Broadening of genetic diversity in spring canola (*Brassica napus* L.) by use of the C genome of *Brassica oleracea* var. *italica* and *Brassica oleracea* var. *capitata*

by

Xin Wang

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 canola *Brassica napus* L. (AACC, 2*n* = 38) is one of the major crops in Canada. A decline in genetic diversity in breeding populations is a threat for continued improvement of this crop from a long-term perspective. Genetic diversity in Canadian spring *B. napus* canola can be broadened through introgression of allelic diversity from its diploid progenitor species *Brassica rapa* L., *Brassica oleracea* L., and other allied species of the family Brassicaceae. This M.Sc. thesis research investigated the feasibility of introgression of new alleles from two variants of *B. oleracea*, viz. *B. oleracea* var. *italica* (broccoli) and var. *capitata* (cabbage) into spring *B. napus* canola. For this, *B. napus* × *B. oleracea* interspecific crosses were made and the F_1 plants were self-pollinated for F_2 seeds as well as backcrossed to the *B. napus* parent for backcross (BC_1) seeds. The F_2 and BC_1 populations were self-pollinated for several generations with selection for canola quality traits for the development of euploid *B. napus* (2*n* = 38) plants. Plant fertility was poor in early generations; however, it improved with the progression of generation. Flow cytometric analysis for nuclear DNA content showed that the majority of the advanced generation plants were similar to the *B. napus* parent. Segregation for erucic acid and glucosinolate contents was found in all populations where selection for zero erucic acid and low glucosinolate content led to the development of canola quality lines in advanced generation. Estimation of genetic diversity in F_4 and BC_1F_3 populations by the use of simple sequence repeats (SSR) markers showed that *B. oleracea* alleles introgressed in the progeny derived from *B. napus* × *B. oleracea* crosses. Thus, the results from this study demonstrated the viability of introducing alleles from broccoli and cabbage into spring *B. napus* canola.

Preface

This M.Sc. thesis reports interspecific hybridization between *B. napus* and *B. oleracea* for the development of genetically diverse canola quality *B. napus* lines for heterosis in *B. napus* hybrid cultivars. The early generation populations reported in this thesis were generated by staff of the Canola Program of the University of Alberta under supervision of Dr. Habibur Rahman. This includes crossing of *B. napus* and *B. oleracea* (broccoli and cabbage) and growing of F_1, F_2, F_4 and F_5, and BC_1 (F_1 × *B. napus*), BC_1F_2, BC_1F_3 and BC_1F_4 populations reported in Chapter 2. Agronomic and seed quality data of these populations were also collected by the Canola Program. I was responsible for growing of the F_6, F_7 and F_8, and BC_1F_5, BC_1F_6 and BC_1F_7 populations and collection of all data.

For the development of inbred lines from the interspecific crosses reported in Chapter 2, experiments were conducted in greenhouse and field. In case of the experiments in greenhouse, I seeded the materials and took all notes; greenhouse staff watered and fertilized the plants. In case of the field experiment conducted in 2014, I got help from the Canola Program for seeding and harvesting. Soil preparation and management of the field plots, such as fertilization and weed control, was done by Mr. Jose Salvador Lopez. I collected all data from this field trial.

In case of the experiment with test hybrids reported in Chapter 2, I produced all test hybrid seeds in greenhouse during 2014-15 winter and laid out the design of the field experiment. I got help from the Canola Program for seeding and harvesting. Soil preparation for the trial and plot management was done by Mr. Jose Salvador Lopez. I was responsible for taking all notes of this hybrid trial.

For genetic diversity analysis with F_4 and BC_1F_3 populations reported in Chapter 3, the leaf samples were collected and stored at -80 ^oC by Rameez Iftikhar. I extracted DNA, identified polymorphic markers, genotyped the populations and analyzed all data. I received training on molecular marker analysis from Dr. Berisso Kebede and Dr. Neil Hobson.

I was responsible for analysis of all data reported in this thesis and interpretation of the results.

Acknowledgements

I would like to express my deep thanks of gratitude to my supervisor Dr. Habibur Rahman who gave me the opportunity to study in Canola Breeding Program of University of Alberta. Thanks to his academic guidance, valuable advice and criticism during my M.Sc. study, I completed my research project and built up canola breeding skills. I am also grateful to Dr. Changxi Li, my committee member, for his great support of my study and helpful suggestions of my research.

I would like to give my heartfelt thanks to Dr. Berisso Kebede, Dr. Neil Hobson and Dr. Rudolph Fredua-Agyeman for their technical support and guidance of my molecular work and An Vo for her guidance of canola quality analysis. I also want to give my sincere thanks to other members of Canola Breeding Program for their support during my research and course study.

I would like to acknowledge Natural Sciences and Engineering Research Council (NSERC) for the financial support to this research, and the University of Alberta for scholarship in the first year of my study.

Finally, I want to give my special thanks of gratitude to my beloved parents who always give me encouragement and moral support.

Table of Contents

List of Tables

List of Figures

List of Symbols and Abbreviations

±	Plus/minus
χ^2	Chi-square test statistics
2*n*	Diploid number of chromosomes
n	Number of observations
ng/µl	Nano gram per micro liter
µl	Microliter
µmol/g	Micromoles per gram
°C	Degrees Celsius
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_n	nth backcross generation
cm	Centimeter
cv.	Cultivar
d.f.	Degree of freedom
DH	Doubled haploid
DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleotide triphosphate
F_1	First filial generation
F_n	nth filial generation
FAO	Food and Agriculture Organization of the United Nations
Fig.	Figure

g	Gram
GCA	General Combining Ability
Gen.	Generation
GSL	Glucosinolate
HEAR	High erucic acid rapeseed
HOLL	Canola oil containing high oleic acid and low linolenic acid
LL	Low linolenic acid (C18:3) canola oil
m	Meter
M	Mole
min	Minute
mm	Millimeter
mM	Millimole
MPH	Mid-parent heterosis
MS	Mean squares
NIRS	Near infra-red spectroscopy
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RAPD	Random amplified polymorphism
RFLP	Restriction fragment length polymorphism
rpm	Revolution per minute
SCA	Specific combining ability
SD	Standard deviation
SE	Standard error
SNP	Single-nucleotide polymorphism
SSR	Simple sequence repeat
Taq polymerase	Thermus aquaticus polymerase

UPGMA	Unweighted pair-group method with arithmetic mean
USDA	United states department of agriculture
var.	Variety

Chapter 1

Literature review

1.1 Introduction

Canola, collectively includes *Brassica rapa* L. (AA, 2*n* = 20), *Brassica napus* L. (AACC, 2*n* = 38) and *Brassica juncea* (L.) Czern (AABB, 2*n* = 36), is one of the most important vegetable oil crops in the world. These crop species belong to the family *Brassicaceae* and contributes about 15% of the total vegetable oil supply in the world (Rahman et al. 2013).

Canola was developed from rapeseed through conventional plant breeding (McInnis 2004). Traditional *Brassica* seed oil contains a high level of erucic acid (> 40%). This fatty acid is considered to be the cause of accumulation of fat in the heart of animals (Heijkenskjold and Ernster 1975). Traditional *Brassica* seed meal also contains a high level of glucosinolates (> 60 µmol/g/seed). This compound is also known to exert adverse effects on animals when fed as protein supplements (European Food Safety Authority 2008). Therefore, efforts have been made by different researchers to eliminate or reduce the contents of erucic acid and glucosinolates from *Brassica* oilseeds. Canada was the first country in the world to introduce low erucic acid *Brassica* oilseed crop for commercial production. This trait was introduced from the European forage rape cultivar Liho to the Canadian *B. napus* in 1964 and the first low erucic acid cultivar Oro was released in 1968. The first double low (low erucic acid, low glucosinolate) *B. napus* cultivar Tower was developed at the University of Manitoba through introduction of low glucosinolate genes from the Polish fodder rape cultivar Bronowski (Stefansson and Downey

1995). This type of double low cultivars of the *Brassica* oilseed crops is generally called 'canola'.

Canola is one of the most important crops in Canada in regard to its acreage and production. Several seed companies are involved in the breeding of canola cultivars. Canola breeding research in the past focused on increasing seed yield, seed quality and resistance to diseases and herbicides. In Canada, three major groups of herbicide-tolerant canola are grown: Roundup Ready, Liberty Link and Clearfield. Roundup Ready and Liberty Link herbicide-tolerance traits were developed through genetic engineering, while Clearfield herbicide-tolerance trait was developed through mutagenesis (Smyth et al. 2011). Currently, hybrid canola cultivars captured more than 90% of the Canadian canola acreage; therefore, research on broadening of genetic diversity in this crop has received attention in the canola breeding programs as genetic diversity between hybrid-parents often show association with heterosis in hybrid cultivars (reviewed in Rahman 2013). The long term focus of this MSc thesis research project is to broaden genetic diversity in spring *B. napus* canola through exploitation of the barely explored C-genome of *Brassica oleracea* L. This section of the thesis reviews the economic importance of this oilseed crop, the use of canola oil and meal, genetic control of the two canola quality traits (erucic acid and glucosinolate), evolution of the three *Brassica* genomes and relationships between the *Brassica* species. This section also reviews the extent of genetic diversity present in *Brassica*, its importance and relationship with heterosis.

1.2 Canola and its economic importance

The oilseed crop *B. napus* exists in three ecotypes based on the different growth habit: winter, semi-winter, and spring or summer types. Winter-type requires vernalization (exposure to

low temperature to induce flowering) for about eight weeks to flower; this type is grown primarily in Europe. Semi-winter type requires vernalization for about four weeks and is grown largely in China. The spring type requires no vernalization for flowering and is grown primarily in Canada, Australia and Northern Europe (Butruille et al. 1999; Ferreira et al. 1995; Qian et al. 2006). The major spring *B. napus* canola growing provinces in Canada include Saskatchewan, Alberta and Manitoba; small cultivation area can also be found in British Columbia and Ontario (LMC International 2013).

The oilseed type *B. rapa* exists in spring and winter forms. The spring-growth form is mainly cultivated in Indian sub-continent and in small acreage in Canada, northern Europe, and China, while the winter-growth form is dominant in China and also grown in small acreage in northern Europe. *B. juncea* exists only in spring growth habit; primarily grown in Indian subcontinent and in limited areas in Canada (Labana and Gupta 1993; Raymer 2002; Prakash 2012).

Large consumption of canola oil needs millions of tons of canola seeds to be produced every year in the world. In 2013-14, global canola oil consumption reached to 25.63 million metric tons (USDA 2015a), and about 71 million metric tons of seeds was produced to meet this demand (Statista 2015). In 2013, Canada was the largest producer of this crop with a production of 17.95 million metric tons, while China and India produced 14.46 and 7.82 million metric tons, respectively (Fig. 1.1).

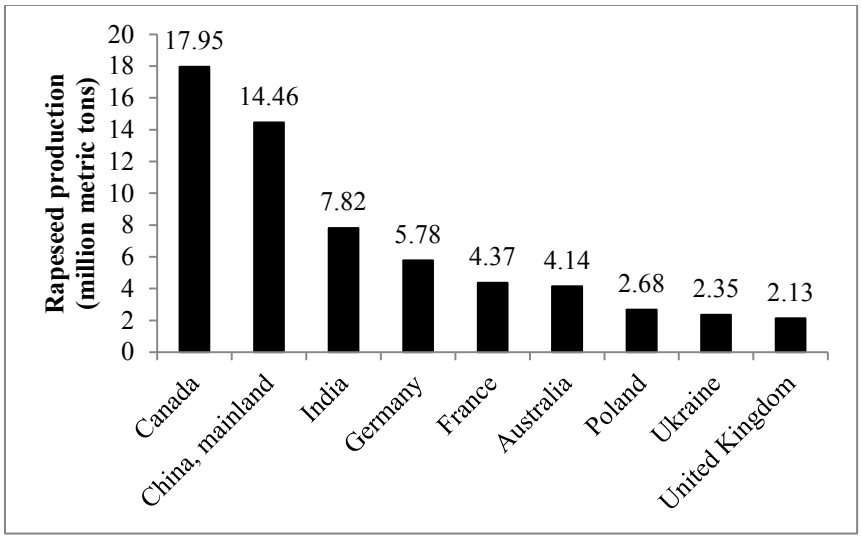

Fig. 1.1 Global production of Brassica oilseeds in 2013 (FAO 2013).

Canada is also the largest exporter of canola in the world (Canadian Agri-Food Trade Allicance 2013, Fig. 1.2). About 90% of the total Canadian canola generally exported as raw seed or oil and meal to about 55 markets all over the world and this brings billions of dollars every year into the country. Raw seeds are exported to China, Japan, Mexico, the United States and the United Arab Emirates (USDA 2015b). Direct and indirect contribution of this crop to the Canadian economy is about $21 billion per year (LMC International 2013).

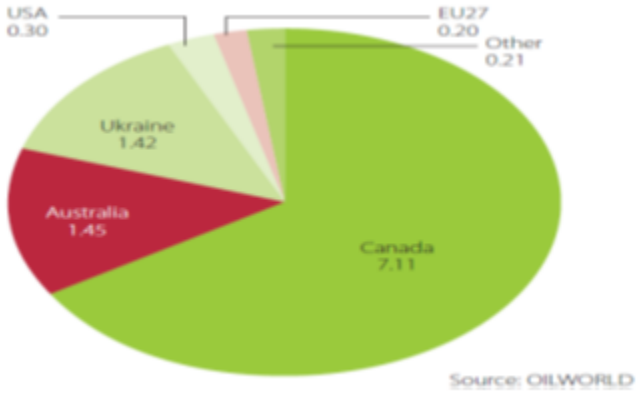

Fig. 1.2 Major canola exporting countries in the world in 2010-11(million metric tons). (http://www.agric.wa.gov.au).

1.3 Canola oil and meal

Traditionally, the use of *Brassica* seed oil in western countries was limited to non-food application, such as lubricants, wood preservatives and lighting fuel. Currently, this oil is primarily used for edible purposes all over the world. However, to meet the increasing demand for fuel, this oil is also used as a feedstock for biodiesel production, especially in Europe (Sheehan et al. 1998). The seed meal remaining after extraction of oil contains about 40% protein and is used as animal feed (Newkirk 2009).

Canola seeds contain about 45% oil (Rahman et al. 2013), which is composed of about 6.0% saturated fatty acids (3.5% palmitic C16:0, 1.5% stearic C18:0, 0.6% arachidic C20:0, and 0.3% behenic C22:0), 62% monounsaturated fatty acids (60.1% oleic C18:1, 1.4% gadoleic C20:1, 0.2% erucic C22:1), and 30% polyunsaturated fatty acids (20.1% linoleic C18:2, and 9.6% linolenic C18:3) (Ackman 1990, cited by Przybylski 2001). Saturated fatty acids are considered unhealthy for human health. For example, diet rich in saturated fatty acids can cause inflammation-related diseases (van Dijk et al. 2009). Fatty acid composition in different types of *Brassica* oil is presented in Table 1.

Table 1.1 Fatty acid composition of different types of *Brassica* oil.

	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1
Rapeseed	4.0	0.3	1.0	15.0	14.1	9.1	1.0	10.0	0.8	45.1
Canola	3.5	0.2	1.5	60.1	20.1	9.6	0.6	1.4	0.3	0.2
LL[a]	3.9	0.2	1.2	61.1	27.1	2.1	0.6	1.5	0.4	0.1
HOLL[b]	3.4	0.2	2.5	76.8	7.8	2.6	0.9	1.6	0.5	0.1

Source: Przybylski 2001.
[a]LL = low linolenic acid (C18:3) canola oil.
[b]HOLL = canola oil containing high oleic acid and low linolenic acid.

Modification of fatty acid profile of *Brassica* seed oil has been made in the past decades to produce oil for specific uses. The first modification was the elimination of erucic acid from traditional rapeseed and the development of canola cultivars free from this fatty acid to meet the

quality standard of the oil for edible purposes. Development of canola oil with high oleic acid and low linolenic acid contents made the oil suitable for frying applications as well as better stability of the oil under storage condition (Przybylski 2001).

Canola meal, normally contain about 36-39% protein (Newkirk 2009). This meal has become an important source of protein in feed for livestock. High glucosinolate content in traditional rapeseed meal is known to cause different adverse effects in animals, such as reduced utilization of protein and growth of chickens (Rutkowski 1971; Fenwick 1982, cited by Khajali and Slominski 2012). However, the feed value of this meal has been significantly improved through reduction of this seed constituent in double low or canola cultivars. Khajali and Slominski (2012) reviewed that canola meal has well-balanced amino acid composition despite this meal has lower protein content than soybean meal. Bell (1993) reviewed that canola meal contains less energy and protein, and higher content of fiber than soybean meal; however, most of the B-vitamins and essential minerals were found to be richer in canola meal than in soybean meal.

Although the importance of canola meal based diets has gradually increased for feeding pigs, poultry and cattle, the presence of some anti-nutritional compounds in this seed meal is still an issue for its use in animal feed. For example, sinapine and phytic acid in canola meal are responsible for "fishy eggs" or an off-flavor in egg produced by susceptible hens and reduced absorption of minerals in animal body, respectively (for review see Bell 1993, and Khajali and Slominski 2012). Another anti-nutrient compound in canola meal is the content of fiber in seed meal. Canola meal contains about three times greater content of fiber than soybean meal (reviewed in Bell 1993). The content of polyphenols in seed coat is associated with the darkness of seed coat color. Seed meal of black seed generally contains higher content of fiber than meal

from yellow-seeded canola (Rahman et al. 2001; Slominski et al. 1994). High content of fiber in canola meal reduces digestibility of this seed meal. Therefore, fiber content in canola seed meal can be reduced through the development of yellow-seeded canola cultivars. For example, Rahman et al. (2001) reported a yellow-seeded *B. napus* line with 55% reduced content of fiber in seed meal.

1.4 *Brassica* genome evolution

1.4.1 Origin of *Brassica* species

Polyploidy or whole genome duplication has played a pivotal role in crop evolution. Most of the present-day crop species evolved through polyploidization of the ancestral genome. According to Trick et al. (2009), soybean, cotton, bread wheat and oilseed canola are relatively recently formed polyploids. Polyploidization is not simply a process of the merger of two genomes; it also includes adjustment of the genomes at molecular and physiological level. Several studies have indicated that gene loss, chromosome rearrangement and change of gene expression usually follow the polyploidy process (Levy and Feldman 2004; Osborn et al. 2003; Schnable et al. 2011), and this can result in novel phenotypes, such as flowering time divergence and size of the plant or parts (Adams and Wendel 2005). Therefore, the knowledge of polyploidization and the level of genome alterations occurred will help to understand the origin of the crop plants, and this knowledge can be applied for the improvement of our crop plants.

Brassica species occur as diploid and allopolyploid. The species *Arabidopsis thaliana* was used to study the process of polyploidization and evolution of the *Brassica* genomes. There is strong evidence to support that the A-genome of *B. rapa*, B- genome of *B. nigra* and the C-genome of *B. oleracea* share homoelologous genomic regions (Parkin et al. 1995; Truco et al.

1996; Xiong and Pires 2011), and a collinear relationship exists between the genes conserved in the *Brassica* genomes and their homoeologous genome segments in *Arabidopsis* (Panjabi et al. 2008; Chalhoub et al. 2014). This indicates that the *Brassica* genomes probably evolved from an *Arabidopsis*-like common ancestor, and the ancestral genome of *Brassica* has been proposed to be a hexaploid (Parkin et al. 2002; Lysak et al. 2005). Chromosome rearrangement played an important role in speciation. For example, Parkin et al. (2002) found that a single inversion led to the formation of *Arabidopsis* chromosome 5 and its homologue in *Brassica*. The *Brassica* and *Abrabidopsis* lineages diverged about 20 Mya (Yang et al. 1999).The *Brassica* lineage was further splitted into *Nigra* lineage and *Rapa*/*Oleracea* lineage about 7.9 Mya (Lysak et al. 2005) as a result of diversification of the A, B, and C genomes (Warwick and Black 1991). Multiple chromosome duplication and chromosome rearrangement occurred during the formation of the three diploid *Brassica* species, as described in U's triangle (U 1935, Fig. 1.3) (reviewed in Prakash et al. 2012). *Brassica napus* was formed about 7,500 years ago through hybridization between *B. rapa* and *B. oleracea* followed by allopolyploidy events (Chalhoub et al. 2014). According to Chalhoub et al. (2014), since the origin of angiosperms, about 72× genome multiplication has occurred for the formation of *B. napus*.

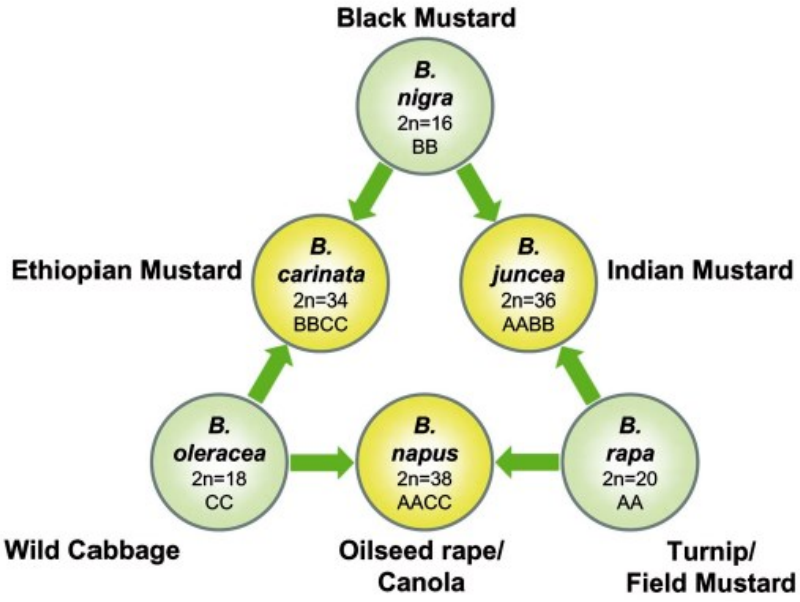

Fig. 1.3 Relationship between different *Brassica* species (U 1935, modified by Ahuja et al. 2010).

1.4.2 Implication of polyploidization in *Brassica napus* improvement

The knowledge of polyploidization and genome rearrangement events occurred during the evolution of the *Brassica* genomes can be valuable information for plant breeders for introgression of favorable genes from allied species into the target crop species. The first *B. napus* was evolved through spontaneous hybridization between only a few genotypes of *B. rapa* and *B. oleracea* during medieval times (cited by Iniguez-Luy and Federico 2011). This indicates that, a large portion of the gene pools of *B. rapa* and *B. oleracea* are not included in *B. napus*, and can be used for genetic improvement of this crop species.

The number of gene copies resulting from polyploidization during the evolution of the crop genomes may be associated with the variation observed in the phenotypes. For example, *A. thaliana* has only a single copy of *FLOWING LOCUS C* (*FLC*) gene controlling flowing time. On the other hand, *Brassica* has multiple copies – at least four *FLC* loci are present in *B. rapa*

(Schranz et al. 2002) while eight loci are present in *B. napus* (reviewed by Pires et al. 2004); this explains the variation of flowering time observed in these crop species.

1.5 Genetics of seed quality traits: Erucic acid and glucosinolates

The quality of *Brassica* seed oil is mainly determined by its fatty acid composition. Erucic acid (*cis*-1, 3-docosenoic acid, C22:1) is one of the major fatty acid in traditional *Brassica* seed oil. For human consumption, low erucic acid (<2%) oil is considered healthy since high content of erucic acid in oil is likely to be associated with myocardial lesions (Charlton et al. 1975). Despite its detrimental effect on human health, high erucic acid oil and the derivatives of erucic acid have valuable commercial applications. For example, high erucic acid oil is used as an additive to lubricants, and the amide derivatives of erucic acid are used in the production of polymers, surfactants, and surface coatings (reviewed in Töpfer et al. 1995; Scarth and Tang 2006).

Erucic acid content in *B. napus* seed oil is governed by two gene loci with additive effect of the genes (Harvey and Downey 1964). These two loci are mapped on the chromosome A8 (N8) and C3 (N13) (Zhang et al. 2008; Rahman et al. 2008). In the diploid species *B. rapa*, this trait is controlled by a single gene locus (Rahman et al. 1994); while in the amphidiploid species *B. juncea*, a two-gene loci model of the control of this trait has been confirmed by Mahmood et al. (2003). The knowledge of the genes and their control on erucic acid content is useful for developing *Brassica* oilseed cultivars with high or low content of erucic acid in seed oil. Rahman et al. (2008) developed high throughput gene-specific markers for the two erucic acid genes, *Bn-FAE1.1* and *Bn-FAE1.2*, of *B. napus*. They also found that a single nucleotide change in *Bn-FAE.1* of the A genome and two nucleotide deletion in *Bn-FAE1.2* of the C genome resulted the

zero erucic acid alleles. These markers can be used in marker-assisted selection for breeding of high- or low-erucic acid *B. napus* cultivars.

Glucosinolates (*β*-thioglucoside-*N*-hydroxysulfates) are sulfur-rich, secondary metabolites of plants. More than 120 types of glucosinolates are identified in the family Brassicaceae (reviewed in Fahey et al. 2001). On the basis of their origin from different amino acids such as methionine, phenylalanine, and tryptophan, glucosinolates are classified as aliphatic, benzyl and indole glucosinolates (reviewed in Rahman et al. 2014). When the plant tissues are damaged, glucosinolates undergo hydrolysis in presence of water and the enzyme myrosinase, and produce various compounds, such as isothiocyanates, thiocyanates and nitriles (Fahey et al. 2001). Some of these products, such as isothiocyanates, are beneficial to plants as these compounds impart resistance to insects and diseases (Bednarek et al. 2009; Hopkins et al. 2009). Similarly, isothiocyanates and indoles can reduce the risk of cancer in human body (Matusheski et al. 2006; Zhang and Talalay 1994; McDanell and McLean 1988; Traka and Mithen 2009). On the other hand, some of the breakdown products of glucosinolates are known to be deleterious to animals (Mawson et al. 1994). For example, the hydrolysis product nitriles are toxic and can cause liver hemorrhage in laying hens (Campbell 1987, cited by Mawson et al. 1994).

Classical genetic analysis of seed glucosinolate content showed that this trait is under polygenic control − at least four gene loci to be involved in the control of total seed glucosinolate content in *B. napus* (Rahman et al. 2001). Howell et al. (2003) detected four QTLs on the chromosomes A9, C2, C7, and C9 of *B. napus*; however, Rahman et al. (2014) detected three QTL on A2, A7 and A9 chromosomes of the A genome of *B. rapa*. Therefore, the number of QTL controlling total seed glucosinolate content in *B. napus* can be greater than four.

1.6 Genetic diversity

1.6.1 Importance of genetic variation in crops

Presence of genetic variation in crop germplasm is needed for the improvement of our crops through breeding. Furthermore, wide genetic variation between the commercial cultivars is also needed for secured crop production under the changing environment as well as biotic and abiotic stresses. However, many of our cultivated crop species have a narrow genetic base. This primarily resulted from the bottleneck during evolution and domestication (Cooper et al. 2001). For example, involvement of limited genetic variation of *B. rapa* and *B. oleracea* during the evolution of *B. napus* is one of the reasons of narrow genetic diversity observed in this species - despite wide genetic diversity exists in its two progenitors (Becker et al. 1995). Intensive plant breeding over a period of time has also narrowed down the genetic variability of *B. napus* (Fu and Gugel 2010). Plant breeders in private companies are usually under pressure to develop new cultivars in a short period of time (Rahman 2013); therefore, they repeatedly use elite lines or cultivars in breeding to develop new cultivars. This results in uniformity in genetic base of the crop and thus increases vulnerability of the crop to biotic and abiotic stresses as well (Cooper et al. 2001). The classic examples are the Irish famine in 1845 resulting from crop loss due to potato leaf blight disease, and the susceptibility of US maize to southern leaf blight disease in 1970. Therefore, it is an urgent and important task for plant breeders to broaden the genetic base of the breeding materials and maintain it for crop improvement.

1.6.2 Assessment of genetic diversity in *Brassica* species

Various methods, such as morphological traits (e.g. Yu et al. 2005; Alemayehu and Becker 2002), enzyme markers, and nuclear DNA markers (e.g. Becker et al. 1995; Hasan et al.

2006), were employed for evaluation of genetic diversity in diploid and amphidiploid *Brassica* species. Among these, nuclear DNA markers is the most commonly used and efficient method. The types of DNA markers used in these studies include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs) or microsatellites. Among these, RFLP is a hybridization based marker (require hybridization between a probe and homologous DNA segment) while the other three are PCR-based markers.

