Broadening of genetic diversity in spring canola (*Brassica napus* L.) by use of yellow sarson and Canadian spring *Brassica rapa* L.

by

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

Master of Science

in

Plant Science

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Abstract

Canada is the top producer of Brassica oilseeds [*B. napus* L. (*n* = 19, AC genome)] in the world. Genetic diversity has declined in this crop in the recent years due to use of only superior and genetically narrow gene pool in breeding. Presence of adequate genetic diversity is important for further improvement of this crop through breeding. Genetically distinct germplasm of *B. napus* or its allied species can be used to broaden genetic diversity in Canadian *B. napus* canola. However, limited efforts have been made to utilize genetic diversity of the progenitor species *B. rapa* (*n* = 10, A genome) and *B. oleracea* (*n* = 9, C genome) in the breeding of this crop as interspecific cross often introduces undesirable traits in the breeding program. This M.Sc. thesis research was undertaken to develop genetically distinct *B. napus* lines through interspecific crosses between *B. napus* canola and *B. rapa.* For this, three genetically distinct *B. rapa* lines were used. The F_1's of *B. napus* × *B. rapa* interspecific crosses were self-pollinated for F_2 as well as backcrossed to the *B. napus* parent for BC_1F_1 progenies. Pedigree breeding was applied where selection for plant fertility and glucosinolate content was done in each generation. SSR marker analysis of the F_4 plants revealed that the three populations derived from *B. napus* × *B. rapa* crosses are genetically distinct from each other as well as from the *B. napus* parent; thus, the advanced generation populations derived from the progeny of these plants expected to carry allelic diversity of the *B. rapa* parents. Plant fertility and glucosinolates content in many of the F_7 and BC_1F_4 families reached close to the *B. napus* parent. Flow cytometric analysis of F_6 and BC_1F_3 families for nuclear DNA content indicated that many families are euploid *B. napus* type. Findings from this thesis research suggest that genetically distinct, fertile, euploid *B. napus* canola lines can be developed from both F_2 and BC_1F_1 of the *B. napus* × *B. rapa* interspecific crosses.

Acknowledgement

I take this as an opportunity to acknowledge the people who have helped me directly or indirectly during my M.Sc. program and because of whom I was able to complete my research project.

I would like to pay my deepest gratitude to my supervisor Dr. Habibur Rahman for his academic guidance and support during the program. Dr. Rahman's dedication for canola research has been an inspiration for me. I can't think about a moment that I asked for his assistance and my request was not ensured by insightful and productive meeting. I am proud to call him my mentor.

I also enjoyed several mind provoking discussions with my committee member Dr. Urmila Basu. I give my sincere thanks to other members of the Canola Program, who have been extremely helpful and kind to me. I am thankful to my friends and colleagues at the department of AFNS for their support during my research and coursework.

I acknowledge financial support from NSERC Discovery and NSERC-CRD grants to Dr. Habibur Rahman.

Last, I would like to thank my family and loved ones for being supportive and patient during my M.Sc. program. I could not find words to convey my feelings for them.

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Symbols and Abbreviations

µmol g^{-1}	Micromoles per gram
µl	Micro liter
ng μL^{-1}	Nano gram per micro liter
A1 to A10	*B. rapa* (A-genome) chromosomes
BC_1F_1	First backcross generation
BC_1F_n	n^{th} backcross generation
F_1	First filial generation
F_n	n^{th} filial generation
FISH	Fluorescence *in situ* hybridization
GCA	General Combining Ability
GSL	Glucosinolate
HEAR	High erucic acid rapeseed
HOLL	High oleic and low linoleic acid
N1 to N10	*B. napus* chromosomes corresponding to *B. rapa* A-genome
N11 to N19	*B. napus* chromosomes corresponding to *B. oleracea* C-genome
NIRS	Near-infrared spectroscopy
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RAPD	Random Amplified Polymorphism
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolution per minute
SD	Standard deviation
SNP	Single-Nucleotide Polymorphism
SSR	Simple sequence repeat
UPGMA	Unweighted pair-group method with arithmetic mean

Chapter 1

Literature review

1.1 Introduction

Oilseed rape, also known as rapeseed or canola, belongs to the genus *Brassica* of the family Brassicaceae. Vast diversity exists in the genus *Brassica*; many of its species are cultivated as vegetables, condiments and oil crops (Rakow 2004, Gupta and Pratap 2007). *Brassica napus* L. (*n* = 19, AC genome), *Brassica rapa* L. (*n* = 10, A genome) and *Brassica juncea* (L) Czern. (*n* = 18, AB genome) are the important oilseed crops of the Brassicaceae family. Collectively *B. napus* and *B. rapa* are called as rapeseed, while *B. juncea* as Indian mustard. Based on growth habit, *B. napus* is classified into: winter, spring and semi-winter types. Winter type requires vernalization for about eight week for flowering and is primarily grown in Europe; the semi-winter type requires shorter period of vernalization (about four weeks) and is grown in China, while the spring type does not require vernalization for flowering and is cultivated in Canada, Australia and USA (Raymer 2002).

Brassica crops have been cultivated and consumed in India since 1500 to 2000 BC (Gupta and Pratap 2007). It was introduced in Europe in 13th century (Bell 1982). The oil from traditional Brassica oilseed contain a high level of erucic acid (>40% of total fatty acid) and therefore it was considered unhealthy for edible purpose Hung et al. (1977). The seed meal remain after oil extraction contains about 40% protein; however, its use in animal feed was restricted due to high content of glucosinolates (>100 μmol g^{-1} seed meal) (Mawson et al. 1993). Traditional rapeseed oil was primarily used as lubricant and to some extent as edible oil in Europe (Bell 1982). As the demand for lubricant oil for steam engines increased during the

World War II, this oilseed was introduced in Canada. However, at the end of the war and with increased use of diesel engines, compared to the steam engines, the demand for this oil reduced markedly, resulting a decrease in its cultivation in Canada (Casséus 2009). Canada, being located in the temperate zone, does not have the climate for cultivation of major oilseed crops, such as soybean and sunflower. Therefore, Canada was heavily dependent on the import of edible oils (Casséus 2009). The ability of the Brassica oilseed crops to withstand and grow under lower temperature makes it perfect oilseed crop for the Canada (Shahidi 1990). To use this oil for edible purpose and the meal as animal feed, cultivars with low level of erucic acid in oil and low content of glucosinolate in seed meal were developed in 1970s (Casséus 2009). These seed - quality improved cultivars termed as CANOLA (Canadian Oil Low Acid) (Casséus 2009). In Canada, three types of canola crops are cultivated: Argentine canola (*B. napus*), Polish canola (*B. rapa*) and brown mustard (*B. juncea*) (Canola Council of Canada 2011a). Intense breeding over the last 50 years has reduced genetic diversity in Canadian canola germplasm significantly (Juska et al. 1997, Fu and Gugel 2010). This study aims to increase genetic diversity in Canadian *B. napus* canola germplasm by introgression of allelic diversity from the A genome of *B. rapa* through interspecific hybridization between these two species.

1.2 Evolution of *Brassica* genome

1.2.1 Relationship of the genus *Brassica* with *Arabidopsis*

The present day *Brassica* genomes believed to have evolved from a *Arabidopsis*-like smaller ancestral genome through polyploidization and chromosome rearrangements (reviewed in Schranz et al. 2006). Qiu et al. (2009) showed that some of the chromosomal segments or genes of *Arabidopsis* are missing in the *Brassica* genomes. Similarly, Trick et al. (2009) reported that

some of the genetic blocks found in *Arabidopsis* genome are missing in the *Brassica* genome. Molecular analysis of the *Arabidopsis* and three *Brassica* genomes, A, B and C, provided insight that in addition to polyploidization of the ancestral genome other events such as chromosome fission, fusion, deletion, insertion and rearrangements may have also took place during the evolution of the three *Brassica* genomes (Parkin et al. 2005, Lagercrantz 1998, Lagercrantz and Lydiate 1996, Lysak et al. 2007, reviewed in Schranz et al. 2006).

1.2.2 Evolution of amphidiploid and their relationship with diploid species

Brassica napus is an amphidiploid species, originated from hybridization between the two diploid species *B. rapa* and *B. oleracea* L. (n = 9, C genome). The relationship between the three diploid species *B. rapa*, *B. nigra* L. (n = 8, B genome) and *B. oleracea*, and the three amphidiploid species *B. napus*, *B. juncea* and *B. carinata* A. Braun (n = 17, BC genome), was first proposed by the Japanese researcher U in 1935 (cited by Chen et al. 2011).

Based on chloroplast and nuclear genome markers, Allender and King (2010) reported that multiple hybridization events between the variants of *B. rapa* and *B. oleracea* resulted the present day *B. napus,* and it is highly likely that *B. rapa* served as the maternal parent. Based on fluorescence *in situ* hybridization (FISH) pattern with 5S, 25S rDNA probes and DAPI staining of metaphase chromosomes, Snowdon et al. (2002) were able to identify the ten chromosomes originating from *B. rapa* and the nine from *B. oleracea* in the amphidiploid species *B. napus*.

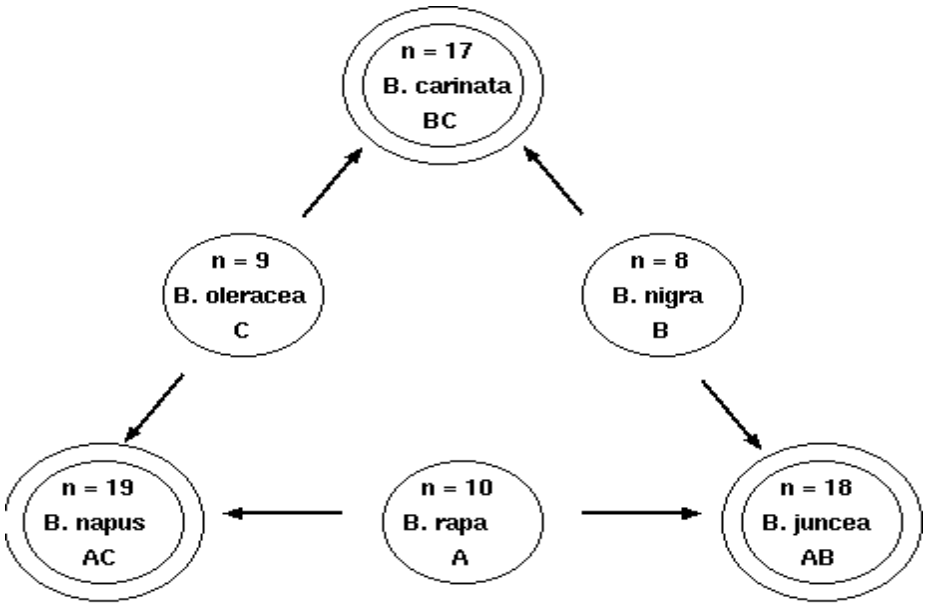


Figure 1.1 Relationship between the diploid and the amphidiploid *Brassica* species (U 1935, cited by Chen et al. 2011).

Similarly, construction of genetic linkage map of *B. napus* (Parkin et al. 1995) and alignment of this map with the genetic maps of *B. rapa* (Suwabe et al. 2008) and *B. oleracea* (Bohuon et al. 1996) further confirmed that the 19 linkage groups of *B. napus* composed of ten linkage groups of *B. rapa* and nine of *B. oleracea.* To support the evolution of the amphidiploid species of the genus Brassica, artificial amphidiploid species were synthesized by different researchers (*B. juncea* and *B. carinata* by Song et al. 1993, *B. napus* by Rahman 2005) through interspecific crosses between their diploid progenitors. The artificial amphidiploid species often show significant difference from the natural amphidiploid species reflecting the evolutionary changes occurred since the formation of the amphidiploid species (Song et al. 1993).

1.3 Oilseed Brassica and its quality

1.3.1 Types of Brassica oilseed based on its oil and meal quality

Brassica oilseed contains about 45% oil, and the oil-free seed meal contain about 35-40% protein (Raymer 2002). Oil of traditional Brassica seed contain about 40-45% erucic acid (Rakow 2011)

and seed meal contain 100-150 µmol glucosinolate (GSL) per gram of dry matter (Canola Council of Canada 2011b). However, most of the oils of the present day Brassica oilseed cultivars used for edible purpose are virtually free (<1%) from erucic acid and seed meal of these cultivars contain less than 30 µmol of total aliphatic glucosinolates per gram of air-dry, oil-free solid. This improved Brassica oilseed crop cultivars are called double low type rapeseed or canola (Raymer 2002, Canola Council of Canada 2011c).

Glucosinolates are the secondary plant metabolites, biosynthesized from amino acids, occur in plants of the order Brassicales. Nearly hundred different types of glucosinolates identified so far (Halkier and Du 1997, Magrath et al. 1994) of which only few are dominating in Brassica (Sang and Salisbury 1988). Table 1.1 and 1.2 show that glucobrassicin is only present in Type 4 *B. napus* and sinigrin is only present in Type 3 and 4 *B. napus* and in Type 3 *B. rapa.*

Table 1.1 Types of glucosinolates (GSL) found in oilseed Brassica

	Trivial name	Semi-systemic name
I	Gluconapin	3-butenyl-GSL
II	Progoitrin	2-hydroxy-3-butenyl-GSL
III	Glucobrassicanapin	4-pentenyl-GSL
IV	Gluconapoleiferin	2-hydroxy-4-pentenyl-GSL
V	Glucobrassicin	3-indolylmethyl-GSL
VI	4-hydroxyglucobrassicin	4-hydroxy – 3- indolylmethyl-GSL
VII	Sinigrin	2-propenyl-GSL
VIII	Gluconasturtiin	2-phenylethyl-GSL

(Adapted from Sang and Salisbury 1988)

Table 1.2 Classification of *Brassica rapa* and *Brassica napus* based on the proportion of individual glucosinolates (GSL) of the total GSL

	Glucosinolates (% of total)								Total GSL
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	µmol g^{-1} air dried oil-free meal
B. napus									
Type 1 (Dominant I, II, III)	38.5	19.0	27	3.5	tr	6.5	tr	5.0	81.0
Type 3 (Dominant I, II)	18.3	62.2	6.8	3.8	tr	4.9	1.8	2.1	116.3
Type 4 (Dominant (I, II, VI)	20.2	48.6	4.8	2.8	2.2	18.4	2.2	1.2	25.6
B. rapa									
Type 1 (Dominant I, II, III)	39.9	23.9	22.3	4.6	tr	5.3	tr	3.4	84.1
Type 2 (Dominant I)	93.3	1.5	1.5	1.0	tr	1.7	tr	tr	152.2
Type 3 (Dominant I, II)	38.5	58.0	6.0	3.0	tr	3.5	2.5	2.0	139.0

(Note: I = Gluconapin, II = Progoitrin, III = Glucobrassicanapin, IV = Gluconapoleiferin, V = Glucobrassicin, VI = 4-hydroxyglucobrassicin, VII = Sinigrin, VIII = Gluconasturtiin; tr = traces) (Adapted from Sang and Salisbury 1988)

Based on fatty acid composition of oil, *B. napus* can be classified into three major types: (i) high erucic acid (>50% erucic acid) (HEAR) type oil for industrial use, (ii) zero or low erucic acid type oil for edible purpose, and (iii) high oleic and low linoleic acid (HOLL) (≥ 75% oleic acid and ≤ 3% linolenic acid) for application in specialty food as high stability oil (Möller 2002).

1.3.2 Quality traits of oilseed Brassica and their genotypes

Seed oil and meal quality are the main decisive factors for a cultivar to be defined as canola, Brassica oilseed canola quality cultivars need to have less than 2% erucic acid in its seed oil and less than 30 μmol of total glucosinolates in its per gram of seed meal (Canola Council of Canada 2011c).

Table 1.3 Fatty acid profile of traditional rapeseed, canola (*B. napus and B. rapa*) and HOLL (High Oleic Low Linolenic) and HEAR (High Erucic Acid Rapeseed) types

Fatty acid	Fatty acid	Traditional rapeseed	*B. napus* canola	*B. rapa* canola	HOLL	HEAR
Saturated	C14:0	-	0.1	0.1	-	-
	C16:0	3	3.9	3.5	4	4
	C18:0	1.5	1.6	1.4	2	1
	C20:0	0.5	0.5	0.4	-	1
	C22:0	-	0.4	0.2	-	<1
Monounsaturated	C16:1	0.5	0.2	0.3	<1	-
	C18:1	20.9	59.1	55.6	79	15
	C20:1	12.2	1.4	1.8	2	10
	C22:1	38.6	0.5	1.6	-	45
Polyunsaturated	C18:2	13.9	18.8	21.9	7	14
	C18:3	9.1	8.8	13.0	5	9

(Adapted from Shahidi 1990, Kimber and McGregor 1995)

High level of erucic acid in edible oil is considered to be nutritionally undesirable, and high content of glucosinolate reduce the value of the protein-rich seed meal. Hung et al. (1977) reported that consumption of oil high in erucic acid can result in greater accumulation of triglycerides in heart and incidence of myocardial necrosis. Similarly, feeding animals with Brassica seed meal with high content of glucosinolates hinders the absorption of other nutrients

by the animal body. Breakdown products of some glucosinolates are goiterogenic and adversely affect the normal functionality of the thyroid gland (Bell 1982). To resolve these problems, intensive breeding efforts are made in Canada in the 1950-1970's to reduce the levels of erucic acid and glucosinolates and that led to the development of the *B. napus* cultivar 'Oro' in 1968 with low level of erucic acid in oil and glucosinolates in seed meal (Slinkard and Knott 1995). This development laid the foundation of the present day '00' or 'canola' cultivars.

The content of erucic acid in Brassica seed oil is determined by the genotype of the embryo (Downey and Craig 1964). Therefore, pollen genotype exerts significant effect on the content of erucic acid content in oil - a phenomenon known as xenia effect (Stefansson and Hougen 1964). Two gene loci, each with two alleles, with additive effect determine the level of erucic acid in oil. However, Jönsson (1977) reported the presence of multiple alleles at these loci which can result in various levels of erucic acid in *B. napus* seed oil. The gene controlling erucic acid also affects the oleic acid content inversely (Chen and Beversdorf 1990, Siebel and Paul 1989, Stefansson and Hougen 1964, Downey and Craig 1964). Genes controlling erucic acid were identified on the linkage group A8 (N8) and C3 (N13) of the Brassica genome (Rahman et al. 2008).

The initial low erucic acid cultivars were developed by crossing high erucic acid cultivars with low erucic acid lines followed by selection for low erucic acid content in the subsequent segregating generations (Downey and Harvey 1963). However, backcross breeding technique can also be used with high efficiency to transfer zero erucic allele into an otherwise desirable cultivar (Rahman et al. 1996). Rahman et al. (2001) reported that the white petal colour allele of the C genome is linked to the high erucic acid allele; therefore, breeding program aimed at developing low erucic acid cultivars should generally not select for white petal colour.

In contrast to erucic acid, which is controlled by the embryonic genotype (Kondra and Stefansson 1965), glucosinolate content is controlled by the genotype of the maternal plant (Kondra and Stefansson 1970). Glucosinolates in seed meal is controlled by at least four gene loci, where the presence of recessive alleles at all loci results low glucosinolate (< 15 umol/g seed) content (Rahman et al. 2001). Uzunova et al. (1995) mapped four quantitative trait loci (QTL) for total seed glucosinolate content on a RFLP map of *B. napus*. On the other hand, Rahman et al. (2014) identified three QTL for this trait on the A genome linkage groups A2, A7 and A9 of *B. rapa*.

Interspecific hybridization is an important technique for broadening of genetic diversity in canola. In Crosses where a non-canola quality species is involved, intensive selection for the canola quality traits (erucic acid and glucosinolates) is needed in the interspecific hybrid progenies to develop canola quality germplasm. Iqbal et al. (2011) showed that canola quality trait can be transferred from *B. napus* to other *Brassica* sp. and vice versa through interspecific crosses. Low erucic acid and high oleic acid traits from *B. napus* were transferred in to *B. juncea* through interspecific crosses (Iqbal et al. 2011). Similarly, Bennett et al. (2012) demonstrated the prospect of developing canola quality *B. napus* from interspecific hybrid progenies of *B. napus* × *B. oleracea.*

1.3.3 Importance of seed meal (protein) in relation to soybean protein-how to improve canola meal quality

Brassica oilseed crop is the second largest source of protein-rich seed meal in the world after soybean. Soybean meal accounts for more than half of the world's total seed meal production. In 2012-13, total production of seed meal in the world was 268 million metric tons, where Brassica

seed meal accounted 37 million metric tons as compared to 180 million metric tons of soybean meal (USDA 2014a). Canola meal is generally considered to be lower in quality as compared to soya meal; however, its importance in the feed meal industry cannot be undermined as this meal is relatively rich in amino acids methionine and cysteine as compared to soybean meal. This attribute makes canola meal a potential source of protein for supplementing soybean meal in animal feed (Khajali and Slominski 2012). The nutritive value of canola and soybean meal in animal feed was compared by various researchers. Claypool et al. (1985) found no significant difference between soybean and canola meal as a protein supplement for weight gain in calf. Lim et al. (1998) showed that canola meal can be used to a limited extend (30% of the diet) to replace the soybean meal without any adverse effect on the growth of channel catfish. Studies conducted at the University of Alberta showed that complete replacement of soybean meal with canola meal possess health risk to the chickens through reducing amino acid digestibility (Summers and Robblee 1985). Bell (1993) reviewed the value of canola meal as protein-rich feed in poultry, pig and cattle diet, and concluded that canola meal is poorer as compared to soybean meal due to its higher fiber and high phenolic compound. Therefore, there is a need to further improve the quality of canola meal. Theodoridou and Yu (2012) reported that meal from yellow-seeded canola contains less fiber and shows higher digestibility as compared to the meal from brown seeded canola.

A yellow-seeded *B. napus* line was developed by Rahman (2001) through interspecific hybridization. The yellow seed colour in this line is controlled by 3 to 4 gene loci where presence of recessive alleles at all loci results in yellow seed (Rahman et al. 2001). However, single and partially dominant gene control of yellow seed colour has also been reported in a *B. napus* line developed in previous studies (Liu et al. 2005, Zhi-wen et al. 2005). Agriculture and Agri Food

Canada (AAFC) has also developed a yellow seeded *B. napus* line through interspecific hybridization (Rakow et al. 1999); however the genetic control of this trait is not known.

1.4 Position of Brassica oilseed in the world and in Canada

1.4.1 Brassica oilseed crops in the world

Brassica oilseed crop is the second largest oilseed crop in the world in terms of acreage and production after soybean (Raymer 2002). Canada and China are the largest producers of Brassica oilseed followed by India (FAOSTAT 2014**)**. This oilseed crop is also grown at a large scale in Europe, Australia and USA (USDA 2012).

Acreage: In 2012, Brassica oilseed crop was harvested from 34.1 million ha, while soybean was harvested from 105 million ha (FAOSTAT 2014)

Table 1.4 Harvested acreage and production of major oilseed crops in the world in 2012

Oilseed Crops	Harvested acreage (million ha)	Production (million metric tonnes)
Canola/Rapeseed	34.1	65.1
Soybean	105.0	241.8
Cottonseed		47.2
Peanut	24.7	41.2
Sunflower	24.8	37.4
Total oilseed	280.5	474.3

(FAOSTAT 2014, USDA 2014b)

Harvested acreage of peanut and sunflower in 2012 was 24.7 and 24.8 million ha, respectively (FAOSTAT 2014). World's total harvested acreage of oilseed crops in 2012 was 280.5 million ha (FAOSTAT 2014).

Production: In terms of production, Brassica oilseed crop is the second largest oilseed crop in the world after Soybean (USDA 2014b). In 2012-13, total oilseed production in the world

was 474.3 million metric tonnes (USDA 2014b), whereas soybean accounted for more than half of the world's total production (241.8 million metric ton). In the same year, world production of Brassica oilseeds was 65.1 million metric tons followed by cottonseed, peanut and sunflower with 47.2, 41.2 and 37.4 million metric tonnes, respectively (FAOSTAT 2014). In 2012, Canada was the largest producer of the Brassica oilseed in the world with a production of 15.4 million metric tons followed by China with a production of 14 million metric tons, and India with 6.8 million metric ton. Collectively, the European Union produced 19.2 million metric tonnes of this oilseed in 2012 (FAOSTAT 2014).

