Evaluation of genetic diversity and performance of the inbred lines derived from *B. napus* × *B. rapa* interspecific crosses and their test hybrids

by

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ABSTRACT

Spring *Brassica napus* L. canola (AACC, *2n* = 38) is the second most important crop in Canada. Genetic diversity in this crop is narrow; therefore, broadening of the genetic base of this crop is needed for continued improvement through breeding. For this, the exotic gene pools of this species, such as winter and semi-winter type, as well as the wide diversity exist in its progenitor species *Brassica rapa* L. and *Brassica oleracea* L. can be utilized. In this MSc thesis research project, potential value of the *B. rapa* gene pool for broadening the genetic base of the Canadian spring *B. napus* canola as well as for increasing the level of heterosis for seed yield in hybrid canola was investigated by developing three inbred populations from two *B. napus* × *B. rapa* interspecific crosses. Also, Genetic diversity analysis of the inbred population derived from the BC_1 of (*B. napus* × *B. rapa*) × *B. napus* by SSR markers showed that genetically distinct spring *B. napus* canola lines carrying *B. rapa* alleles can be achieved from this interspecific cross. Of the theoretical expected number of *B. rapa* alleles, the inbred population carried about 79% of the alleles. Seed yield of the three inbred populations derived from the F_2 and BC_1 of these interspecific crosses was, on average, lower than the *B. napus* canola parent. However, seed yield of the test hybrid populations was significantly higher than the *B. napus* canola parent, and exhibited up to 24% mid-parent heterosis and 20% heterosis over the *B. napus* canola parent. Thus, this study demonstrated the potential of the *B. rapa* gene pool for broadening the genetic base of the Canadian canola as well as for increasing the seed yield in hybrid canola cultivars.

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List of Symbols and Abbreviations

±	Plus/minus
χ^2	Chi-square test statistics
2*n*	Diploid number of chromosomes
n	Number of observations
ng/µl	Nano gram per micro liter
µl	Microliter
µmol/g	Micromoles per gram
°C	Degrees Celsius
AAFC	Agriculture and Agri-Food Canada
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
BC_1	First backcross generation
BC_1F_n	nth backcross generation
B. nap	*Brassica napus*
cm	Centimeter
cv.	Cultivar
d.f.	Degree of freedom
DH	Doubled haploid
DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleotide triphosphate
F_1	First filial generation
F_n	nth filial generation
FAO	Food and Agriculture Organization of the United Nations
Fig.	Figure

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

Taq polymerase	Thermus aquaticus polymerase
UPGMA	Unweighted pair-group method with arithmetic mean
USDA	United States Department of Agriculture
var.	Variety

Chapter 1

Literature review

1.1 Introduction

The genus Brassica of the family *Brassicaceae* (*Cruciferae*) includes several crops of economic importance in the world. Plants of this family have cross-shaped flower with four petals (McVetty and Duncan, 2016). The genus Brassica includes different species, such as *B. oleracea*, a main vegetable crop species which includes kale, broccoli, cauliflower, brussels sprouts and cabbages; *B. rapa*, which includes the vegetable turnip, Chinese cabbage and pakchoi as well as oilseed type; *B. napus*, which is grown mainly as oilseed crop, and *B. carinata, B. nigra* and *B. juncea*, which belongs to oilseed and condiment mustard group (Cartea et al., 2010; Velasco et al., 2016).

The oilseed *B. juncea* is grown mainly in India, and is also suitable for growing in low rainfall region of southern Alberta. This species is tolerant of heat and drought. Oilseed *B. rapa* is mainly cultivated in Indian sub-continent (Mendham and Robertson, 2016). In the very beginning of establishment of Brassica oilseed crop in Canada, this species was mainly cultivated in the Peace River region of northern Alberta. However, the development of early maturing, higher-yielding *B. napus* cultivars has resulted a significant reduction in *B. rapa* average; currently, this species almost disappeared from Canada. Compared to *B. rapa* and *B. juncea, B. napus* has the greatest potential of yield and wide adaptability (Mendham and Robertson, 2016). The winter type of *B. napus* is mainly grown in Europe while the spring type is mostly grown in western Canada and Australia. *B. napus* is an allotetraploid crop species and is the second most important oilseed crop in the world (McVetty and Duncan, 2016). This crop species is evolved in the Mediterranean region from the cross between *B. rapa* and *B. oleracea* (Barthet, 2016).

Brassica napus canola is the second most important crop in Canada after wheat; it is called Canada's Cinderella crop because the development of canola started in Canada (Eskin, 2016). Canada was not growing this oilseed crop at a commercial scale before World War II. Planting of this crop in this country started in 1930`s in Saskatchewan when Fred Solvoniuk, a Polish-Canadian farmer, planted some *B. rapa* seeds introduced from Poland. Also, during this time, *B. napus* was introduced in Canada from Argentina (Eskin, 2016). At that time, the oil of this crop was used to lubricate marine engines. The high content of erucic acid in this oil was the major hindrance for use of this oil in human diet. Fatty acid composition of this oil was modified by the Canadian breeders and made this oil suitable for human consumption (Eskin, 2016). Canola is the improved version of rapeseed, and also called double-low rapeseed, with oil containing less than 2% erucic acid and seed meal with less than 30 μmol glucosinolate per g meal. Canola was developed in Canada through joint effort between Agriculture and Agri-Food Canada and the University of Manitoba (Barthet, 2016). The conversion of canola from rapeseed was an important landmark in Canadian agriculture. The reduction in the level of erucic acid in canola oil also resulted to an increase in the level of monounsaturated fatty acid, the oleic acid (Eskin, 2016). The name canola is derived from the contracted words of "Can" for Canada and "ola" for oil, and this name was officially approved by the Rapeseed Association of Canada in 1978 to trade mark this new type (double-low) of rapeseed (Barthet, 2016).

Seedling vigor, earliness of flowering and maturity, lodging resistance, and high seed yield are the important traits for *B. napus* canola (Jan et al., 2016). Breeding for silique shatter resistance has received much attention in the breeding programs in the recent years as silique shatter can reduce seed yield. Increasing seed oil content is another objective of canola breeding, and this can be achieved by crossing of high oil content lines with selection in the segregating population

(McVetty and Duncan, 2016). The negative correlation between seed oil and protein content (Sernyk and Stefansson, 1983) make it challenging for simultaneous improvement of these two traits. Yellow-seeded *B. napus*, due to its thinner seed coat, indirectly contribute to increased oil content in seed; therefore, the development of yellow seeded cultivars might increase oil content in seed while maintaining its protein content (McVetty and Duncan, 2016). Maintenance of the reduced content of erucic acid and glucosinolate is another objective of canola breeding, especially when exotic germplasm and allied species are used in breeding (reviewed by Rahman, 2013).

On the fatty acid profile of oil, reduction of linoleic acid content from 10% to less than 3% and an increase in oleic acid content over 70% has improved the stability of the canola oil under storage and frying conditions. The development of high oleic low linoleic acid canola (HOLL) is an example of an important achievement of breeding *B. napus* canola (McVetty and Duncan, 2016). In contrary, breeding for increased erucic acid content in oil is an objective for use of the Brassica seed oil in industrial applications. The high erucic acid rapeseed "HEAR" cultivars such as "Hero" with erucic acid content of 50.2% and glucosinolate content of 15µmol/g have been developed through traditional breeding in both winter and spring *B. napus* (Barthet, 2016). These cultivars are not considered as canola but are produced for special oil used in different industrial applications, such as lubricants (Barthet, 2016). Resistances to different diseases, such blackleg caused by *Leptosphaeria maculans*, and clubroot caused by *Plasmodiophora brassicae* are also important objectives in Brassica oilseed crop breeding in Canada (McVetty and Duncan, 2016).

In a breeding program, it is important to evaluate the breeding materials in field trials for reliable data to be used for selection of breeding lines. However, evaluation of the breeding materials before testing in the field trials by molecular markers is gaining increasing interest in

modern breeding (Jan et al., 2016). This may range from selection for a specific trait to selection at the whole genome level, called genomic selection (GS) (Jan et al., 2016). However, GS can be a viable approach if the cost of selection gain can be reduced and maintained at a lower level as compared to the cost of conventional breeding where field trials at various locations and multiple years is needed (Jan et al., 2016). Among the different molecular markers, single-nucleotide polymorphism (SNP) is a powerful tool for genotyping at whole genome level (Jan et al., 2016). Genotyping by microsatellite or simple sequence repeat (SSR) markers is very common in plant science research for the reasons that SSR genotyping can be done at low cost and can be done with inexpensive equipment's (Hobson and Rahman, 2016; Vieira et al., 2016). Almost 1000 articles have been published during the period of 2010 to 2015 by using SSR markers in research (Fig. 1.1). This indicates the worldwide use of SSR markers; however, after 2012 there has been a decline in the number of publications using SSR markers, apparently due to the introduction of SNP markers (Vieira et al., 2016).

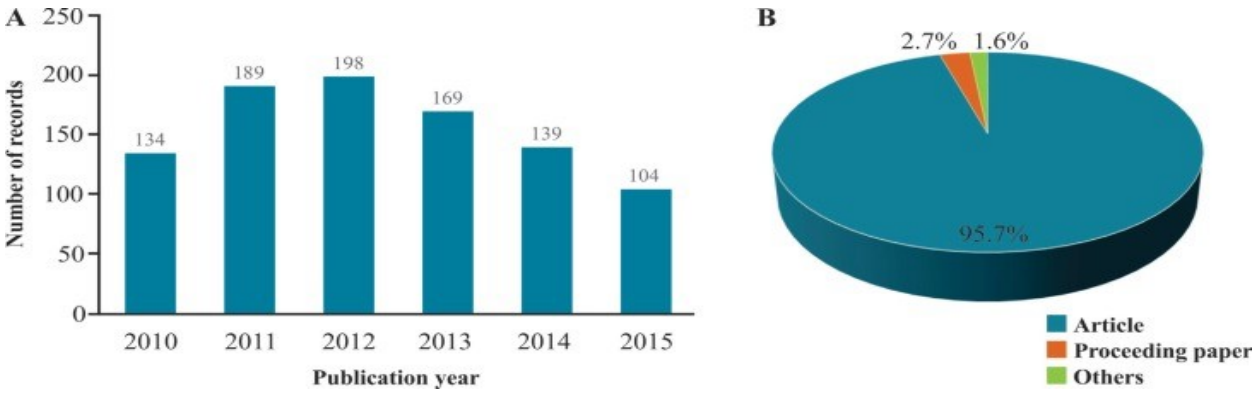

Fig. 1.1 (A) Shows the number of articles published by using SSR markers in plant science research during the period of 2010 to 2015 and (B) indicates that 95.7% of publications were scientific articles (Vieira et al., 2016).

1.2 Economic value of canola

Canola is the second most important oilseed crop in the world after soybean. The high demand for vegetable oil in the world resulted about 89% increased production of canola in the

last decade (McVetty and Duncan, 2016). Brassica oilseed production in the world in 2016-17 was 68.52 million metric tons (Statista, 2017) where Canada was the top of producer of this oilseed (Fig. 1.2 and Fig. 1.3).

In 2014-2015, the direct and indirect contribution of canola to the Canadian economy was estimated to be about $26.7 billion. The province of Saskatchewan gets the highest economic impact from canola with gross revenue of $12.2 billion, followed by Alberta with $7.1 and Manitoba with $4.1 billion (LMC International, 2016). The yield of canola in Canada was 2.37 t/ha in 2016-17. This exceeded the former record of 2.27 t/ha in 2013-14 and 2.21 t/ha in 2015-16. Among the three Prairie Provinces of Canada the highest canola yield often recorded in Alberta (e.g. 2.60 t/ha in 2016; AAFC, 2016).

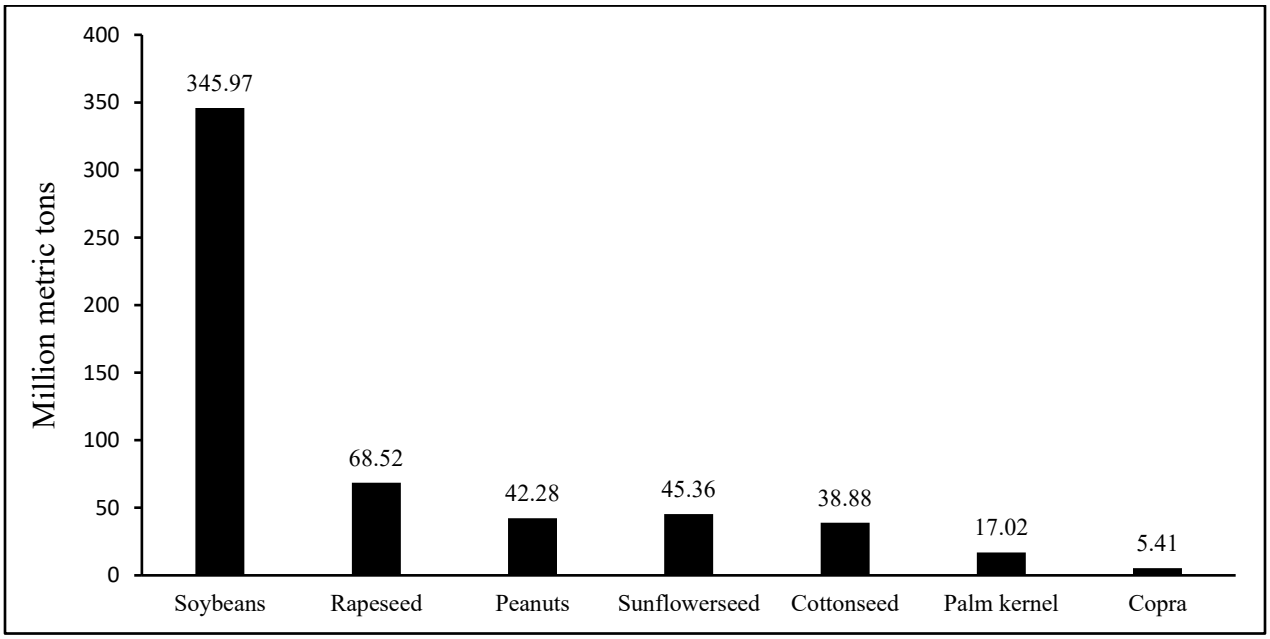

Fig. 1.2 Oilseed production in the world in 2016/17 in million metric tons (Statista, 2017a).

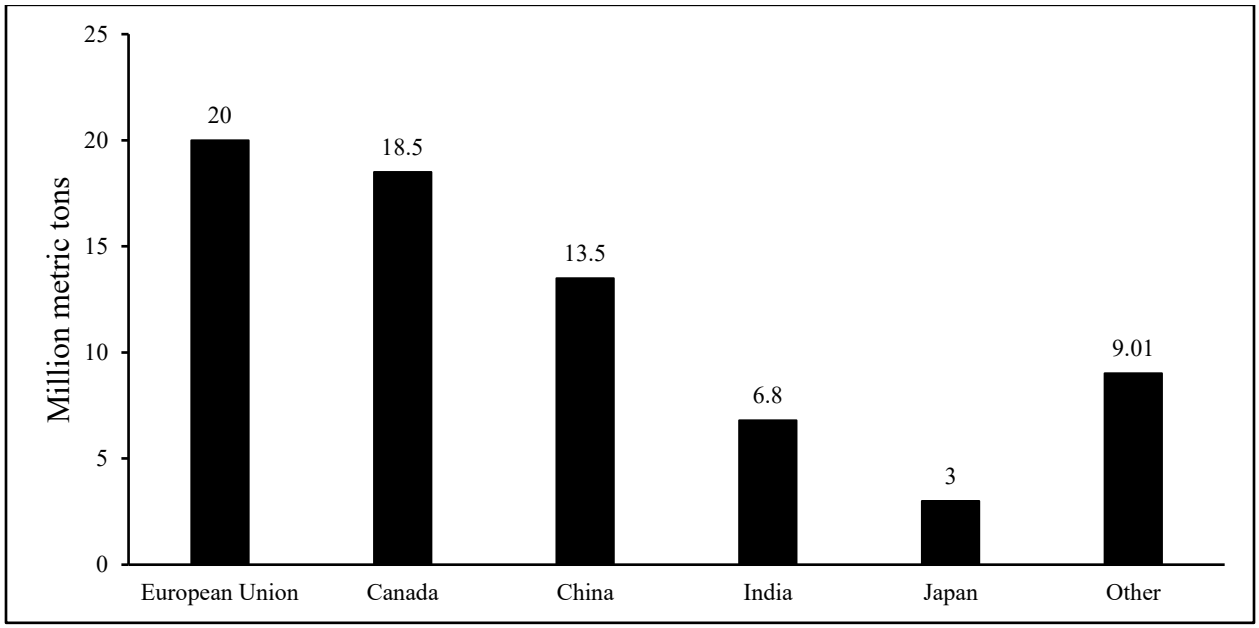

Fig. 1.3 Brassica oilseed production by country in 2016/17 in million metric tons (Statista, 2017b).

Of the total quantity of edible oil consumed in Canada, canola`s share is about 50%; and of the total production of canola oil in Canada, about 65% is being exported to other countries for use as edible oil, such as salad dressings, margarine, and shortening (Eskin, 2016). Canada was the largest exporter of canola in the world in 2014-15 (Gervais, 2015) (Fig. 1.4).

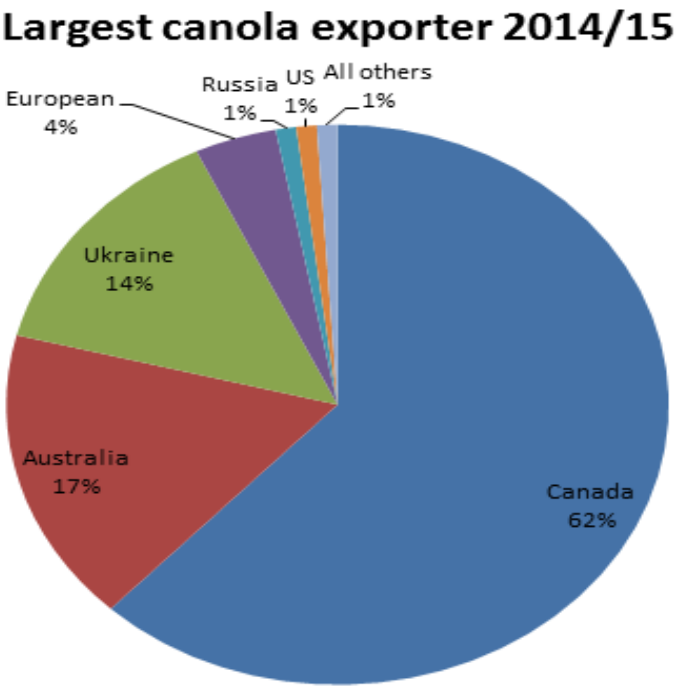

Fig. 1.4 Canola export countries in 2014-15 (Gervais, 2015).

Among the different canola exporting countries, Australia ranked in the second position. In Australia, canola is the third important crop after wheat and barley. In 2016-17, this country produced about 3.3 million tons of canola. Australian canola is mostly exported to Asia for human consumption and to Europe for human consumption and biodiesel production (USDA, 2016).

1.3 Canola oil and its benefit and uses

Canola oil is composed of about 7% saturated fatty acids, 62% monounsaturated fatty acid, 19% omega-6 or linoleic fatty acid, and 9% omega-3 or alpha-linolenic fatty acid. Among the different cooking or salad oils, canola oil has the lowest level of saturated fatty acids (Fig. 1.5) (Canola Council of Canada, 2017). The high omega-3 fatty acid in canola oil is nutritionally desirable as this fatty acid is considered to have anti-inflammatory function and protection of humans from heart attack, while the omega-6 is necessary for human growth and development and is also good for skin health (Canola Council of Canada, 2017; Harris et al., 2008).

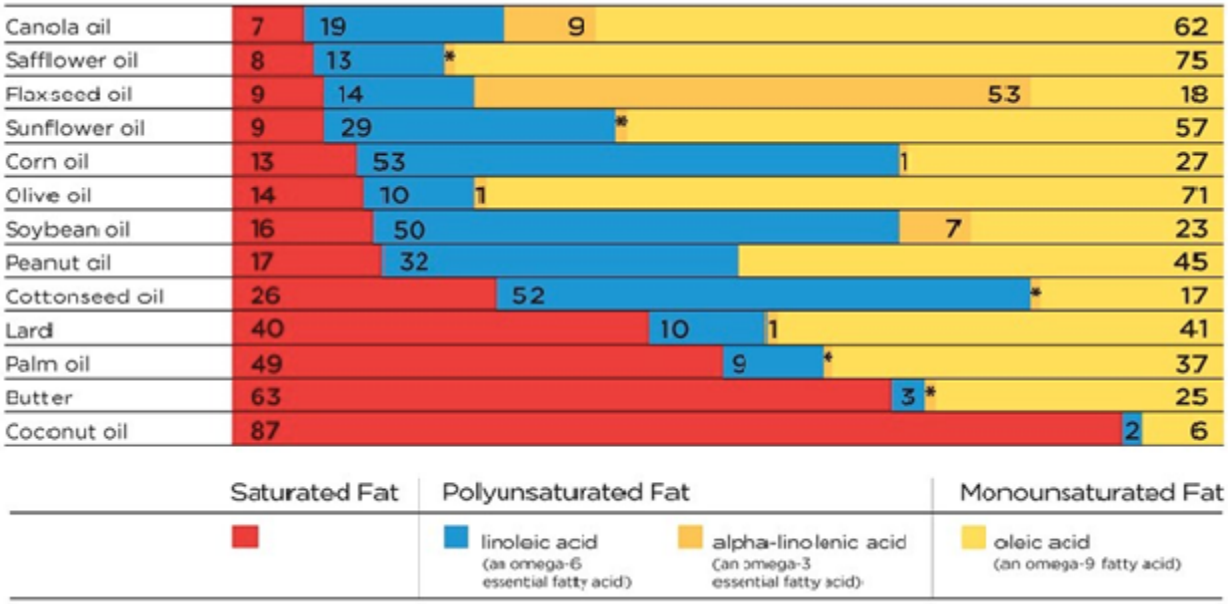

Fig. 1.5 Comparison of fatty acid composition of different oils, fatty acid content expressed as g/100g fat (Canola Council of Canada, 2017).

Canola oil is good for frying and other high heat applications, as it does not degrade at high temperature. Among the different cooking oils, canola oil has the second highest smoke point (242 °C) (the temperature at which oil begins to smoke and degrade) (Table 1.1) (Canola Council of Canada, 2017).

Table 1.1 Smoke point of different vegetable oils (Canola Council of Canada, 2017)

Oil	Smoke Point(°C)
Peanut	244
Canola	242
Sunflower	240
Corn	234
Soybean	234
Safflower	230
Grape seed	224
Olive processed	220
Extra Virgin Olive	166

1.4 Seed quality traits

Canola breeding started in the 1950's with the discovery of low erucic acid seeds in the spring forage cultivar 'Liho' (Cao et al., 2010). Low erucic acid and low glucosinolates contents are two important factors that determine the quality of Brassica seed oil and seed meal respectively (Su et al., 2017). High content of erucic acid in oil is undesirable for use of this oil in food application, and high content of glucosinolates is undesirable for use of this protein-rich seed meal in animal feed (Andersson et al., 2008). Eruric acid content in *B. napus* seed oil is controlled by two loci, one from each of the two genomes (A8 and C3) (Rahman et al., 2008), where the zero erucic acid alleles in canola cultivars are derived from the forage rape cultivar 'Liho' (Cao et al., 2010). Cao et al., (2010) found evidence of a strong linkage drag of low seed erucic acid allele, which originally derived from the cultivar 'Liho', with low oil content. They analyzed 90 *B. napus* cultivars and found this linkage drag on A8 chromosome in more than 46% of the cultivars, and suggested that backcrossing along with marker-assisted selection can be applied to break this linkage. To date, more than 10 QTLs contributing to glucosinolate content have been identified in Brassica (Arasu et al., 2017). Rahman et al. (2014) detected three QTL on A2, A7 and A9 linkage groups of *B. rapa* contributing to seed glucosinolate content in this species. The main GLSs of *B. rapa* and *B. napus* seeds are progoitrin, gluconapin, glucobrassicanapin and napoleiferin (Sang and Salisbury, 1988) of which gluconapin and progoitrin are the dominating compounds in *B. napus* (Chun et al., 2016; Arasu et al., 2017), while the seeds of yellow sarson predominatly contain gluconapin (Rahman et al., 1986).

1.5 Evolution of Brassica species and genetic diversity

B. napus (2*n* = 38) carries the AACC genome and evolved from an interspecific hybridization between *B. rapa* (2*n* = 20, AA) and *B. oleracea* (2*n* = 18, CC) (Diers and Osborn, 1994; reviewed

by Prakash and Hinata, 1980) (Fig. 1.6) The geographical location where the *B. napus* was evolved is not clearly known; however, it is generally accepted that this amphidiploid species originated in the Mediterranean region where its two parents, *B. rapa* and *B. oleracea*, can be found (reviewed by Prakash and Hinata, 1980; Barthet, 2016).