RFLP marker was the first DNA marker used to evaluate genetic variation in *Brassica* species. McGrath and Quiros (1992) used this marker to study genetic variation among 20 accessions of *B. rapa*. In the following two to three years, the same marker technique was used to estimate genetic diversity in *B. napus* (Diers and Osborn 1994; Becker et al. 1995). Given the disadvantages of RFLP marker analysis, such as the need of a large amount of DNA, time and cost, researchers invented more efficient and powerful markers with the development of PCR technique. The PCR-based markers are more sensitive and can detect low-frequency of polymorphism (Brown 1992, cited by Lu et al. 1996); this makes this type of markers useful for construction of genetic linkage maps (e. g. lettuce, Kesseli et al. 1994) and estimation of genetic diversity (e.g. wheat, Plaschke et al. 1995). For instance, AFLP markers were used to study genetic variation in different *Brassica* crops, such as *B. nigra* (Negi et al. 2004), *B. rapa* (Zhao et al. 2005), *B. carinata* (Warwick et al. 2006) and yellow-seeded *B. napus* (Yu et al. 2007). RAPD markers were also used in some cases to study genetic diversity in *Brassica* (Jain et al. 1994; Yu et al. 2005) as it is fast, cheap and can generate large number of data point in short period of time (Williams et al. 1990, cited by Lu et al. 1996). Microsatellites or SSRs developed later and found to be more informative, highly repeatable, and amenable to automation (Velasco and Fernández-

Martínez 2010). For example, by the use of 23 SSR markers, Ciancaleoni et al. (2014) identified the difference among broccoli (*B. oleracea*) landraces and their derived synthetics and F_1 hybrids. Hasan et al. (2006) evaluated genetic diversity in various types of *B. napus* from its primary gene pool by use of 30 SSR markers, and found that the vegetable type of *B. napus* has the greatest genetic diversity, followed by winter fodder and winter oilseed types; the spring oilseed and fodder type were found to have the least diverse genetic base.

Other methods, such as enzyme markers, morphological and agronomic traits, geographic origin, and pedigree information were also used to study genetic variation; this information was also combined with information generated from DNA marker analysis. Allozymes and isozymes markers were used in early studies (McGrath and Quiros 1992; Becker et al. 1995); however, the use of enzyme markers became limited due to lack of sufficient marker loci and low polymorphic information (Melchinger 1999, cited by Bennett 2012). Yu et al. (2005) estimated genetic similarities of the parents to predict heterosis in the hybrids by use of morphological features, and isozymes and RAPD markers.

1.6.3 Improvement of genetic variation in spring *Brassica napus*

Various studies indicated a decline in genetic variation occurred in modern *B. napus* cultivars (Fu and Gugel 2010; Cowling 2007). Genetically distinct germplasm identified by different researchers for broadening of genetic diversity in this crop (Hasan et al. 2006; Zhou et al. 2006).

Two hybridization based approaches, intra- and interspecific hybridization, can be applied to widen the genetic base of the spring *B. napus* canola gene pool. The intra-specific hybridization includes the use of other types of *B. napus*, such as winter and semi-winter types, and rutabagas. Several researchers suggested that these types of *B. napus* can be exploited in

breeding programs to enhance genetic diversity in spring canola (Quijada et al. 2006; Chen et al. 2008; Rahman and Kebede 2012; Rahman 2013). For example, Quijada et al. (2006) developed two doubled haploid (DH) populations of winter × spring type *B. napus* and used these DH's to produce test hybrids by crossing with spring canola. They found that QTL alleles introgressed from the winter parent can increase seed yield in test hybrids. This suggests that the winter *B. napus* germplasm can be used to develop high yielding spring canola hybrids. Kebede et al. (2010) demonstrated that seed yield in open-pollinated spring *B. napus* canola can also be increased through introgression of genetic diversity from European winter *B. napus* canola.

The other approach of broadening genetic diversity in spring *B. napus* canola is the interspecific hybridization with its diploid progenitor species *B. rapa* and *B. oleracea*, as well as with its other allied species, such as *B. juncea* and *B. carinata*. Zou et al. (2010) and Xiao et al. (2010) studied the feasibility of broadening genetic diversity in *B. napus* by use of the genomic components from *B. rapa* and *B. carinata*. They constructed *B. napus* lines ($A^rA^rC^cC^c$) possessing the A^rA^r genome component of *B. rapa* and C^cC^c genome component of *B. carinata*. Zou et al. (2010) studied heterosis for seed yield in hybrids produced by use of this new type of *B. napus* and found significant positive correlation between heterosis and the introgressed genome components. On the other hand, Xiao et al. (2010) found novel traits, such as yellow seed color, in this new type *B. napus*. Some other traits have also been introgressed into *B. napus* through interspecific hybridization. For example, crosses between *B. napus* and *B. rapa* were made to introgress early maturity into Chinese semi-winter *B. napus* (Liu 2000, cited by Xiao et al. 2010), and the early flowering trait was introduced from *B. oleracea* into spring *B. napus* (Rahman et al. 2011). Similarly, blackleg resistance genes have been introgressed into oilseed *B. napus* from its allied species (Delourme et al. 2006). *B. napus* lines resynthesized from *B. rapa*

and *B. oleracea* can also be used to broaden the genetic base of the spring *B. napus* canola (Girke et al. 2012b; Jesske et al. 2013).

The use of genetically distinct *Brassica* germplasm in the breeding of spring *B. napus* can be challenging, because unwanted alleles from exotic germplasm can also be introduced into the breeding population. Interspecific hybridization between spring *B. napus* and allied *Brassica* species can be challenging due to high level of sterility in the hybrid progenies caused by chromosomal anomalies at meiosis, as well as linkage drag (reviewed in Rahman 2013). However, repeated cycle of breeding or backcrossing of the interspecific hybrids to elite *B. napus* cultivars can overcome these difficulties. Therefore, the use of exotic germplasm and allied species in breeding program can benefit from the development of improved *B. napus* canola cultivars from a long-term perspective.

1.6.4 Genetic diversity and heterosis

Heterosis refers to the performance of the heterozygous hybrids over their homozygous parents. This phenomenon is usually measured as mid-parent heterosis, which is calculated as the difference between the F_1 hybrid and the mean of its parents, and high-parent heterosis, which is calculated as the difference between the F_1 hybrid and the high parent. Hybrid cultivars of field crops such as maize, canola, sorghum and rice, and important vegetables are grown commercially in different parts of the world (Duvick 1999).

The relationship between genetic diversity and heterosis has been studied in different crops. Early studies proposed that genetic distance between the parents generally correlates with the performance of F_1 hybrids or heterosis (Griffing and Linstrom 1954, cited by Yu et al. 2005). For example, Ali et al. (1995) studied genetic distance in 30 winter canola cultivars collected

from different sources and found a positive correlation between genetic distance of the parents and heterosis for seed yield. Similar results have also been reported by other researchers in *B. napus* (Riaz et al. 2001) as well as in rice (Xiao et al. 1996). However, some researchers found no strong correlation between genetic distance and hybrid seed yield in *B. napus* (Diers et al. 1996; Girke et al. 2012a), *B. juncea* (Jain et al. 1994) as well as in beans (Ghaderi et al. 1984). Based on hybrids developed from crossing of *B. napus* cultivars and resynthesized *B. napus* lines, where genetic diversity was introgressed from *B. oleracea*, Jesske et al. (2013) even found slightly negative correlation between hybrid seed yield and genetic distance. Fabrizius et al. (1998) reported that only part of the heterosis in spring wheat can be explained by genetic difference between the parental lines indicating that other factors may also have an effect on the expression of heterosis.

Studies on general combining ability (GCA) and specific combining ability (SCA) have been carried out by different researchers to identify good parents for hybrids. GCA is useful for identifying an inbred which has the highest average performance compared to other inbreds in a series of hybrid crosses, while SCA can identify the specific cross which potentially can end up with a commercial hybrid cultivar. Makumbi et al. (2011) found that mid-parent heterosis had a strong correlation with SCA and grain yield but a weak correlation with genetic distance in tropical maize. Similarly, Perenzin et al. (1998) also found that GCA and SCA had a positive effect on hybrids for agronomic traits such as grain yield and plant height in bread wheat. Therefore, heterosis is related to both genetic distance and combining ability of the parental lines, and this agrees with the results reported by Diers et al. (1996) in case of spring canola hybrids.

Widening the genetic base of *B. napus* for the improvement of hybrid performance or heterosis has been the focus of many researchers. According to Seyis et al. (2006), resynthesized *B. napus* lines carry potential for producing good hybrid cultivars. Li et al. (2004) showed that it is possible to develop *B. napus* ($A^rA^rC^cC^c$) lines with genome contents introgressed from *B. rapa* (A^rA^r) and *B. carinata* ($B^cB^cC^cC^c$) through interspecific hybridization between *B. rapa*, *B. carinata* and *B. napus*. These diversified *B. napus* lines showed great potential for use in breeding for the development of high yielding hybrid canola cultivars (Zou et al. 2010). Indeed, Li et al. (2014) found that *B. napus* lines diversified with the alleles introgressed from *B. oleracea* exhibit heterosis in F_1 hybrids.

1.7 Research objectives

Based on the literature reviewed above, it is apparent that efforts have been made to use different types of *B. napus* and its allied *Brassica* species, such as *B. rapa* and *B. carinata*, to broaden the genetic base of *B. napus*; however, limited efforts have been made to improve this crop by use of the C genome of *B. oleracea*. Therefore, this M.Sc. thesis research is focused on the long-term objective to increase allelic variation in the C-genome of spring *B. napus* canola through *B. napus* × *B. oleracea* interspecific crosses. In the short-term, the objectives of this research were:

1) Investigate the feasibility of developing canola quality (< 1% erucic acid content in seed oil and < 15 μmol glucosinolates per gram of seed meal) euploid *B. napus* ($2n = 38$) lines from F_2 and backcross derived populations of *B. napus* × *B. oleracea* interspecific crosses.
2) Study the inheritance of erucic acid and glucosinolate contents as well as agronomic traits in different generations.

3) Assess the extent of allelic variation in the C-genome of the families derived from *B. napus* × *B. oleracea* interspecific crosses by use of simple sequence repeat (SSR) markers.

4) Assess the value of the *B. napus* lines derived from the interspecific crosses for heterosis in F_1 hybrids.

Chapter 2

Development of *B. napus* lines from *B. napus* × *B. oleracea* interspecific crosses and assessment of heterotic potential of these lines

Summary

Interspecific cross between *B. napus* and *B. oleracea* was done and the F_1 plants were self-pollinated for F_2 seeds as well as backcrossed to the *B. napus* parent for backcross (BC_1) seeds for the development of genetically diverse canola quality *B. napus* lines. The F_2 and BC_1 populations were self-pollinated for several generations with selection for zero erucic acid and low glucosinolate contents. Plant fertility in the progenies of the interspecific hybrids was poor in early generations; however, it improved with the progression of generations. Flow cytometric analysis showed that, majority of the advanced generation plants were similar to the *B. napus* parent. Segregation for erucic acid and glucosinolate contents was found in all populations where selection for zero erucic acid and low glucosinolate content led to the development of canola quality lines in advanced generation. Few of the advanced generation lines flowered earlier than the *B. napus* parent suggesting that earliness of flowering has been introgressed from *B. oleracea* into spring *B. napus* canola, and these early flowering lines showed potential for developing early flowering hybrid cultivar.

2.1 Introduction

Oilseed *B. napus* (AACC, $2n = 18$) is an economically important crop in the world. This crop makes significant economic contribution to the countries where it is grown as one of the major crops. For instance, the contribution of canola to the Canadian economy is about $21 billion per year when its direct, indirect and induced impacts are taken into account. This crop-

industry also created about 249,000 jobs in Canada (LMC International 2013). Increasing demand for canola oil in the world market require greater production of this crop, and this can be achieved through developing high yielding cultivars, such as hybrids, with good agronomic and seed quality traits. In the past decade, intensive breeding efforts led to the development of hybrid cultivars with higher seed yield than open-pollinated cultivars (Zand and Beckie 2002); however, further improvement of the hybrid cultivars for seed yield and agronomic traits is still possible through the use of genetically diverse germplasm in breeding (for review see Rahman 2013).

In canola, a decline in genetic diversity has been reported by different researchers. One of the reasons of this is that breeders often prefer using the elite canola lines from a restricted gene pool in breeding to develop a new cultivar in a short period of time. Use of exotic germplasm or allied species is expected to broaden the genetic base of germplasm in a breeding program; however, this is generally avoided due to the risk of introducing undesirable traits from these types in the breeding program (reviewed in Rahman 2013). Cowling (2007) reported a loss of genetic diversity in Australian spring *B. napus* cultivars, and Fu and Gugel (2010) also observed a decline in genetic variation in Canadian *B. napus* germplasm. Indeed, among the different types of *B. napus* canola, the spring type has been reported to possess the least genetic diversity followed by winter and semi-winter types (Hasan et al. 2006; Qian et al. 2006).

The narrow genetic base in spring *B. napus* germplasm has been a concern to the canola breeders as genetic diversity in crop germplasm is essential for the development of improved crop cultivars. Germplasm sources available for use in breeding can be categorized into different groups. The primary gene pool includes different types of *B. napus* where traits and alleles can be introduced into the crop cultivar without much difficulty. On the other hand, the use of secondary gene pool, such as progenitors and other allied species, will face

several challenges in breeding (reviewed in Rahman 2013). Several studies conducted in the past to broaden the genetic base of spring *B. napus* by use of winter *B. napus* (Butruille et al. 1999; Udall et al. 2004; Kebede et al. 2010), Chinese semi-winter *B. napus* (Qian et al. 2007), *B. rapa* (Liu et al. 2002) and *B. carinata* (Li et al. 2006), as well as to transfer agronomic traits, such as earliness of flowering into *B. napus* (Rahman et al. 2011). The diploid progenitor species *B. rapa* has been widely used in *B. napus* breeding programs, especially for the improvement of Chinese semi-winter type (Qian et al. 2006). However, *B. oleracea* has barely been used in the breeding of *B. napus* for many reasons including the difficulty of crossing this species with *B. napus* (Bennett et al. 2008), as well as high content of erucic acid (more than 40%) and glucosinolates (100 µmol/g dry matter) in its seed. According to Bus et al. (2011), the A genome of *B. napus* is more genetically diverse than the C genome. This suggests that broadening of genetic diversity in the C genome is needed for the improvement of *B. napus* canola.

B. oleracea is mostly cultivated as vegetable crop. This species possess vast variation in leaf, stem as well as inflorescence morphology. According to Snogerup (1980) and Dixon (2007), the cultivated *B. oleracea* can be divided into six major groups: kale (var. *acephala*), cabbage (var. *capitata*), kohlrabi (var. *gongylodes*), inflorescence kale (var. *botrytis* and var. *italica*), branching bush kale (var. *fruticosa*), and Chinese kale (var. *alboglabra*) (cited by Prakash 2012). Though *B. oleracea* is grown as a vegetable crop, it carries desired alleles for the improvement of canola. For instance, Rahman et al. (2011) introduced earliness of flowering from the late flowering species *B. oleracea* var. *alboglabra* into Canadian spring *B. napus* canola. Crisp et al.

(1989) and Hasan et al. (2012) identified *B. oleracea* accessions carrying resistance of clubroot disease. Thus, given that great genetic diversity exists in the C genome of *B. oleracea* and alleles desired for canola can be found in this species, *B. oleracea* can certainly be used for the improvement of spring *B. napus* canola.

The objectives of this M.Sc. thesis research project were the following: (1) assess the feasibility of developing euploid ($2n = 38$) *B. napus* lines from *B. napus* × *B. oleracea* (var. *capitata* and var. *italica*) interspecific crosses; (2) study the inheritance of erucic acid and glucosinolate contents and plant fertility in different generation populations of these interspecific crosses; and (3) evaluate the potential of the interspecific-cross-derived lines for use in hybrid canola breeding.

2.2 Materials and methods

2.2.1 Parental materials

The parental materials used in this study were a spring type *B. napus* (AACC, $2n = 38$) elite canola line A04-73NA, and two *B. oleracea* accessions, var. *capitata* cv. Balbro and var. *italica* cv. Premium Crop. A04-73NA was developed by the Canola Program of the University of Alberta. *B. oleracea* var. *capitata*, commonly called as cabbage and *B. oleracea* var. *italica*, commonly called as broccoli, are cultivated as vegetable crops (Prakash et al. 2012). Both broccoli and cabbage are non-canola quality types, i.e. their seed oil contains a high content of erucic acid (> 40%) and seed meal contains a high content of glucosinolates (> 60 μmol/g seed).

2.2.2 Development of F_1 and BC_1 plants

The following crosses were made by the Canola Program using *B. napus* A04-73NA as female and *B. oleracea* as male: A04-73NA × *B. oleracea* var. *italica* and A04-73NA × *B. oleracea* var. *capitata. In vitro* ovule culture technique (Bennett et al. 2008) was applied to produce F_1 plants. The F_1 plants were manually self-pollinated to produce F_2 seeds and also backcrossed to A04-73NA to produce BC_1 seeds of (A04-73NA × *B. oleracea* var. *italica*) × A04-73NA, and (A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA.

2.2.3 F_2- and BC_1-derived population development

The F_2 and BC_1 plants were grown in a greenhouse and self-pollinated by bag isolation, and pedigree breeding was applied for the development of inbred *B. napus* lines (Fig. 2.1). The F_3 and BC_1F_2, F_4 and BC_1F_3, F_5 and BC_1F_4, F_6 and BC_1F_5, and F_8 and BC_1F_7 generation populations were grown in greenhouse (21°/18° ± 2°C day/night,) in spring 2012, winter 2012-13, spring 2013, winter 2013-14, and winter 2014-15, respectively. The F_7 and BC_1F_6 generation populations were grown in field in summer 2014 at the Edmonton Research Station of the University of Alberta. In field, seeding was done in 2 m long single-row plots with 50 cm space between the rows. All early generation populations including F_1 and BC_1, F_2 and BC_1, F_3 and BC_1F_2, and F_4 and BC_1F_3 were grown by the Canola Program. I received F_5 and BC_1F_4 seeds, and collected data from the F_5 and BC_1F_4, F_6 and BC_1F_5, F_7 and BC_1F_6, and F_8 and BC_1F_7 generation populations.

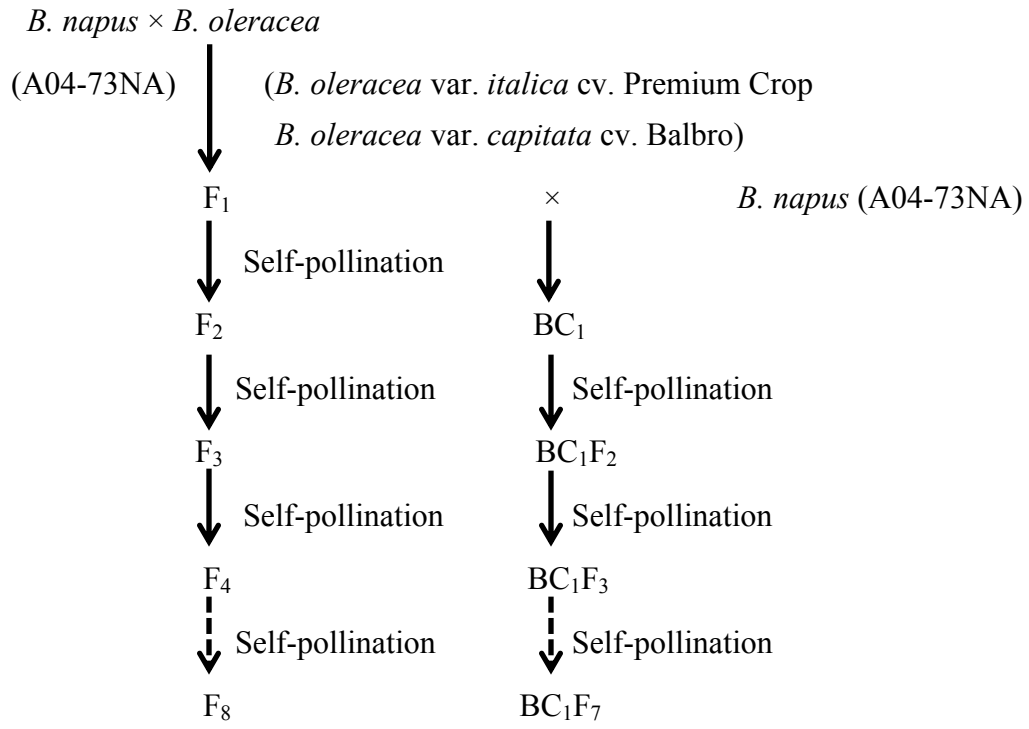

Fig. 2.1 A flow diagram showing the development of *B. napus* lines from *B. napus* × *B. oleracea* interspecific crosses.

2.2.4 Plant fertility and agronomic traits

The following plant fertility, agronomic and seed quality data were collected.

Plant fertility: Plant fertility in F_2 and BC_1, BC_1F_2, and F_4 and BC_1F_3 generations was estimated based on the ability of the plants to produce seed under bag isolation, while in F_3, F_5 and BC_1F_4 and in their progeny generations was estimated based on silique length (mm), number of seeds per silique, and seed yield (g) per plant. For this, length of three to five siliques from the middle of the main raceme were measured and the number seeds produced in these siliques was counted. The mean values of silique length and number of seeds per silique were calculated and compared with the *B. napus* parent A04-73NA.

Days to flowering: Days to flowering of the F_7 and BC_1F_6, and F_9 and BC_1F_8 generation populations grown in field was recorded as the number of days required from seeding to the date when approximately 50% plants in a plot had at least one open flower.

2.2.5 Flow cytometric analysis

The F_6 and BC_1F_5, and F_8 and BC_1F_7 generation plants were analyzed by a flow cytometer for nuclear DNA content (reported as partec value). For this, approximately 0.5 cm^2 fresh leaf tissue from 15-20 days old seedling was collected and chopped with a sharp blade in 400 µl nuclear extraction buffer (Partec GmbH, Münster, Germany). The content was filtered through 50 µm Cell Trics disposable filter and 1.6 ml nuclear fluorochrome DAPI (4,6-diaminido-2-phenylindole, Sigma, product no. D-9542) staining buffer was added. The samples were incubated for 1 minute and analyzed by a Partec Ploidy Analyzer (Partec GmbH, Münster, Germany).

2.2.6 Seed quality traits

Seeds harvested from individual plants of F_2 and BC_1, F_3 and BC_1F_2, F_4 and BC_1F_3, F_5 and BC_1F_4, and F_6 and BC_1F_5 populations grown in greenhouse, and bulk seeds of F_7 and BC_1F_6 families grown in field plots were analyzed for erucic acid and glucosinolate contents. Both analyses were done in the Analytical Laboratory of the Canola Breeding program of the University of Alberta.

Fatty acid analysis for erucic acid content was done using 0.10 to 0.25 g self-pollinated seeds. For this, seeds were crushed in a 50 ml conical tube filled with N-pentane, then centrifuged at 1500 rpm for 15-20 minutes, and the supernatant was transferred into a 10 mm × 75 mm glass tube. The extracted oil, left after evaporation of the N-pentane, was methylated to

produce fatty acid methyl esters and analyzed by gas chromatography for estimation of fatty acid composition of oil (for detail, see Bennett et al. 2008).

Glucosinolate content in seed was estimated by near-infrared spectroscopy (NIRS, Model 6500, Foss North America, Eden Prairie, MN). For this, 2.5 to 4 g self-pollinated seeds harvested from individual plants grown in greenhouse or 5 to 8 g bulk open-pollinated seeds harvested from field plots were used. A calibration equation (unpublished) developed in the Analytical Laboratory of the Canola Program of the University of Alberta using WinISI II (Infrasoft International, LLC.) was used for quantification of GSL content. This laboratory is accredited by the Canadian Grain Commission for analysis of GSL and fatty acid contents. Glucosinolate content was calculated on whole-seed basis at 8.5% moisture content and reported as μ mol/g seed.

2.2.7 Production of test hybrids and field trails

Test hybrid seeds were produced in 2014-15 winter in greenhouse using 77 F_8 and 45 BC_1F_7 lines as male and the *B. napus* line A04-73NA as female. Hybrid seeds were produced manually through emasculation of the female parent followed by pollination with the male lines. The F_8 and BC_1F_7 plants were also self-pollinated to produce F_9 and BC_1F_8 seeds. The 122 test hybrids and their F_9 and BC_1F_8 lines, and the common parent A04-73NA were grown in field plots at the Edmonton Research Station of the University of Alberta during summer 2015. The trial was laid out in an alpha-lattice design with two replications. The two parents and their hybrid constituted an experimental unit of three plots, where the hybrid plot was always located in between the two parents. This layout allowed direct comparison of the hybrids with their respective parents and gave greater precision of the measurement of heterosis. Randomization of

the experimental units within the blocks of each replication was done using CropStat 7.2 (International Rice Research Institute, Los Baños, Philippines). Plot size was 1.0 m in length and 1.2 m in width (1.2 m^2) with 50 cm space between the plots. Each plot consisted of three rows with 25 cm space between the rows. Seeding was done by hand where 44 to 66 seeds were placed at 22 spots in the middle row and 30 seeds were dropped in each of the two side rows. Thinning was done in the middle row where 20 ± 2 plants were retained. The hybrids of the F_2- and BC_1-derived lines and their parents were seeded in separate blocks.

The following agronomic data were collected:

Days to flowering: Days to flowering data collected when approximately 50% plants in the middle row had at least one open flower.

Plant height: Plant height (cm) data was collected at the end of flowering. For this, height of three plants from the middle row was measured from the base to the top of the main branch and the mean values were used for statistical analysis.

2.2.8 Statistical analysis

The computer software program SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used to calculate mean, standard deviation (SD) and standard error (SE) using the feature Proc Means. Comparisons between the means of different generation populations, as well as mean values of these generations and the *B. napus* parent A04-73NA were made using the feature Proc Mixed with the following statement:

```
model response variable = cross generation cross*generation;
lsmeans cross generation cross*generation/adjust = tukey;
repeated/group = cross*generation.
```

The Tukey's test was used to control type-I error and the repeated/group statement was used to compute the generation variance. Proc Ttest was used to compare the mean value of the whole population with the mean value of the selected population. The computer software program Excel was used to calculate confidence interval of the parents for different traits.

Mid-parent heterosis (MPH) for days to flowering and plant height (cm), was calculated using the formula [(Test hybrid - mid-parent value)/mid-parent value] × 100; and heterosis over A04-73NA was calculated using the formula [(Test hybrid – A04-73NA)/ A04-73NA] × 100. Data of the test hybrids were analyzed using restricted maximum likelihood (REML)-analysis of variance (ANOVA), and the mean values were calculated with the Lsmeans option of Proc Mixed by SAS 9.4 (SAS institute Inc., Cary, NC, USA), where replication and block nested in each replication were considered as random effects. The four populations (two F_2-derived and two BC_1-derived) were compared using 'pdiff' command.

2.3 Results

2.3.1 Production of F_1 and BC_1 hybrids and their self-pollinated populations

A total of 26 crosses of A04-73NA × *B. oleracea* var. *italica* (cross ID: 5CA1358) and 15 crosses of A04-73NA × *B. oleracea* var. *capitata* (cross ID: 5CA1392) were made from where 51 and 82 ovules, respectively were obtained and cultured *in vitro* (Table 2.1). These cultured ovules yielded a total of 37 embryos from where 31 plants were obtained. Hybrid nature of these plants was confirmed through comparison with the female *B. napus* parent A04-73NA. Also, the interspecific hybrid plants had very poor fertility. Thus, the number of *B. napus* × *B. oleracea* interspecific hybrids of the two crosses produced through application of ovule culture technique was 0.76 per pollination.

A total of 278 buds of the F_1 plants of 5CA1358 were self-pollinated manually and this produced 321 F_2 seeds. On the other hand, only three F_2 seeds were harvested from the F_1 plants of 5CA1392. These two crosses, on an average, produced 1.17 seeds per self-pollination (Table 2.2). All F_2 seeds of 5CA1392 and sixty seeds of 5CA1358 were grown in greenhouse; 87% of these plants produced viable seeds. In F_3, 340 plants of the two crosses were grown and about 50% of these plants produced F_4 seeds.

In case of the backcross of (A04-73NA × *B. oleracea* var. *italica*) × A04-73NA (cross ID: 5CA1678) and (A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA (cross ID: 5CA1679), a total of 337 crosses were made which resulted 0.28 and 0.26 seeds per pollination, respectively (Table 2.3). Ninety BC_1 plants of the two backcrosses were grown in a greenhouse of which 66 plants produced seeds, i.e. 73.3% of the BC_1 plants were fertile. In BC_1F_2, a total of 350 plants were grown of which 24% plants produced self-pollinated seeds under bag isolation.

Table 2.1 Production of F_1 hybrids of *B. napus* × *B. oleracea* interspecific crosses.

Cross	No. pollination	No. ovules cultured	No. ovules/pollination	No. embryo to solid media	No. embryo/pollination	No. F_1 plantlet to soil	No. F_1/pollination
A04-73NA × *B. oleracea* var. *italica*	26	51	1.96	27	1.04	25	0.96
A04-73NA × *B. oleracea* var. *capitata*	15	82	5.47	10	0.67	6	0.4
Total	41	133	3.24	37	0.90	31	0.76

Table 2.2 Production of F_2 and subsequent generation populations through self-pollination.

Cross	No. bud pollination of F_1 plants	No. F_2 seeds harvested	No. F_2 seeds/self-pollination	No. F_2 plants grown	No. F_2 plants produced seeds	% fertile F_2 plants	No. F_3 plants grown (families)	No. F_3 produced seeds	% fertile F_3 plants
A04-73NA × *B. oleracea* var. *italica*	200	321	1.61	60	53	88.3	290 (53)	127 (26)	48.1
A04-73NA × *B. oleracea* var. *capitata*	78	3	0.04	3	2	66.7	50 (2)	42 (2)	84
Total	278	324	1.17	63	55	87.3	340	169	49.7

Table 2.3 Production of BC_1 hybrids of (*B. napus* × *B. oleracea*) × *B. napus* and BC_1F_2 populations.