1.4.2 Brassica oilseed crops in Canada

Canada is one of the largest producers of canola in the world. It is cultivated primarily in the western Canadian Prairie Provinces, Saskatchewan, Alberta and Manitoba (USDA 2005). Small acreage of this crop can also be found in other provinces, such as Ontario and British Columbia. More than 52,000 Canadian farmers cultivate this crop (Saskatchewan Canola Development Commission, website). Apart from low erucic acid canola, other types, such as high erucic acid rapeseed (HEAR) and high oleic and low linoleic acid (HOLL) cultivars are also produced in Canada. Nearly half of the edible oil consumed in Canada is canola oil (Casséus 2009).

Acreage: In 2012, Canada grew oilseed crops on 10.9 million ha (Agriculture and Agri-Food Canada, 2013) of which brassica oilseed crop accounted 8.8 million ha. This oilseed crop is the second largest crop in Canada after wheat, which was grown on 9.5 million ha. Wheat is primarily cultivated in Alberta, Manitoba and Saskatchewan and on limited scale in the British Columbia, Ontario, New Brunswick, Nova Scotia and Prince Edward Island (Canadian Grain Commission 2013a).

Other oilseed crops grown in Canada are soybean, flax and sunflower with harvested acreage of 1.68, 0.4 and 0.04 million ha, respectively (Statistics Canada 2013a).

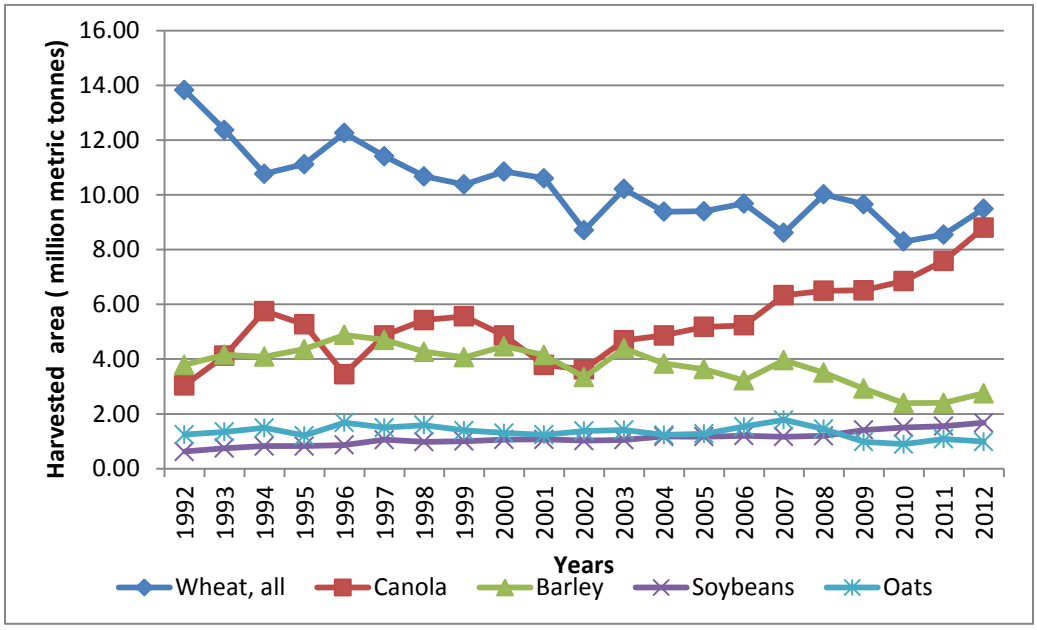


Figure 1.2 Harvested acreage of the major crops in Canada in last one decade (Statistics Canada 2014)

Soybean cultivation is limited to Ontario and Quebec, flax is grown in Prairies and in Atlantic Provinces (Canadian Grain Commission, 2013b) and sunflower is cultivated in Alberta, Manitoba and Saskatchewan (Canadian Grain Commission, 2013c).

Production: Total oilseed (canola, mustard, flax, soybean, sunflower and safflower) production in Canada in 2012 was 19.4 million metric tonnes (Canadian Grain Commission, 2013d and Agriculture and Agri-Food Canada, 2013), of which the share of canola and soybean was 13.9 and 5.09 million tonnes, respectively.

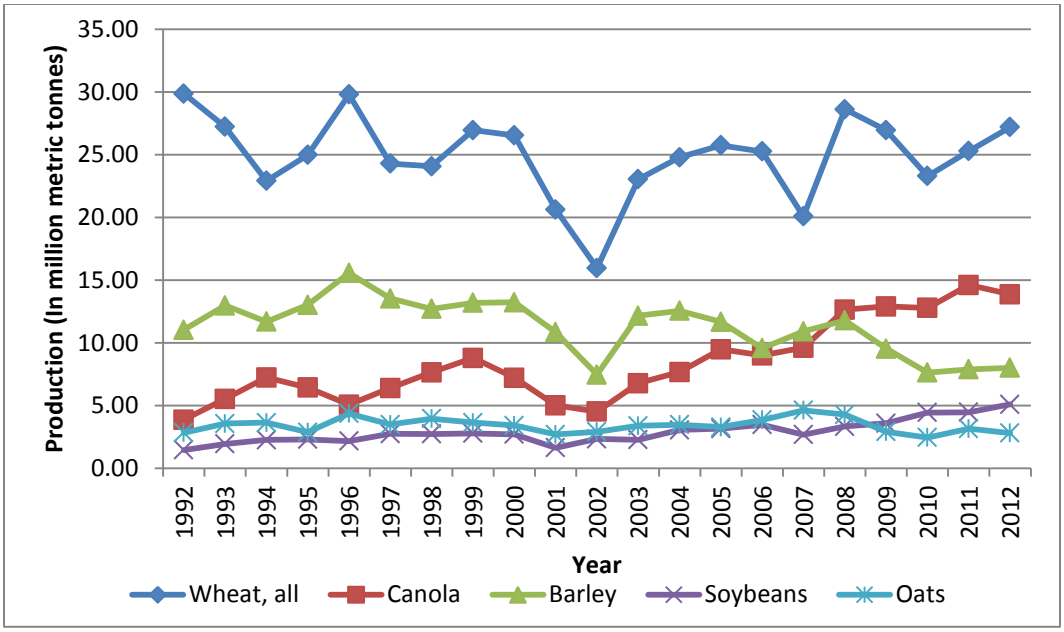


Figure 1.3 Production of major crops in Canada in last one decade (Statistics Canada 2014)

Sunflower is the third largest oilseed crop in Canada with a production of 0.09 million tonnes (Statistics Canada, 2013b). Among all field crops in Canada, wheat is the largest crop with a production of 27.2 million tonnes in 2012.

1.4.3 Canadian canola export and its importance in Canadian economy

Canola plays an important role in Canadian economy. More than 50% of the Canadian canola seed and 84% of the extracted canola oil is exported to other countries, such as Japan, USA and China, and thus Canada is being positioned the largest exporter of canola in the world (Casséus 2009). Revenue generated from Canola export totals about $8.5 billion per year. In addition to this, canola industry has created 228,000 jobs in Canada (Parliament of Canada 2012). Taking all these together, the canola industry contributes more than $19 billion per year to the Canadian economy every year (Canola Council of Canada 2011d). To boost canola production in Canada, it is expected that the Federal government of Canada together with private sector will invest $25 million on research and development of this crop in the period of 2013 to 2018 (Canola Council

of Canada 2011e, Alberta Canola Producers Commission 2013). The importance of the canola industry in Canada can be judged from the fact that any fluctuation in the production of canola in Canada significantly affects the global price of oilseeds, especially the canola price index (USDA 2005).

1.5 Genetic diversity

1.5.1 Genetic diversity in Canadian *Brassica napus* germplasm

In the early 1950s, the Canadian breeders were using very limited germplasm, descended primarily from a few European accessions, in cultivar development, and this has gradually narrowed down genetic diversity in the Canadian *B. napus* gene pool (Juska et al. 1997). Fu and Gugel (2010) found a trend of decreasing allelic diversity in Canadian *B. napus* cultivars bred since 1940's in a Canadian breeding program. Presence of adequate genetic diversity in breeding materials is essentially needed to develop improved open-pollinated as well as hybrid cultivars (reviewed in Rahman 2013). By use of sequence related amplified polymorphic markers, Riaz et al. (2001) reported that significant level of heterosis for seed yield in *B. napus* hybrid cultivars can be achieved by use of genetically diverse lines. However, genetic distance between parents does not always positively correlate with seed yield in hybrid *B. napus*. Yu et al. (2005) found a non-significant correlation between mid-parent heterosis and genetic distance between the parents for seed yield. On the other hand, Diers et al. (1996) reported that inclusion of general combining ability (GCA) with genetic distance of the parents shows significant correlation with seed yield in hybrids. They also reported that GCA of the parents shows stronger correlation with seed yield heterosis as compared to correlation between genetic distance of the parent's lines and heterosis. Thus, inclusion of both genetic diversity and GCA of the parents in statistical analysis

significantly improves the correlation with seed yield hetrosis as compared to inclusion of just one variable. Therefore, both genetic distance and GCA of the parents need to be taken into account in hybrid breeding.

1.5.2 Germplasm sources for increasing genetic diversity in *Brassica napus* canola

Based on RFLP molecular marker analysis, Diers and Osborn (1994) found that oilseed *B. napus* germplasm comprise of three distinct gene pools: winter, spring and Chinese semi-winter types. Qian et al. (2006) also reported similar results. Among these three types of oilseed *B. napus,* the Chinese semi-winter type possesses high genetic diversity as compared to the other types (Qian et al. 2006). Therefore, winter and Chinese semi-winter gene pools can be used to increase genetic diversity of the spring *B. napus* canola gene pool. Indeed, winter and Chinese semi-winter type have been used by different researchers for broadening genetic diversity in spring type *B. napus* (Kebede et al. 2010, Rahman 2011, Udall et al. 2004, Quijada et al. 2004). Rahman and Kebede (2012) developed genetically diverse high yielding spring *B. napus* canola lines from spring × winter *B. napus* crosses. Soengas et al. (2008) reported that rutabaga (*B. napus* var. *napobrassica)* is genetically distinct from oilseed *B. napus,* and this gene pool can also be used to broaden genetic diversity in oilseed *B. napus.*

1.5.3 Interspecific hybridization with allied species

Based on cluster analysis (dendrograms), Thormann et al. (1994) reported that the genome of the two diploid parental species *B. rapa* and *B. oleracea* are genetically distinct from *B. napus.* Liu and Meng (2006) studied different accessions of *B. rapa* and *B. napus,* collected from Europe, China and North America, by use of Restriction fragment length polymorphism (RFLP) and Amplified fragment length polymorphism (AFLP) markers and found the existence of high

genetic diversity in *B. rapa* as compared to *B. napus.* Since evolution of the A genome in nature, vast genetic diversity arose in this genome which resulted in wide morphological diversity in *B. rapa. Brassica rapa* has been used extensively in the breeding of Chinese semi-winter *B. napus* cultivars (Qian et al. 2006), and these semi-winter type display good heterosis when combined with the European winter type (Qian et al. 2009). This suggests that the A genome of *B. rapa* can be exploited to diversify the A genome of the Canadian spring oilseed *B. napus.* Substitution of the whole A genome of *B. napus* with the whole genome of *B. rapa* would invariably increase genetic diversity in this amphidiploid species; however, *B. rapa* also carries several gene/alleles which are undesirable in *B. napus* (reviewed in Rahman 2013). Introgression of these unwanted alleles can be avoided by increasing selection pressure as well as through limited backcrossing approach (Falk 2010). Zou et al. (2010) found that interspecific cross derived *B. napus* lines, carrying the A and C genome components, respectively from *B. rapa* and *B. carinata,* show significant heterosis in *B. napus* hybrids.

Brassica rapa also possesses some traits, such as yellow seed colour and low saturated fatty acid in oil which are desired in *B. napus* canola. Rakow et al. (2007) developed low saturated fatty acid *B. napus* line from *B. napus* × *B. rapa* interspecific crosses. Thus, it is apparent that the A genome of *B. rapa* can also be used to improve some specific traits in *B. napus* in addition to seed yield in hybrid cultivars.

The use of *B. napus* lines resynthesized from *B. rapa* and *B. oleracea* is an another approach of introgressing genetic diversity from these two parental species into oilseed *B. napus.* However, the resynthesized *B. napus* lines are often poor for different agronomic traits including seed yield when compared with the traditional oilseed *B. napus* cultivars (Girke et al. 1999,

Gowers 1989) and often show chromosomal anomalies in meiosis (Gaeta and Pires 2010, Parkin et al. 1995).

1.6 Research objectives

1.6.1 Long-term objectives

The long-term objective of the research is to broaden genetic diversity in Canadian spring *B. napus* canola by use of the *B. rapa* gene pool.

1.6.2 Short-term objectives

In short-term, the following investigations will be made in this M.Sc. research project:

i) Study the feasibility of developing canola quality recombinant *B. napus* ($2n = 38$) inbred lines from *B. napus* × *B. rapa* interspecific crosses through reconstruction of the A genome of *B. napus* with the A genome of *B. rapa.*

ii) Study the inheritance of seed glucosinolate (GSL) content in *B. napus* × *B. rapa* interspecific hybrid progenies and response to selection for low GSL content.

iii) Estimate genetic diversity in the interspecific cross derived from *B. napus* lines by the use of simple sequence repeat (SSR) markers.

1.6.3 Research hypothesis

In this Master's thesis research project, the following research hypothesis will be tested

i) Random assortment of the A genome alleles of *B. rapa* and *B. napus* in the *B. napus* × *B. rapa* interspecific hybrid progeny would lead to the development of canola

quality euploid *B. napus* (2n = 38) lines with A genome component interogressed from *B. rapa.*

ii) The A genome of *B. rapa* is genetically distinct from the A genome of *B. napus,* and this would substantially broaden allelic diversity in spring *B. napus* canola.

iii) Genetic diversity in interspecific cross derived lines will be greater than current spring *B. napus* canola.

iv) Genetic diversity in the interspecific inbred lines developed through self-pollination of F_1 will be greater than the inbred lines derived from backcross (F_1 × *B. napus)* progenies; however, backcross derived lines will be agronomically superior as compared to the F_1 derived lines due to less disruption of the favorable allele combinations in the *B. napus* parent.

Chapter 2

Development of recombinant inbred lines from F_2 and backcross derived population of *B. napus* × *B. rapa* interspecific crosses

2.1 Introduction

Canola [*Brassica napus* L. (*n* = 19, AC genome)] is the second largest oilseed crop in the world (Raymer 2002), and is the major oilseed crop in Canada in regard to production (Statistics Canada, 2013b). It is the most valuable crop in Canada generating more than one fourth of the total farm cash receipt (Canola Council of Canada, 2014a). Canola is not an indigenous crop in Canada; this crop species was introduced from Argentina in 1940's (Canola Council of Canada, 2014b). Through breeding efforts in 1960-70's, the quality of its oil and seed meal has been improved dramatically and the canola quality cultivars were developed. This crop is mainly produced in western Canadian Provinces. Of the total canola production in Canada in 2013, Saskatchewan produced about 50%, Alberta 33% and Manitoba 16% (Statistics Canada, 2014). Average seed yield of this crop is higher in Alberta when compared with yield in other provinces (Statistics Canada, 2014). Since its introduction in 1940's, seed yield of this crop has increased by 2.5 fold (Statistics Canada, 2014). In 2013, average canola yield in Canada was 0.88 t/ac (Statistics Canada, 2014); it is expected to increase yield to 1.18 t/ac by 2025 (Canola Council of Canada, 2014c). To achieve this target, it is important to develop high-yielding cultivars with resistance to biotic and abiotic stresses through breeding.

Canola breeders in the last few decades have made significant improvement in this crop; however, there is scope for further improvement through breeding research (reviewed in Rahman 2013). The three Brassica genomes, A (*n* = 10), B, (*n* = 8) and C (*n* = 9) evolved from a common

progenitor genome and share homoeology to a great extent; therefore, it is possible to introgress desired traits or alleles from allied Brassica species into *B. napus* canola. For example, *B. juncea* (L) Czern. (*n* = 18, AB genome) (Roy 1984, Wang et al. 2007) and *B. carinata* A. Braun (*n* = 17, BC genome) (Fredua-Agyeman et al. 2014) were used for the development of blackleg resistant and silique shatter resistant *B. napus* lines. Rahman et al. (2011) introgressed earliness of flowering from *B. oleracea* into *B. napus*. Similarly, *B. rapa* L. (*n* = 10, A genome) was used for the development of yellow seeded *B. napus* lines (Rahman 2001).

Interspecific crosses of *B. napus* with *B. rapa* have been used extensively to develop semi-winter *B. napus* cultivars in china (Qian et al. 2005). This new type of *B. napus* lines, developed from *B. napus* × *B. rapa* interspecific crosses, exhibited high heterosis for seed yield when crossed with natural *B. napus*. Schelfhout et al. (2008) used *B. napus* × *B. juncea* interspecific crosses to increase genetic diversity in Australian *B. napus* germplasm. Likewise, Bennett et al. (2012) and Rahman et al. (2015) developed genetically diverse canola lines from *B. napus* × *B. oleracea* L. (*n* = 9, C genome) interspecific cross.

To date, very limited effort has been made to improve the Canadian spring *B. napus* canola by the use of the *B. rapa* gene pool. The long term objectives this research is to improve the Canadian spring *B. napus* canola through exploitation of the A genome of *B. rapa* for the development of high-yielding hybrids cultivars. The specific objectives of this study were to investigate the prospect of developing canola quality *B. napus* lines from *B. napus* × *B. rapa* interspecific crosses and to study the inheritance of one of the canola quality trait, the seed glucosinolate (GSL) content, in the interspecific hybrid progeny.

2.2 Material and methods

2.2.1 Parent lines

A spring *B. napus* (*n* = 19, AC genome) canola line A04-73NA and three *B. rapa* (*n* = 10, A genome) lines, YS49, T4-3-3-1 and 3-0026.027 were used as parents. A04-73NA is a canola quality cultivar developed at the University of Alberta. YS49 is a yellow sarson line possessing zero erucic acid (< 2% erucic acid in seed oil) and high GSL (> 60 µmol/g seed meal). T4-3-3-1 is a canola quality line. 3-0026.027 is a F_{10} generation breeding line derived from a cross between *B. rapa* var. yellow sarson line M-91 and Canadian *B. rapa* canola cultivar Tobin (Rahman et al. 1996). *B. napus* is generally treated as self-pollinated crop though about 20% outcrossing can occur between plants under field condition (Rakow and Woods 1987; Becker et al. 1992). On the other hand, *B. rapa* is generally considered as a cross-pollinated crop; however, the 'yellow sarson' variant of this species highly self-compatible (Rahman et al. 1996).

2.2.2 Population development

Following interspecific crosses were made by using A04-73NA as female: A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027. The F_1 plants were grown in a growth chamber (temperature 21/18^{o}C day/night and photoperiod 16 h) and self-pollinated manually through pollination of individual flower with pollen from the same plant to produce F_2 seeds. The F_1 plants of A04-73NA × YS49 were also backcrossed to A04-73NA to produce BC_1F_1 seeds. The F_2 and BC_1F_1 plants were grown in a greenhouse and pedigree breeding was applied to develop canola quality *B. napus* lines from these interspecific crosses (Fig 2.1). To speed up this breeding research, populations were raised in greenhouse (winter) and in field (summer). The F_2, F_3, F_4, F_6, BC_1F_1 and BC_1F_3 generation populations were raised in greenhouse (21/18^{o}C

day/night and 16 h photoperiod), while the F_5, F_7, BC_1F_2 and BC_1F_4 generation populations were grown in field plots of 1.5 m long single row at the Edmonton Research Station of the University of Alberta (U of A). In each generation, individual plants were self-pollinated by bag isolation to produce the next generation seeds. The *B. napus* parent (A04-73NA) was included as check in all greenhouse and field experiments.

The interspecific hybrids and early generation populations were produced by the Canola Program of the University of Alberta. I used the seeds from the following crosses for my thesis research: F_4 seeds of A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027 crosses, and BC_1F_1 seeds of (A04-73NA × YS49) × A04-73NA. F_4 generation onwards, various traits such as silique length, number of seeds per silique, seed glucosinolates content and ploidy level was measured.

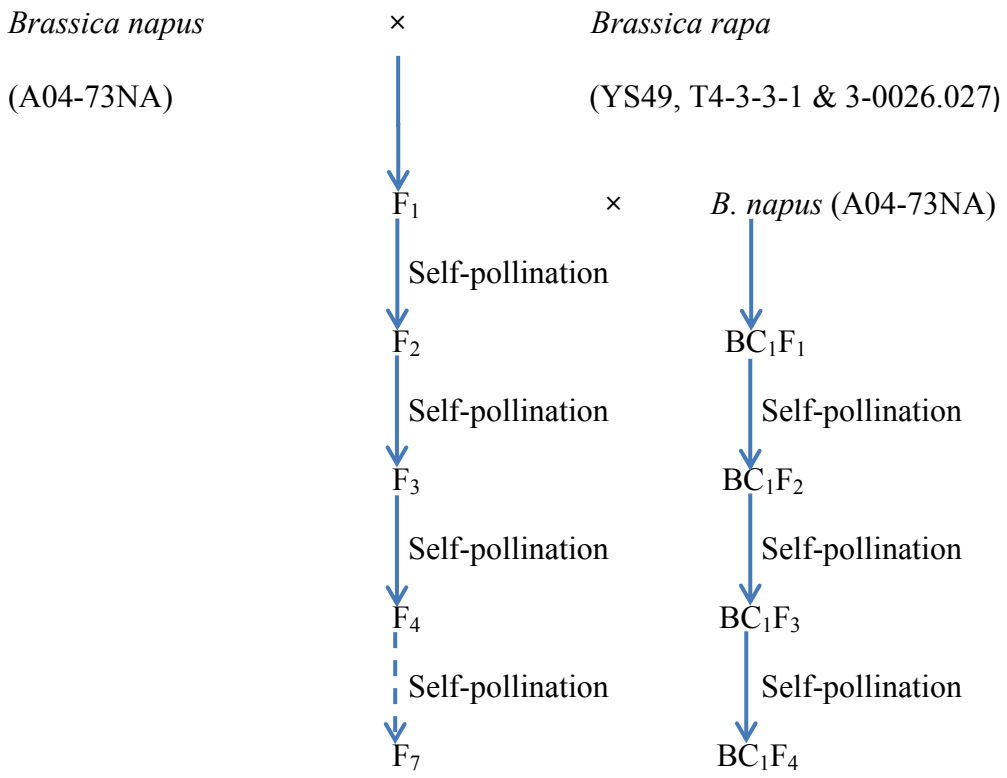


Figure 2.1 Flow diagram of the *B. napus* × *B. rapa* interspecific crossing scheme for the development of genetically diverse *B. napus* lines

2.2.3 Plant fertility evaluation

The F_4, F_5, F_6 and F_7 generation populations of A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027 crosses, and the BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation populations of (A04-73NA × YS49) × A04-73NA backcross were evaluated for silique length (mm) and number of seeds per silique as a measure of estimation of fertility of the plants. Number of plants evaluated for these traits is presented in Tables 2.1, 2.2, 2.3 and 2.4. For this, five siliques from the middle part of the main raceme were measured in mm, and number of seeds in these siliques was counted. In case of the populations (F_4, F_6, BC_1F_1, BC_1F_3) grown in greenhouse, self-pollinated siliques were used for plant fertility estimation, while open-pollinated siliques were used for the field grown populations (F_5, F_7, BC_1F_2 and BC_1F_4). The mean values of these five siliques were used for statistical analysis. Silique measurement was done following the guideline by Canadian Food Inspection Agency (2008).