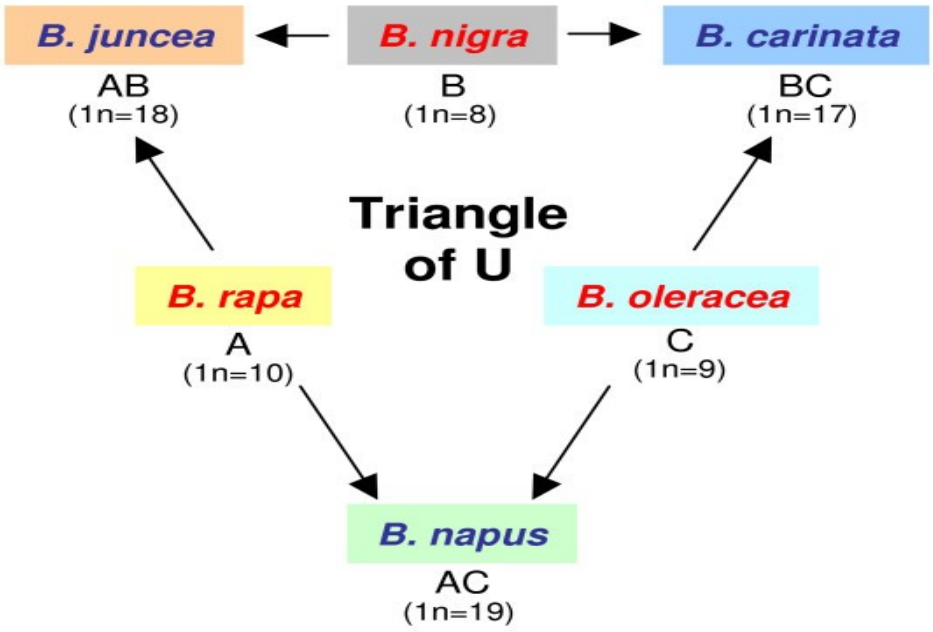

Fig. 1.6 The relationships of the Brassica species of the "Triangle of U" (Østergaard and King, 2008).

B. juncea, an amphidiploid of *B. rapa* and *B. nigra*, is the main Brassica oilseed crop in India; (Singh et al., 2013); while *B. carinata*, an amphidiploid of *B. oleracea* and *B. nigra*, is primarily grown in Africa as leafy vegetable (McVetty and Duncan, 2016). Among the diploid Brassica species, *B. oleracea* has great economic importance as vegetable crop worldwide (El-Esawi et al., 2016a). *B. rapa* is an important oil crop in Indian-subcontinent; this species possess a wide range of genetic and morphological diversity (Thakur et al., 2016) and occur as vegetable form as well. The existence of wide genetic diversity in *B. rapa* also has been confirmed by Hobson and

Rahman (2016) through the analysis of 43 accessions of different variants of *B. rapa* by 730 SSR alleles. This analysis separated the accessions into seven genetically distinct groups: Chinese cabbage, Chinese winter oilseed, European winter oilseed, Canadian spring oilseed, pak-choi, turnip, and yellow sarson (Fig. 1.7).

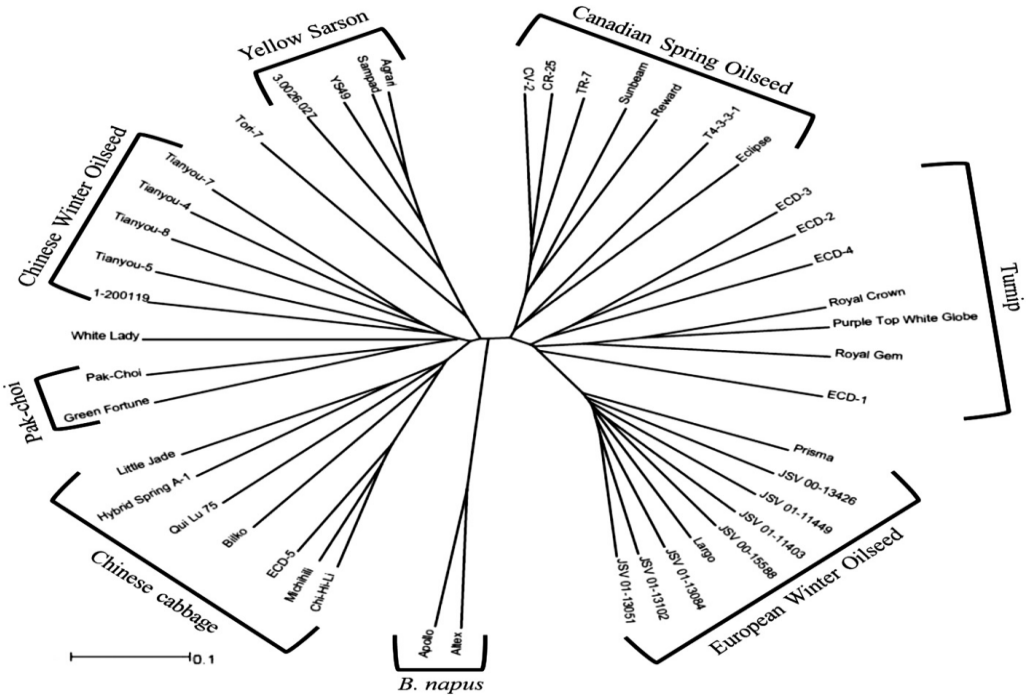

Fig. 1.7 Genetic diversity in *B. rapa* analyzed by use of SSR markers (Hobson and Rahman, 2016).

Tian et al. (2017a) investigated genetic diversity among 127 accessions of *B. napus*, *B. rapa* and *B. juncea* by use of A-genome specific SSR markers, and found that the A genome of these three species are genetically distinct (Fig. 1.8); this suggests that allelic diversity from one of these species can be introduced to the other. However, they also found considerable diversity within the species as shown from within species variance of 63.14%, suggesting that wide diversity present within these species can also be used in breeding. Among these three species, *B. juncea* and *B. napus* have the farthest relationship.

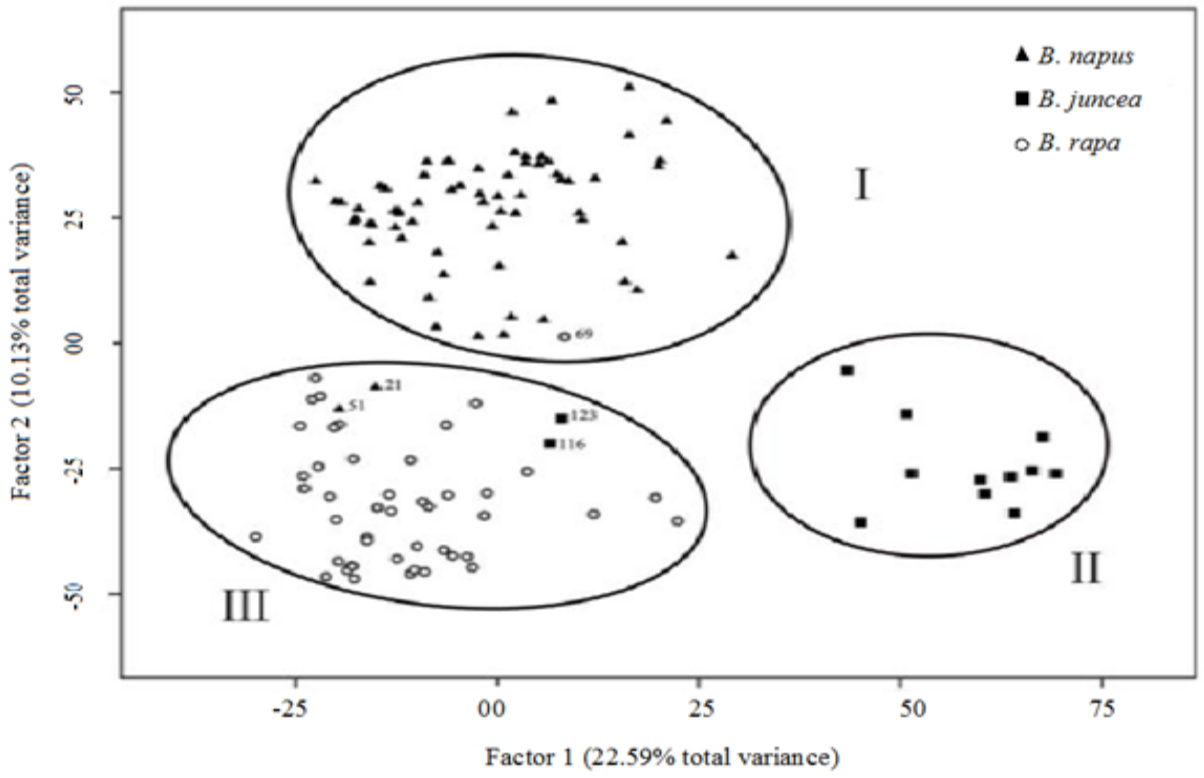

Fig. 1.8 The genetic variation within and between *B. napus*, *B. juncea* and *B. rapa* estimated by use of A genome specific markers. The group I includes 66 *B. napus* and one *B. rapa* accession; Group II includes 11 *B. juncea* accessions, and Group III includes 45 *B. rapa*, two *B. napus* and two *B. juncea* accessions (Tian et al., 2017a).

Based on growth habit, oilseed *B. napus* can be divided into winter, semi-winter and spring types. The winter type is mainly grown in Europe and is the most important oilseed crop of this continent (Gehringer et al., 2007); this type needs vernalization for flowering. The semi-winter type is grown in China and need a short period of vernalization for flowering, while the spring type does not require vernalization for flowering and is grown in Northern Europe, Canada and Australia (Friedt and Snowdon, 2009). Comparison between spring and winter types based on SNP markers revealed that spring oilseed type possess greater level of polymorphism, and linkage disequilibrium decay faster in this type than winter types (Delourme et al., 2013).

While plant biodiversity takes the number of species in an ecosystem into consideration, plant genetic diversity refers to the extent of heritable variation present within and between populations of a plant species. Genetic diversity in a crop species changes over time depending on breeding history and the extent of human intervention as well as ecological and geographical conditions under which the species has been grown (Rao and Hodgkin, 2002). Presence of adequate genetic diversity in a crop species is not only important for further improvement through breeding (reviewed by Rahman, 2013), but also for integrated pest management as well as for the maintenance of the agro-ecosystem (Hajjar et al., 2008). However, the development of new high-yielding cultivars and use of modern technology has narrowed crop genetic diversity (Hawkes, 1991); maintenance of adequate genetic diversity in crop germplasm is vital for present and future human prosperity (Rao and Hodgkin, 2002). Wild species and exotic germplasm are the valuable resources in a breeding program for widening the genetic base of a crop (Hawkes, 1991; reviewed by Rahman, 2013).

Molecular markers are valuable tools for evaluation of genetic diversity within the crop germplasm to investigate the relationship between different genotypes (Mahjoob et al., 2016). Different molecular markers have been used to assess genetic diversity in Brassica. Singh et al. (2013) used 143 A and B genome-specific SSR markers and 12 phenotypic traits to evaluate genetic diversity among 44 *B. juncea* accessions and found that grouping of the accessions based on SSR marker data is more reliable than the phenotype traits. El-Esawi et al. (2016a) used SSR markers to assess genetic diversity and population structure of *B. oleracea* accessions from Ireland and found that SSR markers are efficient for discriminating the accessions. Hassan et al. (2006) investigated genetic relationship among 96 accessions of *B. napus*. Based on SSR marker data, they classified these accessions collected into four groups: spring oilseed and fodder, winter

oilseed, winter fodder, and vegetable type. Thakur et al. (2016) investigated genetic diversity among 28 accessions, belonging to three subspecies of *B. rapa*, such as yellow sarson, toria and brown sarson, based on different agronomic traits and 65 polymorphic SSR markers and found greatest variation for silique angle (coefficient of variation, CV = 30.9%) followed by number of seeds/siliqua (CV = 18.8%), plant height (CV = 16.8%) and leaf length (CV = 10%) in this population. Based on morphological traits and SSR marker data, they classified the accessions into five groups, and also detected 23 unique alleles in 13 *B. rapa* accessions.

The amplified fragment length polymorphism (AFLP) is another type of marker of choice for the assessment of genetic diversity in Brassica (Faltusová et al., 2011). El-Esawi et al. (2016b) used AFLP markers to analyze genetic diversity in 25 accessions of *B. oleracea* collected from Ireland and found that about 90% (423/471) of the AFLP fragments to be polymorphic among the accessions; about 33.7% of the total genetic variance was detected among the accessions and 66.3% variation within the accessions. Havlickova et al. (2014) assessed genetic diversity among 94 accessions of winter *B. napus* by using 89 AFLP, 1003 ISSR and 53 SSR markers, and found that 100%, 53.9%, and 90.6% of the fragments, respectively of these three marker type was polymorphic among the accessions. All three type of markers were capable of differentiating the accessions; however, the greatest genetic distance was detected by ISSRs (62.3%) followed by SSRs (49.4%) and AFLPs (35.5%). Leonte and Arsene (2016) evaluated genetic diversity among 50 *B. napus* cultivars by use of 20 RAPD and 55 SSR markers and 7 phenotypic traits. Based on phenotypic data they were able to group the cultivars into three clusters and two clusters were established based on genotypic data. In addition to our knowledge of the nuclear genome, knowledge of the diversity in the cytoplasmic genome may also be useful for the improvement of our crop plants. Heng et al. (2017) developed molecular markers based on mitochondrial genome

and these markers were capable of detecting different mitochondrial types in *B. napus* for use in breeding.

1.6 Broadening of the genetic base of spring *Brassica napus*

Genetic diversity in spring oilseed *B. napus* is known to be narrow. Different methods, such as interspecific cross between *B. napus* × *B. rapa* or *B. napus* × *B. oleracea* for introgression of exotic alleles from the related species, and crossing of different ecotypes of *B. napus*, such as winter and semi-winter type to spring type can be used to broaden the genetic base of spring *B. napus* (reviewed by Rahman, 2013). Wide genetic diversity exists in *B. rapa* (Hobson and Rahman, 2016); this means that this species can be used as a valuable source of germplasm to broaden the genetic base of *B. napus*. While crossing *B. napus* and *B. rapa*, Nieman et al. (2015) found that greater number of interspecific hybrids can be obtained using of *B. napus* as the male and *B. rapa* ssp. chinensis as the female parent. Mei et al. (2011) demonstrated that the Chinese semi-winter *B. rapa* can be used to widen the genetic base of *B. napus*. They found wide genetic distance between natural oilseed *B. napus* and the *B. napus* lines derived from *B. napus* × semi-winter *B. rapa* interspecific cross. Qian et al. (2006) reported that introgression of genome content of Chinese *B. rapa* into Chinese *B. napus* played an important role in the evolution of semi-winter *B. napus*, and this type of *B. napus* found to be genetically distinct from the spring and winter type. The Chinese semi-winter *B. napus* has shown great potential for the improvement of seed yield in European winter *B. napus* cultivars (Qian et al., 2009). Liu and Meng (2006) used RFLP and an AFLP markers to investigate the extent of genetic variations within and between *B. napus* and *B. rapa* and found that Chinese *B. rapa* were not only more polymorphic than Chinese *B. napus*, but also was more polymorphic than European *B. rapa* (49.2%). This confirmed the value of using *B. rapa* in the breeding of *B. napus*. Liu and Meng (2006) also found

that genetic diversity analysis based on AFLP and RFLP markers correlate well ($r = 0.72$, $P < 0.001$).

Rahman and Kebede, (2012) showed the potential of the use of European winter oilseed *B. napus*, which is known to be genetically distinct from the spring oilseed *B. napus*, for broadening the genetic base of spring *B. napus* canola. They showed that several spring *B. napus* canola lines derived from winter × spring crosses could be genetically close to the winter type, and lines with higher seed yield than the spring parent can be achieved from this program after one cycle of breeding. However, some of these lines found to possess some undesirable traits, such as delay in flowering and maturity; therefore, a repeated cycle of breeding is often needed to improve these traits. The spring canola cultivar UA AlfaGold is an example, which was developed from a winter × spring cross (Rahman, 2016).

Resynthesized *B. napus* is another valuable resource of germplasm for introduction of valuable traits from the parental species *B. rapa* and *B. oleracea* into *B. napus*, as well as for broadening the genetic base of this crop species (Chen and Heneen, 1989; Rahman, 2013). The production of resynthesized *B. napus* has been simplified through the application of cell and tissue culture techniques, such as embryo culture (Rahman, 2004).

1.7 Heterosis

1.7.1 Genetic basis of heterosis

Heterosis or hybrid vigor is a phenomenon in which the progeny of two genetically different inbred lines show greater productivity than the parental lines. This phenomenon has been utilized in the breeding of cultivars of different crops such as maize, rice and sorghum (Lee and Tollenaar, 2007). Hybrid vigor was first recognized by Charles Darwin in 1876 when he noticed that the

offspring of cross-pollinated maize plants become 25% taller and show greater tolerance to cooler growth condition as compared to self-fertilized plants (Duvick, 2001). This phenomenon was rediscovered in maize by George H. Shull in 1910 who coined the term "heterosis" for this phenomenon (Shull, 1948).

Heterosis can be calculated in three ways: Mid parent heterosis (MPH), which estimates the hybrid performance compared to the mean value of its two homozygous parents [MPH = (F_1 – MP)/MP × 100]; better parent heterosis (BPH), which show the performance of the hybrid over the parent with higher value for the trait [BPH = (F_1 – BP)/BP × 100]; and standard heterosis of the F_1-hybrid (SVH), which to demonstrate the performance of the hybrid over the standard check cultivar [SVH= (F_1 – SV)/SV × 100] (Hayes et al., 1955; Hochholdinger and Hoecker, 2007).

Despite many researches proposed different hypothesis for the phenomenon of heterosis, enough evidence cannot be found to completely explain the genetic basis of heterosis (Reif et al., 2005; Hochholdinger and Hoecker, 2007; Shi et al., 2011; Yao et al., 2013; Fu et al., 2015). Several hypotheses have been proposed for this phenomenon, such as dominance, overdominance, pseudo-overdominance and epistasis effect of the genes (Birchler et al., 2010).

i). Dominance hypothesis: This model proposes that the deleterious recessive alleles of the inbred lines are complemented by the dominant alleles in hybrids (Bruce, 1910; Jones, 1917; Xiao et al., 1995). The drawback of this proposal is that, this hypothesis cannot solely support the phenomenon of hybrid vigor in all situations as an inbred line with dominant homozygous condition can be, theoretically, as good as hybrids; however, in reality, the F_1 hybrids often exceed the performance of the inbred lines carrying dominant alleles in homozygous condition at all loci (reviewed by Chen, 2010).

ii). Overdominance hypothesis: In this model, it is assumed that interaction of alleles at many genetic loci in heterozygous state in the hybrid is superior over the homozygous state in the inbred lines. This model could explain heterosis better than the dominance model as performance of a hybrid generally exceeds the performance of the inbred line carrying many dominant alleles (East, 1936; reviewed by Chen, 2010).

iii). Pseudo-over dominance hypothesis: This model explains heterosis due to the linkage of dominant and recessive alleles in repulsion phase. This results in superior fitness of the heterozygous genotypes over the homozygous genotypes (Jones, 1917; reviewed by Chen, 2010).

iv). Epistasis hypothesis: This model explains that heterosis results from positive epistatic interaction between favorable alleles from different loci originating from the two parents (Stuber et al., 1992; Yu et al., 1997). In additive and non-additive epistasis interaction model, hybrid performance from a combination of two loci become greater than the cumulative effect of the loci (1+1>2) (reviewed by Chen, 2010).

Although several researchers explained the phenomenon heterosis based on a single-gene model, however, in most cases this is due to the effect of multiple gene loci (Schnable and Springer, 2013). Lu et al. (2003) reported that 24 of the 28 QTL for grain yield in hybrid maize show overdominance; however, QTL for plant height, grain moisture and lodging resistance display partial to complete dominance. Lariepe et al. (2012) found significant association between heterosis and hybrid heterozygosity, and confirmed the importance of the dominance gene action in heterosis. They hypothesized that the overdominance effect of heterosis results from QTL linked in repulsion phase. According to Springer and Strupar (2007), heterosis or hybrid vigor results from allelic variation between the two parents and this variation in maize results from the

allelic variation in the fragments of the genomic regions, repetitive elements surrounding the genes, gene expression level, transposons and repetitive DNA (Springer and Stupar, 2007).

1.7.2 Heterosis in Brassica

Heterosis, a phenomenon which has been exploited to increase yield in many crops; it has also played an important role in adaption of the crops to abiotic and biotic stresses arising from increased vigor in hybrids (Lee and Tollenaar, 2007; Birchler et al., 2010). Therefore, for the crop species where the development hybrid cultivar is possible, the majority of the commercial cultivars are hybrids. In some crops, the level of heterosis is not high enough to compensate the cost of hybrid seeds, or a suitable pollination control system cannot be found to justify the development of hybrid cultivars (Springer and Stupar, 2007). Hybrid seeds are generally more expensive than seeds of open-pollinated line cultivars; however, the yield advantage of hybrid cultivars, as compared to line cultivars, can justify the growing of hybrid cultivars. For example, approximately 40% greater seed yield in F_1 hybrids of spring *B. napus* has been reported by Sernyk and Stefansson (1983), and up to 72% higher yield in *B. napus* hybrid reported by Grant and Beversdorf (1985). This may justify growers to pay higher price for hybrid seeds. Due to the increased demand for canola oil in world market for edible use or industrial applications, growing of high yielding hybrid cultivars for greater production has become more important than before. However, one of the limitations of the development of a high yielding *B. napus* hybrid cultivars is the narrow genetic base of this crop species (reviewed by Rahman, 2013). While broadening the genetic base of this crop through the use of exotic germplasm undesirable alleles are often introduced along with the desirable ones. Canola breeders need to be aware that repeated cycles of breeding might be needed to eliminate the undesirable alleles (reviewed by Rahman, 2013).

In a breeding program for increasing seed yield, it is important to identify the loci contributing to heterosis and use this knowledge in breeding. Seed yield in *B. napus* is a complex trait contributed by many yield-contributing traits. Therefore, it is also important to understand the genetic control of heterosis of these traits as well (Luo et al., 2016). According to Diepenbrock (2000), number of silique per plant, silique length and number seeds/silique directly or indirectly affect seed in *B. napus*. Rameeh (2014) also demonstrated the importance of number of silique/plant on seed yield in *B. napus*. According to Zhang et al. (2010) number of seeds/silique in *B. napus* is controlled by few (two) major and several minor genes with additive effects along with complete dominance and epistasis. Starmer et al. (1998) found that heavier seeds and a greater number of silique on the main raceme contribute to higher seed yield in spring *B. napus* hybrids. The earliness of flowering and maturity are also important traits of breeding spring *B. napus*; these traits are controlled by several QTL from both A and C genomes with both additive and non-additive effect of the genes (Liu et al., 2016; Rahman et al., 2017).

Radoev et al. (2008) followed quantitative trait loci (QTL) mapping approach to understand the genetic basis of heterosis in *B. napus*. They detected a total of 33 QTL contributing to heterosis for seed yield and yield components of which 10 QTL exhibited dominance effects. They found mostly complete dominance or overdominance effects of the genes contributing to seed yield heterosis while partial dominance effect genes are mostly involved in heterosis for yield components. They also detected a large number of epistasis interactions suggesting that heterosis in *B. napus* results from epistasis, dominance and partial to overdominance effect of the genes. Dong et al. (2007) also reported that overdominance and epistasis play an important role in the genetic control of heterosis in *B. rapa*. Rahman et al. (2016) found that alleles contributing to non-addition effect of heterosis can be found more frequently in *B. oleracea* than in winter *B.*

napus; and the C subgenome of *B. napus* might have contributed to the improved of GCA in hybrid breeding of this crop (Zhao et al., 2016). Noticeable level of heterosis has also been recorded in the hybrids derived from interspecific cross between *B. napus* and *B. rapa*. In this case, genes from the A genome were mainly found contributing to heterosis for growth performance, while genes from the C genome mainly contributing to heterosis for stress tolerance (Zhang et al., 2015).

B. napus canola is an oilseed crop, oil of this crop is used as main product, and the protein-rich seed meal is used as feed as by-product. Therefore, in addition to increasing seed yield, increasing oil as well as protein content in hybrid cultivar is important. Grant and Beversdorf (1985) found a negative heterosis for protein content and no heterosis for oil content. Diers et al. (1996) also found lower protein content in hybrids as compared to the parents. Han-Zhong et al. (2009) suggested that high seed oil content in the hybrid parents is needed to achieve a positive heterosis for oil content. While selecting the parents, it is also important to consider the maternal effect of the traits. For instance, according to Xing et al. (2014) parents with greater combining ability for thousand seed weight or number of seeds per silique or seed oil content should be used as female.

To predict the performance of the test hybrids based on genotyping data, Jan et al. (2016) genotyped 475 spring type *B. napus* pollinator lines by 24,403 genome-wide SNP loci, and evaluated 950 F_1 hybrids derived from cross between the pollinator lines and two testers, for different agronomic and seed quality traits. They found the accuracy of genomic prediction was highest for seed oil content (0.81) followed by oil yield (0.75) and the lowest accuracy was found for seedling emergence (0.29). Prediction accuracies for days to flowering, lodging resistance, seed yield, and seed glucosinolate content was 0.56, 0.39, 0.45 and 0.61, respectively.

1.8 Genetic diversity and heterosis

Many studies showed that a weak or no relationship exists between genetic diversity (GD) and heterosis. For instance, Makumbi et al. (2011) found a weak correlation between genetic distance and mid-parent heterosis in maize. Similarly, Qian et al. (2009), Luo et al. (2016), Kawamura et al. (2016) and Ahmad et al. (2011) also found a low correlation between parental genetic distance and hybrid performance. According to Dhliwayo el al. (2009), genetic diversity between the hybrid parents does not guarantee the level of heterosis in hybrids, but it can give useful information for initial grouping of the breeding germplasm. In contrast, Ali et al. (1995) found a significant positive correlation between genetic distance and heterosis for seed yield, number of silique/plant, and number of seed/silique in winter *B. napus*. They also reported higher heterosis in the hybrids of parents belonging to different clusters as compared to the hybrids of the parents belonging to the same cluster. Lefort-Buson et al. (1987) found that F_1-hybrids derived from cross between European and Asian *B. napus* show higher vigor and seed yield compared to the hybrids derived from European × European or Asian × Asian *B. napus*. Cuthbert et al. (2009) suggested that hybrids derived from parents belonging to different geographical groups display greater vigor compared to the hybrids derived from parents from the same geographical group; however, Cuthbert et al. (2009) and Xing et al. (2014) found that stronger prediction of heterosis can be made by taking the general combing ability (GCA) of the parents as well as GD into account. Xing et al. (2014) reported a significant association between GD and better-parent heterosis for thousand seed weight in *B. napus* and between GD and specific combining ability (SCA) for number seeds per silique, thousand seed weight, and seed yield per plant. Qian et al. (2003) reported that biomass yield in the hybrids of *B. napus* × *B. rapa* cross show a significant positive association with the genetic distance between the parents, and suggested that increasing the

subgenomic portion of A^r (the subgenome from the Asian oil crop *B. rapa*, A^rA^r), and C^c (the subgenome from the African oil crop *B. carinata*) in the new-typed *B. napus* ($A^rA^rC^cC^c$) might increase the level of heterosis for seed yield in *B. napus* (Li et al., 2006). Tian et al. (2017b) found no correlation between genetic distance of the parents and heterosis when estimating the genetic distance by use of molecular markers; however, they found a significant positive relationship between the sum of parental GCA (f + m) and heterosis. Yu et al. (2005) found a positive correlation between heterosis and genetic distance between the parents, estimated based on significant or favoring markers, for plant height, number of seeds per silique and seed yield. Flint-Garcia et al. (2009) attempted to develop a model to predict the level of heterosis in maize based on genetic diversity and maturity; however, the model was able to predict hybrid performance for some traits, but not for the others.