Cross	No. crosses made	No. BC_1 seeds harvested	No. BC_1 seeds/pollination	No. BC_1 plants grown	No. BC_1 plants produced seeds	% fertile BC_1 plants	No. BC_1F_2 plants grown (families)	No. BC_1F_2 plants produced seeds	% fertile BC_1F_2 plants
(A04-73NA × *B. oleracea* var. *italica*) × A04-73NA	220	62	0.28	60	51	85	241 (51)	37 (23)	15.4
(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA	117	30	0.26	30	15	50	109 (14)	47 (10)	43.1
Total	337	92	0.27	90	66	73.3	350	84	24

2.3.2 Plant fertility and agronomic traits

Plant fertility of the population derived from F_2 and BC_1 were evaluated based on silique length (mm) and number of seeds per silique, as well as based on self-pollinated seeds produced under bag isolation. The *B. napus* parent A04-73NA was grown along with each generation population for comparison. A confidence limit of A04-73NA for silique length and number of seeds per silique was calculated to assess fertility of the F_2- and BC_1-derived plants. Silique length and number of seeds per silique in different generation populations of the two crosses presented in Table 2.4 and Table 2.5, respectively, and pooled data of the two crosses presented in Table 2.6.

Silique length

Silique length in F_3 of 5CA1358 ranged from 10.2 to 53.8 mm with a mean of 30.9 ± 1.28 SE mm. About 17% plants of this population had silique size similar or larger than A04-73NA (confident limit, 45.7 - 48.1 mm) (Table 2.4). Similar variation was found in F_3 population of 5CA1392. In this generation, selection focused only on low erucic acid content. The non-significant difference ($p < 0.05$) between the whole population and selected population of the two crosses suggest that fertility of the plants was not affected by erucic acid content in seed oil. In F_5 population of 5CA1392, silique length varied from 12.0 to 60.2 mm with a mean of 37.9 ± 0.8 SE mm (standard deviation SD = 11.0). This population showed greater variation as compared to 5CA1392 (SD = 8.6) as well as had significantly greater size of silique. The selected population of 5CA1358 and 5CA1392 had significantly longer siliques (38.4 ± 1.3 SE mm and 43.2 ± 1.0 SE mm) compared to the whole population. Silique size of the F_6 populations of 5CA1358 and 5CA1392 was statistically similar to A04-73NA. Average silique length of the

F_7 population of 5CA1358 was 51.8 ± 0.5 SE mm while silique length of the selected population of this cross was 54.5 ± 0.6 SE mm; this difference was statistically significant. However, no significant difference between the whole and selected population was found in the case of 5CA1392.

In summary, length of silique in F_3 population of the two crosses, 5CA1358 and 5CA1392, were statistically similar; while 5CA1392 had significantly longer silique than 5CA1358 in F_5 and F_6. However, both populations had similar size silique in F_7. Therefore, no specific trend for the difference between these populations could be found. Silique length significantly increased in each generation; however, both populations had significantly shorter size silique than the *B. napus* parent A04-73NA (Fig. 2.2).

In case of the populations derived from BC_1, the BC_1F_4 population of 5CA1678 had mean length of silique 33.1 ± 0.8 SE mm which was slightly longer than silique size of 5CA1679 (30.8 ± 0.7 SE mm); however, the size of silique of both populations was significantly shorter than the *B. napus* parent (Table 2.4). Selection in this generation performed for higher fertility; therefore, the selected population had significantly longer size siliques than the whole population. Mean silique length in BC_1F_5 and BC_1F_6 generation populations of 5CA1678 was 37.2 ± 0.9 SE mm and 47.9 ± 0.7 SE mm, respectively, which was significantly shorter than the *B. napus* parent A04-73NA. On the other hand, the BC_1F_5 and BC_1F_6 populations of 5CA1679 had significantly longer silique than the two populations of 5CA1678; however, the size of siliques of these two populations was still significantly shorter than A04-73NA.

In summary, the BC_1F_4 population of 5CA1678 had longer silique than the 5CA1679 population; in contrast, the 5CA1679 population had longer silique than 5CA1678 in BC_1F_5 and

BC_1F_6 generation. The length of silique increased with the progression of generation in both cases (Fig. 2.2).

Taken together the F_2- and BC_1-derived populations into account, no consistent pattern was found for the differences between these two populations of the two crosses as well as for the proportion of the plants that became similar to *B. napus* for silique length over the generations (Table 2.4, Fig. 2.2). Also, no consistent difference between the populations possessing the genetic component of *B. oleracea* var. *capitata* or var. *italica* could be found for silique length. A trend of increasing size of silique was found with the advancement of the generations in both populations. Pooled data of the two crosses showed significant difference between the F_5 and BC_1F_4, F_6 and BC_1F_5 populations; however, no significant difference was found between F_7 and BC_1F_6 for silique length (Table 2.6, Fig. 2.4).

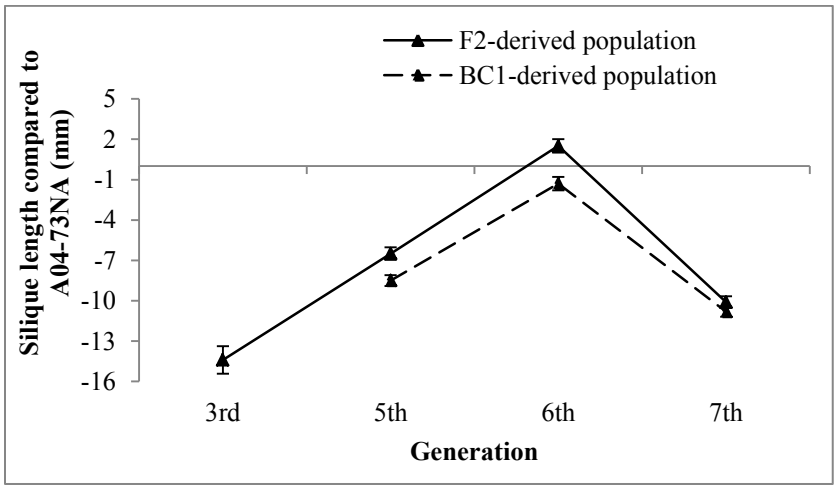

Fig. 2.2 Silique length (mean ± SE mm), compared to the *B. napus* parent A04-73NA, in different generation populations derived from F_2 and BC_1 of *B. napus* × *B. oleracea* interspecific crosses. Pooled data of the F_2-derived populations of *B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata*, and BC_1-derived populations of (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus* presented. 3rd generation = F_3 (greenhouse); 5th generation = F_5/BC_1F_4 (greenhouse); 6th generation = F_6/BC_1F_5 (greenhouse); 7th generation = F_7/BC_1F_6 (field).

Table 2.4 Silique length (mm) in different generation populations derived from F_2 and BC_1 of two *B. napus* × *B. oleracea* interspecific crosses.

			Whole population				Selected population		
Pedigree[1]	Cross ID	Gen.[2]	No. plants (families)	Range (SD)	Mean ± SE[3]	% ≥ *B. napus*[4]	No. plants (families)	Range (SD)	Mean ± SE[5]
B. nap × *B. o. ital*	5CA1358	F_3	95 (28)	10.2 - 53.8 (12.4)	30.9 ± 1.3b	16.8	32 (17)	10.2 - 50 (9.9)	26.1 ± 1.8
B. nap × *B. o. cap*	5CA1392	F_3	43 (2)	9.2 - 65.2 (13.0)	33.2 ± 2.0b	14.0	20 (2)	9.2 - 65.2 (12.7)	31.5 ± 2.8
B. nap[6]			4	45.7 - 48.3 (1.4)	46.9 ± 0.7a				
B. nap × *B. o. ital*	5CA1358	F_5	143 (43)	13.6 - 53.6 (8.6)	30.3 ± 0.7b	19.6	41 (28)	15.6 - 53.6 (8.1)	38.4 ± 1.3*
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_4	146 (25)	11.4 - 48.4 (9.4)	33.1 ± 0.8b	34.9	49 (20)	21.2 - 48.4 (6.4)	40.4 ± 0.9*
B. nap × *B. o. cap*	5CA1392	F_5	184 (56)	12.0 - 60.2 (11.0)	37.9 ± 0.8a	55.4	67 (50)	21.8 - 60.2 (8.3)	43.2 ± 1.0*
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_4	182 (67)	14.2 - 48.4 (8.7)	30.8 ± 0.7b	29.1	47 (36)	21.8 - 48.4 (7.4)	38.6 ± 1.1*
B. nap[6]			6	35.3 - 48.8 (5.0)	42.7 ± 2.1a				
B. nap × *B. o. ital*	5CA1358	F_6	90 (39)	18.7 - 61.3 (9.3)	39.5 ± 1.0ab	51.1	76 (36)	18.7 - 61.3 (8.9)	40.5 ± 1.0
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_5	103 (47)	19.0 - 63.0 (9.0)	37.2 ± 0.9a	43.7	63 (35)	20.7 - 54.0 (8.4)	37.06 ± 1.1
B. nap × *B. o. cap*	5CA1392	F_6	131 (66)	18.0 - 66.7 (9.0)	45.6 ± 0.8c	80.2	70 (43)	30.0 - 66.7 (8.2)	44.1 ± 1.0
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_5	134 (44)	18.7 - 64.7 (10.5)	41.6 ± 0.9b	57.5	114 (43)	18.7 - 64.7 (10.5)	42.1 ± 1.0
B. nap[6]			5	35.3 - 45.7 (4.5)	44.7 ± 2.0abc				
B. nap × *B. o. ital*	5CA1358	F_7	171 (68)	22.3 - 67.7 (7.1)	51.8 ± 0.5b	4.1	86 (49)	43.7 - 67.7 (5.1)	54.5 ± 0.6*
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_6	124 (54)	26.7 - 67.7 (7.6)	47.9 ± 0.7c	2.4	35 (22)	33.0 - 57.0 (5.9)	48.0 ± 1.0
B. nap × *B. o. cap*	5CA1392	F_7	105 (61)	27.0 - 73.0 (7.9)	51.2 ± 0.8b	8.6	34 (22)	37.3 - 62.7 (6.6)	51.2 ± 1.1
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_6	249 (101)	18.7 - 68.3 (7.6)	52.5 ± 0.5b	10.0	81 (71)	43.0 - 67.0 (6.2)	54.8 ± 0.7*
B. nap[6]			35	48.0 - 78.3 (6.2)	63.6 ± 1.1a				

[1]*B. nap* = *B. napus* line A04-73NA, *B. o. ital* = *B. oleracea* var. *italica* cv. Premium Crop, *B. o. cap*= *B. oleracea* var. *capitata* cv. Balbro.
[2]F_7 and BC_1F_6 generation populations were grown in field, other generations were grown in greenhouse.
[3]Comparison between the populations grown under the same environment, values following with the different letter indicates significant difference, p-value < 0.05.
[4]Proportion of the population falling within the confidence limit of the *B. napus* parent A04-73NA. This also includes the population with greater size siliuqe than A04-73NA, if any.
[5]Asterisks indicate the selected population was significantly different from the whole population at p < 0.05.
[6]Confidence limits of *B. napus* parent A04-73NA for silique length were 45.7 - 48.1 mm, 39.0 - 46.3 mm, 38.3 - 44.7 mm and 61.6 - 65.7mm while grown along with F_3, F_5 and BC_1F_4, F_6 and BC_1F_5, F_7 and BC_1F_6, respectively.

Number of seeds per silique

The mean number of seeds per silique of 5CA1358 in F_3 was 5.5 ± 0.6 SE, which was similar to 5CA1392 but significantly lower than *B. napus* (21.7 ± 1.0 SE). In this generation, less than 3% plants had number seeds per silique similar to *B. napus*. The number of seeds per silique in F_5 of 5CA1392 was 11.1 ± 0.5 SE which was significantly greater than the number of seeds per silique in 5CA1358 (3.6 ± 0.3 SE) (Table 2.5); the selected populations of both crosses had significantly greater number of seeds per silique than the whole population. In F_6, the population of the cross 5CA1395 produced significantly greater number of seeds per silique compared to 5CA1358; however, under field growing condition, the F_7 of 5CA1358 produced significantly greater number of seeds per silique than that of 5CA1392. Therefore, no consistent difference between the crosses can be found over the generations. About 32% plants of the F_7 plants of 5CA1358 produced similar number of seeds per silique as A04-73NA; however, both populations, on average, produced significantly fewer seeds per silique than A04-73NA.

In summary, number of seeds per silique in F_3 populations of 5CA1358 and 5CA1392 were statistically similar; however, 5CA1392 produced significantly greater number of seeds per silique than 5CA1358 in F_5 and F_6 (Table 2.5). In case of F_7, the 5CA1358 population produced greater number of seeds per silique than 5CA1392. Thus, no specific pattern for the difference in number of seeds per silique between 5CA1358 and 5CA1392 could be found. Overall, the number of seeds per silique increased in both populations with the progression of generation; however, average seed set in advanced generation populations of both crosses was till significantly lower than A04-73NA (Fig. 2.3).

In case of the populations derived from backcross, the number of seeds per silique in BC_1F_4 of 5CA1678 of was 8.1 ± 0.6 SE while it was 5.8 ± 0.5 SE in 5CA1679; this difference was statistically significant. On the other hand, the BC_1F_5 and BC_1F_6 populations of 5CA1679 had significantly greater number of seeds per silique than 5CA1678. Thus, no trend for the difference between the two crosses over generations was found for this trait. Like silique length, the number of seeds per silique in the selected populations of BC_1F_4 and BC_1F_6 was greater than the whole populations. About 24% of the BC_1F_6 plants of 5CA1679 produced similar or greater number of seeds per silique than the *B. napus* parent.

In summary, the 5CA1678 population produced greater number of seeds per silique than 5CA1679 in BC_1F_4 generation; however, in BC_1F_5 and BC_1F_6, the 5CA1679 population produced greater number of seeds per silique than 5CA1678. Thus, no consistent pattern for the difference between 5CA1678 and 5CA1679 was found for the number of seeds per silique. Like the F_2-derived populations, the populations derived from BC_1 also had fewer seeds per silique than the *B. napus* parent A04-73NA (Fig. 2.3).

Taking the F_2- and BC_1-derived populations into account, no consistent trend for the difference between these two types of populations of these two crosses could be found over the generations for number of seeds per silique as well as for percentage of plants producing number of seeds per silique similar to the *B. napus* parent (Table 2.5, Fig. 2.3). Also, no consistent difference for the number of seeds per silique was found between the populations possessing the genetic component of *B. oleracea* var. *capitata* or var. *italica*. An increase in the number of seeds per silique was found with the advancement of generation. Pooled data of the two crosses showed no significant difference between the populations derived from F_2 or BC_1 (Table 2.6, Fig. 2.5).

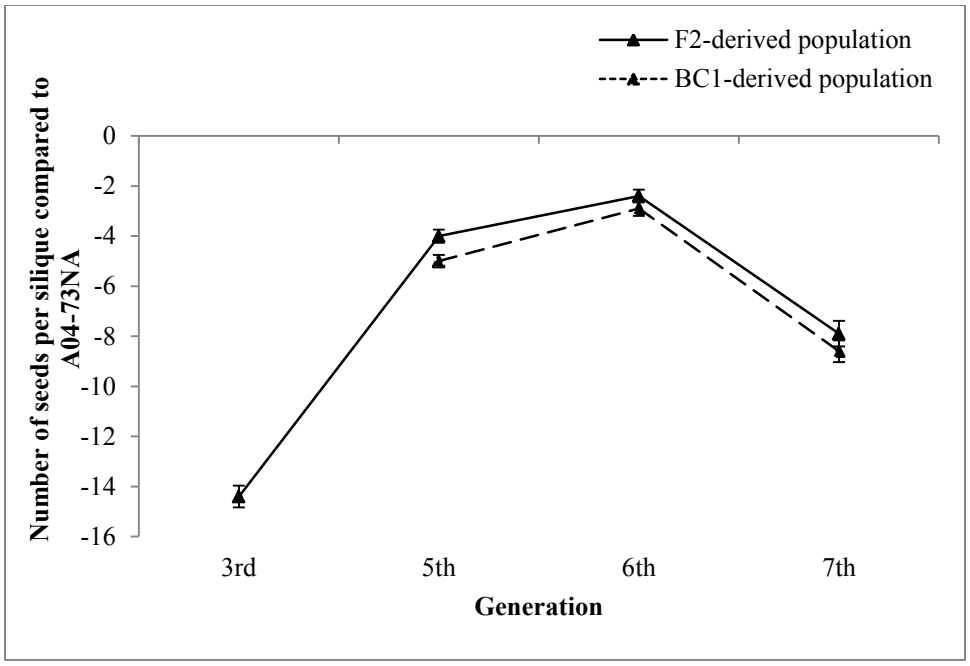

Fig. 2.3 Number of seeds per silique (mean ± SE), compared to the *B. napus* parent A04-73NA, in different generation populations derived from F_2 and BC_1 of *B. napus* × *B. oleracea* interspecific crosses. Pooled data of the F_2-derived populations of *B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata*, and BC_1-derived populations of (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus* presented. 3rd generation = F_3 (greenhouse); 5th generation = F_5/BC_1F_4 (greenhouse); 6th generation = F_6/BC_1F_5 (greenhouse); 7th generation = F_7/BC_1F_6 (field).

Table 2.5 Number of seeds per silique in different generation populations derived from F_2 and BC_1 of two *B. napus* × *B. oleracea* interspecific crosses.

			Whole population				Selected population		
Pedigree[1]	Cross ID	Gen.[2]	No. plants (families)	Range (SD)	Mean ± SE[3]	% ≥ *B. napus*[4]	No. plants (families)	Range (SD)	Mean ± SE[5]
B. nap × *B. o. ital*	5CA1358	F_3	95 (28)	0 - 20.6 (5.4)	5.5 ± 0.6b	2.1	32 (17)	0.2 - 10.0 (2.5)	3.33 ± 0.5*
B. nap × *B. o. cap*	5CA1392	F_3	43 (2)	0 - 25.2 (5.2)	6.2 ± 0.8b	2.3	20 (2)	0 - 17.8 (4.8)	6.03 ± 1.1
B. nap[6]			4	20 - 24.0 (2.0)	21.7 ± 1.0a				
B. nap × *B. o. ital*	5CA1358	F_5	143 (43)	0 - 17.6 (3.6)	3.6 ± 0.3b	7.0	41 (28)	1.6 - 17.6 (3.6)	7.9 ± 0.6*
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_4	146 (25)	0 - 22.8 (7.4)	8.1 ± 0.6c	38.4	49 (20)	2.0 - 22.8 (6.5)	14.1 ± 0.9*
B. nap × *B. o. cap*	5CA1392	F_5	184 (56)	0 - 25.4 (7.1)	11.1 ± 0.5a	53.3	67 (50)	2.0 - 24.0 (6.1)	13.2 ± 0.7*
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_4	182 (67)	0 - 23.6 (6.6)	5.8 ± 0.5d	17.6	47 (36)	2.4 - 23.6 (8.6)	11.7 ± 1.3*
B. nap[6]			6	8.3 - 20.7 (5.2)	14.1 ± 2.1a				
B. nap × *B. o. ital*	5CA1358	F_6	90 (39)	0 - 23.3 (5.7)	9.0 ± 0.6b	24.4	76 (36)	0 - 23.3 (5.7)	9.4 ± 0.7
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_5	103 (47)	0.7 - 27.7 (6.1)	9.1 ± 0.6b	27.2	63 (35)	0.7 - 27.7 (6.4)	9.0 ± 0.8
B. nap × *B. o. cap*	5CA1392	F_6	131 (66)	0 - 24.0 (5.1)	12.3 ± 0.5a	58.8	70 (43)	3.3 - 23.3 (4.6)	11.9 ± 0.6
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_5	134 (44)	0.7 - 25.3 (6.7)	11.7 ± 0.6a	53.0	114 (43)	0.7 - 25.3 (6.8)	11.9 ± 0.6
B. nap[6]			5	10.0 - 20.7 (4.8)	15.2 ± 2.2a				
B. nap × *B. o. ital*	5CA1358	F_7	171 (68)	0.7 - 39.3 (9.1)	25.4 ± 0.7b	32.2	86 (49)	15.3 - 37.3 (5.1)	29.9 ± 0.6*
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_6	124 (54)	1.3 - 39.3 (8.6)	21.7 ± 0.8c	7.3	35 (22)	9.3 - 35.3 (5.8)	24.5 ± 1.0*
B. nap × *B. o. cap*	5CA1392	F_7	105 (61)	2.0 - 35.3 (8.0)	21.2 ± 0.8c	9.5	34 (22)	10.7 - 33.3 (6.0)	21.2 ± 1.0
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_6	249 (101)	0 - 38.7 (8.7)	23.7 ± 0.6b	24.1	81 (71)	14.7 - 38.0 (6.5)	27.7 ± 0.7*
B. nap[6]			35	24.7 - 37.3 (3.7)	32.5 ± 0.6a				

[1]*B. nap* = *B. napus* line A04-73NA, *B. o. ital* = *B. oleracea* var. *italica* cv. Premium Crop, *B. o. cap*= *B. oleracea* var. *capitata* cv. Balbro.
[2]F_7 and BC_1F_6 generation populations were grown in field, other generations were grown in greenhouse.
[3]Comparison between the populations grown under the same environment, values following with the different letter indicates significant difference, p-value < 0.05.
[4]Proportion of the population falling within the confidence limit of the *B. napus* parent A04-73NA. This also includes the population with greater number of seeds per silique than A04-73NA, if any.
[5]Asterisks indicate the selected population was significantly different from the whole population at $p < 0.05$.
[6]Confidence limits of *B. napus* A04-73NA for number seeds per silique were 20.0 - 23.4, 10.3 - 17.8, 11.8 - 18.7 and 31.3 - 33.7 while grown along with F_3, F_5 and BC_1F_4, F_6 and BC_1F_5, F_7 and BC_1F_6, respectively

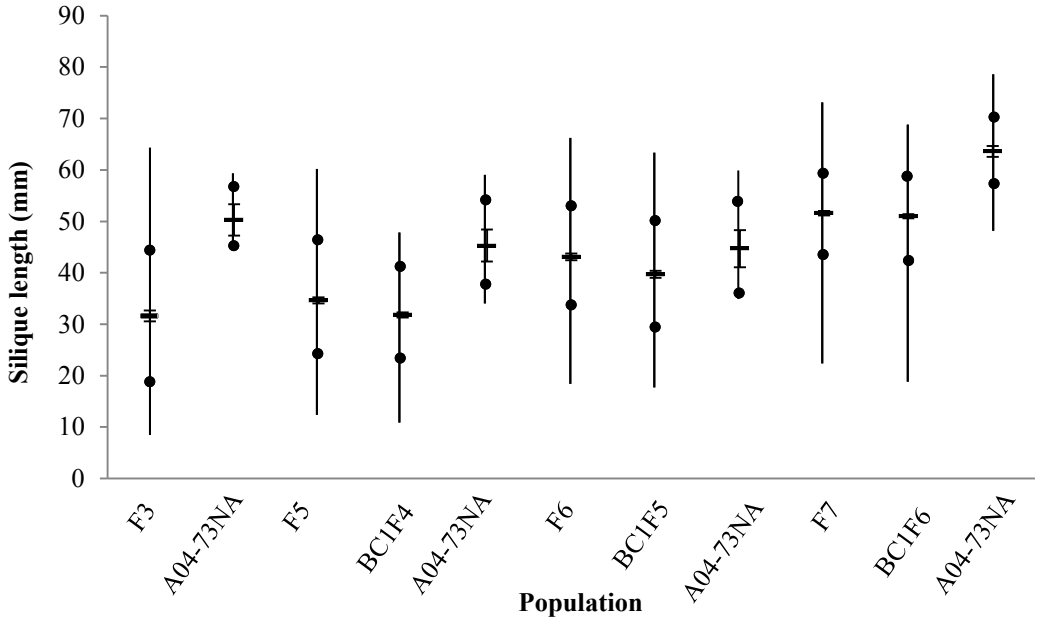

Fig. 2.4 Diagram showing range (——), mean with standard error (⊢⊣), and standard deviation (•⊢⊣•) for silique length in *B. napus* × *B. oleracea* interspecific crosses. Pooled data of *B. napus* × *B. oleracea* var. *italica*, *B. napus* × *B. oleracea* var. *capitata*, (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*, presented.

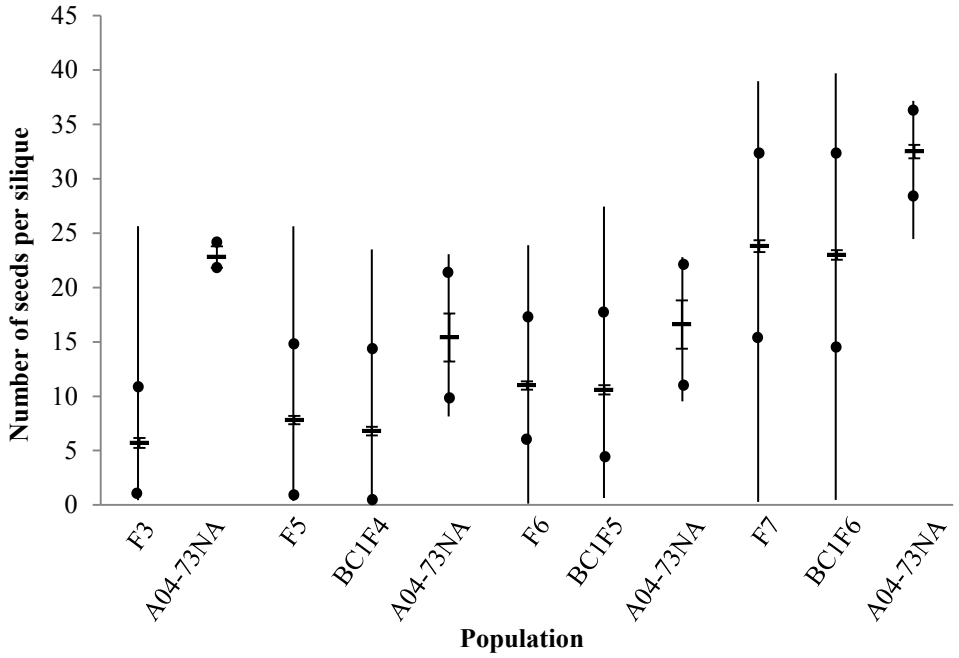

Fig. 2.5 Diagram showing range (——), mean with standard error (⊢⊣), and standard deviation (•⊢⊣•) for number of seeds per silique in *B. napus* × *B. oleracea* interspecific crosses. Pooled data of *B. napus* × *B. oleracea* var. *italica*, *B. napus* × *B. oleracea* var. *capitata*, (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*, presented.

Table 2.6 Plant fertility in different generation populations derived from F_2 and BC_1 of *B. napus* × *B. oleracea* interspecific crosses. Pooled data of the two crosses (*B. napus* × *B. oleracea* var. *italica*, *B. napus* × *B. oleracea* var. *capitata*) presented.

Generation[1]	No. plants grown[2]	No. plants produced seeds[2]	% fertile plants	Silique length (mm)		No. seeds per silique	
				Range (SD)	Mean ± SE	Range (SD)	Mean ± SE
F_3	340 (50)	169 (30)	50	9.2 - 65.2 (12.6)	31.6 ± 1.1	0 - 25.2 (5.3)	5.7 ± 0.5
BC_1F_2	350 (66)	84 (33)	24	–	–	–	–
F_4	328 (62)	217 (57)	66	–	–	–	–
BC_1F_3	310 (86)	221 (79)	71	–	–	–	–
F_5	502 (114)	327 (99)	65	12.0 - 60.2 (10.7)	34.6 ± 0.6**	0 - 25.4 (6.9)	7.8 ± 0.4
BC_1F_4	443 (101)	328 (92)	74	11.4 - 48.4 (9.1)	31.8 ± 0.5	0 - 23.6 (7.1)	6.8 ± 0.4
F_6	256 (108)	221 (105)	86	18.0 - 66.7 (9.6)	43.1 ± 0.7**	0 - 24.0 (5.6)	11.0 ± 0.4
BC_1F_5	280 (98)	237 (91)	85	18.7 - 64.7 (10.1)	39.7 ± 0.7	0.7 - 27.7 (6.6)	10.6 ± 0.4
F_7	288 (130)	276 (129)	96	22.3 - 73.0 (7.4)	51.6 ± 0.5	0.7 - 39.3 (8.9)	23.8 ± 0.5
BC_1F_6	381 (156)	373 (155)	98	18.7 - 68.3 (7.9)	51.0 ± 0.4	0 - 39.3 (8.7)	23.0 ± 0.5

[1]F_7 and BC_1F_6 generation populations were grown in field, other generations were grown in greenhouse.
[2]Number families given in brackets.
**Significantly different at $p < 0.01$.

Correlation of silique length and number of seeds per silique

Correlation between silique length and number of seeds per silique was calculated for the populations derived from F_2, such as in F_3, F_5, F_6 and F_7, and the populations derived from BC_1, such as BC_1F_4, BC_1F_5 and BC_1F_6. Positive correlation between these two traits was found in both F_2- ($r = 0.66 – 0.81$, $p < 0.01$) (Fig. 2.6) and backcross-derived populations ($r = 0.64 – 0.82$, $p < 0.01$) (Fig. 2.7) grown under field or greenhouse conditions.

