversity of UK barley. Theor. Appl. Genet. 106: 550-558.

Kondra, Z. P., and B. R. Stefansson, 1970: Inheritance of the major glucosinolates of rapeseed (*Brassica napus*) meal. Can. J. Plant Sci. 50: 643-647.

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

Kresovich, S., A. K. Szewc-McFadden, S. M. Bliek, and J. R. McFerson, 1995: Abundance and characterization of simple-sequence repeats (SSRs) isolated from a size-fractionated genomic library of *Brassica napus* L. (rapeseed). Theor. Appl. Genet. 91: 206-211.

Lagercrantz, U., and D. J. Lydiate, 1996: Comparative genome mapping in *Brassica*. Genetics 144: 1903-1910.

Lammerink, J., 1970. Inter-specific transfer of clubroot resistance from *Brassica campestris* L. to *B. napus* L. New Zealand J. Agric. Res. 13: 105-110.

Lan, T. H., T. A. DelMonte, K. P. Reischmann, J. Hyman, S. P. Kowalski, J. McFerson, S. Kresovich, and A. H. Paterson, 2000: An EST-enriched comparative map of *Brassica oleracea* and *Arabidopsis thaliana*. Genome Res. 10: 776-788.

Leflon, M., H. Brun, F. Eber, R. Delourme, M. Lucas, P. Vallée, M. Ermel, M. Balesdent, and A. Chèvre, 2007: Detection, introgression and localization of genes conferring specific resistance to *Leptosphaeria maculans* from *Brassica rapa* into *B. napus*. Theor. Appl. Genet. 115: 897-906.

Li, G., M. Gao, B. Yang, and C. F. Quiros, 2003: Gene for gene alignment between the *Brassica* and *Arabidopsis* genomes by direct transcriptome mapping. Theor. Appl. Genet. 107: 168-180.

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

Li, Q. F., J. Q. Mei, Y. J. Zhang, J. N. Li, X. H. Ge, Z. Y. Li, and W. Qian, 2013: A large-scale introgression of genomic components of *Brassica rapa* into *B. napus* by the bridge of hexaploid derived from hybridization between *B. napus* and *B. oleracea*. Theor. Appl. Genet. 126: 2073-2080.

Li, Q., Q. Zhou, J. Mei, Y. Zhang, J. Li, Z. Li, X. Ge, Z. Xiong, Y. Huang and W. Qian, 2014: Improvement of *Brassica napus* via interspecific hybridization between *B. napus* and *B. oleracea*. Mol. Breeding 1-9.

Liu, H., 1985: Rapeseed genetics and breeding. Shanghai Science and Technology Press, Shanghai pp 556-559.

Liu, R., W. Qian, and J. Meng, 2002: Association of RFLP markers and biomass heterosis in trigenomic hybrids of oilseed rape (*Brassica napus* × *B. campestris*). Theor. Appl. Genet. 105: 1050-1057.

Lukens, L., F. Zou, D. Lydiate, I. Parkin, and T. Osborn, 2003: Comparison of a *Brassica oleracea* genetic map with the genome of *Arabidopsis thaliana*. Genetics 164: 359-372.

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

Mahmood, T., U. Ekuere, F. Yeh, A. G. Good, and G. R. Stringam, 2003: RFLP linkage analysis and mapping genes controlling the fatty acid profile of *Brassica juncea* using reciprocal DH populations. Theor. Appl. Genet. 107: 283-290.

Mason, A. S., V. Huteau, F. Eber, O. Coriton, G. Yan, M. N. Nelson, W. A. Cowling, and A. M. Chevre, 2010: Genome structure affects the rate of autosyndesis and allosyndesis in AABC, BBAC and CCAB *Brassica* interspecific hybrids. Chromosome Res. 18: 655-666.

McCouch, S., 2004: Diversifying selection in plant breeding. PLoS Biology 2: e347

Mei, J., Q. Li, L. Qian, Y. Fu, J. Li, M. Frauen, and W. Qian, 2011: Genetic investigation of the origination of allopolyploid with virtually synthesized lines: application to the C subgenome of *Brassica napus*. Heredity 106: 955-961

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

Muangprom, A., S. G. Thomas, T. Sun and T. C. Osborn, 2005: A novel dwarfing mutation in a green revolution gene from *Brassica rapa*. Plant Physiol. 137: 931-938.