1.9 Research objectives

The long-term objective of this research is to increase the level of heterosis for seed yield in hybrid *B. napus* canola cultivars through the use of the gene pool of its allied species, *B. rapa*.

In the short-term, the following investigations were made in this MSc thesis research project:

1) Evaluate the performance of the inbred lines derived from *B. napus* × *B. rapa* interspecific crosses for agronomic and seed quality traits.
2) Evaluate performance of the test hybrids developed based on the above-mentioned inbred lines for agronomic and seed quality traits.
3) Estimate genetic diversity among the above-mentioned inbred lines using simple sequence repeat (SSR) markers, and study the relationship between genetic distance of the parents and heterosis.

Chapter 2

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

2.1 Introduction

The genus Brassica comprises various species, such as *B. oleracea* (*2n* = 18, CC), *B. rapa* (*2n* = 20, AA), *B. napus* (*2n* = 38, AACC), *B. carinata* (*2n* = 34, BBCC), *B. nigra* (*2n* = 16, BB) and *B. juncea* (*2n* = 36, AABB) of which *B. napus* is the main oilseed crop species (Cartea et al., 2010; Velasco et al., 2016) and is the second most important oilseed crop in the world (McVetty and Duncan, 2016). Compared to *B. rapa* and *B. juncea, B. napus* has greater yield potential (Mendham and Robertson, 2016). *B. napus* is an allotetraploid species originated from the cross between *B. rapa* and *B. oleracea* (Barthet, 2016; McVetty and Duncan, 2016).

Based on growth habit, the oilseed *B. napus* can be divided into winter, semi-winter and spring types (Gehringer et al., 2007) of which the spring type is grown in western Canada (McVetty and Duncan, 2016). Canola is a type of *B. napus* whose seed oil and meal quality was improved by Canadian breeders to make the oil suitable for human consumption and the seed meal for use in animal feed. This improved version of *B. napus*, has oil containing less than 2% erucic acid and seed meal containing less than 30 μ glucosinolate per g seed meal (Stefansson and Kondra, 1975; Barthet, 2016; Eskin, 2016), is known as double low rapeseed or canola. The main objective of canola breeding is to develop high yielding cultivar with earliness of flowering and maturity, and good resistance to lodging, silique shatter, disease and insect pests. Most of these traits are under polygenic control; therefore, existence of wide allelic diversity for these traits in this crop is essential for further improvement (McVetty and Duncan, 2016; Rao and Hodgkin, 2002). Genetic diversity in *B. napus* canola is narrow (reviewed by Rahman, 2013), therefore,

several researchers suggested crossing of this species with *B. rapa* and *B. oleracea*, or cross with different eco-types of *B. napus* for introgression of new alleles in this important oilseed crop (Wolko, 2012; Rahman et al., 2015). Wide morphological and genetic diversity can be found in *B. rapa*; this makes this progenitor species a valuable resource of alleles for broadening the genetic base of oilseed *B. napus* (Elling et al., 2010). Most semi-winter *B. napus* cultivars in China were, indeed, developed through interspecific crossing of *B. napus* with *B. rapa* (Qian et al., 2005).

Heterosis or hybrid vigor is the increased productivity of the F_1 hybrid compared to the parents (Duvick, 2001). Heterosis can be estimated as mid parent heterosis (MPH), which indicates the performance of the F_1 compared to the mean value of its two parents, or as better parent heterosis (BPH), which indicates the performance of the F_1 over the better parent, or as standard heterosis (SVH), which indicates the performance of the F_1 over the standard check cultivar (Hayes et al., 1955, Hochholdinger and Hoecker, 2007). Many hypotheses, such as dominance, overdominance, pseudo-overdominance and epistasis, have been proposed by different researchers; however, there is no enough evidence to completely explain the genetic basis of heterosis (Reif et al., 2005; Hochholdinger and Hoecker, 2007; Birchler et al., 2010; Shi et al., 2011; Yao et al., 2013; Fu et al., 2015).

Heterosis has been recorded in canola hybrids; particularly, while using genetically distinct inbred lines as parents (Sernyk et al., 1983; Lefort-Buson et al., 1987; Ali et al., 1995). In hybrid breeding, it is important to evaluate a large number of hybrids in multiple trials to achieve reliable data on heterosis and to select the best hybrid for commercialization. The magnitude of heterosis varies for different traits; a high level of heterosis can be found for seed yield and morphological traits, such as plant height, which is often controlled by both non-additive and additive effect of the genes. In contrast, the seed quality traits, such as oil content, which are

mainly controlled by additive genes, show a low level of heterosis (Shen et al., 2005; Delourme et al., 2006). Han-Zhong et al. (2009) suggested that selection of parents with high oil content might result heterosis for seed oil content in *B. napus*. In general, the level of heterosis in *B. napus* is lower than maize; for example, Radoev et al. (2008) reported about 30% heterosis for seed yield in *B. napus*, while more than 100% heterosis for seed yield was reported by Flint-Garcia (2009) in maize.

The objective of this study was to evaluate the performance of a set of recombinant inbred lines derived from *B. napus* × *B. rapa* interspecific cross for different agronomic and seed quality traits, and to understand the level of heterosis can be achieved in hybrid *B. napus* canola by using the *B. rapa* gene pool in breeding.

2.2 Materials and Methods

2.2.1 Parental materials

The Canola Program of the University of Alberta developed several inbred lines from two *B. napus* × *B. rapa* interspecific crosses: Spring *B. napus* line A04-73NA × *B. rapa* line YS49 and A04-73NA × *B. rapa* line T4-3-3-1. In both crosses, A04-73NA (a canola quality line with zero erucic acid in oil and <15 μmol glucosinolate (GSL) per g/seed) was used as female and the *B. rapa* lines YS49 (zero erucic acid in oil and high GSL in seed meal; yellow seed) and T4-3-3-1 (a canola quality line; zero erucic acid in oil and low GSL in seed meal; yellow seed) were used as the male parent.

B. napus (2*n* = 38, CC) is self-compatible; however, about 20% outcrossing can occur in this species under field condition (Rakow et al., 1987; Becker et al., 1992). On the other hand, *B. rapa* (2*n* = 20, AA) is generally self-incompatible; however, the two lines used in this research were self-compatible (Rahman et al., 1996; Rahman et al., 2014). The F_1 plants of these two interspecific crosses (A04-73NA × YS49 and A04-73NA × T-4-3-3-1) were self-pollinated for

F_2 seeds, and the F_1 of A04-73NA × YS49 was backcrossed to A04-73NA to produce BC_1 seeds. F_9 and BC_1F_6 generation seeds were produced from the F_2 and BC_1 plants through self-pollination of single plants with selection for canola quality traits and euploid (*2n* = 38) *B. napus* plants.

2.2.2 F_9 and BC_1F_6 populations

The material used in this study includes 93 lines: 31 F_9 lines derived from A04-73NA × YS49 (cross ID: 5CA1257), 31 F_9 lines derived from A04-73NA × T4-3-3-1 (cross ID: 5CA1299) and 31 BC_1F_6 lines derived from (A04-73NA × YS49) × A04-73NA (cross ID: 5CA1680). I received all these lines from the Canola Program; all these lines confirmed to be euploid *B. napus* (*2n* =38, AACC).

2.2.3 Test hybrid production

By using the 93 inbred lines, 93 test hybrids were produced in a greenhouse during 2015-16 and 2016-17 winter, using A04-73NA as female and the inbred lines as male. Test hybrids were grown in field plots together with their parents (A04-73NA and the inbred lines from *B. napus* × *B. rapa* crosses).

2.2.4 Field trials with the inbred lines

Field trials with the inbred lines were conducted at the following locations: Edmonton Research Station 1 (ERS-1, Michener), Edmonton Research Station 2 (ERS-2, West-240) and St. Albert Research Farm of the University of Alberta, and in Killam, Alberta. The design of the experiment was alpha lattice with two replications. Each plot was 5 m long and 1.8 m wide with 6 rows; 8.6 g seed was seeded per plot.

2.2.5 Field trials with the test hybrids

The field trial with the test hybrids was conducted at the St. Albert Research Farm of the University of Alberta in 2016 and 2017. The design of the experiment was alpha lattice with two replications. The trial was laid out in a way that the test hybrid is located in between its two

parents. Each plot was 2-3 m long with 4 rows and 1.3 m wide; 2 g seed was seeded per plot. In addition to the above-mentioned trials, data for the same set of the test hybrids (tested at ERS-1 in 2015) was obtained from the canola program and included in the statistical analysis.

2.2.6 Data collection

The following agronomic and seed quality traits were recorded for the inbred lines and test hybrids.

- **Plot establishment**: Scored on a 1-9 scale; 1 = very poor and 9 = very good establishment. Seed yield data from the plots with a score of < 5 was not included in statistical analysis.
- **Early vigour:** Taken before bolting on a 1-9 scale, where 1 = poor and 9 = very good vigour.
- **Days to flowering**: Recorded when about 50% of the plants in a plot had at least one open flower.
- **Plant height:** Measured in cm from the ground to the tip of the main raceme at the end of flowering.
- **Lodging:** Recorded at the time of taking maturity notes. This trait was recorded on a 1-9 scale, where 1 = erect and 9 = totally lodged.
- **Days to maturity:** Recorded on a 1-9 scale based on color change of the silique, where 9 = very early and 1 = very late.
- **Seed Yield:** Measured by an automated computerized system on the plot harvester. The seed yield per plot data was converted to kg/ha at 8.5% moisture basis.
- **1000-seed weight:** Extrapolated by weighing 250 randomly selected seeds and recorded in g.

2.2.7 Seed quality analysis

Seeds harvested from the field plots were analyzed for oil, protein and GSL content by near-infrared spectroscopy (NIRS, FOSS NIR system model 6500) method. For this, 2 g bulk seed harvested from the field plots was used. Oil and protein contents are reported as percentage of whole-seed while GSL content is reported as μmol/g seed at 8.5% moisture basis.

2.2.8 Statistical analysis

Statistical analysis of agronomic and seed quality data of the inbred lines and test hybrids was done by using R program. The design of the experiments was incomplete block where environment (year and location), replication and block were considered as random effect and the type of cross (cross with YS49 or T4-3-3-1), type of population (F_2 or BC_1 derived) and inbred lines were considered as fixed effects. In addition, for the test hybrid trials, mid-parent heterosis (MPH) and heterosis over the parent A04-73NA (73NAH) were calculated for yield, days to flowering and maturity, plant height, seed oil, protein and GSL contents using the following two formulae:

$$\text{MPH} : \frac{\text{Test hybrid - mid-parent value}}{\text{mid-parent value}} \times 100$$

$$\text{73NAH} : \frac{\text{Test hybrid – A04-73NA}}{\text{A04-73NA}} \times 100$$

The mean value for each population were calculated by the lsmeans model of R and SE (Standard Error) was calculated using STD function of R. In addition, Pearson's simple correlation coefficients were calculated for agronomic and seed quality traits of inbred lines, test hybrids populations.

The means of inbred lines, test hybrids, MPH and 79NAH for agronomic traits and seed quality traits in three populations are available in Appendix 2.1 to 2.12.

2.3 Results

2.3.1 Inbred line trials

Days to flowering

Days of flowering of the two F_2- and one BC_1-derived populations (F_9 and BC_1F_6) as well as the *B. napus* parent A04-73NA is presented in Table 2.1. The parent A04-73NA took 50.6 ± 0.2 days to flower; the F_9 populations of A04-73NA × YS49 and A04-73NA × T-4-3-3-1 took almost similar number of days to flower (50.4 ± 0.2 and 50.2 ± 0.1) as the *B. napus* parent A04-73NA. In contrast, the BC_1-derived population of (A04-73NA × YS49) × A04-73NA took significantly less number of days to flower (49.6 ± 0.3) as compared to the *B. napus* parent as well as the two F_9 populations ($P < 0.01$).

Days to maturity

The *B. napus* parent A04-73NA took 103.4 ± 1.0 days to mature. The F_9 population of A04-73NA × YS49 also took similar number of days to mature (103.0 ± 0.5). Among the three populations, the BC_1F_6 population of (A04-73NA × YS49) × A04-73NA matured the earliest with a mean of 101.5 ± 0.5 days. Days to maturity for the inbred lines in the three populations varied from 100 to 105 days. In summary, similar to days to flowering, of the three populations, the BC_1F_6 population of (A04-73NA × YS49) × A04-73NA matured the earliest followed by the F_9 population of A04-73NA × T-4-3-3-1 (table 2.1). More than 40% lines of these two populations mature in 100 days, while only 13% F_9 lines of A04-73NA × YS49 matured in 100 days (Fig 2.1).

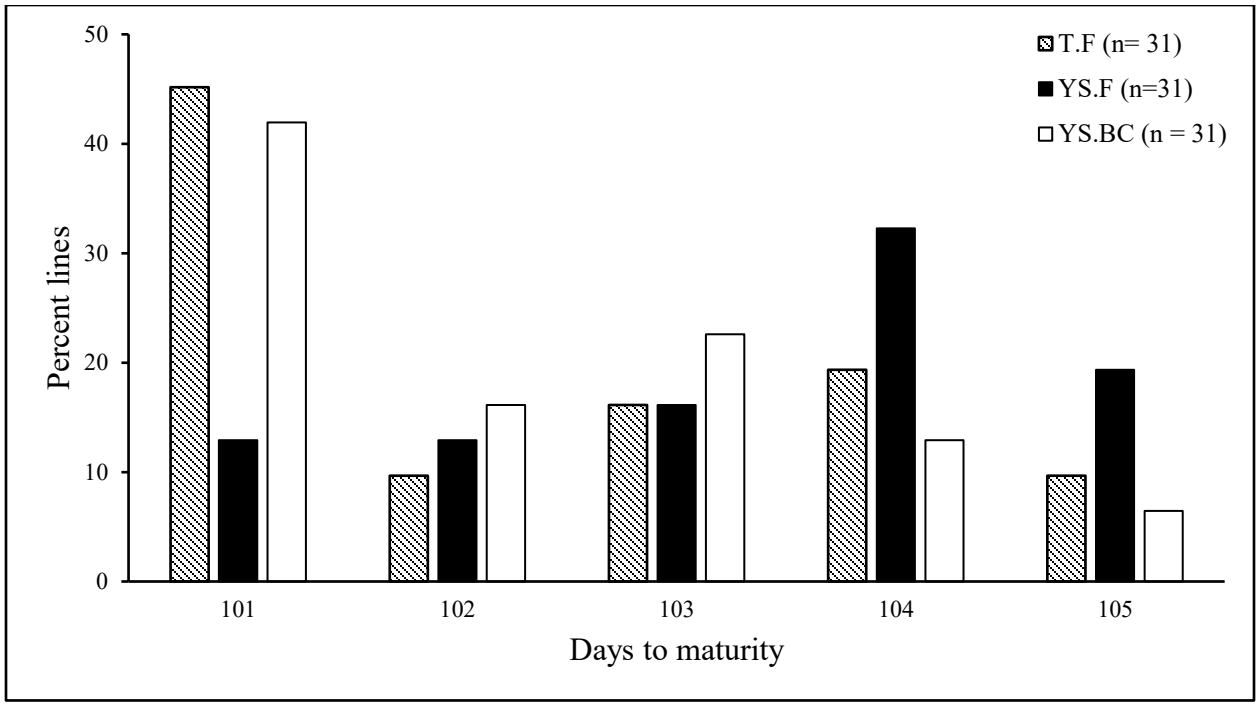

Fig. 2.1 Frequency distribution of the F_9 inbred lines derived from *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (T.F) and *B. napus* A04-73NA × *B. rapa*YS49 (YS.F), and BC_1F_6 lines derived from (*B. napus* A04-73NA × YS49) × A04-73NA (YS.BC) interspecific cross for days to maturity. Days to maturity of the *B. napus* parent A04-73NA was 103.4 ± 1.0 days.

Plant height

Mean height of the *B. napus* parent A04-73NA was 126.3 ± 1.2 cm; all three interspecific cross-derived populations had significantly lower height than the *B. napus* parent (Table 2.1). The lowest height was recorded for BC_1F_6 population of (A04-73NA × YS49) × A04-73NA with a mean of 117.4 ± 0.7 cm (range 110 to 127 cm). The mean height of the F_9 population of A04-73NA × YS49 was 119.6 ± 0.7 cm (range 115 to 129 cm) and the F_9 population of A04-73NA × T-4-3-3-1 was 120.2 ± 0.9 cm (range 114 to 130 cm); these two populations were significantly taller than the BC_1F_6 population.

Yield

The *B. napus* parent A04-73NA yielded 3460.0 ± 56.1 kg/ha. The F_9 population of A04-73NA × T-4-3-3-1 gave higher yield (3503.0 ± 22.6 kg/ha; range 3292 to 3696) than the *B. napus* parent; however the difference between this population and A04-73NA was not statistically significant. The F_9 and BC_1F_6 populations gave significantly lower yield than the *B. napus* parent as well as the F_9 population of A04-73NA × T-4-3-3-1 ($P < 0.01$).

Frequency distribution of the three populations for yield is presented in Fig. 2.2. About 77% of the F_9 lines of A04-73NA × T-4-3-3-1 yielded more than 3400 kg/ha; none of the lines of gave less than 3200 kg/ha. This shows that the line T-4-3-3-1 has the greater capability of producing higher yielding lines as compared to YS49. In case of the A04-73NA × YS49 cross, only 19% of the F_9 lines yielded more 3400 kg/ha. Thus, an effect of the *B. rapa* parent on the interspecific cross derived population is evident.

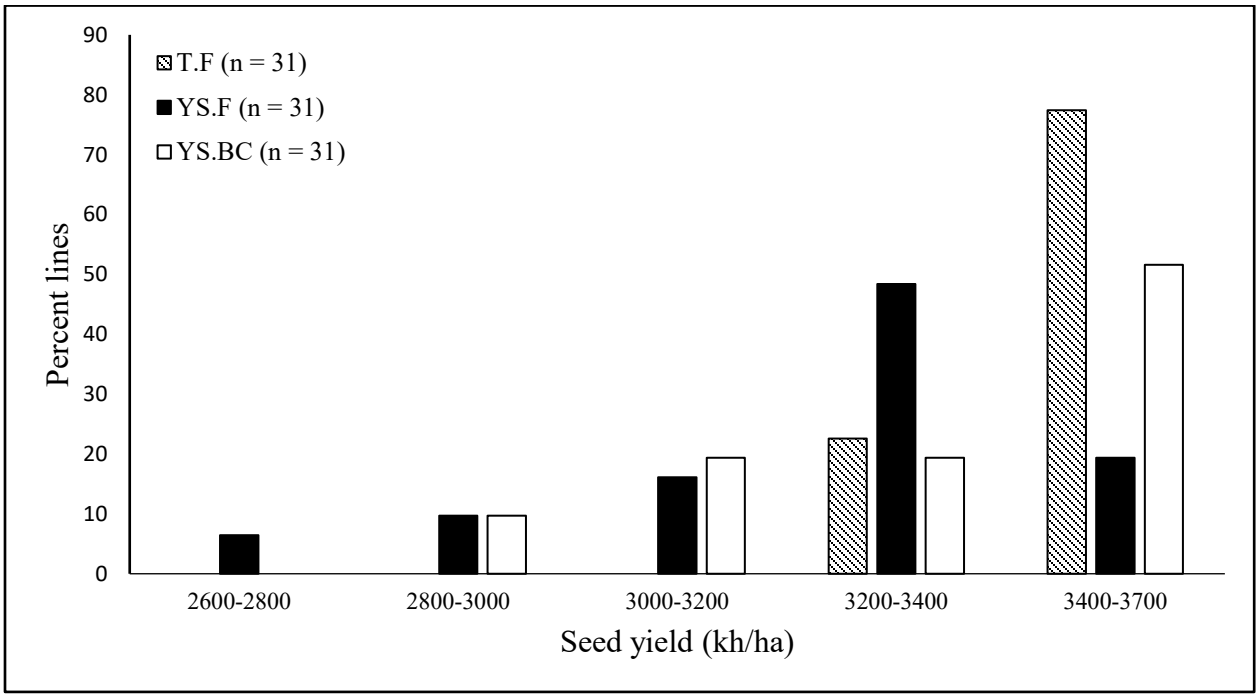

Fig. 2.2 Frequency distribution of the F_9 inbred lines derived from *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (T.F) and *B. napus* A04-73NA × *B. rapa*YS49 (YS.F), and the BC_1F_6 lines derived from (*B. napus* A04-73NA × YS49) × A04-73NA (YS.BC) interspecific cross. Seed yield of the *B. napus* parent A04-73NA was 3460 ± 56.1 kg/ha.

Seed oil content

Seed oil content (%) of the *B. napus* parent A04-73NA was 47.4 ± 0.2%. Only the F_9 population of A04-73NA × T-4-3-3-1 had similar oil content (47.3 ± 0.1%; range 45 to 49%) as the *B. napus* parent, while the F_9 and BC_1F_6 populations of the A04-73NA × YS49 cross had significantly lower seed oil content than the *B. napus* parent and the F_9 population of A04-73NA × T-4-3-3-1 ($P < 0.01$). The BC_1F_6 population of (A04-73NA × YS49) × A04-73NA had significantly higher oil content (46.6 ± 0.1%; range 44 to 48%) than the F_9 population of A04-73NA × YS49 (46.2 ± 0.1%; range 45 to 48) ($P < 0.01$), as expected; however, oil content of this population was significantly lower than the F_9 population of A04-73NA × T-4-3-3-1.

Thus, seed oil content showed a similar trend as seed yield. Of the three populations, the F_9 population of A04-73NA × T-4-3-3-1 gave the highest yield and also had the highest oil content while the F_9 population of A04-73NA × YS49 gave the lowest yield and also had the lowest oil content. Also, similar to seed yield, the BC_1F_6 population had higher oil content than the F_9 population derived from same cross (A04-73NA × YS49).

Seed protein content

In contrast to seed oil content (%), the F_9 population of A04-73NA × YS49 had the highest seed protein content with a mean of 25.3 ± 0.1% (range 24 to 27%) followed by the BC_1F_6 population of A04-73NA × YS49 with a mean of 25.1 ± 0.1% (range 24 to 27%). Protein content of the *B. napus* parent was 24.9 ± 0.2% which was not significantly different from the above-mentioned two populations. The lowest seed protein was recorded for the F_9 population of A04-73NA × T-4-3-3-1 (24.6 ± 0.1%; range 24-26%); this population in fact had the highest oil content. Thus, the F_9 population of A04-73NA × T-4-3-3-1 produced the highest yield and had the highest seed oil content but lowest seed protein content, while the F_9 population of A04-73NA ×

YS49 produced the lowest yield and had the lowest seed oil but the highest seed protein content. This indicated a trend of negative relationship between seed oil and protein content, but a positive relationship of seed oil with seed yield in these populations.

Seed glucosinolate (GSL) content

Of the three populations, the F_9 population derived from A04-73NA × T-4-3-3-1 had the lowest seed GSL with a mean of 14.6 ± 0.3 μmol/g seed (range 12 to 21 μmol/g seed), while the BC_1F_6 population derived from (A04-73NA × YS49) × A04-73NA had the highest seed GSL content, which was about 2 μmol/g seed higher than the above-mentioned F_9 population. The F_9 population of A04-73NA × T-4-3-3-1 indeed had the highest (71%) proportion of lines with GSL content ≤ 15 μmol/g seed. In contrast, the BC_1F_6 population of the A04-73NA × YS49 cross had only 16% of the lines with GSL content ≤ 15 μmol/g seed, while the F_9 population of the same cross had about (45% of the lines with GSL content ≤ 15 μmol/g seed (Fig. 2.3).