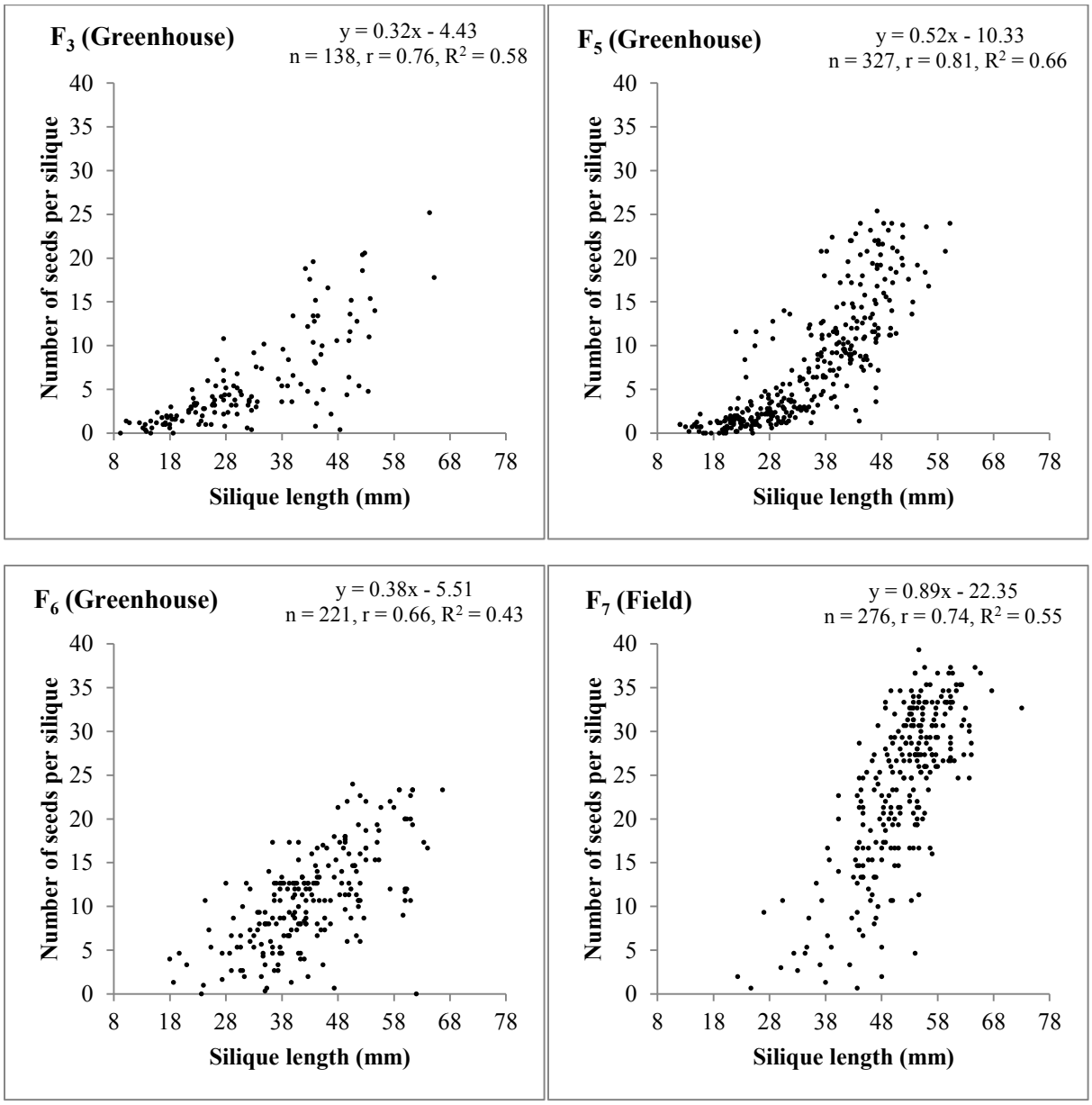

Fig. 2.6 Scatter diagram for silique length and number of seeds per silique in different generation populations derived from *B. napus* × *B. oleracea* interspecific crosses. Pooled data of the two crosses, *B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata*, presented.

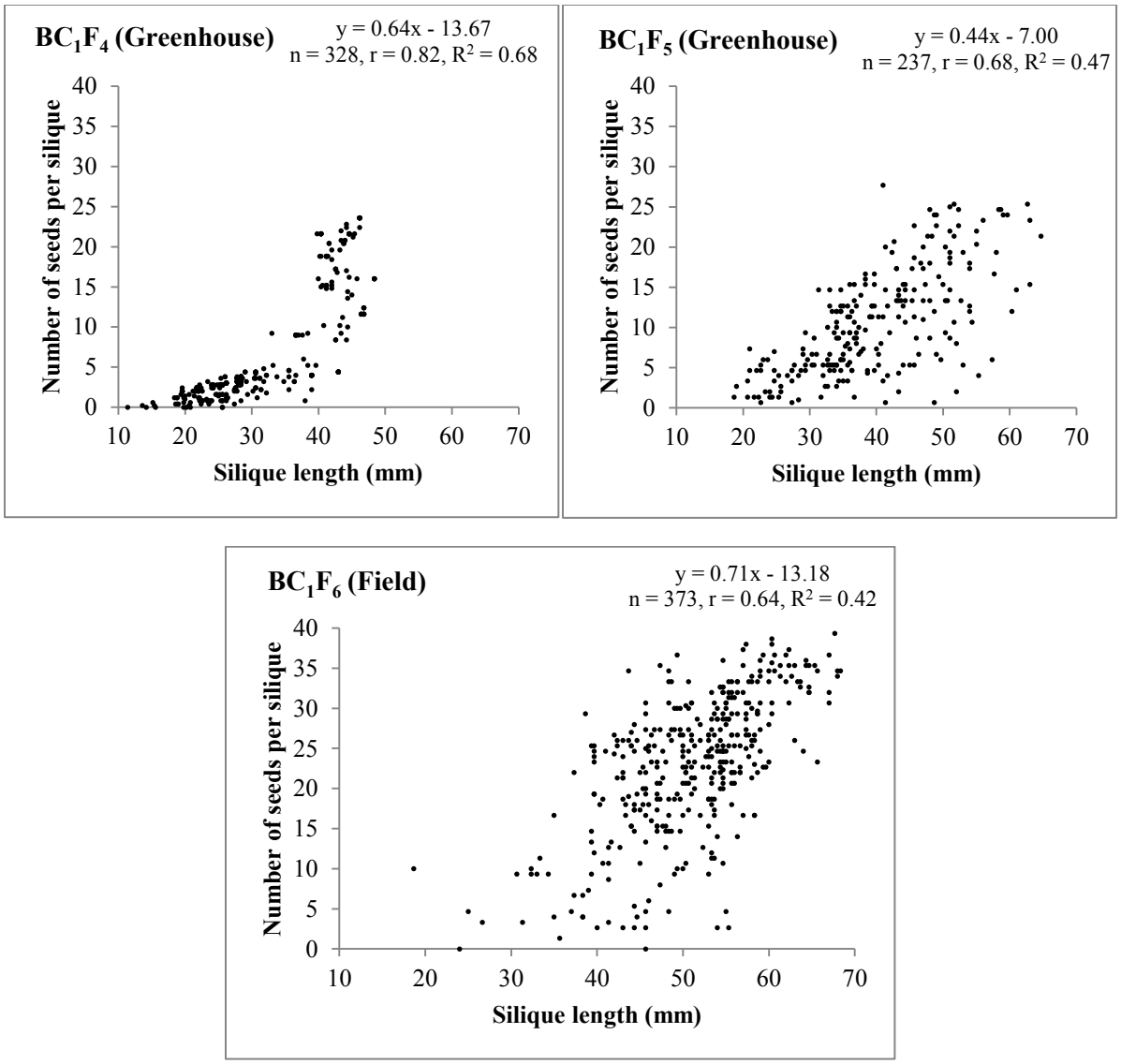

Fig. 2.7 Scatter diagram for silique length and number of seeds per silique in different generation populations derived from (*B. napus* × *B. oleracea*) × *B .napus* interspecific crosses. Pooled data of the two crosses, (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*, presented.

Days to flowering

Days to flowering data of the F_7 and BC_1F_6, and F_9 and BC_1F_8 generation populations grown in field 2014 and 2015 were collected and presented in Table 2.7. The confidence limit of A04-73NA for days to flowering was 43.6 - 44.2 days. Based on this, 59% F_7 families of 5CA1358 and 23% families of 5CA1392, and 35% BC_1F_6 families of 5CA1678 and 55% families of 5CA1679 flowered earlier than A04-73NA (Fig. 2.8). Among these, one F_7 family of 5CA1392 and two BC_1F_6 families of 5CA1679 flowered one week earlier than A04-73NA. The F_9 and BC_1F_8 generation families flowered 43 to 62 days after seeding (Table 2.7). The confidence limit of A04-73NA, grown with F_9 and BC_1F_8 populations in field 2015, for days to flowering was 46.8 - 47.7 days. Based on this, 31% F_9 families of 5CA1358 and 17% families of 5CA1392, and 15% BC_1F_8 families of 5CA1678 and 13% families of 5CA1679 flowered earlier than A04-73NA (Fig. 2.8). Among these, four F_9 families of 5CA1358 and two F_9 families of 5CA1392, and one BC_1F_8 family of 5CA1679 flowered three days earlier than A04-73NA.

Table 2.7 Days to flowering in advanced generation populations of *B. napus* × *B. oleracea* interspecific crosses.

Pedigree	Cross	Gen.	No. families	Range (SD)	Mean ± SE[2]	Gen.	No. families	Range (SD)	Mean ± SE[2]
B. nap × *B. o. ital*	5CA1358	F_7	76	39 - 57 (2.9)	43.8 ± 0.3ac	F_9	48	43 - 56 (2.7)	47.6 ± 0.6a
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_6	63	38 - 58 (3.7)	44.8 ± 0.5c	BC_1F_8	13	45 - 62 (4.4)	48.6 ± 0.7b
B. nap × *B. o. cap*	5CA1392	F_7	70	37 - 59 (3.9)	46.5 ± 0.5b	F_9	29	44 - 53 (2.1)	47.9 ± 0.7ab
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_6	112	37 - 53 (3.4)	43.3 ± 0.3a	BC_1F_8	32	44 - 59 (3.2)	49.2 ± 0.9b
B. nap	A04-73NA		35	41 - 46 (0.9)	43.9 ± 0.2a		93	43 - 54 (2.2)	47.3 ± 0. 2a

[1]*B. nap* = *B. napus* line A04-73NA, *B. o. ital* = *B. oleracea* var. *italica* cv. Premium Crop, *B. o. cap*= *B. oleracea* var. *capitata* cv. Balbro.
[2]Values followed by the different letter indicate significant difference, $p < 0.05$.

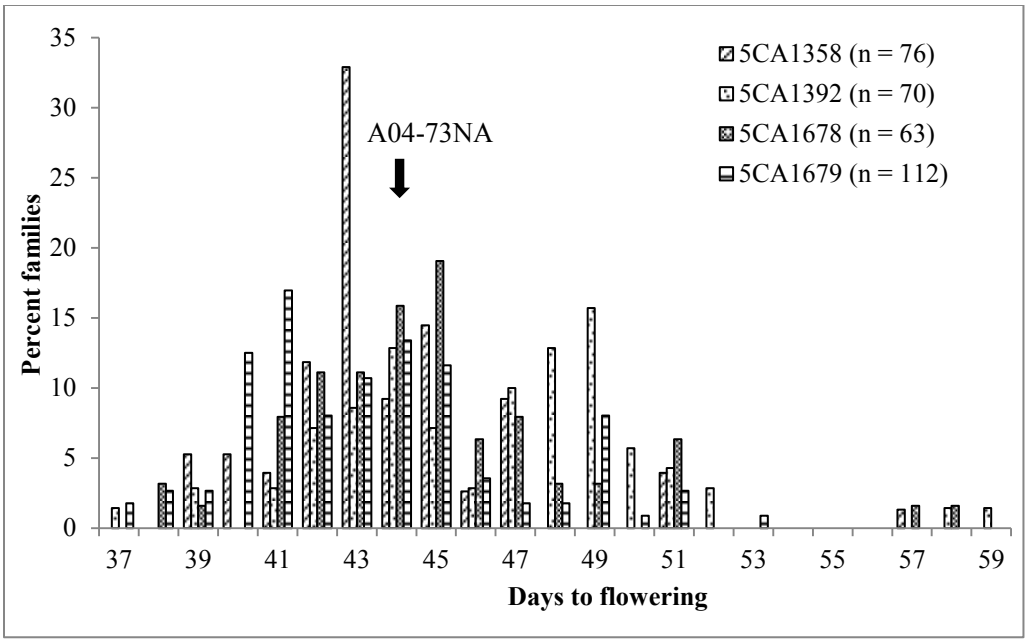

Fig. 2.8 Frequency distribution of the F_7 and BC_1F_6 families of *B. napus* × *B. olercea* interspecific crosses for days to flowering. Confidence limit of *B. napus* parent A04-73NA for days to flowering was 43.6 – 44.2 days. Cross 5CA1358 = *B. napus* × *B. oleracea* var. *italica*; 5CA1392 = *B. napus* × *B. oleracea* var. *capitata*; 5CA1678 = (*B. napus* × *B. oleracea* var. *italica*) × *B. napus*; 5CA1679 = (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*.

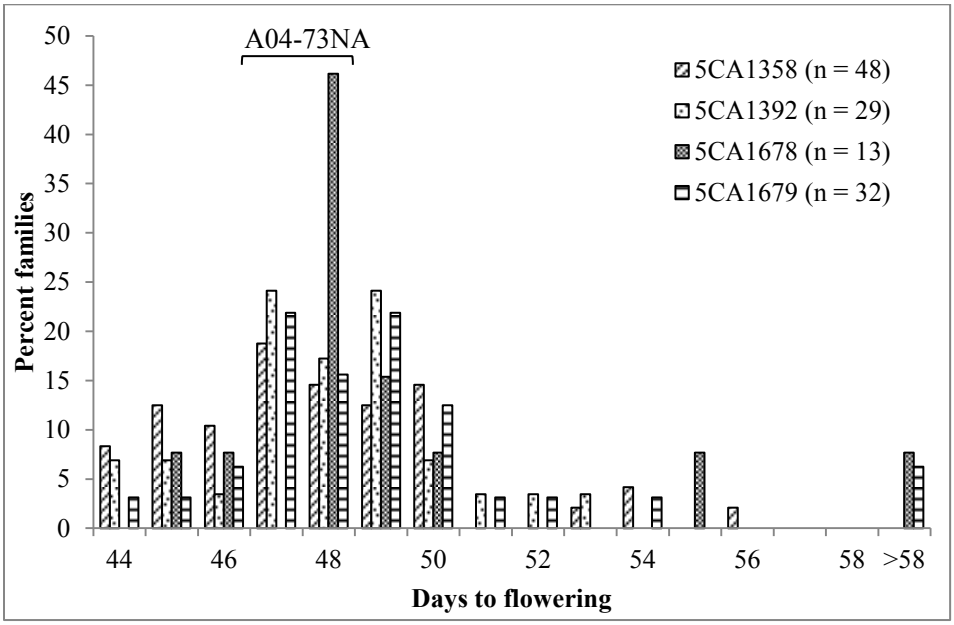

Fig. 2.9 Frequency distribution of the F_9 and BC_1F_8 families of *B. napus* × *B. olercea* interspecific crosses for days to flowering. Confidence limit of *B. napus* parent A04-73NA for days to flowering was 46.8 – 47.7 days. Cross 5CA1358 = *B. napus* × *B. oleracea* var. *italica*; 5CA1392 = *B. napus* × *B. oleracea* var. *capitata*; 5CA1678 = (*B. napus* × *B. oleracea* var. *italica*) × *B. napus*; 5CA1679 = (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*.

Correlation between generations for days to flowering

Coefficient of correlations between F_7 (parent) and F_9 (offspring) generation populations of the two crosses, and between BC_1F_6 (parent) and BC_1F_8 (offspring) generation populations of the two crosses were calculated for days to flowering. Significant positive correlation between parent and offspring generations was found in case of F_2- derived populations (F_7 and F_9) of 5CA1358 ($r = 0.359$, $p < 0.05$), while correlation was not significant for the other three crosses ($p > 0.05$) (Fig. 2.10, 2.11).

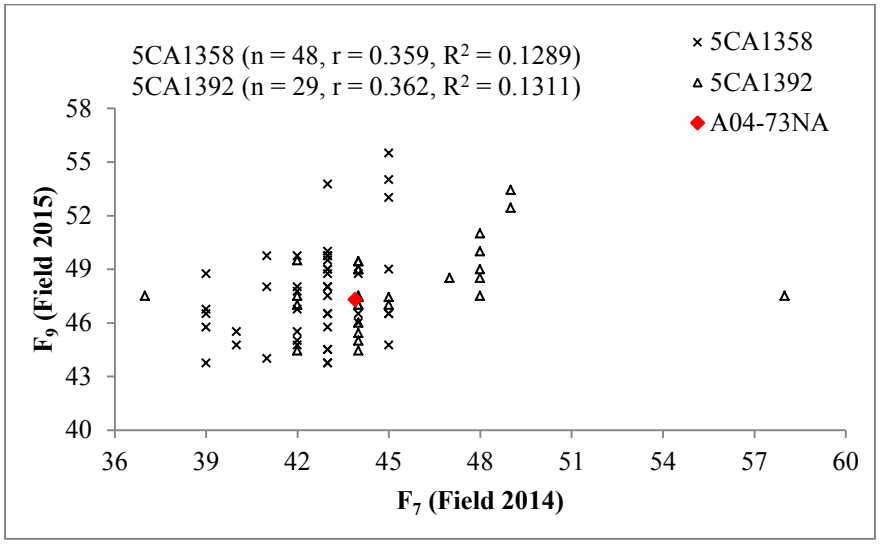

Fig. 2.10 Scatter diagram of parent (F_7) vs. offspring (F_9) generation families derived from *B. napus* × *B. oleracea* var. *italica* (cross ID 5CA1358) and *B. napus* × *B. oleracea* var. *capitata* (cross ID 5CA1392) interspecific crosses for days to flowering. Days to flowering of the *B. napus* parent also shown.

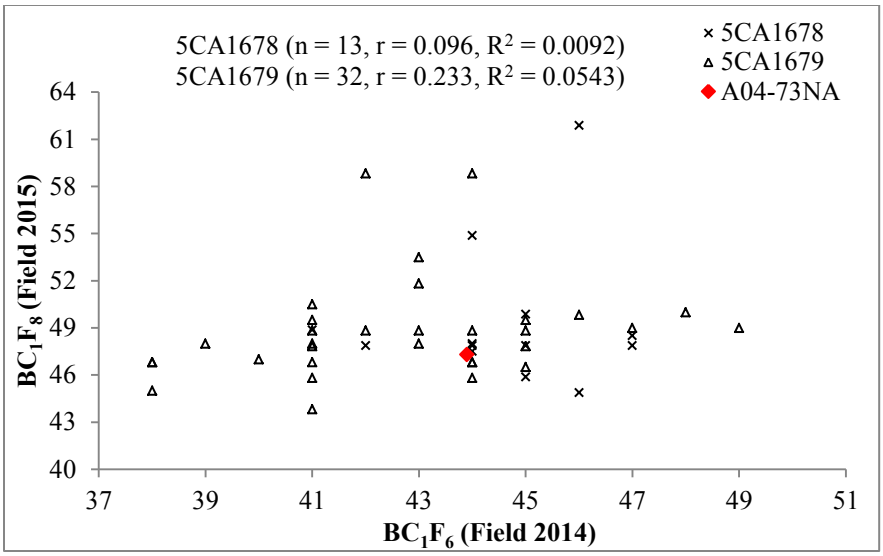

Fig. 2.11 Scatter diagram of parent (BC_1F_6) vs. offspring (BC_1F_8) generation families derived from (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* (cross ID 5CA1678) and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus* (cross ID 5CA1679) interspecific crosses for days to flowering. Days to flowering of the *B. napus* parent also shown.

2.3.3 Flow cytometric analysis

Confidence limits for partec values of *B. napus* and *B. oleracea* were 376 – 386 and 207 – 220 with mean values of 381 ± 2.90 SE and 214 ± 3.46 SE, respectively. Based on this, the F_6 and BC_1F_5 populations were classified into three groups: Group I, included the plants with partec value less than 376; Group II included the plants with partec value of 376 – 386, i.e. *B. napus* type; and Group III included the plants with partec value greater than 386.

In F_6, 18.5% plants fall into Group I with a mean partec value of 354 ± 4.06 SE; 93% plants of this group produced viable seeds (Table 2.8). Mean partec value of the plants of Group II was 382 ± 0.44 SE where 93% plants were fertile. More than 50% F_6 plants had partec value greater than 386 (mean 413 ± 3.05 SE); this group of plants had lower fertility than the plants belonging to Group I and Group II. In case of backcross, 24.2%, 20.3%, 55.5% plants fell into Group I, II, III, respectively. Among the 46 plants (20.3% of the total) of Group II, 96% plants produced silique with viable seed, while 79% of plants of Group III were fertile. Based on this, it

can be anticipated that, at least 22.9% of the F_6 plants and 20.3% of the BC_1F_5 plants had nuclear DNA content similar to *B. napus*.

Table 2.8 Nuclear DNA content in F_6 and BC_1F_5 generation plants of *B. napus* × *B. oleracea* interspecific crosses and their fertility.

Group	Gen.	No. plants (families)	Partec value		Percent fertile plants
			Range (SD)	Mean ± SE	
Group I	F_6	46 (43)	237 - 374 (27.3)	354 ± 4.1	93
	BC_1F_5	55 (47)	321 - 374 (11.9)	362 ± 1.6	85
Group II	F_6	57 (53)	375 - 386 (3.3)	382 ± 0.4	93
	BC_1F_5	46 (44)	375 - 386 (3.2)	381 ± 0.5	96
Group III	F_6	146 (121)	387 - 568 (36.7)	413 ± 3.1	83
	BC_1F_5	126 (99)	387 - 479 (16.7)	408 ± 1.5	79
B. napus[1]		22	356 - 402 (13.3)	381 ± 2.9	100
B. oleracea[1]		13	196 - 229 (12.0)	214 ± 3.5	-

[1]Confidence limits of *B. napus* was 376 – 386 and *B. oleracea* 207 – 220.

In F_8 and BC_1F_7 generation, two leaf samples were collected from each plant for measurement of nuclear DNA content and average value of the two samples was used for data analysis. Partec value of the F_8 plants varied between 340 and 400 with a mean of 364 ± 1.01 SE (Table 2.9). In case of BC_1F_7, partec value ranged from 342 to 397 with a mean of 363 ± 1.09 SE. Although, only 24.0% of the F_8 and 28.6% of the BC_1F_7 plants fall within the confidence limit of *B. napus* A04-73NA, more than 90% plants of these two populations had partec value falling within the range of A04-73NA (346 – 409). Moreover, the average partec value of these two populations was statistically similar to *B. napus* (368 ± 2.43 SE) and 100% plants of these two populations were fertile and produced viable seeds. This indicates that the F_8 and BC_1F_7 generation populations derived from *B. napus* × *B. oleracea* interspecific crosses reached to *B. napus* for chromosome number ($2n = 38$).

Table 2.9 Nuclear DNA content in F_8 and BC_1F_7 generation populations of *B. napus* × *B. oleracea* interspecific crosses.

Generation	Observation	Range (SD)	Partec value (Mean ± SE)[2]	Percent fertile plants
F_8	129	340 - 400 (11.5)	364 ± 1.0a	100
BC_1F_7	126	342 - 397 (12.3)	363 ± 1.1a	100
B. napus	42	346 - 409 (15.8)	368 ± 2.4a	100
B. oleracea[1]	15	191 - 229 (11.6)	209 ± 3.0b	-

[1]Pooled data of *B. oleracea* var. *italica* and var. *capitata*.
[2]Sharing the same letter indicate no significant difference at p-value > 0.05.

2.3.4 Seed quality analysis

Erucic acid content in BC_1 population

Seeds of the two *B. oleracea* parents used in this study contain more than 40% erucic acid in seed oil, while the *B. napus* parent A04-73NA is almost free from this fatty acid(< 1% erucic acid) (Table 2.10). In BC_1 population, 90 plants of the two crosses were grown of which 55 plants produced sufficient amount of seeds for fatty acid analysis. Erucic acid content in seeds harvested from BC_1 plants varied from 0.1% to 21.6% where seeds of six plants were free from this fatty acid (< 1%); the content of this fatty acid in the remaining 49 plants ranged from 5.5% to 21.6%. Thus, the observed segregation of 49:6 for presence vs. absence of erucic acid was significantly different from the expected 1:1 segregation ($\chi^2 = 33.6$, $p < 0.01$) (Fig. 2.12).

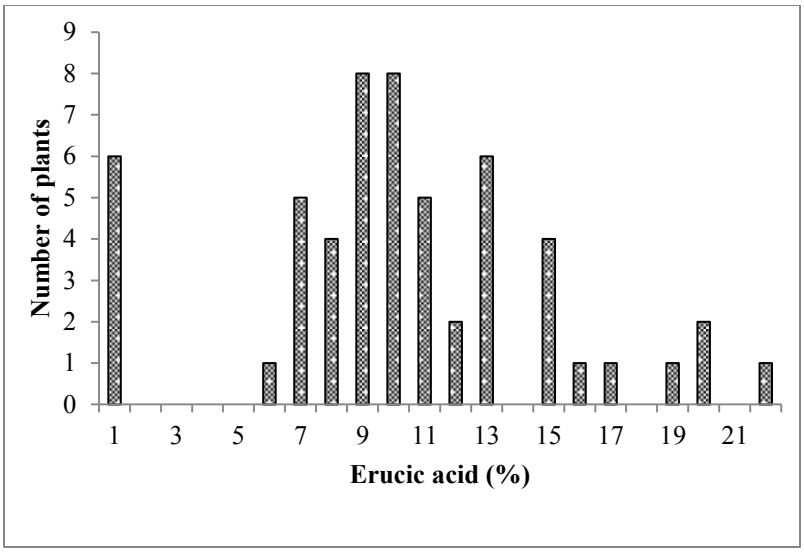

Fig. 2.12 Frequency distribution for erucic acid content in seeds harvested from BC_1 (n = 55) plants of (*B. napus* × *B. oleracea*) × *B. napus* interspecific crosses. Pooled data of two crosses, (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*, presented.

Erucic acid in F_2- and BC_1-derived populations

Progeny of all F_2 and BC_1 plants were grown. Erucic acid content in seeds harvested from F_3 plants of 5CA1358 ranged from 0 to 30.7 % with a mean 9.0% ± 0.9 SE, and in case of 5CA1392, it ranged from 0 to 20.9% with a mean of 9.9% ± 1.0 SE (Table 2.10). The mean values of the two crosses was not significantly different ($t = 0.7$, $p > 0.05$). Selection in this generation was performed for lower content of this fatty acid. Erucic acid content in selected populations of these two crosses was reduced to about half (5CA1358 = 4.9% ± 0.9 SE; 5CA1359 = 5.0% ± 1.0 SE).

Seeds harvested from 183 F_4 plants analyzed for erucic acid. Mean erucic acid content in seeds of 74 plants of 5CA1358 was 5.6% ± 0.9 SE; and in case of 5CA1392, the content of this fatty acid in 109 plants was 3.9% ± 0.6 SE. The BC_1F_3 population had higher content of erucic acid than F_4 (11.0% ± 1.1 SE for BC_1F_3 of 5CA1678 and 6.8% ± 0.72 SE for BC_1F_3 of 5CA1679). This is apparently due to the reason that selection for low content of this fatty acid

was performed in F_3 but not in BC_1F_2 due to poor seed set in this population. Selection for low erucic acid content in F_4 and BC_1F_3 population resulted significantly reduced content of this fatty acid in the progeny generations (Fig. 2.13, 2.14, 2.15).

In F_5, the mean erucic acid content of 5CA1358 was 0.4% ± 0.1 SE (0 – 10.3%) and in case of 5CA1392 it was 1.7% ± 0.3 SE. In this generation, only the plants free from erucic acid were selected for growing the next generation population. Mean erucic acid content in BC_1F_4 generation was about 2 – 3% higher than F_5; however, variation for this fatty acid (0.0 – 28.8%) was found in this population from where only the zero-erucic (< 1% erucic acid) plants were selected for growing BC_1F_5 generation populations.

In summary, selection for low erucic acid content was effective in this study where the proportion of plants having less than 1% erucic acid was increased in each generation. From F_3 to F_5, the proportion of zero-erucic acid (< 1%) plants increased by three fold (Fig. 2.13) As for the backcross population, the proportion of plants with erucic acid content less than 1% increased from 43% in BC_1F_3 to 77% BC_1F_4 (Fig. 2.14). In F_6 and BC_1F_5 generation, all families were free from erucic acid.

