Potential of the *Brassica oleracea* gene pool for the improvement of spring *B. napus* canola

by

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Abstract

Canola (*Brassica napus* L.) is an amphidiploid or allotetraploid (AACC, 2*n* = 4*x* = 38) crop plant and it is one of the most important oilseed crops in the world. The narrow genetic base of this crop, especially in its C genome, is not only a major impediment for its continued improvement but also for mapping and identification of all loci and alleles for a trait that could be found in the *Brassica* genomes. Currently, hybrid canola cultivars have taken the majority of market share in different countries including Canada. To increase the yield of hybrid canola, there is a need for increasing the level of heterosis or hybrid vigor in this type of cultivar for which presence of adequate genetic diversity in hybrid parental lines is needed. In this study, the value of the C genome of six *B. oleracea* L. (CC, 2*n* = 18) accessions belonging to four variants of this species, viz. vars. *alboglabra*, *botrytis*, *capitata* and *italica*, was investigated for broadening the genetic base of spring *B. napus* canola. Six *B. napus* canola inbred populations developed from six *B. napus* × *B. oleracea* interspecific crosses and two breeding methods (F_2- and BC_1-derived inbred lines) were used. Test hybrids were produced by crossing the inbred lines to the *B. napus* canola line and the inbred and test hybrid populations were evaluated in replicated field trials for different agronomic and seed quality traits including yield. Inbred lines were also analyzed by SSR and SNP markers to assess genetic diversity of the inbred populations and the effect of the C genome alleles in the inbred and hybrid populations as well as for QTL mapping of different traits.

Analysis of the parents and the inbred populations using 95 SSR markers showed the existence of wide diversity among the *B. oleracea* accessions; several canola lines derived from the six crosses tended to group together with their *B. oleracea* parent demonstrating that the diversity of the *B. oleracea* gene pool can be exploited for broadening the genetic base of the C genome of *B. napus* canola. However, loss of some *B. oleracea* alleles occurred in the

inbred populations during the development of these lines and this loss occurred to a greater extent in the F_2-derived population as compared to the BC_1-derived population, which might be due to a stronger selection by breeders for the two canola quality traits (zero erucic acid in seed oil and low glucosinolate in seed meal) in the F_2-derived population. Evaluation of the inbred populations in 10 field trials showed that the population derived from the cross involving var. *italica* gave the greatest yield, while the population derived from the cross with var. *botrytis* had the highest seed oil content; this population also gave high seed yield. In regard to the performance of the test hybrids, population derived from the cross involving var. *alboglabra* gave the greatest mid-parent heterosis (MPH) (11.1 ± 2.2 S.E. %) while the population derived from the cross involving var. *italica* gave the lowest MPH (4.0 ± 2.2 S.E. %); however, individual test hybrid exhibiting upto 82.7% MPH was found in this population. Multivariate analysis showed that inbred lines or test hybrids with high seed yield and oil content, and earliness of flowering and maturity with longer grain-filling period can be obtained from this population.

A genome-wide association study using the above-mentioned inbred populations and 3,131 SNP markers detected 18 QTLs for three agronomic and seed quality traits; this included the QTLs located on chromosome C2, C3, C5 and C6 affecting days to flowering, QTLs on C1, C3, C5, C7 and C8 affecting seed oil content and QTLs on C1, C2, C3, C5 and C6 affecting seed glucosinolate content. Novel QTLs and alleles, which have not been reported previously, were also identified in this study, e.g. the C5 QTL affecting days to flowering; in this case, the alternative allele was derived from *B. oleracea* var. *capitata* cv. Bindsachsener and this allele improved the earliness flowering. Thus, the results from this study provided substantial evidence that the *B. oleracea* gene pool can be used to broaden the genetic base of *B. napus* canola for the improvement of inbred and hybrid cultivars of this crop as well as for identification of novel QTLs and alleles for different traits.

Preface

This dissertation is submitted by Azam Nikzad for the degree of Doctor of Philosophy. Azam Nikzad conducted all the experiments and analysed all the data and prepared the draft of all the chapters of this thesis and incorporated all comments, suggestions and editorial revisions provided by Dr. Habibur Rahman, her supervisor.

A version of chapter 2 of this dissertation has been submitted as: Nikzad A, Kebede B, Pinzon J, Bhavikkumar J and Rahman H (2020) Study of genetic structure of a *Brassica napus* canola population derived from six interspecific crosses of *B. napus* × *B. oleracea*. Can. J. Plant Sci.

Azam Nikzad conducted all experiments for chapter 2, such as planting inbred lines in greenhouse, collecting the leaves, extracting DNA, genotyping the inbred lines with SSR markers, conducted genetic diversity and genetic structure analysis, and wrote the first draft of the manuscript. Dr. Habibur Rahman developed the research plan, helped in interpretation of the results and provided feedback on the manuscript for further improvement. Dr. Berisso Kebede helped in data analysis. Jani Bhavikkumar helped in genotyping a small portion of the inbred lines.

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Azam Nikzad conducted all field experiments for chapter 3. She also helped to design the experiment, seed the trials and harvest the plots. She collected all data, analyzed them, wrote the first draft of the manuscript. Dr. Habibur Rahman developed the research plan, edited the

manuscript, and helped in interpretation of the results. Dr. Berisso Kebede helped in data analysis. Dr. Jaime Pinzon taught multivariate analysis and provided comments on the multivariate analysis. Jani Bhavikkumar helped in collecting a small portion of the data in 2016. Rong-Cai Yang provided advice on data analysis.

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Azam Nikzad conducted all experiments in chapter 4, such as producing hybrid seeds in the greenhouse, helped in seeding and harvesting the experiments including management of the plots, collected and analyzed data, and wrote the first draft of the manuscript. Dr. Habibur Rahman developed the research plan, helped in interpretation of the results and provided feedback on the manuscript for further improvement. Dr. Berisso Kebede helped in data analysis. Dr. Jaime Pinzon taught multivariate analysis and provided editorial comments on the manuscript. Jani Bhavikkumar and Xianping Wang helped in collecting a small portion of the data in 2015. Rong-Cai Yang provided advice on data analysis.

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Azam Nikzad conducted all experiments in chapter 5, such as helped in seeding and harvesting the experiments including management of the plots, collected and analyzed phenotypic data, extracted DNA, conducted association analysis of SNP markers and wrote the first draft of the manuscript. Dr. Habibur Rahman developed the research plan, helped in

interpretation of the results and provided feedback on the manuscript for further improvement. Dr. Berisso Kebede helped in data analysis. Dr. Miles Buchwaldt and Dr. Isobel Parkin conducted SNP analysis of the tGBS data.

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List of Symbols and Abbreviations

±	Plus/minus
=	Equal
≥	Greater than or equal to
>	Greater than
≤	Less than or equal to
<	Less than
%	Percent
⊗	Self-pollination
~	About
°C	Degrees Celsius
2n	Diploid number of chromosomes
µmol/g seed	Micromoles per gram *per se*ed
σ^2_{ε}	Residual variance
σ^2_{G}	Genotypic variance
$\sigma^2_{G\times E}$	Genotype × environment variance
AAFC	Agriculture and Agri-Food Canada
ABI	Applied biosystem
AFLP	Amplified fragment length polymorphism
AFNS	Agriculture, Food and Nutritional Science
AM	Association mapping
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
BBSRC	Biological Science Research Council
BC_1	First backcross generation
BC_1F_x	x^{th} generation of BC_1-derived population
BIC	Bayesian information criteria
BLUP	Best linear unbiased prediction
bp	Base pair
BT	Breeding technique (method)
Canola	Canadian oil low acid
CLUMPAK	Cluster markov packager across K
cm	Centimeter
cM	Centimorgan

cv.	Cultivar
cvs.	Cultivars
df	Degree of freedom
DH	Doubled haploid
DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleotide triphosphate
DOF	Duration of flowering
DOGF = GFP	Grain-filling period
DTF	Days to flowering
DTM	Days to maturity
e.g.	For example
ERS	Edmonton research station
F_1	First generation
FAO	Food and Agriculture Organization of the United Nations
FarmCPU	Fixed and random model circulating probability unification
Fig.	Figure
F_x	x^{th} generation of F_2-derived population
g	Gram
G	Group
GAPIT	Genome Association and Prediction Integrated Tool
GCA	General combining ability
GFP	Grain-filling period
GWAS	Genome-wide association study
H	Broad sense heritability
Hi-Di	Highly deionized formamide
I	Shannon's Information index
i.e.	That is
K	Number of subgroups
kg ha^{-1}	Kilogram per hectare
LG	Linkage group
LnP(D))	Log likelihood of *K*
LOD	Logarithm of odds
LSmeans	Least-squares means
MAF	Minor allele frequency
Mb	Mega base pair

MLM	Mixed linear model
MPH	Mid-parent heterosis
MS	Mean squares
n	Haploid number of chromosomes
n	Number of observations
Na	Number of alleles
Ne	Effective number of alleles
N_E	Number of replications
NIRS	Near infrared reflectance spectroscopy
NPH	Heterosis over *B. napus* parent
N_R	Number of environments
NSERC	Engineering Research Council of Canada
NTSYS-pc	Numerical taxonomy and multivariate analysis system software
Ol.alb.nrc	*B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI
Ol.bot.cau cauliflower-1	*B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI
Ol.cap.bad	*B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper
Ol.cap.bal	*B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro
Ol.cap.bin	*B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener
Ol.ita.pre	*B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop
PCA	Principle component analysis
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
PCs	Principle component
PH	Plant height
PIC	Polymorphic information content
Pop	Population
P-value	Probability value
Q	Probability of membership
QTL	Quantitative trait loci
r	Pearson's correlation coefficient
R	R project for statistical computing
RAPD	Random amplified polymorphism DNA
REML	Residual maximum liklihood
RFLP	Restriction fragment length polymorphism

r_g	Genotypic correlation coefficient
r_p	Phenotypic correlation coefficient
S.E.	Standard Error
SAS	Statistical analysis software
Sd	Standard deviation
SGC = SG	Seed glucosinolate content
SNP	Single nucleotide polymorphism
SOC = SO	Seed oil content
SPC = SP	Seed protein content
SRAP	Sequence related amplified polymorphism
SSR	Simple sequence repeat
SY	Seed yield
tGBS	Tunable genotyping-by-sequencing
t-test	Test statistic for t-test
UPGMA	Unweighted pair-group method with arithmetic mean
USDA	United States Department of Agriculture
var.	Variety
vs.	Versus
WinISI	Infrasoft International

Chapter 1

Introduction and literature review

1.1 General introduction

The family Brassicaceae (Cruciferae) includes several economically important species, such as *Brassica napus* L. (AACC, 2*n* = 38), *B. oleracea* L. (CC, 2*n* = 18), *B. rapa* L. (AA, 2*n* = 20) and *B. juncea* (L.) Czern. (AABB, 2*n* = 36). *B. napus* is an amphidiploid species carry the A genome of *B. rapa* and the C genome of *B. oleracea*. Winter and summer types of *B. napus* are the most important *Brassica* oilseed crops worldwide. *B. oleracea* are used for their edible foliage, heads, axillary buds, above ground thickened stem and inflorescences; this species includes different variants, such as kales (*B. oleracea* viz. var. *viridis*, var. *costata*, var. *medullosa* and var. *sabellica*), cabbages (*B. oleracea* viz. var. *capitata* and var. *sabauda*), brussel sprouts (*B. oleracea* var. *gemmifera*), kohlrabi (*B. oleracea* var. *gongyloides*), cauliflower (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*) and Chinese kale (*B. oleracea* var. *alboglabra*). Various subspecies of *B. rapa* are used for their edible root or leaf as vegetables or grown as an oilseed crop; for example, turnip (*B. rapa* ssp. *rapifera*), Chinese cabbage (*B. rapa* ssp. *pekinensis*) and Pak choi (*B. rapa* ssp. *chinensis*) are used as vegetables and spring and winter types black seeded *B. rapa* and yellow sarson (yellow seeded; *B. rapa* ssp. *trilocularis*) are used for oil extraction (Warwick 2010).

Canola (*B. napus*) is a modified form of rapeseed. Cultivation of rapeseed can be traced back to early 2000 BC in India and 0 AD in China and Japan (Edwards and Hertel 2011). In Europe and Australia, cultivation of this crop started in the 13th and in the mid 20th century, respectively. The oil of traditional rapeseed contains > 40% erucic fatty acid, which is undesired for edible purposes, and the seed meal contain > 100 µmol glucosinolates per gram of dry matter. A high content of glucosinolate is undesired for use of this protein-rich meal in

animal feed. Intensive breeding in 1960's to 1970's resulted the removal of the erucic fatty acid from its oil and reduced the content of glucosinolate to < 30 μmol/g of seed meal (Downey and Harvey 1963; Jonsson 1978; Kirk and Oram 1981; Stefansson and Downey 1995). This improved version of rapeseed is named as Canola (Canadian oil low acid); currently, canola is the third most important oil crop in the world after soybean and palm (reviewed in Lin et al. 2013, and Maheshwari and Kovalchuk 2016). Conventional breeding methods have been applied to combine the zero erucic acid and low glucosinolates traits with high seed yield, blackleg resistance and other superior agronomic traits, and this was the turning point of the high demand of this crop worldwide (Edwards and Hertel 2011).