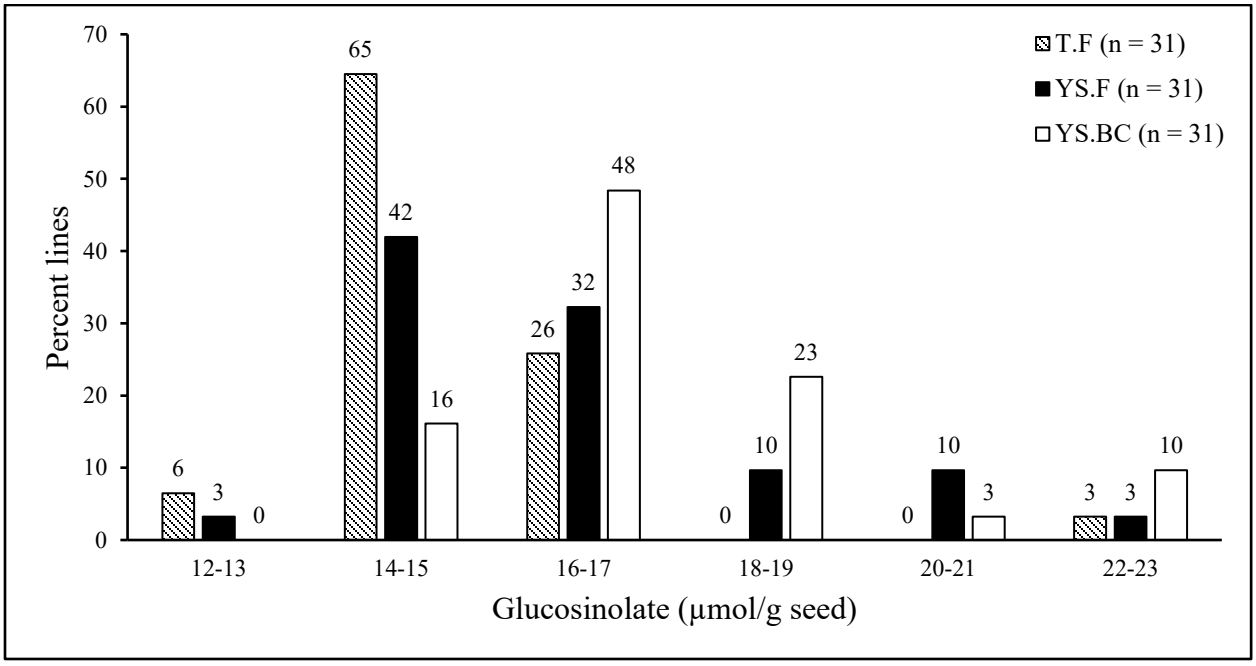

Fig. 2.3 Frequency distribution of the F_9 inbred lines derived from *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (T.F) and *B. napus* A04-73NA × *B. rapa*YS49 (YS.F), and the BC_1F_6 lines derived from (*B. napus* A04-73NA × YS49) × A04-73NA (YS.BC) interspecific cross for seed glucosinolate (GSL) (μmol/g seed) content. GSL content of the *B. napus* parent A04-73NA was 21.4 ± 0.3 μmol/g seed.

Correlation between the traits in the inbred line populations

A positive correlation between days to flowering and days to maturity was found in pooled data of the three populations (r = 0.74, p < 0.01) as well as individually in the F_9 population of A04-73NA × YS49 (r = 0.80, p < 0.01), BC_1F_6 population of (A04-73NA × YS49) × A04-73NA (r = 0.67, p < 0.01) and F_9 population of A04-73NA × T-4-3-3-1 (r = 0.75; p < 0.01); and both traits showed a positive correlation with plant height in all three populations. Seed yield did not show significant correlation with the above-mentioned three agronomic traits (Table 2.2). However, seed yield showed a positive correlation with seed oil content in the whole population (r = 0.55, p < 0.01) (Table 2.2, Fig 2.4) as well as in the F_9 population of A04-73NA × YS49 (r = 0.66, p < 0.01) and BC_1F_6 population of (A04-73NA × YS49) × A04-73NA (r = 0.41, p < 0.05). On the other hand, seed yield showed a negative correlation with seed protein content in the whole population (r = - 0.62, p < 0.01) (Table 2.2, Fig 2.5) as well as in the F_9 population of A04-73NA × YS49 (r = - 0.60, p < 0.01) and BC_1F_6 population of (A04-73NA × YS49) × A04-73NA (r = - 0.67, p < 0.01), but not in the F_9 population of A04-73NA × T-4-3-3-1 (r = - 0.14). Seed yield also showed a weak negative correlation with GSL content in the whole population (r = - 0.34, p < 0.01) (Table 2.2, Fig 2.6); however, this relationship was not consistent in the three populations individually. Correlation between seed oil and protein content was negative in the whole population (r = - 0.75, p < 0.01) (Table 2.2, Fig 2.7), as well as, individually, in all three populations (F_9 population of A04-73NA × YS49, r = - 0.83, p < 0.01; BC_1F_6 population of (A04-73NA × YS49) × A04-73NA, r = - 0.72, p < 0.01; and the F_9 population of A04-73NA × T-4-3-3-1, r = - 0.50, p < 0.01).

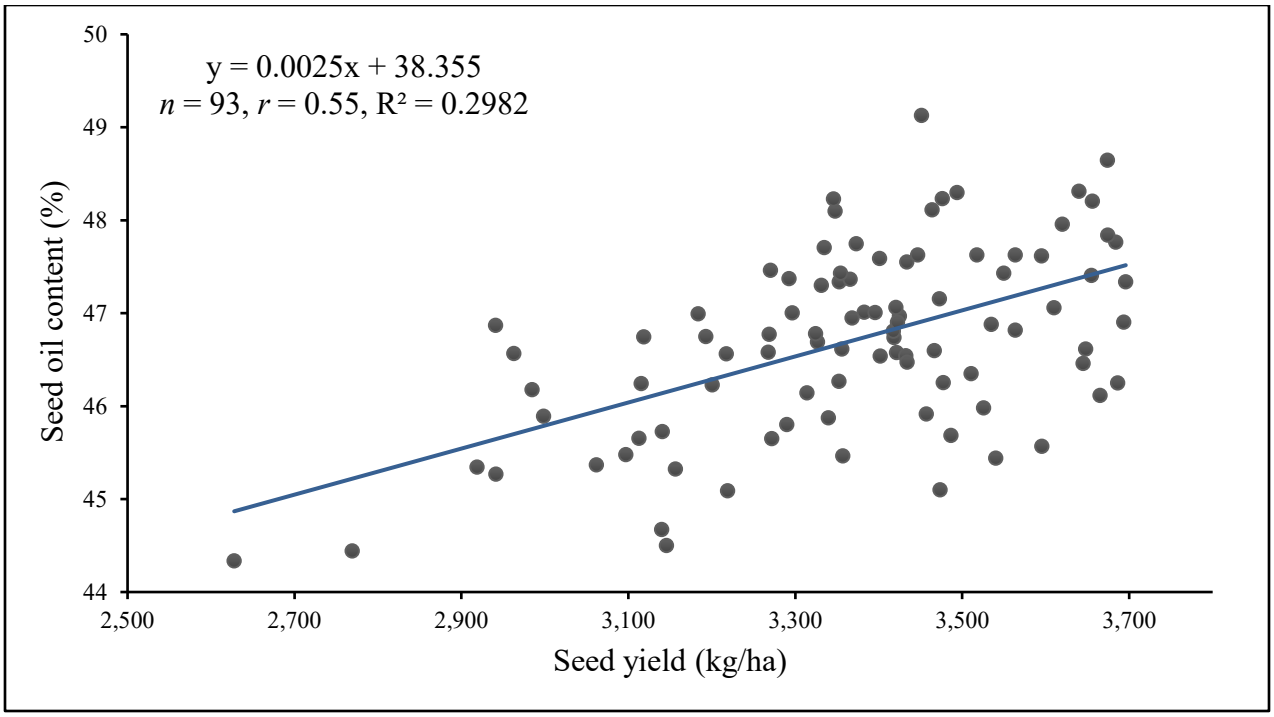

Fig. 2.4 Scatter diagram for seed oil content (%) and yield (kg/ha) in the whole inbred line population derived from *B. napus* A04-73NA × *B. rapa* interspecific crosses.

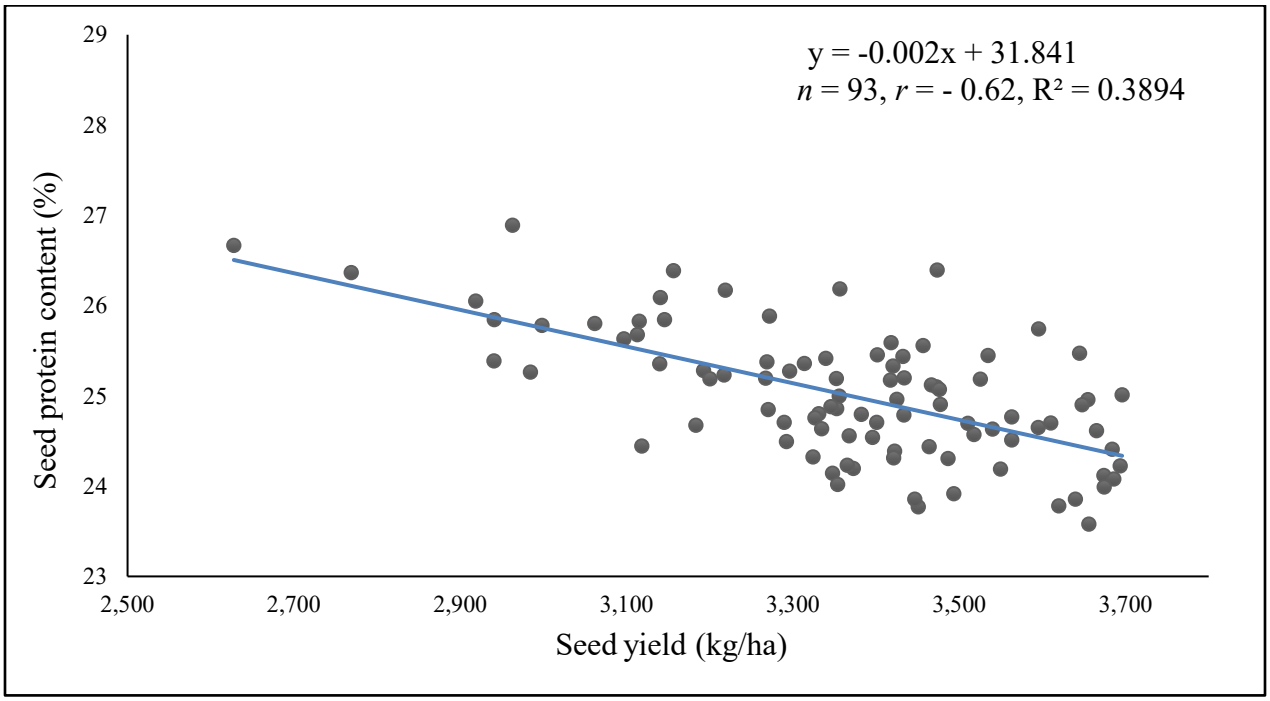

Fig. 2.5 Scatter diagram for seed protein content (%) and yield (kg/ha) in the whole inbred line population derived from *B. napus* A04-73NA × *B. rapa* interspecific crosses.

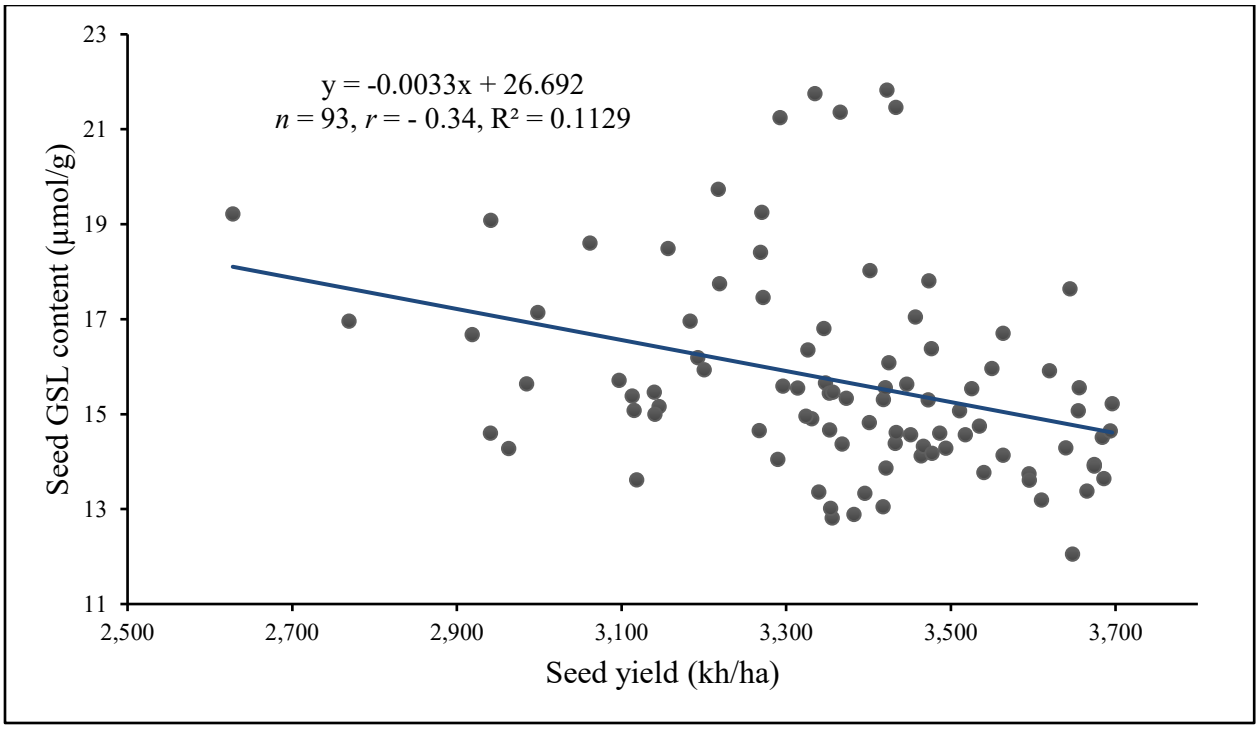

Fig. 2.6 Scatter diagram for seed glucosinolate (GSL) content (%) and yield (kg/ha) in the whole inbred lines population derived from *B. napus* A04-73NA × *B. rapa* interspecific crosses.

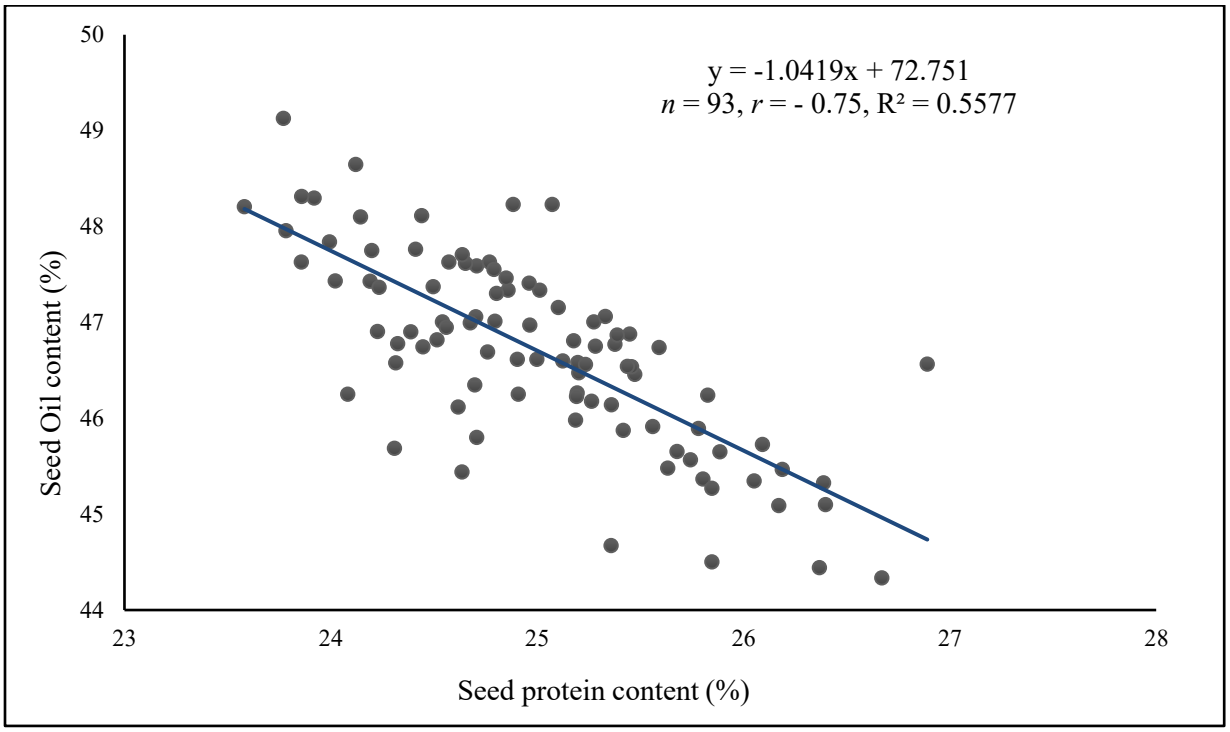

Fig. 2.7 Scatter diagram for seed protein (%) and seed oil content (%) in the whole inbred lines population derived from *B. napus* A04-73NA × *B. rapa* interspecific crosses.

Table 2.1 Agronomic and seed quality traits (mean ± SE and range) of the *B. napus* parent A04-73NA (check) and two F_9 populations derived from *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (T.F.) and *B. napus* A04-73NA × *B. rapa* YS49 (YS.F.) and the single BC_1F_6 population derived from (*B. napus* A04-73NA × *B. rapa* YS49) × *B. napus* A04-73NA (YS.BC.) interspecific cross.

Cross[1]	Pop.	No. lines	DTF	DTM	Height	Yield (kg/ha)	Oil (%)	Protein (%)	GSL
B. nap × *B. rapa*T-4-3-31	T.F.	31	50.2 ± 0.1 b (49 to 52)	102.0 ± 0.3 b (100 to 105)	120.2 ± 0.9 b (114 to 130)	3503.0 ± 22.6 a (3292 to 3696)	47.3 ± 0.1 a (45 to 49)	24.6 ± 0.1 b (24 to 26)	14.6 ± 0.3 d (12 to 21)
B. nap × *B. rapa*YS49	YS.F.	31	50.4 ± 0.2 a (48 to 53)	103.0 ± 0.5 a (100 to 105)	119.6 ± 0.7 b (115 to 129)	3248.3 ± 31.7 c (2628 to 3674)	46.2 ± 0.1 c (44 to 48)	25.3 ± 0.1 a (24 to 27)	15.6 ± 0.1 c (13 to 22)
(B. nap × *B. rapa*YS49*)* × *B. nap*	YS.BC.	31	49.6 ± 0.3 c (47 to 51)	101.5 ± 0.5 b (100 to 105)	117.4 ± 0.7 c (110 to 127)	3355.7 ± 30.5b (2941 to 3693)	46.6 ± 0.1b (45 to 48)	25.1 ± 0.1 a (24 to 27)	16.4 ± 0.1 b (14 to 22)
A04-73NA	Check	7	50.6 ± 0.5 a	103.4 ± 1.0 a	126.3 ± 1.2 a	3460.0 ± 56.1 a	47.4 ± 0.2 a	24.9 ± 0.2 ab	21.4 ± 0.3 a

[1]*B. nap* = *B. napus* (A04-73NA)

Note: DTF = days to flowering; DTM = days to maturity; GSL = glucosinolate (μmol/g seed).

Within the column, values followed by the same letter indicate no significant difference.

Table 2.2 Correlation between agronomic and seed quality traits in the inbred F_9 population of *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (uppermost row) and *B. napus* A04-73NA × *B. rapa*YS49 (second row), and in the BC_1F_6 population of (*B. napus* A04-73NA × YS49) × A04-73NA (third row) interspecific crosses. Coefficient of correlation values of the pooled data of the three populations presented in the lowest line and also marked by bold font.

Trait	DTM	Height	Yield	Oil	Protein	GSL
DTF	0.80***	0.50**	0.23	0.37*	-0.53**	-0.38*
	0.67***	0.71***	0.26	0.19	-0.092	0.27
	0.75***	0.76***	0.039	-0.61***	0.032	-0.018
	0.74* **	**0.62*** **	**0.12**	**0.24**	**-0.23***	**-0.16**
DTM		0.44**	0.14	-0.22	-0.34	-0.60***
		0.80***	0.34	0.17	-0.26	0.25
		0.80***	-0.047	-0.71***	0.27	-0.013
		0.69* **	**0.0084**	**-0.21***	**-0.062**	**-0.031**
Height			-0.063	0.064	-0.22	0.23
			0.31	0.3	-0.27	0.46**
			0.031	-0.76***	0.3	-0.057
			0.12	**-0.12**	**-0.11**	**0.11**
Yield				0.66***	-0.60***	-0.44*
				0.41*	-0.65***	0.012
				-0.016	-0.14	-0.48**
				0.55* **	**-0.62*** **	**-0.34*** **
Oil					-0.83***	0.53**
					-0.72***	-0.21
					-0.50**	-0.022
					-0.75* **	**0.22***
Protein						-0.29
						0.19
						0.14
						-0.12

Note: DTF = days to flowering; DTM = days to maturity; GSL = glucosinolate (μmol/g seed).
* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)
*** Correlation is significant at the 0.001 level (2-tailed)

2.3.2 Test hybrid trials

2.3.2.1 *Per se* performance of the test hybrids

Days to flowering

The *B. napus* parent A04-73NA took 48.7 ± 0.5 days to flower. All three test hybrids populations took significantly lower number of days to flower than the *B. napus* parent. The mean days to flowering of the hybrids of the F_9 inbred lines of A04-73NA × YS49 was 47.1 ± 0.2 (range 45 to 49 days), while the hybrids of the BC_1F_6 lines of (A04-73NA × YS49) × A04-73NA took about 0.5 days less time to flower (47.5 ± 0.2, range 45 to 50). The hybrids of the F_9 lines of T-4-3-3-1 × A04-73NA took 47.6 ± 0.2 days to flower (range 46 to 50 days). The difference between the three test hybrid populations for days to flowering was not significant (Table 2.3)

Days to Maturity

The *B. napus* parent A04-73NA took 104.9 ± 0.2 days to mature. All three test hybrids populations took similar number of days to mature (Table 2.3).

Plant Height

Height of the *B. napus* parent A04-73NA was 124.8 ± 1.7 cm. Plant height of the test hybrid population of the F_9 inbred lines of A04-73NA × T-4-3-3-1 (124.8 ± 1.9 cm; range 117 to 134 cm), was not significantly different from the *B. napus* parent. On the other hand, plant height of the test hybrid populations of the F_9 inbred lines of A04-73NA × YS49, and BC_1F_6 lines of (A04-73NA × YS49) × A04-73NA were significantly shorter than the *B. napus* parent. Thus, it is apparent that the *B. rapa* parent YS49 exerted a significant effect for reduced plant height in the inbred (Table 2.1) as well as in the test hybrid populations (Table 2.3).

Yield

All three test hybrid populations yielded significantly greater than the *B. napus* parent A04-73NA (4154.0 ± 76.4 kg/ha). This is a contrast to the inbred lines which, on average, gave lower seed yield than the *B. napus* A04-73NA (Table 2.1). However, the test hybrids of these lines A04-73NA gave higher yield than this *B. napus* parent. Among the three test hybrid populations, the test hybrids of the F_9 inbred lines of A04-73NA × YS49 (4801.7 ± 138.5 kg/ha; range 3691 to 5386 kg/ha) gave the greatest yield followed by the test hybrids of the F_9 inbred lines of A04-73NA × T-4-3-3-1 (4590.7 ± 124.2 kg/ha; range 3792 to 5541 kg/ha) and the test hybrids of the BC_1F_6 inbred lines of (A04-73NA × YS49) × A04-73NA (4553.2 ± 138.9 kg/ha; range 3806-5219 kg/ha) (Table 2.3). Some of the test hybrids showed about 30% higher seed yield than *B. napus* parent.

Seed oil content

Seed oil content (%) of the *B. napus* parent A04-73NA was 48.5 ± 0.1 %. Seed oil content of the test hybrid population of the F_9 inbred lines of A04-73NA × T-4-3-3-1 was statistically similar to A04-73NA (47.6 ± 0.1%; range 46 to 49%). On the other hand, seed oil content of the test hybrid populations of the F_9 inbred lines of A04-73NA × YS49 was 46.4 ± 0.2% (range 44 to 49%) and of the BC_1F_6 inbred lines of (A04-73NA × YS49) × A04-73NA was 46.6 ± 0.2% (range 43 to 49%); these values were significantly lower than the *B. napus* parent. However, variation for oil content was found in all three test hybrid populations where oil content of some of the hybrids was similar to the *B. napus* parent.

Seed protein content

Of the three test hybrid populations, the highest seed protein content (%) was found in the test hybrid population of the F_9 inbred lines of A04-73NA × YS49 (25.3 ± 0.2%; range 23 to

27%) and the lowest protein was recorded for the test hybrid population of the F_9 inbred lines of A04-73NA × T-4-3-3-1 (24.5 ± 0.1%; range 23 to 26%); these two populations, respectively had the lowest and highest oil content; thus, an inverse relationship between these two seed quality traits was evident in the test hybrid population (Table 2.3), as was found in the inbred population (Fig. 2.7).

Seed glucosinolate (GSL) content

Of the three populations, the test hybrid population of the F_9 inbred lines of A04-73NA × T-4-3-3-1 had the lowest seed GSL with a mean of 14.7 ± 0.2 μmol/g seed (range 12 to 18 μmol/g seed) followed by the test hybrid population of the F_9 inbred lines of A04-73NA × YS49 with a mean of 15.0 ± 0.2 μmol/g (range 12 to 21 μmol/g seed) and the BC_1F_6 inbred lines of (A04-73NA × YS49) × A04-73NA with a mean of 16.2 ± 0.2 μmol/g seed (range 14 to 21 μmol/g seed).

Thus, the trend of seed GSL content in the hybrid populations was similar to the inbred lines populations where the F_9 inbred lines of A04-73NA × T-4-3-3-1 as well as their test hybrids had the lowest GSL content, while the BC_1F_6 inbred lines of (A04-73NA × YS49) × A04-73NA as well as their test hybrids had the highest mean GSL content (Table 2.3).

Correlation between the traits in the test hybrids populations

Correlation between the agronomic and seed quality traits in the test hybrid population (pooled data of three populations) (Table 2.4) was not always consistent with the correlation between these traits in the inbred line population (Table 2.2). For example, days to flowering, days to maturity and plant showed a strong positive correlation in the inbred population (Table 2.2), but not in the test hybrid population (Table 2.4). In contrast, plant height showed a significant positive correlation with seed yield in the test hybrid population, but not in the inbred population. Significant positive correlation between oil content and seed yield was found in the

inbred population but not in the test hybrid population; however, oil content showed a strong negative correlation with protein content in both inbred and test hybrids populations.