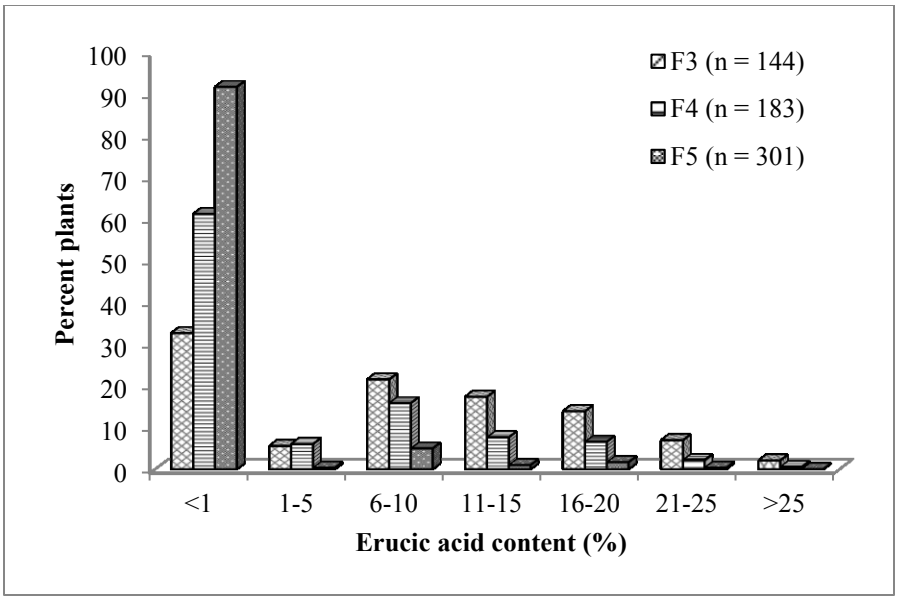

Fig. 2.13 Frequency distribution for erucic acid content (% of total fatty acids) in different generation population of *B. napus* × *B. oleracea* interspecific crosses. Pooled data of two crosses, *B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata*, presented.

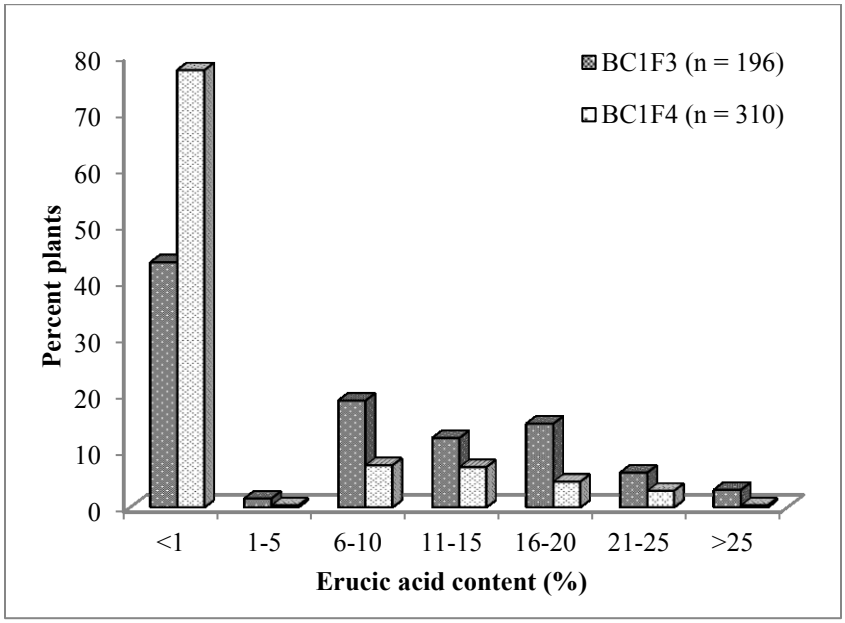

Fig. 2.14 Frequency distribution for erucic acid content (% of total fatty acids) in different generation populations of (*B. napus* × *B. oleracea*) × *B. napus* interspecific crosses. Pooled data of two crosses, (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*, presented.

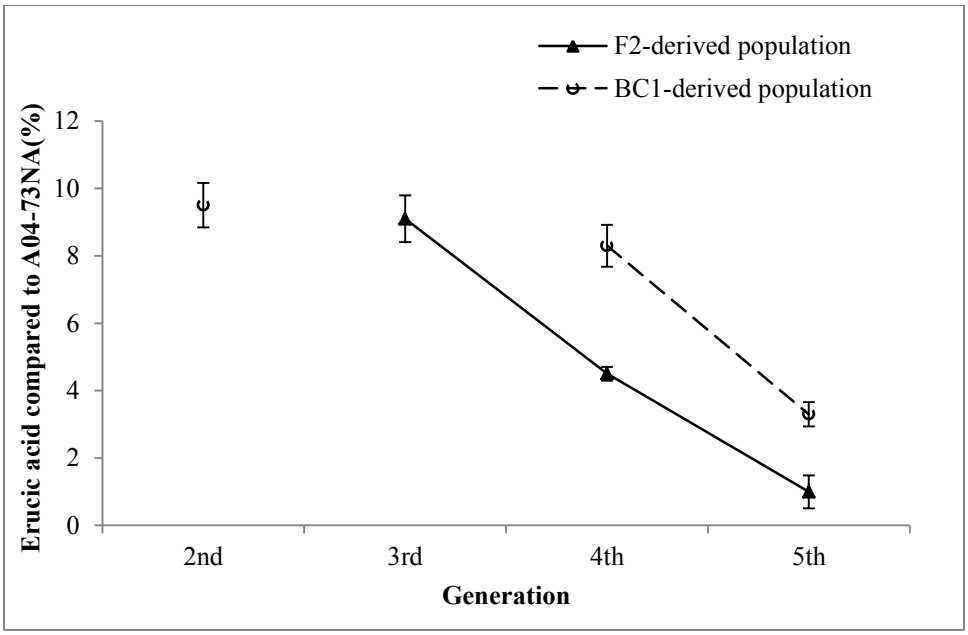

Fig. 2.15 Erucic acid content (% of total fatty acids, mean ± SE), compared to the *B. napus* parent A04-73NA, in different generation populations derived from F_2 and BC_1 of *B. napus* × *B. oleracea* interspecific crosses. Pooled data of the F_2-derived populations of *B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata*, and BC_1-derived populations of (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus* presented. 2nd generation = F_2 (greenhouse); 3rd generation = F_3 (greenhouse); 4th generation = F_4/BC_1F_3 (greenhouse); 5th generation = F_5/BC_1F_4 (greenhouse).

Table 2.10 Erucic acid content (% of total fatty acids) in different generation populations derived from F_2 and BC_1 of two *B. napus* × *B. oleracea* interspecific crosses.

			Whole population			Selected population		
Pedigree[1]	Cross ID	Gen.[2]	No. plants (families)	Range (SD)	Mean ± SE[3]	No. plants (families)	Range (SD)	Mean ± SE[4]
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1	41	0.2 - 21.6 (4.9)	10.6 ± 0.8c		all to next generation	
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1	14	0.1 - 15.0 (3.6)	7.0 ± 1.0b		all to next generation	
B. nap			7	0 - 0.3 (0.1)	0.1 ± 0.0a			
B. nap × *B. o. ital*	5CA1358	F_3	107 (22)	0 - 30.7 (8.8)	9.0 ± 0.9b	26 (13)	0 - 13.7 (4.6)	4.9 ± 0.9*
B. nap × *B. o. cap*	5CA1392	F_3	37 (2)	0 - 20.9 (6.3)	9.9 ± 1.0b	18 (2)	0 - 9.8 (4.1)	5.0 ± 1.0*
B. nap			8	0 - 0.3 (0.1)	0.1 ± 0.0a			
B. nap × *B. o. ital*	5CA1358	F_4	74 (27)	0 - 29.2 (7.5)	5.6 ± 0.9bc	46 (20)	0 - 11.4 (3.6)	2.2 ± 0.5*
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_3	81 (32)	0.1 - 33.7 (9.5)	11.0 ± 1.1d	28 (13)	0.1 - 12.7 (4.9)	4.4 ± 0.9*
B. nap × *B. o. cap*	5CA1392	F_4	109 (20)	0 - 19.9 (5.9)	3.9 ± 0.6b	68 (17)	0 - 13.3 (4.0)	2.6 ± 0.5
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_3	115 (47)	0 - 27.0 (7.7)	6.8 ± 0.7c	73 (31)	0 - 20.9 (6.2)	3.9 ± 0.7*
B. nap			8	0 - 0.3 (0.1)	0.1 ± 0.0a			
B. nap × *B. o. ital*	5CA1358	F_5	124 (37)	0 - 10.3 (1.1)	0.4 ± 0.1b	41 (28)	0 - 0.7 (0.2)	0.2 ± 0.0
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_4	134 (24)	0.1 - 23.3 (5.6)	3.4 ± 0.5d	49 (20)	0.1 - 0.9 (0.2)	0.3 ± 0.0*
B. nap × *B. o. cap*	5CA1392	F_5	177 (56)	0 - 21.3 (4.3)	1.7 ± 0.3c	67 (50)	0 - 0.7 (0.2)	0.2 ± 0.0*
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_4	176 (65)	0 - 28.8 (6.9)	3.5 ± 0.5d	47 (36)	0 - 0.6 (0.2)	0.2 ± 0.0*
B. nap			7	0 - 0.2 (0.1)	0.1 ± 0.0a			

[1]*B. nap* = *B. napus* line A04-73NA, *B. o. ital* = *B. oleracea* var. *italica* cv. Premium Crop, *B. o. cap*= *B. oleracea* var. *capitata* cv. Balbro.
[2]All generations were grown in greenhouse.
[3]Comparison between the populations grown under the same environment, values followed with the different letter indicates significant difference, $p < 0.05$.
[4]Asterisks indicate the selected population was significantly different from the whole population at $p < 0.05$.
Note: From F_6 and BC_1F_5 generation, all plants were free from erucic acid.

Seed glucosinolate content

The different generation populations derived from F_1 and BC_1 of the two crosses as well as the *B. napus* parent A04-73NA grown along with these generations were analyzed for total seed glucosinolates (GSL) content. GSL data of the individual crosses presented in Table 2.11 and pooled data of the two crosses presented in Table 2.12 and Fig. 2.18. The confidence limits of A04-73NA, grown along with F_3, F_4 and BC_1F_3, F_5 and BC_1F_4, F_6 and BC_1F_5, and F_7 and BC_1F_6, for GSL content were 7.3 - 8.2, 9.2 - 10.4, 9.0 - 11.2, 8.3 - 11.0, 18.4 - 20.0 μmol/g seed, respectively. Based on this, the interspecific cross derived plants falling within the confidence limit of A04-73NA or lower than this was considered as low GSL type.

GSL content in F_3 generation population of 5CA1358 ranged from 5.2 to 77.4 μmol/g seed with a mean of 24.0 ± 2.0 SE, and the mean GSL content in 5CA1392 were similar to the F_3 of 5CA1358. Both F_3 populations had significantly higher GSL content than the *B. napus* parent A04-73NA. Selection in this generation was focused for low erucic acid content only; therefore, GSL content in selected population of F_3 was not significantly different from the whole population. Mean GSL content in F_4 population was higher than the F_3 population (Table 2.11). This was probably due to the difference in growth condition as evident from about 2 μmol higher GSL per gram of seed found in A04-73NA when this parent was grown together with F_4. Mean GSL in F_5 population was similar to the earlier generations (F_4 and F_3). This was for the reason that selection up to this generation focused mainly for reduction of erucic acid content.

In case of the populations derived from BC_1, GSL content was first measured in BC_1F_3 grown along with F_4. GSL content in BC_1F_3 populations varied from about 8 to 80 μmol/g seed. Selection in this generation significantly reduced GSL content, where mean GSL content of

5CA1678 reduced from 42.16 ± 2.46 SE to 22.32 ± 1.73 SE µmol/g seed, and in case of 5CA1679 this reduced from 32.15 ± 1.98 SE to 20.1 ± 1.11 SE µmol/g seed. GSL content in BC_1F_4 generation was similar to the selected population of BC_1F_3; no selection for low GSL was done in this generation.

Table 2.8 shows that the selected F_5 and BC_1F_4 plants, i.e. the F_6 and BC_1F_5 generation populations were free from erucic acid. Therefore, selection in F_6 and BC_1F_5 and in subsequent generations focused on reducing the content of seed GSL. This is evident from significant difference between the selected population and the whole population. The selected populations of F_7 and BC_1F_6 had GSL content below 20 µmol/g seed, and this indicate that large number of the advanced generation families had low content of GSL in seed.

In Summary, selection for low GSL content in F_2- and BC_1- derived populations was effective where the proportion of the populations with low content of GSL increased steadily with the advancement of the generation (Fig. 2.16, 2.17, 2.18). Of the 145 F_7 plants belonging to 137 families, 71 plants produced seeds with GSL content of ≤ 15 µmol/g seed. In case of BC_1F_6, 86 of the 174 plants (154 families) had GSL content of ≤ 15 µmol/g seed.

Table 2.11 Glucosinolate content (μmol/g seed) in different generation populations derived from F_2 and BC_1 of two *B. napus* × *B. oleracea* interspecific crosses.

			Whole population				Selected population		
Pedigree[1]	Cross ID	Gen.[2]	No. plants (families)	Range (SD)	Mean ± SE[3]	% ≤ *B. napus*[4]	No. plants (families)	Range (SD)	Mean ± SE[5]
B. nap × *B. o. ital*	5CA1358	F_3	76 (18)	5.2 - 77.4 (17.1)	24.02 ± 2.0b	14.5	18 (8)	8.6 - 51.2 (13.1)	23.5 ± 3.1
B. nap × *B. o. cap*	5CA1392	F_3	25 (2)	6.9 - 49.3 (12.5)	26.28 ± 2.5b	8.0	13 (2)	7.9 - 49.3 (10.8)	24.9 ± 3.0
B. nap[6]			5	7.2 - 9.0 (0.7)	7.85 ± 0.3a				
B. nap × *B. o. ital*	5CA1358	F_4	55 (22)	9.5 - 63.0 (13.4)	27.58 ± 1.8b	1.8	45 (19)	9.5 - 44.9 (11.3)	24.0 ± 1.7
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_3	81 (32)	8.2 - 81.7 (22.1)	42.16 ± 2.5c	2.5	28 (13)	8.2 - 37.3 (9.2)	22.3 ± 1.7*
B. nap × *B. o. cap*	5CA1392	F_4	82 (17)	10.3 - 57.7 (10.6)	31.27 ± 1.2b	2.4	65 (17)	10.3 - 45.8 (8.7)	29.1 ± 1.1
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_3	115 (47)	8.1 - 80.4 (21.3)	32.15 ± 2.0b	7.0	73 (31)	8.1 - 39.3 (9.4)	20.1 ± 1.1*
B. nap[6]			8	8.4 - 11.2 (1.0)	9.81 ± 0.4a				
B. nap × *B. o. ital*	5CA1358	F_5	37 (20)	8.5 - 61.7 (14.8)	23.64 ± 2.4b	8.1	24 (17)	8.5 - 61.7 (15.0)	23.4 ± 3.1
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_4	28 (13)	8.1 - 51.0 (11.9)	24.18 ± 2.3b	10.7	21 (10)	8.1 - 51.0 (12.1)	26.9 ± 2.6
B. nap × *B. o. cap*	5CA1392	F_5	99 (43)	8.5 - 61.1 (13.8)	31.69 ± 1.4c	6.1	50 (37)	8.5 - 60.0 (13.2)	31.0 ± 1.9
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_4	15 (11)	10.0 - 57.4 (13.3)	22.73 ± 3.4b	13.3	9 (7)	10.9 - 57.4 (15.8)	24.7 ± 5.3
B. nap[6]			6	9.1 - 13.1 (1.5)	10.08 ± 0.6a				
B. nap × *B. o. ital*	5CA1358	F_6	85 (39)	6.2 - 58.7 (11.7)	17.30 ± 1.3b	32.9	75 (36)	6.2 - 41.7 (5.8)	13.8 ± 0.7*
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_5	88 (45)	5.8 - 78.8 (15.3)	26.17 ± 1.6c	14.8	55 (33)	5.8 - 34.1 (6.9)	16.0 ± 0.9*
B. nap × *B. o. cap*	5CA1392	F_6	127 (66)	6.2 - 66.7 (14.5)	28.55 ± 1.3c	12.6	68 (43)	6.2 - 45.1 (10.1)	18.6 ± 1.2*
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_5	130 (44)	6.6 - 79.9 (11.9)	18.31 ± 1.1b	23.1	110 (43)	6.6 - 40.7 (6.2)	14.3 ± 0.6*
B. nap[6]			6	7.5 - 12.8 (1.9)	9.61 ± 0.8a				
B. nap × *B. o. ital*	5CA1358	F_7	75 (72)	11.7 - 49.4 (7.1)	17.27 ± 0.8b	78.7	49 (49)	11.7 - 20.6 (2.2)	14.6 ± 0.3*
(*B. nap* × *B. o. ital*) × *B. nap*	5CA1678	BC_1F_6	63 (54)	9.5 - 46.6 (11.1)	22.21 ± 1.4c	58.7	23 (23)	9.5 - 22.1 (3.4)	13.7 ± 0.7*
B. nap × *B. o. cap*	5CA1392	F_7	70 (65)	12.0 - 45.3 (11.8)	24.71 ± 1.3c	45.7	25 (25)	12.0 - 24.4 (2.8)	15.0 ± 0.6*
(*B. nap* ×*B. o. cap*) × *B. nap*	5CA1679	BC_1F_6	112 (100)	11.8 - 39.9 (6.6)	17.53 ± 0.6b	82.1	70 (70)	11.8 - 19.9 (1.8)	14.7 ± 0.2*
B. nap[6]			35	12.7 - 22.8 (2.4)	19.18 ± 0.4a				

[1]*B. nap* = *B. napus* line A04-73NA, *B. o. ital* = *B. oleracea* var. *italica* cv. Premium Crop, *B. o. cap* = *B. oleracea* var. *capitata* cv. Balbro.
[2]F_7 and BC_1F_6 generation populations were grown in field, other generations were grown in greenhouse.
[3]Comparison between the populations grown under the same environment, values followed with the same letter indicates no significant difference, $p > 0.05$.
[4]Proportion of the population within the confidence limit of the *B. napus* parent A04-73NA. This also includes the population with less GSL than A04-73NA.
[5]Asterisks indicate the selected population was significantly different from the whole population at $p < 0.05$.
[6]Confidence limits of A04-73NA for GSL were 7.3 - 8.2, 9.2 - 10.4, 9.0 - 11.2, 8.3 - 11.0, 18.4 - 20.0 μmol/g seed while grown along with F_3, F_4 and BC_1F_3, F_5 and BC_1F_4, F_6 and BC_1F_5, and F_7 and BC_1F_6, respectively.

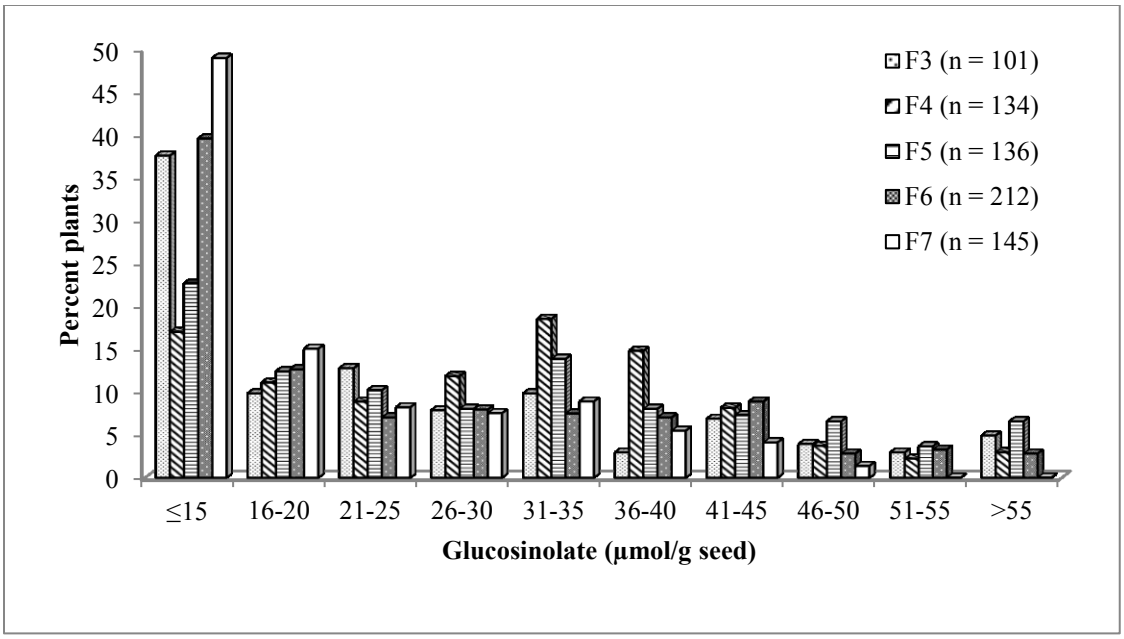

Fig. 2.16 Frequency distribution of different generation populations of *B. napus* × *B. oleracea* interspecific crosses for seed glucosinolate content. Pooled data of two crosses, *B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata*, presented.

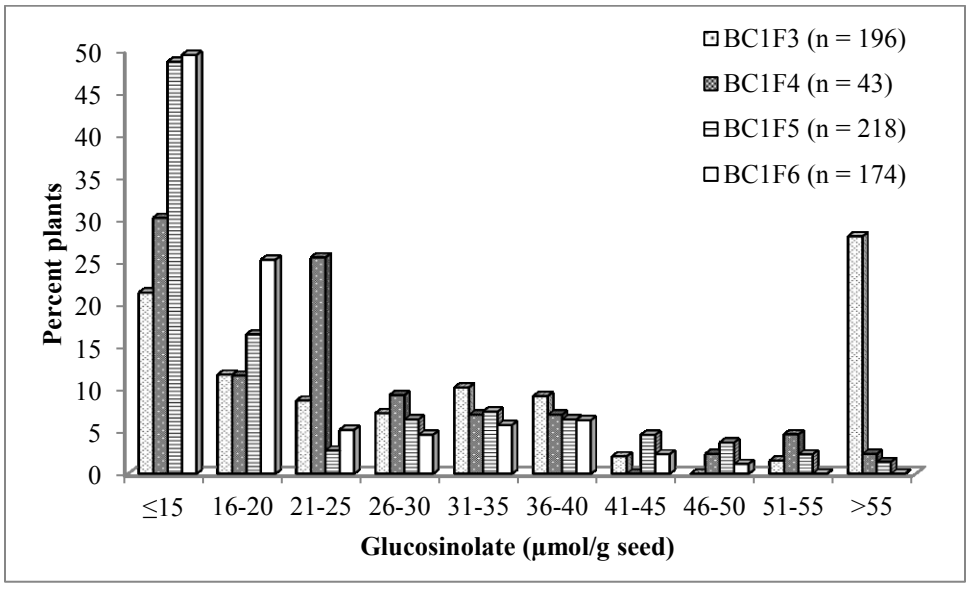

Fig. 2.17 Frequency distribution of glucosinolate content in different generation populations of (*B. napus* × *B. oleracea*) × *B. napus* interspecific crosses for seed glucosinolate content. Pooled data of two crosses, (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*, presented.

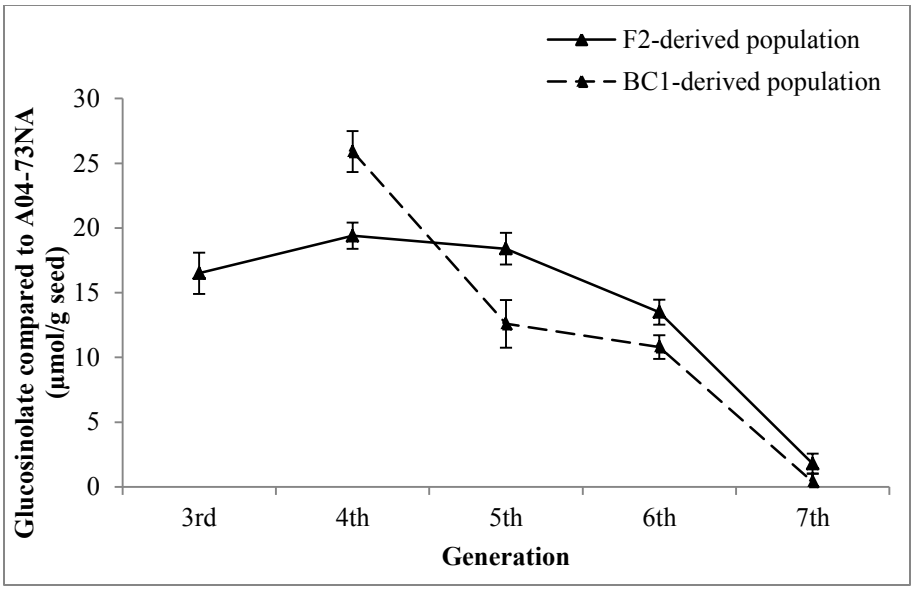

Fig. 2.18 Seed glucosinolate content (mean ± SE), compared to the *B. napus* parent A04-73NA, in different generation populations derived from F_2 and BC_1 of *B. napus* × *B. oleracea* interspecific crosses. Pooled data of the F_2-derived populations of *B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata*, and BC_1-derived populations of (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus* presented. 3rd generation = F_3 (greenhouse); 4th generation = F_4/BC_1F_3 (greenhouse); 5th generation = F_5/BC_1F_4 (greenhouse); 6th generation = F_6/BC_1F_5 (greenhouse); 7th generation = F_7/BC_1F_6 (field).

Double low or canola quality plants

The proportion of the F_3, F_4, F_5, F_6, F_7 and BC_1F_3, BC_1F_4, BC_1F_5, BC_1F_6 generation populations having low content of erucic acid in seed oil and low content of GSL in seed meal was estimated. The proportion of double low or canola quality plants obtained in different generations presented in Table 2.12. The mean GSL content in F_4 and BC_1F_3 generation was 29.8 ± 1.0 SE and 36.3 ± 1.6 SE µmol/g seed, respectively which reduced to 20.9 ± 0.8 SE and 19.2 ± 0.7 SE µmol/g seed in F_7 and BC_1F_6 generations. Overall, the proportion of canola quality plants increased with the advancement of generation in most cases. For example, about 49% plants were canola quality type in F_7 and BC_1F_6, while only 14.6 – 16.8% plants were of this type in F_4/BC_1F_3 generation.

Table 2.12 Occurrence of canola quality plants in different generation populations derived from F_2 and BC_1 of two *B. napus* × *B. oleracea* interspecific crosses.

Generation[1]	Erucic acid (%)			Glucosinolate (µmol/g seed)			% canola quality plants[2]
	No. plants	Range	Mean ± SE	No. plants	Range	Mean ± SE	
F_3	144	0 - 30.7	9.2 ± 0.7	101	5.2 - 77.4	24.6 ± 1.6	27.7
F_4	183	0 - 29.2	4.6 ± 0.5	137	9.5 - 63.0	29.8 ± 1.0	14.6
BC_1F_3	196	0 - 33.7	8.5 ± 0.6	196	8.1 - 81.7	36.3 ± 1.6	16.8
F_5	301	0 - 21.3	1.2 ± 0.2	136	8.5 - 61.7	29.5 ± 1.2	20.6
BC_1F_4	310	0 - 28.8	3.4 ± 0.4	43	8.1 - 57.4	23.7 ± 1.9	16.3
F_6	212	<1	<1	212	6.2 - 66.7	24.0 ± 1.0	39.6
BC_1F_5	218	<1	<1	218	5.8 - 79.9	21.5 ± 0.9	48.6
F_7	145	<1	<1	145	11.7 - 49.4	20.9 ± 0.8	49.0
BC_1F_6	175	<1	<1	175	9.5 - 46.6	19.2 ± 0.7	49.1

[1]F_7 and BC_1F_6 generation populations were grown in field; other generations were grown in greenhouse. Pooled data of *B. napus* × *B. oleracea* var. *italica*, *B. napus* × *B. oleracea* var. *capitata*, (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus* presented.
[2]Percent canola quality type plants = less than 1% erucic acid in seed oil and ≤ 15µmol glucosinolate per gram seed.

2.3.5 Performance of the test hybrids

Due to damage of the crop by wind storm prior to harvest, yield data was not used. Mid-parent heterosis (MPH) and heterosis over *B. napus* A04-73NA for days to flowering and plant height are presented in Table 2.13.

For days to flowering, both MPH and heterosis over A04-73NA showed wide variation - ranging from negative to positive heterosis; however, mean heterosis in most cases was negative. The F_8 lines of 5CA1358 and BC_1F_7 lines of 5CA1678 showed significant difference for MPH as well as for heterosis over A04-73NA; however, pooled data of the two crosses showed no significant difference for MPH as well as heterosis over A04-73NA. For plant height, mean heterosis in three of the four populations was positive, while it was negative in case of the hybrids based on F_9 lines of 5CA1358. On average, height of the hybrids derived from BC_1F_8 was significantly greater than the hybrids developed based on F_9 ($p < 0.01$).

Table 2.13 Heterosis for days to flowering and plant height (cm) in test hybrids produced from cross between the *B. napus* parent A04-73NA and the inbred lines derived from A04-73NA × *B. oleracea* interspecific crosses.