Navabi, Z. K., T. Huebert, A. G. Sharpe, C. M. O. Neill, I. Bancroft, and I. A. P. Parkin, 2013: Conserved microstructure of the *Brassica* B Genome of *Brassica nigra* in relation to homologous regions of *Arabidopsis thaliana*, *B. rapa* and *B. oleracea*. Genomics 14:1-15.

Nei, M., and W. H. Li, 1979: Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269-5273.

Nicolas, S. D., M. Leflon, H. Monod, F. Eber, O. Coriton, V. Huteau, and E. Jenczewski, 2009: Genetic regulation of meiotic cross-overs between related genomes in *Brassica napus* haploids and hybrids. Plant Cell 21: 373-385.

Olin-Fatih, M., 1994: A new method for differential staining of *Brassica* metaphase chromosomes, and karyotypes of *B. campestris*, *B. oleracea*, and *B. napus*. Hereditas 120: 253-259.

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

Palmer, J. D., C. R. Shields, D. B. Cohen, and T. J. Orton, 1983: Chloroplast DNA evolution and the origin of amphidiploid *Brassica* species. Theor. Appl. Genet. 65: 181-189.

Pandey, Sharad, Manoj Kabdal, and M. K. Tripathi, 2013: Study of inheritance of erucic acid in Indian mustard (*Brassica juncea* L. Czern & Coss). Octa J. Biosci. 1: 77-84.

Parkin, I. A. P., A. G. Sharpe, D. J. Keith, and D. J. Lydiate, 1995: Identification of the A and C genomes of the amphidiploid *Brassica napus* (oilseed rape). Genome 38: 1122-1131.

Parkin, I., and D. J. Lydiate, 1997: Conserved patterns of chromosome pairing and recombination in *Brassica napus* crosses. Genome 40: 496-504.

Parkin, I. A. P., S. M. Gulden, A. G. Sharpe, L. Lukens, M. Trick, T. C. Osborn, and D. J. Lydiate, 2005: Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. Genetics 171: 765-781.

Peakall, R., and P. E. Smouse, 2006: GENALEX 6: Genetic analysis in Excel-Population genetic software for teaching and research. Mol. Ecol. Notes 6: 288-295.

Pikaard, C. S., 2001: Genomic change and gene silencing in polyploids. Trends Genet. 17: 675-677.

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

Powell, W., G. C. Machray, and J. Provan, 1996: Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 1: 215-222.

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

Prakash, S., and L. V. Chopra, 1990: Reconstruction of alloploid *Brassicas* through non-homologous recombination: introgression of resistance to pod shatter in *Brassica napus*. Genet. Res. Camb. 56: 1-2.

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

Przybylski, R., and T. Mag, 2002: Canola/rapeseed oil. Vegetable oils in food technology: Composition, properties and uses: Blackwell publishing, CRC Press, USA/ Canada, pp. 98-127.

Qian, W., R. Liu, and J. Meng, 2003: Genetic effects on biomass yield in interspecific hybrids between *Brassica napus* and *B. rapa*. Euphytica 134: 9-15.

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

Qian, W., J. Meng, M. Li, M. Frauen, O. Sass, J. Noack, and C. Jung, 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.

Quijada, P. A., J. A. Udall, B. Lambert, and T. C. Osborn, 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.

Rahman, M. H., L. Rahman, O. Stùlen, and H. Sorensen, 1994: Inheritance of erucic acid content in yellow- and white-flowered yellow sarson × Canadian *Brassica campestris* L. Acta Agric. Scand., Sect. B 44: 94 -97.

Rahman, M. H., 2001: Production of yellow seeded *Brassica napus* through interspecific crosses. Plant Breed. 120: 463-472.

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

Rahman, M. H., 2002: Fatty acid composition of resynthesized *Brassica napus* and trigenomic *Brassica* void of genes for erucic acid in their A genomes. Plant Breed. 121: 357-359.

Rahman M. H., 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, M. H., 2005: Resynthesis of *Brassica napus* for self-incompatibility: Self-incompatibility reaction, inheritance and breeding potential. Plant Breed. 124: 13-19.