A positive correlation between inbred line yield and test hybrid yield was found (r = 0.11); however, the strength of this relationship was not significant (Fig. 2.8). Among the three populations, the F_9 inbred population of A04-73NA × YS49 had highest correlation with test hybrids yield (r = 0.29).

Table 2.3 Agronomic and seed quality traits (mean ± SE and range) of the *B. napus* parent A04-73NA (check) and the test hybrids populations of the two F_9 populations derived from *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 and *B. napus* A04-73NA × *B. rapa* YS49 and one BC_1F_6 population derived from (*B. napus* A04-73NA × *B. rapa* YS49) × *B. napus* A04-73NA interspecific crosses. Test hybrid populations were produced by crossing the inbred lines to A04-73NA.

Inbred lines	Test hybrid pop.[1]	No. lines	DTF	DTM	Height	Yield	Oil	Protein	GSL
F_9: (A04-73NA × T-4-3-3-1)	TC.T.F	31	47.6 ± 0.2 b (46 to 50)	104.5 ± 0.2 a (103 to 108)	124.8 ± 1.9a (117 to 134)	4590.7 ± 124.2 ab (3792 to 5541)	47.6 ± 0.1 a (46 to 49)	24.5 ± 0.1 b (23 to 26)	14.7 ± 0.2 c (12 to 18)
F_9: (A04-73NA × YS49)	TC.YS.F	31	47.1 ± 0.2 b (45 to 49)	104.6 ± 0.2 a (103 to 106)	121.0 ± 2.0 b (110 to 130)	4801.7 ± 138.5 a (3691 to 5386)	46.4 ± 0.2 c (44 to 49)	25.3 ± 0.2 a (23 to 27)	15.0 ± 0.2 c (12 to 21)
BC_1F_6: (A04-73NA × YS49) × A04-73NA	TC.YS.BC	31	47.5 ± 0.2 b (45 to 50)	104.7 ± 0.2 a (102 to 107)	121.3 ± 2.1 b (113 to 131)	4553.2 ± 138.9 b (3806 to 5219)	46.6 ± 0.2 b (43 to 49)	25.2 ± 0.2 a (22 to 27)	16.2 ± 0.2 b (14 to 21)
(A04-73NA)	Check	7	48.7 ± 0.5 a	104.9 ± 0.2 a	124.0 ± 1.6 a	4154.0 ± 76.4 c	48.5 ± 0.1 a	24.4 ± 0.1 b	18.2 ± 0.1 a

Note: DTF = days to flowering; DTM = days to maturity; GSL = glucosinolate (µmol/g seed).
[1] TC.T.F = A04-73NA × (A04-73NA × T-4-3-3-1); TC.YS.F = A04-73NA × (A04-73NA × YS49); TC.YS.BC = A04-73NA × [(A04-73NA × YS49) × A04-73NA].
Within the column, values followed by the same letter indicate no significant difference.

Table 2.4 Coefficient of correlations between agronomic and seed quality traits in the test hybrid population of the inbred lines derived from *Brassica napus* × *B. rapa* interspecific crosses.

Traits	DTM	Height	Yield	Oil	Protein	GSL
DTF[1]	0.1	0.14	-0.06	0.1	-0.06	0.02
DTM[2]		0.12	-0.03	0.05	-0.02	-0.07
Height			0.34***	0.30**	-0.29**	-0.24*
Yield				0.15	-0.21*	-0.34***
Oil					-0.87***	-0.28**
Protein						0.36***

[1] DTF = days to flowering; [2] DTM = days to maturity; GSL = glucosinolate
* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level
*** Correlation is significant at the 0.001 level

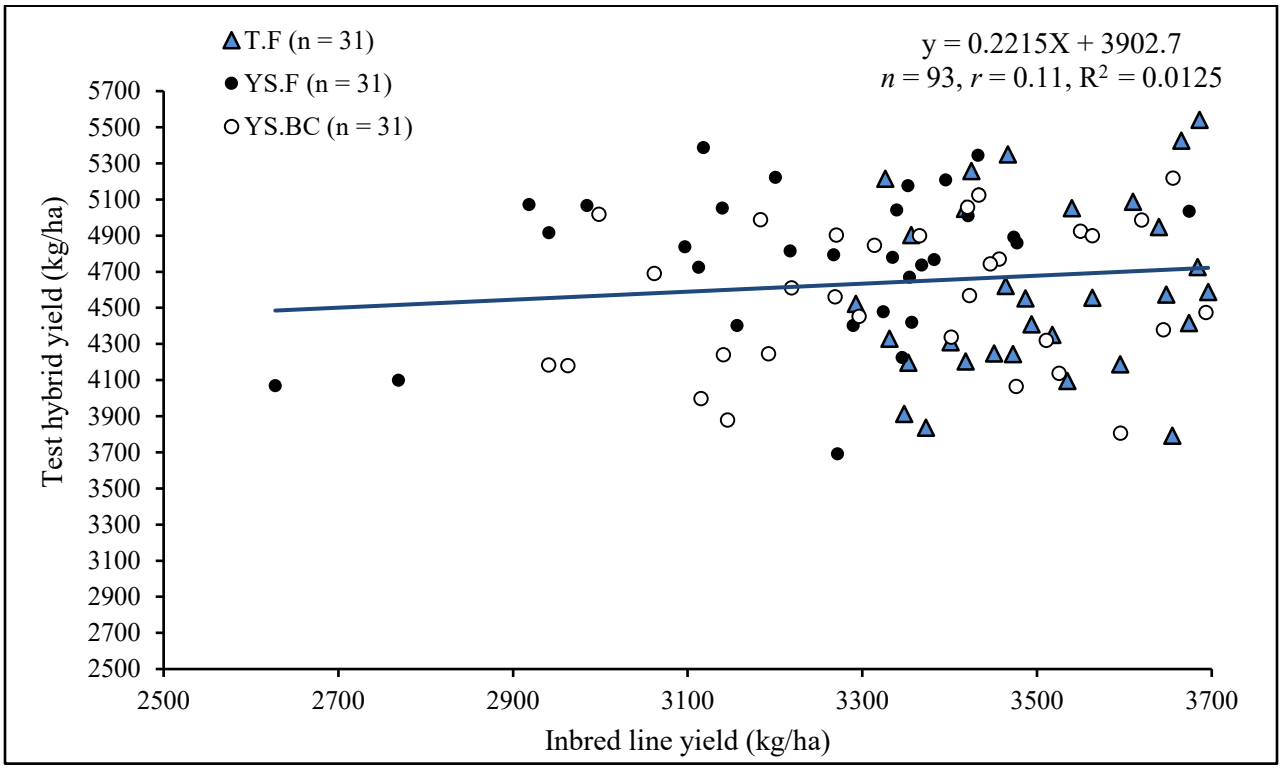

Fig. 2.8 Scatter diagram for test hybrid yield (kg/ha) vs. inbred line yield (kg/ha) in the whole population derived from *B. napus* × *B. rapa* interspecific crosses. T.F = A04-73NA × T-4-3-3-1; YS.F = A04-73NA × YS49; YS.BC = (A04-73NA × YS49) × A04-73NA.

2.3.2.2 Mid parent heterosis (MPH) and heterosis over A04-73NA (73NAH)

Days to flowering

MPH and 73NAH for days to flowering (Table 2.5) was low and the values were negative in all three populations (MPH: -2.1 ± 0.4 to -3.3 ± 0.4; 73NAH: -2.1 ± 0.4 to -4.3 ± 0.5). This indicates that, compared to the inbred lines, the hybrids tend to flower earlier, which apparently resulted from the effect of the alleles introgressed from *B. rapa.* Among, the three test hybrid populations, the lowest negative value for MPH (-9 days) was found in the test hybrid population of the F_9 inbred line of A04-73NA × T-4-3-3-1.

Days to maturity

Similar to days to flowering, the MPH and 73NAH for days to maturity (Table 2.5) were low in all three test hybrid populations. This further confirm the effect of *B. rapa* alleles on earliness in the test hybrids.

Height

The average MPH and 73NAH values for plant height (Table 2.5) was low and negative in all three test hybrid populations (MPH: -0.2 ± 0.4 to -0.6 ± 0.5; 73NAH: -0.5 ± 0.5 to -2.4 ± 0.6), and the difference between the mean values was not significant.

Yield

The highest MPH and 73NAH for seed yield was found in the test hybrid population of the F_9 inbred lines of A04-73NA × YS49 with a mean of 24.2 ± 1.7% (range -2 to 56%) for MPH and 20.6 ± 2.0% (range -12 to 46%) for 73NAH followed by the test hybrid population of the BC_1F_6 inbred lines of (A04-73NA × YS49) × A04-73NA with a mean of 13.0 ± 2.0% (range -12 to 51%) for MPH, and 11.4 ± 1.8% (range -16 to 45%) for 73NAH. The lowest heterosis was found in case of the F_9 inbred lines of A04-73NA × T-4-3-3-1 with a mean of 10.5 ± 1.6% (range -12 to 46%)

for MPH and 11.4 ± 1.2% (range -12 to 34%) for 73NAH. The level of MPH and 73NAH in test hybrids of the A04-73NA × YS49 population was significantly greater than the other two populations.

More than 70% test hybrids of the F_9 lines of A04-73NA × YS49 exhibited more than 20% MPH, while only 15% of the test hybrids of the BC_1F_6 lines of (A04-73NA × YS49) × A04-73NA exhibited more than 20% MPH (Fig. 2.9). In the test hybrids of the F_9 lines of A04-73NA × T-4-3-3-1, about 25% of the hybrids exhibited more than 20% MPH. Likewise, 45% test hybrids of the F_9 lines of A04-73NA × YS49 exhibited more than 20% 73NAH, while only 19% of the test hybrids of the BC_1F_6 lines of (A04-73NA × YS49) × A04-73NA exhibited greater than 20% 73NAH (Fig 2.10).

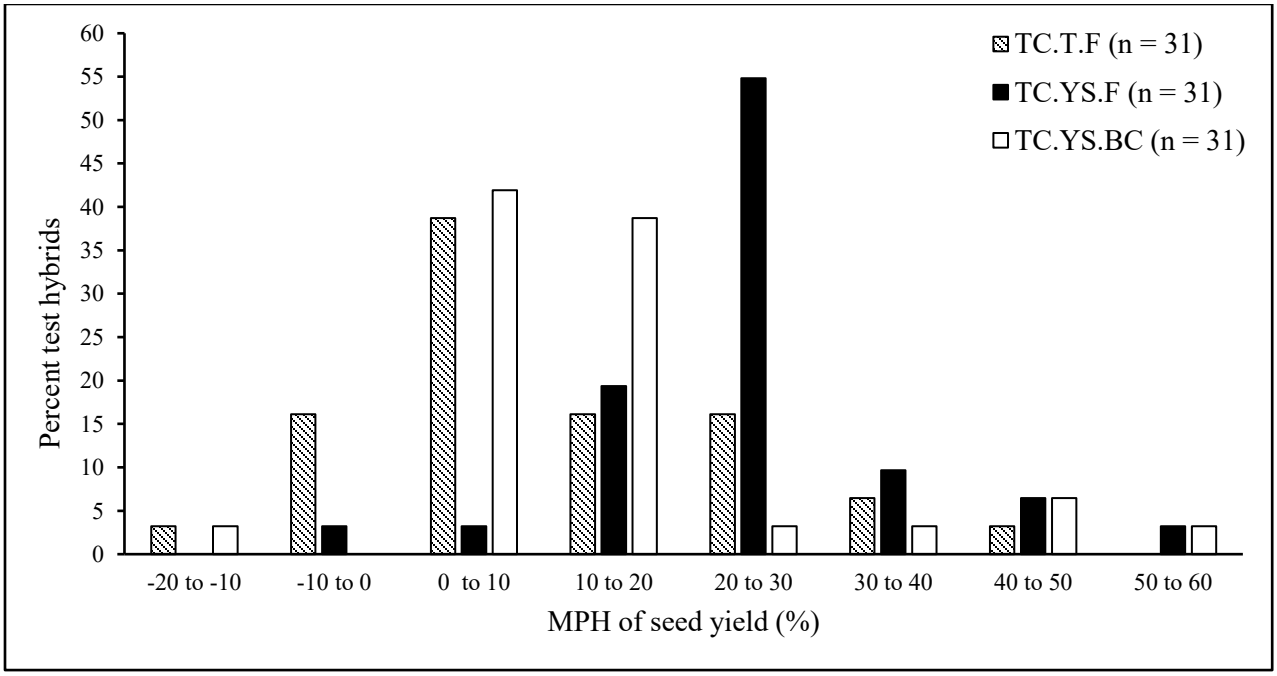

Fig. 2.9 Frequency distribution of the test hybrids populations of the F_9 inbred lines of *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (TC.T.F) and *B. napus* A04-73NA × *B. rapa*YS49 (TC.YS.F), and the BC_1F_6 inbred lines of (*B. napus* A04-73NA × YS49) × A04-73NA (TC.YS.BC) interspecific crosses for mid-parent heterosis (MPH) for seed yield.

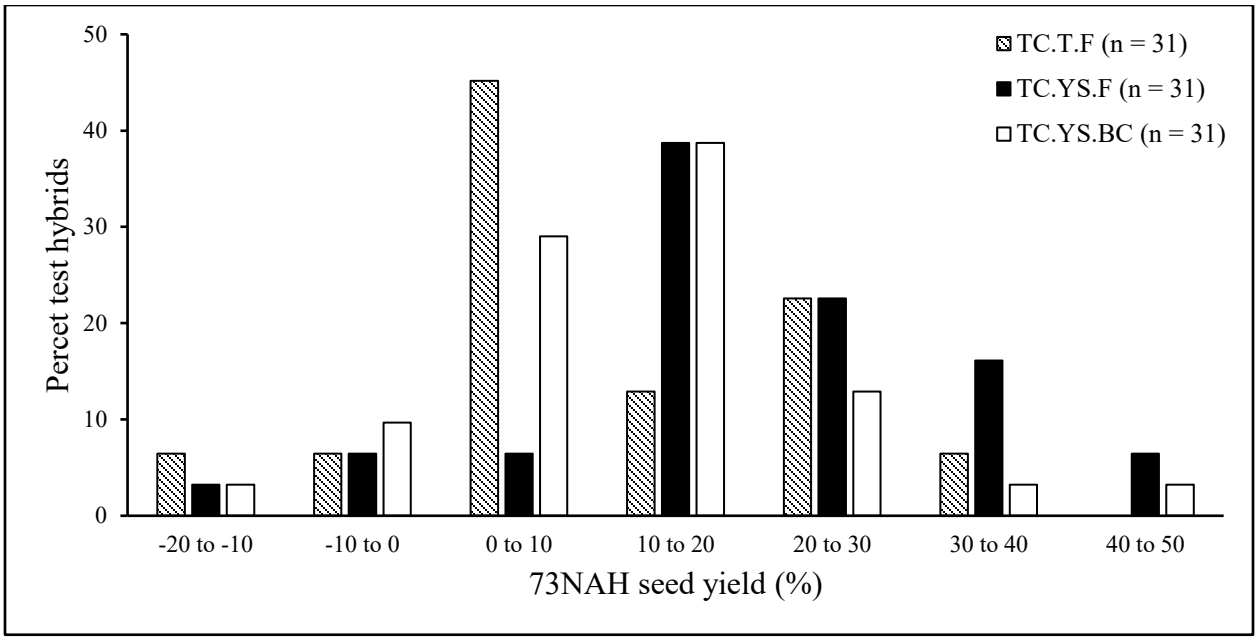

Fig. 2.10 Frequency distribution of the test hybrids populations of the F_9 inbred lines of *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (TC.T.F) and *B. napus* A04-73NA × *B. rapa*YS49 (TC.YS.F), and the BC_1F_6 inbred lines of (*B. napus* A04-73NA × YS49) × A04-73NA (TC.YS.BC) interspecific crosses for heterosis over A04-73NA (73NAH) for seed yield.

Seed oil content

Almost no MPH and 73NAH was found for seed oil content (Table 2.5) and the average values were negative in all three test hybrid populations. The mean MPH in the three populations varied from -1.0 ± 0.2% in the test hybrids of the F_9 lines of A04-73NA × YS49 to -1.9 ± 0.3% in the test hybrids of the BC_1F_6 lines of (A04-73NA × YS49) × A04-73NA. The mean 73NAH in the three test hybrid populations varied from -2.0 ± 0.3% to -3.8 ± 0.5%.

Seed protein content

Like heterosis for seed oil content, almost no heterosis for seed protein content was found in three test hybrid populations. MPH in the three populations varied from -0.4 ± 0.5% to 0.6 ± 0.4% and this difference was not significant (Table 2.5). The mean 73NAH in these three population varied from 0.7 ± 0.7% to 3.0 ± 0.7.

Seed glucosinolate (GSL) content

MPH and 73NAH for seed GSL content was negative in all three populations (Table 2.5); the lowest MPH and 73NAH was found in the test hybrid population of the F_9 lines of A04-73NA × YS49 with a mean of -13.0 ± 1.1% (range - 27 to 8%) for MPH and -17.4 ± 1.5% (range - 34 to 6%) for 73NAH, followed by the test hybrids of the F_9 lines of A04-73NA × T-4-3-3-1 with a mean of -9.9 ± 1.0% (range - 30 to 5%) for MPH and -16.1 ± 1.4% (range - 40 to 4%) for 73NAH. The test hybrid population of the BC_1F_6 lines of (A04-73NA × YS49) × A04-73NA exhibited MPH of -7.2 ± 1.0% (range - 36 to 8%) and 73NAH of -9.3 ± 1.4% (range - 40 to 4%).

Table 2.5 Heterosis over mid-parent (MPH) and over A04-73NA (73NAH) for different agronomic and seed quality traits (mean ± SE and range) of the test hybrid populations of the two F_9 inbred populations derived from *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 and *B. napus* A04-73NA × *B. rapa* YS49 and one BC_1F_6 inbred population derived from (*B. napus* A04-73NA × *B. rapa* YS49) × *B. napus* A04-73NA interspecific crosses. Test hybrid population were produced by crossing the inbred lines to A04-73NA.

Test hybrid Pop.[1]	DTF		DTM		Height		Yield		Oil		Protein		GSL	
	MPH	73NAH	MPH	73NAH	MPH	73NAH	MPH	73NAH	MPH	73NAH	MPH	73NAH	MPH	73NAH
TC.T.F	-2.1 ± 0.4a (-9 to 3)	-2.5 ±0.4a (-8 to 2)	-0.4 ± 0.2a (-2 to 1)	-0.6 ± 0.2a (-3 to 2)	-0.2 ± 0.4a (-4 to 6)	-0.5 ± 0.5a (-6 to 7)	10.5 ± 1.6b (-12 to 46)	11.4 ± 1.2b (-12 to 34)	-1.0 ± 0.2a (-4 to 3)	-2.0 ± 0.3a (-4 to 1)	0.6 ± 0.3a (-3 to 5)	1.1 ± 0.3b (-4 to 6)	-9.9 ± 1.0ab (-30 to 5)	-16.1 ± 1.4b (-40 to 4)
TC.YS.F	-2.1 ± 0.2a (-5 to 0)	-2.1 ± 0.4a (-6 to 2)	-0.7 ± 0.2a (-2 to 1)	-0.7 ± 0.2a (-3 to 1)	-0.6 ± 0.5a (-6 to 5)	-2.4 ± 0.6b (-11 to 5)	24.2 ± 1.7a (-2 to 56)	20.6 ± 2.0a (-12 to 46)	-1.3 ± 0.3a (-3 to 1)	-3.7 ± 0.5b (-7 to 2)	0.6 ± 0.4a (-5 to 4)	3.0 ± 0.7a (-5 to 9)	-13.0 ± 1.1b (-27 to 8)	-17.4 ± 1.5b (-34 to 6)
TC.YS.BC	-3.3 ± 0.4b (-7 to 0)	-4.3 ± 0.5b (-8 to -1)	-0.5 ± 0.2a (-4 to 1)	-0.8 ± 0.2a (-3 to 2)	-0.4 ± 0.5a (-6 to 9)	-1.8 ± 0.6ab (-10 to 8)	13.0 ± 2.0b (-12 to 51)	11.4 ± 1.8b (-16 to 45)	-1.9 ± 0.3a (-6 to 2)	-3.8 ± 0.5b (-9 to 1)	-0.4 ± 0.5a (-5 to 7)	0.7 ± 0.7b (-7 to 11)	-7.2 ± 1.0a (-36 to 8)	-9.3 ± 1.4a (-22 to 8)

Note: DTF = days to flowering; DTM = days to maturity; Oil and protein = per cent; GSL = glucosinolate (µmol/g seed).
[1] TC.T.F = A04-73NA × (A04-73NA × T-4-3-3-1); TC.YS.F = A04-73NA × (A04-73NA × YS49); TC.YS.BC = A04-73NA × [(A04-73NA × YS49) × A04-73NA].
Within the column, values followed by the same letter indicate no significant difference.

2.3.2.3 Correlation of MPH with inbred and test hybrids

Seed yield of the inbred lines showed a significant negative correlation (r = - 0.39, p < 0.01) with MPH for seed yield (Fig 2.11); however, test hybrid yield showed a significant positive correlation with MPH for seed yield (r = 0.57, p < 0.01) (Fig 2.12). No significant correlation between 73NAH and inbred yield was found (Fig 2.13); however, 73NAH showed a significant positive correlation with test hybrid yield (Fig 2.13).

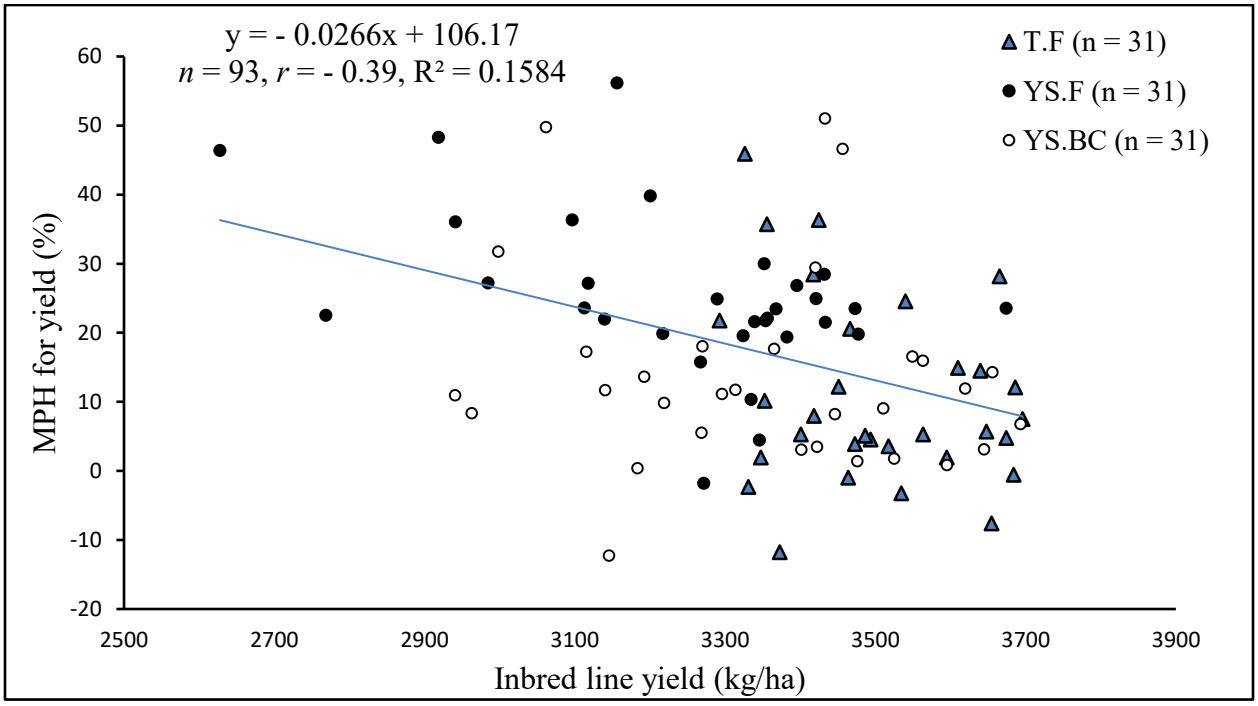

Fig. 2.11 Scatter diagram of MPH (%) for seed yield in the test hybrid population vs. inbred line yield of the three inbred populations derived from *B. napus* × *B. rapa* interspecific crosses. Pooled data of the F_9 inbred lines of *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (T.F) and *B. napus* A04-73NA × *B. rapa*YS49 (YS.F), and the BC_1F_6 inbred lines of (*B. napus* A04-73NA × YS49) × A04-73NA (YS.BC) interspecific crosses is presented.