			Days to flowering					Plant height			
			MPH (%)[4]		A04-73NA(%)[3]			MPH (%)[4]		A04-73NA(%)[4]	
Cross ID[1]	Gen.[2]	No. TC[3]	Range	Mean ± SE[5]	Range	Mean ± SE[5]	No. TC[3]	Range	Mean ± SE[5]	Range	Mean ± SE[5]
5CA1358	F_9	47	-9.7 - 6.8	-2.0 ± 0.7a	-12.6 - 6.7	-1.8 ± 1.7a	28	-24.4 - 9.1	-4.1 ± 1.4c	-26.8 - 5.8	-9.6 ± 2.1c
5CA1678	BC_1F_8	13	-5.3 - 8.9	1.0 ± 1.2b	-5.2 - 10.5	3.0 ± 2.1b	9	-4.8 - 9.3	4.8 ± 2.4ab	-3.1 - 14.6	7.0 ± 3.3ab
5CA1392	F_9	29	-8.8 - 5.6	-0.7 ± 0.8ab	-7.2 - 8.6	-0.1 ± 1.7ab	27	-13.0 - 25.3	0.4 ± 1.4b	-14.3 - 21.7	0.7 ± 2.0b
5CA1679	BC_1F_8	32	-7.4 - 10.6	-1.1 ± 0.8ab	-12.4 - 6.9	-0.1 ± 1.7ab	25	-5.8 - 20	5.5 ± 1.5a	-6.0 - 27.9	8.2 ± 2.2a
Pooled	F_9	76	-9.7 - 6.8	-1.2 ± 0.4x	-12.6 - 8.6	-1.2 ± 0.5x	55	-24.4 - 25.3	-1.8 ± 1.2x	-26.8 - 21.7	-4.4 ± 2.5x
Pooled	BC_1F_8	45	-7.4 - 10.6	-0.1 ± 0.6x	-12.4 - 10.5	0.9 ± 0.7x	34	-5.8 - 20	5.3 ± 1.5y	-6.0 - 27.9	7.9 ± 2.9y

[1]5CA1358 = *B. napus* × *B. oleracea* var. *italica* cv. Premium Crop, 5CA1392 = *B. napus* × *B. oleracea* var. *capitata* cv. Balbro, 5CA1678= (*B. napus* × *B. oleracea* var. *italica* cv. Premium Crop) × *B. napus*, 5CA1679 = (*B. napus* × *B. oleracea* var. *capitata* cv. Balbro) × *B. napus*.
[2]Generation of the inbred lines used to produce test hybrids.
[3]Number of test hybrids.
[4]Heterosis over mid-parent (MPH) and over A04-73NA.
[5]Comparison between crosses: values sharing the same letter (a, b, c) indicates no significant difference at $p > 0.05$; comparison between pooled data of F_8 and BC_1F_7: values sharing the same letter (x, y) indicate no significant difference at $p > 0.05$.

2.4 Discussion

The short domestication history of *B. napus* and intensive breeding emphasis on selection for canola quality traits are partly responsible for the narrow genetic base in current canola germplasm (Becker et al. 1995; Cooper et al. 2001). Among the two genomes of *B. napus*, genetic diversity in the C genome is narrower than the A genome (Bus et al. 2011). However, the progenitor species *B. oleracea*, which generally grown as a vegetable crop, possesses wide genetic variation and this genome is distinct from the C genome of *B. napus* for allelic diversity (Mei et al. 2010; Jesske et al. 2013). The C genome of broccoli (*B. oleracea* var. *italica*) and white cabbage (*B. oleracea* var. *capitata*) are known to be genetically distinct (Song et al. 1990; Lu et al. 2009). This study aimed to introgress the genomic component from these two variants of *B. oleracea* into spring *B. napus* canola.

It is difficult to produce viable hybrid seeds from many interspecific crosses, such as *B. napus* × *B. oleracea*, *B. juncea* × *B. oleracea*, *B. oleracea* × *B. rapa* (reviewed by Rahman 2013; Wen et al. 2008). In such cases, application of embryo culture (Rahman 2004) or ovule culture (Bennett et al. 2008) techniques are needed to increase the chance of producing interspecific hybrid plants. In the present study, application of *in vitro* ovule culture technique (Bennett et al. 2008) was effective for production of interspecific hybrid plants of the two crosses involving the *B. napus* cultivar A04-73NA as female and the two *B. oleracea* var. *italica* and var. *capitata* cultivars as male.

High sterility in early generations of the interspecific hybrid progenies was found in this study. Chromosome anomalies at meiosis generally result sterility in the plants (Kianian and Quiros 1992; Bennett 2012). The level of sterility in interspecific hybrid progeny often depends

on genome composition of the interspecific hybrids. For example, the AC genome hybrids usually show high sterility while the AAC hybrids show partial fertility (reviewed in Rahman 2013). In case of *B. napus* ($A^nA^nC^nC^n$) × *B. oleracea* (C^oC^o) cross, segregation and recombination of the A and C genome chromosome in the progeny of F_1 ($A^nC^nC^o$) hybrids can result plants with different genome composition (e.g. $A^nC^oC^o$, $A^nA^nC^nC^o$, $A^nC^nC^n$, C^nC^n). This suggests that aneuploid plants can occur frequently in the progeny of this interspecific cross. In this case, plants carrying greater number of A and C chromosome seems to show higher fertility (Bennett et al. 2008, 2012; Rahman et al. 2015). Therefore, flow cytometric analysis was done on the advanced generation populations to identify the plants with $2n = 38$ chromosomes. As expected, plants with nuclear DNA content similar to *B. napus* ($2n = 38$) had higher fertility than the plants with lower or greater DNA content (Table 2.6). Rahman et al. (2015) found good seed set in euploid ($2n = 38$) plants derived from *B. napus* × *B. oleracea* interspecific cross. Therefore, silique length and number of seeds per silique was recorded to measure fertility of the plants which expected to reflect the chromosome composition of the plants. As mentioned above, most of the advanced generation plants had good fertility as well as had nuclear DNA content similar to the *B. napus* parent A04-73NA. It was found that open-pollinated plants tend to produce greater number of seeds per silique than self-pollinated plants. Similar result also reported by Li et al. (2013) in case of resynthesized *Brassica* hexaploid plants (AABBCC).

The two *B. oleracea* parents used in this study contained more than 40% erucic acid (22:1), while the *B. napus* parent was almost free from this fatty acid. Erucic acid content in *B. napus* seed oil is controlled by two major gene loci with additive effect (Harvey and Downey 1964), and the two genes are located on chromosome A8 (N8) and C3 (N13) (Zhang et al. 2008; Rahman et al. 2008; Li et al. 2014). Therefore, segregation for erucic acid content in the present

study involved segregation of the erucic acid alleles of the C genome only. Based on this, a 1:1 segregation for presence vs. absence of erucic acid was expected in BC_1 population. However, strong segregation distortion was found in the present study. Theoretically, the BC_1 population should fall into three groups for erucic acid content: 15%, 10%, and < 1% erucic acid in 1:1:2 ratio (Iftikhar 2015). Indeed, the BC_1 population roughly falls into three groups: 14.3 – 21.6%, 5.5 – 12.7%, and < 1% erucic acid; however, the observed segregation of 10:39:6 deviated significantly from the expected 1:1:2 ratio ($\chi^2 = 64.2$, $p < 0.01$). Rahman et al. (2015) and Bennett et al. (2008) also reported segregation distortion for erucic acid alleles in the progeny of *B. napus* × *B. oleracea* var. *alboglabra* interspecific cross. Despite segregation distortion for erucic acid observed in *B. napus* × *B. oleracea* cross, selection for zero erucic acid *B. napus* plants from these interspecific crosses was achievable where all F_6 and BC_1F_5 generation plants were free from this fatty acid.

Like erucic acid, the inheritance of seed GSL content also involved the C genome gene loci only. At least three loci controlling seed GSL content are identified in the C genome chromosomes (Howell et al. 2003; Li et al. 2014). In the present study, involvement of more than one gene loci in the inheritance of this trait make selection for low GSL content a more difficult task compared to selection for low etucic acid content. Furthermore, seed GSL content is highly influenced by environment (Josefsson and Appelqvist 1968) and this also reduces the efficiency of selection for low GSL content. In present study, all F_6 and BC_1F_5 generation families were free from erucic acid; however, some of the families still contained more than 40 µmol GSL per gram of seed (Table 2.9). In F_7 and BC_1F_6, more than 60% of the population had GSL content similar to the *B. napus* parent A04-73NA.

The flowering time in *Brassica* species is a quantitative trait (Axelsson et al. 2001). Several quantitative trait loci (QTL) involved in the control of days to flowering has been reported by different researchers in *B. rapa* (Teutonico and Osborn 1995; Osborn et al. 1997), *B. oleracea* (Okazaki et al. 2007), and *B. napus* (Teutonico and Osborn 1995), however, only a few of these found to have major effect. These genes act either in additive or dominant manner in *B. napus* (Ringdahl et al. 1986). In the present study, a few advanced generation lines of 5CA1358 and 5CA1392 found to flower earlier than the *B. napus* parent A04-73NA. This indicates that, alleles for earliness of flowering have been introduced from *B. oleracea* var. *italica* and var. *capitata* into spring *B. napus* canola. This agrees with the results reported by Rahman et al. (2011) that the late flowering species *B. oleracea* carry alleles which can improve the earliness in *B. napus*.

In conclusion, present study demonstrated that canola quality *B. napus* ($2n = 38$) inbred lines can be achieved from *B. napus* × *B. oleracea* (var. *capitata* and var. *italica*) interspecific crosses. Some of the inbred lines derived from the two interspecific crosses showed potential for developing early flowering/maturing hybrid cultivar. However, the potential of these lines for use in hybrid breeding needs to be investigated.

Chapter 3

Assessment of allelic variation introgressed from *B. oleracea* var. *italica* and var. *capitata* into spring *B. napus*

Summary

Genetic diversity in F_4 and BC_1F_3 populations derived from *B. napus* × *B. oleracea* interspecific crosses was estimated by use of simple sequence repeat (SSR) markers. A total of 146 alleles were detected in these populations by use 48 polymorphic SSR markers, of which 75 alleles were specific to the *B. oleracea* parents. Genetic similarity coefficient between the two *B. oleracea* parents, var. *italica* cv. Premium Crop and var. *capitata* cv. Balbro, was 0.24, while the coefficients of similarity of these two parents with the *B. napus* parent A04-73NA was 0.14 and 0.09, respectively. In general, the BC_1-derived population clustered together and was close to the *B. napus* parent compared to the F_2-derived population. A small group of the plants clustered close to *B. oleracea*, while large number of the plants falls in between the *B. napus* and *B. oleracea* parents. Thus, the result demonstrates the viability of introducing alleles from broccoli and cabbage into spring *B. napus* canola.

3.1 Introduction

Scientific evidences supports that the amphidiploid species *Brassica napus* L. (AACC, 2*n* = 38) evolved in nature about 0.7 – 1 Mya (reviewed in Ford et al. 2012) and it was domesticated about 400 years ago (Gómez-Campo and Prakash 1999). Breeding in the last few decades for the development of canola quality cultivars as well as intensive breeding by use of limited genetic variation has narrowed down the genetic base of current *B. napus* canola breeding materials. For instance, Fu and Gugel (2010) reported that genetic diversity in Canadian spring oilseed *B. napus*

cultivars has been decreased over the years. Hasan et al. (2006) found that the spring type *B. napus* has the lowest genetic diversity compared to winter and vegetable types while evaluating different accessions of winter and spring oilseed, and fodder and vegetable types of *B. napus* collected from gene bank and analyzed by use of simple sequence repeat (SSR) markers. Bus et al. (2011) found the evidence that the C genome of *B. napus* is genetically less diverse compared to its A genome. By use of high density genome-wide SNP markers, Qian et al. (2014) found that larger size of SNP haplotype blocks are associated with the genes controlling erucic acid and glucosinolates in the C genome as compared to the size of the haplotype blocks associated with these traits in the A genome of Chinese semi-winter *B. napus*. This indicates that the A genome of Chinese semi-winter *B. napus* is more diverse than the C genome. Hence, there is a need of broadening genetic diversity in the C genome of *B. napus* canola germplasm for further improvement of this crop.

Molecular markers are important tools in plant breeding since they reveal variation at molecular level and provide accurate information without the interference of environment. Thus, the use of this tool in breeding can increase the efficiency of selection (Flint-Garcia et al. 2003). Different types of DNA-based molecular markers are available today; however, the following four types of markers being widely used by different researchers: Restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs) or microsatellites. RFLP is a non-PCR (polymerase chain reaction) based, co-dominant markers, RAPD and AFLP are PCR-based dominant markers, and SSR is a PCR-based co-dominant markers (Vignal et al. 2002). Recently, single nucleotide polymorphism (SNP) markers received much attention to the

researchers and breeders (Vignal et al. 2002; Qian et al. 2014) and becoming new markers of choice.

Several researchers compared the various types of genetic markers and suggested their implications in genetic research (Vignal et al. 2002, Nybom 2004, Semagn et al. 2006, Abdel-Mawgood 2012). SSR markers were widely used in the last two decades before SNP markers become available. These are highly informative and economical as polymorphism can be detected through gel electrophoresis. In case of SNP, genotyping arrays containing thousands of markers from the entire genome is now available for many crops including canola, and this makes this marker type a powerful tool for use in association mapping, fine mapping of traits, and genomic selections (Chen and Sullivan 2003, Ganal et al. 2012). Hamblin et al. (2007) found that, compared to the number of polymorphic SSR markers to be required for estimation of genetic relatedness between maize lines, much greater number of SNPs will be required for this.

The objective of this study was to evaluate the extent of *B. oleracea* alleles introgressed into the *B. napus* canola lines derived from *B. napus* × *B. oleracea* interspecific crosses, and identify lines which are genetically diverse from *B. napus* A04-73NA.

3.2 Materials and methods

3.2.1 Plant materials

A set of 89 plants derived from *B. napus* × *B. oleracea* interspecific crosses were genotyped by SSR markers to evaluate the extent of allelic diversity introgressed from *B. oleracea* into these plants. This plant population included 19 F_4 plants derived from A04-73NA × broccoli (*B. oleracea* var. *italica*) cv. Premium Crop, 24 F_4 plants from A04-73NA × white cabbage (*B. oleracea* var. *capitata*) cv. Balbro, 15 BC_1F_3 plants derived from [A04-73NA ×

broccoli (*B. oleracea* var. *italica*) cv. Premium Crop] × A04-73NA, and 31 BC_1F_3 plants from [A04-73NA × white cabbage (*B. oleracea* var. *capitata*) cv. Balbro] × A04-73NA. Seeds harvested from these were plants grown to generate the advanced generation families reported in Chapter 2. List of these plant materials and their genotyping codes is presented in Appendix 3.1, and the list of polymorphic SSR markers used for genotyping is presented in Appendix 3.2.

3.2.2 DNA extraction and PCR

Young, healthy leaf samples of F_4 and BC_1F_3 plants were collected from the plants grown in a greenhouse and stored at -80 ^oC. For DNA extraction, about 40 mg frozen leaf sample of each plant was placed in a 1.5 ml Eppendorf tube and immersed in liquid nitrogen for one minute and the samples were grounded with a micropestal. Genomic DNA was extracted from the grounded leaf samples using a SIGMA DNA extraction kit ((Sigma-Aldrich, St. Louis, MO) following manufacturer instruction with some modifications. The DNA extraction protocol was as following: 400 µl nuclei lysis buffer was added to the grounded leaf samples, and the samples were incubated at 65 ^oC (water bath) for 15 – 20 min and 400 µl chloroform was added. The samples were centrifuged at 12,000 rpm for 5 min, and 300 µl supernatant was transferred to a new tube and 300 µl isopropanol was added and mixed by inverting the tubes. The samples were kept at room tempreture (20 ^oC) for 5 min and then centrifuged at 12,000 rpm for 3 min. The supernatant was discarded and 500 µl ethanol (70%) was added, and this followed centrifuging for 2 min. Samples were air-dried and suspended in 100 µl TE buffer (mixture of 5 ml 1 M Tris-HCl, 1 ml 0.5 M EDTA and 494 ml milliQ water, pH = 8.0). The concentration and quality of the genomic DNA was measured using NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Finally, the DNA of each sample was diluted to 15 ± 5 ng/µl with TE buffer and stored at 4 ^oC for use.

PCR amplification of genomic DNA was carried out in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA) with a total volume of 12.75 µl, containing about 22.5 ng of template DNA, 0.25 µl of 10 µM primer (for each forward and reverse primer), 0.38 µl of 10 mM dNTPs mix (Invitrogen Life Technologies Inc., Burlington, ON), 1.0 µl of 25 mM $MgCl_2$, 2.5 µl of 5x PCR reaction buffer, 0.125 units of Taq DNA polymerase (Promega Corporation, Madison, WI) and 6.745 µl milliQ water. PCR cycle was initial denaturation for 5 min at 95 ^{o}C, 35 cycles of denaturation for 1 min at 95 ^{o}C, annealing for 1 min at 58 ^{o}C and extension for 1 min 30 seconds at 72 ^{o}C and final extension for 30 min at 72 ^{o}C.

3.2.3 Genotyping by polymorphic SSR markers

Three parental lines (A04-73NA, *B. oleracea* var. *italica* cv. Premium Crop and *B. oleracea* var. *capitata* cv. Balbro) were screened with 454 SSR markers through gel electrophoresis for identification of polymorphic markers. Sequence of 396 markers obtained from Agriculture and Agri-Food Canada (AAFC) through a material transfer agreement, and 58 markers obtained from the papers published by Cheng et al. (2009) and Li et al. (2011).

For gel electrophoresis, 10× TBE buffer was prepared as follows: 107 g Tris base, 54.5 g Boric acid and 3.7g EDTA were mixed and MilliQ water was added to a final volume of 1000 ml. The 10× TBE buffer was diluted to 1× TBE. The 3% agarose gel was prepared using 300 ml of 1× TBE buffer, 4 µl of SYBR safe and 9 g agarose (Invitrogen, Carlsbad, CA). For gel electrophoresis, a mixture of 2 µl PCR product and 3 µl loading buffer was loaded on each well of the agarose gel, and the gel run in TBE buffer at 90 volts for about 20 min and then 120 volts for 2h. The gel image was scanned using Typhoon FLA 9500 scanner (GE Healthcare Bio-

Sciences AB, Piscataway, NJ). Based on polymorphism between the parents, 48 markers with even distribution throughout the nine C genome linkage groups (C1 to C9) were selected for genotyping the F_4 and BC_1F_3 plants.

Forty three F_4 and 46 BC_1F_3 plants were genotyped using a capillary ABI sequencer No. 3730 (Applied Biosystems, Foster City, CA). All SSR primers were labelled following the M13-tailing technique as described by Schuelke (2000). The forward primer of each SSR was appended with the universal M13 primer sequence 5′-CACGACGTTGTAAAACGAC-3′ labelled with fluorescent dyes FAM, VIC, NED and PET (Applied Biosystems, Foster City, CA).

3.2.4 Data analysis

The amplified marker fragments were scored for polymorphic bands using GeneMarker v2.4.0 (SoftGenetics) where a score of 1 was used for presence and 0 for absence of a band/fragment. A data matrix for the F_4 and BC_1F_3 plants of the two crosses was prepared for analysis.

The percentage of *B. oleracea* alleles present in a F_4 or BC_1F_3 plant was calculated based on the number of alleles of the *B. oleracea* parent detected in the plant divided by total number of alleles of this *B. oleracea* parent expected to be present in the population and multiplied by hundred.

Dice (Nei and Li 1979) genetic similarity coefficients were calculated between pairs of plants using the software program Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.2 Rohlf 2000), and the distances were calculated by subtracting the similarity scores from 1. A UPGMA cluster analysis was done to construct the dendrograms of F_4 and BC_1F_3 populations with the Tree plot module of the same software. Analysis of molecular

variance (AMOVA) was done using the software program Arlequin version 3.5 (Excoffier and Lischer 2010) to determine the extent of genetic differentiation exist among the populations. Significance was determined with 1023 permutations. The criteria proposed by Wright (1978) was used to categorize the genetic differentiation, where F_{st} value of 0 – 0.05 indicate little genetic difference, a value of 0.05 – 0.15 indicate moderate, 0.15 – 0.25 high, and a value of greater than 0.25 indicate very high difference (reviewed by Lopes et al. 2007).

3.3 Results

3.3.1 Introgression of alleles from *B. oleracea*

A total of 146 alleles were amplified by 48 polymorphic SSR markers from the C genome, and thus, the average number of alleles detected by a SSR marker was 3.04 (range 2.5 – 4.5) (Table 3.1). However, 75 of the total of 146 alleles were polymorphic between the *B. napus* and *B. oleracea* parents; thus the average number of *B. oleracea* alleles detected by these 48 SSR markers was 1.56 per SSR (Table 3.2). Of the 75 *B. oleracea* alleles, 27 were specific to var. *italica*, 38 were specific to var. *capitata*, and 10 alleles were common to both *B. oleracea* parents.

Among the nine C genome chromosomes, C6 showed greatest polymorphism as evident from the greater number of alleles per SSR marker. The number of alleles introduced from *B. oleracea* into F_4 and BC_1F_3 plants varied widely – ranged from 0 to 18 (Fig. 3.1, Table 3.2). Four F_4 plants of 5CA1358 lack *B. oleracea* var. *italica* alleles, while one plant of the same population carried 18 alleles of this *B. oleracea* parent. On average, the F_4 population carried greater proportion of *B. oleracea* alleles compared to the BC_1F_3 population, as expected. For example, the occurrence of *B. oleracea* var. *italica* alleles in the F_4 plants of *B. napus* × *B. oleracea* var.

italica cross varied from 0 to 48.6% of the total number of alleles detected in the *B. oleracea* parent with a mean of 18.2 ± 3.9% SE, and in case of BC_1F_3, it varied from 4.2 to 29.2% with a mean of 16.8 ± 2.0% SE (Table 3.2); however, the difference between the two populations for the average proportion of *B. oleracea* alleles was not statistically significant. Similar extent of introgression of *B. oleracea* var. *capitata* alleles was also found in the F_4 and BC_1F_3 populations of *B. napus* × *B. oleracea* var. *capitata* cross. Pooled data of the two crosses also showed that the F_4 population carried greater proportion of *B. oleracea* alleles compared to the BC_1F_3 populations; however, the difference was not statistically significant ($t = 1.06$, $p > 0.05$). Of the 10 alleles common to the two *B. oleracea* parents, only about 4.1% of these alleles were found in F_4 while 3.7% alleles found in BC_1F_3 population.

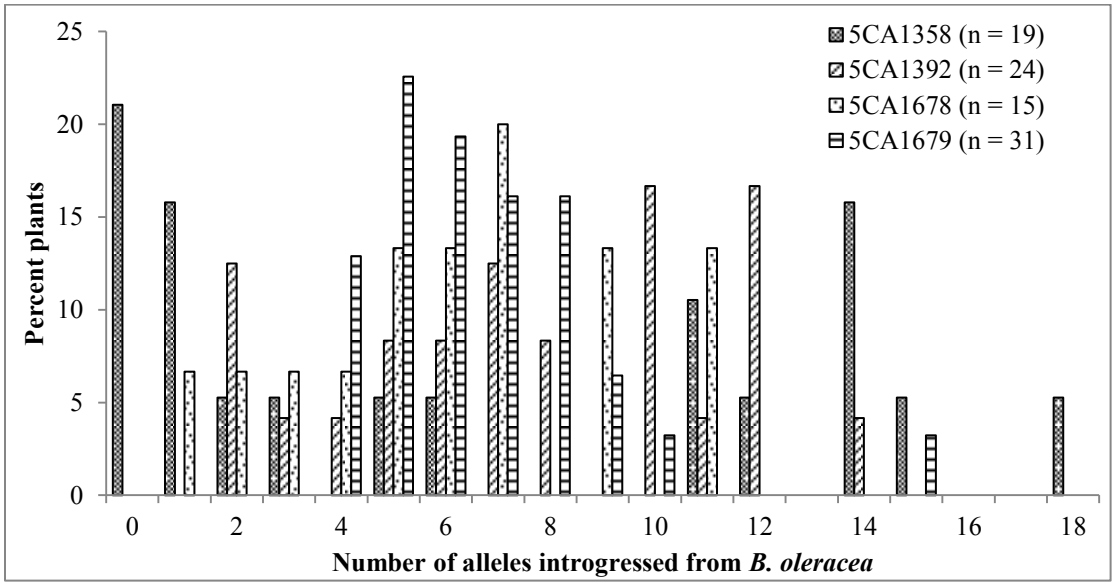

Fig. 3.1 Frequency distribution of F_4 and BC_1F_3 generation populations of *B. napus* × *B. oleracea* crosses for the number of alleles introgressed from *B. oleracea*. 5CA1358 = *B. napus* × *B. oleracea* var. *italica*; 5CA1392 = *B. napus* × *B. oleracea* var. *capitata*; 5CA1678 = (*B. napus* × *B. oleracea* var. *italica*) × *B. napus*; 5CA1679 = (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*.

Table 3.1 Occurrence of polymorphic alleles and alleles specific to *B. oleracea* in F_4 and BC_1F_3 population of *B. napus* × *B. oleracea* interspecific crosses detected by 48 SSR markers from nine C genome linkage groups.

Linkage group	No. SSR markers	Total no. alleles[1]	No. alleles/SSR	Total *B. oleracea* alleles	No. *B. oleracea* alleles/SSR
C1	5	15	3.0	8	1.6
C2	5	17	3.4	7	1.4
C3	4	10	2.5	5	1.3
C4	5	13	2.6	5	1.0
C5	9	25	2.8	15	1.7
C6	6	27	4.5	15	2.5
C7	6	16	2.7	7	1.2
C8	4	12	3.0	7	1.8
C9	4	11	2.8	6	1.5
Total	48	146	3.04	75	1.56

[1]Total number of alleles detected in *B. napus* and *B. oleracea* parents.

Table 3.2 Occurrence of *B. oleracea* alleles in F_4 and BC_1F_3 populations derived from two *B. napus* × *B. oleracea* interspecific crosses.

Cross[1]	Gen.	No. SSR	Total *B. oleracea* alleles[2]	No. plants	No. alleles/plant		% alleles/plant	
					Range	Mean ± SE	Range	Mean ± SE
a) Alleles detected in two *B. oleracea* parents								
B. nap × *B. o. ital*	F_4	48	37 (27)	19	0 - 18	6.7 ± 1.4	0.0 - 48.6	18.2 ± 3.9
	BC_1F_3	48	37 (27)	15	1 - 11	6.2 ± 0.8	2.7 - 29.7	16.8 ± 2.0
B. nap × *B. o. cap*	F_4	48	48 (38)	24	2 - 14	7.7 ± 0.7	4.2 - 29.2	16.1 ± 1.5
	BC_1F_3	48	48 (38)	31	4 - 15	6.6 ± 0.4	8.3 - 31.3	13.8 ± 0.8
Both crosses	F_4	48	75	43	0 - 18	7.3 ± 0.8	0.0 - 48.6	17.0 ± 1.9
	BC_1F_3	48	75	46	1 - 15	6.5 ± 0.4	2.7 - 31.3	14.7 ± 0.9
b) Alleles common to both *B. oleracea* parents								
Both crosses	F_4	48	10	43	0 - 6	1.7 ± 0.3	0.0 - 16.2	4.1 ± 0.7
	BC_1F_3	48	10	46	0 - 5	1.5 ± 0.2	0.0 - 13.5	3.7 ± 0.4

[1]*B. napus* line A04-73NA, *B. o. ital* = *B. oleracea* var. *italica* cv. Premium Crop, *B. o. cap* = *B. oleracea* var. *capitata* cv. Balbro.
[2]Number alleles specific to the *B. oleracea* parent given in brackets.

3.3.2 Genetic diversity among and within populations

Analysis of molecular variance (AMOVA) revealed that variation within the F_4 and BC_1F_3 populations accounted 86% of the total variation estimated in the whole population while only 14% of the total variation ($p = 0.001$) was accounted by the difference between the four populations of the two crosses (*B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata*) (Table 3.3). The F-statistic of 0.14 also indicates that moderate genetic difference existed among the four populations. Therefore, cluster analysis was done to further group the individuals of the F_4 and BC_1F_3 populations of the two crosses.

Table 3.3 Analysis of molecular variance (AMOVA) of the F_4 and BC_1F_3 populations derived from *B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata* crosses.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F_{st}
Among populations	3	242.49	2.91	14	0.14
Within populations	85	1493.32	17.57	86	
Total	88	1735.81	20.48	100	

Genetic similarity coefficient of the two *B. oleracea* parents var. *italica* cv. Premium Crop and var. *capitata* cv. Balbro was 0.238 and the coefficient of similarity of the two parents with the *B. napus* parent A04-73NA was 0.135 and 0.088, respectively. Cluster analysis showed that, the population derived from *B. napus* × *B. oleracea* var. *italica* cross could be divided into two distinct groups at a genetic similarity coefficient of 0.53 (Fig. 3.2). Group I included only five plants (F_Ita_2, F_Ita_4, F_Ita_5, F_Ita_6, and F_Ita_12), while Group II included 29 plants where similarity coefficients varied between 0.358 and 0.945. Four plants of this group (F_Ita_9, F_Ita_10, F_Ita_11, and F_Ita_13) were genetically close to the spring *B. napus* parent. Small grouping of the plants derived from *B. napus* × *B. oleracea* var. *capitata* can also be found. For example, three plants (F_Cap_28, F_Cap_42 and F_Cap_41) of this population were genetically

distinct from others and were close to the *B. oleracea* var. *capitata* cv. Balbro. In general, the BC_1 derived population clustered together and were closer to the *B. napus* parent compared the F_2 derived population in both crosses. Among the 89 analyzed plants, the plant F_Cap_42 showed lowest similarity (0.31) with *B. napus* A04-73NA.