2.2.4 Seed quality analysis

Seeds harvested from F_4, F_5, F_6, F_7, BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation plants were analyzed by use of near infrared spectroscopy (NIRS) (FOSS NIR system model 6500) for GSL content. For this, 4-5g seed were used and results reported as µmol/g at 8% moisture. Number of samples analyzed of these populations is presented in Table 2.5 and Table 2.6.This analysis was done in the analytical laboratory of the Canola Program of the U of A.

2.2.5 Flow cytometric analysis

The F_5, F_6, BC_1F_2 and BC_1F_3 generation plants along with the *B. napus* (A04-73NA) parent were analyzed by a CyFlow Space flow cytometer (Partec GmbH, Münster, Germany) for relative nuclear DNA content. Number of samples analyzed is presented in Table 2.7 and Table 2.8.

Fresh leaf sample of approximately 0.5 cm^2 size from 15-20 days old seedlings was used. Leaf tissues were finely chopped with a sharp blade in 400 µl nuclear extraction buffer (Partec GmbH, Münster, Germany). Chopped leaf samples were incubated for 30 to 60 seconds, filtered through 50 µm Cell Trics disposable filter and 1.6 ml of staining buffer (Partec GmbH, Münster, Germany) was added. The samples were incubated again for 30 seconds or for 5 minutes before analysis by the flow cytometer.

2.2.6 Statistical analysis

Statistical analysis was done using SAS software version 9.2 and 9.3 (SAS Institute Inc., Cary, NC). Proc MIXED was used to compare means of different generation populations with the *B. napus* parent A04-73NA, while Proc TTEST was used to compare means of the whole population with the means of selected population for a given generation of a cross. Confidence interval for A04-73NA grown with different generation population was calculated using Proc UNIVARIATE. Based on this, the segregating generation plants falling within the confidence limit of A04-73NA were considered *B. napus* type. Proc CORR was used to calculate spearman correlation coefficient for glucosinolates content among the different generation populations.

2.3 Results

2.3.1 Plant fertility in populations derived from *B. napus* × *B. rapa* interspecific crosses

Silique length

Frequency distribution of silique length in F_4, F_5, F_6 and F_7 plants of the three crosses, A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027, are presented in Fig 2.2 and for BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 plants of the (A04-73NA × YS49) × A04-73NA

backcross are presented in Fig 2.3. Confidence limits for the *B. napus* parent for silique length was 43.8–58.3 (no. of plants 7), 43.0–63.1 (no. of plants 3), 54.4–57.7 (no. of plants 44), 47.0–57.1 (no. of plants 8) and 61.8–71.4 mm (no. of plants 12), respectively for the plants grown in 2012 greenhouse (grown along with F_4,), in 2012 greenhouse (grown along with BC_1F_1), in 2013 field (grown along with F_5, BC_1F_2), in 2013 greenhouse (grown along with F_6, BC_1F_3), and in 2014 field (grown along with F_7, BC_1F_4). Based on this, the proportion of the F_4, F_5, F_6 and F_7 generation plants that were similar to the *B. napus* parent for silique length was 6.7, 5.2, 14.5 and 13.5% for A04-73NA × YS49 cross, 13.2, 11.1, 19.5 and 6.7% for A04-73NA × T4-3-3-1 cross, and 17.9, 48.9, 13.9 and 0% for A04-73NA × 3-0026.027 cross, respectively (Table 2.1). Similarly, proportion of BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation plants that were similar to *B. napus* parent for silique length was 4.5, 2.8, 26.3 and 23.8% respectively (Table 2.2). When considering the cross A04-73NA × YS49, which had both F_2 and BC_1F_1 derived populations, 13.5% of the F_7 plants were *B. napus* type. In contrast, 23.8% of the BC_1F_4 generation plants were similar to the *B. napus* parent. This apparently resulted from higher contribution of the *B. napus* parent genome in BC_1F_1 derived population.

A summary of silique length in F_4, F_5, F_6 and F_7 generation plants of A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027 crosses are presented in Table 2.1 and in BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation plants of (A04-73NA × YS49) × A04-73NA cross in Table 2.2 along with *B. napus* parent A04-73NA as check. Average silique length of A04-73NA measured in 2012 greenhouse (grown along with F_4), in 2012 greenhouse (grown along with BC_1F_1), in 2013 field (grown along with F_5 and BC_1F_2), in 2013 greenhouse (grown along with F_6 and BC_1F_3) and in 2014 field (grown along with F_7 and BC_1F_4) was 51.1 ± 7.8 SD, 53.1 ± 4.0 SD, 55.3 ± 7.1 SD, 52.0 ± 6.0 and 66.6 ± 7.6 SD mm, respectively (Table 2.1 and 2.2).

Average, length of silique of the F_4, F_5, F_6 and F_7 populations of all three crosses was significantly shorter than the *B. napus* parent ($P < 0.05$) (Table 2.1); however, approximately 5-15% of the plants in these populations had silique size similar to the *B. napus* parent (Fig 2.2, Table 2.1). Mean silique length of the selected population in all cases was still significantly shorter than A04-73NA ($P < 0.05$) (Table 2.1). This is mainly due to inclusion of several plants with shorter silique during selection to capture greater allelic diversity in the euploid *B. napus* lines derived from these crosses. In F_7, 13.5 and 6.7% plants, from A04-73NA × YS49 and A04-73NA × T4-3-3-1 crosses respectively had silique size similar to A04-73NA; however, all plants of A04-73NA × 3-0026.027 had shorter silique than the *B. napus* parent. The *B. rapa* parent 3-0026.027 used in this cross had very short silique (1.9 cm, Kebede and Rahman 2014); therefore, an influence of this parent in this population cannot be ruled out. In backcross derived population of (A04-73NA × YS49) × A04-73NA, average silique length of BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 populations of backcross was significantly shorter than their *B. napus* parent ($P < 0.05$) (Table 2.2). In BC_1F_1 and BC_1F_3, silique length of the selected population was not significantly different from the whole population; however, in BC_1F_2 and BC_1F_4 silique length of the selected population was significantly greater ($P < 0.05$) than the whole population (Table 2.2). All selected populations of all generations were significantly ($P < 0.05$) shorter than A04-73NA. In BC_1F_4 generation, 23.8% of the plants have silique size similar to the *B. napus* parent.

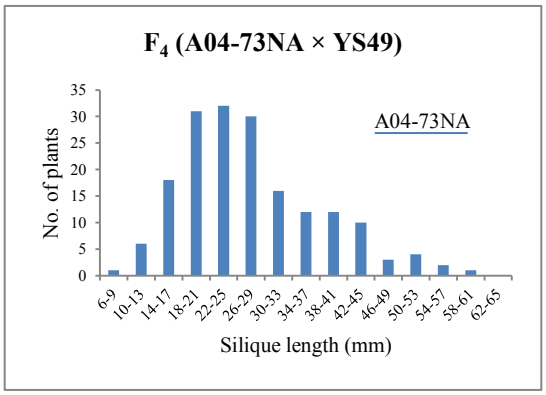
Number of seeds per silique

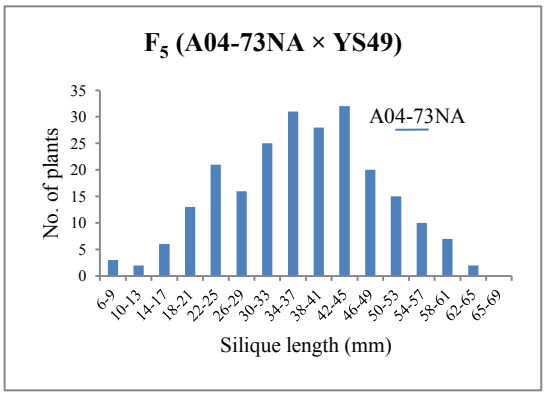
Confidence limits for the *B. napus* parent for number of seeds per silique was 12.4–22.3 (no. of plants 7), 15.0–19.4 (no. of plants 3), 31.1–33.9 (no. of plants 44), 15.0–25.6 (no. of plants 8) and 26.6–34.6 (no. of plants 12), respectively for the plants grown in 2012 greenhouse (grown along with F_4), in 2012 greenhouse (grown along with BC_1F_1), in 2013 field (grown along with

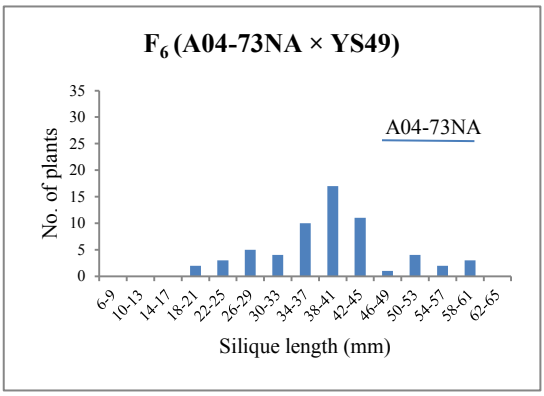
F_5, BC_1F_2), in 2013 greenhouse (grown along with F_6, BC_1F_3) and in 2014 field (grown along with F_7, BC_1F_4). Based on this, the proportion of *B. napus* type plants for number of seeds per silique in F_4, F_5, F_6 and F_7 generation populations was 1.1, 12.5, 25.2 and 17.3% for A04-73NA × YS49 cross, 5.7, 1.6, 28.7 and 44.2% for A04-73NA × T4-3-3-1 cross, and 2.8, 49.6, 16.3 and 19.1% for A04-73NA × 3-0026.027 cross, respectively (Table 2.3). Similarly, the proportion of BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation plants that were similar to the *B. napus* parent for number of seeds per silique was 0.9, 0.5, 15.2 and 0.9%, respectively (Table 2.4).

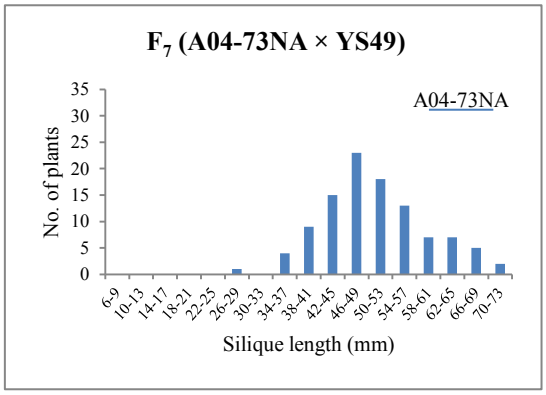
Number of seeds per silique in F_4, F_5, F_6 and F_7 generation plants of A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027 crosses are presented in Table 2.3, and in BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation plants of (A04-73NA × YS49) × A04-73NA presented in Table 2.4 along with the *B. napus* parent A04-73NA as check. Average number of seeds per silique of A04-73NA measured in 2012 greenhouse (grown along with F_4), in 2012 greenhouse (grown along with BC_1F_1), in 2013 field (grown along with F_5, BC_1F_2), in 2013 greenhouse (grown along with F_6, BC_1F_3) and in 2014 field (grown along with F_7, BC_1F_4) was 17.3 ± 5.4 SD, 17.2 ± 0.9 SD, 32.3 ± 4.8 SD, 20.3 ± 6.4 SD and 30.6 ± 6.3 SD seeds per silique, respectively (Table 2.3 and 2.4). The average number of seeds per silique in whole F_4, F_5, F_6 and F_7 populations as well as selected populations of all three crosses was significantly lower than the *B. napus* parent (P <0.05) (Table 2.3). However, an improvement in the number of seeds per silique over the generations was found. In F_7 generation, 17.3, 44.2 and 19.1% plants, respectively of the A04-73NA × YS49, A4-73NA × T43-3-1 and A04-73NA × 3-0026.027 crosses were similar to the *B. napus* parent for this trait. The average number of seeds per silique in BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 populations was significantly lower than the *B. napus* parent

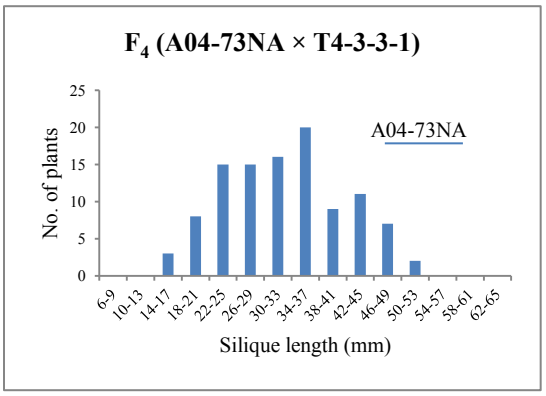
A04-73NA ($P < 0.05$). In BC_1F_4, about 1% of the population produced number of seeds per silique similar to the *B. napus* parent.

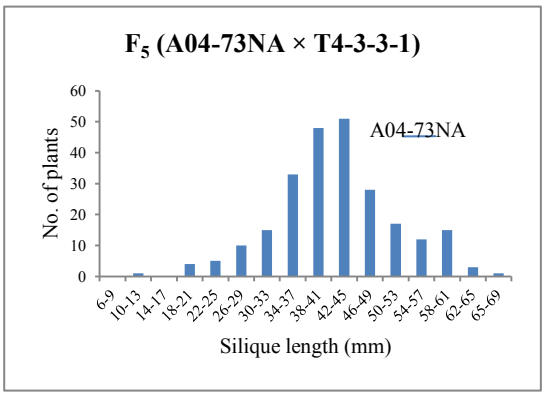












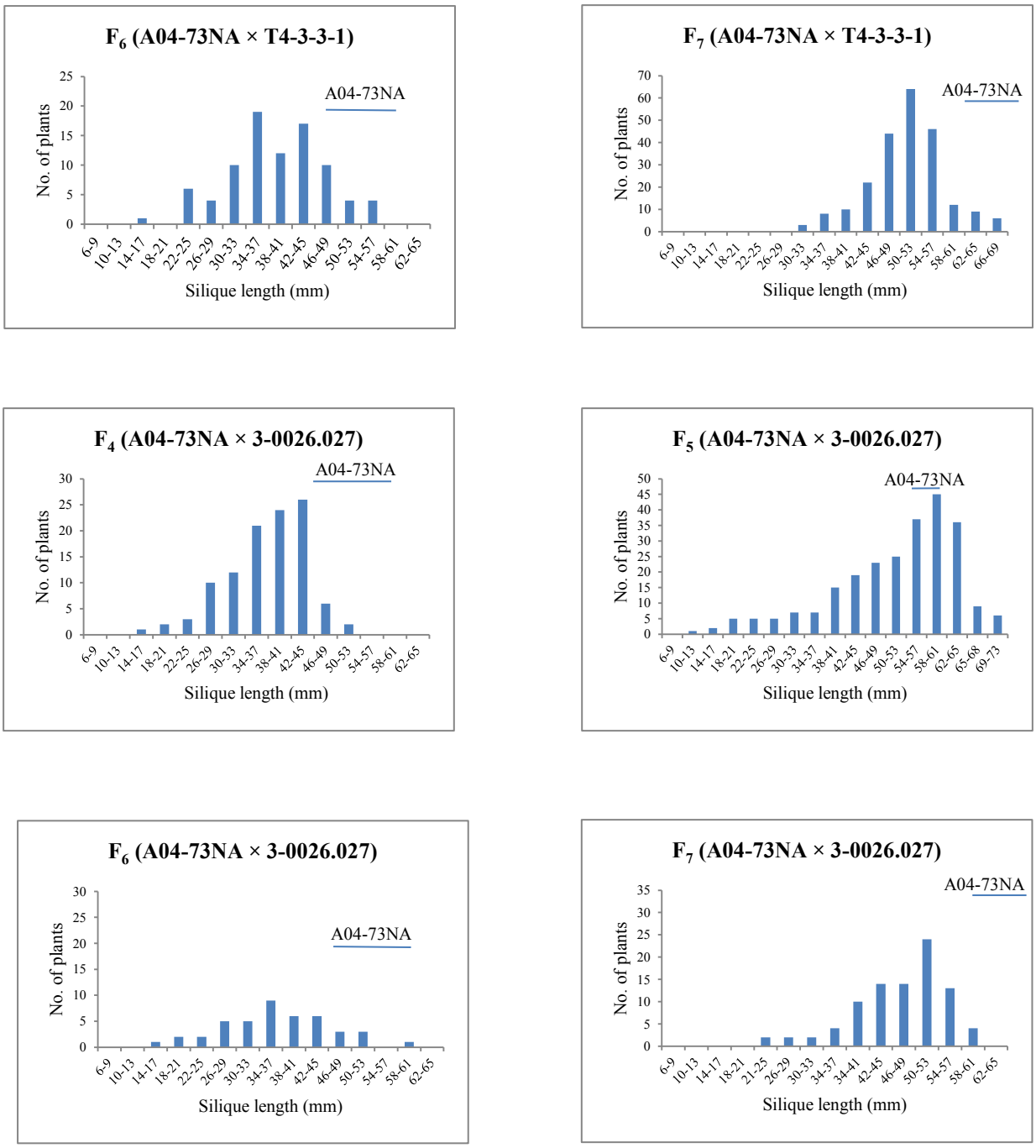


Figure 2.2 Frequency distribution of F_4, F_5, F_6 and F_7 populations, derived from different *Brassica napus* × *B. rapa* interspecific crosses, for silique length. A04-73NA = *B. napus*; YS49, T4-3-3-1 and 3-0026.027 = *B. rapa*

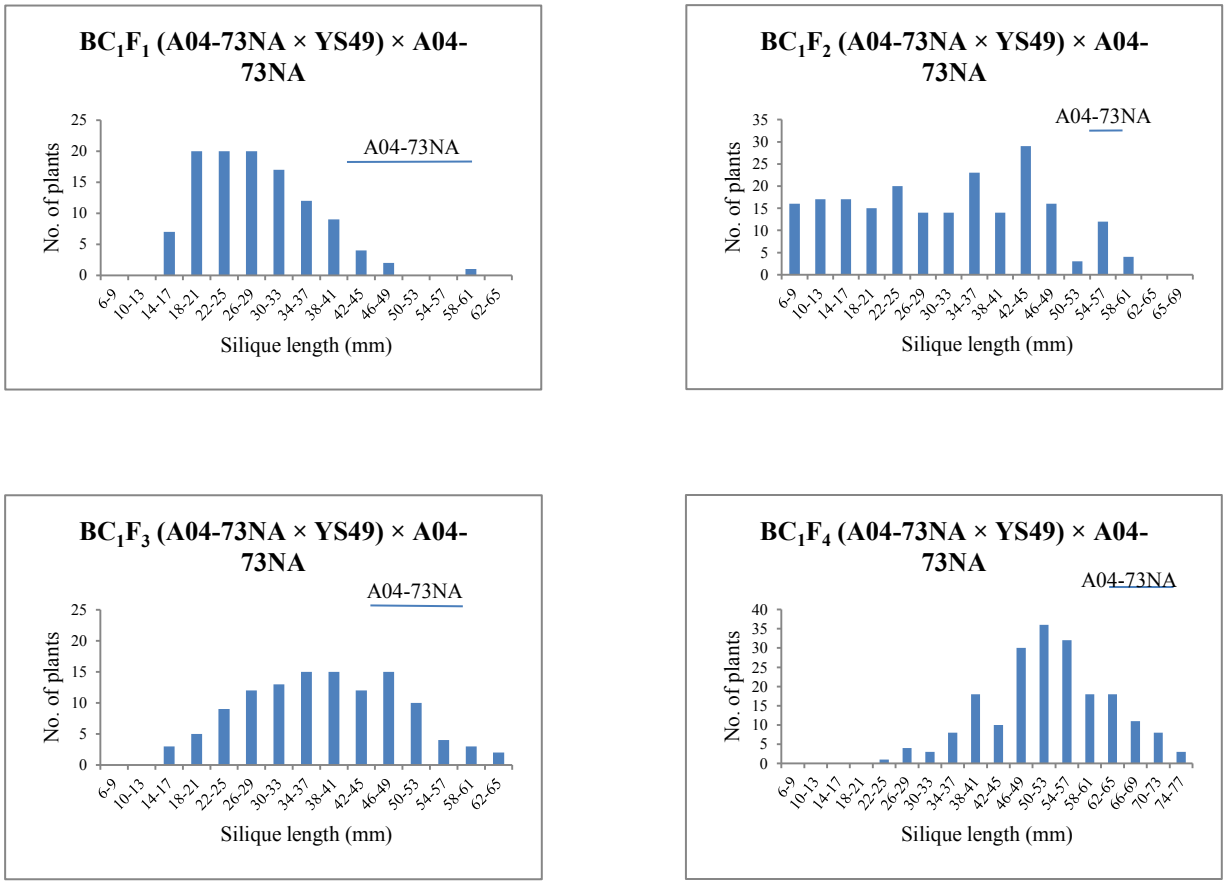


Figure 2.3 Frequency distribution of BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 populations, derived from (*B. napus* × *B. rapa*) × *B. napus* backcross, for silique length. A04-73NA = *B. napus*; YS49 = *B. rapa*

Table 2.1 Silique length (mm) in F_4, F_5, F_6 and F_7 generation populations of *B. napus* × *B. rapa* interspecific crosses

Cross[1]	F_4 (Greenhouse)			F_5 (Field)		
	Whole population	Percent *B. napus* type	Selected population	Whole Population	Percent *B. napus* type	Selected Population
	Range[2]		Range[2]	Range[2]		Range[2]
	Mean ± S.D.		Mean ± S.D.	Mean ± S.D.		Mean ± S.D.
A04-73NA × YS49	9–59 (178) 27.5 ± 10.0†	6.7	14–59 (85) 33.2 ± 9.8$^{\dagger\dagger a}$	8–64 (231) 36.7 ± 11.8†	5.2	20–64 (44) 40.4 ± 12.4$^{\dagger\dagger a}$
A04-73NA × T4-3-3-1	14–52 (106) 32.5 ± 8.7†	13.2	20–52 (85) 35.0 ± 7.6$^{\dagger\dagger a}$	12–65 (244) 41.9 ± 9.3†	11.1	24–65 (87) 44.3 ± 8.5$^{\dagger\dagger a}$
A04-73NA × 3-0026.027	21–50 (107) 37.3 ± 6.9†	17.9	19–50 (85) 38.2 ± 6.5^{a}	13–73 (247) 48.1 ± 12.2†	48.9	13–71 (39) 47.1 ± 14.4^{a}
A04-73NA (Check)	37–59 (7) 51.1 ± 7.8		37–59 (7) 51.1 ± 7.8	41–68 (44) 55.3 ± 7.1		41–68 (44) 55.3 ± 7.1

Cross[2]	F_6 (Greenhouse)			F_7 (Field)		
	Whole population	Percent *B. napus* type	Selected population	Whole population	Percent *B. napus* type	Selected population
	Range[1]		Range[1]	Range[1]		Range[1]
	Mean ± S.D.		Mean ± S.D.	Mean ± S.D.		Mean ± S.D.
A04-73NA × YS49	17–59 (62) 39.0 ± 8.9†	14.5	20–59 (59) 39.2 ± 8.8^{a}	26–72 (104) 50.6 ± 8.8†	13.5	34–72 (60) 51.9 ± 8.3^{a}
A04-73NA × T4-3-3-1	17–56 (87) 38.8 ± 8.4†	19.5	17–56 (87) 38.8 ± 8.4$^{\dagger a}$	30–68 (224) 50.7 ± 7.0†	6.7	40–63 (88) 52.3 ± 6.1$^{\dagger\dagger a}$
A04-73NA × 3-0026.027	15–60 (43) 38.0 ± 9.5†	13.9	15–60 (35) 36.8 ± 10.4^{a}	23–61 (89) 46.9 ± 7.8†	0	28–58 (48) 45.6 ± 7.0^{a}
A04-73NA (Check)	43–60 (8) 52.0 ± 6.0		43–60 (8) 52.0 ± 6.0	47–79 (12) 66.6 ± 7.6		60–79 (11) 66.6 ± 7.6

[1] A04-73NA = *B. napus*; YS49, T4-3-3-1, 3-0026.027 = *B. rapa*; [2] In brackets, number of plants examined given

† Whole population significantly different than the *B. napus* parent (A04-73NA) at $P < 0.05$

†† Selected population significantly different than whole population at $P < 0.05$

^{a} Selected population significantly different than the *B. napus* parent (A04-73NA) at $P < 0.05$