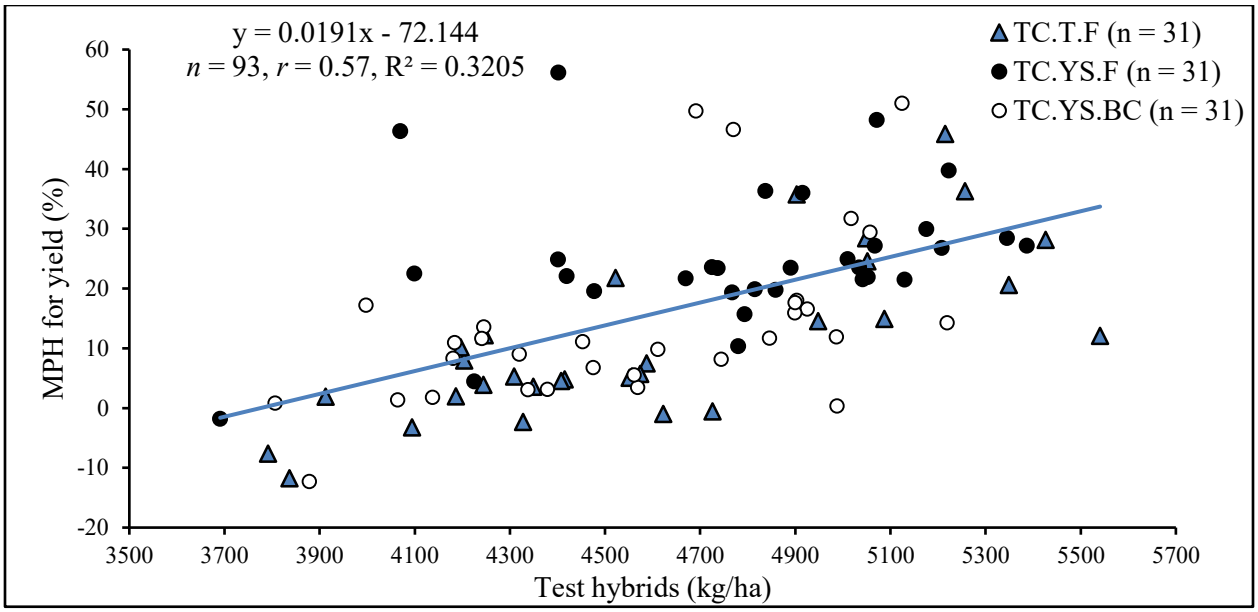

Fig. 2.12 Scatter diagram of MPH (%) for seed yield (%) in the test hybrid population vs. test hybrids yield of three inbred populations derived from *B. napus* × *B. rapa* interspecific crosses. Pooled data of the F_9 inbred lines of *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (TC.T.F) and *B. napus* A04-73NA × *B. rapa* YS49 (TC.YS.F), and the BC_1F_6 inbred lines of (*B. napus* A04-73NA × YS49) × A04-73NA (TC.YS.BC) interspecific crosses is presented.

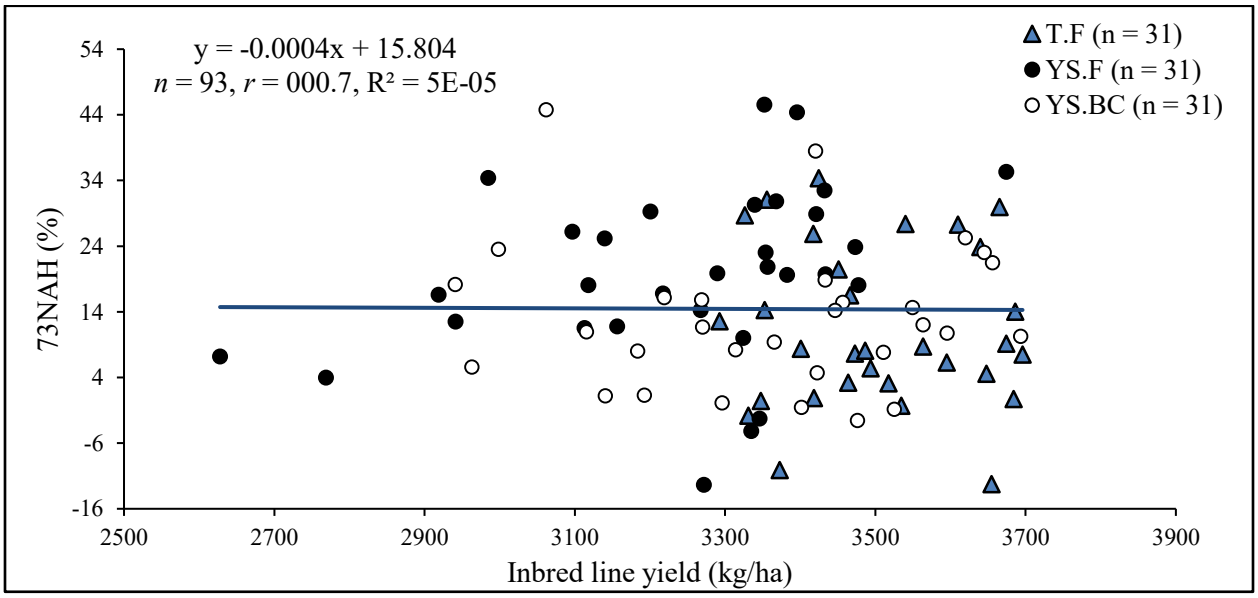

Fig. 2.13 Scatter diagram of 73NAH (%) for seed yield in the test hybrid population vs. test hybrids yield of the three inbred populations derived from *B. napus* × *B. rapa* interspecific crosses. Pooled data of the F_9 inbred lines of *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (TC.T.F) and *B. napus* A04-73NA × *B. rapa* YS49 (TC.YS.F), and the BC_1F_6 inbred lines of (*B. napus* A04-73NA × YS49) × A04-73NA (TC.YS.BC) interspecific crosses is presented.

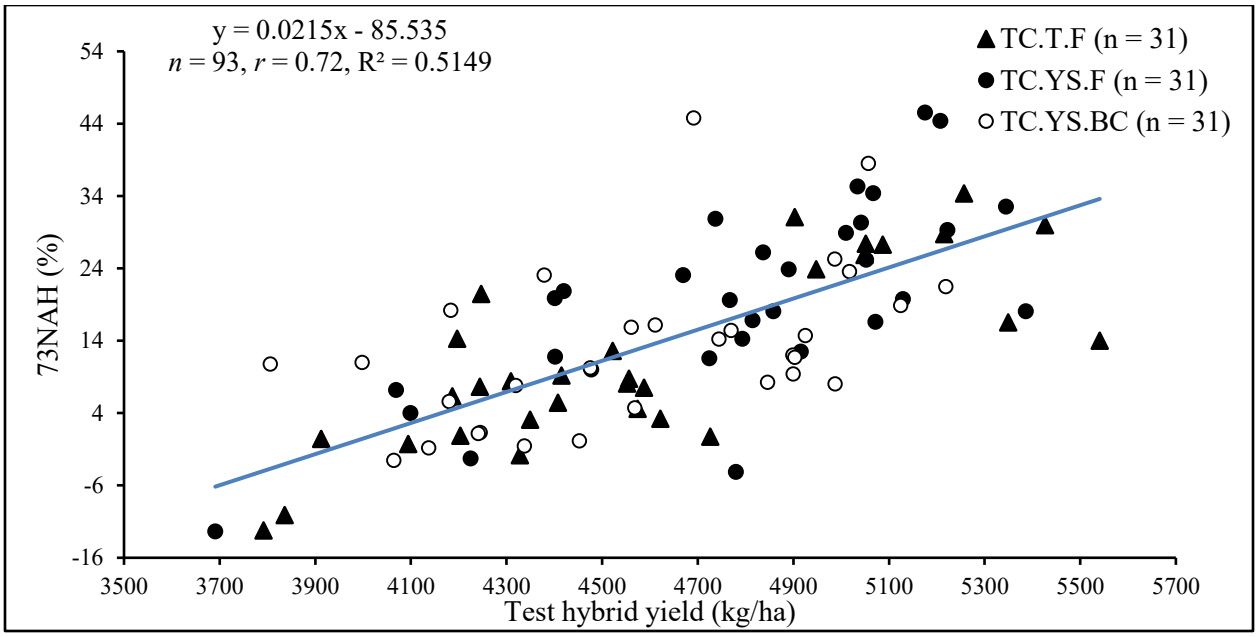

Fig. 2.14 Scatter diagram of 73NAH (%) for seed yield in the test hybrid population vs. test hybrids yield of the three inbred populations derived from *B. napus* × *B. rapa* interspecific crosses. Pooled data of the F_9 inbred lines of *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (TC.T.F) and *B. napus* A04-73NA × *B. rapa* YS49 (TC.YS.F), and the BC_1F_6 inbred lines of (*B. napus* A04-73NA × YS49) × A04-73NA (TC.YS.BC) interspecific crosses is presented.

2.4 Discussion

Rahman (2013) reviewed that genetic diversity in spring *B. napus* canola is low. Canola is one of the most important crop in Canada; therefore, broadening the genetic base of this crop is needed. Studies have showed that genetically the A genome of *B. rapa* is very distinct from the A genome of *B. napus* (Thormann et al. 1994 and Tian et al., 2017a), and the wide diversity present in the *B. rapa* gene pool (Annisa and Cowling, 2013; Hobson and Rahman, 2016) can contribute valuable alleles in *B. napus*. Mei et al. (2011) and Qian et al. (2006) demonstrated the value of Chinese *B. rapa* the broadening of the genetic base of Chinese *B. napus*. Qian et al. (2005) also reported heterosis for seed yield in the hybrids developed by use of the *B. napus* lines, derived from *B. napus* × *B. rapa* and (*B. napus* × *B. rapa*) × *B. rapa* interspecific crosses, and cultivated *B. napus*.

The MPH and 73NAH values obtained in this study for days to flowering were negative. This has important implication in breeding as earliness of flowering is an important trait in canola. Days to flowering is quantitative trait (Axelsson et al. 2001) controlled by dominance, additive and epistasis effects of the genes (Long et al., 2007). This negative MPH and 73NAH for days to flowering might be due to the effect of the dominant genes involved in earliness of flowering.

A positive correlation of plant height with seed yield ($r = 0.34$, $P < 0.01$) and oil content ($r = 0.30$, $P < 0.05$) but a negative correlation with protein content ($r = -0.29$, $P < 0.05$) was found in the present study in the test hybrid population. Girke et al. (2012) also found a positive correlation of plant height with seed yield ($r = 0.74$, $P < 0.01$) and oil content ($r = 0.61$, $P < 0.01$), but a negative correlation with protein content ($r = -0.70$, $P < 0.01$). Positive relationship of plant height with seed yield have also been reported in other crops, such as wheat (Law et al., 1978) and common bean (Habibi, 2011). This positive relationship between height and yield

might be due to tight linkage of the genes exerting positive effect on these two traits, or pleiotropic effect of the genes, as has been reported by Law et al. (1978) in wheat.

All the three populations used in this study demonstrated seed yield advantage over the mid parents and over the *B. napus* parent A04-73NA, and up to 24% MPH and up to 20% 73NAH was found. This shows potential value of the *B. rapa* yellow sarson and Canadian *B. rapa* gene pools for increasing seed yield in Canadian spring *B. napus* canola hybrids. Radoev et al. (2008) reported 30% mid parent heterosis for seed yield in winter *B. napus*, while Sernyk and Stefansson (1983) reported more than 40% heterosis and Grant and Beversdorf (1985) reported up to 72% heterosis for yield in spring *B. napus* hybrids.

The higher MPH and 73NAH for seed yield in the test hybrids of F_9 inbred lines derived from *B. napus* A04-73NA × *B. rapa* YS49 as compared to the test hybrids of the F_9 inbred lines of A04-73NA × T-4-3-3-1 suggests that greater number of heterotic alleles for seed yield can be found in yellow sarson and compared to the Canadian *B. rapa*. Among the three inbred line populations, the F_9 lines of A04-73NA × YS49 gave the lowest yield while the F_9 lines of A04-73NA × T-4-3-3-1 gave the highest yield. The occurrence of the greatest level of MPH in the test hybrids of the inbred lines of A04-73NA × YS49 suggests that alleles displaying overdominance effect of heterosis can be found frequently in yellow sarson. Radoev et al. (2008) also reported the involvement of the overdominance effects of the genes in the genetic control of contributing to heterosis. Introduction of alleles, which are unfavorable for inbred lines cultivars but favorable for heterosis has apparently occurred in the inbred lines derived from *B. napus* × *B. rapa* interspecific crosses. This is also evident from almost no correlation between the inbred and hybrid yield ($r = 0.11$). However, the trend of positive correlation between the inbred and hybrid yield in all three populations indicate the importance of general combining ability of the parents

for increasing seed yield in hybrid. The negative correlation found in the study between the MPH of seed yield and seed yield of the inbred lines (r = - 0. 40, $p < 0.001$) is very similar to the results reported by Rahman et al. (2016).

The lower seed yield in majority of the inbred lines derived from *B. napus* × *B. rapa* interspecific crosses as compared to the *B. napus* parent could be due to the introduction of unfavorable alleles from *B. rapa*. Introgression of undesirable alleles along with the desired alleles from the related species, such as *B. oleracea* into *B. napus* has also been reported by Rahman et al. (2016).

In this study, significant negative correlation between seed oil and protein content was found in both inbred line and test hybrid populations. Sernyk and Stefansson (1983) also found a negative association between these traits in F_1 hybrids of spring *B. napus*. Several other researchers, such as Qian et al. (2009), Girke et al. (2012) and Rahman et al. (2016) also reported significant negative correlation between oil and protein content. The negative correlation between oil and protein content might be due to a tight linkage between oil and protein in repulsion phase.

Protein content also showed a significant negative correlation with seed yield, and seed yield showed a significant negative correlation with glucosinolate content in both inbred line and test hybrid populations. Qian et al. (2009) and Girke et al. (2012) also reported negative correlation between yield and protein contents. No consistent correlation between the other agronomic and seed quality traits was found in both inbred line and test hybrid populations.

In conclusion, this study demonstrates the potential of the *B. rapa* alleles for increasing seed yield and heterosis in hybrid cultivars as well as for improving the earliness flowering/maturity in hybrid *B. napus* cultivars. High heterosis observed for seed yield

apparently resulted from the non-additive effect of the genes in the genetic control of heterosis, while the lack of high MPH for other traits, such as days to flower and maturity, plant height and seed oil and protein contents suggests that these traits are mostly under additive genetic control in the populations used in this study.

Chapter 3

Detection and estimation of allelic diversity introgressed from *B. rapa* into *B. napus* in the BC_1F_6 population using SSR markers

3.1 Introduction

Brassica. napus (2*n* = 38, AACC) is an allotetraploid species, originated from interspecific hybridization between *B. rapa* (2*n* = 20, AA) and *B. oleracea* (2*n* = 18, CC) (reviewed by Prakash and Hinata, 1980). It is the main oilseed crop species of the genus Brassica (Velasco et al., 2016) and is the second most important oilseed crop in the world (McVetty and Duncan, 2016).

Existence of genetic diversity in a breeding population of a crop is important for further improvement through breeding (Rao and Hodgkin, 2002). Genetic diversity in many crops has declined due to the introduction of new high-yielding cultivars, and intensive breeding over a period of time within the restricted gene pool (Hawkes, 1991; reviewed by Rahman, 2013). Hence, conservation of germplasm resources and use of these in breeding is important for broadening the genetic base of crops and further improvement from a long-term perspective (Rao and Hodgkin, 2002). In case of spring *B. napus* canola, allele diversity in this economically important crop is narrow; to address this limitation, introgression of new alleles from its related species and exotic germplasm is needed (reviewed by Rahman, 2013). Among the different Brassica germplasm resources, *B. rapa* is genetically distinct from *B. napus*, and wide diversity exists in this diploid species (Elling et al., 2010; Thakur et al., 2016; Hobson and Rahman, 2016). The value of this diploid species in the breeding of *B. napus* has been confirmed by many researchers (Liu and Meng, 2006; Qian et al. 2006; Mei et al., 2011; Attri and Rahman, 2017).

Molecular markers have been used to assess the extent of genetic diversity present in different plant species (Mahjoob et al., 2016). Various types of molecular markers, such as simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), and single

nucleotide polymorphism (SNP), have been used for this purpose. The SSR or microsatellites are short tandem repeats of nucleotides, such as $(AT)_n$, $(GT)_n$, $(TTC)_n$ or $(GACA)_n$, which are spread widely throughout the genome (Kresovich et al., 1995; Broun and Tanksley, 1996). The SSR markers are highly informative, co-dominant, robust and reproducible (Powell et al., 1996). High polymorphism of SSR markers, due to variation in the number of repeat units, makes this marker suitable for assessment of genetic diversity in a crop and mapping of traits (Hearne et al., 1992; Hobson and Rahman, 2016; Rahman et al., 2016, 2017). Hamblin et al. (2007) compared SSR and SNP markers and found that a large number of SNPs is required to replace the highly polymorphic SSRs in genetic diversity analysis. Worldwide use of SSR markers in different research, such as population genetics study, assessment of genetic diversity, and mapping of traits has increased over the years (Cieslarová, 2011; Vieira et al., 2016), which is primarily due to its reliability in genotyping and low cost (Hobson and Rahman, 2016; Vieira et al., 2016). For instance, Tian et al. (2017a) used a set of A genome specific SSR markers to investigate the extent of genetic diversity exist among 127 accessions of *B. napus*, *B. rapa*, and *B. juncea*. Hobson and Rahman (2016) used SSR markers, designed from *B. rapa* genome sequence information, to genotype 43 accessions of *B. rapa* and found that these markers can be used reliably to study the genetic variation in Brassica.

The objective of this study was to assess the extent of *B. rapa* alleles (A genome) introgressed into a BC_1F_6 population of *B. napus* derived from a (*B. napus* × *B. rapa*) × *B. napus* interspecific cross, and to identify the genetically diverse *B. napus* lines for use in canola breeding.

3.2 Materials and Methods

3.2.1 Plant germplasm

Thirty BC_1F_6 plants, derived from (*B. napus* A04-73NA × *B. rapa* Y49) × A04-73NA

interspecific cross were genotyped by SSR markers to detect the extent of allelic diversity introgressed into these plants from *B. rapa*.

3.2.2 DNA extraction

The BC_1F_6 plants were grown in a greenhouse in 2016-17 winter at 18-20 ºC with 18 h photoperiod. About 100 mg leaf sample was collected from each plant in aluminum foil and stored at -80 ºC for extraction of DNA. A Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI) was used for extraction of DNA as described below.

The frozen leaf samples were crushed into fine powder by a microcentrifuge tube pestle. Approximately 40 mg of this leaf powder was placed in 1.5 ml microcentrifuge tube and 600 µl Nuclei Lysis Solution was added to the powdered tissue and the samples vortexed for 10 sec. After that, samples were incubated in a water bath at 65 ºC for 15 min and 200 µl Protein Precipitation Solution was added to the contents, and the samples vortexed vigorously at high speed for 20 sec. After that, 400 µl chloroform was added, and the samples were centrifuged at 13,000 rpm for 3 min. At this stage, the precipitated proteins formed a light pellet. The supernatant was carefully transferred to a new 1.5 ml microcentrifuge tube containing 600 µl of room temperature isopropanol. The solution was gently mixed by inversion until the thread-like strand of DNA became visible. The samples were kept at -20 ºC for 10 min, and centrifuged at 13,000 rpm for 2 min. The supernatant was decanted and 600 µl ethanol (70%) was added to it; the tube was gently inverted several times to wash the DNA and centrifuged at 13,000 rpm for 1 min. The ethanol was carefully aspirated and the tube was inverted onto a clean absorbent paper and was air dried. After drying, the pellet was suspended in 300 µl of elution buffer.

The quality and concentration of the DNA was estimated by a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Samples having low quality and/or low concentration of DNA were discarded and DNA extraction was repeated. Finally, the DNA was diluted to 10 ng/µl with TE buffer and stored at 4 ºC.

3.2.3 Screening for parental polymorphism

The parental lines of *B. napus* (A04-73NA) and *B. rapa* (Y49) were screened by Attri (2015) with 397 SSR markers from A1 to A10 linkage group of the Brassica A genome for identification of polymorphic markers. For the present study, 55 markers which were found to be polymorphic between the parents (A04-73NA and Y49) were used to genotype the 30 BC_1F_6 plants. The list of polymorphic SSR markers used for genotyping is presented in Appendix 3.1.

3.2.4 PCR reaction

Polymerase chain reaction (PCR) is a method to amplify a specific segment of DNA into millions of copies. The copy number of the target DNA doubles at every cycle; thus, 35 cycles of PCR can produce a million-fold amplification (2^{35}). The PCR cycling process includes template DNA denaturation, annealing of the primers, and extension of the annealed primers by DNA polymerase until the DNA fragment is grown and extension at the 5′ end is terminated; the process is repeated until abundant copies of the DNA fragment is produced (Erlich, 1989). In this study, PCR was done in a reaction volume of 12.025 µl containing 10 ng of template DNA, 5 µM of each forward and reverse primers, 10 mM of each dNTP (Invitrogen Life Technologies Inc., Burlington, ON), 25 mM of MgCl2, 1x PCR reaction buffer, 0.125 units of Taq DNA polymerase (Promega Corporation, Madison, WI) and 4.9 µl distilled water. After initial denaturation for 5 min at 95 ºC, 35 PCR cycles performed using the following temperature profile: Denaturation for 1 min at 95 ºC, primer annealing for 1 min at 56 ºC, and primer extension for 1.30 min at 72 ºC. Cycling concluded with a final extension for 30 min at 72 ºC.

3.2.5 ABI (Applied Biosystem Instruments) sequencing

Genotyping the BC_1F_6 population was done by a ABI sequencer No. 3730 (Applied Biosystems, Foster City, CA). The SSR primers were labelled following the M13-tailing technique of Schuelke (2000). The forward primer of each SSR was appended with the universal

M13 primer sequence 5'-CACGACGTTGTAAAACGAC-3' labelled with fluorescent dyes FAM, VIC, NED and PET (Applied Biosystems, Foster City, CA).

3.2.6 Data analysis

Scoring the peaks from the ABI sequencer was done manually; each peak was given a score of 1 and the absence of a peak was given a score of 0. In the analysis, only the sharp and well-defined peaks were considered, and ambiguous or weak peaks were discarded. Dice genetic similarity coefficients (Nei and Li, 1979) were calculated between the pairs of the plants from the data matrix using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.2; Rohlf, 2000). The similarity coefficients were used to develop a dendogram using the unweighted pair-group method with arithmetic mean (UPGMA).

The proportion of the SSR loci carrying *B. rapa* alleles observed in a BC_1F_6 plant was calculated using the following formula: (Number of loci carrying *B. rapa* alleles detected in the plant / the total number of loci carrying *B. rapa* alleles detected in the population) × 100. SSR markers can be used to distinguish between the homozygous and heterozygous genotypes. In case of the SSR markers amplifying a single locus, a single peak in the electropherogram represents the homozygous condition, while two peaks indicate the heterozygous condition. Based on this, the SSR markers which amplified one locus as well as the markers which amplified more than one locus, however, the marker genotype of these loci can be deduced, were used for estimation of the occurrence of *B. rapa* alleles in the population. The number of *B. rapa* allele observed in the BC_1F_6 population was compared with the number of alleles, theoretically, expected to be present in the population. To calculate the total observed number of *B. rapa* alleles, the following formula was used: (number loci homozygous for *B. rapa* alleles × 2) + number heterozygous loci); and to calculate of expected number of *B. rapa* alleles, the following formula was used: (total number of SSR loci in the population × 2) × 0.25.

Chi-square test for goodness-of-fit was done to compare the observed number of loci carrying *B. rapa* alleles with the expected number of loci carrying *B. rapa* alleles in the population. For this, the following marker genotypes were used: (i) loci homozygous for *B. rapa* allele, (ii) loci heterozygous for *B. rapa* and *B. napus* alleles, and (ii) loci homozygous for *B. napus* allele. Chi-square test for goodness-of-fit was also done to compare the observed number *B. rapa* alleles with the expected number of *B. rapa* allele; Chi square test for 1:3 segregation of (i) *B. rapa* alleles and (ii) non-*B. rapa* alleles (includes *B. napus* alleles and loci with no *B. rapa* or *B. napus* alleles) was also done to study the inheritance of *B. rapa* alleles.

3.3 Results

3.3.1 Occurrence of *B. rapa* alleles in the BC_1F_6 population

The 55 polymorphic SSR markers from 10 A genome chromosome amplified a total of 111 loci in the two parents A04-73NA and YS49, of which 56 loci were amplified in the *B. rapa* parent YS49. The frequency distribution of the 30 BC_1F_6 plants based on the number of loci carrying *B. rapa* alleles is shown in Fig. 3.1. About 23% of the plants carried more than 20 SSR loci with *B. rapa* alleles, while 7% of the plants carried less than 7 loci with *B. rapa* alleles; the majority (76%) of the plants carried 12 to 24 SSR loci with *B. rapa* alleles.

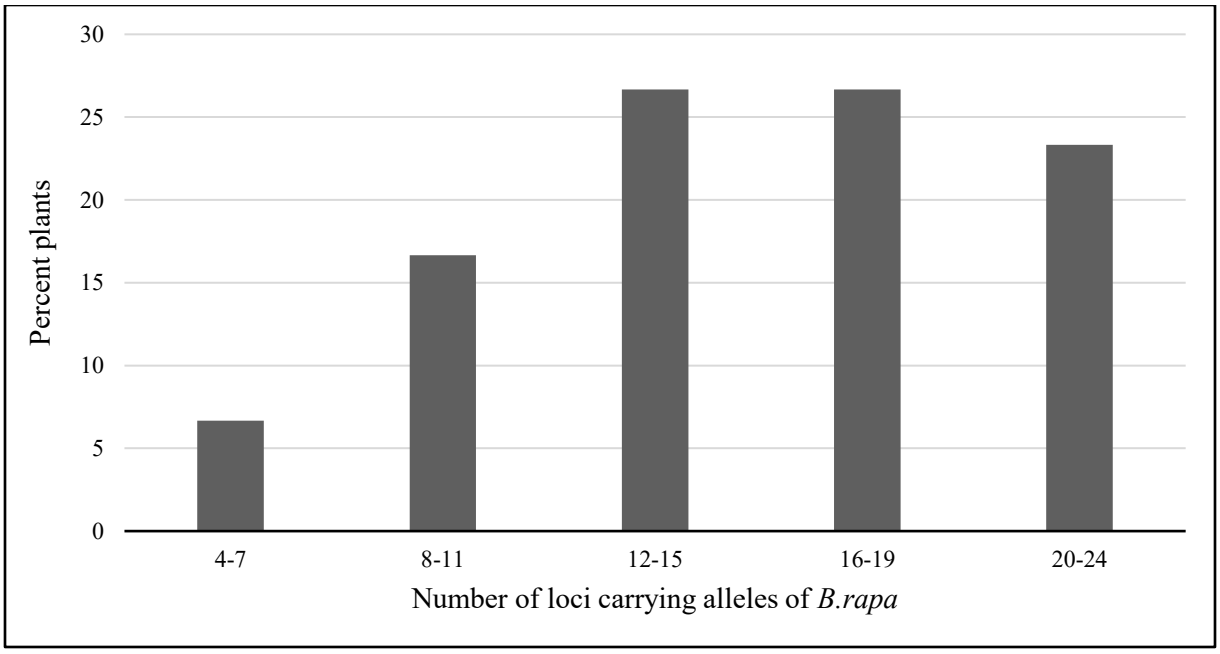

Fig. 3.1 Frequency distribution of the 30 BC_1F_6 plants based on the number of loci carrying *B. rapa* alleles

3.3.2 Genetic diversity within the population

Based on genetic similarity among the 30 BC_1F_6 plants, UPGMA cluster analysis was done to partition the population into different groups. Genetic similarity coefficient of the two parents YS49 (*B. rapa*) and A04-73NA (*B. napus*) was 0.26. The BC_1F_6 population was genetically, closer to the *B. napus* parent A04-73NA (similarity coefficient coefficients of 0.62 to 0.88) as compared to the *B. rapa* parent YS49 (similarity coefficient 0.26). Cluster analysis showed that the BC_1F_6 population could be divided into 3 groups at a genetic similarity coefficient of 0.76 (Fig. 3.2). The Group I included 25 plants with similarity coefficients of 0.76 to 0.88 with A04-73NA, where the plant 1680-386 showed the greatest similarity (0.88) with this *B. napus* parent. The Group II included 3 plants (1680-421, 1680-369, 1680-366) with similarity coefficient of 0.66 with A04-73NA and the Group III included only two plants (1680-417, 1680-377) with similarity coefficient of 0.62 with A04-73NA.