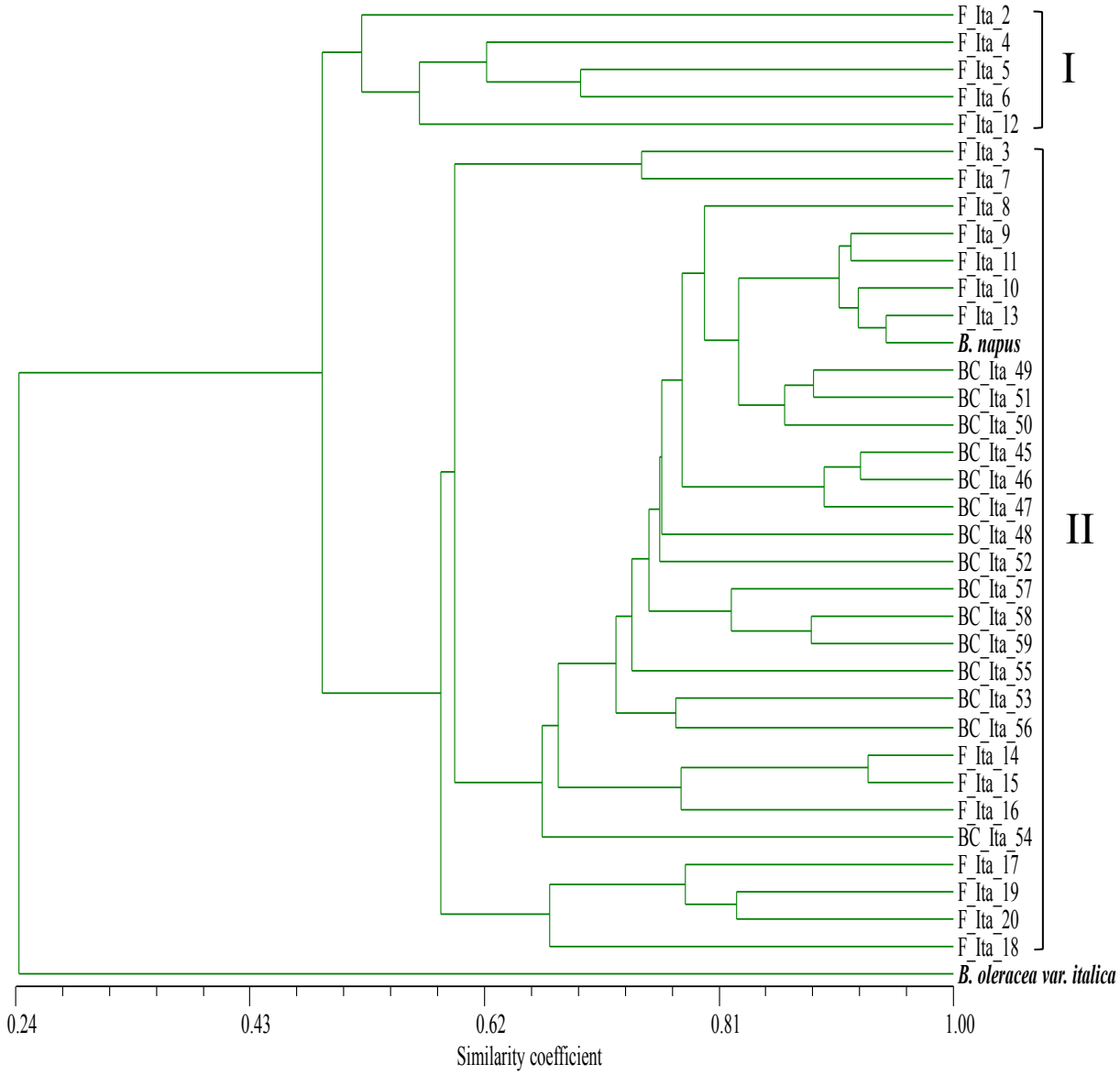

Fig. 3.2 Dendrogram showing genetic similarity among 19 F_4 and 15 BC_1F_3 plants derived from *B. napus* × *B. oleracea* var. *italica* interspecific cross as revealed by UPGMA clustering by use of 48 polymorphic SSR markers.

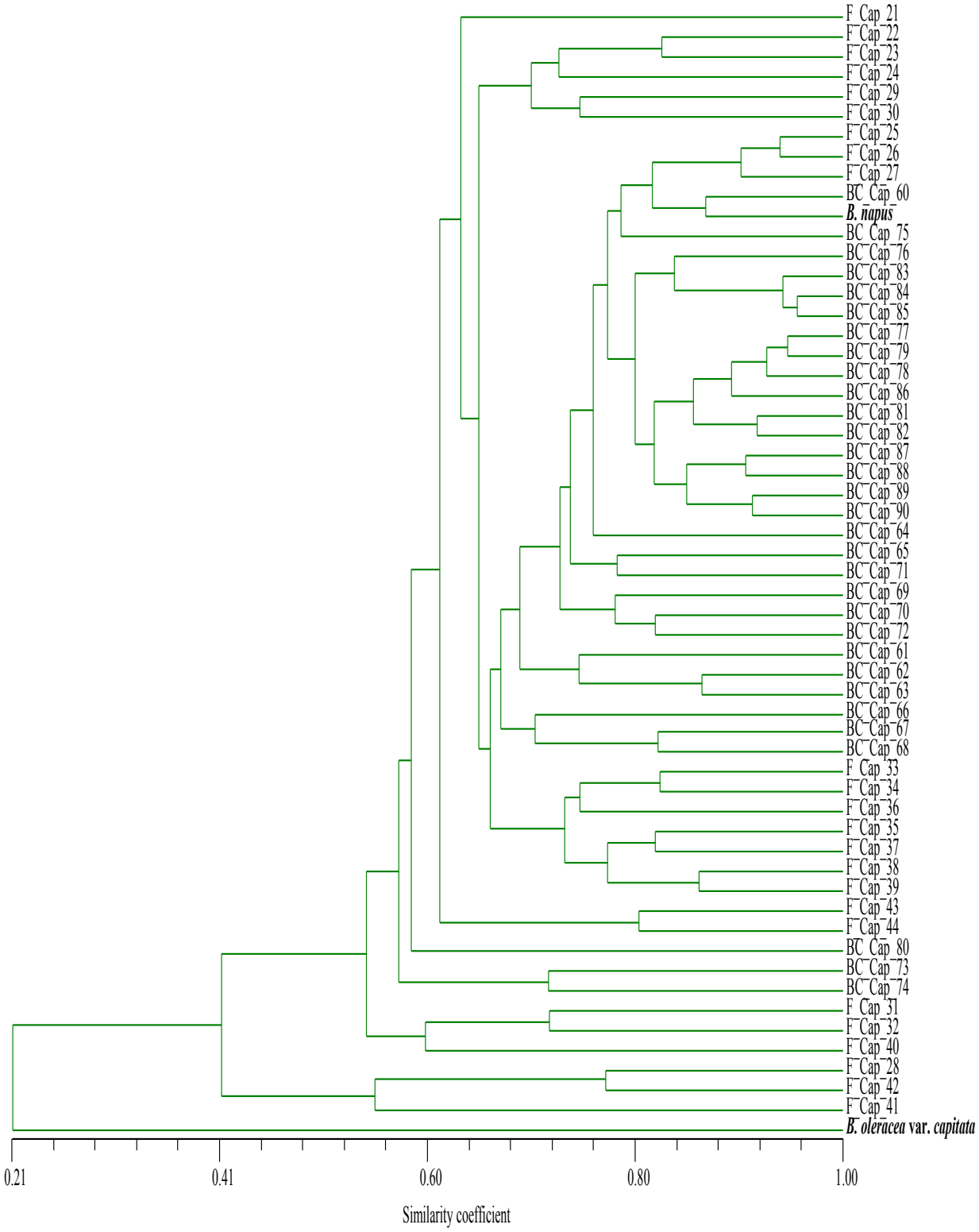

Fig. 3.3 Dendrogram showing genetic similarity among 24 F_4 and 31 BC_1F_3 plants derived from *B. napus* × *B. oleracea* var. *capitata* interspecific cross as revealed by UPGMA clustering by use of 48 polymorphic SSR markers.

3.4 Discussion

Several researchers indicated that allelic diversity in the C genome of *B. napus* is low when compared with its A genome (Bus et al. 2011; Wang et al. 2014; Snowdon et al. 2015). Research conducted to date has broadened allelic diversity in the A genome of *B. napus* to a greater extent than the C genome. This has been achieved through utilization of the diploid progenitor species *B. rapa* in breeding of *B. napus* (Qian et al. 2006; Chen et al. 2010) or through the use of resynthesized *B. napus* created from its progenitor species *B. rapa* and *B. oleracea* (Girke et al. 2012a; Jesske et al. 2013). However, limited studies have been conducted to broaden the genetic base of the C genome of spring type *B. napus* canola by the use of its diploid progenitor *B. oleracea* (Bennett et al. 2012; Rahman et al. 2015). Also, these research projects used only one variant of *B. oleracea* despite vast diversity exists in this species (Song et al. 1990; Lu et al. 2009).

In this study, one *B. oleracea* var. *italica* (broccoli) and one *B. oleracea* var. *capitata* (cabbage) were used to introduce exotic alleles into Canadian spring *B. napus* canola. These two variants of *B. oleracea* known to be genetically distinct (Song et al. 1990).Results from this study showed that allelic variation from these two variants of *B. oleracea* can be introgressed into spring *B. napus* canola through interspecific hybridization between the two species.

The average proportion of the *B. oleracea* alleles introgressed into the F_4 and BC_1F_3 population was 17.0% and 14.7%, respectively; however, a F_4 plant found to carry as high as 48.6% of the *B. oleracea* alleles. In a similar study using *B. oleracea* var. *alboglabra*, Rahman et al. (2015) found that an advanced generation family derived from F_2 of *B. napus* × *B. oleracea* cross can carry up to 53.8% of the *B. oleracea* alleles; however, the average proportion of *B.*

oleracea alleles in this population was 19.0%, which is very similar to the results of my study. The reason of slightly lower introgression of *B. oleracea* alleles in the present study was probably for the reason that the C genome has gone through intensive selection in the earlier generations for the canola quality traits. Rahman et al. (2015) also found lower number (10% of the total number) of alleles in canola quality lines compared to the lines having more than 30 µmol glucosinolate per gram seeds (27.3% of the total number of alleles).

In conclusion, results from this study demonstrated that genetic diversity in Canadian spring *B. napus* canola can be broadened though interspecific hybridization with the diploid species *B. oleracea* var. *italica* and var. *capitata*, and the materials from this study can be used in breeding for broadening of the genetic base of Canadian canola.

Chapter 4
General discussion and conclusions

4.1 General discussion

B. napus canola is a relatively young but an important oilseed crop in the world. This species originated from a recent polyploidization event and domesticated about 400 years ago (Trick et al. 2009; Gómez-Campo and Prakash 1999). This short domestication history and reproductive isolation of this species from its progenitor species *B. rapa* and *B. oleracea* partly responsible for the narrow genetic base seen today in its germplasm, and increased genetic variation found between the genomes of *B. napus* and its two progenitor species (Becker et al. 1995; Thormann et al. 1994; Cooper et al. 2001). Therefore, researchers put efforts to broaden the genetic base of *B. napus* canola through utilization of exotic germplasm of *B. napus* (Udall et al. 2006; Kebede et al. 2010) as well as its allied species *B. rapa* and *B. oleracea* (reviewed in Rahman 2013). Of the two parental species of *B. napus*, *B. rapa* has been used for broadening the genetic base of oilseed *B. napus* germplasm, for example, the Chinese semi-winter type *B. napus* (Qian et al. 2006). This is probably due to the reason that interspecific hybrids of *B. napus* × *B. rapa* can easily be obtained through sexual hybridization (Downey et al. 1980) while it is quite difficult in case of *B. napus* × *B. oleracea* cross (Downey et al. 1980; Bennett et al. 2008). Thus, genetic diversity of the A genome in *B. napus* has been enriched to some extent; however, this found to be narrow in the C genome of *B. napus* (Bus et al. 2011). Some researchers have also resynthesized *B. napus* from *B. rapa* × *B. oleracea* interspecific crosses for utilization of allelic diversity of the two parental species for the improvement of *B. napus* (Schranz and Osborn 2000; Seyis et al. 2003; Girke et al. 2012a; Jesske et al. 2013). However, interspecific

hybridization between *B. oleracea* and *B. napus* has barely been done for introgression of genome components of the C genome of *B. oleracea* into the C genome of *B. napus*.

B. oleracea is mostly grown as vegetable crop where morphologically different types have been developed for different uses. The cultivated *B. oleracea* includes kale (var. *acephala*), cabbage (var. *capitata*), kohlrabi (var. *gongylodes*), inflorescence kale (var. *botrytis*, var. *italica*), branching bush kale (var. *fruticosa*), and Chinese kale (var. *alboglabra*) (reviewed by Prakash 2012). However, only a few variants of *B. oleracea* have been used for the improvement of oilseed *B. napus*. For example, Rahman et al. (2011, 2015) introgressed earliness of flowering and allelic diversity from Chinese kale into Canadian spring *B. napus*. Li et al. (2014) introgressed genome components of *B. oleracea* var. *acephala* into Chinese semi-winter *B. napus* for increasing seed yield in hybrids cultivars. In the present research, *B. oleracea* var. *italica* and var. *capitata* were used to widen the genetic base of spring *B. napus* canola through interspecific hybridization between these two species.

Several challenges encountered in the present study for the development of canola quality euploid ($2n = 38$) *B. napus* lines from *B. napus* × *B. oleracea* interspecific crosses. First, it was difficult to produce F_1 hybrids from *B. napus* × *B. oleracea* crosses. Second, high sterility was observed in the interspecific hybrid progenies, especially in the early generations, and high erucic acid and glucosinolate alleles were introduced into the interspecific hybrid progenies from the *B. oleracea* parents. Several researchers also faced extreme difficulty to obtain hybrids from *B. napus* × *B. oleracea* interspecific crosses (Quazi 1988; Bennett et al. 2008). To overcome this obstacle, researchers have applied different embryo rescue techniques, such as embryo culture (Zhang et al. 2003; Rahman

2004) and ovule culture (Bennett et al. 2008), to produce hybrid plants of this interspecific cross. In this research, *in vitro* ovule culture technique was employed which found to be quite effective for production of *B. napus* × *B. oleracea* interspecific hybrids.

The hybrid progenies of both interspecific crosses showed high sterility in early generations as evident from poor silique and seed set (Table 2.6). Quazi (1988) also found high sterility in interspecific hybrid plants derived from *B. napus* × *B. oleracea* cross. Sterility in early generations of *B. napus* × *B. oleracea* interspecific cross generally arise from abnormal paring of homologous or homoeologous chromosomes in meiosis (Chiang et al. 1980; Mason et al. 2010), and therefore, aneuploid plants can occur at a high frequency. However, Rahman et al. (2011, 2015) found that self-pollination of *B. napus* × *B. oleracea* var. *alboglabra* plants stabilizes into *B. napus* type; this was also evident in the present study where the advanced generation plants (F_8 and BC_1F_7) developed through self-pollination showed high fertility as well as had nuclear DNA content similar to the *B. napus* parent (Table 2.9).

It is well established that a major gene locus is involved in the control of erucic acid in the C genome (Chen et al. 1988; Rahman et al. 2001) and multiple gene loci are involved in the control of GSL content (Rahman et al. 2001; Howell et al. 2003). In this regard, a simple Mendelian inheritance for erucic acid content was expected in F_2 and BC_1 populations. However, strong segregation distortion for erucic acid alleles was found in both populations of both crosses. This is apparently due to variable chromosome composition and differential viability of the gametes as has been suggested by Rahman et al. (2015) based on a study with an F_2 population of *B. napus* × *B. oleracea* var. *alboglabra* cross. However, selection for low

erucic acid and low GSL content was effective in both F_2 and BC_1 derived populations of both crosses.

In the present study, two variants of *B. oleracea*, var. *italica* and var. *capitata*, were used to introduce exotic alleles in Canadian spring *B. napus*. These two variants of *B. oleracea* known to be genetically distinct (Song et al. 1990).The results of genotypic analysis of the populations derived from two crosses also confirmed this. Of the total 75 SSR alleles, 27 (36%) were specific to var. *italica*, 38 (50.7%) specific to var. *capitata*, while only 10 (13.3%) were common to both *B. oleracea* parents. This suggests that allelic variation from these two variants of *B. oleracea* can be introgressed into spring *B. napus* canola through interspecific hybridization between the two species. Li et al. (2014) and Rahman et al. (2015) also reported introgression genome component of *B. oleracea* var. *acephala* and var. *alboglabra* into *B. napus*.

The reconstructed *B. napus* lines derived from interspecific crosses often show heterotic potential for seed yield. For instance, Zou et al. (2010) found high heterosis for seed yield in hybrids produced by the use of reconstituted *B. napus* lines derived from *B. napus* × *B. rapa* interspecific cross. Li et al. (2014) developed semi-winter *B. napus* lines through reconstitution of its C genome with the C genome of *B. oleracea* var. *acephala* and found that the hybrids exhibit heterosis for seed yield. This suggests that the lines derived from the present study may show potential for increasing seed yield in hybrid spring canola cultivars.

4.2 Conclusions

The following conclusions were drawn from this thesis research:

- Canola quality (< 1% erucic acid content in seed oil and < 15 μmol glucosinolate per gram of seed meal) euploid *B. napus* ($2n = 38$) lines can be achieved from both F_2 and

BC_1 derived populations of *B. napus* × *B. oleracea* var. *italica* and *B. napus* × *B. oleracea* var. *capitata* interspecific crosses.

- Low plant fertility in early generations suggests the occurrence of aneuploid plants resulting from anomalies in meiosis; however, plant fertility improved in advanced generation populations developed through self-pollination. Chromosome number of these plants was close to *B. napus* as demonstrated by flow cytometric analysis of F_8 and BC_1F_7 generation populations.
- Segregation for erucic acid and GSL contents involved segregation of the C genome alleles for these two traits; therefore, selection for low erucic acid and low GSL lines was quite effective in both F_2 and BC_1 derived populations.
- Genomic contents of *B. oleracea* var. *italica* and var. *capitata* were detected in the progeny derived from both *B. napus* × *B. oleracea* interspecific crosses by use of SSR markers. This revealed that allelic variation from the two *B. oleracea* variants can be introduced into spring *B. napus* canola to broaden the genetic base of this crop.
- Earliness of flowering exhibited partial dominance effect over late flowering. This suggests that early flowering hybrids can be produced through the use of early flowering *B. napus* lines derived from *B. napus* × *B. oleracea* interspecific crosses.

4.3 Future research

The reconstructed *B. napus* lines developed from *B. napus* × *B. oleracea* interspecific crosses need to be evaluated for agronomic performance. These lines also need to be evaluated for heterotic potential in test hybrids with the *B. napus* parent A04-73NA for identification of the genomic regions contributing to heterosis. Furthermore, heterosis potential of these lines also needs to be evaluated through crossing with other *B. napus* lines to identify general and specific

combining ability of these lines. It is also probable that the reconstituted *B. napus* lines derived from *B. napus* × *B. oleracea* interspecific crosses carry several undesired alleles introduced from *B. oleracea*; in this case, performance of these lines need to be improved through crossing with the same and/or other elite lines and selection for genome contents introgressed from *B. oleracea*.

References

Abdel-Mawgood AL (2012) DNA-based techniques for studying genetic diversity. In: Genetic Diversity in Microorganisms (edited by Mahmut C). In Tech, Croatia, pp 95-122

Ackman RG (1990) Canola fatty acids – an ideal mixture for health, nutrition, and food use. In: Canola and Rapeseed: Production, Chemistry, Nutrition, and Processing Technology (edited by Shahidi F). Van Nostrand Reinhold, New York, pp 81-98

Adams KL, Wendel JF (2005) Polyploidy and genome evolution in plants. Current Opinion in Plant Biology 8:135-141

Ahuja I, Rohloff J, Bones AM (2010) Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management. A review. Agronomy for Sustainable Development 30: 311-348

Alemayehu N, Becker H (2002) Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (*Brassica carinata* A. Braun). Genetic Resources and Crop Evolution 49:573-582

Ali M, Copeland LO, Elias SG (1995) Relationship between genetic distance and heterosis for yield and morp hological traits in winter canola (*Brassica napus* L.). Theoretical and Applied Genetics 91:118-121

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

Becker HC, Engquvist GM, Karlsson B (1995) Comparison of rapeseed cultivars and re-synthesized lines based on allozyme and RFLP markers. Theoretical and Applied Genetics 91:62-67

Bednarek P, Pislewska-Bednarek M, Svatos A, Schneider B, Doubsky J, Mansurova M, Humphry M, Consonni C, Panstruga R, Sanchez-Vallet A, Molina A, Schulze-Lefert P (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science 323:101-106

Bell JM (1993) Factors affecting the nutritional value of canola meal: A review. Canadian Journal of Animal Science 73:679-697

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

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

Bennett RA, Séguin-Swartz G, Rahman H (2012) Broadening Genetic Diversity in Canola Using the C-Genome Species *Brassica oleracea* L. Crop Science 52: 2030-2039

Brown PTH (1992) DNA fingerprinting in plant breeding. Agro-Food-Industry Hi-Tech 6:8-11

Bus A, Körber N, Snowdon RJ, Stich B (2011) Patterns of molecular variation in a species-wide germplasm set of *Brassica napus*. Theoretical and Applied Genetics 123:1413-1423

Butruille DV, Guries RP, Osborn TC (1999) Increasing yield of spring oilseed rape hybrids through introgression of winter germplasm. Crop Science 39:1491-1496

Campbell LD (1987) Intact glucosinolates and glucosinolate hydrolysis products as causative agents in liver hemorrhage in laying hens. Canadian Journal of Animal Science 36:491-496

Canadian Agri-food Trade Allicance (CAFTA) (2013), Canola. Retrieved from http://cafta.org/pages/agri-food-exports/canola/ (Accessed: 20 November 2015)

Chalhoub B et al. (2014) Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science 345:950-953

Charlton KM, Corner AH, Davey K, Kramer JK, Mahadevan S, Sauer FD (1975) Cardiac lesions in rats fed rapeseed oils. Canadian Journal of Comparative Medicine 39:261-269

Chen BY, Heneen WK, Jönsson R (1988) Independent Inheritance of Erucic Acid Content and Flower Colour in the C-Genome of *Brassica napus* L. Plant Breeding 100:147-149

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

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

Chen X, Sullivan P (2003) Single nucleotide polymorphism genotyping: biochemistry, protocol, cost and throughput. The Pharmacogenomics Journal 3:77-96

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

Chiang B, Chiang M, Grant W, Crete R (1980) Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* spp. *capitata*). IV. A resistant 18-chromosome B1 plant and its B2 progenies. Euphytica 29:47-55

Ciancaleoni S, Chiarenza GL, Raggi L, Branca F, Negri V (2014) Diversity characterisation of broccoli (*Brassica oleracea* L. var. *italica* Plenck) landraces for their on-farm (*in situ*) safeguard and use in breeding programs. Genetic Resources and Crop Evolution 61:451-464

Cooper HD, Spillane C, Hodgkin T (2001) Broadening the genetic base of crops: an overview. In: Broadening the Genetic Base of Crop Production (edited by Cooper HD, Spillane C, Hodgkin T). IPGRI and FAO, CABI publishing, Wallingford, Oxon, UK, pp 1-23

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 Crops Research 104:103-111

Crisp P, Crute I, Sutherland R, Angell S, Bloor K, Burgess H, Gordon P (1989) The exploitation of genetic resources of *Brassica oleracea* in breeding for resistance to clubroot (*Plasmodiophora brassicae*). Euphytica 42:215-226

Delourme R, Chèvre AM, Bran H, Rouxel T, Balesdent M-H, Dias JS et al. (2006) Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). European Journal of Plant Pathology 114:41-52

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

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

Dixon GR (2007) Vegetable Brassicas and related crucifers. CABI, Willingford, UK

Downey RK, Klassen AJ, Stringam GR (1980) Rapeseed and mustard. In: Hybridization of Crop Plants (edited by Fehr WR, Hadley HH). ASA, CSSA, Madison, WI, pp 495-509

Duvick DN (1999) Heterosis: feeding people and protecting natural resources. In: The Genetics and Exploitation of Heterosis in Crops (Coors JG, Pandey S). ASSA/CSSA/SSSA, Madison, WI, pp 19-29

European Food Safety Authority (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. The EFSA Journal 590:1-76

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567

Fabrizius MA, Bush RH, Khan K, Huckle L (1998) Genetic diversity and heterosis of spring wheat crosses. Crop Science 38:1108-1112

Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocynanates among plants. Phytochemistry 56:5-51

Fenwick GR (1982) The assessment of a new protein source—Rapeseed. Proceedings of the Nutrition Society 41:277-288

Ferreira ME, Satagopan J, Yandell BS, Williams PH, Osborn TC (1995) Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theoretical and Applied Genetics 90: 727-732

Flint-Garcia SA, Thornsberry JM, IV B (2003) Structure of linkage disequilibrium in plants*. Annual Review of Plant Biology 54: 357-374

Food and Agriculture Organization of the United Nations (FAO) (2013). Retrieved from http://faostat3.fao.org/download/Q/QC/E (Accessed: 16 January 2016)

Ford BA, Ernest JR, Gendall AR (2012) Identification and characterization of orthologs of *AtNHX5* and *AtNHX6* in *Brassica napus*. Frontiers in Plant Science 3

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

Ganal MW, Polley A, Graner E-M, Plieske J, Wieseke R, Luerssen H, Durstewitz G (2012) Large SNP arrays for genotyping in crop plants. Journal of Biosciences 37:821-828

Ghaderi A, Adams MW, Nassib AM (1984) Relationship between genetic distance and heterosis for yield and morphological traits in dry edible bean and faba bean. Crop Science 24:37-42

Girke A, Schierholt A, Becker HC (2012a) Extending the rapeseed genepool with resynthesized *Brassica napus* L. I: Genetic diversity. Genetic Resources and Crop Evolution 59:1441-1447

Girke A, Schierholt A, Becker HC (2012b) Extending the rapeseed gene pool with resynthesized *Brassica napus* II: Heterosis. Theoretical and Applied Genetics 124:1017-1026

Gómez-Campo C, Prakash S (1999) Origin and domestication. Biology of Brassica Coenospecies:33-58

Griffing B, Linstrom EW (1954) A study of the combining ability of corn inbreds having varying proportions of corn belt and noncorn belt germplasm. Agronomy Journal 46:545-552

Hamblin MT, Warburton ML, Buckler ES (2007) Empirical comparison of simple sequence repeats and single nucleotide polymorphisms in assessment of maize diversity and relatedness. PloS one 2:e1367

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

Hasan M, Seyis F, Badani AG, Kuhnemann JP, Lühs W, Friedt W, Snowdon RJ (2006) Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genetic Resources and Crop Evolution 53:793-802

Hasan MJ, Strelkov SE, Howard RJ, Rahman H (2012) Screening of *Brassica* germplasm for resistance to *Plasmodiophora brassicae* pathotypes prevalent in Canada for broadening diversity in clubroot resistance. Canadian Journal of Plant Science 92:501-515

Heijkenskjold L, Ernster L (1975) Studies of the mode of action of erucic acid on heart metabolism. Acta Medica Scandinavica 198:75-83

Hopkins RJ, van Dam NM, van Loon JJA (2009) Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annual Review of Entomology 54:57-83

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

Iftikhar R (2015) Broadening genetic diversity in spring canola (*Brassica napus* L.) by the use of the C-genome of *B. oleracea* var. *alboglabra* and *B. oleracea* var. *botrytis*. MSc. thesis. University of Alberta, Edmonton, Canada

Iniguez-Luy FL, Federico ML (2011) The genetics of *Brassica napus* L. In: Genetics and Genomics of the Brassicaceae (edited by Bancroft I, Schmidt R). Springer, New York, Dordrecht, Heidelberg, London, pp 291-322

Jain A, Bhatia S, Banga SS, Prakash S, Lakshmikumaran M (1994) Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. Theoretical and Applied Genetics 88:116-122

Jesske T, Olberg B, Schierholt A, Becker HC (2013) Resynthesized lines from domesticated and wild *Brassica* taxa and their hybrids with *B. napus* L.: genetic diversity and hybrid yield. Theoretical and Applied Genetics 126:1053-1065

Josefsson E, Appelqvist L-A (1968) Glucosinolates in seed of rape and turnip rape as affected by variety and environment. Journal of the Science of Food and Agriculture 19:564-570

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

Kesseli RV, Paran I, Michelmore RW (1994) Analysis of a detailed genetic linkage map of *Lactuca sativa* (lettuce) constructed from RFLP and RAPD markers. Genetics 136:1435-1446

Khajali F, Slominski BA (2012) Factors that affect the nutritive value of canola meal for poultry. Poultry Science 91:2564-2575

Kianian SF, Quiros CF (1992) Trait inheritance, fertility, and genomic relationships of some n= 9 *Brassica* species. Genetic Resources and Crop Evolution 39:165-175

Labana KS, Gupta ML (1993) Importance and Origin. In: Breeding Oilseed Brassicas (edited by Labana KS, Banga SS, Banga SK). Springer-Verlag, Berlin, Germany, pp 1-7

Levy AA, Feldman M (2004) Genetic and epigenetic reprogramming of the wheat genome upon allopolyploidization. Biological Journal of the Linnean Society 82:607-613

Li F, Chen B, Xu K, Wu J, Song W, Bancroft I, Harper AL, Trick M, Liu S, Gao G (2014) Genome-wide association study dissects the genetic architecture of seed weight and seed quality in rapeseed (*Brassica napus* L.). DNA Research 21:355-367