The most productive *Brassica* oilseed crop species is *B. napus*; this species is adapted to different environmental and regional climatic conditions and has three different growth habit types: (i) the spring type, which flowers without vernalization and is primarily grown in Canada, Australia and northern Europe, (ii) the winter type, which need vernalization for flowering and are predominantly grown in Europe, and eastern USA, and (iii) the semi-winter type, which are grown in geographical regions with moderate winter temperature, such as China. Higher seed yield of the winter type *B. napus* was the primary reason of growing this type as compared to spring type and winter type of *B. rapa* in Europe and eastern USA (Kimber and McGregor 1995; Kumar et al. 2015). Spring type of *B. rapa* and *B. juncea* are predominantly grown in the Indian subcontinent as winter crop.

The annual world production of *Brassica* oilseed is about 71 million metric tons (Statista 2018a). The global demand for canola oil is increasing; therefore, there is a demand for increasing its production through breeding of high-yielding cultivars. However, intensive breeding over the last years has narrowed down genetic diversity in *B. napus* cultivars (Cowling 2007; Fu and Gugel 2009); this is a bottleneck for continued improvement through breeding. To improve this crop for seed yield and agronomic traits, broadening the genetic

base of this crop through introducing allelic diversity from exotic germplasm and its allied species such as, *B. rapa* and *B. oleracea*, would be needed (for review, see Downey et al. 1980 and Rahman 2013; Zhang et al. 2015).

Broadening of genetic diversity in *B. napus* canola is also important for the development of competitive hybrid cultivars. There are evidences that the hybrid cultivars offer advantage over the line cultivars for seed yield as well as for different agronomic traits, such as early-season vigor, speed of crop development, and stress tolerances (for review, see Brichler et al. 2003). Superior hybrids cultivars of *B. napus* canola can be produced through exploitation of the huge genetic diversity exist in *Brassica* gene pool (for review, see Rahman 2013).

The focus of this Ph.D. thesis research was to study the impact of the allelic diversity of the C genome of *B. oleracea* introgressed into spring *B. napus* canola on agronomic and seed quality traits including heterosis in hybrid cultivars. This chapter of the thesis reviews the importance of canola worldwide and in Canada, its seed quality traits, evolution of the *Brassica* species, the importance of genetic diversity in canola breeding, genetic diversity analysis through molecular markers and its relationship with heterosis, and finally the heterosis in canola, as well as in maize where hybrid cultivars are well established.

1.2 Importance of canola

1.2.1 Canola production in the world and in Canada

Canola production in the world has increased rapidly over the past 40 years, and currently, it is the second largest oil crop after soybean (Statista 2018a; USDA 2016) (Fig. 1.1). The climatic condition of the temperate zone is suitable for growing this crop; however, its production area is extended to the sub-tropical zone, such as the Indian sub-continent, where it is grown as a winter crop. Canola seed can germinate at a soil temperature of 5°C while the

optimum temperature for seed germination is 10°C. The minimum, optimum and maximum temperature for growth of this crop is about 0°C, 20°-25°C, 35°C, respectively (Oplinger et al. 1989). In Europe, winter type *B. napus* canola is planted in mid-August to early September when temperature is favourable for growth and the crop can reach to a rosette stage before winter. In winter, the crop gets vernalized and they flower in spring. Seed yield of the winter type is about 2.5-3.0 tons/ha, while spring type yield 1.0-1.5 tons/ha in Canada and the United States of America (for review, see McVetty et al. 2016). In North America, the spring type is planted in May and is harvested in September.

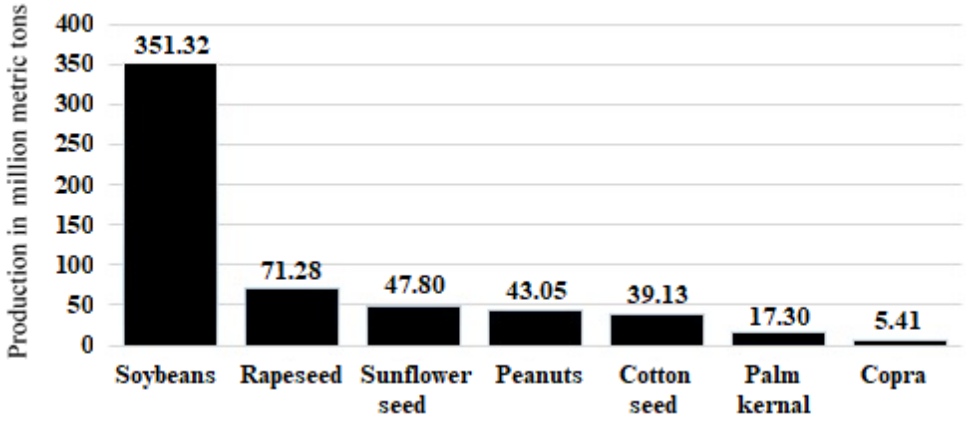


Fig. 1.1 World Production of oilseeds in 2016/2017 (Statista 2017a)

In 2014, Canada was the largest producer of canola in the world with a production of 16.41 million metric tons seed followed by China producing 14.77 million metric tons (FAO 2018). In 2018, this crop was produced on 9.11 million hectares in Canada (Statista 2018b) with a production of 21.33 million metric tons (FAO 2018). Canada exports about 90 % of its production as oil for human consumption and industrial uses, meal for livestock feed and as raw seed to 50 countries in the world. The main export markets are being the United States of America, China, Japan and Mexico (Canola Council of Canada 2016).

1.2.2 Seed quality traits and breeding canola quality cultivars

The low erucic acid and low glucosinolate (double-low) rapeseed cultivars, which were developed in 1970's, were branded as "canola" by the Western Canadian Crushers Association in 1978 (USDA 2016) to take the advantage of the nutritional features of this seed oil and seed meal. Canola seeds must contain less than 2% erucic fatty acid in its oil and less than 30 μmol glucosinolates g^{-1} of seed meal (Canola Council of Canada 2018).

Canola seed contain about 44% (range 38-50%) oil, 26% (range 20-32%) protein, 8% carbohydrates, 14% fibre, 5% ash and 15% hull (Crum et al. 1993). Oil is the most important component of the canola seed and is composed of about 93% polyunsaturated and monounsaturated fatty acids and 7% saturated fatty acids. The major unsaturated fatty acids are oleic acid (C18:1, 60%), linoleic acid (C18:2, 20%) and linolenic acid (C18:3, 10%), while the major saturated fatty acids are palmitic acid (C16:0, 4%) and stearic acid (C18:0, 2%) (The Paleo Diet 2018; for review, see Nath et al. 2016). Fatty acid composition of canola oil in comparison with other oils is presented in Fig. 1.2 (Canola Council of Canada 2018).

The traditional rapeseed oil contains more than 45% erucic acid (cis-1,3-docosenoic acid) (Sharafi et al. 2015). High content of erucic acid in diet was found to be associated with deposition of fat in heart and skeletal muscles, and adrenal glands of rodents (Christophersen and Bremer 1972; Clandinin and Yamashiro 1982; Sauer and Kramer 1983); therefore, removal of this fatty acid from edible oil was desired. However, erucic acid has several industrial applications, such as anti-block and slip agent of plastic films. It is also used for production of paints, surface coatings, nylon, inks, polymers and high pressure grease (Przybylski et al. 2005). Therefore, development of *Brassica* cultivar with high content of erucic acid is also desired for industrial application of this oil (for review, see Scarth and Tang 2006).

The seed meal is the solid component after extraction of oil; this typically contain about 35 - 40% protein, 12 - 15% crude fibre, 3.5% oil, and 15% starch, free sugars and soluble non-starch polysaccharides (Newkirk et al. 2003; Newkirk 2009). Canola meal is one of the most important sources of protein in animal feed and aquaculture industries after soybean meal. Amino acid profile of this seed meal is excellent. It contains a higher content of methionine and cystine than soybean meal (for review, see Wickramasuriya et al. 2015); therefore, there is a growing demand for this protein also for production of food for human. However, this is limited by the presence of some anti-nutritional compounds in this seed meal, such as glucosinolates, sinapine, phytic acid and tannins (Newkirk 2009).

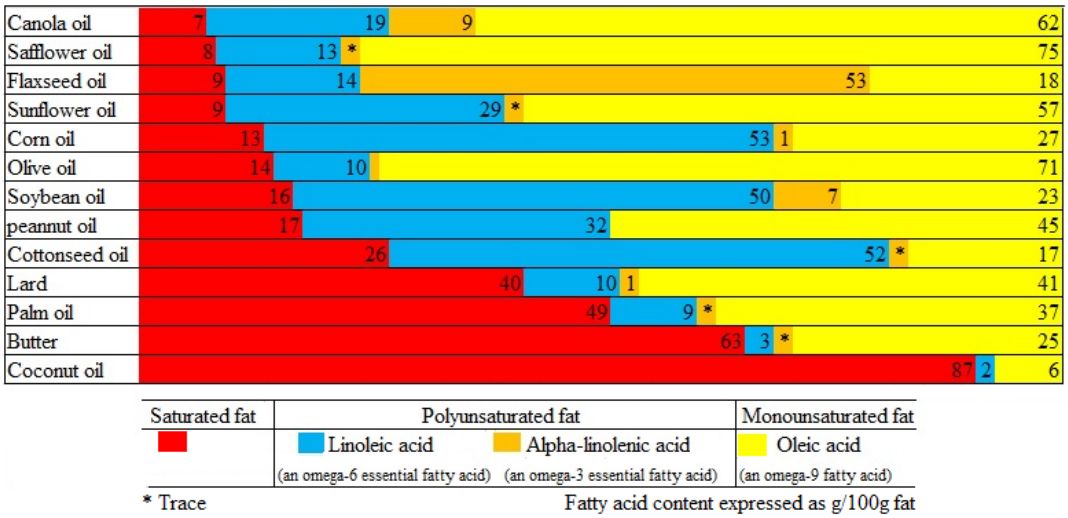


Fig. 1.2 Comparison of fatty acids composition in canola and other oils adopted from Canola Council of Canada (2018)

Prior to the development of canola from rapeseed, all *Brassica* oilseed cultivars contained a high level (>100µmol/g seed) of glucosinolates in their seed meal; this was the main limitation for use of rapeseed meal in livestock feed (Bell 1984). A high level of glucosinolates in feed tends to increase mortality of chickens and decrease egg production (for review, see Khajali and Slominski 2012). The toxic effect of glucosinolates is, in fact, due to its hydrolysis products, such as thiocyanate, isothiocyanate, oxazolidinethione and

nitriles, which are produced in presence of myrosinase in seed or in microflora of animal's gut (for review, see Tripathi and Mishra 2007).

The traditional methods of selection within the existing *B. napus* and *B. rapa* gene pools to develop better quality germplasm with a low level of erucic acid and glucosinolate in seed were applied in the early 1960's and 1970's (Downey and Harvey 1963; Jonsson 1978). The first zero erucic acid *B. napus* plant was identified in the German cv. Liho, which was introduced in 1961 by Stefansson et al. (reviewed in Lammerink and Morice 1971) and the first zero erucic acid *B. rapa* plant was identified by Downey (1964) through selection within the *B. rapa* cv. Polish. A zero erucic acid *B. juncea* line was also developed through selection in yellow-seeded *B. juncea* (Kirk and Oram 1981). The first genetic resource of low seed glucosinolate content was identified in the Polish forage *B. napus* cv. Bronowski by Agriculture and Agri-Food Canada (AAFC); the low glucosinolate genes of this cultivar has been used to develop all low glucosinolate *B. napus* and *B. rapa* cultivars across the world (Stefansson and Downey 1995; for review, see McVetty et al. 2011). The first *B. napus* canola cultivar Tower with less than 1% erucic acid in seed oil and less than 30 μmol glucosinolate g^{-1} oil-free meal was developed through pedigree selection in the progeny of the cross (Bronowski × [Turret × Turret]) × (Liho × [Turret × Turret]) at the University of Manitoba in 1974 (Stefansson and Kondra 1975). The first double low or canola quality *B. rapa* cultivar Candle was developed in 1978 by AAFC from an interspecific cross involving *B. rapa*, *B. juncea* and *B. napus* (Stefansson and Downey 1995, cited by Tahir et al. 2012).