Rahman, M. H., R. A. Bennett, R-C Yang, B. Kebede, and M. R. Thiagarajah, 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, M. H., R. A. Bennett, R-C Yang, B. Kebede, and M. R. Thiagarajah, 2011a: 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, M. H., A. Shakir, and M. J. Hasan, 2011b: Breeding for clubroot resistant spring canola (*Brassica napus* L.) for the Canadian prairies: Can the European winter canola cv. Mendel be used as a source of resistance? Can. J. Plant Sci. 91: 447-458.

Rahman, H., and B. Kebede, 2012: Improvement of spring canola *Brassica napus* (L.) by use of winter canola. J. Oilseed Brassica 3: 1-17.

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

Rahman, H., B. Kebede, C. Zimmerli, and R. C. Yang, 2014: Genetic study and QTL mapping of seed glucosinolate content *Brassica rapa* L. Crop Sci. 54: 1-7.

Rahman, H., R. A. Bennett, and G. Séguin-Swartz, 2015: Broadening genetic diversity in *Brassica napus* canola: Development of canola-quality spring *B. napus* from *B. napus* × *B. oleracea* var. a*lboglabra* interspecific crosses. Can. J. Plant Sci. 95: 1-13.

Reif, J. C., P. Zhang, S. Dreisigacker, M. L. Warburton, M. van Ginkel, D. Hoisington, M. Bohn and A. E. Melchinger, 2005: Wheat genetic diversity trends during domestication and breeding. Theor. Appl. Genet. 110: 859-864.

Riaz, A., G. Li, Z. Quresh, M. S. Swati, and C. F. Quiros, 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.

Rich, T. C. G., 1991: Crucifers of Great Britain and Ireland. Botanical Society of the British Isles, London, pp. 1–336.

Rohlf, F. J., 2000: NTSYS-pc numerical taxonomy and multivariate analysis system. Exeter Software, New York.

Rosillo-Calle, F., Pelkmans L., and A. Walter, 2009: A global overview of vegetable oils, with reference to biodiesel. A Report for the IEA bioenergy task 40, IEA bioenergy.

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

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

Sacristán, M. D., and M. Gerdemann, 1986: Different behavior of *B. juncea* and *B. carinata* as sources of *Phoma lingam* resistance in experiments of interspecific transfer to *B. napus*. Plant Breed. 97: 304-314.

Seyis, F., R. J. Snowdon, W. Luhs, and W. Friedt, 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.

Seyis, F., W. Friedt, and W. Lühs, 2006: Yield of *Brassica napus* L. hybrids developed using resynthesized rapeseed material sown at different locations. Field Crops Res. 96: 176-180.

Shaked, H., K. Kashkush, H. Ozkan, M. Feldman, and A. A. Levy, 2001: Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell 13: 1749-1759.

Sharma, S. R., P. K. Singh, V. Chable, and S. K. Tripathi, 2005: A review of hybrid cauliflower development. J. New Seeds 6: 151-193.

Siebel, J., and K. P. Pauls, 1989: A comparison of anther and microspore culture as a breeding tool in *Brassica napus*. Theor. Appl. Genet.78: 473-479.

Snowdon, R. J., T. Friedrich, W. Friedt, and W. Köhler, 2002: Identifying the chromosomes of the A and C genome diploid *Brassica* species *B. rapa* and *B. oleracea* in their amphidiploid *B. napus*. Theor. Appl. Genet. 104: 533-538.

Song, K. M., T. C. Osborn, and P. H. Williams, 1988: *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theor. Appl. Genet. 75: 784-794.

Song, K, P. Lu, K. Tang, and T. C. Osborn, 1995: Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proceedings of the National Academy of Sciences 92: 7719-7723.

Song, K. and T. C. Osborn, 1992: Polyphyletic origins of *Brassica napus*: new evidence based on organelle and nuclear RFLP analyses. Genome 35: 992-1001.

Sønderby, I. E., F. Geu-Flores, and B. A. Halkier, 2010: Biosynthesis of glucosinolates-gene discovery and beyond. Trends Plant Sci. 15: 283-290.

Starmer, K. P., J. Brown, and J. B. Davis, 1998: Heterosis in spring canola hybrids grown in Northern Idaho. Crop Sci. 38: 376-380.

Stefansson, B. R., 1983: The development of improved rapeseed cultivars. In: Kramer JKG, Sauer FD and Pigden EJ (eds) High and low erucic acid rapeseed oils: Production, Usage, Chemistry, and Toxicological Evaluation. Academic Press, New York, pp 143-159.