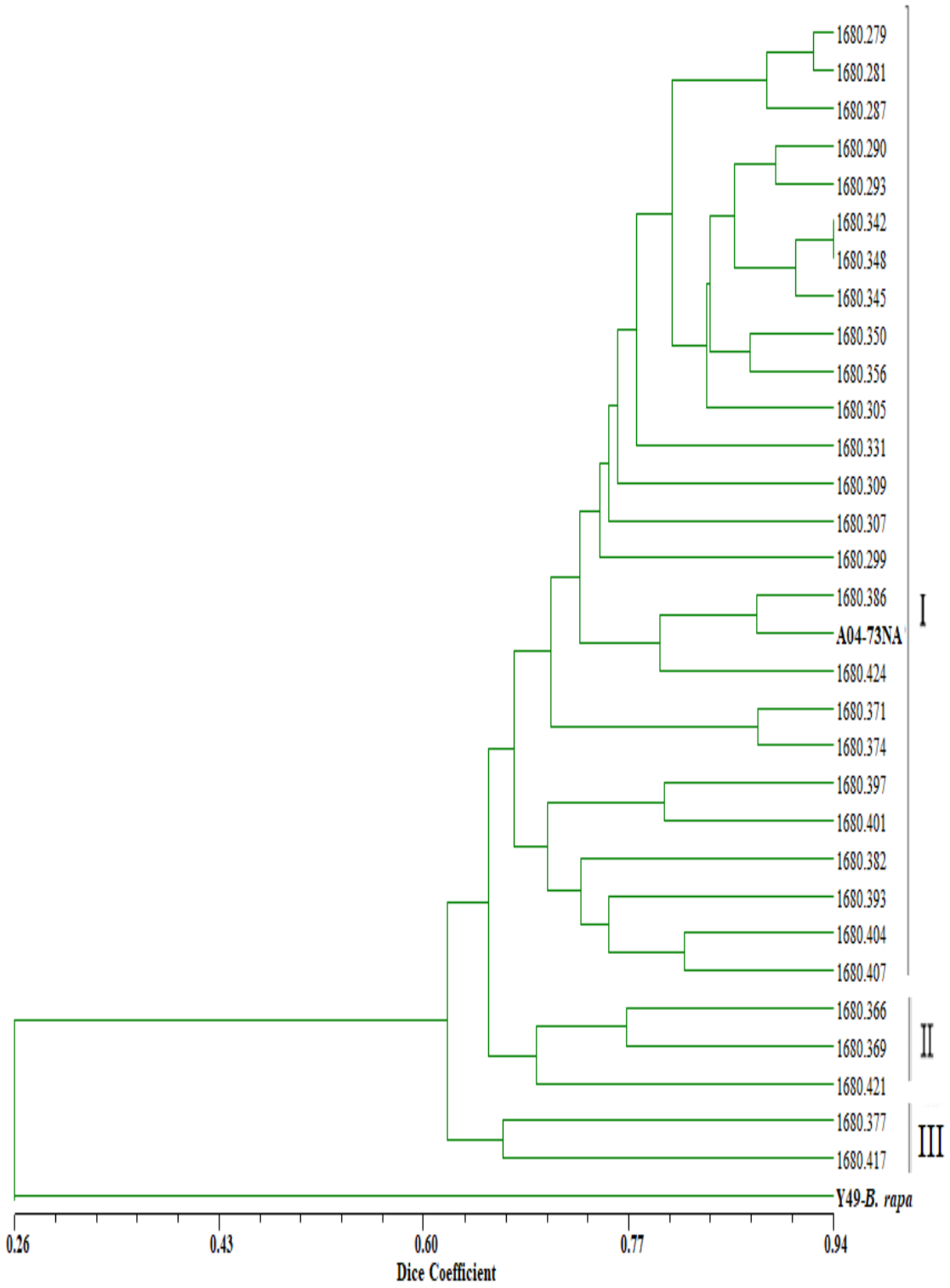

Fig. 3.2 Dendrogram showing genetic similarity among 30 BC_1F_6 plants derived from (*B. napus* × *B. rapa*) × *B. napus* interspecific cross.

3.3.3 Inheritance of SSR alleles in the BC_1F_6 population

To investigate the inheritance of SSR marker alleles of *B. rapa*, 1,320 SSR loci amplified by 44 SSR markers, which genotype in respect to the *B. napus* and *B. rapa* alleles could be determined, were used. Of 1320 SSR loci detected in the 30 BC_1F_6 plants, 24.21%, 1.36% and 74.21% of the loci were expected to be homozygous for *B. rapa* alleles, heterozygous for *B. rapa* and *B. napus* alleles and homozygous for *B. napus* alleles, respectively. However, the proportion of loci homozygous for *B. rapa* and *B. napus* alleles was 14.2% and 69.3%, respectively; while the proportion of heterozygous loci was 9.2%. Chi-square tests for goodness of fit showed that the obseved number of loci deviate significantly from the expected number (Table 3.1). This deviation was found for the marker alleles from most of the chromosomes.

The number of loci detected the population (1,320) multiplied by two gives total number of alleles in the population; while the number of loci in the population multiplied by 0.25 gives the greatest number of *B. rapa* alleles to be expected in the BC_1F_6 population. Chi-square tests was also done for segregation of the non-*B. rapa* alleles, which includes *B. napus* allels and the loci whitout *B. rapa* or *B. napus* alleles, and the *B. rapa* alleles. In this case also, deviation from the expected 3:1 segregation of the *B. napus* : *B. rapa* alleles was found in the BC_1F_6 population. Of the total expected number of *B. rapa* alleles, 78.6% alleles were found in the populaion suggesting a loss of about 20% SSR alleles occurred during the development of the BC_1F_6 population. The greatest loss of alleles occurred from the chromosomes A9 and A2.

Table 3.1 Inheritance of SSR marker alleles in BC_1F_6 population populations of (*B. napus* × *B. rapa*) × *B. napus* interspecific cross

Linkage group	No. polymorphic SSR markers	No. polymorphic loci studied	No. plants	Total no. of SSR loci[1]	No. loci homo. for *B. rapa* alleles (%)	No. het. loci (%)	No. loci homo. for *B. napus* alleles (%)	No. loci with no *B. napus* and *B. rapa* alleles (%)	Chi-square[2]	Total obs. *B. rapa* alleles[3]	Maximum exp. No. of *B. rapa* alleles	% of the exp. no. of *B. rapa* alleles obs.[5]	Chi-square (segr. for alleles)[6]
A1	3	3	30	90	9 (10)	19 (21.1)	60 (66.7)	2 (2.2)	233.5*	37	45	82.2	1.9
A2	2	2	30	60	2 (3.3)	8 (13.3)	49 (81.7)	1 (1.7)	65.5*	12	30	40	14.4*
A3	7	7	30	210	34 (16.2)	14(6.7)	143 (68.1)	19 (9.0)	45.0*	82	105	78.1	6.7*
A4	8	7	30	210	36 (17.1)	11(5.2)	161 (76.7)	2 (1.0)	22.9*	83	105	79	6.2*
A5	7	4	30	120	23 (19.2)	9 (7.5)	85 (70.8)	3 (2.5)	29.2*	55	60	91.7	0.6
A6	8	6	30	180	25 (13.8)	33 (18.3)	117 (65)	5 (2.8)	343.4*	83	90	92.2	0.7
A7	5	4	30	120	29 (24.2)	4 (3.3)	86 (72)	1 (0.8)	2.5	62	60	103.3	0.1
A8	10	7	30	210	38 (18.1)	6 (2.9)	164 (78.1)	2 (1.0)	6.0*	82	105	78.1	6.7*
A9	3	3	30	90	0 (0.0)	12 (13.3)	73 (81.1)	5 (5.6)	107.9*	12	45	26.7	32.3*
A10	2	1	30	30	0(0.0)	11 (36.7)	19 (63.3)	0 (0.0)	244.3*	11	15	73.3	1.4
Total	55	44	30	1320	196(14.2)	127(9.2)	957(69.3)	40(2.9)	614.4*	519	660	78.6	40.2*

[1] The number of polymorphic loci studied multiplied total number of plants (30).

[2]Chi-square test for goodness of fit for the observed vs. expected number loci (i) homozygous for *B. rapa* allele, (ii) heterozygous for *B. rapa* and *B. napus* alleles, and (iii) and homozygous for *B. napus* allele. Asterisk indicate *p*-values <0.05.

[3] (Number loci homozygous for *B. rapa* alleles × 2) + number of loci heterozygous for *B. rapa* and *B. napus* alleles.

[4] [(Total number of SSR loci in the population) × 2] × 0.25.

[5] (Total number of observed *B. rapa* alleles / Maximum expected number of *B. rapa* alleles) × 100.

[6] Chi-square test for goodness of fit was done based on the observed and expected number of *B. rapa* and other (*B. napus* alleles and loci without *B. rapa* or *B. napus* alleles) alleles. Asterisk indicate *p*-values <0.05.

Note: homo.= homozygous; het.= heterozygous; Obs. = observed; Exp. = expected.

3.4 Discussion

The present study was conducted to evaluate the prospect of using *B. rapa* to broaden the genetic base of Canadian spring *B. napus* canola through *B. napus* × *B. rapa* interspecific cross. The *B. rapa* line used in this study is genetically distinct from *B. napus* canola (Hobson and Rahman, 2016). As expected, the BC_1F_6 plants were genetically diverse from the *B. napus* and *B. rapa* parents; however, most of the plants clustered closer to the *B. napus* parent compared to the *B. rapa* parent (Fig. 3.2), which is apparently due to the backcross of the F_1 to the *B. napus* parent. Genetic diversity analysis by SSR markers showed that the BC_1F_6 population had a similarity coefficient of 0.75 with the *B. napus* parent. Attri and Rahman (2017) also found a similar similarity coefficient of a F_8 population derived from the same cross, with the *B. napus* parent. They also found that the progeny derived from this interspecific cross stabilized into *B. napus* type plants carrying about 45% of the expected number of *B. rapa* alleles. In contrast, the BC_1F_6 population used in the present study carried about 79% of the expected number of *B. rapa* alleles. This difference is apparently due to the effect of backcross. Wang (2016) also found that the BC_1F_3 population of (*B. napus* × *B. olerecea*) *B. napus* was closer to *B. napus* than the F_2 derived population.

In the present study, a deviation from the expected segregation of *B. rapa* alleles or the number of loci carrying *B. rapa* allele was found in the BC_1F_6 population. This deviation might have resulted from greater viability of the gametes carrying greater genome content of *B. napus*. Attri and Rahman (2017) also reported a deviation from normal segregation of *B. rapa* alleles in F_4 and F_8 population derived from *B. napus* × *B. rapa* cross. A loss of about 3% SSR loci was found in the BC_1F_6 population. This might be due to homoeologous pairing and rearrangements of the A and C chromosomes (Tian et al. 2010).

In conclusion, this study confirmed that it is possible to introgress exotic alleles from the A genome of *B. rapa* into Canadian spring *B. napus* canola through interspecific cross between

these two species followed by backcrossing of the F_1 to the *B. napus* parent. Vast diversity exists in the gene pool of *B. rapa* (Hobson and Rahman, 2016); use of these genetically distinct *B. rapa* in the breeding of *B. napus* canola is expected to broaden the gene pool of this important crops. The materials developed in this study can be used in canola breeding programs for the improvement of Canadian canola.

Chapter 4

General discussion and conclusions

4.1 General discussion

Interspecific hybridization is one of the approaches to broaden the genetic base of a crop species. Mallet (2005) reported that at least 25% of plant species undergo natural hybridization in the wildlife. In case of Brassica, Jørgensen et al. (1998) demonstrated that spontaneous hybridization between *B. napus* and *B. rapa* as well as backcrossing of the hybrids to *B. napus* can occur under natural condition. Genetic distance between the A genome of *B. napus* and the A genome of *B. rapa* is wide (Tian et al., 2017a); therefore, the wide diversity present in the *B. rapa* gene pool (Annisa and Cowling, 2013; Hobson and Rahman, 2016), can be used to broaden the genetic base of the A genome of *B. napus*. Mei et al. (2011) and Qian et al. (2006) also demonstrated the value of Chinese *B. rapa* in broadening the genetic base of Chinese *B. napus.* Also, heterosis for seed yield in the hybrids involving *B. napus* lines derived from *B. napus* × *B. rapa* and (*B. napus* × *B. rapa*) × *B. rapa* interspecific cross has been reported by Qian et al. (2005).

In the present study, the yellow sarson *B. rapa* accession, YS49, and the Canadian *B. rapa* line T-4-3-3-1 were used to introduce exotic alleles from these accession into Canadian spring *B. napus* canola. Analysis of yellow sarson and Canadian *B. rapa* by use of SSR markers showed that these two types of *B. rapa* are genetically quite distinct (Hobson and Rahman, 2016).

Also, positive relation between plant height and yield was recorded in this study. This result is similar to other studies such as Law et al. (1978) in wheat, Habibi (2011) in common

beans and Girke et al. (2012) in canola. This positive relationship between these two traits might be because of tight coupling linkage and pleiotropy.

Results from the present study showed that the gene pool of yellow sarson can contribute alleles to *B. napus* which can exhibit almost 2 times greater heterosis for seed yield compared to Canadian *B. rapa* T-4-3-3-1. Also, the population derived from F_2 exhibited twice greater heterosis than the population derived from BC_1 indicating that some of the exotic alleles contributing to heterosis might have been depleted from the population due to backcrossing of the F_1 to the *B. napus* parent A04-73NA. The trend of positive correlation between inbred and hybrid yield in all three populations represents the importance of general combining ability of the parents for increasing seed yield in hybrid. Rahman et al. (2016) also found low positive correlation between test hybrids and inbred lines yield.

In this study, correlation between seed yield and protein content was negative. Qian et al. (2009) and Girke et al. (2012) also reported a negative correlation between yield and protein contents. The negative correlation between these two traits might be due to an increase in seed yield which may cause a reduction in the biosynthesis of amino acids and proteins to be translocated to grain and these results in reduced protein content.

Earliness of flowering is one of the main goals of breeding of spring *B. napus* canola. In this study, negative MPH and 73NAH for days to flowering was found; which might be due to dominant or partially dominant genes controlling earliness of flowering. Long et al. (2007) reported dominance, additive and epistasis effects of the genes involved in the control of days to flowering (Long et al., 2007). The results from this study is also similar to the results reported by Rahman et al. (2016).

In this study correlation between seed protein and oil content was negative in both inbred and test hybrid populations. This result is similar to the results reported by other researchers, such as Sernyk and Stefansson (1983), Qian et al. (2009), Girke et al. (2012) and Rahman et al. (2016). Tight linkage in repulsion phase between the positive and negative alleles of these two seed quality traits might be the reason of this negative correlation of these two traits.

The present study demonstrated the prospect of introgression of exotic alleles from the A genome of *B. rapa* into the A genome of Canadian spring *B. napus* canola through *B. napus* × *B. rapa* interspecific cross followed by backcrossing of the F_1 to the *B. napus* parent. SSR markers shows that the BC_1F_6 population investigated in the present study carried about 79% of the expected number of *B. rapa* alleles while the BC_1F_6 population showed a similarity coefficient of 0.75 with the *B. napus* parent. This result is similar to Wang (2016) in case of the BC_1F_3 population derived from (*B. napus* × *B. oleracea*) × *B. napus* interspecific cross. Loss of about 3% SSR loci was also found in the BC_1F_6 population which might have resulted from homoeologous pairing and rearrangements between the A and C chromosomes. Attri and Rahman (2017) also reported loss of about 30% marker loci in F_4 and about 25% in F_8. Song et al. (1995) and Tian et al. (2010) also reported loss of marker loci in Brassica.

In conclusion, this study demonstrates the capability of the *B. rapa* alleles for increasing seed yield and heterosis in hybrid cultivars as well as for improving the earliness in hybrid *B. napus* cultivars. High heterosis observed for seed yield apparently resulted from the non-additive effect of the genes in the genetic control of heterosis, while the lack of high MPH for other traits, such as days to flowering and maturity, plant height and seed oil and protein contents suggests that these traits are mostly under additive genetic control in the population used in this study.

4.2 Conclusions

The following conclusions were drawn from this thesis research:

- Genetically diverse canola quality *B. napus* lines can be developed from (*B. napus* × *B. rapa*) × *B. napus* interspecific crosses through reconstitution of the A genome of *B. napus* with the A genome of *B. rapa*.
- *Brassica napus* inbred lines derived from *B. napus* × *B. rapa* interspecific cross can exhibit high heterosis for seed yield in *B. napus* hybrids.
- The *B. rapa* alleles can increase seed yield and the level of heterosis for seed yield as well as can improve the earliness of flowering/maturity in hybrid *B. napus*.
- The lack of high heterosis for days to flower, plant height and seed oil and protein contents suggest that these traits are mostly under additive genetic control.
- The negative correlation between oil and protein content suggests tight linkage between the alleles for seed oil and protein contents in repulsion phase.
- Compared to the Canadian *B. rapa* gene pool, the yellow sarson gene pool showed higher heterotic potential in the *B. napus* hybrids.

4.3 Future research

- On average, the *B. napus* inbred lines derived from the *B. napus* × *B. rapa* interspecific crosses gave low yield and had lower oil content as compared to the *B. napus* parent; this might be due to introduction of undesired alleles from *B. rapa* into these lines. These traits need to be improved through crossing with the same and/or other elite lines.
- Heterotic potential of these inbred lines was only evaluated in test hybrids with the *B. napus* parent A04-73NA; therefore, evaluation of these inbred lines in test hybrids with

other *B. napus* lines will be needed to understand the general and specific combining ability of these lines.

- The molecular genetic basis of the phenomenon heterosis is still unknown. Research would be needed to understand this phenomenon. For this, QTL mapping of genomic regions contributing to heterosis can be done by use of the recombinant *B. napus* inbred lines used in this study.
- There is a need to find a relationship between the performance of the inbred lines and the performance of the hybrids. Establishment of such a model for prediction of heterosis would reduce the cost of hybrid breeding.

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Appendix 2.1 Means of the inbred lines agronomic traits and seed quality traits in the F_9 of A04-73NA × *B. rapa* T-4-3-3-1 (T.F) interspecific cross.

Reg. number of inbred lines	Inbred line ID	DTF	DTM	Height	Yield (kg/ha)	Oil (%)	Protein (%)	GSL (%)
5CA1299.319-A1295	T.F.01	49	101	114	3451	49	24	15
5CA1299.321-A1295	T.F.02	49	100	118	3674	49	24	14
5CA1299.330-A1295	T.F.03	50	101	115	3464	48	24	14
5CA1299.333-A1295	T.F.04	50	102	120	3418	47	26	15
5CA1299.343-A1295	T.F.05	50	100	114	3494	48	24	14
5CA1299.346-A1295	T.F.06	51	101	117	3401	48	25	15
5CA1299.348-A1295	T.F.07	50	102	118	3473	47	25	15
5CA1299.354-A1295	T.F.08	50	100	116	3348	48	24	16
5CA1299.357-A1295	T.F.09	49	101	114	3353	47	25	15
5CA1299.361-A1295	T.F.10	50	101	119	3696	47	25	15
5CA1299.369-A1295	T.F.11	50	101	114	3595	48	25	14
5CA1299.371-A1295	T.F.12	51	101	116	3684	48	24	15
5CA1299.374-A1295	T.F.13	50	102	115	3563	48	25	14
5CA1299.387-A1295	T.F.14	50	101	116	3640	48	24	14
5CA1299.412-A1295	T.F.15	51	103	118	3373	48	24	15
5CA1299.413-A1295	T.F.16	49	100	115	3331	47	25	15
5CA1299.414-A1295	T.F.17	49	102	121	3655	47	25	15
5CA1299.425-A1295	T.F.18	49	102	118	3535	47	25	15
5CA1299.430-A1295	T.F.19	49	100	115	3518	48	25	15
5CA1299.446-A1295	T.F.20	50	104	127	3356	47	25	13
5CA1299.450-A1295	T.F.21	51	105	124	3648	47	25	12
5CA1299.457-A1295	T.F.22	51	103	126	3610	47	25	13
5CA1299.462-A1295	T.F.23	51	104	130	3417	47	25	13
5CA1299.465-A1295	T.F.24	51	103	125	3425	47	25	16
5CA1299.470-A1295	T.F.25	51	104	126	3540	45	25	14
5CA1299.485-A1295	T.F.26	52	104	128	3487	46	24	15
5CA1299.493-A1295	T.F.27	50	104	124	3686	46	24	14
5CA1299.500-A1295	T.F.28	51	103	130	3665	46	25	13
5CA1299.506-A1295	T.F.29	50	101	126	3466	47	25	14
5CA1299.513-A1295	T.F.30	51	104	126	3292	47	24	21
5CA1299.528-A1295	T.F.31	51	105	123	3326	47	25	16

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.2 Means of the inbred lines agronomic traits and seed quality traits in the F_9 of *B. napus* A04-73NA × *B. rapa*YS49 (YS.F) interspecific cross.

Reg. number of inbred lines	Inbred line ID	DTF	DTM	Height	Yield (kg/ha)	Oil (%)	Protein (%)	GSL (%)
5CA1257.672-A1295	YS.F.01	50	103	122	3097	45	26	16
5CA1257.673-A1295	YS.F.02	51	104	118	3421	47	24	14
5CA1257.674-A1295	YS.F.03	51	103	117	3477	46	25	14
5CA1257.675-A1295	YS.F.04	51	104	119	3324	47	24	15
5CA1257.682-A1295	YS.F.05	50	101	119	2941	45	26	19
5CA1257.683-A1295	YS.F.06	48	103	118	3156	45	26	18
5CA1257.686-A1295	YS.F.07	49	100	118	3113	46	26	15
5CA1257.688-A1295	YS.F.08	49	103	117	2769	44	26	17
5CA1257.691-A1295	YS.F.09	50	102	116	2919	45	26	17
5CA1257.697-A1295	YS.F.10	49	102	119	2628	44	27	19
5CA1257.698-A1284	YS.F.11	48	101	120	3272	46	26	17
5CA1257.706-A1295	YS.F.12	50	101	118	3346	48	25	17
5CA1257.707-A1295	YS.F.13	53	105	129	3217	47	25	20
5CA1257.708-A1295	YS.F.14	49	103	124	3335	48	25	22
5CA1257.711-A1295	YS.F.15	50	103	118	3357	45	26	15
5CA1257.713-A1295	YS.F.16	49	102	116	3473	45	26	18
5CA1257.717-A1295	YS.F.17	52	103	117	3339	46	25	13
5CA1257.718-A1295	YS.F.18	51	104	118	3433	47	25	14
5CA1257.719-A1295	YS.F.19	52	105	122	2985	46	25	16
5CA1257.720-A1295	YS.F.20	52	105	119	3200	46	25	16
5CA1257.722-A1295	YS.F.21	52	104	123	3434	46	25	15
5CA1257.724-A1295	YS.F.22	51	104	118	3396	47	25	13
5CA1257.726-A1295	YS.F.23	51	104	117	3368	47	25	14
5CA1257.727-A1295	YS.F.24	50	103	120	3267	47	25	15
5CA1257.728-A1295	YS.F.25	50	102	115	3382	47	25	13
5CA1257.732-A1295	YS.F.26	51	104	122	3674	48	24	14
5CA1257.733-A1295	YS.F.27	51	103	122	3352	46	25	15
5CA1257.734-A1295	YS.F.28	51	103	120	3354	47	24	13
5CA1257.735-A1295	YS.F.29	53	104	126	3118	47	24	14
5CA1257.738-A1295	YS.F.30	49	102	121	3290	46	25	14
5CA1257.743-A1295	YS.F.31	51	104	128	3140	45	25	15

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.3 Means of the inbred lines agronomic traits and seed quality traits in the BC_1F_6 (*B. napus* A04-73NA × YS49) × A04-73NA (YS.BC) interspecific cross.