Identification of zero erucic acid natural mutants of *B. juncea* in early 1980's was the starting point towards the development of canola quality *B. juncea* (Kirk and Oram 1981; for review, see Canola Council of Canada 1999). The first low glucosinolate *B. juncea* line 1058, with less than 10 μmol glucosinolate g^{-1} meal was developed from an interspecific cross of Indian *B. juncea* × low glucosinolate (zero erucic acid) *B. rapa* line carrying the low glucosinolate

genes of the cv. Bronowski followed by backcrossing of the interspecific hybrid to the Indian *B. juncea* (Love 1988; Love et al. 1990). Canola quality *B. juncea* cultivars were developed by the use of zero erucic acid and low glucosinolate lines in cross-breeding and selection (Rakow et al. 1995).

1.3 Evolution of *Brassica* species

The genus *Brassica* belongs to the family Brassicaceae (Cruciferae) which comprises about 3000 species belonging to 360 genera including six economically important species, viz. *B. nigra* (2*n* = 16, genome BB), *B. oleracea* (2*n* = 18, genome CC), *B. rapa* (2*n* = 20, AA), *B. carinata* (2*n* = 34, BBCC), *B. juncea* (2*n* = 36, AABB), and *B. napus* (2*n* = 38, AACC) (for review, see Shavorskaya 2004). The cytogenetic relationship between these six *Brassica* species was first illustrated by Nagaharu U (1935). In this *B. nigra*, *B. oleracea*, and *B. rapa* are three diploid species, where hybridization between these diploid species resulted the three amphidiploid species, *B. carinata*, *B. juncea*, and *B. napus* (Fig. 1.3).

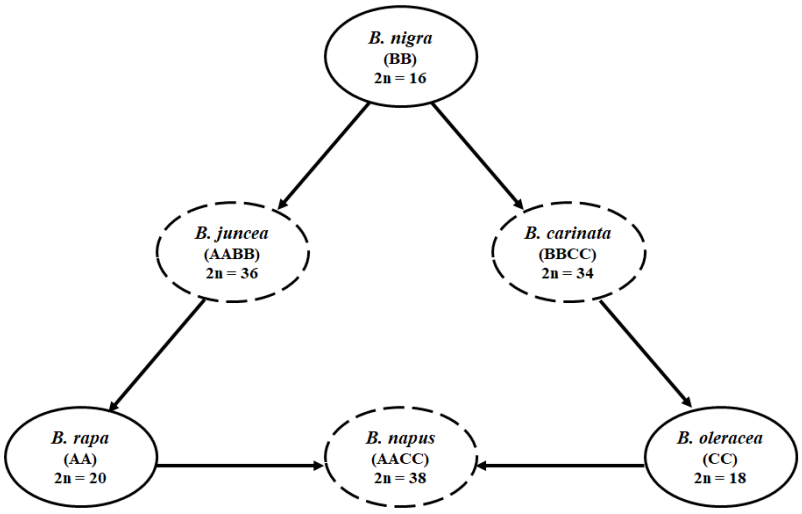


Fig. 1.3 Triangle of U showing the cytogenetic relationship among the six *Brassica* species. Adapted from Nagaharu U (1935)

Of the two diploid parental species of *B. napus*, *B. oleracea* possesses wide morphological diversity and includes different variants such as kales (var. *acephala*), cabbages (var. *capitata*

and var. *sabauda*), kohlrabi (var. *gongylodes*), brussel sprouts (var. *gemmifera*), cauliflower (var. *botrytis*), broccoli (var. *italica*) and Chinese kale (var. *alboglabra*) (Warwick 2010). Similarly, *B. rapa* (*B. campestris*) also possess considerable morphological diversity and this species share the same evolutionary pathway with *B. oleracea* (Warwick 2010). *B. napus* seems to have a polyphyletic evolutionary background – originated from interspecific crosses involving more than one type of *B. rapa* and *B. oleracea* (Song and Osborn 1992; Allender and King 2010). However, it is very unlikely that all variants of the two parental species has been included in the evolution of this amphidiploid species (for review, see Rahman 2013). It is generally accepted that *B. napus* was originated on the coast of northern Europe or the Mediterranean region where the two parental species *B. oleracea* and *B. rapa* grow wild (for review, see Rakow 2004).

Comparative analysis of restriction fragment length polymorphism markers of the three diploids species of the U triangle indicated that their genomes had a triplicated structure (Lagercrantz and Lydiate 1996) suggesting that the diploid *Brassica* genomes descended from a hexaploid ancestor. This was further supported by Lysak et al. (2005) who found that *Brassica* species contain three or six copies of a genomic region similar to that of *Arabidopsis thaliana*. Thus, the phylogenetic studies provided evidence for a whole-genome triplication in the ancestry of *Brassica* after the divergence from *Arabidopsis* followed by chromosome fission, fusion and rearrangements leading to the evolution of the diploid *Brassica* species (Fig. 1.4) (for review, see Shavorskaya 2004; Cheng et al. 2013; reviewed in Zou, Hu et al. 2016).

The genus *Brassica* has been subjected to various fundamental studies, such as genome change and the effect of polyploidy, primarily for the occurrence of closely related genomes and their natural and artificial amphidiploid species (for review, see Ziolkowski et al. 2012).

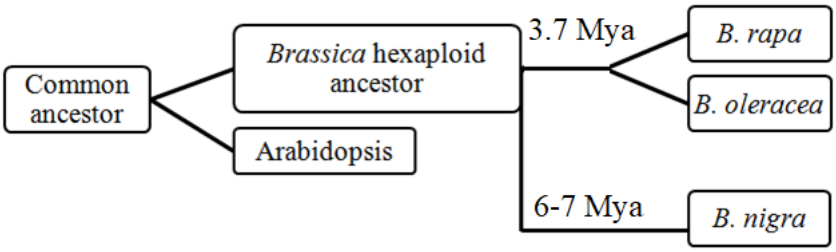


Fig. 1.4 Schematic diagram showing hypothesized genome evolution of the *Brassica* diploid species. Mya = Million years ago (for review, see Shavorskaya 2004; Cheng et al. 2013; reviewed in Zou, Hu et al. 2016).

1.4 Genetic diversity in *Brassica*

1.4.1 Importance of genetic diversity in canola breeding

Genetic diversity in crop germplasm is needed to develop new and improved cultivars with desirable traits through combining desirable alleles from the parents. Till modern times, the best plants/seeds were selected from natural population by plant breeders. Genetic variability in natural population has been generated through recombination of genes during sexual reproduction, spontaneous mutation, polyploidy, as well as spontaneous hybridization between related plant species. The extent to which a cultivar could be improved was limited during the pre-Mendelian time of plant breeding because breeders then did not have the scientific knowledge on genetics and the ability to manipulate natural genetic diversity in a planned and organized way to produce a new cultivar. Rediscovery of Mendel's laws of inheritance in the beginning of 1900's followed by establishment of plant breeding enterprises in Europe was the turning point to modern plant breeding. That was the time of creating new genetic variation through application of scientific knowledge, such as artificially induced variation through mutation and variation created by gene manipulation (for review, see Anio 2001).

Like any other crop species, presence of sufficient genetic diversity is needed to increase seed yield and improve agronomic and seed quality traits of *Brassica* oilseed crop species. *B.*

napus is the most widely cultivated *Brassica* oilseed crop species. It is a new species that most likely originated from spontaneous interspecific hybridization between turnip rape (*B. rapa*) and cabbages (*B. oleracea*) during medieval times (Iniguez-Luy and Federico 2011). The evolution of this species from a limited number of variants of its progenitor species (for review, see Abbadi and Leckband 2011 and Rahman 2013) as well as the occurrence of two bottlenecks during intensive breeding in the recent years for the improvement of the two seed quality traits (low erucic acid and low glucosinolate content) resulted a low genetic diversity in this crop species (Becker et al. 1995; Cowling 2007; Bus et al. 2011). This narrow genetic diversity can limit continued improvement of this crop through breeding and it can limit F_1 hybrid vigour as well (Jesske et al. 2013; Rahman et al. 2016). Therefore, several researchers suggested increasing genetic diversity in *B. napus* by introducing new alleles from exotic germplasm including its parental species *B. rapa* and *B. oleracea* through interspecific hybridization (for review, see Downey et al. 1980; Qian et al. 2006; Jesske et al. 2013; for review, see Rahman 2013; Zhang et al. 2015).

1.4.2 Broadening of genetic diversity in canola breeding

The gene pool of *B. rapa* and *B. oleracea*, the two ancestor species of *B. napus*, has great potential to contribute allelic diversity for genetic improvement of *B. napus*; these two gene pools are quite distinct (Abel et al. 2005), and their A and C genomes are also distinct from the corresponding A and C genomes of *B. napus* (Thormann et al. 1994). Broadening the genetic base of *B. napus* through interspecific cross with its allied species is a challenging task for various reasons, such as the difficulty of producing viable interspecific hybrids, hybrid sterility, linkage drag, introduction of undesired alleles from the allied species, and disturbance of the desired allele combinations of the crop species (Bennett et al. 2008; Falk 2010; for review, see Rahman 2013). Application of embryo rescue techniques has been

found to increase the efficiency of production of the interspecific hybrids (Rahman 2004; Bennett et al. 2008), and limited backcrossing of the interspecific hybrids to the crop species (Falk 2010) can mitigate some of the above-mentioned other constraints. Resynthesis of *B. napus* from its parental species, which utilizes the diversity of both parental species, has also been done by several researchers to broaden the genetic base of *B. napus* canola (Becker et al. 1995; Girke et al. 2012a; Guo et al. 2016).

Qian et al. (2006) demonstrated higher genetic diversity in new type *B. napus* derived from *B. napus* × Chinese *B. rapa* interspecific cross. In fact, introgression of genome contents of Chinese *B. rapa* into Chinese semi-winter *B. napus* has made this crop genetically distinct from the spring and winter type *B. napus* (Qian et al. 2006; Chen et al. 2008). Li et al. (2013) found high allelic variation in the *B. napus* lines derived from crossing of AACCCC digenomic allohexaploids to *B. rapa*. While working with new type *B. napus* derived from *B. napus* × *B. rapa* interspecific crosses, Attri and Rahman (2018) found a loss about half of the alleles in F_8 as compared to the alleles found in F_4, suggesting the need of selection for the exotic alleles in early generation.

When comparing with *B. oleracea*, it is relatively easy to transfer alleles from *B. rapa* into *B. napus*; this primarily relates to the ease of production of the interspecific hybrids of *B. napus* × *B. rapa* (Qian et al. 2006; Chen, Zou et al. 2010; Mei et al. 2011). This might be one of the reasons for the lower genetic diversity observed in the C genome of *B. napus* as compared to its A genome (Bus et al. 2011), and this highlight the need of increasing allele diversity in the C genome of this crop species (Rahman et al. 2011). To broaden the genetic base of the C genome of the spring *B. napus* canola, Rahman et al. (2015) crossed *B. oleracea* var. *alboglabra* to *B. napus* and developed several genetically diverse *B. napus* canola lines from this interspecific cross. Similarly, Iftikhar et al. (2018) developed genetically diverse canola lines from different *B. napus* × *B. oleracea* interspecific crosses involving different variants

of this diploid species, such as var. *alboglabra, botrytis, italica* and *capitata*. Li, Zhou et al. (2014) found that the inbred lines derived from *B. napus* × *B. oleracea* var. *acephala* to be genetically distinct from the available winter and spring type *B. napus* lines. While using *B. oleracea* in the breeding of *B. napus*, non-canola quality traits are also introduced into the interspecific hybrid progeny. Erucic acid content is controlled by one gene locus in each of the A and C genomes (Fourmann et al. 1998) while glucosinolate content is controlled by more than one gene loci in each of these two genomes (Rahman et al. 2001; reviewed in Rahman et al. 2015). Given this genetic control of these two quality-traits, canola quality plants could be selected from the progeny of *B. napus* × *B. oleracea* crosses (Rahman et al. 2015; Iftikhar et al. 2018).

1.4.3 Analysis of genetic diversity by use of molecular markers

Genetic diversity study by use of molecular markers can help breeder to understand the extent of allelic diversity present within a breeding population, identify the changes in allele frequency in a population, assign the breeding lines and germplasm to different heterotic groups, understand the relatedness of a crop species with its wild relatives, and study the genetic structure of germplasm to identify the parents for cross to produce superior progeny (Warburton and Hoisington 2001; Hu et al. 2007). The non-DNA based marker technologies, such as isozyme markers have been used with some success (Becker et al. 1995); however, the emergence of DNA-based markers has changed the practice of genetic diversity studies. Many DNA-based marker technologies have been successfully used to assess genetic diversity and phylogenetic relationship in *Brassica* crops; this includes the use of restriction fragment length polymorphism (RFLP) markers (Diers and Osborn 1994; Pradhan et al. 2003), followed by polymerase chain reaction (PCR)-based markers such as, random amplified polymorphic DNA (RAPD) (Lázaro and Auginagalde 1998; Saha et al. 2008) and

amplified fragment length polymorphism (AFLP) makers (Lombard et al. 2000; Pradhan et al. 2003; Christensen et al. 2011). Simple sequence repeat (SSR) marker, alternatively known as microsatellites, was the next simple, reliable, codominant, and inexpensive marker technology used to amplify DNA repeat sequences in *Brassica* (Powell et al. 1996; Batley et al. 2007; Hopkins et al. 2007; Wu et al. 2014; Hobson and Rahman 2016; Attri and Rahman 2018; for review, see El-Esawi 2017).