Szadkowski, E., F. Eber, V. Huteau, M. Lode, C. Huneau, H. Belcram, O. Coriton, M. J. Manzanares- Dauleux, R. Delourme, G. J. King, B. Chalhoub, E. Jenczewski, and A. M. Chèvre, 2010: The first meiosis of resynthesized *Brassica napus*, a genome blender. New Phytol. 186: 102-112.

Tallury, S. P., and M. M. Goodman, 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.

Thormann, C. E., J. Romero, J. Mantet, and T. C. Osborn, 1996: Mapping loci controlling the concentrations of erucic and linolenic acids in seed oil of *Brassica napus* L. Theor. Appl. Genet. 93: 282-286.

Tian, E., Y. Jiang, L. Chen, J. Zou, F. Liu, and J. Meng, 2010: Synthesis of a *Brassica* trigenomic allohexaploid (*B. carinata* × *B. rapa*) de novo and its stability in subsequent generations. Theor. Appl. Genet. 121: 1431-1440.

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

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

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

Udall, J. A., P. A. Quijada, H. Polewicz, R. Vogelzang, and T. C. Osborn, 2004: Phenotypic effects of introgressing Chinese winter and resynthesized *Brassica napus* L. germplasm into hybrid spring canola. Crop Sci. 44: 1990-1996.

Udall, J. A., P. A. Quijada, and T. C. Osborn, 2005: Detection of chromosomal rearrangements derived from homeologous recombination in four mapping populations of *Brassica napus* L. Genetics 169: 967-979.

Udall, J. A., P. A. Quijada, B. Lambert, and T. C. Osborn, 2006: Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (*Brassica napus* L.): 2. Identification of alleles from unadapted germplasm. Theor. Appl. Ge net. 113: 597-609.

United States Department of Agriculture report 2012 available at http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx#.UWbBYLUxDA4.

United State Department of Agricultuere, economic research service, http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx

United States Department of Agriculture (USDA), publicated in March 2014. Available at http://www.statista.com/statistics/263937/vegetable-oils-global-consumption/

van de Wouw, M., C. Kik, T. van Hintum, R. van Treuren, and B. Visser, 2010: Genetic erosion in crops: concept, research results and challenges. Plant Genet. Resour. 8: 1-15.

von Bothmer, R., M. Gustafsson, and S. Snogerup, 1995: *Brassica* sect. *Brassica* (Brassicaceae). II. Inter- and intraspecific crosses with cultivars of *B. oleracea*. Genet. Resour. Crop Evol. 42: 165-178.

Velasco, P., P. Soengas, M. Vilar, and M. E. Cartea, and M. del Rio, 2008: Comparison of glucosinolate profiles in leaf and seed tissues of different *Brassica napus* crops. JASHS 133: 551-558.

Wang, N., F. Li, B. Chen, K. Xu, G. Yan, J. Qiao, J. Li, G. Gao, I. Bancroft, J. Meng, G. J. King, and X. Wu, 2014: Genome-wide investigation of genetic changes during modern breeding of *Brassica napus*. Theor. Appl. Genet. 127: 1817-1829.

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

Warwick, S. I., A. Francis, and I. A. Al-Shehbaz, 2006: Brassicaceae: species checklist and database on CD-Rom. Pl. Syst. Evol. 259: 249-258.

Warwick, S. I., A. Francis, R. K. Gugel, 2009: Guide to wild germplasm of *Brassica* and allied crops (tribe *Brassiceae*, *Brassicaceae*) 3rd edn. Agriculture and Agri-Food Canada Techical Bulletin, Ottawa, Canada.

Wikipedia, retrived in Feb 2014. Available at http://en.wikipedia.org/wiki/Triangle-of-U

Wroblewski, T., S. Coulibaly, J. Sadowski, and C. F. Quiros, 2000: Variation and phylogenetic utility of the *Arabidopsis thaliana* Rps2 homolog in various species of the tribe *Brassiceae*. Mol. Phylog. Evol. 16: 440-448.

Xiao, J., S. Grandillo, S. N. Ahn, S. R. McCouch, S. D. Tanksley, J. Li, L. Yuan, L. Stenflo and M. Y. Yu, 1996: Genes from wild rice improve yield. Nature 384: 223-224.