Table 2.2 Silique length (mm) in BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation populations of (*B. napus* × *B. rapa*) × *B. napus* interspecific cross

Cross[1]	BC_1F_1 (Greenhouse)			BC_1F_2 (Field)		
	Whole population	Percent *B. napus* type	Selected population	Whole population	Percent *B. napus* type	Selected population
	Range[2]		Range[2]	Range[2]		Range[2]
	Mean ± S.D.		Mean ± S.D.	Mean ± S.D.		Mean ± S.D.
(A04-73NA × YS49) × A04-73NA	14–58 (112) 28.2 ± 8.1[†]	4.5	15–58 (85) 30.0 ± 8.1[a]	5.3–61 (214) 30.7 ± 14.3[†]	2.8	7–54 (53) 34.2 ± 12.2[††a]
A04-73NA (Check)	50–58 (3) 53.1 ± 4.0		50–58 (3) 53.1 ± 4.0	41–68 (44) 55.3 ± 7.1		41–68 (44) 55.3 ± 7.1

Cross[1]	BC_1F_3 (Greenhouse)			BC_1F_4 (Field)		
	Whole population	Percent *B. napus* type	Selected population	Whole population	Percent *B. napus* type	Selected population
	Range[2]		Range[2]	Range[2]		Range[2]
	Mean ± S.D.		Mean ± S.D.	Mean ± S.D.		Mean ± S.D.
(A04-73NA × YS49) × A04-73NA	17–62 (118) 38.2 ± 11.1[†]	26.3	17–62 (101) 38.2 ± 11.1[a]	22–77 (202) 52.3 ±10.3[†]	23.8	33–77 (94) 55.9 ± 9.1[††a]
A04-73NA (Check)	43–60 (8) 52.0 ± 6.0		43–60 (8) 52.0 ± 6.0	47–79 (12) 66.6 ± 7.6		60–79 (11) 66.6 ± 7.6

[1] A04-73NA = *B. napus*; YS49= *B. rapa*; [2] In brackets, number plants examined given

[†] Whole population significantly different than the *B. napus* parent A04-73NA at $P < 0.05$

[a] Selected population significantly different than the *B. napus* parent (A04-73NA) at $P < 0.05$

[††] Selected population mean significantly different than whole population mean at $P < 0.05$

Table 2.3 Seeds per silique in F_4, F_5, F_6 and F_7 generation populations of *B. napus* × *B. rapa* interspecific crosses

Cross[1]	F_4 (Greenhouse)			F_5 (Field)		
	Whole population	Percent *B. napus* type	Selected population	Whole population	Percent *B. napus* Type	Selected population
	Range[2]		Range[2]	Range[2]		Range[2]
	Mean ± S.D.		Mean ± S.D.	Mean ± S.D.		Mean ± S.D.
A04-73NA × YS49	0–15 (178) 3.5 ± 3.5[†]	1.1	0–15 (85) 5.1 ± 3.5[††a]	1–80 (231) 20.4 ± 9.1[†]	12.5	7–37 (44) 21.9 ± 7.1[a]
A04-73NA × T4-3-3-1	0–23 (106) 7.1 ± 4.9[†]	5.7	0–23 (85) 8.5 ± 4.5[††a]	0–35 (244) 18.0 ± 7.9[†]	1.6	15–35 (87) 23.6 ± 4.6[††a]
A04-73NA × 3-0026.027	0–15 (107) 7.0 ± 3.4[†]	2.8	1–15 (85) 7.7 ± 3.2[a]	1–41 (247) 27.9 ± 8.8[†]	49.6	7–13 (39) 27.5 ± 8.7[a]
A04-73NA (Check)	15–21 (7) 17.3 ± 5.4		15–21 (7) 17.3 ± 5.4	18–43 (44) 32.3 ± 4.8		18–43 (44) 32.3 ± 4.8

Cross[1]	F_6 (Greenhouse)			F_7 (Field)		
	Whole population	Percent *B. napus* type	Selected population	Whole population	Percent *B. napus* Type	Selected population
	Range[2]		Range[2]	Range[2]		Range[2]
	Mean ± S.D.		Mean ± S.D.	Mean ± S.D.		Mean ± S.D.
A04-73NA × YS49	1–28 (62) 10.8 ± 5.7[†]	25.2	1–28 (59) 10.9 ± 5.6[a]	0–38 (104) 20.5 ± 7.1[†]	17.3	0–38 (60) 21.2 ± 7.0[a]
A04-73NA × T4-3-3-1	1–22 (87) 11.0 ± 5.2[†]	28.7	1–22 (87) 11.0 ± 5.2[†a]	8–38 (224) 24.8 ± 5.9[†]	44.2	14–38 (88) 26.8 ± 4.9[††]
A04-73NA × 3-0026.027	1–19 (43) 9.3 ± 4.5[†]	16.3	1–19 (35) 9.1 ± 4.8[a]	1–31 (89) 18.5 ± 7.1[†]	19.1	3–29 (48) 19.2 ± 6.8[a]
A04-73NA (Check)	13–31 (8) 20.3 ± 6.4		13–31 (8) 20.3 ± 6.4	18–39 (12) 30.6 ± 6.3		18–39 (11) 30.6 ± 6.3

[1] A04-73NA = *B. napus*; YS49, T4-3-3-1, 3-0026.027 = *B. rapa*; [2] In brackets, number plants examined given

[†] Whole population significantly different than the *B. napus* parent (A04-73NA) at $P < 0.05$, [††] Selected population significantly different than whole population at $P < 0.05$, [a] Selected population significantly different than the *B. napus* parent (A04-73NA) at $P < 0.05$

Table 2.4 Seeds per silique in BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation populations of (*B. napus* × *B. rapa*) × *B. napus* interspecific cross

Cross[1]	BC_1F_1			BC_1F_2		
	Whole population	Percent *B. napus* type	Selected population	Whole population	Percent *B. napus* type	Selected population
	Range[2]		Range[2]	Range[2]		Range[2]
	Mean ± S.D		Mean ± S.D	Mean ± S.D		Mean ± S.D
(A04-73NA × YS49) × A04-73NA	0–19 (112) 3.5 ± 3.2[†]	0.9	0–19 (85) 4.2 ± 3.3[a]	0–33 (214) 11.4 ± 7.2[†]	0.5	0–33 (53) 13.4 ± 6.7[††a]
A04-73NA (Check)	16–18 (3) 17.2 ± 0.9		16–18 (3) 17.2 ± 0.9	18–43 (44) 32.3 ± 4.8		18–43 (44) 32.3 ± 4.8

Cross[1]	BC_1F_3			BC_1F_4		
	Whole population	Percent *B. napus* type	Selected population	Whole population	Percent *B. napus* type	Selected population
	Range[2]		Range[2]	Range[2]		Range[2]
	Mean ± S.D		Mean ± S.D	Mean ± S.D		Mean ± S.D
(A04-73NA × YS49) × A04-73NA	0–25 (118) 8.8 ± 5.6[†]	15.2	0–25 (101) 8.8 ± 5.6[a]	0–36 (202) 21.4 ± 7.0[†]	0.9	11–36 (94) 24.2 ± 5.8[††a]
A04-73NA (Check)	11–31 (8) 20.3 ± 6.4		11–31 (8) 20.3 ± 6.4	18–39 (12) 30.6 ± 6.3		18–39 (12) 30.6 ± 6.3

[1] A04-73NA = *B. napus*; YS49= *B. rapa*; [2] In brackets, number plants examined given

[†] Whole population significantly different than the *B. napus* parent A04-73NA at $P < 0.05$

[a] Selected population significantly different than the *B. napus* parent (A04-73NA) at $P < 0.05$

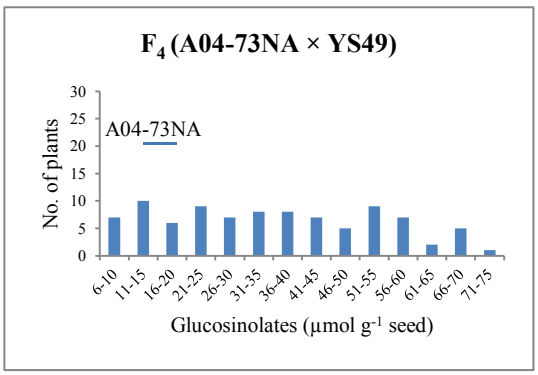
[††] Selected population mean significantly different than whole population mean at $P < 0.05$

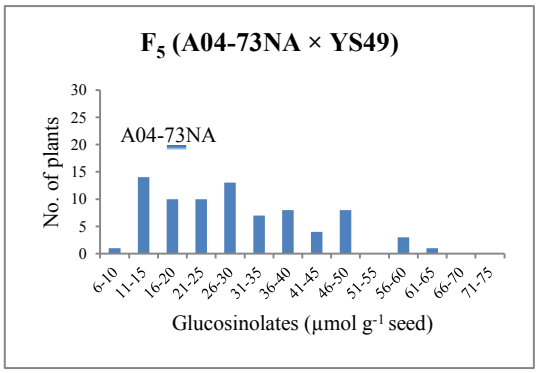
2.3.2 Glucosinolates content in population derived from *B. napus* × *B. rapa* interspecific crosses

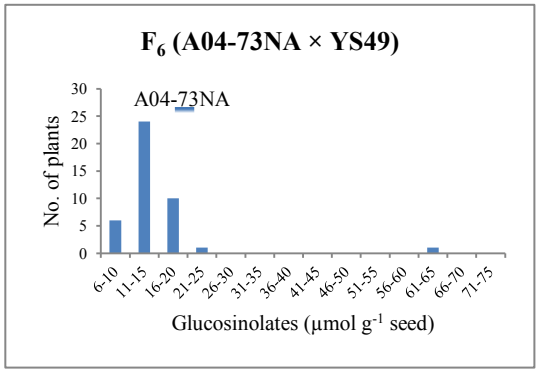
Frequency distribution of the F_4, F_5, F_6 and F_7 plants and of BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 plants of *B. napus* × *B. rapa* crosses for GSL content, presented in Fig 2.4 and 2.5. Confidence limits for the *B. napus* parent for GSL content were 10.7–16.5 (no. of plants 6), 15.7–17.0 (no. of plants 111), 10.8–12.9 (no. of plants 7) and 17.0–17.8 (no. of plants 93) μmol g^{-1} seed respectively for the plants grown in 2012 greenhouse (grown along with F_4, BC_1F_1), 2013 field (grown along with F_5, BC_1F_2), in 2013 greenhouse (grown along with F_6, BC_1F_3) and in 2014 field (grown along with F_7, BC_1F_4). Based on this, the proportion of F_4, F_5, F_6 and F_7 generation plants of A04-73NA × YS49 that were either similar or better than the *B. napus* parent was 23.1, 10.2, 27.6 and 48.2% respectively. On the other hand, 76.8, 53.5 and 96.6% plants of A04-73NA × T4-3-3-1 and 9.4, 33.3 and 42.8% plants of A04-73NA × 3-0026.027 were either similar or better than A04-73NA in F_5, F_6 and F_7 generations cross, respectively (Table 2.5). In BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4, the proportion, of plants that were similar to the *B. napus* parent for GSL content was 16.3, 8.3, 28.8 and 50%, respectively.

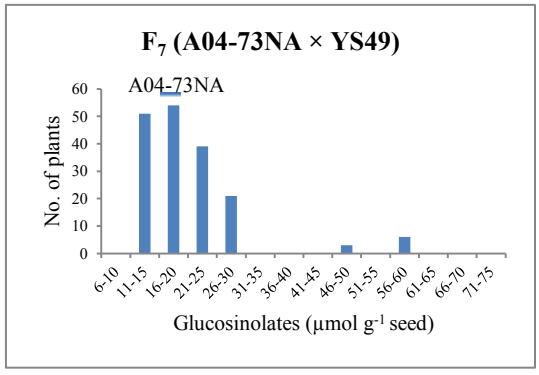
A summary of GSL content in F_4, F_5, F_6 and F_7 generation plants of A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027 crosses are presented in Table 2.5, and in BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation plants of (A04-73NA × YS49) × A04-73NA are presented in Table 2.6 along with the *B. napus* par ent A04-73NA as check. Average GSL content of A04-73NA measured in seeds harvested from 2012 greenhouse (grown along with F_4, BC_1F_1), in 2013 field (grown along with F_5, BC_1F_2), in 2013 greenhouse (grown along with F_6, BC_1F_3) and in 2014 field (grown along with F_7, BC_1F_4) was 13.6 ± 2.5 SD, 16.2 ± 1.5 SD, 11.9 ± 1.2 SD and 17.4 ± 1.8 SD μmol g^{-1} seed, respectively (Table 2.5 and 2.6). Glucosinolate content

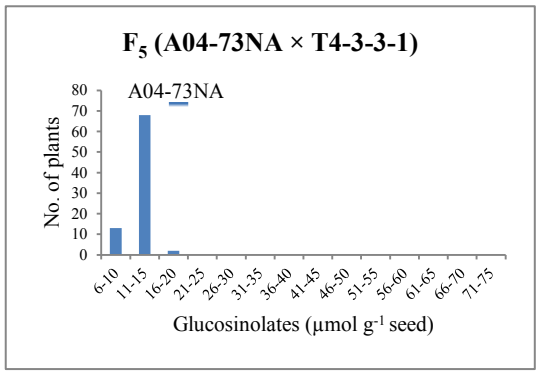
of F_4 and F_5 population of A04-73NA × YS49 and F_5 and F_7 population of A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027 was significantly higher than the *B. napus* parent ($P < 0.05$). In case of the cross A04-73NA × T4-3-3-1, GSL content in F_5 population was significantly lower than the *B. napus* parent. In F_7 generation, 48.6, 96.6 and 42.8% plants respectively of A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027 crosses had GSL content either similar or lower than the *B. napus* parent. The average glucosinolates content in BC_1F_1, BC_1F_2 and BC_1F_3 populations was significantly higher than the *B. napus* parent ($P < 0.05$); however, no significant difference ($P < 0.05$) was found in case of BC_1F_4 population (Table 2.6). In BC_1F_4 generation, 50% of the plants had GSL content either similar or lower than the *B. napus* parent.

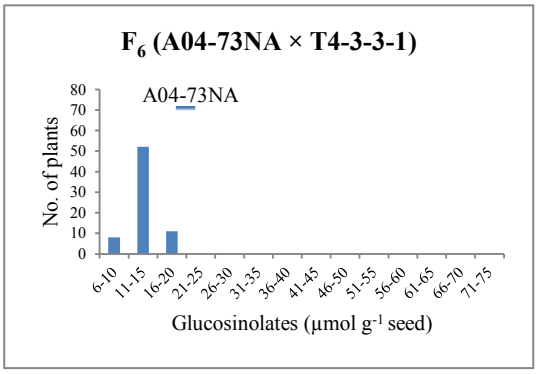


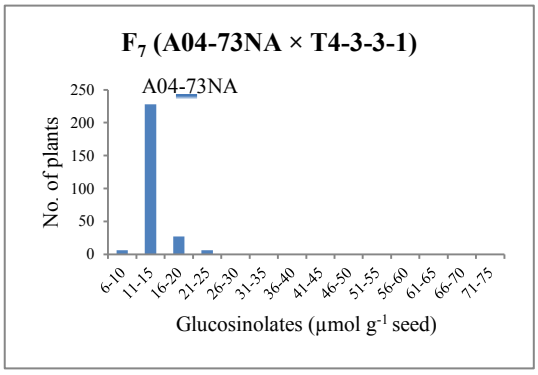


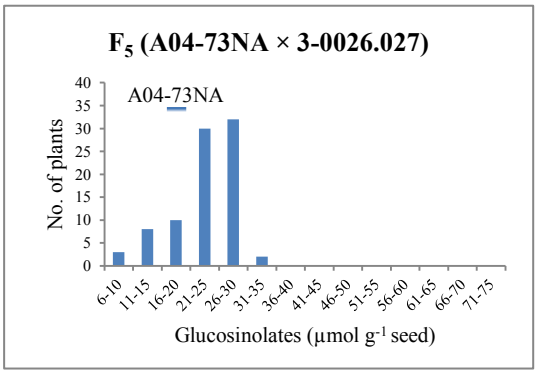


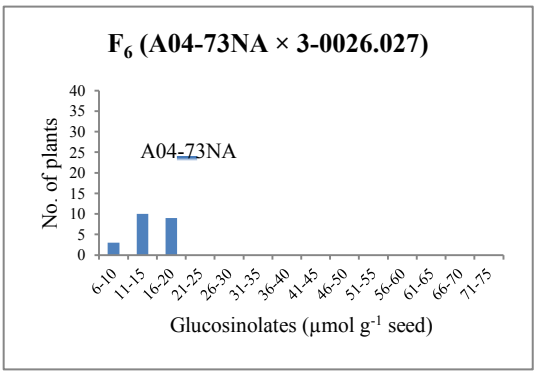












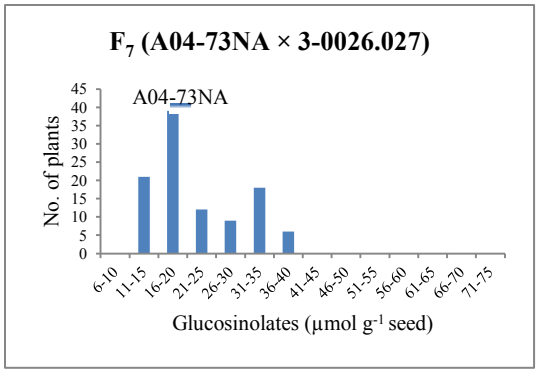


Figure 2.4 Frequency distribution of F_4, F_5, F_6 and F_7 populations, derived from different *B. napus* × *B. rapa* interspecific crosses, for glucosinolates content. A04-73NA = *B. napus*; YS49, T4-3-3-1 and 3-0026.027 = *B. rapa*

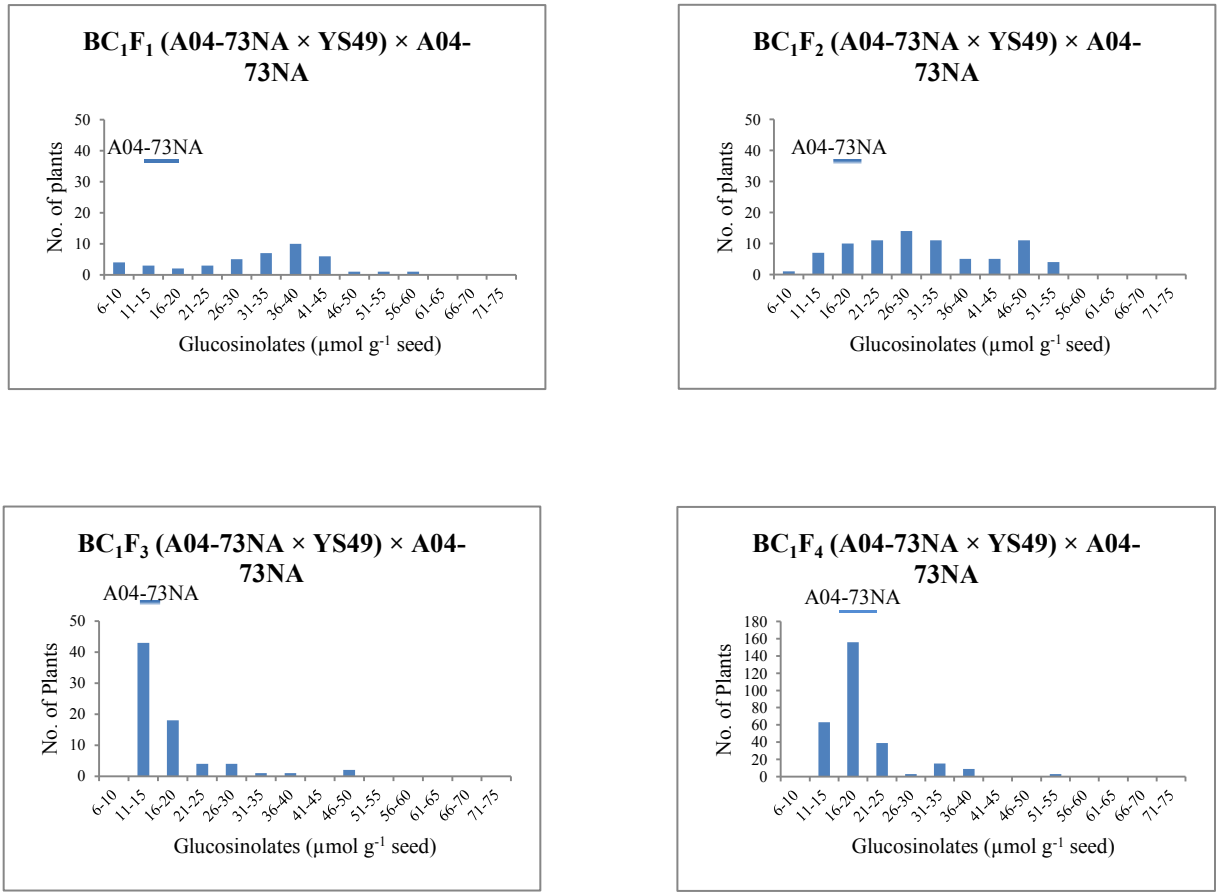


Figure 2.5 Frequency distribution of BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 populations, derived from (*B. napus* × *B. rapa*) × *B. napus*, for glucosinolates content. A04-73NA = *B. napus*; YS49 = *B. rapa*

Table 2.5 Glucosinolates (GSL) (µmol g^{-1} seed) content in F_4, F_5, F_6 and F_7 generation populations derived from *B. napus* × *B. rapa* interspecific crosses

Cross[1]	F_4 (Greenhouse)				F_5 (Field)			
	Whole population	Percent *B. napus* type	Percent better than *B. napus*	Selected population	Whole population	Percent *B. napus* type	Percent better than *B. napus*	Selected population
	Range[2]			Range[2]	Range[2]			Range[2]
	Mean ± S.D			Mean ± S.D	Mean ± S.D			Mean ± S.D
A04-73NA × YS49	7–70 (91) 35.2 ± 18.3†	13.2	9.9	7–39 (55) 22.7 ± 10.3††a	9–61 (237) 29.0 ± 13.0†	8.9	1.3	9–19 (44) 15.1 ± 3.0††
A04-73NA × T4-3-3-1	No Data	No Data	No Data	No Data	8–18 (246) 12.9 ± 4.9†	62.2	14.6	8–16 (86) 12.3 ± 1.6a
A04-73NA × 3-0026.027	No Data	No Data	No Data	No Data	8–32 (255) 22.7 ± 5.5†	5.9	3.5	8–26 (40) 16.6 ± 5.2††
A04-73NA (Check)	10–17 (6) 13.6 ± 2.5			10–17 (6) 13.6 ± 2.5	13–19 (37) 16.2 ± 1.5			13–19 (37) 16.2 ± 1.5

Cross[1]	F_6 (Greenhouse)				F_7 (Field)			
	Whole population	Percent *B. napus* type	Percent better than *B. napus*	Selected population	Whole population	Percent *B. napus* type	Percent better than *B. napus*	Selected population
	Range[2]			Range[2]	Range[2]			Range[2]
	Mean ± S.D			Mean ± S.D	Mean ± S.D			Mean ± S.D
A04-73NA × YS49	7–64 (42) 14.9 ± 8.4	33.3	14.3	7–19 (39) 13.4 ± 3.0	11–59 (174) 20 ± 9.6	1.7	46.5	11–20 (71) 14.6 ± 2.4††a
A04-73NA × T4-3-3-1	9–19 (71) 13.3 ± 2.3	42.2	11.3	9–19 (71) 13.3 ± 2.3	9–23(267) 13.2 ± 2.1†	1.1	95.5	9–19 (94) 12.8 ± 1.6††a
A04-73NA × 3-0026.027	7–20.4 (24) 18.9 ± 14.8	20.8	12.5	7–18 (20) 14.1 ± 2.9	10–40 (105) 21.9 ± 7.9†	11.4	31.4	10–22 (43) 16.5 ± 2.7††
A04-73NA (Check)	10–13 (7) 11.9 ± 1.2			10–13 (7) 11.9 ± 1.2	14–20 (93) 17.4 ± 1.8			14–20 (93) 17.4 ± 1.8