Microsatellites or simple sequence repeat (SSR) are segments of DNA consisting of tandemly repeated nucleotides that generally occur throughout the whole genome. Reproducibility of the genotyping results of SSR markers is high due to the use of long primer pairs. They are codominant markers; however, mutation in the primer binding site may result the failure of amplification of the targeted alleles, i.e. the occurrence of null alleles (For review, see Carlsson 2008; Kumar et al. 2009). SSR markers were used in various breeding applications, such as for assessing genetic diversity in *B. napus* (Hasan et al. 2006; Gyawali et al. 2013), *B. rapa* (Fu and Gugel 2009; Hobson and Rahman 2016) and *B. oleracea* (Louarn et al. 2007; El-Esawi et al. 2016), evaluation of allelic diversity introgressed from exotic germplasm into cultivated crop species (Kebede et al. 2010; Attri and Rahman 2018), gene mapping studies (Kapoor et al. 2009; Kebede and Rahman 2014), and association mapping of traits (Gyawali et al. 2016).

1.4.3.1 Analysis of genetic diversity by SSR markers in *Brassica*

Broadening the genetic base of spring and winter *B. napus* is the important goal of today's breeding programs. Several researchers conducted genetic diversity analysis in *B. napus* using SSR markers. For example, Chen et al. (2008) characterized 72 *B. napus* accessions collected from different countries and classified the Chinese germplasm into two distinct groups; one of this group was quite distinct from the accessions collected from India,

Australia and Canada. The study conducted by Hasan et al. (2006) placed the spring and winter *B. napus*, and the vegetables types into three genetically distinct groups; they found the greatest allelic diversity in the vegetable types. Similarly, Bus et al. (2011) also found distinct clustering of spring and winter *B. napus* using SSR markers. While comparing genetic diversity in the A and C genomes of *B. napus* using SSR markers, Bus et al. (2011) and Wu et al. (2014) found lower diversity in the C genome as compared to the A genome; the lowest diversity in the C genome was found in Chinese semi-winter type. The lower diversity in the C genome of Chinese semi-winter type might be the consequence of the use of *B. rapa* in the breeding of this type (Qian et al. 2006; Chen, Zou et al. 2010; Mei et al. 2011).

SSR marker analysis has also demonstrated the existence of wide genetic diversity in the two diploid parental species of *B. napus*. For example, genetic diversity analysis of *B. rapa* by Hobson and Rahman (2016) using SSR marker developed based on genome sequence information of *B. rapa* placed the Chinese cabbage, Chinese winter oilseed, European winter oilseed, Canadian spring oilseed, pak-choi, turnip, and yellow sarson into distinct group; this provided strong evidence for the existence of wide diversity in this species. Similarly, Annisa et al. (2013) also found wide diversity in this species while studying a world collection of 164 oilseed *B. rapa* accessions. According to this research group, accessions from south Asia, southern and eastern Europe (mostly winter and semi-winter types) and northern Europe are genetically quite distinct.

SSR marker analysis also demonstrated that *B. oleracea* is a great reservoir of allelic diversity (Louarn et al. 2007; El-Esawi et al. 2016; Tortosa et al. 2017); this is also evident from wide morphological diversity in this species (Warwick 2010; for review, see El-Esawi 2017). Louarn et al. (2007) found that accessions of red cabbage, savoy cabbage, broccoli and cauliflower are generally quite distinct. El-Esawi et al. (2016) found that kale and Brussels

sprouts are genetically distinct; they also found the existence of high genetic variation within the spring cabbage accessions to be used in breeding. Tortosa et al. (2017) provided evidence that accessions collected from different geographic regions can also be genetically distinct. Thus, these studies using molecular markers provided substantial evidence of the existence of genetic diversity in *Brassica* which can be used in breeding for broadening the genetic base of *B. napus* canola.

1.5 Molecular mapping of traits in *Brassica*

1.5.1 Quantitative trait loci (QTL) mapping in *Brassica*

Genetic studies on traits in *Brassica* can be traced back to the early 20th century; however, research on identification of a genomic region controlling a trait entered into a new era in early 1990's with the evolution of DNA marker technologies (for review, see Branca and Cartea 2011). Research efforts resulted the construction of linkage maps for different crops, including the *Brassica* crops, using molecular makers (Quiros and Paterson 2004; Snowdon and Friedt 2004; Parkin et al. 2005), and the linkage maps have been used to identify the genomic regions carrying the genes of interest for use of the flanking markers in marker assisted breeding. The advent of genome sequencing along with significant advances in molecular biology and bioinformatics have increased the efficiency of the molecular marker technology for use in breeding (for review, see Duran et al. 2009a, 2009b).

Over the past two decades, a large number of genetic maps of *B. napus* have been constructed by researchers across the world by use of different mapping populations and markers to locate the genes and quantitative trait loci (QTL) controlling different agronomic and seed quality traits. For example, Sun et al. (2007) constructed a genetic linkage map with a marker density of 8.45 sequence related amplified polymorphism (SRAP) markers per cM by use of a doubled haploid population derived from crossing of a resynthesized *B. napus* line (derived

from *B. rapa* × *B. oleracea*) to a natural *B. napus*. They used this map as a platform for map-based cloning of different genes such as, blackleg disease resistance, yellow seed colour and male sterility. Kebede et al. (2012) constructed a linkage map based on a *B. rapa* recombinant inbred line population and SSR markers. They mapped one major and three minor QTL on chromosome A3, A5, and A9 which collectively explained 67% of the total phenotypic variance for seed colour. Using the same linkage map and the mapping population, Kebede and Rahman (2014) detected three QTL on chromosome A3, A5, and A7 responsible for silique length and one QTL on A2 for petal colour, and Rahman et al. (2014) detected three QTL on A2, A7 and A9 for total seed glucosinolate content. These reports demonstrated that the same map can be used for mapping of multiple traits and identification of markers for use in marker assisted selection. Delourme et al. (2013) developed an integrated genetic map of *B. napus* using four segregating populations; genome coverage of this map was 3.27 markers per cM. They found a high collinearity between the four maps; the A genome linkage groups of the map included a greater number of markers as compared to the C genome linkage groups. This also provide further evidence that greater diversity of alleles exists in the A genome as compared to the C genome.

To date, the results of QTL mapping based on linkage map seems to have little impact on breeding primarily due to the large confidence intervals of these QTL markers (Van Inghelandt et al. 2012). Emergence of a large amount of genomic data from sequencing of *B. rapa* (Wang et al. 2011), *B. oleracea* (Liu et al. 2014; Parkin et al. 2014) and *B. napus* (Chalhoub et al. 2014) genomes has provided opportunity for mining single nucleotide polymorphism (SNP) markers to get better insight into the *Brassica* genomes. SNP markers, due to their abundance in the genome, has been found to be a very efficient for use in genetic diversity analysis (Pelc et al. 2015; Su et al. 2018; Yousef et al. 2018), construction of genetic and physical maps (Bus et al. 2012; Clarke et al. 2016; Huang et al. 2017), QTL mapping

(Wang et al. 2015; He et al. 2017; Li, Jeong et al. 2018) as well as fine mapping of traits (Zhang et al. 2018) in *Brassica*.

1.5.2 Association mapping in *Brassica*

Association mapping (AM), which is based on historical recombination and linkage disequilibrium between marker and trait, is a powerful method of scanning the whole genome for identification of the alleles associated with phenotypic traits of interest (Huang et al. 2013; Brown et al. 2014; Raman et al. 2014a). In other words, association mapping is a form of genome-wide association study (GWAS) where scanning of the entire genome is done and markers associated with trait of interest is identified (Meuwissen and Goddard 2000). AM has several advantages over traditional QTL mapping: (i) high mapping resolution due to historical recombination accumulated in natural population with diverse genetic backgrounds, (ii) ability to identify multiple alleles at a genetic loci in a population, and (iii) time saving in QTL mapping without the need to develop special mapping populations (Flint-Garcia et al. 2003; for review, see Soto-Cerda and Cloutier 2012). AM has been extensively used in different crops, such as rice (Agrama et al. 2007), maize (Yan et al. 2011) as well as *B. napus* (Zou, Jiang et al. 2010; Cai et al. 2014).

The development of array-based marker technology with high genome coverage and high marker density has increased the power of detection of QTL through association mapping. For example, the *B. napus* 6 K SNP array and later the 60 K array have been used by different researchers (Li, Chen et al. 2014; Clarke et al. 2016; Lu et al. 2017; Mason et al. 2017). Using 6 K array and 509 inbred lines, Bus et al. (2014) identified 29 loci associated with variation for shoot ionome. Körber et al. (2015) detected 63 SNP markers associated with seedling development traits. Using the same array Körber et al. (2016) identified association of 112 SNPs with seed quality traits including an association of a single SNP with

sulfur concentration in seeds. They also identified 46 loci associated with different agronomic traits including a single SNP for color variation.

By use of the 60K SNP array, Li, Chen, Geng et al. (2010) identified two genomic regions on chromosome A8 and C3 associated with the oil content, and Qu et al. (2015) detected 11 SNP markers associated with seed glucosinolate content including two SNPs for candidate genes involved in biosynthesis and accumulation of seed glucosinolate content. Luo et al. (2015) detected nine SNPs in the C genome significantly associated with harvest index including five loci simultaneously associated with harvest index and seed yield per plant. Qu et al. (2017) identified 62 loci associated with the concentration of seven fatty acids, and 24 orthologs of the functional candidate genes involved in fatty acid biosynthesis. Chen et al. (2018) detected 98 SNPs from 25 QTL associated with cadmium accumulation at seedling stage; they also identified 32 candidate genes located 0.33 – 497.97 kb away from the SNPs.

Genome-wide association study (GWASs) has also been done to detect marker-trait association for many other traits in *B. napus*, such as seed oil content (Zou, Jiang et al. 2010), seed weight (Li, Chen et al. 2014), phenolic compounds (Rezaeizad et al. 2011), seed tocopherol content and composition (Fritsche et al. 2012), and fatty acid composition (Qu et al. 2017) as well as several agronomic traits, such as seed coat color (Wang, Xian et al. 2017), plant height (Sun et al. 2016), primary branch number (Li et al. 2016), flowering time (Xu et al. 2016), silique shatter resistance (Raman et al. 2014b), and resistance to several diseases, such as stem canker caused by *Leptosphaeria maculans* (Jestin et al. 2011; Fopa-Fomeju et al. 2014) and *Sclerotinia* stem rot (Wei, Jian et al. 2016).

1.6 Heterosis or hybrid vigour

Heterosis for a trait indicate the higher performance of the hybrid than the performances of its parents; the degree of heterosis is often calculated from the difference between the

performance of hybrid and average of the performances of its two parents (Hochholdinger and Baldauf 2018; for review, see Fujimoto et al. 2018). The first exploitation of heterosis was reported by Shull in 1908 in maize. They observed that crossing two unrelated inbred lines increases the growth and yield of the F_1 hybrids as compared to the better parent (Shull 1908, 1911). Cultivation of hybrid maize has grown very fast because of higher seed yield of these types of cultivars as compared to open-pollinated cultivars. Seed price of hybrid cultivars is more expensive than the open-pollinated cultivars and growers are not be able to save seeds of this type of cultivar due to inbreeding depression in the subsequent generations (Kutka 2011). However, the popularity of these types of cultivars has increased rapidly since launch of the maize hybrids in 1930's (for review, see Phillips 2010) primarily due to the yield advantage associated with these types of cultivars. Maize has been subjected to intensive investigation of the phenomenon known as heterosis – primarily because of its high economic importance and greater expression of this phenomenon for different traits (Flint-Garcia et al. 2009; Guo and Rafalski 2013; Samayoa et al. 2017; Li, Coffey et al. 2018). Compared to many other cross-pollinated crops, hybrid breeding is well established in maize for the reason that it is easier to develop hybrid parent lines through self-pollination and produce F_1 hybrid seeds through controlled cross-pollination; this crop has also enormous natural genetic diversity which is required for the development of a competitive hybrid cultivar (for review, see Reif et al. 2005).