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

Yang, Y. W., K. N. Lai, P. Y. Tai, and W. H. Li, 1999: Rates of nucleotide substitution in angiosperm mitochondrial DNA sequence and dates of divergence between *Brassica* and other angiosperm lineages. J. Mol. Evol. 48: 597-604.

Zhang, J. F., Q. I. Cun-Kou, P.U. Hui-Ming, C. Song, C. Feng, G. A. O. Jian-Qin, C. Xin-Jun, G. U. Hui, and F. U. Shou-Zhong, 2008: QTL identification for fatty acid content in rapeseed (*Brassica napus* L.). Acta Agron. Sin. 34: 54-60.

Ziolkowski, P. A., M. Kaczmarek, D. Babula, and J. Sadowski, 2006: Genome evolution in *Arabidopsis*/*Brassica*: conservation and divergence of ancient rearranged segments and their breakpoints. Plant J. 47: 63-74.

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

Zou, J., D. Fu, H. Gong, W. Qian, W. Xia, J. C. Pires, R. Y. Li, Y. Long, A. S. Mason, T. J. Yang, Y. P. Lim, B. S. Park, and J. L. Meng, 2011: De novo genetic variation associated with retrotransposon activation, genomic rearrangements and trait variation in a recombinant inbred line population of *Brassica napus* derived from interspecific hybridization with *Brassica rapa*. Plant J. 68: 212-224.

Appendices

Supplementary table from Chapter 3

Table S3.1. Pedigree of F_4 plants derived from A04-73NA × *B. oleracea* var. *alboglabra* (Cross ID 5CA1300) and A04-73NA × *B. oleracea* var. *botrytis* (Cross ID 5CA1343) and BC_1F_3 plants derived from (A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA (Cross ID 5CA1676) and (A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA (Cross ID 5CA1677) used for genetic fingerprinting by use of simple sequence repeat (SSR) markers.

Cross ID	F_2/ BC_1 plant	F_3/ BC_1F_2 plant	Plants Genotyped gen: F_4/BC_1F_3	Gen.	Sample code
5CA1300	5CA1300.002-A1220 P15	5CA1300.010-A1231 P2	5CA1300.098-A1242P02	F_4	Albo-F-67
5CA1300	5CA1300.002-A1220 P15	5CA1300.010-A1231 P2	5CA1300.098-A1242P03	F_4	Albo-F-85
5CA1300	5CA1300.002-A1220 P15	5CA1300.010-A1231 P5	5CA1300.099-A1242P04	F_4	Albo-F-74
5CA1300	5CA1300.002-A1220 P17	5CA1300.012-A1231 P1	5CA1300.102-A1242P03	F_4	Albo-F-92
5CA1300	5CA1300.002-A1220 P27	5CA1300.025-A1231 P2	5CA1300.122-A1242P03	F_4	Albo-F-72
5CA1300	5CA1300.002-A1220 P27	5CA1300.025-A1231 P5	5CA1300.123-A1242P04	F_4	Albo-F-76
5CA1300	5CA1300.002-A1220 P34	5CA1300.031-A1231 P1	5CA1300.128-A1242P03	F_4	Albo-F-77
5CA1300	5CA1300.002-A1220 P34	5CA1300.031-A1231 P1	5CA1300.128-A1242P04	F_4	Albo-F-86
5CA1300	5CA1300.002-A1220 P34	5CA1300.031-A1231 P5	5CA1300.129-A1242P01	F_4	Albo-F-70
5CA1300	5CA1300.002-A1220 P35	5CA1300.033-A1231 P4	5CA1300.132-A1242P03	F_4	Albo-F-73
5CA1300	5CA1300.002-A1220 P35	5CA1300.035-A1231 P2	5CA1300.137-A1242P01	F_4	Albo-F-66
5CA1300	5CA1300.002-A1220 P35	5CA1300.035-A1231 P2	5CA1300.137-A1242P04	F_4	Albo-F-90
5CA1300	5CA1300.002-A1220 P7	5CA1300.040-A1231 P4	5CA1300.142-A1242P03	F_4	Albo-F-87
5CA1300	5CA1300.002-A1220 P7	5CA1300.040-A1231 P5	5CA1300.143-A1242P03	F_4	Albo-F-69
5CA1300	5CA1300.002-A1220 P7	5CA1300.040-A1231 P5	5CA1300.143-A1242P01	F_4	Albo-F-71
5CA1300	5CA1300.002-A1220 P7	5CA1300.040-A1231 P5	5CA1300.143-A1242P04	F_4	Albo-F-82
5CA1343	5CA1343.002-A1220 P11	5CA1343.006-A1231 P3	5CA1343.095-A1242P05	F_4	Boty-F-78
5CA1343	5CA1343.002-A1220 P12	5CA1343.007-A1231 P1	5CA1343.097-A1242P03	F_4	Boty-F-83
5CA1343	5CA1343.002-A1220 P19	5CA1343.014-A1231 P3	5CA1343.106-A1242P03	F_4	Boty-F-79
5CA1343	5CA1343.002-A1220 P19	5CA1343.014-A1231 P3	5CA1343.106-A1242P02	F_4	Boty-F-91