Li H, Chen X, Yang Y, Xu J, Gu J, Fu J, Qian X, Zhang S, Wu J, Liu K (2011) Development and genetic mapping of microsatellite markers from whole genome shotgun sequences in *Brassica oleracea*. Molecular Breeding 28:585-596

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

Li M, Chen X, Meng J (2006) Intersubgenomic heterosis in rapeseed production with a partial new-typed *Brassica napus* containing subgenome A^r from *B. rapa* and C^c from *Brassica carinata*. Crop Science 46:234-242

Li Q, Mei J, Zhang Y, Li J, Ge X, Li Z, Qian W (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*. Theoretical and Applied Genetics 126:2073-2080

Li Q, Zhou Q, Mei J, Zhang Y, Li J, Li Z, Ge X, Xiong Z, Huang Y, Qian W (2014) Improvement of *Brassica napus* via interspecific hybridization between *B. napus* and *B. oleracea*. Molecular Breeding 34:1955-1963

Liu H (2000) Genetic and breeding in rapeseed. Chinese Agricultural University, Beijing, pp 182-188

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

LMC International (2013) The Economic Impact of Canola on the Canadian Economy. Report for Canola Council of Canada Winnipeg, MB. Retrieved from http://www.canolacouncil.org/media/545722/lmc_economic_impact_of_canola_on_the_canadian_economy_october_2013.pdf (Accessted: 20 November 2015)

Lopes CM, Almeida FSd, Orsi ML, Britto SGdC, Sirol RN, Sodré LMK (2007) Fish passage ladders from Canoas Complex-Paranapanema River: evaluation of genetic structure maintenance of *Salminus brasiliensis* (Teleostei: Characiformes). Neotropical Ichthyology 5:131-138

Lu X, Liu L, Gong Y, Zhao L, Song X, Zhu X (2009) Cultivar identification and genetic diversity analysis of broccoli and its related species with RAPD and ISSR markers. Scientia Horticulturae 122:645-648

Lu J, Knox MR, Ambrose MJ, Brown JKM, Ellis THN (1996) Comparative analysis of genetic diversity in pea assessed by RFLP- and PCR-based methods. Theoretical and Applied Genetics 93:1103-1111

Lu X, Liu L, Gong Y, Zhao L, Song X, Zhu X (2009) Cultivar identification and genetic diversity analysis of broccoli and its related species with RAPD and ISSR markers. Scientia Horticulturae 122:645-648

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

Mahmood T, Ekuere U, Yeh F, Good AG, Stringam GR (2003) RFLP linkage analysis and mapping genes controlling the fatty acid profile of *Brassica juncea* using reciprocal DH populations. Theoretical and Applied Genetics 107:283-290

Makumbi D, Betrán JF, Bänziger M, Ribaut JM (2011) Combining ability, heterosis and genetic diversity in tropical maize (*Zea mays* L.) under stress and non-stress conditions. Euphytica 180:143-162

Mason AS, Huteau V, Eber F, Coriton O, Yan G, Nelson MN, Cowling WA, Chèvre A-M (2010) Genome structure affects the rate of autosyndesis and allosyndesis in AABC, BBAC and CCAB Brassica interspecific hybrids. Chromosome Research 18:655-666

Matusheski NV, Swarup R, Juvik JA, Mithen R, Bennett M, Jeffery EH (2006) Epithiospecifier protein from broccoli (*Brassica oleracea* L. ssp. *italica*) inhibits formation of the anticancer agent sulforaphane. Journal of Agricultural and Food Chemistry 54:2069-2076

Mawson R, Heaney RK, Zdunczyk Z, Kozlowska H (1994) Rapeseed meal-glucosinolates and their antinutritional effects Part 4. Goitrogenicity and internal organs abnormalities in animals. Die Nahrung 38:178-191

McDanell R, McLean AEM, Hanley AB, Heaney RK, Fenwick GR (1988) Chemical and biological properties of indole glucosinolates (glucobrassicins): a review. Food and Chemical Toxicology 26:59-70

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

McInnis M (2004) The Transformation of Rapeseed Into Canola: A Cinderella Story. The Saskatchewan Story exhibit, North Battleford WDM

Mei J, Li Q, Yang X, Qian L, Liu L, Yin J, Frauen M, Li J, Qian W (2010) Genomic relationships between wild and cultivated *Brassica oleracea* L. with emphasis on the origination of cultivated crops. Genetic Resources and Crop Evolution 57:687-692

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

Negi MS, Sabharwal V, Bhat SR, Lakshmikumaran M (2004) Utility of AFLP markers for the assessment of genetic diversity within *Brassica nigra* germplasm. Plant Breeding 123:13-16

Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76:5269-5273

Newkirk RW (2009) Canola meal feed industry guide. 4th ed. Publication of Canola Council of Canada, Winnipeg, MB, Canada

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology 13:1143-1155

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

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

Osborn TC, Pires JC, Birchler JA, Auger DL, Chen ZJ, Lee H-S, Comai L, Madlung A, Doerge RW, Colot V et al. (2003) Understanding mechanisms of novel gene expression in polyploids. Trends in Genetics 19:141-147

Panjabi P, Jagannath A, Bisht NC, Padmaja KL, Sharma S, Gupta V, Pradhan AK, Pental D (2008) Comparative mapping of *Brassica juncea* and Arabidopsis thaliana using Intron Polymorphism (IP) markers: homoeologous relationships, diversification and evolution of the A, B and C *Brassica* genomes. BMC Genomics 9:113

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

Parkin IAP, Lydiate DJ, Trick M (2002) Assessing the level of collinearity between *Arabidopsis thaliana* and *Brassica napus* for *A. thaliana* chromosome 5. Genome 45:356-366

Perenzin M, Corbellini M, Accerbi M, Vaccino P, Borghi B (1998) Bread wheat: F1 hybrid performance and parental diversity estimates using molecular markers. Euphytica 100:273–279

Pires JC, Zhao J, Schranz ME, Leon EJ, Quijada PA, Lukens LN, Osborn TC (2004) Flowering time divergence and genomic rearrangements in resynthesized *Brassica* polyploids (Brassicaceae). Biological Journal of the Linnean Society 82:675-688

Plaschke J, Ganal MW, Roeder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theoretical and Applied Genetics 91:1001-1007

Prakash S, Wu XM, Bhat S (2012) History, evolution, and domestication of Brassica crops. Plant Breeding Reviews, Volume 35:19-84

Przybylski R (2001) Canola Oil: Physical and Chemical Properties. Retrieved from http://www.canola-council.org/pubs/physprop.htm (Accessed: 27 October 2015)

Qian L, Qian W, Snowdon RJ (2014) Sub-genomic selection patterns as a signature of breeding in the allopolyploid *Brassica napus* genome. BMC Genomics 15:1170

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

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

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

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

Rahman H, Rahman L, Stolen O, Sorensen H (1994) Inheritance of Erucic Acid Content in Yellow-and White-Flowered Yellow Sarson × Canadian *Brassica campestris* L. Acta Agriculturae Scandinavica B-Plant Soil Sciences 44:94-97

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

Rahman H (2004) Optimum age of siliques for rescue of hybrid embryos from crosses between *Brassica oleracea*, *B. rapa* and *B. carinata*. Canadian Journal of Plant Science 84:965-969

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

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

Rahman H (2013) Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic germplasm. Canadian Journal of Plant Science 93:363-373

Rahman H, Harwood J, Weselake R (2013) Increasing seed oil content in *Brassica* species through breeding and biotechnology. Lipid Technology 25:182-185

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

Rahman H, Bennett RA, Séguin-Swartz G (2015) Broadening genetic diversity in *Brassica napus* canola: Development of canola-quality spring *B. napus* from *B. napus* × *B. oleracea* var. *alboglabra* interspecific crosses. Canadian Journal of Plant Science 95:29-41

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

Raymer PL (2002) Canola: An emerging oilseed crop. In: Trends in New Crops and New Uses (edited by Janick J and Whipkey A). ASHS Press, Alexandria, VA, pp 122-126

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

Ringdahl E, McVetty P, Sernyk J (1986) Inheritance of earliness, height, and leaf number in crosses of early maturing rapeseed. Canadian Journal of Genetics and Cytology 28:1009-1015

Rohlf FJ (2000) NTSYS-pc numerical taxonomy and multivariate analysis system. Exeter Software, New York

Rutkowski A (1971) The feed value of rapeseed meal. Journal of the American Oil Chemists' Society 48:863-868

Scarth R, Tang J (2006) Modification of oil using conventional and transgenic approaches. Crop Science 46:1225-1236

Schnable JC, Springer NM, Freeling M (2011) Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss. Proceedings of the National Academy of Sciences 108:4069-4074

Schranz M, Osborn T (2000) Novel flowering time variation in the resynthesized polyploid *Brassica napus*. Journal of Heredity 91:242-246

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

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18: 233-234

Semagn K, Bjørnstad A, Ndjiondjop M (2006) An overview of molecular marker methods for plants. African Journal of Biotechnology 5: 2540-2568

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

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

Sheehan J, Camobreco V, Duffield J, Graboski M, Shapouri H (1998) Life cycle inventory of biodiesel and petroleum diesel for use in an urban bus. Final Report, National Renewable Energy Laboratory

Slominski BA, Campbell LD, Guenter W (1994) Carbohydrates and dietary fiber components of yellow-and brown-seeded canola. Journal of Agricultural and Food Chemistry 42:704-707

Smyth SJ, Gusta M, Belcher K, Phillips PWB, Castle D (2011) Environmental impacts from herbicide tolerant canola production in Western Canada. Agricultural Systems 104:403-410

Snogerup S (1980) The wild forms of the *Brassica oleracea* group (2*n* = 18) and their possible relations to the cultivated ones. In: Brassica Crops and Wild Allies: Biology and Breeding (Tsunoda S, Hinata K, Gómez-Campo C). Japanese Science Society Press, Tokyo, pp 121–132

Snowdon RJ, Abbadi A, Kox T, Schmutzer T, Leckband G (2015) Heterotic Haplotype Capture: precision breeding for hybrid performance. Trends in Plant Science 20:410-413

Song K, Osborn TC, Williams PH (1990) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 3. Genome relationships in *Brassica* and

related genera and the origin of *B. oleracea* and *B. rapa* (syn. *campestris*). Theoretical and Applied Genetics 79:497-506

Statista (2015) Worldwide oilseed production from 2008/2009 to 2014/2015. Retrieved from http://www.statista.com/statistics/267271/worldwide-oilseed-production-since-2008 (Accessed: 21 August 2015)

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

Teutonico R, Osborn T (1995) Mapping loci controlling vernalization requirement in *Brassica rapa*. Theoretical and Applied Genetics 91:1279-1283

Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994) Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theoretical and Applied Genetics 88: 973-980

Traka M, Mithen R (2009) Glucosinolates, isothiocyanates and human health. Phytochemistry Reviews 8:269-282

Trick M, Long Y, Meng J, Bancroft I (2009) Single nucleotide polymorphism (SNP) discovery in the polyploid *Brassica napus* using Solexa transcriptome sequencing. Plant Biotechnology Journal 7:334-346

Truco MJ, J Hu, Sadowski J, Quiros CF (1996) Inter- and tance gene in *Brassica* and *Arabidopsis*. Proceeding of the National Academy of Sciences 95:15843-15848

Töpfer R, Martini N, Schell J (1995) Modification of plant lipid synthesis. Science 268:681-686

U Nagaharu (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilisation. Journal of Japanese Botany 7:389-452

Udall JA, Quijada PA, Polewicz H, Vogelzang R, Osborn TC (2004) Phenotypic effects of introgressing chinese winter and resynthesized *Brassica napus* L. germplasm into hybrid spring canola. Crop science 44:1990-1996

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

United States Department of Agriculture (USDA), (2015a) Global consumption of vegetable oils from 1995/1996 to 2014/2015. Retrieved from http://www.statista.com/statistics/263937/vegetable-oils-global-consumption (Accessed: 6 August 2015)

United States Department of Agriculture (USDA), (2015b) Canada oilseeds and products annual (FAS GAIN Report CA15032). Washington, DC: US Department of Agriculture

van Dijk SJ, Feskens EJ, Bos MB, Hoelen DW, Heijligenberg R, Bromhaar MG et al. (2009) A saturated fatty acid–rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome. The American Journal of Clinical Nutrition 90:1656-1664

Velasco L, Fernández-Martínez JM (2010) Other Brassicas. In: Oil Crops Handbook of Plant Breeding (edited by Vollmann J, Rajcan I). Springer, New York, pp 127-153

Vignal A, Milan D, SanCristobal M, Eggen A (2002) A review on SNP and other types of molecular markers and their use in animal genetics. Genetics Selection Evolution 34:275-306

Wang N, Li F, Chen B, Xu K, Yan G, Qiao J, Li J, Gao G, Bancroft I, Meng J (2014) Genome-wide investigation of genetic changes during modern breeding of *Brassica napus*. Theoretical and Applied Genetics 127:1817-1829

Warwick SI, Black LD (1991) Molecular systematics of *Brassica* and allied genera (subtribe Brassicinae, Brassiceae)—chloroplast genome and cytodeme congruence. Theoretical and Applied Genetics 82:81-92

Warwick SI, Gugel RK, McDonald T, Falk KC (2006) Genetic variation of Ethiopian mustard (*Brassica carinata* A. Braun) germplasm in western Canada. Genetic Resources of Crop Evolution 53:297-312

Wen J, Tu J-x, Li Z-y, Fu T-d, Ma C-z, Shen J-x (2008) Improving ovary and embryo culture techniques for efficient resynthesis of *Brassica napus* from reciprocal crosses between yellow-seeded diploids *B. rapa* and *B. oleracea*. Euphytica 162:81-89

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535

Wright S (1978) Variability within and among natural populations. In: Evolution and the Genetics of Populations. The University of Chicago Press, Chicago 4:79-103

Xiao J, Li J, Yuan L, McCouch S, Tanksley S (1996) Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. Theoretical and Applied Genetics 92:637-643

Xiao Y, Chen L, Zou J, Tian E, Xia W, Meng J (2010) Development of a population for substantial new type *Brassica napus* diversified at both A/C genomes. Theoretical and Applied Genetics 121:1141-1150

Xiong ZY, Pires JC (2011) Karyotype and identification of all homoeologous chromosomes of allopolyploid *Brassica napus* and its diploid progenitors. Genetics 187:37-49

Yang YW, Lai KN, Tai PY, Li WH (1999) Rates of nucleotide substitution in angiosperm mitochondrial DNA sequences and dates of divergence between *Brassica* and other angiosperm lineages. Journal of Molecular Evolution 48:597-604

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

Yu CY, Leišova L, Kučera V, Vyvadilová M, Ovesná J, Dotlačil L, Hu SW (2007) Assessment of genetic diversity of yellow-seeded rapeseed (*Brassica napus* L.) accessions by AFLP markers. Czech Journal of Genetics and Plant Breeding 43:105-112

Zand E, Beckie HJ (2002) Competitive ability of hybrid and open-pollinated canola (*Brassica napus*) with wild oat (*Avena fatua*). Canadian Journal of Plant Science 82:473-480

Zhang G, Zhou W, Gu H, Song W, Momoh E (2003) Plant regeneration from the hybridization of *Brassica juncea* and *B. napus* through embryo culture. Journal of Agronomy and Crop Science 189:347-350

Zhang JF, Qi CK, Pu HM, et al. (2008) QTL identification for fatty acid content in rapeseed (*Brassica napus* L.). Acta Agronomica Sinica 34:54-60

Zhang Y, Talalay P (1994) Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. Cancer research 54 (Supplement), pp 1976s-1981s

Zhao J, Wang X, Deng B, Lou P, Wu J, Sun R, Xu Z, Vromans J, Koornneef M, Bonnema G (2005) Genetic relationships within *Brassica rapa* as inferred from AFLP fingerprints. Theoretical and Applied Genetics 110:1301-1314

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

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

Appendices

Table A3.1 Pedigree of the F_4 plants derived from *B. napus* × *B. oleracea* var. *italica* (cross ID: 5CA1358) and *B. napus* × *B. oleracea* var. *capitata* (cross ID: 5CA1392), and BC_1F_3 plants derived from (*B. napus* × *B. oleracea* var. *italica*) × *B. napus* (cross ID: 5CA1678) and (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus* (cross ID: 5CA1679) used to genotype by SSR markers.

Cross ID	F_2/BC_1 Registration No.	F_3/BC_1F_2 Registration No.	F_4/BC_1F_3 Registration No. (genotyped by SSR)	Genotyping Code
5CA1358	5CA1358.002-A1220 P16	5CA1358.011-A1231P12	5CA1358.116-A1242P04	F_Ita_2
5CA1358	5CA1358.002-A1220 P18	5CA1358.013-A1231 P3	5CA1358.120-A1242P02	F_Ita_3
5CA1358	5CA1358.002-A1220 P24	5CA1358.020-A1231 P5	5CA1358.123-A1242P01	F_Ita_4
5CA1358	5CA1358.002-A1220 P24	5CA1358.020-A1231 P5	5CA1358.123-A1242P03	F_Ita_5
5CA1358	5CA1358.002-A1220 P26	5CA1358.022-A1231 P4	5CA1358.124-A1242P03	F_Ita_6
5CA1358	5CA1358.002-A1220 P40	5CA1358.038-A1231 P2	5CA1358.131-A1242P01	F_Ita_7
5CA1358	5CA1358.002-A1220 P41	5CA1358.039-A1231 P2	5CA1358.133-A1242P02	F_Ita_8
5CA1358	5CA1358.002-A1220 P41	5CA1358.039-A1231 P2	5CA1358.133-A1242P04	F_Ita_9
5CA1358	5CA1358.002-A1220 P41	5CA1358.039-A1231 P3	5CA1358.134-A1242P01	F_Ita_10
5CA1358	5CA1358.002-A1220 P41	5CA1358.039-A1231 P3	5CA1358.134-A1242P02	F_Ita_11
5CA1358	5CA1358.002-A1220 P41	5CA1358.039-A1231 P3	5CA1358.134-A1242P03	F_Ita_12
5CA1358	5CA1358.002-A1220 P41	5CA1358.039-A1231 P4	5CA1358.135-A1242P01	F_Ita_13
5CA1358	5CA1358.002-A1220 P42	5CA1358.040-A1231P07	5CA1358.137-A1242P01	F_Ita_14
5CA1358	5CA1358.002-A1220 P42	5CA1358.040-A1231P07	5CA1358.137-A1242P04	F_Ita_15
5CA1358	5CA1358.002-A1220 P42	5CA1358.040-A1231P08	5CA1358.138-A1242P02	F_Ita_16
5CA1358	5CA1358.002-A1220 P47	5CA1358.045-A1231 P4	5CA1358.143-A1242P04	F_Ita_17
5CA1358	5CA1358.002-A1220 P47	5CA1358.045-A1231P07	5CA1358.144-A1242P04	F_Ita_18
5CA1358	5CA1358.002-A1220 P47	5CA1358.045-A1231P14	5CA1358.145-A1242P02	F_Ita_19
5CA1358	5CA1358.002-A1220 P47	5CA1358.045-A1231P14	5CA1358.145-A1242P04	F_Ita_20
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P15	5CA1392.023-A1242P03	F_Cap_21
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P16	5CA1392.024-A1242P05	F_Cap_22
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P16	5CA1392.024-A1242P08	F_Cap_23
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P17	5CA1392.025-A1242P02	F_Cap_24
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P18	5CA1392.026-A1242P01	F_Cap_25
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P18	5CA1392.026-A1242P07	F_Cap_26
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P18	5CA1392.026-A1242P08	F_Cap_27
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P3	5CA1392.028-A1242P08	F_Cap_28
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P6	5CA1392.030-A1242P04	F_Cap_29
5CA1392	5CA1392.002-A1220 P1	5CA1392.004-A1231 P6	5CA1392.030-A1242P07	F_Cap_30
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P18	5CA1392.033-A1242P02	F_Cap_31
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P18	5CA1392.033-A1242P08	F_Cap_32
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P19	5CA1392.034-A1242P05	F_Cap_33
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P19	5CA1392.034-A1242P06	F_Cap_34
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P19	5CA1392.034-A1242P08	F_Cap_35

5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P2	5CA1392.035-A1242P04	F_Cap_36
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P2	5CA1392.035-A1242P06	F_Cap_37
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P21	5CA1392.037-A1242P04	F_Cap_38
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P21	5CA1392.037-A1242P06	F_Cap_39
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P22	5CA1392.038-A1242P01	F_Cap_40
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P25	5CA1392.039-A1242P01	F_Cap_41
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P25	5CA1392.039-A1242P06	F_Cap_42
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P4	5CA1392.040-A1242P01	F_Cap_43
5CA1392	5CA1392.002-A1220 P2	5CA1392.005-A1231 P4	5CA1392.040-A1242P05	F_Cap_44
5CA1678	5CA1678.003-A6220 P11	5CA1678.059-A1231P02	5CA1678.004-A1242P02	BC_Ita_45
5CA1678	5CA1678.003-A6220 P11	5CA1678.059-A1231P02	5CA1678.004-A1242P03	BC_Ita_46
5CA1678	5CA1678.003-A6220 P11	5CA1678.059-A1231P02	5CA1678.004-A1242P04	BC_Ita_47
5CA1678	5CA1678.003-A6220 P11	5CA1678.059-A1231P03	5CA1678.005-A1242P04	BC_Ita_48
5CA1678	5CA1678.003-A6220 P11	5CA1678.059-A1231P04	5CA1678.006-A1242P01	BC_Ita_49
5CA1678	5CA1678.003-A6220 P11	5CA1678.059-A1231P04	5CA1678.006-A1242P03	BC_Ita_50
5CA1678	5CA1678.003-A6220 P11	5CA1678.059-A1231P04	5CA1678.006-A1242P04	BC_Ita_51
5CA1678	5CA1678.003-A6220 P21	5CA1678.070-A1231P03	5CA1678.011-A1242P04	BC_Ita_52
5CA1678	5CA1678.003-A6220 P24	5CA1678.073-A1231P01	5CA1678.013-A1242P01	BC_Ita_53
5CA1678	5CA1678.003-A6220 P36	5CA1678.086-A1231P03	5CA1678.026-A1242P02	BC_Ita_54
5CA1678	5CA1678.003-A6220 P45	5CA1678.096-A1231P04	5CA1678.035-A1242P02	BC_Ita_55
5CA1678	5CA1678.003-A6220 P6	5CA1678.104-A1231P01	5CA1678.040-A1242P03	BC_Ita_56
5CA1678	5CA1678.003-A6220 P6	5CA1678.104-A1231P05	5CA1678.041-A1242P01	BC_Ita_57
5CA1678	5CA1678.003-A6220 P6	5CA1678.104-A1231P05	5CA1678.041-A1242P02	BC_Ita_58
5CA1678	5CA1678.003-A6220 P6	5CA1678.104-A1231P05	5CA1678.041-A1242P04	BC_Ita_59
5CA1679	5CA1679.003-A6220 P1	5CA1679.006-A1231P10	5CA1679.006-A1242P03	BC_Cap_60
5CA1679	5CA1679.003-A6220 P3	5CA1679.008-A1231P01	5CA1679.012-A1242P02	BC_Cap_61
5CA1679	5CA1679.003-A6220 P3	5CA1679.008-A1231P02	5CA1679.013-A1242P01	BC_Cap_62
5CA1679	5CA1679.003-A6220 P3	5CA1679.008-A1231P02	5CA1679.013-A1242P02	BC_Cap_63
5CA1679	5CA1679.003-A6220 P3	5CA1679.008-A1231P03	5CA1679.014-A1242P03	BC_Cap_64
5CA1679	5CA1679.003-A6220 P3	5CA1679.008-A1231P05	5CA1679.016-A1242P01	BC_Cap_65
5CA1679	5CA1679.003-A6220 P5	5CA1679.010-A1231P07	5CA1679.026-A1242P02	BC_Cap_66
5CA1679	5CA1679.003-A6220 P5	5CA1679.010-A1231P09	5CA1679.028-A1242P01	BC_Cap_67
5CA1679	5CA1679.003-A6220 P5	5CA1679.010-A1231P09	5CA1679.028-A1242P03	BC_Cap_68
5CA1679	5CA1679.003-A6220 P7	5CA1679.012-A1231P07	5CA1679.034-A1242P01	BC_Cap_69
5CA1679	5CA1679.003-A6220 P7	5CA1679.012-A1231P07	5CA1679.034-A1242P02	BC_Cap_70
5CA1679	5CA1679.003-A6220 P7	5CA1679.012-A1231P08	5CA1679.035-A1242P02	BC_Cap_71
5CA1679	5CA1679.003-A6220 P7	5CA1679.012-A1231P09	5CA1679.036-A1242P02	BC_Cap_72
5CA1679	5CA1679.003-A6220 P7	5CA1679.012-A1231P10	5CA1679.037-A1242P02	BC_Cap_73
5CA1679	5CA1679.003-A6220 P7	5CA1679.012-A1231P10	5CA1679.037-A1242P03	BC_Cap_74
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P01	5CA1679.041-A1242P01	BC_Cap_75
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P01	5CA1679.041-A1242P03	BC_Cap_76

5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P02	5CA1679.042-A1242P01	BC_Cap_77
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P02	5CA1679.042-A1242P02	BC_Cap_78
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P02	5CA1679.042-A1242P03	BC_Cap_79
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P03	5CA1679.043-A1242P03	BC_Cap_80
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P05	5CA1679.045-A1242P01	BC_Cap_81
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P05	5CA1679.045-A1242P02	BC_Cap_82
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P06	5CA1679.046-A1242P01	BC_Cap_83
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P06	5CA1679.046-A1242P02	BC_Cap_84
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P06	5CA1679.046-A1242P03	BC_Cap_85
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P07	5CA1679.047-A1242P01	BC_Cap_86
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P08	5CA1679.048-A1242P01	BC_Cap_87
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P08	5CA1679.048-A1242P03	BC_Cap_88
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P09	5CA1679.049-A1242P02	BC_Cap_89
5CA1679	5CA1679.003-A6220 P11	5CA1679.016-A1231P09	5CA1679.049-A1242P03	BC_Cap_90

Table A3.2 List of SSR markers used for genotyping of the F_4 and BC_1F_3 plants derived from *B. napus* × *B. oleracea* interspecific crosses

Source	Primer no.	Name	Linkage group	Total alleles	Alleles specific to *B. oleracea*
AAFC	109	sN2305	C1	1	0
AAFC	2287	sN0983F	C1	3	1
AAFC	2297	sN11657	C1	2	0
AAFC	2299	sR1078	C1	4	2
AAFC	2302	sN12790	C1	5	4
AAFC	2068	sN1848 (bNP)	C2	2	1
AAFC	2069	sR2028 (aNP)	C2	5	3
AAFC	2072	sS2206 (aNP)	C2	3	1
AAFC	2081	sN3682 (aNP)	C2	6	2
AAFC	2085	sN3549R (a)	C2	1	0
AAFC	2088	sS1879	C3	2	0
AAFC	2089	sN2034 (NP)	C3	4	2
Cheng et al. 2009 TAG	2208	BnGMS426	C3	1	1
Cheng et al. 2009 TAG	2221	BnGMS631	C3	3	2
AAFC	2097	sORB30	C4	2	1
AAFC	2099	sR0357	C4	4	1
AAFC	2100	sORG31	C4	2	1
AAFC	2463	sORD34	C4	2	1
Cheng et al. 2009 TAG	2200	BnGMS347	C4	3	1
AAFC	303	sORH13	C5	3	2
AAFC	584	sN2036Fa	C5	3	2
AAFC	611	sN11729bNP	C5,C3	1	1
AAFC	710	sS1854(c)	C5,C4,C9	3	2
AAFC	2416	sS1732	C5	4	3
AAFC	2446	sN2046R	C5	2	1
AAFC	2452	sN11661	C5	5	3
AAFC	2461	sN12804	C5	3	1
AAFC	2464	sN12572	C5	1	1
AAFC	2362	sR0472	C6	1	0
AAFC	2365	sN11904	C6	6	2
AAFC	2372	sORF89	C6	5	4
AAFC	2378	sR2319	C6	9	5
Cheng et al. 2009 TAG	2191	BnGMS205	C6	3	2
Cheng et al. 2009 TAG	2213	BnGMS491	C6	3	1
AAFC	2386	sN7407	C7	2	1
AAFC	2399	sS1709	C7	1	1
AAFC	2401	sNRA84	C7	1	0
AAFC	2402	sNRH63	C7	3	1
AAFC	2428	sN3825J	C7	6	2
AAFC	2432	sN12750	C7	3	1
Cheng et al. 2009 TAG	2209	BnGMS439	C8	3	1
Li et al. 2011 Mol Breed	2244	BoGMS0468	C8	2	1
Li et al. 2011 Mol Breed	2245	BoGMS0631	C8	4	3
Li et al. 2011 Mol Breed	2248	BoGMS0868	C8	3	2
Cheng et al. 2009 TAG	2185	BnGMS85	C9	1	0

Table A3.2 continued.

Source	Primer no.	Name	Linkage group	Total alleles	Alleles specific to *B. oleracea*
Cheng et al. 2009 TAG	2193	BnGMS213	C9	2	2
Cheng et al. 2009 TAG	2204	BnGMS385	C9	3	1
Li et al. 2011 Mol Breed	2256	BoGMS0624	C9	5	3