Attempts to understand the genetic basis of hybrid vigour began with the proposal of the dominance hypothesis in 1908. This hypothesis assumes that the superior dominant or functional and the deleterious recessive alleles of a gene are contributed by the different parents and the genetic effect of the deleterious recessive allele is cancelled by the dominant alleles in heterozygous F_1 plants (Bruce 1910; Jones 1917; Collins 1921, for review, see Lippman and Zamir 2006; Chen 2010). The overdominance hypothesis states that the

combination of two alleles at a given locus exerts superior genetic effect as compared to the homozygous genotypes (East 1936; Hull 1945; Crow 1948; Hollick and Chandler 1998). The epistasis hypothesis attributes heterosis to interaction between the genes at two or more loci (Richey 1942; Schnell and Cockerham 1992) (Fig. 1.5). The pseudo-overdominance is a simple case of dominance complementation, in which complementation occur for the alleles that are linked in repulsion (for review, see Lippman and Zamir 2006; Chen 2010).

Molecular markers and the development of linkage maps have facilitated QTL mapping of the loci contributing to heterosis and understanding gene action of these loci, and thus, accelerated the understanding of the genetic basis of heterosis in different crops, such as maize (Lu et al. 2003; Frascaroli et al. 2007), rice (Hua et al. 2002), tomato (Semel et al. 2006) and oilseed *B. napus* (Radoev et al. 2008; Basunanda et al. 2010). Most of these studies focused on yield-related traits. For example, QTL mapping of seed yield heterosis in maize indicated that overdominance or pseudo-overdominance is the main cause of heterosis (Stuber et al. 1992; Lu et al. 2003; Li et al. 2017), while other researchers found that dominance or epistatic effect of the genes is the main cause of this phenomenon (Cockerham and Zeng 1996; Frascaroli et al. 2007). High coverage of the crop genome using a large number of molecular markers would enable fine mapping of the genomic regions contributing to heterosis, and identification and characterization of the underlying genetic architecture behind this phenomenon (for review, see Reif et al. 2005). The genetic basis of heterosis is not limited to a single cause – this can vary depending on the species, traits and parental combination used (Li et al. 2008). Recent, advances in sequencing technologies and bioinformatics analysis of sequence/marker data have greatly increased our knowledge on structural diversity in the maize (Messing and Dooner 2006; Schnable et al. 2009; Unterseer et al. 2014) and *B. napus* (Wei et al. 2017; Zheng et al. 2017) genomes, and this may help to better understand the genetic factors involved in heterosis.

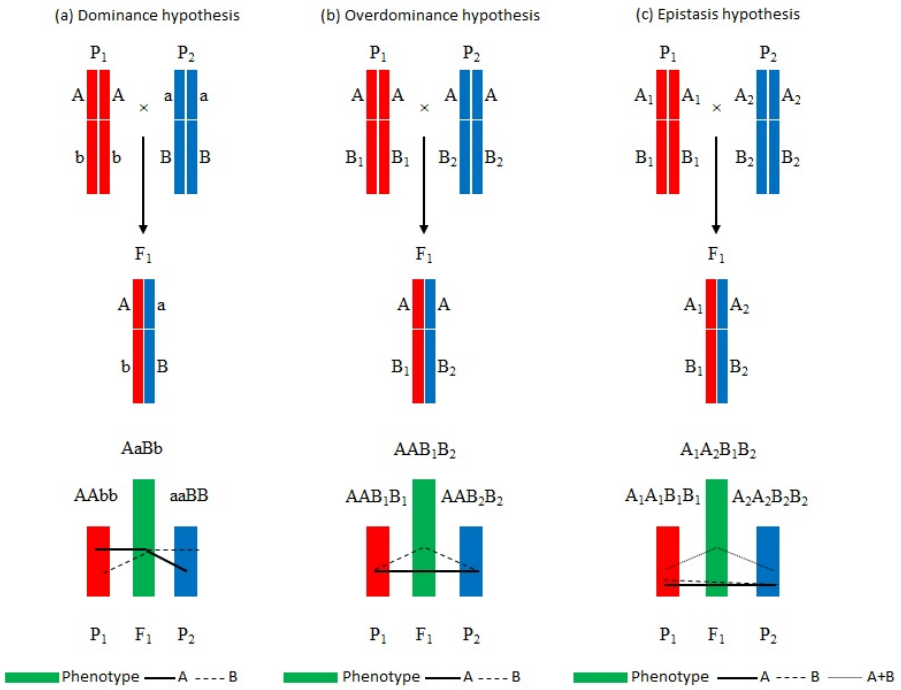


Fig. 1.5 Schematic diagram of the hypotheses of the genetic basis of heterosis (Fujimoto et al. 2018). A and B represent the dominant alleles; a and b represent the recessive alleles; A_1/A_2 and B_1/B_2 represent the heterozygous alleles.

1.6.2 Heterosis in *Brassica*

Heterosis for seed yield and different agronomic and seed quality traits has been reported by different researchers, such as Brandle and McVetty (1989), Butruille et al. (1999); Shen et al. (2005), Basunanda et al. (2007), Qian et al. (2007), and Channa et al. (2018). The effect of abiotic stresses, such as heat, has also been studied on *B. napus* hybrid canola by Koscielny et al. (2018). They found that the hybrids are more tolerant to heat stress than the inbred lines, and yield reduction in inbred is about 5% greater than the hybrids under heat stress condition. Superior hybrid *B. napus* cultivars can be produced through exploitation of the huge genetic diversity exist in the *Brassica* gene pool (for review, see Rahman 2013). For example, Li et al. (2006) found inter-subgenomic heterosis of the A and C genomes for seed yield in *B. napus* hybrids developed by use of *B. napus* lines derived from interspecific cross between *B. rapa* (A genome) and *B. carinata* (BC genome) and natural *B. napus* line. Liu et al. (2002)

found heterosis for biomass yield in trigenomic hybrids of *B. napus* × *B. rapa* interspecific cross and provided evidence that the use of the genomes of the allied species can increase heterosis in *B. napus* hybrids. Indeed, Qian et al. (2005) demonstrated by developing *B. napus* lines from *B. napus* × *B. rapa* interspecific cross that introgression of genome contents of *B. rapa* into *B. napus* can exhibit heterosis for seed yield in *B. napus* F_1 hybrids. They also found some of the DNA segments introgressed from *B. rapa* show positive effect on seed yield and yield components. Similarly, Li, Zhou et al. (2014) found that the inbred lines derived from *B. napus* × *B. oleracea* interspecific cross show wide genetic variation and potential for the development of high yielding Chinese semi-winter *B. napus* hybrids. Rahman et al. (2016) also reported that the alleles of *B. oleracea* as well as the European winter *B. napus* have great potential for increasing seed yield in spring *B. napus* hybrid cultivars. They also reported that alleles exerting non-additive effect in the genetic control of heterosis can be found frequently in *B. oleracea* while the alleles for general combining ability (GCA) of the hybrid parents can be found frequently in winter *B. napus*. Starmer et al. (1998) found that increased number of silique on main raceme and larger size seed primarily contribute to increased seed yield in hybrids; however, hybrids tend to mature later than the parents.

The superiority of the *B. napus* F_1 hybrids derived from crossing of parental lines from different geographic origins have been reported a few decades ago (Sernyk and Stefansson 1983; Lefort-Buson et al. 1987). However, the extent of correlation between genetic distance of the parents and heterosis varied for different traits and in different studies. Ali et al. (1995), Diers et al. (1996), Tan et al. (2007) and Sang et al. (2015) observed significant correlation between genetic distance of the parents and heterosis for seed yield. In contrast, Yu et al. (2005), Qian et al. (2007, 2009), and Luo et al. (2016) found no significant correlation between genetic distance of the parents and heterosis for seed yield, while Kaur et

al. (2007) found negative correlation between genetic distance of the parents and hybrid performance. Recent studies have provided evidence of the occurrence of significant correlation between GCA of the parents and heterosis for seed yield in *B. napus* (Tian et al. 2017). Bansal et al. (2012) found no correlation between heterosis and genetic diversity for seed yield in *B. juncea* and its progenitor diploid species, *B. rapa* and *B. nigra*; however, hybrid performance for biomass yield was correlated with genetic diversity in *B. juncea* and *B. rapa*. They found significant correlation of GCA with heterosis in *B. rapa* for biomass yield; biomass yield was found to exhibit association with seed yield in *B. napus* (Zhang and Flottmann 2016). Thus, it is apparent that genetic distance alone may not be able to predict heterosis or hybrid performance; GCA also plays an important role in this together with genetic diversity of the parents, and vigorous hybrids may produce greater yield.

In the last years, there has been a growing interest to identify the genomic regions contributing to heterosis for different traits in *B. napus* due to increasing interest of the development of hybrid cultivars. Zhang et al. (2015) conducted a comparative transcriptome analysis to understand the molecular mechanism of heterosis at the gene expression level in hybrids of *B. napus* × *B. rapa* and found that both dominance and overdominance effect of the genes contribute to heterosis for growth performance and stress tolerance. Based on differentially expressed genes, they also found that the genes from the A genome to be associated with metabolism and development, while those from the C genome participate in stress tolerance. Whether this is the general feature of intraspecific hybrids involving *B. napus* cultivars and lines would need further investigation. Using the heterosis-associated genes of Arabidopsis, Jeong et al. (2017) identified their orthologues in *B. oleracea*; many of these gene showed greater expression in hybrids of this crop and were associated with yield contributing traits. According to Chen (2013), interaction of alleles of the two parental

genomes can result altered programming of the genes involved in growth, stress tolerance and fitness in hybrids, and this can increase plant vigor.

Following a QTL mapping approach, Radoev et al. (2008) identified 33 loci contributing to heterosis for seed yield and yield-related traits in *B. napus*. Among these, the QTL associated with seed yield often exhibited dominance or overdominance effects, while the QTL for yield contributing traits, such as number of seeds per silique and seed weight often exhibited partial dominance effect. Epistatic interactions of the genes contributing to heterosis have also been reported by several researchers (Radoev et al. 2008; Basunanda et al. 2010; Shi et al. 2011). Basunanda et al. (2010) found co-localization of several QTL involved in per se performance as well as heterosis for different traits in *B. napus.* Advances in molecular marker technologies and QTL mapping of heterosis using high-density map with full coverage of the genome, and in-depth understanding of the *Brassica* genomes, it is expected that this phenomenon in *B. napus* can be better understood and markers closely linked to the heterotic QTL can be identified for use in breeding to improve the accuracy of prediction of superior hybrids (for review, see Wang, Zhang et al. 2017).

1.7 Research objectives

As reviewed in this section, it is apparent that broadening of the genetic base of *B. napus* canola is needed; this can be achieved by introducing exotic alleles from the primary gene pool of *B. napus* as well as from its progenitor species *B. oleracea* and *B. rapa*. This Ph.D. thesis research was focused on understanding the *B. oleracea* gene pool for the improvement of *B. napus* canola. For this, six *B. napus* canola populations derived from crossing a *B. napus* line to six *B. oleracea* accessions were used.

1.7.1 Long-term objectives

The long-term objective of this research project is to utilize the allelic diversity of different variants of *B. oleracea* gene pools to broaden the genetic base of the C genome of *B. napus* canola for improved per se performance of this crop as well as for heterosis for different agronomic and seed quality traits including seed yield in hybrid canola cultivars.

1.7.2 Short-term objectives

In the short term, this Ph.D. thesis research is focused on the following objectives:

i) Investigate the impact of allelic diversity of the C genome of *B. oleracea* for broadening the genetic base of *B. napus* canola, and the effect of these exotic alleles on different agronomic and seed quality traits including seed yield, and to identify QTLs for these traits using SNP and SSR markers;

ii) Investigate the effect of the allelic diversity of the C genome of *B. oleracea* introgressed into *B. napus* on heterosis for different agronomic and seed quality traits including seed yield;

1.8 Research hypothesis

The following hypotheses were tested in this Ph.D. thesis research project:

i) Alleles introgressed from *B. oleracea* into *B. napus* will broaden the genetic base of spring *B. napus* canola;

ii) The introgressed alleles of *B. oleracea* will affect the agronomic and seed quality traits in *B. napus*;

ii) Alleles introgressed from *B. oleracea* into *B. napus* will contribute to heterosis for different traits in *B. napus*;

iii) Genetic distance of the hybrid parental lines will show a positive correlation with heterosis and performance of the hybrids.

Chapter 2

Study of the genetic structure of a *Brassica napus* canola population derived from six interspecific crosses of *B. napus* × *B. oleracea*[1]

2.1 Introduction

The well-known *Brassica* U-triangle includes three diploid species *B. nigra* (BB, 2*n* = 16), *B. oleracea* (CC, 2*n* = 18) and *B. rapa* (AA, 2*n* = 20), and three amphidiploid species *B. carinata* (BBCC, 2*n* = 34), *B. juncea* (AABB, 2*n* = 36) and *B. napus* (AACC, 2*n* = 38) (Nagaharu U 1935). The amphidiploid species *B. napus* carries two homoeologous but divergent sub-genomes: the A genome of *B. rapa* and the C genome of *B. oleracea*. These two genomes had previously been triplicated after divergence from *A. thaliana*, and therefore, often carry multiple copies of a gene (Parkin et al. 2005; Cheung et al. 2009). The genetic distance of *B. napus* from *B. oleracea* (similarity coefficient 0.549) and *B. rapa* (similarity coefficient 0.566) is very similar (Thakur et al. 2018) suggesting that the A and C genomes contributed almost equally to the amphidiploid species *B. napus*; however, the C genome of *B. napus* remained more conserved than its A genome (Bus et al. 2011; Thakur et al. 2018) advocating the need for broadening the genetic base of this genome in *B. napus.* This is important not only for using the genetically diverse parents in crosses for the development of improved open-pollinated canola cultivars, but also for broadening the genetic base of the hybrid parent lines for the development of improved hybrid cultivars (for review, see Rahman 2013).