5CA1343	5CA1343.002-A1220 P31	5CA1343.028-A1231 P4	5CA1343.123-A1242P02	F_4	Boty-F-81
5CA1343	5CA1343.002-A1220 P31	5CA1343.028-A1231 P4	5CA1343.123-A1242P03	F_4	Boty-F-84
5CA1343	5CA1343.002-A1220 P32	5CA1343.029-A1231 P1	5CA1343.124-A1242P01	F_4	Boty-F-68
5CA1343	5CA1343.002-A1220 P32	5CA1343.029-A1231 P1	5CA1343.124-A1242P03	F_4	Boty-F-80
5CA1343	5CA1343.002-A1220 P34	5CA1343.031-A1231 P4	5CA1343.126-A1242P05	F_4	Boty-F-75
5CA1343	5CA1343.002-A1220 P4	5CA1343.033-A1231 P1	5CA1343.127-A1242P04	F_4	Boty-F-89
5CA1343	5CA1343.002-A1220 P8	5CA1343.037-A1231 P1	5CA1343.129-A1242P01	F_4	Boty-F-88
5CA1343	5CA1343.002-A1220 P8	5CA1343.037-A1231 P1	5CA1343.129-A1242P02	F_4	Boty-F-93
5CA1676	5CA1300.003-A6220 P13	5CA1300.045-A1231P03	5CA1676.003-A1242p02	BC_1F_3	Albo-BC-56
5CA1676	5CA1300.003-A6220 P16	5CA1300.048-A1231P02	5CA1676.005-A1242p01	BC_1F_3	Albo-BC-62
5CA1676	5CA1300.003-A6220 P16	5CA1300.048-A1231P05	5CA1676.007-A1242p01	BC_1F_3	Albo-BC-37
5CA1676	5CA1300.003-A6220 P16	5CA1300.048-A1231P05	5CA1676.007-A1242p02	BC_1F_3	Albo-BC-40
5CA1676	5CA1300.003-A6220 P21	5CA1300.054-A1231P01	5CA1676.011-A1242p01	BC_1F_3	Albo-BC-35
5CA1676	5CA1300.003-A6220 P22	5CA1300.055-A1231P02	5CA1676.013-A1242p03	BC_1F_3	Albo-BC-29
5CA1676	5CA1300.003-A6220 P22	5CA1300.055-A1231P02	5CA1676.013-A1242p01	BC_1F_3	Albo-BC-33
5CA1676	5CA1300.003-A6220 P22	5CA1300.055-A1231P02	5CA1676.013-A1242p02	BC_1F_3	Albo-BC-44
5CA1676	5CA1300.003-A6220 P22	5CA1300.055-A1231P03	5CA1676.014-A1242p01	BC_1F_3	Albo-BC-8
5CA1676	5CA1300.003-A6220 P24	5CA1300.057-A1231P03	5CA1676.017-A1242p01	BC_1F_3	Albo-BC-38
5CA1676	5CA1300.003-A6220 P24	5CA1300.057-A1231P03	5CA1676.017-A1242p02	BC_1F_3	Albo-BC-63
5CA1676	5CA1300.003-A6220 P24	5CA1300.057-A1231P05	5CA1676.018-A1242p01	BC_1F_3	Albo-BC-59
5CA1676	5CA1300.003-A6220 P26	5CA1300.059-A1231P02	5CA1676.021-A1242p01	BC_1F_3	Albo-BC-52
5CA1676	5CA1300.003-A6220 P27	5CA1300.060-A1231P02	5CA1676.025-A1242p03	BC_1F_3	Albo-BC-34
5CA1676	5CA1300.003-A6220 P27	5CA1300.060-A1231P02	5CA1676.025-A1242p01	BC_1F_3	Albo-BC-61
5CA1676	5CA1300.003-A6220 P27	5CA1300.060-A1231P03	5CA1676.026-A1242p01	BC_1F_3	Albo-BC-42
5CA1676	5CA1300.003-A6220 P3	5CA1300.063-A1231P01	5CA1676.030-A1242p02	BC_1F_3	Albo-BC-3
5CA1676	5CA1300.003-A6220 P3	5CA1300.063-A1231P01	5CA1676.030-A1242p03	BC_1F_3	Albo-BC-45
5CA1676	5CA1300.003-A6220 P3	5CA1300.063-A1231P04	5CA1676.032-A1242p01	BC_1F_3	Albo-BC-27
5CA1676	5CA1300.003-A6220 P3	5CA1300.063-A1231P04	5CA1676.032-A1242p03	BC_1F_3	Albo-BC-41
5CA1676	5CA1300.003-A6220 P31	5CA1300.065-A1231P02	5CA1676.033-A1242p03	BC_1F_3	Albo-BC-6