[1] A04-73NA = *B. napus*; YS49, T4-3-3-1 and 3-0026.027 = *B. rapa*; [2] In brackets, number plants examined given; † Whole population significantly different than the *B. napus* parent A04-73NA at $P < 0.05$;
†† Selected population significantly different than whole population mean at $P < 0.05$; [a] Selected population significantly different than the *B. napus* at $P < 0.05$

Table 2.6 Glucosinolate (GSL) (μmol g^{-1} seed) content in BC_1F_1, BC_1F_2, BC_1F_3 and BC_1F_4 generation population derived from (*B. napus* × *B. rapa*) × *B. napus* interspecific cross

Cross[1]	BC_1F_1 (Greenhouse)				BC_1F_2 (Field)			
	Whole population	Percent *B.napus* type	Percent better than *B.napus*	Selected population	Whole population	Percent *B.napus* type	Percent better than *B.napus*	Selected population
	Range[2]			Range[2]	Range[2]			Range[2]
	Mean ± S.D.			Mean ± S.D.	Mean ± S.D.			Mean ± S.D.
(A04-73NA × YS49)	7–57 (43)	7.0	9.3	7–48 (40)	9–54 (79)	7.1	1.2	9–26 (53)
× A04-73NA	31.0 ± 12.4[†]			29.5 ± 11.3[a]	30.2 ± 11.9[†]			18.3 ± 4.8[††]
A04-73NA (Check)	10–17 (6)			10–17 (6)	13–19 (111)			13–19 (111)
	13.6 ± 2.5			13.6 ± 2.5	16.2 ± 1.5			16.2 ± 1.5

Cross[1]	BC_1F_3 (Greenhouse)				BC_1F_4 (Field)			
	Whole population	Percent *B.napus* type	Percent better than *B.napus*	Selected population	Whole population	Percent *B.napus* type	Percent better than *B.napus*	Selected population
	Range[2]			Range[2]	Range[2]			Range[2]
	Mean ± S.D.			Mean ± S.D.	Mean ± S.D.			Mean ± S.D.
(A04-73NA × YS49)	11–48 (73)	28.8	0.0	11–20 (61)	12–53 (288)	8.3	41.7	12–21 (100)
× A04-73NA	17.1 ± 7.3[†]			14.5 ± 2.3[††a]	18.8 ± 6.3			15.6 ± 1.9[††]
A04-73NA (Check)	10–13 (7)			10–13 (7)	14–20 (93)			14–20 (93)
	11.9 ± 1.2			11.9 ± 1.2	17.4 ± 1.8			17.4 ± 1.8

[1]A04-73NA = *B. napus*; YS49 = *B. rapa*; [2] In brackets, number plants examined given

[†] Whole population significantly different than the *B. napus* parent A04-73NA at $P < 0.05$

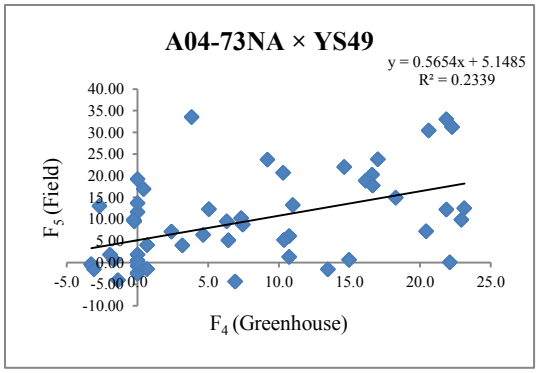
[a] Selected population significantly different than the *B. napus* parent A04-73NA at $P < 0.05$

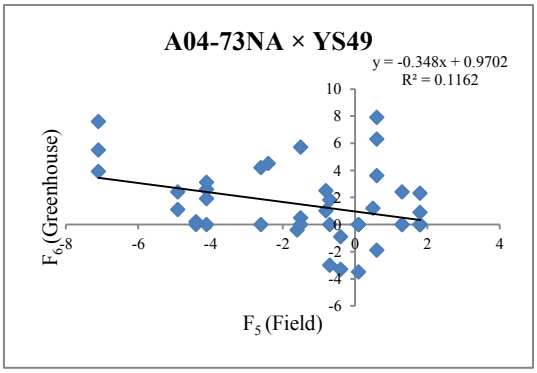
[††] Selected population lower than the whole population at $P < 0.05$

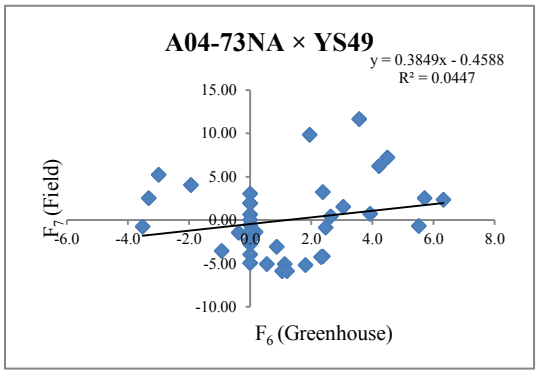
Relationship between parent and offspring populations of *B. napus* × *B. rapa* crosses

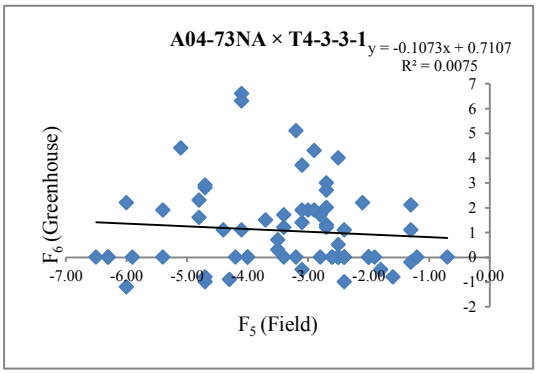
Glucosinolate content in seed of the *B. napus* parent A04-73NA harvested from greenhouse or field in different year varied between 11.9 and 17.4 μmol/g seed. To compare GSL content in given generation population harvested from a given growth condition, GSL data was adjusted to reflect the content relative to A04-73NA. For this, GSL content of A04-73NA was subtracted from the GSL content of the interspecific cross derived plants grown in the same environment. This gave '+ ive' or '- ive' or '0' values reflecting the difference of these plants from A04-73NA for GSL content.

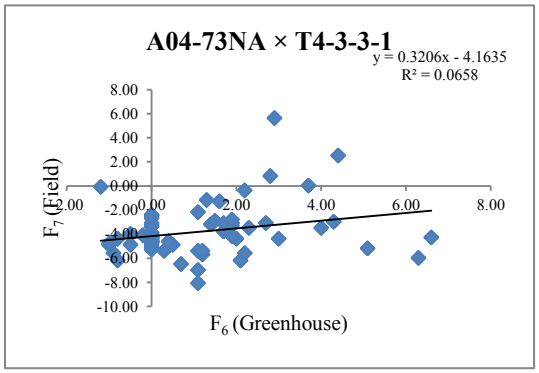
Scattered diagram and correlation between different generation populations derived from F_2 are presented in Fig 2.6, and for the population derived from BC_1F_1 are presented in Fig 2.7. No consistent correlation between parent and offspring generation was found for GSL content in these interspecific cross derived plants. For example, correlation between F_4 and F_5 generation of A04-73NA × YS49 ($r = 0.47$, $P < 0.05$), F_6 and F_7 generation of A04-73NA × YS49 ($r = 0.23$, $P < 0.05$) , A04-73NA × T4-3-3-1 ($r = 0.24$, $P < 0.05$) and A04-73NA × 3-0026.027 ($r = 0.51$, $P < 0.05$) crosses were positive and significant; however, no significant correlation was found between F_5 and F_6 generations of A04-73NA × YS49 (r = -0.30), A04-73NA × T4-3-3-1 (r = -0.16) and A04-73NA × 3-0026.027 (r = 0.16) crosses. Similarly, no significant correlation found between BC_1 and BC_1F_2 (r = 0.17), and BC_1F_3 and BC_1F_4 (r = 0.034) generations; however, a weak significant negative correlation was found between BC_1F_2 and BC_1F_3 ($r = -0.50$, $P < 0.05$) generation populations. Several F_2 and BC_1F_1 derived families had GSL content lower than the *B. napus* parent (Table 2.5 and 2.6).

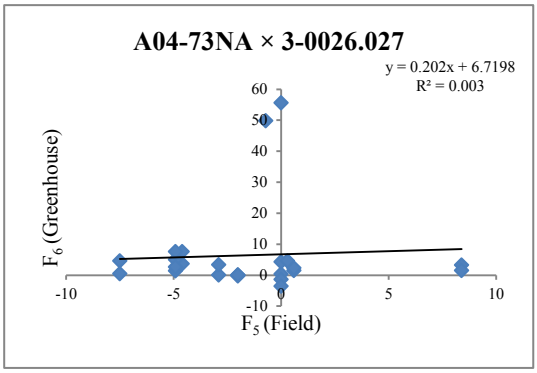












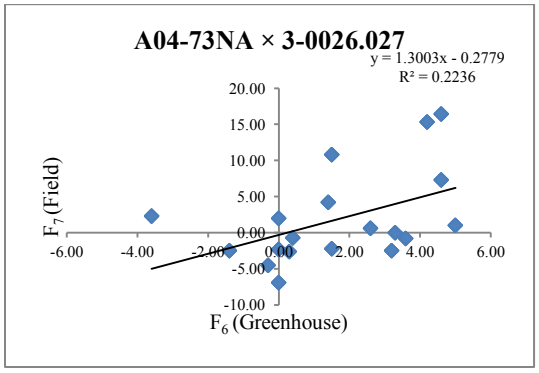
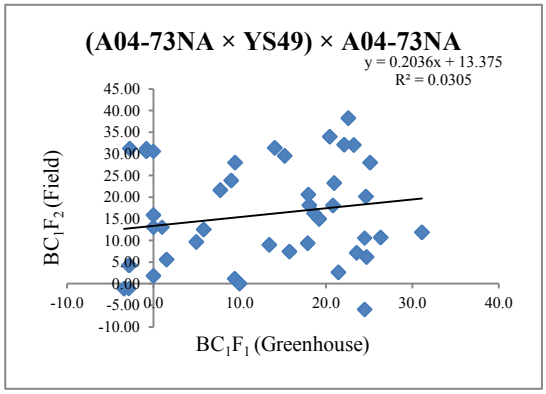
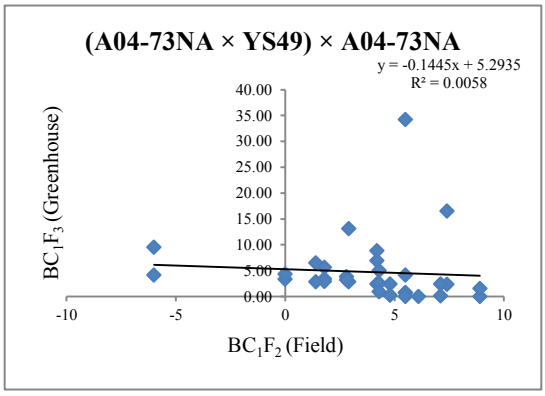


Figure 2.6 Scattered plot diagrams of parent vs offspring generation of *B. napus* × *B. rapa* interspecific crosses, for glucosinolates content. A04-73NA = *B. napus*; YS49, T4-3-3-1 and 3-0026.027 = *B. rapa*





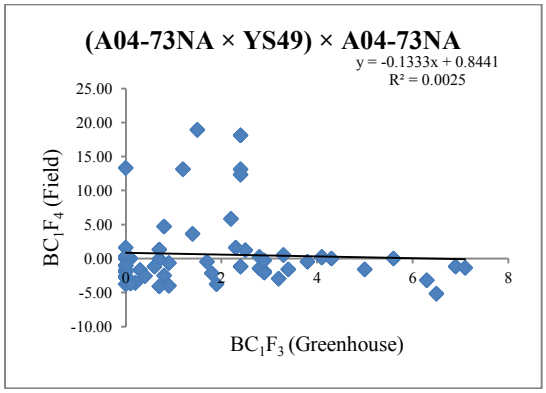


Figure 2.7 Scattered plot diagrams of parent vs offspring generation (*B. napus* × *B. rapa*) × *B. napus* interspecific cross for glucosinolates content. A04-73NA = *B. napus*; YS49 = *B. rapa*

2.3.3 Flow cytometric analysis of population derived from *B. napus* × *B. rapa* interspecific crosses

Confidence limits for the *B. napus* parent for nuclear DNA content was 395–396 (no. of plants 8) and 395–397 (no. of plants 8) respectively, for the plants grown in 2013 field (grown along with F_5, BC_1F_2) and in 2013 greenhouse (grown along with F_6, BC_1F_3). Based on this, 1-2% of the F_5 plants of the three crosses were similar to *B. napus* parent for nuclear DNA content; however, none of the F_6 generation plant had DNA content similar to the *B. napus* parent (Table 2.7). In backcross population 0 and 1.5% of the BC_1F_2 and BC_1F_3 plants were similar to the *B. napus* parent (Table 2.8).

Relative nuclear DNA content in F_5 and F_6 generation plants of A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027 crosses, and in BC_1F_2 and BC_1F_3 generation plants of (A04-73NA × YS49) × A04-73NA cross are presented in Table 2.7 and 2.8. The mean relative nuclear DNA content of A04-73NA measured in 2013 field (grown along with F_5, BC_1F_2) and in 2013 greenhouse (grown along with F_6, BC_1F_3) was 395.4 ± 0.9 SD and 396.2 ± 0.9 SD respectively (Table 2.7 and 2.8). Nuclear DNA content in the whole as well as selected F_5 populations of A04-73NA × YS49 and F_6 populations of A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027 cross was significantly lower than their *B. napus* parent ($P < 0.05$) (Table 2.7). Nuclear DNA content in several F_2 and BC_1F_1 derived plants had reached close to the *B. napus* DNA content. This variation in F_2 and BC_1F_1 derived population can be used to select the *B. napus* type plants.

Mean nuclear DNA content of BC_1F_2 population is significantly lower than the *B. napus* parent ($P < 0.05$); however, no significant difference was found between BC_1F_3 population and the *B. napus* parent ($P < 0.05$) (Table 2.8).

Table 2.7 Relative nuclear DNA content in F_5 and F_6 generation populations of *B. napus* × *B. rapa* interspecific crosses

Cross[1]	F_5 (Field)			F_6 (Greenhouse)		
	Whole population	Percent *B. napus* type	Selected population	Whole population	Percent *B. napus* type	Selected population
	Range[2]		Range[2]	Range[2]		Range[2]
	Mean ± S.D.		Mean ± S.D.	Mean ± S.D.		Mean ± S.D.
A04-73NA × YS49	204–462 (61)	1.6	236–364 (12)	117–387 (59)	0	117–387 (45)
	322.8 ± 63.3[†]		293.0 ± 47.4[a]	359.4 ± 35.0[†]		361.2 ± 38.9[††a]
A04-73NA × T4-3-3-1	220–452 (135)	2.2	286 –421 (43)	101–379 (98)	0	327–379 (89)
	367.7 ± 31.1		380.3 ± 60.3[††]	349.7 ± 26.3[†]		352.7 ± 6.9[a]
A04-73NA × 3-0026.027	208–413 (108)	0.9	300–413 (14)	70–373 (62)	0	333–364 (38)
	338.8 ± 52.8[†]		361.0 ± 29.0[††]	348.0 ± 37.0[†]		350.3 ± 8.0[a]
A04-73NA (Check)	394–397 (8)		394–397 (8)	394–397 (8)		394–397 (8)
	395.4 ± 0.9		395.4 ± 0.9	396.2 ± 0.9		396.2 ± 0.9

[1]A04-73NA = *B. napus*; YS49, T4-3-3-1 and 3-0026.027 = *B. rapa*; [2]In brackets, number plants examined given
[†]Whole population significantly less than the *B. napus* parent A04-73NA at $P < 0.05$
[a] Selected population less than the *B. napus* parent A04-73NA at $P < 0.05$
[††] Selected population mean more than whole population mean at $P < 0.05$

Table 2.8 Relative nuclear DNA content in BC_1F_2 and BC_1F_3 generation populations of (*B. napus* × *B. rapa*) × *B. napus* interspecific cross

Cross[1]	BC_1F_2			BC_1F_3		
	Whole population Range[2]	Percent *B. napus* type	Selected population Range[2]	Whole population Range[2]	Percent *B. napus* type	Selected population Range[2]
	Mean ± S.D.		Mean ± S.D.	Mean ± S.D.		Mean ± S.D.
(A04-73NA × YS49) × A04-73NA	128–499 (42)	0	254 – 458 (9)	289–468 (135)	1.5	321 – 463 (90)
	359.4 ± 67.4[†]		344.6 ± 60.5[a]	373.3 ± 32.3		367.5 ± 26.4
A04-73NA (Check)	394– 397 (8)		394– 397 (8)	394–397 (8)		394–397 (8)
	395.4 ± 0.9		395.4 ± 0.9	396.2 ± 0.9		396.2 ± 0.9

[1] A04-73NA = *B. napus*; YS49 = *B. rapa*; [2] In brackets, number plants examined given
[†] Whole population significantly different from the *B. napus* parent A04-73NA at $P < 0.05$
[††] Selected population significantly different than whole population mean at $P < 0.05$
[a] Selected population significantly different than the *B. napus* parent A04-73NA at $P < 0.05$

2.4 Discussion

The three Brassica genomes, A; B and C are believed to have evolved from a *Arabidopsis*-like ancestral species (Lagercrantz 1998, Lysak et al. 2007, reviewed in Schranz et al. 2006). Although, these genomes and different Brassica species evolved over different period of time, some similarity exists between these Brassica species. Therefore, it is possible to obtain hybrids from most interspecific crosses in Brassica (Röbbelen et al. 1989), with or without application of cell and tissue culture techniques (Rahman 2004, Bennett et al. 2008). However, development of canola quality *B. napus* lines from interspecific crosses are labour and resource intensive (Rahman 2013), as early generation plants show high sterility due to chromosomal anomalies (Kianian and Quiros 1992).

Plant fertility was assessed based on agronomic traits, such as silique length and number of seeds per silique. Estimation of silique length and number of seeds per silique gives idea of the extent of viable male and female gametes produced in the interspecific cross derived plants. Cytological study of the plants derived from an interspecific cross can provide knowledge of chromosomal number in the plants and their behavior in meiosis. However, this is labour intensive and time consuming. On the other hand, flow cytometeric analysis of nuclear DNA content can provide approximate estimate of chromosome number of the plants (Arumuganathan and Earle 1991). The combined knowledge of plant fertility, estimated based on silique length and number of seeds per silique, and relative nuclear DNA content can be used to identify euploid plants (Rahman 2001, Bennett et al. 2012). Many F_6 and BC_1F_4 generation plants had plant fertility and nuclear DNA content close to the *B. napus* parent. This was achieved through selection of fertile plants in each generation. Relative nuclear DNA content data also shows that the population progressively become close to *B. napus.* Rahman (2001) and Zaman (1989)

reported that interspecific hybrid progenies derived from amphidiploid and diploid species often stabilize into amphidiploid type. In this regard, the present result also agrees with the earlier findings. Relative nuclear DNA content data also shows that both F_2 and BC_1F_1 derived populations progressively become similar to the *B. napus* parent with each passing generation.

One of the *B. rapa* parent YS49 used in this study contains high GSL in seed; therefore, inheritance study of this trait in the population derived from A04-73NA × YS49 cross was especially interesting. Seed GSL content in *B. napus* is a quantitative trait and controlled by at least four loci whereas recessive alleles at all loci resulting low GSL content (Rahman et al. 2001). In this context, selection for low GSL content in the interspecific cross derived progeny would resulting lower GSL content in the subsequent generations. Data from this study also revealed that GSL content in both F_2 and BC_1F_1 derived populations reduced in each generation, whereas the F_6 and BC_1F_3 population were statistically similar to the *B. napus* parent. Correlation between parent and offspring generation populations was very weak or non- significant for this trait. Bahrani and McVetty (2008) reported a moderate correlation for this trait while comparing GSL content in oilseed *B. napus* seeds harvested from field and greenhouse. The discrepancy between the present study and the study conducted by Bahrani and McVetty (2008) is due to the type of material used. The present study was conducted using segregating population derived from interspecific crosses where wide variation for seed set was found due to sterility. (Holm et al. 1985, cited by Rahman et al. 2014) found higher GSL content in seeds harvested from poorly pollinated (low seed set) *B. rapa* plants as compared to well pollinated plants with good seed set; this might explain the difference between my study and the study conducted by Bahrani and McVetty (2008). For the same reason, some of the interspecific cross derived plants which had low GSL content produced progeny with higher GSL content.

According to Falk (2010) limited backcross approach could be used to develop elite lines from crosses involving genetically distant parent; this approach was also studied in *B. napus* × *B. rapa* interspecific cross in this thesis research. The BC_1F_1 derived population was expected to be agronomically superior compared to F_2 derived population as much greater content of the *B. napus* genome was introduced into the segregating population. Similarly, BC_1F_1 derived population was expected to have less meiotic anomalies to F_2 derived population. Indeed, a sharp increase in the percent of *B. napus* type plants was observed in very early generation of BC_1F_1 derived population compared to F_2 derived populations.

In conclusion, progeny derived from *B. napus* × *B. rapa* interspecific crosses not only segregate for different agronomic and quality traits, but also for plant sterility. Intensive breeding efforts over many generations will be needed to develop canola quality and fully fertile *B. napus* germplasm from these interspecific cross. Potential of these newly developed euploid *B. napus* lines needs to be investigated for use in breeding of hybrid cultivars.

Chapter 3

Study of allelic diversity in F_2 derived population of *B. napus* × *B. rapa* interspecific cross by SSR markers

3.1 Introduction

Genetic improvement in a crop through breeding requires adequate genetic diversity in its germplasm (Hoisington et al. 1999). Lower genetic diversity in a crop increases the risk of susceptibility to insect and diseases; and also a challenge for further improvement of the crop (Cowling 2007**,** Juska et al. 1997). Genetic diversity in a crop can be broadened though crossing of elite cultivars or lines with germplasm belonging to different forms of the same species or with its allied species (reviewed in Rahman 2013). Phenotypic traits and enzyme- or DNA-based molecular markers can be used to estimate genetic diversity in a crop gerpmpalsm for use in breeding. Some researchers, such as Ali et al. (1995) used phenotypic traits to estimate genetic diversity in *B. napus*; however, this approach has limitation as many of the traits measured by human observations and environment can exert significant effect on the trait as well. Estimation of genetic diversity based on enzyme (protein) markers does not require extraction of DNA; therefore, this technique is relatively fast. However, enzyme based markers are low in abundance and may change with the change in environment and the stage of the plant and plant parts used (McClean 1998, Wageningen UR, website). Therefore, DNA-based molecular markers techniques gained much interest to study genetic diversity of germplasm as they do not rely on phenotypic traits and also not influenced by environment or growth stage of the plant. Molecular markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and single-nucleotide polymorphism

(SNP) provide the estimate of genetic diversity based on variation at the DNA level (coding and non-coding). Some of the advantages of DNA based molecular markers are high accuracy and abundance in the plant genome.

Brassica napus L. (*n* = 19, AC genome) is one of the most important crop in Canada. Fu and Gugel (2010) reported a trend of decreasing genetic diversity in its germplasm. Therefore, broadening of genetic diversity in its germplasm is needed. Genetic diversity in Canadian spring *B. napus* canola can be increased by using different variant of this species such as, winter and Chinese semi-winter types, and rutabagas which are genetically distinct from spring *B. napus* canola germplasm (Diers and Osborn 1994, Bus et al. 2011). Some efforts have already been made using these gemplasm in Canadian canola breeding programs (Kebede et al. 2010, Rahman et al. 2014). Other approach would be the use of its allied species, such as *B. rapa* L. (*n* = 10, A genome) and *B. oleracea* L. (*n* = 9, C genome). Based on molecular marker analysis, Thorman et al. (1994) reported that the genome of the two diploid parental species *B. rapa* and *B. oleracea* are genetically distinct from the genomes of *B. napus*; thus these two species can be used as source allelic diversity for the improvement of spring *B. napus* canola. This thesis research is aimed at increasing the genetic diversity in the A genome of *B. napus* using A genome of *B. rapa* through interspecific cross between these two species. Genetic diversity in the interspecific cross derived population was estimated by use of SSR markers. The extent of the A genome alleles of *B. rapa* introgressed into the three populations derived from *B. napus* × *B. rapa* interspecific crosses was estimated based on the alleles detected in the respective *B. rapa* parents.