[1] A version of this chapter 2 of this dissertation has been submitted as: Nikzad A, Kebede B, Pinzon J, Bhavikkumar J and Rahman H (2020) Study of genetic structure of a *Brassica napus* canola population derived from six interspecific crosses of *B. napus* × *B. oleracea*. Can. J. Plant Sci.

B. oleracea L., carrying the C genome, is an important vegetable crop species in the world; this includes cauliflower (*B. oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*), broccoli (*B. oleracea* var. *italica*), Brussels sprouts (*B. oleracea* var. *gemmifera*), kale (*B. oleracea* var. *acephala*), and kohlrabi (*B. oleracea* var. *gongylodes*). *B. rapa*, the other parental species of *B. napus*, is considered the most primitive ancestor of all diploid *Brassica* species (Thakur et al. 2018). This species also includes different varieties, such as turnip (*B. rapa* var. *rapifera*), Chinese cabbage (*B. rapa* var. *pekinensis*) and Pak choi (*B. rapa* var. *chinensis*) which are used as vegetables, and oilseed winter and spring type (*B. rapa* var. *oleifera*) and yellow sarson (*B. rapa* var. *trilocularis*) (Warwick 2010). Evaluation of this wide genetic variation is important for use in the breeding of *B. napus* for improved agronomic and seed quality traits including seed yield.

Molecular markers are powerful tools for evaluation of genetic variation within and between *Brassica* gene pools. Among the different types of molecular markers, the simple sequence repeat (SSR) is simple, reliable and co-dominant multi-allelic in nature which exhibits high polymorphism; this type of marker is also less expensive for PCR-based assay. SSR markers also show high reproducibility, and therefore, has been used to assess genetic diversity in *B. napus* (Hasan et al. 2006; Gyawali et al. 2013; Leonte and Arsene 2016; Tian et al. 2017), *B. rapa* (Fu and Gugel 2009; Hobson and Rahman 2016; Tian et al. 2017), *B. oleracea* (Louarn et al. 2007; El-Esawi et al. 2016; Tortosa et al. 2017), and *B. juncea* (Singh et al. 2017; Tian et al. 2017; Patel et al. 2018), as well as to evaluate the extent of allelic diversity introgressed from exotic germplasm into cultivated crop species (Kebede et al. 2010; Attri and Rahman 2018), and to understand the relationship between the different *Brassica* gene pools (Thakur et al. 2018).

Genome content and the order of the genes in *B. napus* remained highly conserved in relation to its two progenitor species (for review, see Cheung et al. 2009). Conserved sequence homology in the flanking regions of SSR loci from closely related *Brassica* species has been reported by several researchers, such as Westman and Kresovich (1998), Gao et al. (2014) and Thakur et al. (2015) (also for review, see Suwabe et al. 2006). Due to the conserved nature of the flanking sequences of SSR loci, primer pairs designed based on one species can be used in related species to detect the same microsatellite loci (for review, see Kalia et al. 2011). A large number of SSR markers have been developed in *Brassica* (Suwabe et al. 2002; Piquemal et al. 2005; Iniguez-Luy et al. 2008; Cheng et al. 2009; Li et al. 2011; Gyawali et al. 2013; reviewed in Gyawali et al. 2016) for application in breeding and research. Recently, Thakur et al. (2018) reported 100% cross-transferability of 124 SSR loci between *B. juncea* and *B. rapa*, while 98% cross-transferability of the markers was found between the six *Brassica* species and *Eruca sativa*. They also found that the number of alleles per SSR locus can range from one to six with an average of 3.41, where the greatest number of alleles per primer pair found in *B. napus* and the least in *B. rapa*.

Assessment of genetic diversity in *B. napus* has been done by several research groups, such as Hasan et al. (2006), Wang et al. (2009), Qu et al. (2012), Chen et al. (2011) and Chen et al. (2017). Using a limited number (18 to 55) of SSR markers, these research groups were able to generate knowledge for use in breeding. For example, using only 18 markers, Wang et al. (2009) demonstrated that the Australian canola accessions could be grouped according to their pedigree relationship and their origin from breeding programs. Similarly, Chen et al. (2011) showed that the Indian accessions to be genetically distinct from the Chinese and Australian accessions using 55 markers, and Chen et al. (2017) showed, using 30 SSR markers, that the Japanese accessions

constitute a genetically distinct pool. Thus, the genetic fingerprint generated using very limited number of SSR markers can generate valuable information of genetic relatedness among the accessions. The knowledge of genetic diversity within the *B. napus* germplasm is important not only for identification of parental combinations to produce better offspring but also for the development of efficient strategy for conservation of germplasm for future use.

The objective of this study was to evaluate a set of 227 inbred lines derived from interspecific cross between a *B. napus* line and six different *B. oleracea* cultivars/lines belonging to four varieties of this species for genetic diversity, as well as to access the extent of alleles introgressed from these *B. oleracea* accessions into spring *B. napus* canola lines. To our knowledge, no study has so far been conducted to provide a comprehensive view of the *B. oleracea* gene pool using different varieties of this species for broadening the genetic base of the C genome of *B. napus*.

2.2 Materials and Methods

2.2.1 Plant materials

One zero erucic acid, low glucosinolate (< 15 μmol g^{-1} seed) spring *B. napus* line A04-73NA and six high-erucic (40% erucic acid), high glucosinolate (> 60 μmol g^{-1} seed) *B. oleracea* cultivars/lines, viz. var. *alboglabra* line-NRC (PBI), var. *botrytis* cv. BARI cauliflower-1, var. *capitata* cvs. Badger Shipper, Bindsachsener and Balbro and var. *italica* cv. Premium Crop, were used as parents. A total of 110 F_{10} and 117 BC_1F_9 inbred lines (Supplemental Table 2.1), developed from F_2 and backcross (F_1 × *B. napus* parent) of six *B. napus* × *B. oleracea* interspecific crosses, were used in this study.

The following interspecific crosses were made using A04-73NA as female:

- A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI (Ol.alb.nrc, Chinese kale)
- A04-73NA × *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1 (Ol.bot.cau, cauliflower)
- A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper (Ol.cap.bad, cabbage)
- A04-73NA × *B. oleracea* var. *capitata* cv. Balbro (Ol.cap.bal, cabbage)
- A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener (Ol.cap.bin, cabbage)
- A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop (Ol.ita.pre, broccoli)

The F_1 plants were self-pollinated for F_2 seeds and backcrossed to the *B. napus* parent for BC_1 seeds. The F_2 and BC_1 population were subjected to pedigree breeding with selection for the two canola quality traits (zero erucic acid in oil and low glucosinolate in meal). The details of the development of canola quality advanced generation *B. napus* lines from these crosses are described in Chapter 3.

2.2.2 DNA extraction

Young leaves were collected from the 227 F_{10} and BC_1F_9 lines and their seven parents (*B. napus* and *B. oleracea* cultivars/lines) grown in a greenhouse. About 200 mg bulk leaf sample from three plants of a line was placed in 2 ml safe-lock Eppendorf tube and stored in –80 ˚C for one night prior to crushing using a Mixer Mill (TissueLyser II, Qiagen, Germany). Genomic DNA was extracted using SIGMA DNA extraction kit (Sigma-Aldrich, St. Louis, MO, USA) following manufacture's instruction. DNA concentration and purity of the samples was assessed using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and the extracted DNA was diluted to 20 ng μL^{-1} and stored at –20 ˚C until use.

2.2.3 Primer sequences

A total of 418 SSR markers from nine C-genome linkage groups (LG) were used for screening the parental lines (*B. napus* line A04-73NA and six *B. oleracea* cultivars/lines) to identity the polymorphic markers. Sequences of these markers were obtained from Agriculture and Agri-Food Canada (AAFC), through a material transfer agreement (currently available in http://aafc-aac.usask.ca/BrassicaMAST/), and Biotechnology and Biological Science Research Council (BBSRC), UK (http://brassica.nbi.ac.uk/BrassicaDB/), as well as the markers published by Piquemal et al. (2005), Suwabe et al. (2002), Iniguez-Luy et al. (2008), Cheng et al. (2009), Li et al. (2011) and Hobson and Rahman (2016). The forward primer of each SSR marker was labelled with M13 tail at its 5′ end (5′-CACGACGTTGTAAAACGAC-3′) as described by Schuelke (2000), and they were labeled with universal fluorescent dyes FAM, VIC, NED and PET (Applied Biosystems, Foster City, CA). Based on polymorphism and repeatability of genotyping, 95 markers were selected for genotyping the 110 F_8 and 117 BC_1F_7 inbred lines (Supplemental Table 2.2).

2.2.4 Polymerase chain reactions (PCR)

PCR amplification of genomic DNA was performed in a total volume of 15.5 µl, which included 20 ng genomic DNA, 5× PCR reaction buffer, 25 mM $MgCl_2$, 0.6 unit *Taq* DNA polymerase (Promega Corporation, Madison, WI), 10 mM each dNTP (Invitrogene Life Technologies Inc., Burlington, ON), 5 µM of each forward and reverse primer, and 5 µM tag F (fluorescent dyes FAM, VIC, NED, and PET). PCR was carried out in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA) with the following program: 1 cycle of 5 min at 95 °C for initial denaturation; 35 cycles where each cycle consisted of 1 min at 95 °C for

denaturation; 1 min at 58 °C for annealing and 1.5 min at 72 °C for extension; and the final extension time was 15 min at 72 °C.

2.2.5 Fragment analysis

Size-based separation of the amplified DNA fragments was done using a capillary electrophoresis AB Genetic Analyzer No. 3730 (Applied Biosystems, Foster City, CA). For this, the ABI plates were prepared using 0.8 µl PCR amplification product of each of four different primers with four fluorescent dyes mixed with 8 µl highly deionized (Hi-Di) formamide and 0.06 µl 500 LIZ size standard (GeneScan^{TM} 500 LIZ®). The loaded ABI plate was incubated at 95 ˚C for two minutes followed by cooling on ice for two minutes to denature the samples prior to analysis for detection of the amplification products.

2.2.6 Genetic diversity analysis

The fragment analysis results from the ABI were scored for presence or absence of the alleles using the software program GeneMarker® version 2.4.0 (SoftGenetics LLC, State College, PA); however, all genotyping results were confirmed manually as well. The absence (0) or presence (1) of the polymorphic amplification products were scored based on fragment length, and data was recorded in a 0/1 matrix for absence/presence of the marker amplicons.

To examine the genetic relationship among the 227 inbred lines, the 0/1 matrix was used to calculate Dice similarity coefficient (Nei and Li 1979) between the lines in all possible pairwise comparison using the software program NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System software, version 2.01, Exeter Software, Setauket, NY, USA; Rohlf 2004) with the SIMQUAL model (Nei and Li 1979). Principal coordinates analysis (PCoA) was done to

compute the first two principal components of the similarity matrix data using GENALEX 6 software (Peakall and Smouse, 2006).

Polymorphic information content (PIC) for each marker locus was calculated to estimate the extent of variation for SSR marker allele present in the 227 inbred lines based on the following formula (Xu 2010): $PIC = 1 - \sum_{n=1}^{i} P_i^2$, where P_i is the frequency of the *i*th allele for the individual *P*, and *n* is the total number of alleles (Boopathi 2013). PIC was estimated for inbred lines where heterozygotes were absent and thus it is essentially a measure of allelic diversity (Shete et al. 2000).

Analysis of Molecular Variance (AMOVA) was done to estimate the extent of genetic variation present within the population and among the populations of the six crosses using GenAIEx 6.5 software (Peakall and Smouse, 2006). Calculation of genetic information statistics, such as allele frequency ($\text{FreqAllele}_X = \frac{2(\text{No.of homozygotes for allele X}) + \text{No.of heteozygotes containing the allele X}}{2(\text{total No.of samples})}$), number of alleles (*Na*), effective number of alleles (*Ne*), and Shannon's Information index (*I*) per locus were done using POPGEN 1.31 (Yeh et al. 1999).