5CA1676	5CA1300.003-A6220 P31	5CA1300.065-A1231P03	5CA1676.034-A1242p02	BC_1F_3	Albo-BC-49
5CA1676	5CA1300.003-A6220 P32	5CA1300.066-A1231P05	5CA1676.038-A1242p02	BC_1F_3	Albo-BC-28
5CA1676	5CA1300.003-A6220 P36	5CA1300.070-A1231P05	5CA1676.049-A1242p02	BC_1F_3	Albo-BC-48
5CA1676	5CA1300.003-A6220 P37	5CA1300.071-A1231P02	5CA1676.051-A1242p01	BC_1F_3	Albo-BC-17
5CA1676	5CA1300.003-A6220 P38	5CA1300.072-A1231P04	5CA1676.055-A1242p01	BC_1F_3	Albo-BC-50
5CA1676	5CA1300.003-A6220 P38	5CA1300.072-A1231P05	5CA1676.056-A1242p02	BC_1F_3	Albo-BC-15
5CA1676	5CA1300.003-A6220 P4	5CA1300.074-A1231P01	5CA1676.058-A1242p01	BC_1F_3	Albo-BC-32
5CA1676	5CA1300.003-A6220 P40	5CA1300.075-A1231P03	5CA1676.060-A1242p03	BC_1F_3	Albo-BC-23
5CA1676	5CA1300.003-A6220 P42	5CA1300.078-A1231P04	5CA1676.066-A1242p01	BC_1F_3	Albo-BC-53
5CA1676	5CA1300.003-A6220 P42	5CA1300.078-A1231P05	5CA1676.067-A1242p01	BC_1F_3	Albo-BC-57
5CA1676	5CA1300.003-A6220 P5	5CA1300.085-A1231P01	5CA1676.076-A1242p01	BC_1F_3	Albo-BC-21
5CA1676	5CA1300.003-A6220 P5	5CA1300.087-A1231P01	5CA1676.079-A1242p02	BC_1F_3	Albo-BC-18
5CA1676	5CA1300.003-A6220 P7	5CA1300.090-A1231P04	5CA1676.085-A1242p03	BC_1F_3	Albo-BC-55
5CA1676	5CA1300.003-A6220 P9	5CA1300.092-A1231P02	5CA1676.090-A1242p01	BC_1F_3	Albo-BC-58
5CA1676	5CA1300.003-A6220 P9	5CA1300.092-A1231P03	5CA1676.091-A1242p01	BC_1F_3	Albo-BC-54
5CA1677	5CA1343.003-A6220 P20	5CA1343.051-A1231P01	5CA1677.014-A1242p03	BC_1F_3	Boty-BC-46
5CA1677	5CA1343.003-A6220 P22	5CA1343.053-A1231P02	5CA1677.016-A1242p02	BC_1F_3	Boty-BC-2
5CA1677	5CA1343.003-A6220 P25	5CA1343.056-A1231P01	5CA1677.018-A1242p02	BC_1F_3	Boty-BC-19
5CA1677	5CA1343.003-A6220 P25	5CA1343.056-A1231P01	5CA1677.018-A1242p03	BC_1F_3	Boty-BC-26
5CA1677	5CA1343.003-A6220 P26	5CA1343.057-A1231P01	5CA1677.019-A1242p01	BC_1F_3	Boty-BC-60
5CA1677	5CA1343.003-A6220 P27	5CA1343.058-A1231P03	5CA1677.022-A1242p01	BC_1F_3	Boty-BC-43
5CA1677	5CA1343.003-A6220 P27	5CA1343.058-A1231P04	5CA1677.023-A1242p03	BC_1F_3	Boty-BC-13
5CA1677	5CA1343.003-A6220 P3	5CA1343.061-A1231P01	5CA1677.026-A1242p01	BC_1F_3	Boty-BC-64
5CA1677	5CA1343.003-A6220 P34	5CA1343.066-A1231P03	5CA1677.034-A1242p01	BC_1F_3	Boty-BC-1
5CA1677	5CA1343.003-A6220 P34	5CA1343.066-A1231P04	5CA1677.035-A1242p02	BC_1F_3	Boty-BC-16
5CA1677	5CA1343.003-A6220 P34	5CA1343.066-A1231P05	5CA1677.036-A1242p02	BC_1F_3	Boty-BC-30
5CA1677	5CA1343.003-A6220 P34	5CA1343.066-A1231P05	5CA1677.036-A1242p01	BC_1F_3	Boty-BC-31
5CA1677	5CA1343.003-A6220 P34	5CA1343.066-A1231P05	5CA1677.036-A1242p03	BC_1F_3	Boty-BC-39