3.2 Material and methods

3.2.1 Plant material

Three different F_4 generation populations developed from *B. napus* × *B. rapa* crosses, were used in this study. The detail of the research material development is described in Material and Methods section of Chapter 2 of this thesis. The list of material is presented in Tables A.1, A.2 and A.3 as appendix.

3.2.2 DNA extraction

Leaf samples of 100 mg were collected from 2-week old F_4 and parent plants (grown in greenhouse) in aluminum foils and stored at -80 ^{o}C. Approximately 40 mg of frozen leaf samples were taken in 1.5 ml Eppendorf tube and immersed in liquid nitrogen for a minute, after which the samples were grounded with micropestal. DNA was extracted from crushed leaf samples using Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI) and following manufacturer instruction with some modifications. In brief, 600 µl nuclei lysis buffer was added to the crushed leaf sample and vortexed for 20 seconds. The samples were incubated at 65 ^{o}C (in hot water tub) for 15-30 min and 100 µl protein precipitation solution was added. After vortexing for 20 seconds**,** 400 µl of chloroform was added, and the samples were centrifuged @ 10,000 rpm for 6 min. The supernatant was transformed to a new tube and 550 µl of isopropanol was added to it and mixed by inversion. The samples were kept at -20 ^{o}C for 5 min, and centrifuged @ 10,000 rpm for 6 min. The supernatant was removed and 300 µl ethanol was added to it. Samples were air dried and suspended in 300 µl of elution buffer. The quality and concentration of the DNA was measured using NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Extraction steps were repeated for the samples having low concentration of DNA. Finally, DNA was diluted to 10 ng μL^{-1} with TE buffer and stored at 4 ^{o}C until use.

3.2.3 Identification of polymorphic SSR markers and genotyping the F_4 populations

Marker Source

A total of 397 SSR markers from A1 to A10 linkage group of the Brassica A genome were used. These markers were obtained from various sources: 324 markers from Agriculture and Agri-Food Canada (AAFC) through a material transfer agreement, 12 from Cheng et al. (2009), 7 from Suwabe et al. (2006), 3 by UK Crop Net. (Website) and 60 markers were internally designed by Dr. Neil Hobson (Postdoctoral Fellow, Canola Program, University of Alberta) from *Brassica rapa* genome sequence.

PCR Reaction

Polymerase chain reaction (PCR) was carried out in a reaction volume of 12.025 µl containing 10 ng of template DNA, 5 pmol of each forward and reverse primers, 10 mM of dNTPs (Invitrogen Life Technologies Inc., Burlington, ON), 25 mM of $MgCl_2$, 1x PCR reaction buffer, and 0.125 units of *Taq* DNA polymerase (Promega Corporation, Madison, WI) and 4.9 µl of distilled water. PCR cycle conditions were 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 56 ^{o}C and extension for 1.30 min at 72 ^{o}C and final extension for 30 min at 72 ^{o}C.

Gel electrophoresis and ABI sequencing

Identification of polymorphic markers (see appendix Table A.4) and genotyping of the F_4 population was done by gel electrophoresis and ABI sequencer. Molecular markers (MM) with expected fragment size greater than 30 base pairs were analyzed using gel electrophoresis (Figure 2.8), if fragment size difference was less than 30 base pairs, ABI sequencer No. 3730 (Applied Biosystems, Foster City, CA) was used. For gel electrophoresis, 3% agarose gel was

prepared using 300 ml of TE buffer and 3 µl of syber safe (Invitrogen, Carlsbad, CA). Samples for gel electrophoresis were prepared by using 2 µl of PCR product and 5 µl of loading buffer and the samples were loaded on the agarose gel. Gel was run in TE buffer at 180 volts for about 140 minutes and was scanned using Typhoon FLA 9500 scanner (GE Healthcare Bio-Sciences AB, Piscataway, NJ). Markers with fragment size difference less than 30 base pairs were run on ABI sequencer 3730. To reduce the cost of primer labelling in ABI sequencing, 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). Polymorphic markers found among the parents were used to genotype the F_4 generation population.

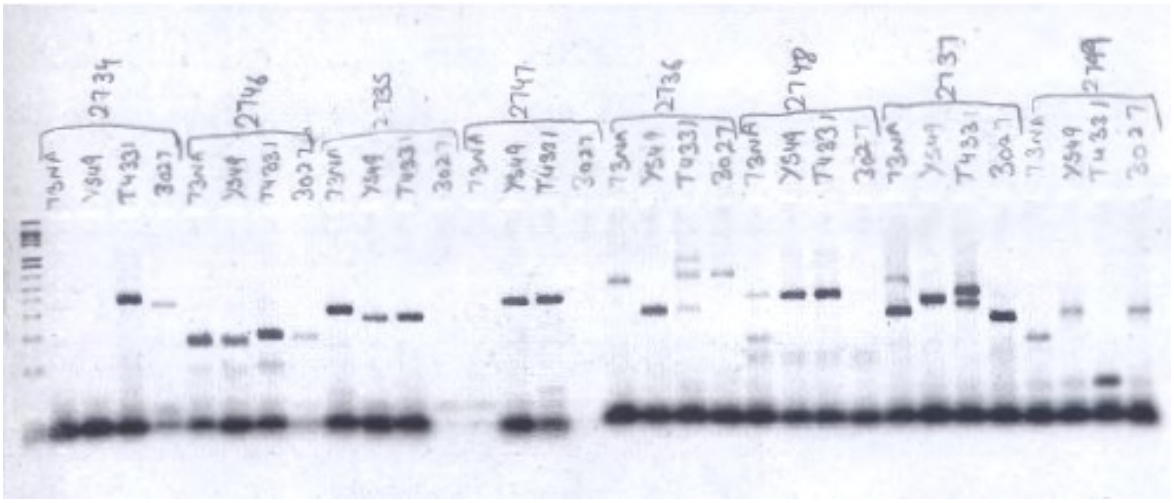


Figure 2.8 Gel electrophoresis image of SSR markers analysis showing polymorphism between four parents used in this study. Parents: 73NA = A04-73NA (*B. napus*), YS49 (*B. rapa*), T4331 = T4-3-3-1 (*B. rapa*) and 3027 = 3-0026.027 (*B. rapa*).

3.2.4 Data scoring and analysis

A combined matrix of F_4 generation of three crosses along with their four parents was developed by scoring the bands from gel electrophoresis and the peak from ABI sequencer. The presence of a band or a peak in a population was marked as 1 while absence was marked as 0. Based on this matrix, Dice similarity coefficient (Nei and Li 1979) calculated by using the computer software program Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.2; Rohlf, 2000). The similarity coefficients were used for cluster analysis through unweighted pair-group method of arithmetic mean, or UPGMA (it reflects genotypic similarities) and using the computer software program Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.2; Rohlf, 2000). Principle coordinate analysis was also done using NTSYSpc 2.2 (Rohlf, 2000). The proportion of *B. rapa* alleles (gel band or ABI peak) detected in each of the three F_4 population was calculated; this gave the estimate of introgression of A genome content of *B. rapa* in these populations.

3.3 Results

3.3.1 Parental polymorphism

A summary of SSR markers for polymorphism between the *B. napus* and *B. rapa* parents is presented in Table 3.1. Of the total markers tested, the proportion of polymorphic markers for linkage group A1-A10 varied from 5.9 to 39.4% with a mean of 23.7% between the parents A04-73NA and YS49, 7.5 to 35.3% with a mean of 23.7% between A04-73NA and T4-3-3-1, and 7.5 to 36.4% with a mean of 24.4% between A04-73NA and 3-0026.027, and 8.9 to 39.4% with a mean of 25.7%, between A04-73NA and *B. rapa* (combined). Thus the extent of polymorphism between *B. napus* and all three *B. rapa* parents were very similar. Among the ten linkage groups

Table 3.1 Summary of simple sequence repeat (SSR) markers from the A genome linkage group A1 to A10 tested for polymorphism between the *B. napus* and *B. rapa* parents

LG[1]	Total SSR tested	A04-73NA vs. YS49			A04-73NA vs. T4-3-3-1		
		No. polymorphic[a]	No. monomorphic	No. *B. rapa* specific	No. polymorphic[a]	No. monomorphic	No. *B. rapa* specific
A1	40	10 (25.0)	30	0	12 (30.0)	28	2
A2	67	4 (5.9)	63	0	5 (7.5)	62	1
A3	47	9 (19.1)	38	0	9 (19.1)	38	0
A4	33	13 (39.4)	20	2	11 (33.3)	22	0
A5	46	14 (30.4)	32	0	14 (30.4)	32	0
A6	32	9 (28.1)	23	2	8 (25.0)	24	1
A7	37	10 (27.0)	27	0	11 (29.7)	26	1
A8	34	12 (35.3)	22	0	12 (35.3)	22	0
A9	37	7 (18.9)	30	0	7 (18.9)	30	0
A10	24	6 (25.0)	18	1	6 (25.0)	18	1
Total	397	94 (23.7)	303	5	95 (23.9)	302	6

LG[1]	Total SSR tested	A04-73NA vs. 3-0026.027			*B. napus* vs. *B. rapa* (combined)		
		No. polymorphic[a]	No. monomorphic	No. *B. rapa* specific	No. polymorphic[a]	No. monomorphic	No. *B. rapa* specific
A1	40	10 (25.0)	30	0	12 (30.0)	28	2
A2	67	5 (7.5)	62	1	6 (8.9)	61	2
A3	47	10 (21.3)	37	1	10 (21.3)	37	1
A4	33	12 (36.4)	21	1	13 (39.4)	20	2
A5	46	14 (30.4)	32	0	14 (30.4)	32	0
A6	32	9 (28.1)	23	1	9 (28.1)	23	2
A7	37	11 (29.7)	26	1	11 (29.7)	26	1
A8	34	12 (35.3)	22	0	12 (35.3)	22	0
A9	37	7 (18.9)	30	0	7 (18.9)	30	0
A10	24	7 (29.2)	17	2	8 (33.3)	16	3
Total	397	97 (24.4)	300	7	102 (25.7)	295	13

[1] LG = Linkage group; [a] In brackets, percent of the total tested markers.

markers from A2 was least polymorphic while markers from A4, A5 and A6 were highly polymorphic (Table 3.1). Of the total 102 polymorphic markers, 13 (12.7%) were found to be specific to *B. rapa*; five of which were detected in YS49, six in T4-3-3-1 and seven in 3-0026.027.

3.3.2 Genetic diversity in F_4 population

A total of 102 SSR markers producing distinct fragments were used to genotype the 44 F_4 generation plants. This included 15 plants from A04-73NA × YS49, 16 from A04-73NA × T4-3-3-1 and 13 from A04-73NA × 3-0026.027.

Cluster analysis, based on genetic similarity among the 44 F_4 plants identified three genetically distinct groups. Group I consist of 14 plants from A04-73NA × T4-3-3-1 cross and Group II consisted of one plant derived from A04-73NA × T4-3-3-1 and 13 from A04-73NA × 3-0026.027 cross. The Group I and II had coefficient of genetic similarity of 0.19 with the *B. napus* parent A04-73NA. The Group III consisted of 15 plants derived from A04-73NA × YS49 cross, and had coefficient of genetic similarity of 0.32 with its *B. napus* parent.

Principal coordinate analysis showed that the first and second principle coordinate explained 16.1 and 10.1% of genetic variation, respectively in the F_4 populations and the parents (Fig 3.2). Based on this analysis, the whole population was divided into four groups. Group I consisted of only the *B. rapa* parent YS49. The Group II consisted of 15 plants derived from A04-73NA × YS49 and also included the A04-73NA parent. The group III consisted of 14 plants derived from A04-73NA × T4-3-3-1, and group IV consisted of 14 plants derived from A04-73NA × 3-0026.027.

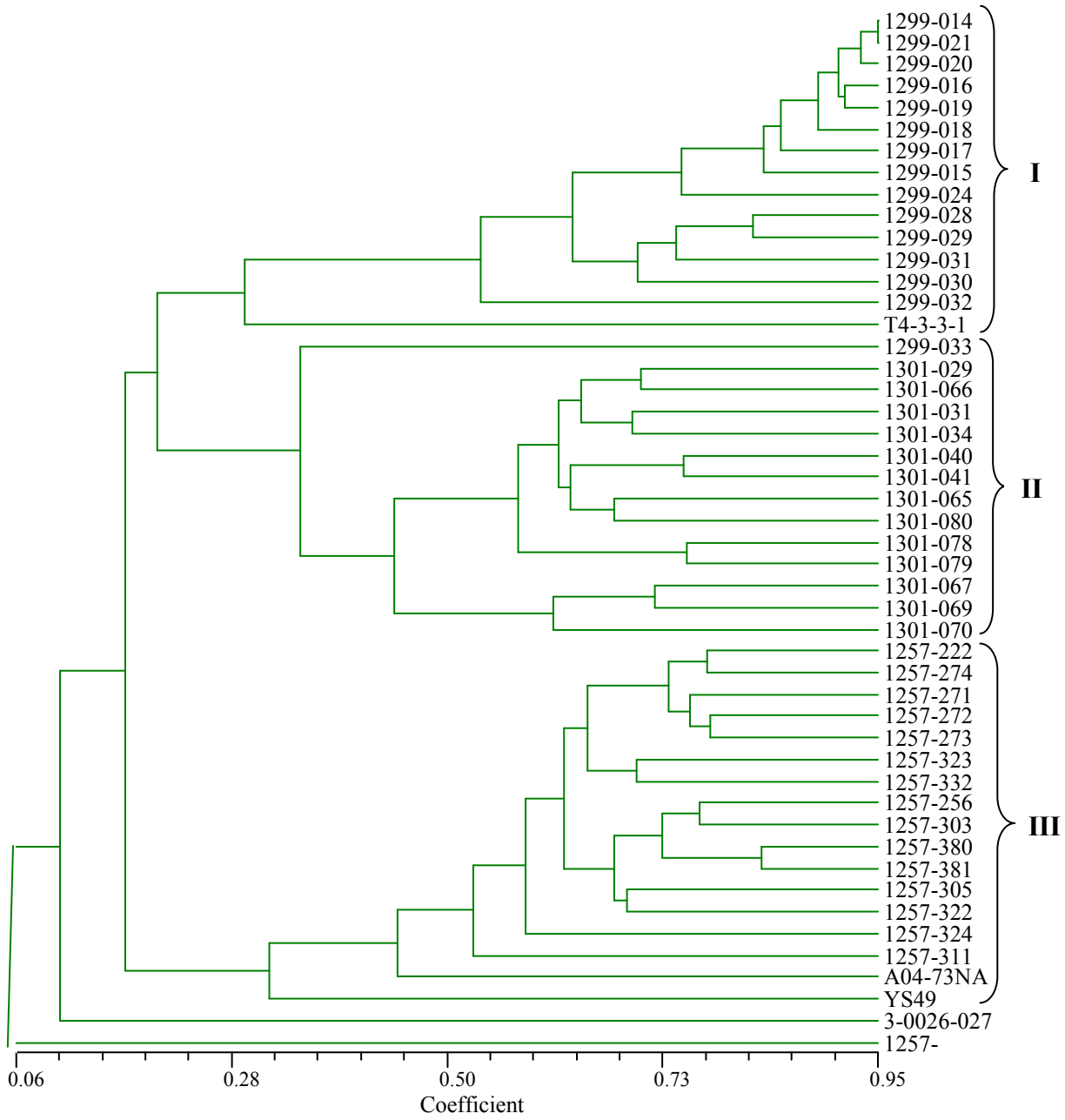


Figure 3.1 Dendrogram showing genetic similarity among 44 F_4 plants derived from three *B. napus* × *B. rapa* interspecific crosses (A04-73NA × YS49, A04-73NA × T4-3-3-1 and A04-73NA × 3-0026.027). Plant ID starting with 1299 are derived from A04-73NA × T4-3-3-1, 1301 derived from A04-73NA × 3-0026.027, and 1257 derived from A04-73NA × YS49 cross

Thus, results of PCoA are in conjugation with UPGMA analysis, and confirmed that three genetically distinct populations were developed from three different *B. rapa* parents in interspecific crosses.

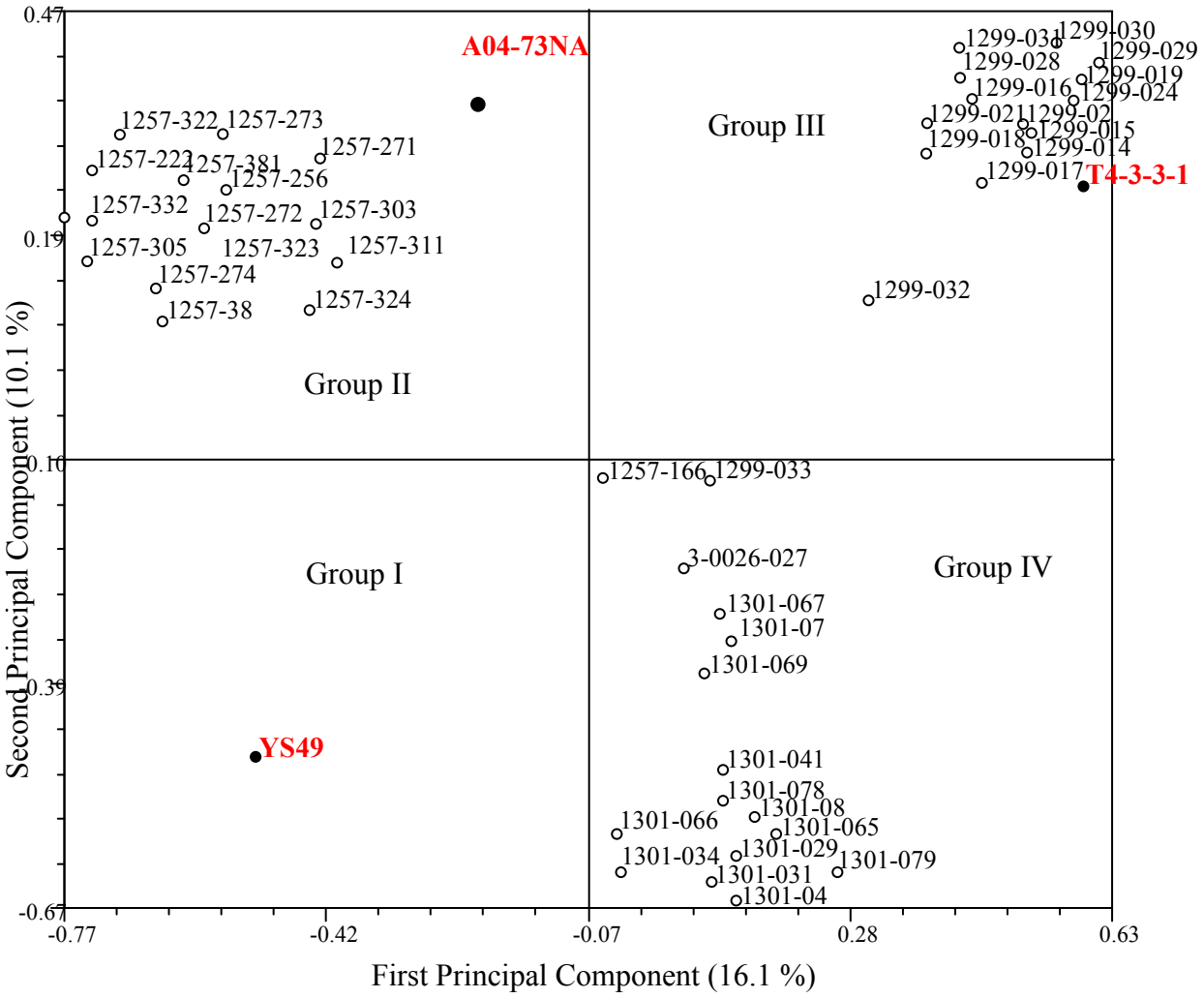


Figure 3.2 Distribution of 44 F_4 plants derived from three *B. napus* × *B. rapa* interspecific crosses by principle coordinate analysis. Plant ID starting with 1299 are derived from A04-73NA × T4-3-3-1, 1301 derived from A04-73NA × 3-0026.027 and 1257 derived from A04-73NA × YS49 cross

Introgression of the A genome SSR alleles of *B. rapa* into F_4 population

A summary of the proportion of the A genome alleles of *B. rapa* introgressed into their corresponding F_4 generation plants is presented in Table 3.2

Table 3.2 Summary of introgression of the A genome SSR alleles of *B. rapa* into F_4 population derived from three *B. napus* × *B. rapa* interspecific crosses

F_4 ID[1]	Percent *B. rapa* alleles	F_4 ID[2]	Percent *B. rapa* allels	F_4 ID[3]	Percent *B. rapa* alleles
1299-014	9.5	1301-029	41.7	1257-166	7.6
1299-015	9.5	1301-031	41.7	1257-222	33.3
1299-016	9.5	1301-034	25.0	1257-256	21.2
1299-017	9.5	1301-040	41.7	1257-271	31.8
1299-018	14.3	1301-041	25.0	1257-272	28.8
1299-019	19.0	1301-065	50.0	1257-273	37.9
1299-020	11.9	1301-066	25.0	1257-274	28.8
1299-021	11.9	1301-067	16.7	1257-303	13.6
1299-024	30.9	1301-069	16.7	1257-305	25.7
1299-028	40.5	1301-070	16.7	1257-311	16.7
1299-029	47.7	1301-078	16.7	1257-322	24.2
1299-030	45.2	1301-079	66.7	1257-323	31.8
1299-031	54.8	1301-080	50.0	1257-324	21.2
1299-032	11.9			1257-332	45.4
1299-033	0.0			1257-380	22.7
				1257-381	25.7
Avg	21.7		33.3		26.0

[1] Plants derived from cross A04-73NA × T4-3-3-1
[2] Plants derived from cross A04-73NA × 3-0026.027
[3] Plants derived from cross A04-73NA × YS49

On average, 21.7, 33.3 and 26.0 % of the polymorphic alleles detected in the *B. rapa* parents were introgressed into the F_4 populations derived from A04-73NA × T4-3-3-1, A04-73NA × 3-0026.027 and A04-73NA × YS49 crosses, respectively. The F_4 plants derived from A04-73NA × T4-3-3-1 carried 0.0 to 54.8% of the polymorphic alleles detected in T4-3-3-1. In case of A04-73NA × 3-0026.027 cross, it varied from 16.7 to 66.7 %, and in A04-73NA × YS49 it varied from 7.6 to 45.4%.

In case of the F_4 population derived from A04-73NA × T4-3-3-1, the plants 1299-014, 1299-015, 1299-016 and 1299-017 had the lowest (4 of 42, i.e. 9.5%) number of *B. rapa* alleles, while the plant no 1299-31 had the greatest (23 of 42, i.e. 54.8%) number of *B. rapa* alleles. For the cross A04-73NA × 3-0026.027, lowest number of *B. rapa* alleles detected in plants 1301-67,

1301-69 and 1301-70 (2 of 12, i.e. 16.6%) and the greatest number in plant 1301-79 (8 of 12, i.e. 66.6%). For A04-73NA × YS49, the lowest number of *B. rapa* alleles were detected in plant 1257-166, 5 of 66, i.e 7.6% and greatest number in plant no 1257-332 (30 of 66, i.e. 45.4%).