2.2.7 Occurrence of *B. oleracea* alleles in F_{10} and BC_1F_9 populations

The occurrence of the SSR marker alleles of *B. oleracea* in the inbred population was calculated to study the inheritance of these alleles based on simple Mendelian segregation. For this, the total observed number of SSR loci in a population and the expected number of *B. oleracea* alleles in the population of the six crosses was calculated. The following formula was used to calculate the total observed number of SSR loci in a population: Number of polymorphic loci × the number of plants in that population. The expected number of *B. oleracea* alleles in the population was calculated using the following procedure: [(the total number of SSR loci in the population –

number of loci missing amplification) × 2], and the value was multiplied by 0.5 in the case of the F_2-derived population and multiplied by 0.25 in case of the BC_1-derived population. The following formula was used to calculate the observed number of *B. oleracea* alleles in the F_2 and BC_1 population: (number homozygous loci for *B. oleracea* × 2) + number of heterozygous loci for *B. oleracea* and *B. napus*.

2.2.8 Genetic structure analysis

The STRUCTURE v2.3.4 software (Pritchard et al. 2000) was used to assess the population structure of the 227 inbred lines and the parents. The run length of burn-in period and the number of MCMC (Markov Chain Monte Carlo) replications was set to 10,000 and 100,000, respectively. The number of subgroups (*K*) tested in the analysis was 2 to 14 with 10 runs per *K* value. The optimal number of subgroups (*K* value) was determined based on Δ*K* values estimated using Structure Harvester v0.6.94 (Earl and VonHoldt 2012) with the input of the log likelihood of *K* (LnP(D)) values from STRUCTURE, as described by Evanno et al. (2005). The probability of membership (Q) ≥ 60% was considered as a threshold for inclusion of the individuals to their assigned group (Lu et al. 2009; Miranda et al. 2010; Ertiro et al. 2017). The Software CLUMPAK (Cluster Markov Packager Across K) was used to identify the optimal alignment across runs within the selected *K* (Kopelman et al. 2015).

2.3 Results

2.3.1 Polymorphism of SSR markers

The 95 polymorphic SSR markers from the nine C genome chromosomes (Supplemental Table 2.2) amplified a total of 340 alleles (Supplemental Table 2.3) in the *B. napus* and *B. oleracea* parents. The number of alleles (*Na*) per locus varied from 2 to 7 with an average of 3.58,

whereas the effective number of alleles (*Ne*) per locus varied from 1.00 to 3.16 with a mean of 1.44. The Shannon's Information index (*I*) of these markers ranged from 0.00 to 0.97 with an average of 0.42 (Supplemental Table 2.4). The frequency of occurrence of an allele in the inbred population varied widely, ranging from 0.026 (rare alleles) to 0.928 (frequent alleles) with an average of 0.316 (Supplemental Table 2.3). Polymorphic information content (PIC) of these SSR markers in the 227 inbred lines varied from 0.24 for the marker BnGMS43 to 0.99 for BoGMS0081 with an average of 0.70 for all markers (Supplemental Table 2.4). Of the total 95 markers, 86 were highly informative (PIC ≥ 0.5), eight were moderately informative (PIC > 0.25 – 0.5) and only one was low informative (PIC < 0.25). Correlation between PIC value of the markers tested on the parents and PIC value of the markers tested on the inbred population was $r = 0.64$ ($R^2 = 0.41$; $P < 0.001$).

2.3.2 Occurrence of SSR alleles in the parents and inbred populations

Of the total 340 alleles, 57.9% (197/340) of the alleles could be detected in the six *B. oleracea* parents, 16.8% only in the *B. napus* parent, and 25.3% in both *B. napus* and *B. oleracea* parents; thus, a greater number of alleles was detected in *B. oleracea* as compared to *B. napus*. The 197 alleles detected only in *B. oleracea* were amplified by 94 SSR markers (excluding BnGMS681); size of these alleles varied from 100 to 494 bp, however, majority of the alleles was about 130 to 350 bp size. Of the 197 *B. oleracea* SSR alleles, some of the alleles could be detected in more than one *B. oleracea* parent and this included a total of 118 (60%) alleles; while the remaining 79 (40%) alleles could be detected in only one of the six *B. oleracea* parents, and these alleles, therefore, were considered as 'unique *B. oleracea*-parent alleles'. Of the total 197 *B. oleracea* alleles, 20 (10.2%) could not be detected in the 227 inbred lines derived from the six

interspecific crosses. In case of the unique 79 alleles of the *B. oleracea* parents, 59 (75%) were found to be introgressed in the inbred population (Table 2.1).

The AMOVA for 95 SSR markers indicated that significant (F_{ST} = 0.174, $P < 0.001$) genetic variation existed among the 227 inbred lines derived from the six crosses. About 17% of the total variation was accounted by the populations derived from the six crosses and following two breeding methods, while variation within the whole population accounted the remaining 83% of the total variation (Supplemental Table 2.5). The greater variation within the inbred population might have resulted from the wide genetic difference between the C genome of *B. napus* and *B. oleracea*; selection performed for the two canola quality traits and spring growth habit apparently did not eliminated the large genetic variation of the two parents in the inbred population.

2.3.3 Population structure analysis based on 340 SSR alleles

The statistic ΔK analysis depicted a sharp peak at K = 11 with the greatest ΔK (9.54) (Fig. 2.1B) and the smallest standard deviation (Fig. 2.1A) suggesting that the 227 inbred lines and their parents can be placed in 11 subgroups (Fig. 2.2); the individuals which could not be strongly assigned to any of the 11 subgroups due to their probability membership (Q) values less than 60% was assigned to mixed group. Most of the *B. oleracea* parents included in group G9 while var. *alboglabra* included in G3. The group G8 was the largest and included the *B. napus* parent and 60 inbred lines from the six crosses (Fig. 2.2); the remaining inbred lines formed several diverse groups which were distinct from the groups where the *B. napus* and *B. oleracea* parents are included. For example, the G1 included 53.3% of the F_2- and 38.1% BC_1-derived lines of the cross involving Chinese kale and 23.5% of the F_2- and 40.9% BC_1-derived lines of the cross

involving cauliflower, and 3.8% of the F_2-derived line of the cross involving broccoli (Supplementary Fig. 2.1). The G2 included 50.0% of the BC_1-derived lines of the cross involving cauliflower. The F_2- and BC_1-derived lines of the cross involving cabbage cv. Balbro fell into two groups, G4 and G11, where the G4 included 81.3% F_2- and 46.2% BC_1-derived lines, and G11 included 12.5% F_2- and 11.5% BC_1-derived lines. The group G5 included 46.2% of the BC_1-derived lines of the cross involving cabbage cv. Badger Shipper. About 35% F_2-derived lines of the cross involving cabbage cv. Bindsachsener, 73.7% F_2-derived lines of the cross involving the cabbage cv. Badger Shipper, and 88.5% BC_1-derived lines of the cross involving cabbage cv. Bindsachsener clustered in G6, G7 and G10, respectively (Fig. 2.2). A summarized version of the Fig. 2.2 is presented as Supplemental Fig. 2.1.

Principal coordinate analysis (PCoA) was done to further confirm the results from structure analysis. PCoA displayed 10 overlapping groups (Supplemental Fig. 2.2) without clear differentiation. However, this broadly agreed with the grouping based on structure analysis excluding the grouping of the *B. oleracea* parents which in PCoA analysis, all *B. oleracea* parents fell into one group (G9, Supplemental Fig. 2.2). The PCoA explained a total of 70.6% of the total variation where the 1st, 2nd and 3rd coordinates explained 29.6%, 26.2% and 14.8% variation, respectively. Of the 227 inbred lines, 18 included in G7 which positioned closest to the *B. oleracea* parents; this group included 14 F_2-derived lines the cross involving cabbage cv. Badger Shipper (Supplemental Fig. 2.2).

2.3.4 Population structure analysis based on *B. oleracea* alleles

The population structure of the 227 inbred lines and their six parents based on 197 *B. oleracea* alleles was assessed using STRUCTURE. This analysis depicted a sharp peak at $K = 5$ with the

greatest ΔK (9.23) (Supplemental Fig. 2.3B) and the lowest variance (Supplemental Fig. 2.3A) indicating that the 227 inbred lines and their parents can be placed in five groups (Fig. 2.3).

In contrast to structure analysis using the 340 SSR alleles of *B. oleracea* and *B. napus* (Fig. 2.2), there was a tendency of clustering the inbred lines with their respective *B. oleracea* parent when the analysis was done using only the *B. oleracea* alleles (Fig. 2.3). For example, of the total 36 inbred lines derived from the cross involving Chinese kale, 52.8% of the lines clustered in G1, and 41.7% clustered in G3 together with their *B. oleracea* parent (Supplementary Fig. 2.4). Similarly, 50% of the BC_1-derived lines of the cross involving cauliflower, and more than 85% of the lines derived from the crosses involving cabbage cvs. Badger Shipper and Bindsachsener clustered, respectively, in G5, G4 and G2 together with their respective *B. oleracea* parent. In contrast, 69.1% of the inbred lines derived from the cross involving cabbage cv. Balbro clustered in G1 while their *B. oleracea* parent fell in a mixed group. A summarized version of the Fig. 2.3 is presented as Supplemental Fig. 2.4.

2.3.5 Inheritance of *B. oleracea* alleles in F_{10} and BC_1F_9 populations

Theoretically, without any selection, it was expected that the F_{10} and BC_1F_9 generation populations would carry about 50% and 25% of the *B. oleracea* alleles, respectively. However, the occurrence of *B. oleracea* allele's in the F_2-derived inbred populations was significantly less than the expected number. Based on pooled data of the six crosses, the observed number of *B. oleracea* alleles in the F_2-derived population was 48.1% (4109/8548) and in BC_1-derived population it was 92.4% (4256/4605) of the expected number of alleles (Table 2.2). Among the F_2-derived populations of the six crosses, populations derived from the crosses involving cabbage cvs. Badger Shipper and Bindsachsener carried greater proportion (61.0 – 72.6%) of the

expected number of alleles, while the population based on broccoli carried the least proportion (16.2%) of the *B. oleracea* alleles (Table 2.2). However, this contrasting difference could not be found among the six BC_1-derived populations.

2.4 Discussion

In the present study, analysis of the *B. napus* canola recombinant inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses revealed that about 75% (59/79) of the unique SSR alleles of the *B. oleracea* parents were introgressed in this population; the greatest introgression occurred in the population derived from the cross involving var. *capitata* cv. Bindsachsener and the lowest in the population derived from the cross involving var. *italica*. According to Chen et al. (2008), the frequency of occurrence of the unique alleles can be used to describe the genetic distinctness of the *B. napus* germplasm, and the occurrence of unique alleles among the *B. napus* populations, collected from different parts of the world, has been reported to be rare (Gyawali et al. 2013). In this regard, the unique *B. oleracea* alleles introgressed in this study, indeed, will broaden the genetic base of spring *B. napus* canola. However, a loss of about 25% unique alleles occurred during the development of the inbred lines from these interspecific crosses. Attri and Rahman (2018) reported that a loss of about 55% *B. rapa* alleles can occur in the F_8 progeny of *B. napus* × *B. rapa* interspecific cross, and this loss primarily occur in early generation. Therefore, selection for unique alleles will be needed in early generations to accumulate a greater number of these alleles in the progeny of interspecific crosses involving *B. napus* and its parental species.

Introgression of unique alleles, specifically from *B. oleracea* into *B. napus*, would broaden the genetic base of *B. napus* canola, especially its C genome which is known to have a narrow

genetic base as compared to its A genome (Bus et al. 2011). The narrow genetic diversity in canola germplasm apparently resulted from the bottleneck during the evolution of *B. napus* from a limited number of variants of its progenitor species (*B. rapa*, A-genome and *B. oleracea*, C-genome) as well as from intensive breeding for canola quality cultivars over the last few decades (for review, see Rahman 2013).

STRUCTURE analysis based on 340 SSR alleles placed the 227 lines into nine groups, while the remaining two groups were represented by the *B. oleracea* parents. One of these group included the var. *alboglabra* (G3) and the other group (G9) included three *capitata* and the *italica* parents; the parent var. *botrytis*, shared a probability of membership 58% and 38%, respectively, with G9 and G2. This indicate that the var. *alboglabra* to be distinct from var. *botrytis*, var. *capitata* and var. *italica*. This was also evident from STRUCTURE analysis based on *B. oleracea* alleles, where a clear genetic difference between these four varieties of *B. oleracea* could be established (Fig. 2.3). Genetic distinctness between these four varieties of *B. oleracea* has also been reported by Louarn et al. (2007), Izzah et al. (2013) and Pelc et al. (2015). According to Izzah et al. (2013) var. *botrytis* and var. *italica* show a high degree of similarity; our results from STRUCTURE analysis based on 340 alleles as well as the analysis based on only the *B. oleracea* alleles also support this (Fig. 2.2 and 2.3). According to Branca (2008), the var. *botrytis* probably originated from var. *italica* and has been introduced in west Europe from the east side of the Mediterranean coast which is believed to be its center of origin (Quiros and Farnham 2011). The genetic distinctiveness of var. *alboglabra* from other accessions of *B. oleracea* could be due to its independent origin from *B. cretica* ssp. *nivea* (Snogerup 1980). *B. oleracea* var. *alboglabra* has been domesticated in China (Pua 1993; Quiros and Farnham 2011) from early introductions from Europe (Herve 2003), and currently it is an important vegetable in this country. Taken

together, these lines of evidence support the results from our study that the var. *alboglabra* to be genetically distinct from other varieties of this species.