5CA1677	5CA1343.003-A6220 P36	5CA1343.068-A1231P02	5CA1677.038-A1242p02	BC_1F_3	Boty-BC-5
5CA1677	5CA1343.003-A6220 P36	5CA1343.068-A1231P02	5CA1677.038-A1242p03	BC_1F_3	Boty-BC-25
5CA1677	5CA1343.003-A6220 P36	5CA1343.068-A1231P05	5CA1677.040-A1242p02	BC_1F_3	Boty-BC-4
5CA1677	5CA1343.003-A6220 P36	5CA1343.068-A1231P05	5CA1677.040-A1242p03	BC_1F_3	Boty-BC-36
5CA1677	5CA1343.003-A6220 P39	5CA1343.071-A1231P01	5CA1677.042-A1242p03	BC_1F_3	Boty-BC-9
5CA1677	5CA1343.003-A6220 P39	5CA1343.071-A1231P02	5CA1677.043-A1242p02	BC_1F_3	Boty-BC-7
5CA1677	5CA1343.003-A6220 P39	5CA1343.071-A1231P03	5CA1677.044-A1242p01	BC_1F_3	Boty-BC-10
5CA1677	5CA1343.003-A6220 P39	5CA1343.071-A1231P04	5CA1677.045-A1242p02	BC_1F_3	Boty-BC-24
5CA1677	5CA1343.003-A6220 P39	5CA1343.071-A1231P04	5CA1677.045-A1242p03	BC_1F_3	Boty-BC-65
5CA1677	5CA1343.003-A6220 P41	5CA1343.074-A1231P03	5CA1677.050-A1242p02	BC_1F_3	Boty-BC-22
5CA1677	5CA1343.003-A6220 P46	5CA1343.079-A1231P03	5CA1677.056-A1242p01	BC_1F_3	Boty-BC-51
5CA1677	5CA1343.003-A6220 P48	5CA1343.081-A1231P05	5CA1677.061-A1242p03	BC_1F_3	Boty-BC-47
5CA1677	5CA1343.003-A6220 P52	5CA1343.086-A1231P01	5CA1677.065-A1242p01	BC_1F_3	Boty-BC-12
5CA1677	5CA1343.003-A6220 P52	5CA1343.086-A1231P01	5CA1677.065-A1242p03	BC_1F_3	Boty-BC-20
5CA1677	5CA1343.003-A6220 P6	5CA1343.087-A1231P05	5CA1677.070-A1242p03	BC_1F_3	Boty-BC-14
5CA1677	5CA1343.003-A6220 P7	5CA1343.088-A1231P05	5CA1677.073-A1242p02	BC_1F_3	Boty-BC-11
			B. oleracea var. *botrytis*		*B. botry*
			B. napus		*B. napus*
			B. oleracea var. *alboglabra*		*B. albo*