3.4 Discussion

Interspecific cross involving cultivated and allied species has the potential for introgression of allelic diversity from allied species into crop germplasm. *B. napus* and its allied species evolved from a common progenitor and therefore the three Brassica genomes A, B and C share homoeology to some extent (Lagercrantz 1998). Interspecific cross between Brassica species can be made and hybrids can be obtained (Downey et al. 1980) and this opens the avenue for introgression of allelic diversity from allied species into *B. napus* canola. Efforts by other researchers have demonstrated the prospect of introgression of allelic diversity in spring *B. napus* from its allied species, such *B. oleracea* (Bennett et al. 2012), and in Chinese semi-winter *B. napus* from *B. rapa* (Qian et al. 2006). This study focused on introgression of allelic diversity into Canadian spring *B. napus* from three different *B. rapa* lines (YS49, T4-3-3-1 and 3-0026.027).

Analysis of genetic diversity carried out on a set of *B. rapa* lines by the Canola Program of the University of Alberta (Dr. Neil Hobson and Dr. Habibur Rahman, personal communication) revealed that the three *B. rapa* lines used in this study are genetically distinct from each other. The present study revealed that among these three *B. rapa* parents, 3-0026.027 is most distinct from *B. napus* followed by T4-3-3-1 and YS49. Genetically distinct populations were achieved from all three interspecific crosses. Based on SSR markers, the F_4 plants derived from A04-73NA × 3-0026.027 and A04-73NA × T4-3-3-1 were found to be most distinct.

Interspecific crosses have been successfully used in the past to transfer allelic diversity or a specific trait from allied Brassica species into *B. napus*. Various approaches such as, use of its diploid progenitors for artificial resynthesize of *B. napus* or crossing of this diploid to *B. napus* can be used to develop genetically diverse *B. napus* germplasm. Seyis et al. (2003) and Rygulla et al. (2007) found that the resynthesized *B. napus* lines are genetically distinct from existing *B. napus* cultivars. Diploid species such as, *B. rapa* has been successfully used by Qian et al. (2006) to introgress allelic diversity in Chinese semi-winter *B. napus*. Similarly Bennett et al. (2012) and Rahman et al. (2015) used *B. oleracea* to diversify Canadian spring *B. napus* canola. Molecular analysis by SSR markers confirmed that these newly developed *B. napus* lines are genetically distinct from existing Canadian *B. napus*. Amphidiploid species are also a great source of allelic variation for the improvement of *B. napus.* Choudhary and Joshi (2001) developed genetically distinct F_4 population and Roy (1984) developed blackleg resistant germplasm from *B. juncea* (L) Czern. (n = 18, AB genome) × *B. napus* crosses. Rashid et al. (1994) transferred yellow seed colour genes from *B. juncea* and *B. carinata* A. Braun (n =17, BC genome) into *B. napus.*

In the present study, the extent of *B. rapa* allele introgressed into the F_4 population ranged from 0.0 to 66.7%, with a mean of 26.7%. In a similar study with *B. napus* × *B. oleracea* interspecific crosses, Bennett et al. (2012) found up to 58.3% of the *B. oleracea* allele introgressed into BC_1S_5 ($\approx BC_1F_6$) population, and working with the same cross, Rahman et al. (2015) found 0-54% (mean 19%) of the *B. oleracea* alleles in F_8 population. Thus, slightly higher level of introgression was observed in the population derived from *B. napus* × *B. rapa* crosses compared to *B. napus* × *B. oleracea* cross reported by Bennett et al. (2012) and Rahman et al. (2015).

Results from this study confirm that it is possible to introgress allelic diversity from *B. rapa* into *B. napus* through interspecific cross between these two species. Different variants of *B. rapa* are known to be genetically distinct (Zhao et al. 2005), therefore, different types of *B. rapa* are expected to contribute different allelic diversity in the interspecific cross derived populations. This is also evident from the present study that the three populations developed in this study were found to be genetically distinct. This research also suggests that other variants of *B. rapa* can be used to broaden genetic diversity in the A genome of *B. napus*. Agronomic performance and heterotic potential of these lines developed in this research need to be investigated.

Chapter 4

Summary and general discussion

4.1 Overview

Brassica crops account for 14% of the total global oilseed production. Canada is the largest producer of this crop followed by China and India (FAOSTAT 2014). Oilseed *B. napus* L. (*n* = 19, AC genome) was introduced in Canada during the World War II; repeated breeding on this crop using limited introduced germplasm, mainly from Europe, reduced genetic diversity in this crop germplasm (Juska et al. 1997, Kneen 1992). Therefore, it is important to broaden allelic diversity in its germplasm, which is essential for progress in breeding (reviewed in Rahman 2013), as well as for greater heterosis for seed yield (Riaz et al. 2001).

According to Thorman et al. (1994), the A genome of *B. napus* and *B. rapa* L. (*n* =10, A genome) are genetically distinct, and thus can be used for broadening of genetic diversity in *B. napus.* This M.Sc. thesis research is focused on increasing genetic diversity in the A genome of Canadian spring *B. napus* canola using the alleles from the A genome of *B. rapa.* For this, the following objectives were laid out in this thesis research project (i) Study the feasibility of developing canola quality recombinant *B. napus* inbred lines from *B. napus* × *B. rapa* interspecific crosses through reconstitution of the A genome of *B. napus* with the A genome of *B. rapa.* (ii) Study the inheritance of seed glucosinolate (GSL) content in *B. napus* × *B. rapa* interspecific hybrid progenies and response to selection for low GSL content. (iii) Estimate genetic diversity in the interspecific cross derived populations by the use of simple sequence repeat (SSR) markers.

4.2 Major findings

4.2.1 Families developed from *B. napus* × *B. rapa* interspecific crosses

- Fertile F_7 families with nuclear DNA content similar to the *B. napus* parent were developed.
- Nuclear DNA content in BC_1F_1 derived population was close to the *B. napus* parent already in BC_1F_3 generation.
- Repeated selection for glucosinolates content lead to the development of canola quality families.
- No consistent correlation was found between the parent and offspring generation for GSL content in these interspecific cross-derived population.

4.2.2 SSR marker analysis

- Cluster analysis revealed that the *B. rapa* parent 3-0026.027 is most distinct from the *B. napus* parent with genetic similarity of coefficient of 0.10 followed by T4-3-3-1 with 0.19 and YS49 with 0.32.
- Use of the three genetically distinct *B. rapa* parents in crossing lead to the development of three distinct populations. Populations derived from A04-73NA × 3-0026.027 and A04-73NA × T4-3-3-1 were most distinct from the *B. napus* parent with genetic similarity coefficient of 0.19, followed by the population developed from A04-73NA × YS49 (genetic similarity coefficient 0.32).

4.3 Discussion

Genetic gain in crop germplasm achieved through interspecific crosses comes at a price as these crosses lead to high sterility, poor seed set as well as, often introgress undesirable traits (Falk 2010). However, limited backcrossing of the interspecific hybrids to the cultivated species,

intensive selection for different traits and use of molecular markers can increase the efficiency in breeding with interspecific crosses. In *Brassica*, interspecific crosses have been successful in many cases, such as *B. napus* × *B. carinata* A. Braun (*n* =17, BC genome) cross for introgression of disease resistance from *B. carinata* into *B. napus* (Navabi et al. 2011) and *B. napus* × *B. oleracea* L. (*n* = 9, C genome) cross for introgression of allelic diversity from *B. oleracea* into *B. napus* (Bennett et al. 2012, Rahman et al. 2015).

Poor fertility in the initial generations of *B. napus* × *B. rapa* crosses was observed in this study. This is not uncommon in progeny derived from interspecific crosses. Nishiyama et al. (1991) also reported poor fertility and seed set in F_1 generation of *B. napus* × *B. rapa* interspecific crosses, and no seed set was observed in *B. oleracea* or *B. nigra* L. (*n* = 8, B genome) crosses to *B. napus*. Tu et al. (2009) explained that poor set in distant crosses is due to meiotic irregularities in hybrid progenies. In the present study, improvement in the plant fertility traits was observed with advancement of generation in all *B. napus* × *B. rapa* interspecific crosses. Similar results were also reported by Rahman et al. (2015) and Qian et al. (2005) for the *B. napus* × *B. oleracea* and *B. napus* × *B. rapa* crosses respectively. This improvement in plant fertility is due to balanced chromosome combination in the gametes with the progression of generation (Kato and Tokumasu 1983).

All the *B. rapa* parents used in this study are genetically distinct from *B. napus*. Use of genetically very distinct intercrossing parents can lead to poor agronomic performance of progenies even in later generations (Tu et al. 2009). Studies in other crops also gave similar results, such as in rice by (Li et al. 1997) and maize (Moll et al. 1965). Agronomic performance of the lines derived from *B. napus* × *B. rapa* crosses needs to be evaluated In field trails.

Glucosinolates content, a quantitative trait, is controlled by at least four genes loci and presence of recessive alleles at all loci results low in glucosinolates (< 30 μmol g^{-1} seed) phenotype (Rahman et al. 2001). Howell et al. (2003) identified four QTL's responsible for more than 76 % of phenotypic variation for total glucosnolate content in *B. napus*. Kondra and Stefenson (1970) reported that each of the different individual glucosinolates, such as gluconapin, glucobrassicanapin and progoitrin are controlled by three to five loci, and the inheritance of these loci are not independent. Although quantitative traits such as glucosinolates show complex inheritance, still these traits can be improved in a breeding program for which intensive selection over many generations may be needed (Fleury et al. 2012). In the present study, selection over segregating generations leads to the development of low glucosinolate plants. However, strong correlation between the parent progeny generation raised alternatively in greenhouse and field conditions could not be found in the present study. This is in contrast, to Bahrani and McVetty (2008) who reported moderate correlation for GSL content in *B. napus* between the population grown in field and greenhouse. The difference in correlation for glucosinolates content between greenhouse and field experiment is probably due to different types of plant material used in these two experiments.

Earlier studies in *B. napus* × *B. oleracea* cross by Rahman et al. (2015) and in *B. napus* × *B. rapa* cross by Qian et al. (2005) demonstrated that progenies derived from interspecific crosses involving amphidiploid and diploid species often stabilizes into amphidiploid types in advanced generation. Results obtained from this study agree with earlier studies. This is evident from nuclear DNA content in both F_2 and BC_1 derived populations which become similar to the *B. napus* parent. Nuclear DNA content in backcross derived population reached close to the *B.*

napus parent in BC_1F_3 generation, this apparently resulted from introgression of more genome content from the recurrent parent (Vogel 2009).

4.4 Conclusion

The results presented in this thesis suggest that genetically distinct, fertile and canola quality germplasm can be developed from *B. napus* × *B. rapa* interspecific crosses. High sterility and poor seed set was observed in the early generation population; however, selection for fertile plants and low GSL content led to the development of canola quality germplasm from these interspecific crosses. Nuclear DNA content in backcross derived population reached close to the *B. napus* parent already in early segregating generation; suggesting that the limited backcrossing to *B. napus* can be applied (Falk 2010) for the development of *B. napus* lines from *B. napus* × *B. rapa* crosses for introgression of desired traits and allelic diversity from *B. rapa* into *B. napus.*

4.5 Future research

B. napus germplasm developed from the *B. napus* × *B. rapa* crosses need to be evaluated in field trials for seed yield and agronomic properties. These lines can be used for crossing with elite *B. napus* lines to broaden genetic diversity in Canadian *B. napus* canola. The potential of these germplasm in hybrid breeding need to be tested through producing test hybrids with available canola lines. Backcross derived population also need to be genotyped with SSR markers to investigate the extent of *B. rapa* alleles introgressed into this population; and based on this, the value of the use of limited backcrossing for introgression of genetic diversity can be assessed. The knowledge gained from this research can also be used in breeding for introgression of allelic diversity from other *B. rapa* into *B. napus* canola.

References

Agriculture and Agri-Food Canada, 2013: http://www.agr.gc.ca/eng/industry-markets-and-trade/statistics-and-market-information/by-product-sector/crops/crops-market-information-canadian-industry/canada-outlook-for-principal-field-crops/canada-outlook-for-principal-field-crops-2013-10-16/?id=1382366483303 (Retrieved on February 12, 2014)

Alberta Canola Producers Commission, 2013: http://canola.ab.ca/federal_research_investment_will_drive_canola_industry_growth_news_07_22_2013.aspx (Retrieved on November 24, 2013)

Ali, M., L. O. Copeland, S. G. Elias, and J. D. Kelly, 1995: Relationship between genetic distance and heterosis for yield and morphological traits in winter canola (*Brassica napus* L.). Theor. Appl. Genet. 91, 118–121.

Allender, C. J., and G. J. King, 2010: Origins of the amphiploid species *Brassica napus* L. investigated by chloroplast and nuclear molecular markers. BMC Plant Biol. 10, 54.

Arumuganathan, K., and E. D. Earle, 1991: Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol. Biol. Rep. 9, 229–241.

Bahrani, J., and P. B. E. McVetty, 2008: Relationship of seed quality traits for greenhouse-grown versus field-grown high erucic acid rapeseed: Is seed quality trait selection for greenhouse-grown seed worthwhile?. Can. J. Plant Sci. 88, 419–423.

Becker, H. C., C. Damgaard, and B. Karlsson, 1992: Environmental variation for outcrossing rate in rapeseed (*Brassica napus*). Theor. Appl. Genet. 84, 303–306.

Bell, J. M., 1982: From rapeseed to canola: A brief history of research for superior meal and edible oil. Poultry Sci. 61, 613–622.

Bell, M., 1993: Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci. 73, 679–697.

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

Bennett, R. A., M. R. Thiagarajah, J. R. King, and M. H. Rahman, 2008: Interspecific cross of *Brassica oleracea* var. *alboglabra* and *B. napus*: effects of growth condition and silique age on the efficiency of hybrid production, and inheritance of erucic acid in the self-pollinated backcross generation. Euphytica, 164, 593–601.

Bohuon, E. J. R., D. J. Keith, I. A. P. Parkin, A. G. Sharpe and D. J. Lydiate, 1996: Alignment of the conserved C genomes of *Brassica oleracea* and *Brassica napus*. Theor. Appl. Genet. 93, 833–839.

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

Canadian Food Inspection Agency, 2008: Cruciferous crop inspection procedures: Appendix VII – Plant descriptions and illustrations of mustard and canola. http://www.inspection.gc.ca/english/plaveg/seesem/man/swi-crue.shtml#a22 (Retrieved on July 30, 2014)

Canadian Grain Commission, 2013a: http://www.grainscanada.gc.ca/wheat-ble/wbm-mwb-eng.htm (Retrieved on April 09, 2013)

Canadian Grain Commission, 2013b: http://www.grainscanada.gc.ca/flax-lin/flm-mfl-eng.htm (Retrieved on July 07, 2014)

Canadian Grain Commission, 2013c: http://www.grainscanada.gc.ca/sunflower-tournesol/stm-mst-eng.htm (Retrieved on July 7, 2014)

Canadian Grain Commission, 2013d: http://www.grainscanada.gc.ca/grainsc-cgrains/om-mo-eng.htm (Retrieved on April 09, 2014)

Canola Council of Canada, 2011a: http://www.canolacouncil.org/crop-production/canola-grower%27s-manual-contents/chapter-2-canola-varieties/canola-varieties (Retrieved on December 10, 2013)

Canola Council of Canada, 2011b: http://www.canolacouncil.org/oil-and-meal/canola-meal/nutrient-composition-of-canola-meal/glucosinolate-content/ (Retrieved on February 04, 2013)

Canola Council of Canada, 2011c: http://www.canolacouncil.org/oil-and-meal/what-is-canola/ (Retrieved on February 04, 2013)

Canola Council of Canada, 2011d: http://www.canolacouncil.org/markets-stats/industry-overview/economic-impact-of-the-canola-industry/ (Retrieved on February 04, 2013)

Canola Council of Canada, 2011e: http://www.canolacouncil.org/news/historic-investment-in-canola-research/ (Retrieved on February 04, 2013)

Canola Council of the Canada, 2014a: http://www.canolacouncil.org/markets-stats/industry-overview/ (Retrieved on June 25, 2014)

Canola Council of Canada, 2014b: http://www.canolacouncil.org/crop-production/canola-grower's-manual-contents/chapter-2-canola-varieties/canola-varieties (Retrieved on June 27, 2014)

Canola Council of Canada, 2014c: http://www.canolacouncil.org/oil-and-meal/what-is-canola/the-history-of-canola/ (Retrieved on June 25, 2014)

Casséus, L., 2009: Canola: a Canadian success story. Statistics Canada. http://www.statcan.gc.ca/pub/96-325-x/2007000/article/10778-eng.pdf (Retrived on January 04, 2014)

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

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

Cheng, X., J. Xu, S. Xia, J. Gu, Y. Yang, J. Fu, X. qian, S. Zhang, J. Wu, and L. Kede, 2009: Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus.* Theor. Appl. Genet. 118, 1121–1131.

Choudhary, B. R., and P. Joshi, 2001: Genetic diversity in advanced derivatives of Brassica interspecific hybrids. Euphytica, 121, 1–7.

Claypool, D. W., C. H. Hoffman, J. E. Oldfield, and H. P. Adams, 1985: Canola Meal, Cottonseed, and Soybean Meals as Protein Supplements for Calves. J. Dairy Sci. 68, 67–70.

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

Diers, B. W., P. B. E McVetty, and T. C. Osborn, 1996: Relationship between heterosis and genetic distance based on restriction fragment length polymorphism markers in oilseed rape (*Brassica napus* L.). Crop sci. 36, 79–83.

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

Downey, R. K., and B. M. Craig, 1964: Genetic control of fatty acid biosynthesis in rapeseed (*Brassica napus* L.). J. Am. Oil Chem. Soc. 41, 475–478.

Downey, R. K., and B. L. Harvey, 1963: Methods of breeding for oil quality in rape. Can. J. Plant Sci. 43, 271–275.

Downey, R. K., A. J. Klassen, and G. R. Stringam, 1980: Rapeseed and mustard. In: Fehr, W. R. and H. H. Hadley. Hybridization of crop plants. American Society of Agroonomy, Crop Science Society of America. (pp. 495–509).

Falk, D. E., 2010: Generating and maintaining diversity at the elite level in crop breeding. Genome 53, 982–991.

FAOSTAT, 2014: http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E (Retrieved on December 20, 2014)

Fleury, D., X. Delannay, P. Langridge, 2012: Quantitative Trait Loci and Breeding. In: eLS. John Wiley and Sons, Ltd: Chichester

Fredua-Agyeman, R., O. Coriton, V. Huteau, I. A. P. Parkin, A. M Chèvre, and H. Rahman, 2014: Molecular cytogenetic identification of B genome chromosomes linked to blackleg

disease resistance in *Brassica napus* × *B. carinata* interspecific hybrids. Theor. Appl. Genet. 1305–1318.

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

Gaeta, R. T., and J. Chris Pires, 2010: Homoeologous recombination in allopolyploids: the polyploid ratchet. New Phytologist 186, 18–28.

Girke, A., H. C. Becker and G. Enqvist, 1999: Resynthesized rapeseed as a new gene pool for hybrid breeding. In: 10th International Rapeseed Congress, Canberra, Australia. 26, pp. 29.

Gowers, S., 1989: Self-incompatibility interactions in *Brassica napus*. Euphytica. 42, 99–103.

Gupta, S. K., and A. Pratap, 2007: History, origin, and evolution. Adv. Bot. Res. 45, 1–20.

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

Hoisington, D., M. Khairallah, T. Reeves, J. M. Ribaut, B. Skovmand, S. Taba, and M. Warburton, 1999: Plant genetic resources: What can they contribute toward increased crop productivity?. Proc. Natl. Acad. Sci. 96, 5937–5943.

Holm, S.N., M.H. Rahman, O. Stolen, and H. Sorensen, 1985: Studies on pollination requirement in rapeseed (*Brassica campestris*). In: H. Sorensen, editor, Advances in the production and utilization of cruciferous crops. Martinus Nijhoff Publ., Dordrecht, the Netherlands. pp. 245–253.

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

Hung, S., T. Umemura, S. Yamashiro, S. J. Slinger, and B. J. Holub, 1977: The effects of original and randomized rapeseed oils containing high or very low levels of erucic acid on cardiac lipids and myocardial lesions in rats. Lipids 12, 215–221.

Iqbal, M. C. M., S. R. Weerakoon, H. D. N. Geethanjalie, P. K. D. Peiris, and O. V. D. S. J. Weerasena, 2011: Changes in the fatty acids in seeds of interspecific hybrids between *Brassica napus* and *Brassica juncea*. Crop Pasture Sci. 62, 390–395.

Jönsson, R., 1977: Erucic-acid heredity in rapeseed:(*Brassica napus* L. and *Brassica campestris* L.). Hereditas, 86, 159–170.

Juska, A., L. Busch, and F. H. Wu, 1997: Producing genetic diversity in crop plants: The case of Canadian Rapeseed, 1954–1991. J. Sustain. Agric. 9, 5–23.

Kato, M., and S. Tokumasu, 1983: The stabilization of chromosome numbers and the maintenance of euploidy in *Brassicoraphanus*. Euphytica, 32, 415–423.

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

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

Khajali, F., and B. A. Slominski, 2012: Review factors that affect the nutritive value of canola meal for poultry. Poultry Sci. 91, 2564–2575.

Kianian, S. F., and C. F. Quiros, 1992: Trait inheritance, fertility, and genomic relationships of some n = 9 *Brassica* species. Genet. Resour. Crop. Ev. 39, 165–175.

Kimber, D. S., and D. I. McGregor, 1995: Brassica oilseeds: production and utilization. Cab international, Wallingford, UK.

Kneen, B., 1992: The rape of canola. New Canada Publications.

Kondra, Z. P., and B.R. Stefansson, 1965: Inheritance of erucic and eicosenoic acid content of rapeseed oil (*Brassica napus*). Can. J. Genet. Cytol. 7, 500–510.

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

Lagercrantz, U., 1998; Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that Brassica genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. Genetics 150, 1217–1228.

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

Li, Z., S. R. Pinson, A. H. Paterson, W. D. Park, and J. W. Stansel, 1997: Genetics of hybrid sterility and hybrid breakdown in an intersubspecific rice (*Oryza sativa* L.) population. Genetics 145, 1139-1148.

Lim, C., P. H. Klesius, and D. A. Higgs, 1998: Substitution of canola meal for soybean meal in diets for channel catfish *Ictalurus punctatus*. J. World Aquac. Soc. 29, 161–168.

Liu, R. H., and J. L. Meng, 2006: RFLP and AFLP analysis of inter-and intraspecific variation of *Brassica rapa* and *B. napus* shows that *B. rapa* is an important genetic resource for *B. napus* improvement. Acta Genet. Sin. 33, 814–823.

Liu, X. P., J. X. Tu, B.Y. Chen, and T. D. Fu, 2005: Identification and inheritance of a partially dominant gene for yellow seed colour in *Brassica napus*. Plant Breeding 124, 9–12.

Lysak, M. A., K. Cheung, M. Kitschke, and P. Bureš, 2007: Ancestral chromosomal blocks are triplicated in Brassiceae species with varying chromosome number and genome size. Plant Physiol. 145, 402–410.

Magrath, R., F. Bano, M. Morgner, I. Parkin, A. Sharpe, C. Lister, C. Dean, J. Turner, D. Lydiate and R. MITHEN, 1994: Genetics of aliphatic glucosinolates. I. Side chain elongation in *Brassica napus* and *Arabidopsis thaliana*. Heredity 72, 290–299.

Mawson, R., R. K. Heaney, Z. Zdunczyk, and H. Kozlowska, 1993: Rapeseed meal-glucosinolates and their antinutritional effects. Part II. Flavour and palatability. Die Nahrung 37, 336–344.

McClean, P., 1998; Mapping plant genomes with molecular marker. http://www.ndsu.edu/pubweb/~mcclean/plsc731/mapping/mapping1.htm (Retrieved on February 26, 2015)

Moll, R. H., J. H. Lonnquist, J. V. Fortuno, and E. C. Johnson, 1965: The relationship of heterosis and genetic divergence in maize. Genetics 52, 139–144.

Möllers, C., 2002: Development of high oleic acid oilseed rape. In: 8th International Conference for Renewable Resources and Plant Biotechnology, NAROSSA, Magdeburg, Germany. pp. 10–11

Navabi, Z. K., K. E. Stead, J. C. Pires, Z. Xiong, A. G. Sharpe, I. A. P. Parkin, M. H. Rahman, and A. G. Good, 2011: Analysis of B-genome chromosome introgression in interspecific hybrids of *Brassica napus* × *B. carinata*. Genetics 187, 659–673.