Reg. number of inbred lines	Inbred line ID	DTF	DTM	Height	Yield (kg/ha)	Oil (%)	Protein (%)	GSL (%)
5CA1680.279-A1265	YS.BC.01	50	105	122	3550	47	24	16
5CA1680.281-A1265	YS.BC.02	50	103	124	3620	48	24	16
5CA1680.287-A1265	YS.BC.03	50	102	118	3656	48	24	16
5CA1680.290-A1265	YS.BC.04	49	101	116	3563	47	25	17
5CA1680.293-A1265	YS.BC.05	49	101	115	3457	46	26	17
5CA1680.299-A1265	YS.BC.06	50	104	119	3595	46	26	14
5CA1680.305-A1265	YS.BC.07	49	99	114	3422	47	24	22
5CA1680.307-A1265	YS.BC.08	51	101	116	3270	47	25	19
5CA1680.309-A1265	YS.BC.09	50	102	120	2998	46	26	17
5CA1680.331-A1265	YS.BC.10	49	101	115	3314	46	25	16
5CA1680.342-A1265	YS.BC.11	51	103	127	3433	48	25	21
5CA1680.345-A1265	YS.BC.12	51	102	117	3693	47	24	15
5CA1680.348-A1265	YS.BC.13	49	102	116	3446	48	24	16
5CA1680.350-A1265	YS.BC.14	50	103	124	3645	46	25	18
5CA1680.356-A1265	YS.BC.15	49	101	115	3193	47	25	16
5CA1680.366-A1265	YS.BC.16	50	103	123	3062	45	26	19
5CA1680.369-A1265	YS.BC.17	51	102	119	3183	47	25	17
5CA1680.371-A1265	YS.BC.18	50	103	121	3269	47	25	18
5CA1680.374-A1265	YS.BC.19	50	100	120	3525	46	25	16
5CA1680.377-A1265	YS.BC.20	47	100	111	3115	46	26	15
5CA1680.382-A1265	YS.BC.21	50	101	115	3219	45	26	18
5CA1680.386-A1265	YS.BC.22	50	101	115	3476	48	25	16
5CA1680.393-A1265	YS.BC.23	48	100	116	2941	47	25	15
5CA1680.397-A1265	YS.BC.24	49	101	111	3146	45	26	15
5CA1680.401-A1265	YS.BC.25	48	100	111	3296	47	25	16
5CA1680.404-A1265	YS.BC.26	50	104	126	3366	47	24	21
5CA1680.407-A1265	YS.BC.27	51	104	124	3402	47	25	18
5CA1680.417-A1265	YS.BC.28	48	100	110	3141	46	26	15
5CA1680.421-A1265	YS.BC.29	49	101	116	3511	46	25	15
5CA1680.424-A1265	YS.BC.30	51	102	120	3420	47	25	16
5CA1680.426-A1265	YS.BC.31	50	100	117	2963	47	27	14

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.4 Means of the test hybrids agronomic traits and seed quality traits in the F_9 of (*B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (TC.T.F) interspecific cross.

Test hybrids ID	DTF	DTM	Height	Yield (kg/ha)	Oil (%)	Protein (%)	GSL (%)
TC.T.F.01	46	105	126	4247	49	24	14
TC.T.F.02	46	104	125	4415	49	24	14
TC.T.F.03	46	103	120	4622	48	25	16
TC.T.F.04	48	105	120	4204	47	25	16
TC.T.F.05	48	106	125	4408	49	23	14
TC.T.F.06	48	105	122	4309	47	25	15
TC.T.F.07	48	106	122	4244	48	24	14
TC.T.F.08	49	104	121	3913	47	24	16
TC.T.F.09	46	105	117	4197	48	24	14
TC.T.F.10	48	104	125	4587	49	24	16
TC.T.F.11	49	104	120	4186	48	25	15
TC.T.F.12	49	103	121	4726	48	24	15
TC.T.F.13	49	104	122	4556	47	26	16
TC.T.F.14	46	104	119	4948	48	24	15
TC.T.F.15	46	105	128	3836	48	24	18
TC.T.F.16	47	106	118	4328	47	25	16
TC.T.F.17	48	103	120	3792	47	26	16
TC.T.F.18	47	105	132	4094	48	24	15
TC.T.F.19	47	105	125	4349	48	24	16
TC.T.F.20	48	103	125	4903	47	25	13
TC.T.F.21	48	106	134	4574	48	24	12
TC.T.F.22	47	105	127	5087	47	24	13
TC.T.F.23	49	104	132	5048	48	24	15
TC.T.F.24	47	104	123	5257	47	25	15
TC.T.F.25	48	104	130	5051	46	26	15
TC.T.F.26	46	105	131	4552	46	25	14
TC.T.F.27	47	103	131	5541	47	24	13
TC.T.F.28	50	104	130	5426	46	25	13
TC.T.F.29	48	108	131	5349	46	26	13
TC.T.F.30	49	105	129	4522	47	24	14
TC.T.F.31	47	105	120	5215	48	24	14

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.5 Means of the test hybrids agronomic traits and seed quality traits in the F_9 of (*B. napus* A04-73NA × *B. rapa*YS49) × A04-73NA (TC.YS.F) interspecific cross.

Test hybrids ID	DTF	DTM	Height	Yield (kg/ha)	Oil (%)	Protein (%)	GSL (%)
TC.YS.F.01	48	103	120	4837	48	24	18
TC.YS.F.02	47	105	124	5010	46	25	14
TC.YS.F.03	48	105	121	4858	47	25	15
TC.YS.F.04	47	104	117	4477	44	27	17
TC.YS.F.05	46	104	123	4915	46	26	18
TC.YS.F.06	47	104	110	4401	44	27	17
TC.YS.F.07	46	103	121	4724	46	26	13
TC.YS.F.08	48	103	115	4099	45	27	16
TC.YS.F.09	47	104	119	5071	46	25	16
TC.YS.F.10	47	104	115	4069	45	26	16
TC.YS.F.11	48	104	124	3691	45	27	21
TC.YS.F.12	48	105	126	4225	47	25	16
TC.YS.F.13	49	105	128	4815	47	24	17
TC.YS.F.14	47	106	121	4780	47	26	18
TC.YS.F.15	48	106	121	4419	46	26	15
TC.YS.F.16	46	104	122	4890	46	26	16
TC.YS.F.17	48	105	126	5041	46	26	14
TC.YS.F.18	47	106	126	5345	47	26	13
TC.YS.F.19	46	105	130	5067	46	26	15
TC.YS.F.20	48	106	126	5222	47	25	14
TC.YS.F.21	46	105	118	5129	47	26	14
TC.YS.F.22	48	105	124	5207	48	24	13
TC.YS.F.23	47	104	120	4737	47	24	13
TC.YS.F.24	47	105	117	4793	48	24	14
TC.YS.F.25	48	103	116	4767	46	26	14
TC.YS.F.26	48	105	127	5034	47	25	14
TC.YS.F.27	46	105	123	5175	49	23	12
TC.YS.F.28	48	103	124	4669	48	24	14
TC.YS.F.29	48	103	125	5386	48	24	13
TC.YS.F.30	45	106	119	4401	46	24	13
TC.YS.F.31	47	104	124	5052	46	25	14

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.6 Means of the test hybrids agronomic traits and seed quality traits in the BC_1F_6 (*B. napus* A04-73NA × YS49) × A04-73NA (TC.YS.BC) interspecific cross.

Test hybrids ID	DTF	DTM	Height	Yield (kg/ha)	Oil (%)	Protein (%)	GSL (%)
TC.YS.BC.01	50	106	122	4925	24	48	17
TC.YS.BC.02	47	105	124	4986	25	48	16
TC.YS.BC.03	45	105	120	5219	24	48	16
TC.YS.BC.04	47	104	122	4899	22	49	17
TC.YS.BC.05	47	102	122	4770	25	48	16
TC.YS.BC.06	46	104	113	3806	26	46	16
TC.YS.BC.07	46	106	126	4568	26	46	21
TC.YS.BC.08	48	105	117	4903	25	47	17
TC.YS.BC.09	47	104	122	5017	26	46	16
TC.YS.BC.10	46	104	120	4846	26	46	15
TC.YS.BC.11	48	106	128	5124	24	48	15
TC.YS.BC.12	49	105	120	4475	26	46	15
TC.YS.BC.13	46	105	123	4744	25	47	16
TC.YS.BC.14	46	104	119	4379	27	44	17
TC.YS.BC.15	49	106	115	4245	26	46	18
TC.YS.BC.16	48	105	123	4691	26	47	16
TC.YS.BC.17	48	104	122	4987	24	48	19
TC.YS.BC.18	47	103	131	4561	26	46	18
TC.YS.BC.19	50	105	121	4137	25	48	17
TC.YS.BC.20	46	104	122	3998	25	47	16
TC.YS.BC.21	46	105	121	4611	26	45	18
TC.YS.BC.22	50	106	123	4064	25	48	16
TC.YS.BC.23	48	106	118	4184	24	48	14
TC.YS.BC.24	48	105	121	3878	26	45	17
TC.YS.BC.25	48	106	116	4453	24	47	15
TC.YS.BC.26	48	104	126	4899	24	47	16
TC.YS.BC.27	47	105	122	4338	25	46	16
TC.YS.BC.28	47	105	117	4241	25	47	15
TC.YS.BC.29	48	105	121	4320	25	46	14
TC.YS.BC.30	49	105	123	5057	25	47	15
TC.YS.BC.31	47	107	123	4180	26	47	14

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.7 Means of mid parent heterosis (MPH) in the test hybrids for agronomic traits and seed quality traits in the F_9 of *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (TC.T.F) interspecific cross.

Test hybrids ID	MPH DTF	MPH DTM	MPH Height	MPH Yield	MPH Oil	MPH Protein	MPH GSL
TC.T.F.01	-2	-1	6	12	1	-2	-10
TC.T.F.02	-5	-1	1	5	-2	3	-13
TC.T.F.03	-5	0	-3	-1	0	-1	-3
TC.T.F.04	-2	0	2	8	-2	-1	-5
TC.T.F.05	-1	0	0	5	-1	0	-9
TC.T.F.06	-1	1	5	5	-2	0	-7
TC.T.F.07	3	1	-2	4	0	-3	-12
TC.T.F.08	2	0	-4	2	-2	0	-6
TC.T.F.09	-5	0	-3	10	0	-1	-14
TC.T.F.10	-3	0	-3	8	0	0	-10
TC.T.F.11	-1	-1	-1	2	-1	1	-9
TC.T.F.12	-1	0	-2	-1	-1	-1	-10
TC.T.F.13	3	-1	-2	5	-2	4	-6
TC.T.F.14	-2	0	-3	15	-1	0	-6
TC.T.F.15	-1	1	2	-12	-2	4	6
TC.T.F.16	-1	-1	-4	-2	-4	5	-9
TC.T.F.17	-1	-1	-2	-8	-2	2	-8
TC.T.F.18	-3	0	3	-3	0	1	-7
TC.T.F.19	-2	0	0	4	-1	1	2
TC.T.F.20	0	-1	-3	36	-1	-1	-20
TC.T.F.21	-2	-1	3	6	-1	-2	-30
TC.T.F.22	-4	-1	1	15	-2	1	-11
TC.T.F.23	-2	-2	1	28	-1	2	-7
TC.T.F.24	-4	-2	-2	36	-1	1	-13
TC.T.F.25	-2	-1	2	25	-1	3	-9
TC.T.F.26	-9	-2	-3	5	-4	4	-13
TC.T.F.27	-3	-2	2	12	0	-2	-13
TC.T.F.28	-3	1	-2	28	0	-1	-15
TC.T.F.29	-3	0	5	21	-1	0	-20
TC.T.F.30	-1	-1	-3	22	-2	2	-14
TC.T.F.31	-6	0	0	46	3	-2	-18

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.8 Means of mid parent heterosis (MPH) in the test hybrids for agronomic traits and seed quality traits in the F_9 of *B. napus* A04-73NA × *B. rapa*YS49 (TC.YS.F) interspecific cross.

Test hybrids ID	MPH DTF	MPH DTM	MPH Height	MPH Yield	MPH Oil	MPH Protein	MPH GSL
TC.YS.F.01	-1	-1	0	36	0	-1	8
TC.YS.F.02	-2	0	2	25	-1	1	-13
TC.YS.F.03	-2	-2	-3	20	-2	2	-1
TC.YS.F.04	-1	-1	-3	20	-1	3	-17
TC.YS.F.05	-5	-1	1	36	-1	0	-1
TC.YS.F.06	-1	-1	-6	56	-3	4	-13
TC.YS.F.07	-3	-1	-4	24	-1	-3	-26
TC.YS.F.08	-4	-1	-1	23	-4	3	-6
TC.YS.F.09	-3	-1	-4	48	-1	-1	-16
TC.YS.F.10	-1	-1	1	46	-3	2	-8
TC.YS.F.11	-2	-1	2	-2	-3	4	4
TC.YS.F.12	-1	-1	-2	5	-1	1	-14
TC.YS.F.13	0	0	0	20	-3	0	-20
TC.YS.F.14	-4	0	-4	10	0	0	-12
TC.YS.F.15	0	0	5	22	-1	0	-16
TC.YS.F.16	-1	0	0	24	-1	0	-13
TC.YS.F.17	-1	0	1	22	-3	2	-19
TC.YS.F.18	-1	0	-1	29	-2	4	-15
TC.YS.F.19	-4	-1	4	27	-1	3	-13
TC.YS.F.20	-2	0	2	40	0	0	-9
TC.YS.F.21	-2	-1	1	22	0	3	-9
TC.YS.F.22	-2	-1	4	27	-1	1	-12
TC.YS.F.23	-1	0	1	23	-1	-2	-26
TC.YS.F.24	-1	-2	-3	16	0	-1	-12
TC.YS.F.25	-1	-1	-5	19	-3	3	-14
TC.YS.F.26	-1	1	2	24	-2	4	-8
TC.YS.F.27	-4	-1	-1	30	2	-5	-27
TC.YS.F.28	-2	-1	0	22	0	-2	-14
TC.YS.F.29	-4	0	1	27	1	-2	-20
TC.YS.F.30	-4	-1	-1	25	-2	-1	-21
TC.YS.F.31	0	-2	-1	22	-1	-2	-19

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.9 Means of mid parent heterosis (MPH) in the test hybrids for agronomic traits and seed quality traits in the BC_1F_6 (*B. napus* A04-73NA × YS49) × A04-73NA (TC.YS.BC) interspecific cross.

Test hybrids ID	MPH DTF	MPH DTM	MPH Height	MPH Yield	MPH Oil	MPH Protein	MPH GSL
TC.YS.BC.01	-3	-1	0	17	-3	-3	-6
TC.YS.BC.02	-4	-1	4	12	-2	1	-13
TC.YS.BC.03	-7	-1	-6	14	0	-2	-8
TC.YS.BC.04	-3	-1	1	16	-2	-1	-8
TC.YS.BC.05	-3	-2	2	47	2	-4	-11
TC.YS.BC.06	-7	0	-5	1	-3	4	-11
TC.YS.BC.07	-4	-1	5	4	-3	1	8
TC.YS.BC.08	-2	-1	-2	18	-2	2	-10
TC.YS.BC.09	-2	-1	0	32	-1	-3	-4
TC.YS.BC.10	-3	0	-2	12	-3	2	-13
TC.YS.BC.11	-4	-1	-1	51	1	-4	-13
TC.YS.BC.12	-3	0	-4	7	-2	-2	-9
TC.YS.BC.13	-7	-1	1	8	-2	4	-4
TC.YS.BC.14	-7	0	-6	3	-6	7	6
TC.YS.BC.15	-1	-1	-3	14	-1	1	3
TC.YS.BC.16	-2	-2	-2	50	-3	3	-5
TC.YS.BC.17	0	-4	-2	0	-1	-4	-36
TC.YS.BC.18	-3	0	9	6	-3	2	-3
TC.YS.BC.19	-1	0	-1	2	-1	1	-3
TC.YS.BC.20	-5	-1	0	17	0	-1	-15
TC.YS.BC.21	-3	1	4	10	-2	-1	-2
TC.YS.BC.22	-1	1	2	1	-1	-4	-6
TC.YS.BC.23	-3	-1	-1	11	-3	-5	-15
TC.YS.BC.24	-3	1	3	-12	-4	-4	-6
TC.YS.BC.25	-1	-2	-4	11	-2	0	-4
TC.YS.BC.26	-3	-1	0	18	-2	1	-6
TC.YS.BC.27	-3	-1	1	3	-2	-1	-10
TC.YS.BC.28	-2	0	1	12	2	-5	-9
TC.YS.BC.29	-1	1	-2	9	-4	1	-17
TC.YS.BC.30	-4	1	-1	29	-3	-4	-10
TC.YS.BC.31	-5	-2	-4	8	-2	2	-15

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.10 Means of 73NAH in the test hybrids for agronomic traits and seed quality traits in the F_9 of *B. napus* A04-73NA × *B. rapa* T-4-3-3-1 (TC.T.F) interspecific cross.

Test hybrids ID	73NAH DTF	73NAH DTM	73NAH Height	73NAH Yield	73NAH Oil	73NAH Protein	73NAH GSL
TC.T.F.01	-4	-1	7	21	1	-3	-12
TC.T.F.02	-7	-1	-1	9	-2	3	-23
TC.T.F.03	-5	0	0	3	-1	0	-13
TC.T.F.04	-3	0	-3	1	-4	2	-4
TC.T.F.05	-4	0	0	5	-2	0	-17
TC.T.F.06	-3	1	2	8	-3	2	-16
TC.T.F.07	2	1	-3	8	-1	-2	-15
TC.T.F.08	0	1	-3	0	-3	1	-9
TC.T.F.09	-5	-1	-6	14	-1	-1	-16
TC.T.F.10	-4	0	1	8	1	-1	-13
TC.T.F.11	-1	-2	-5	6	-2	1	-16
TC.T.F.12	-1	-1	-4	1	-1	0	-14
TC.T.F.13	-2	-1	0	9	-2	2	-17
TC.T.F.14	-1	0	-5	24	-1	0	-14
TC.T.F.15	-1	1	3	-10	-2	3	4
TC.T.F.16	-1	-1	-6	-2	-4	6	-18
TC.T.F.17	-1	-1	-4	-12	-4	5	-11
TC.T.F.18	-4	0	3	0	-1	2	-9
TC.T.F.19	-4	-1	-1	3	-1	0	-3
TC.T.F.20	-1	0	-1	31	-4	3	-33
TC.T.F.21	-3	-2	3	5	0	-4	-40
TC.T.F.22	-2	-2	1	27	-3	2	-22
TC.T.F.23	-1	-2	-1	26	-1	1	-13
TC.T.F.24	-1	-1	-3	34	-2	1	-19
TC.T.F.25	-1	0	2	27	-3	4	-15
TC.T.F.26	-8	-3	-2	8	-4	3	-19
TC.T.F.27	-4	-3	3	14	-1	-2	-27
TC.T.F.28	0	2	5	30	-4	3	-17
TC.T.F.29	-3	-1	6	17	-4	4	-32
TC.T.F.30	0	0	1	13	-4	1	-22
TC.T.F.31	-6	1	-3	29	-1	0	-26

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.11 Means of 73NAH in the test hybrids for agronomic traits and seed quality traits in the F_9 of *B. napus* A04-73NA × *B. rapa*YS49 (TC.YS.F) interspecific cross.

Test hybrids ID	73NAH DTF	73NAH DTM	73NAH Height	73NAH Yield	73NAH Oil	73NAH Protein	73NAH GSL
TC.YS.F.01	-1	0	-1	26	-2	1	6
TC.YS.F.02	-2	1	0	29	-2	-1	-25
TC.YS.F.03	-2	-3	-2	18	-2	2	2
TC.YS.F.04	-1	-1	-2	10	-6	6	-6
TC.YS.F.05	-6	-2	-6	13	-4	4	0
TC.YS.F.06	-2	-1	-11	12	-6	9	-9
TC.YS.F.07	-2	-1	-7	12	-5	2	-28
TC.YS.F.08	-2	-1	-1	4	-6	5	-19
TC.YS.F.09	-4	-1	-9	17	-7	7	-15
TC.YS.F.10	-1	0	-4	7	-7	6	-4
TC.YS.F.11	-2	-1	5	-12	-5	7	5
TC.YS.F.12	-2	-1	-3	-2	-3	3	-17
TC.YS.F.13	2	1	0	17	-5	0	-11
TC.YS.F.14	-4	0	-5	-4	-3	4	-10
TC.YS.F.15	0	-1	0	21	-6	7	-20
TC.YS.F.16	-3	1	-3	24	-4	4	-15
TC.YS.F.17	-1	0	-3	30	-5	5	-24
TC.YS.F.18	-1	-1	-2	33	-4	8	-27
TC.YS.F.19	-5	-1	2	34	-4	6	-16
TC.YS.F.20	-1	1	0	29	-3	3	-14
TC.YS.F.21	-2	1	-2	20	-3	5	-17
TC.YS.F.22	-3	-2	-2	44	-1	-1	-19
TC.YS.F.23	-1	0	-4	31	-2	-1	-32
TC.YS.F.24	-2	-1	-5	14	-1	-1	-24
TC.YS.F.25	0	1	-6	20	-4	5	-21
TC.YS.F.26	-1	-1	0	35	-2	4	-22
TC.YS.F.27	-4	-2	-3	46	2	-5	-34
TC.YS.F.28	-2	0	2	23	-4	3	-15
TC.YS.F.29	-2	1	5	18	-2	-3	-32
TC.YS.F.30	-6	-2	-4	20	-5	3	-29
TC.YS.F.31	0	-3	-3	25	-3	-1	-29

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 2.12 Means of 73NAH in the test hybrids for agronomic traits and seed quality traits in the BC_1F_6 (*B. napus* A04-73NA × YS49) × A04-73NA (TC.YS.BC) interspecific cross.

Test hybrids ID	73NAH DTF	73NAH DTM	73NAH Height	73NAH Yield	73NAH Oil	73NAH Protein	73NAH GSL
TC.YS.BC.01	-5	-3	0	15	-5	-5	-8
TC.YS.BC.02	-5	-1	3	25	-4	2	-19
TC.YS.BC.03	-7	-1	-10	22	-1	0	-14
TC.YS.BC.04	-4	-1	0	12	-3	-2	-11
TC.YS.BC.05	-4	-2	-1	15	-1	-2	-14
TC.YS.BC.06	-8	-1	-8	11	-7	8	-5
TC.YS.BC.07	-6	-1	2	5	-5	4	8
TC.YS.BC.08	-3	-1	-4	12	-5	6	-11
TC.YS.BC.09	-4	-2	-3	24	-2	1	-9
TC.YS.BC.10	-4	0	1	8	-5	4	-12
TC.YS.BC.11	-3	0	2	19	-1	-3	-12
TC.YS.BC.12	-5	0	-4	10	-2	-4	-9
TC.YS.BC.13	-7	0	-2	14	-4	4	-8
TC.YS.BC.14	-8	-2	-4	23	-9	11	0
TC.YS.BC.15	-1	-1	-7	1	-3	4	4
TC.YS.BC.16	-4	-3	0	45	-2	-2	-13
TC.YS.BC.17	-1	-2	-3	8	-6	6	8
TC.YS.BC.18	-4	-1	8	16	-4	2	-7
TC.YS.BC.19	-1	0	-1	-1	-2	1	-10
TC.YS.BC.20	-8	-2	-1	11	-4	2	-11
TC.YS.BC.21	-5	-1	0	16	-3	-4	-9
TC.YS.BC.22	-3	1	-2	-3	-3	-4	-10
TC.YS.BC.23	-4	-2	-1	18	-4	-7	-20
TC.YS.BC.24	-6	1	-2	-16	-9	-5	-8
TC.YS.BC.25	-3	-3	-8	0	-4	0	-7
TC.YS.BC.26	-1	-1	-3	9	-6	4	-9
TC.YS.BC.27	-4	1	2	-1	-6	2	-17
TC.YS.BC.28	-3	-1	-4	1	1	-2	-8
TC.YS.BC.29	-2	1	-1	8	-6	3	-19
TC.YS.BC.30	-6	2	0	39	-6	-6	-11
TC.YS.BC.31	-5	-2	-5	6	-3	7	-22

Note: DTF = Days to flowering, DTM = days to maturity

Appendix 3.1 List of the 55 polymorphic SSR markers used to genotype the BC_1F_6 population derived from (*B. napus* × *B. rapa*) × *B. napus* interspecific crosses.

Primer name	Primer code	Linkage group	Expected size
sN2837	122	6	154-165
sNRG67	160	3	229
sN2551	176	10	367
sN13075(aNP)	235	3	249
sNRB88(b)	246	8	-
sR12095	259	2	310-350
sR12015	266	3	299-380
sN2025	271	4	125-136
sNRD03	275	5	93-99
sR9555	278	5	246-279
sS1949	280	6	185-208
sR0282R	281	7	251-267
sR3688	288	8	251-277
sR12384I	322	10	186-195
sS2165b	571	5	186
sN1088b	572	5	178
sN3521Fla	585	5	332
sN7634a	589	9	253
sN11651l	590	9	179
sNRF22b	597	9	153
sN3809(aNP)	815	7	384
sN11682	830	7	340
sS1905a	1996	1	116
sN0990 (bNP)	2001	1	245
sN7974 (cNM)	2004	1	242
sN1939 F(b)	2026	6	312
sN1503 (bNP)	2036	6	254
sN12215	2331	8	358

Primer name	Primer code	Linkage group	Expected size
sNRF19	2336	8	115
sR1868	2337	8	108
sN0809	2343	8	359
sN4145	2344	8	299
sS1921	2346	8	240
sN13082	2480	4	395
sN11639	2487	4	415
sN11719	2496	4	166
A02_13002	2717	2	346
A03_5090	2720	3	283
A03_6890	2721	3	333
A03_12095	2722	3	327
A03_15652	2723	3	317
A04_905	2724	4	251
A04_5262	2727	4	301
A04_7287	2728	4	344
A04_9012	2729	4	256
A05_1116	2730	5	216
A05_10574	2735	5	263
A06_133	2736	6	221
A06_2300	2737	6	241
A06_4740	2738	6	253
A06_9380	2740	6	290
A07_5462	2743	7	153
A07_10877	2746	7	163
A08_1864	2749	8	170
A08_9655	2753	8	160