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

Nishiyama, I., M. Sarashima, and Y. Matsuzawa, 1991: Critical discussion on abortive interspecific crosses in Brassica. Plant breeding 107, 288–302.

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

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

Parliament of Canada, 2012: http://www.parl.gc.ca/Content/HOC/Committee/411/FINA/WebDoc/WD5709773/411_FINA_PBC2012_Briefs%5CCanolaCouncilofCanadaE.pdf (Retrieved on November 24, 2013,)

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

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

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

Qiu, D., M. Gao, G. Li, and C. Quiros, 2009: Comparative sequence analysis for *Brassica oleracea* with similar sequences in *B. rapa* and *Arabidopsis thaliana*. Plant Cell Rep. 28, 649–661.

Quijada, P. A., J. A. Udall, H. Polewicz, R. D. Vogelzang and T. C. Osborn, 2004: Phenotypic effects of introgressing French winter germplasm into hybrid spring canola. Crop Sci. 44, 1982–1989.

Rahman, H., 2011: Use of European winter canola *Brassica napus* L. for the improvement of Canadian spring canola: A practical breeding example. In: 13th Int. Rapeseed Congress, Prague, Czech Republic. pp. 875–878.

Rahman, H., 2013: Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic germplasm. Can. J. Plant Sci. 93, 363–373.

Rahman, H., R. A. Bennett, and G. Séguin-Swartz, 2015: Broadening genetic diversity in *Brassica napus* canola: Development of canola-quality spring *B. napus* from *B. napus* × *B. oleracea* var. alboglabra interspecific crosses. Can. J. Plant Sci. 95, 29–41.

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

Rahman, H., B. Kebede, C. Zimmerli, and R.C. Yang, 2014: Genetic Study and QTL Mapping of Seed Glucosinolate Content in *Brassica rapa* L. Crop Sci. 54, 537–543.

Rahman, H., G. Peng, F. Yu, K. C. Falk, M. Kulkarni, and G. Selvaraj, 2014: Genetics and breeding for clubroot resistance in Canadian spring canola (*Brassica napus* L.). Can. J. Plant Pathol. 36, 122–134.

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

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

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

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

Rahman, M. H., R. A. Bennett, R. C. Yang, B. Kebede, and M. R. Thiagarajah, 2011: Exploitation of the late flowering species *Brassica oleracea* L. for the improvement of earliness in *B. napus* L.: an untraditional approach. Euphytica 177, 365–374.

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

Rahman, M. H., O. Stølen, H. Sørensen, and L. Rahman, 1996: Recurrent backcross: a method for transferring erucic acid allele into Brassica oilseed cultivars, *B. campestris* L. yellow sarson as an example. Acta Agric. Scand. B- Soil Plant Sci. 46, 68–73.

Rakow, G., 2004: Species origin and economic importance of Brassica. In: Springer-Verlag Berlin Heidelberg. 3–11.

Rakow, G., 2011: Classical genetics and traditional breeding. In: Edwards, D., J. Batley, I. Parkin and C. Kole, Genetics genomics and breeding of oilseed *Brassicas*, Science Publisher Inc, Lebanon, USA. pp. 73–84.

Rakow, G., J. Relf-Eckstein, P. Raney, and R. Gugel, 1999: Development of high yielding, disease resistant, yellow-seeded *Brassica napus*. In: 10th International Rapeseed Congress, Canberra, Australia. pp. 26–29.

Rakow, G., J. A. Relf-Eckstein and J. P. Raney, 2007: Rapeseed genetic research to improve its agronomic performance and seed quality. Helia 30, 199–206.

Rakow, G., and D. L. Woods, 1987: Outcrossing in rape and mustard under Saskatchewan prairie conditions. Can. J. Plant Sci. 67, 147–151.

Rashid, A., G. Rakow, and R. K. Downey, 1994: Development of yellow seeded *Brassica napus* through interspecific crosses. Plant breeding 112, 127–134.

Raymer, P. L., 2002, Canola: An emerging oilseed crop. In: J. Janick and A. Whipkey, Trends in new crops and new uses. ASHS Press. Alexandria, VA, pp. 122–126.

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

Röbbelen, S., R. K. Downey, and A. Ashri, 1989: Oilcrops of the world: their breeding and utilization. McGraw-Hill Publishing Company.

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

Rygulla, W., R. J. Snowdon, C. Eynck, B. Koopmann, A. V. Tiedemann, W. Lühs, and W. Friedt, 2007: Broadening the genetic basis of *Verticillium longisporum* resistance in *Brassica napus* by interspecific hybridization. Phytopathol. 97, 1391–1396.

Sang, J. P., and P. A. Salisbury, 1988: Glucosinolate profiles of international rapeseed lines (*Brassica napus* and *Brassica campestris*). J. Sci. Food Agric. 45, 255–261.

Saskatchewan Canola Development Commission, http://www.saskcanola.com/industry/index.php (Retrieved on November 24, 2013)

Schelfhout, C. J., J. M. Wroth, G. Yan and W. A. Cowling, 2008: Enhancement of genetic diversity in canola-quality *Brassica napus* and *B. juncea* by interspecific hybridisation. Aust. J. Agric. Res. 59, 918–925.

Schranz, M. E., M. A. Lysak, and T. Mitchell-Olds, 2006: The ABC's of comparative genomics in the Brassicaceae: Building blocks of crucifer genomes. Trends Plant Sci. 11, 535–542.

Schuelke, M., 2000: An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. 18, 233–234.

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

Shahidi, F, 1990: Canola and rapeseed: Production, chemistry, nutrition and processing technology, Springer Science and Bussiness Media. Van Nostrand Reinhold, New York, USA.

Siebel, J., and K. P. Pauls, 1989: Inheritance pattern of erucic acid content in population of *Brassica napus* microspore- derived spontaneous diploids. Theor. Appl. Genet. 77, 489–494.

Slinkard, A. E., and D.R. Knott, 1995: Harvest of gold: the history of field crop breeding in Canada. University Extension Press, University of Saskatchewan, Saskatoon, Canada.

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

Soengas, P., M. E. Cartea, P. Velasco, G. Padilla, and A. Ordas, 2008: Morphologic and agronomic diversity of *Brassica napus* crops. J. Am. Soc. Hort. Sci. 133, 48–54.

Song, K., K. Tang, and T. C Osborn, 1993: Development of synthetic *Brassica* amphidiploids by reciprocal hybridization and comparison to natural amphidiploids. Theor. Appl. Genet. 86, 811–821.

Statistics Canada, 2013a:

http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/prim11a-eng.htm (Retrieved on November 24, 2013)

Statistics Canada, 2013b: http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/prim11b-eng.htm (Retrieved on January 29, 2014)

Statistics Canada, 2014: Table 001-0010 - Estimated areas, yield, production and average farm price of principal field crops, in metric units, annual, CANSIM. http://www5.statcan.gc.ca/cansim/a26?lang=eng&retrLang=eng&id=0010010&paSer=&pattern=&stByVal=1&p1=1&p2=37&tabMode=dataTable&csid= (Retrieved on February 19, 2014)

Stefansson, B. R., and F. W. Hougen, 1964: Selection of rape plants (*Brassica napus*) with seed oil practically free from erucic acid. Can. J. Plant Sci. 44, 359–364.

Summers, D. J., and A. R. Robblee, 1985: Comparison of apparent amino acid digestibilities in anesthetized versus sacrificed chickens using diets containing soybean meal and canola meal. Poultry Sci. 64, 536–541.

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

Suwabe, K., H. Tsukazaki, H. Iketani, K. Hatakeyama, M. Kondo, M. Fujimura, T. Nunome, H. Fukuoka, M. Hirai, and S. Matsumoto, 2006: Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: the genetic origin of clubroot resistance. Genetics 173, 309–319.

Theodoridou, K., and P. Yu, 2012: Effect of processing conditions on the nutritive value of canola meal and presscake. Comparison of the yellow and brown-seeded canola meal with the brown-seeded canola presscake. J. Sci. Food Agric. 93, 1986–1995.

Thormann, C.E., M. E. Ferreira, L. E. A. Camargo, J. G. Tivang, T. C. Osborn, 1994: Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theor. Appl. Genet. 88, 973–980.

Trick, M., S. J. Kwon, S. R. Choi, F. Fraser, E. Soumpourou, N. Drou, Z. Wang, S. Y. Lee, T. J. Yang, J. H. Mun, A. H. Paterson, C. D. Town, J. C. Pires, Y. P. Lim, B. S. Park, and I. Bancroft, 2009: Complexity of genome evolution by segmental rearrangement in *Brassica rapa* revealed by sequence-level analysis. BMC genomics. 10, 539.

Tu, Y., J. Sun, X. Ge, and Z. Li, 2009: Chromosome elimination, addition and introgression in intertribal partial hybrids between *Brassica rapa* and *Isatis indigotica*. Ann. Bot. 103, 1039–1048.

U, N., 1935: Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Japanese J. Botany 7, 389–452.

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

UK crop net. http://brassica.nbi.ac.uk/ (Retrieved on December 16, 2012)

United States Department of Agriculture, 2005: Foreign Agricultural Service. http://www.fas.usda.gov/remote/Canada/can_rsd.htm (Retrieved on November 24, 2013)

United States Department of Agriculture, 2012: Economic Research Service. http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx (Retrieved on November 22, 2013)

United States Department of Agriculture, 2014a: Foreign Agricultural Service. Table 02: Major Protein Meals: World Supply and Distribution (Commodity View). http://www.fas.usda.gov/psdonline/psdreport.aspx?hidReportRetrievalName=BVS&hidReportRetrievalID=701&hidReportRetrievalTemplateID=5 (Retrieved on February 19, 2014)

United States Department of Agriculture, 2014b: Foreign Agricultural Service. http://apps.fas.usda.gov/psdonline/psdReport.aspx?hidReportRetrievalName=Table+01%3a+Major+Oilseeds%3a+World+Supply+and+Distribution+(Commodity+View)++++++++++++++++++++++++&hidReportRetrievalID=531&hidReportRetrievalTemplateID=5 (Retrieved on February 14, 2014)

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

Vogel, K. E., 2009: Backcross breeding. In: Scott, M. P Transgenic Maize methods and protocols. Humana Press c/o Springer Science+ Bussiness Media. (pp. 161–169).

Wageningen UR, https://www.wageningenur.nl/en/show/Allozymes.htm (Retrieved on Feburary 16, 2015)

Wang, R., V. L. Ripley and G. Rakow, 2007: Pod shatter resistance evaluation in cultivation and breeding lines of *Brassica napus, B. juncea* and *Sinapsis alba.* Plant Breeding 126, 588–595

Yu, C. Y., S. W. Hu, H. X. Zhao, A. G. Guo, and G. L. Sun, 2005: Genetic distances revealed by morphological characters, isozymes, proteins and RAPD markers and their relationships with hybrid performance in oilseed rape (*Brassica napus* L.). Theor. Appl. Genet. 110, 511–518.

Zaman, M. W, 1989: Introgression in *Brassica napus* for adaptation to the growing conditions in Bangladesh. Theor. Appl. Genet. 77, 721–728.

Zhao, J., X. Wang, B. Deng, P. Lou, J. Wu, R. Sun, Z. Xu, J. Vromans, M. Koornneef and G. Bonnema, 2005: Genetic relationships within *Brassica rapa* as inferred from AFLP fingerprints. Theor. Appl. Genet. 110, 1301–1314.

Zhi-wen, L., F. Ting-Dong, T. Jin-Xing, and C. Bao-Yuan, 2005: Inheritance of seed colour and identification of RAPD and AFLP markers linked to the seed colour gene in rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 110, 303–310.

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

Appendix

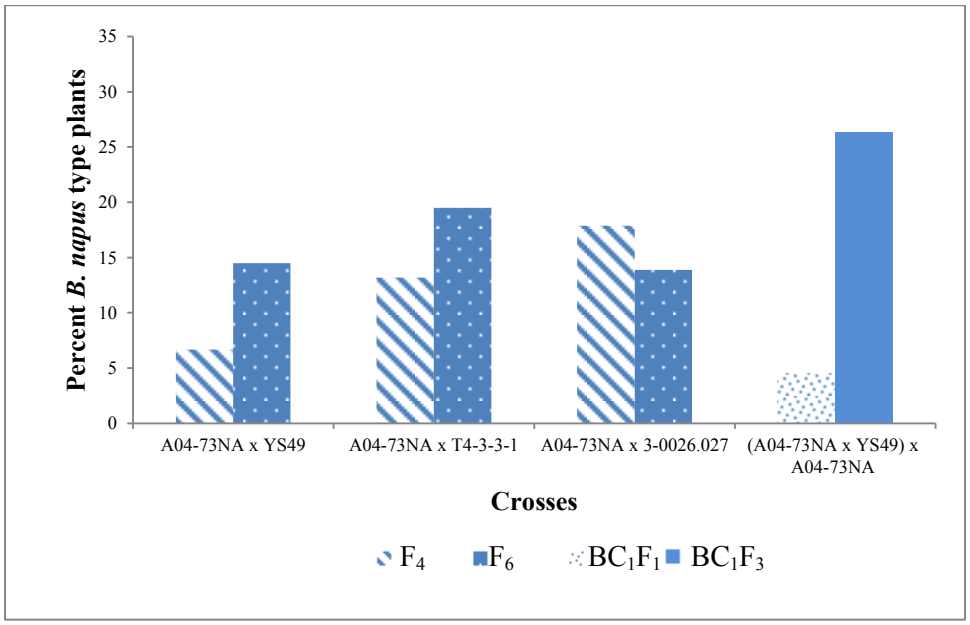


Figure A.1 Percent *B. napus* type plants for silique length in F_2- and BC_1F_1-derived population grown in greenhouse

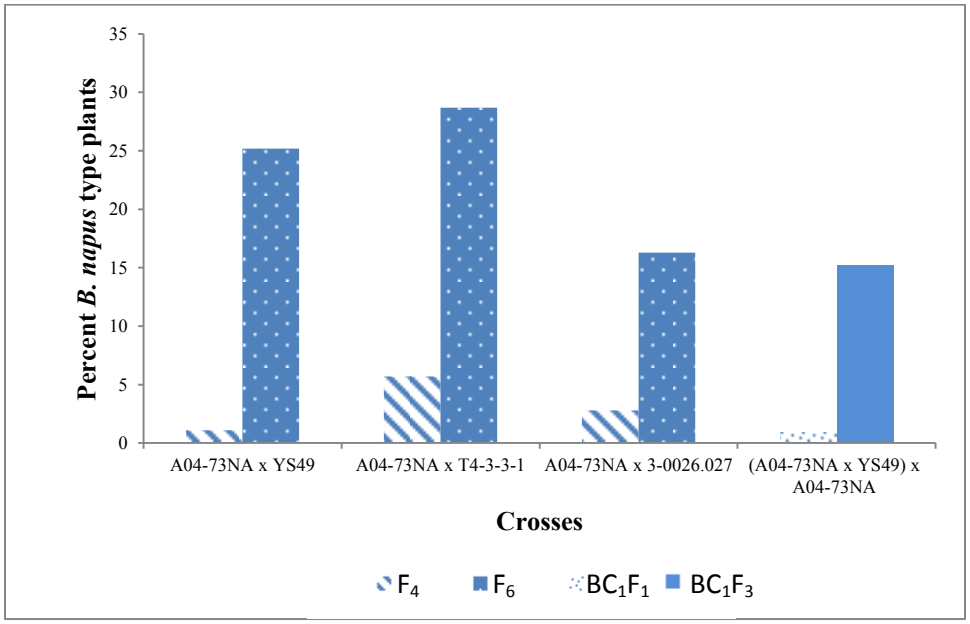


Figure A.2 Percent *B. napus* type plants for number of seeds per silique in F_2- and BC_1F_1-derived populations grown in greenhouse

Table A.1 Pedigree of F_4 plants derived from A04-73NA × T4-3-3-1 cross

Cross	F_3 Registration No.	F_4 Registration No.	F_4 ID
A04-73NA × T4-3-3-1	1299.003-A1231P02	1299.014-A1242P07	1299-014
A04-73NA × T4-3-3-1	1299.003-A1231P03	1299.015-A1242P01	1299-015
A04-73NA × T4-3-3-1	1299.003-A1231P04	1299.016-A1242P01	1299-016
A04-73NA × T4-3-3-1	1299.003-A1231P05	1299.017-A1242P02	1299-017
A04-73NA × T4-3-3-1	1299.003-A1231P06	1299.018-A1242P01	1299-018
A04-73NA × T4-3-3-1	1299.003-A1231P07	1299.019-A1242P01	1299-019
A04-73NA × T4-3-3-1	1299.003-A1231P08	1299.020-A1242P02	1299-020
A04-73NA × T4-3-3-1	1299.003-A1231P09	1299.021-A1242P02	1299-021
A04-73NA × T4-3-3-1	1299.006-A1231P04	1299.024-A1242P01	1299-024
A04-73NA × T4-3-3-1	1299.008-A1231P01	1299.028-A1242P02	1299-028
A04-73NA × T4-3-3-1	1299.008-A1231P03	1299.029-A1242P04	1299-029
A04-73NA × T4-3-3-1	1299.008-A1231P05	1299.030-A1242P03	1299-030
A04-73NA × T4-3-3-1	1299.009-A1231P01	1299.031-A1242P02	1299-031
A04-73NA × T4-3-3-1	1299.010-A1231P01	1299.032-A1242P01	1299-032
A04-73NA × T4-3-3-1	1299.003-A1231P10	1299.033-A1242904	1299-033

Table A.2 Pedigree of F_4 plants derived from A04-73NA × 3-0026.027 cross

Cross	F_3 Registration No.	F_4 Registration No.	F_4 ID
A04-73NA × 3-0026.027	1301.003-A1231P01	1301.029-A1242P01	1301-029
A04-73NA × 3-0026.027	1301.004-A1231P02	1301.031-A1242P02	1301-031
A04-73NA × 3-0026.027	1301.006-A1231P02	1301.034-A1242P02	1301-034
A04-73NA × 3-0026.027	1301.010-A1231P04	1301.040-A1242P02	1301-040
A04-73NA × 3-0026.027	1301.010-A1231P05	1301.041-A1242P01	1301-041
A04-73NA × 3-0026.027	1301.018-A1231P01	1301.065-A1242P03	1301-065
A04-73NA × 3-0026.027	1301.018-A1231P02	1301.066-A1242P01	1301-066
A04-73NA × 3-0026.027	1301.019-A1231P01	1301.067-A1242P02	1301-067
A04-73NA × 3-0026.027	1301.019-A1231P03	1301.069-A1242P01	1301-069
A04-73NA × 3-0026.027	1301.021-A1231P02	1301.070-A1242P03	1301-070
A04-73NA × 3-0026.027	1301.024-A1231P03	1301.078-A1242P02	1301-078
A04-73NA × 3-0026.027	1301.024-A1231P04	1301.079-A1242P02	1301-079
A04-73NA × 3-0026.027	1301.025-A1231P01	1301.080-A1242P01	1301-080

Table A.3 Pedigree of F_4 plants derived from A04-73NA × YS49 cross

Cross	F3 Registration No.	F4 Registration No.	F4 ID
A04-73NA × YS49	1257.018-A1230P01	1257.166-A1242P01	1257-166
A04-73NA × YS49	1257.065-A1230P02	1257.222-A1242P01	1257-222
A04-73NA × YS49	1257.081-A1230P02	1257.256-A1242P01	1257-256
A04-73NA × YS49	1257.092-A1230P01	1257.271-A1242P01	1257-271
A04-73NA × YS49	1257.092-A1230P04	1257.272-A1242P02	1257-272
A04-73NA × YS49	1257.092-A1230P05	1257.273-A1242P01	1257-273
A04-73NA × YS49	1257.093-A1230P01	1257.274-A1242P01	1257-274
A04-73NA × YS49	1257.108-A1230P02	1257.303-A1242P01	1257-303
A04-73NA × YS49	1257.108-A1230P04	1257.305-A1242P01	1257-305
A04-73NA × YS49	1257.110-A1230P01	1257.311-A1242P01	1257-311
A04-73NA × YS49	1257.113-A1230P03	1257.322-A1242P02	1257-322
A04-73NA × YS49	1257.113-A1230P04	1257.323-A1241P01	1257-323
A04-73NA × YS49	1257.113-A1230P05	1257.324-A1242P01	1257-324
A04-73NA × YS49	1257.118-A1230P01	1257.332-A1242P01	1257-332
A04-73NA × YS49	1257.149-A1230P01	1257.380-A1242P01	1257-380
A04-73NA × YS49	1257.149-A1230P02	1257.381-A1242P02	1257-381

Table A.4. List of SSR marker found to be polymorphic between *B. napus* and *B. rapa* parents

Sr No.	MM Code[1]	Source[2]
1	122	AAFC
2	142	AAFC
3	143	AAFC
4	144	AAFC
5	160	AAFC
6	165	AAFC
7	173	AAFC
8	176	AAFC
9	177	AAFC
10	195	AAFC
11	217	AAFC
12	218	AAFC
13	220	AAFC
14	235	AAFC
15	246	AAFC
16	251	AAFC
17	255	AAFC
18	257	AAFC
19	258	AAFC
20	259	AAFC
21	260	AAFC
22	266	AAFC
23	271	AAFC
24	273	AAFC
25	275	AAFC
26	276	AAFC
27	277	AAFC
28	278	AAFC
29	280	AAFC
30	281	AAFC
31	283	AAFC
32	288	AAFC
33	296	AAFC
34	322	AAFC
35	567	AAFC
36	571	AAFC
37	572	AAFC
38	577	AAFC
39	580	AAFC
40	585	AAFC
41	589	AAFC
42	590	AAFC
43	594	AAFC
44	596	AAFC
45	597	AAFC
46	640	Cheng et al. (2009)
47	812	AAFC
48	814	AAFC
49	815	AAFC
50	816	AAFC
51	823	AAFC
52	830	AAFC
53	832	AAFC
54	1997	AAFC
55	2001	AAFC
56	2004	AAFC
57	2026	AAFC
58	2036	AAFC
59	2331	AAFC
60	2336	AAFC
61	2337	AAFC
62	2343	AAFC
63	2344	AAFC
64	2346	AAFC
65	2478	AAFC
66	2480	AAFC
67	2487	AAFC
68	2491	AAFC
69	2496	AAFC
70	2708	Designed by Canola program
71	2710	Internally designed by Canola program
72	2712	Internally designed by Canola program
73	2717	Internally designed by Canola program
74	2718	Internally designed by Canola program
75	2719	Internally designed by Canola program
76	2720	Internally designed by Canola program

Sr No.	MM code[1]	Source[2]
77	2721	Internally designed by Canola program
78	2722	Internally designed by Canola program
79	2723	Internally designed by Canola program
80	2724	Internally designed by Canola program
81	2725	Internally designed by Canola program
82	2726	Internally designed by Canola program
83	2727	Internally designed by Canola program
84	2728	Internally designed by Canola program
85	2729	Internally designed by Canola program
86	2730	Internally designed by Canola program
87	2731	Internally designed by Canola program
88	2732	Internally designed by Canola program
89	2733	Internally designed by Canola program
90	2735	Internally designed by Canola program
91	2736	Internally designed by Canola program
92	2737	Internally designed by Canola program
93	2738	Internally designed by Canola program
94	2739	Internally designed by Canola program
95	2740	Internally designed by Canola program
96	2743	Internally designed by Canola program
97	2746	Internally designed by Canola program
98	2747	Internally designed by Canola program
99	2749	Internally designed by Canola program
100	2750	Internally designed by Canola program
101	2751	Internally designed by Canola program
102	2753	Internally designed by Canola program

[1]Code used internally by the Canola Program of U of A

[2] AAFC: Agriculture and Agri-Food Canada, obtained through a material transfer agreement; Cheng et al. (2009) (Cheng, X., J. Xu, S. Xia, J. Gu, Y. Yang, J. Fu, X. Qian, S. Zhang, J. Wu, and L. Kede, 2009: Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus.* Theor. Appl. Genet. 118, 1121–1131).