Lack of the inbred lines with probability membership (Q) ≥60% in the above-mentioned two groups, G3 and G9, where the *B. oleracea* parents were included, could be related to the number of alleles originating from the *B. napus* parent. Of the 340 SSR alleles used in this analysis, about 42% (16.8 + 25.3%) of the alleles could potentially have originated from the *B. napus* parent; this significant contribution of alleles from the *B. napus* parent and selection for canola quality traits during the development of these lines might have resulted in the inclusion of about a quarter (60/227) of the inbred lines from all six crosses in G8 where the *B. napus* parent was included. However, the remaining 167 lines formed several groups, which were genetically distinct from G8; this indicates that these lines are genetically distinct from *B. napus*. Introgression of alleles from *B. oleracea* into *B. napus* through interspecific cross between these two species has also been reported by Rahman et al. (2015) using a single variety of *B. oleracea*. However, the results from STRUCTURE analysis showing a tendency of clustering the inbred lines with their respective *B. oleracea* parent (Fig. 2.3) demonstrate that the wide diversity exists in this diploid species can be used for broadening the genetic base of *B. napus* canola.

While comparing the populations developed through two different breeding methods, the F_2-derived population carried about half (48.1%) of the expected number of *B. oleracea* alleles, while the BC_1-derived population received almost full complement (92.4%) of the expected number of the *B. oleracea* alleles. This difference might have resulted from stronger selection for the two canola quality traits (zero erucic acid in seed oil and low glucosinolate in seed meal) in the F_2-derived population as compared to the BC_1-derived population during the development of these inbred lines in early generations; all inbred lines used in this study were canola quality

type. Furthermore, higher meiotic anomalies and sterility in F_2 as compared to BC_1 population (Iftikhar et al. 2018) might have resulted in an elimination of a greater number of plants and consequently greater number of *B. oleracea* alleles. Rahman et al. (2015) reported introgression of up to 54% alleles of var. *alboglabra* in F_8 families, while Li, Zhou et al. (2014) reported introgression of 29.9% genome components of var. *acephala* in F_4 population derived from *B. napus* × *B. oleracea* interspecific crosses. Similarly, Attri and Rahman (2018) found about 45% alleles of *B. rapa* in F_4 and F_8 populations of *B. napus* × *B. rapa* interspecific cross. Thus, there are evidences to support that a large proportion of the alleles of the diploid species can get eliminated during the development of inbred *B. napus* lines from interspecific cross involving this amphidiploid species and its diploid progenitor species. However, the results from our study shows that the loss of alleles occurs at a relatively lower frequency in the BC_1-derived populations than in the F_2-derived populations.

In conclusion, results from this study demonstrated that the wide diversity exists in *B. oleracea* gene pool can be exploited for broadening the genetic base of *B. napus* canola. The novel alleles, which do not exist in *B. napus*, can be introgressed from *B. oleracea*; however, loss of some of the *B. oleracea* alleles can occur during the development of canola quality inbred lines from *B. napus* × *B. oleracea* interspecific crosses, where a greater loss apparently occurs in F_2-derived population as compared to BC_1-derived population. Therefore, selection in early generation for *B. oleracea* alleles will be needed to retain a greater number of alleles in the reconstituted *B. napus* lines derived from *B. napus* × *B. oleracea* interspecific crosses.

2.5 Tables

Table 2.1 Occurrence of 79 SSR alleles unique to the *Brassica oleracea* parents in the inbred *B. napus* lines derived from six *B. napus* × *B. oleracea* interspecific cross progenies

Populations	No. inbred lines	SSR marker	No. alleles unique to the *B. oleracea* parent	Total no. unique alleles detected in the population	Frequency of unique alleles in the population[1]
Ol.alb.nrc	36	sN2087 - **sN3734** - sN0691 - sS2268 - sORE66 - sR1863 - BoGMS0570 - MR140 - sNRG34 - BoGMS0632 - sORF37 - CB10028 - BnGMS352	14	83	0.16
Ol.bot.cau	39	sN2087 - CB10036 - BoGMS0953 - MR140 - Ol11H02a - sNRG34 - **BnGMS347** - BoGMS0836 - sN0761 - BoGMS0590 - sORB17 - sNRD41 - sN3825J - sN0706	15	83	0.14
Ol.cap.bad	33	sN3734 - Sn11675 - CB10036 - BoGMS0819 - BoGMS0570 - BoGMS0953 - BoGMS0081 - BnGMS347 - sN0761 - sN11661 - BnGMS386 - BnGMS4 - BoGMS0468 - **BoGMS0741**	15	92	0.19
Ol.cap.bal	42	sN1834 - Sn11675 - sS2206 - sR1863 - sN2316 - sN2052 - sN11661 - BoGMS0632 - sN12743J - sN0706 - BoGMS0468 - BoGMS0845	12	31	0.06
Ol.cap.bin	43	sORE66 - sR1863 - BoGMS0570 - BoGMS1360 - BoGMS0081 - sN2052 - sN12503 - sN0706 - BoGMS0741	9	108	0.28
Ol.ita.pre	34	Sn11675 - sNRE74 - sN1937 - sS2206 - CB10057 - BoGMS0819 - CB10493 - sR0357 - sNRG34 - sN0761 - **sORB17** - sN3825J - sN0706	14	15	0.03
Total	227		79		

Note: SSR markers amplified more than one allele are bolded; they amplified different size alleles in the same parents, e.g. the marker sN3734 amplified 282 and 291 bp alleles in *B. oleracea* var *alboglabra*. Alleles of the SSR markers could not be detected in the inbred population are underlined.

Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop

[1] Frequency of unique alleles in the individual population was calculated based on Total number of unique alleles detected in the population divided by No. inbred lines × No. alleles unique to the *B. oleracea* parent

Table 2.2 Inheritance of 340 SSR alleles based on 95 SSR markers in the 227 inbred *B. napus* lines derived from six *B. napus* × *B. oleracea* interspecific crosses and following two breeding methods

Breed method[1]	Cross[2]	Number lines	No polymorphic loci	Total no. of SSR loci[3]	No. loci homo. for B.o. allele (%)	No. loci het. for B.o. and B.n. alleles (%)	No. loci homo. for B.n. alleles (%)	No. loci missing ampli-fication (%)	Total obs. B.o. alleles[4]	Total exp. B.o. alleles[5]	% of the exp. no. of B.o. alleles obs.[6]	Chi-square (Segr. for alleles)[7]
F_2	Ol.alb.nrc	15	80	1200	230(19.2)	51(4.3)	845(70.4)	74(6.2)	511	1126	45.4	671.8*
	Ol.bot.cau	17	77	1309	208(15.9)	26(2.0)	1001(76.5)	74(5.7)	442	1235	35.8	1018.4*
	Ol.cap.bad	19	90	1710	529(30.9)	202(11.8)	910(53.2)	69(4.0)	1260	1641	76.8	176.9*
	Ol.cap.bal	16	85	1360	249(18.3)	62(4.6)	982(72.2)	67(4.9)	560	1293	43.3	831.1*
	Ol.cap.bin	17	87	1479	395(26.7)	157(10.6)	865(58.5)	62(4.2)	947	1417	66.8	311.8*
	Ol.ita.pre	26	73	1898	191(10.1)	7(0.4)	1638(86.3)	62(3.3)	389	1836	21.2	2280.8*
Total									4109	8548	48.1	
BC_1	Ol.alb.nrc	21	80	1680	357(21.3)	87(5.2)	1116(66.4)	120(7.1)	801	780	102.7	3037.1*
	Ol.bot.cau	22	77	1694	353(20.8)	114(6.7)	1091(64.4)	136(8.0)	820	779	105.3	2956.3*
	Ol.cap.bad	14	90	1260	235(18.7)	59(4.7)	948(75.2)	18(1.4)	529	621	85.2	2879.3*
	Ol.cap.bal	26	85	2210	395(17.9)	116(5.2)	1591(72.0)	108(4.9)	906	1051	86.2	4824.0*
	Ol.cap.bin	26	87	2262	409(18.1)	156(6.9)	1634(72.2)	63(2.8)	974	1100	88.6	4928.6*
	Ol.ita.pre	8	73	584	99(17.0)	28(4.8)	422(72.3)	35(6.0)	226	275	82.3	1309.1*
Total									4256	4605	92.4	

[1] Breeding method: F_2 = F_2-derived population; BC_1 = BC_1-derived population.

[2] Ol.alb.nrc = *B. napus* × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* × *B. oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = *B. napus* × *B. oleracea* var. *italica* cv. Premium Crop.

[3] Number of polymorphic loci × number of lines.

[4] Calculated based on the following formula: (number homozygous loci for *B. oleracea* × 2) + number of heterozygous loci for *B. oleracea* and *B. napus*.

[5] (Total number SSR loci in the population – number loci missing amplification) × 2] × 0.5 for F_2-derived population, and (Total number SSR loci in the population – number loci missing amplification) × 2] × 0.25 for BC_1-derived population.

[6] Calculated based on the following formula: (total number of observed *B. oleracea* alleles / total expected number of *B. oleracea* alleles) × 100.

[7] Chi-square test for goodness of fit was done based on the observed and expected number of *B. oleracea* and *B. napus* alleles. Asterisk indicates *p*-values <0.05.

2.6 Figures

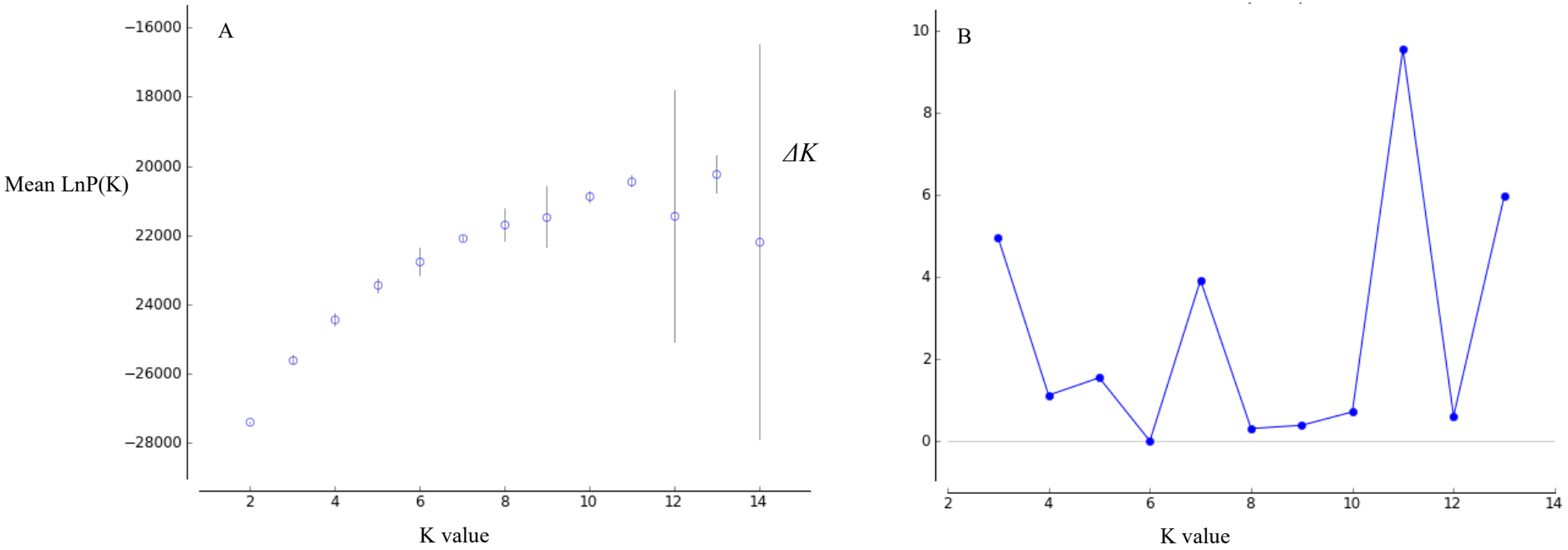


Fig. 2.1 The estimated mean log-likelihood of *K* values with standard deviation (A) and *ΔK* values (B) for the population of 227 *Brassica napus* inbred lines derived from *B. napus* × *B. oleracea* interspecific crosses and estimated using 340 SSR alleles. Analysis was done following Evanno et al. (2005) to examine the rate of change of the slope of the log probability curve over the range of *K* values.

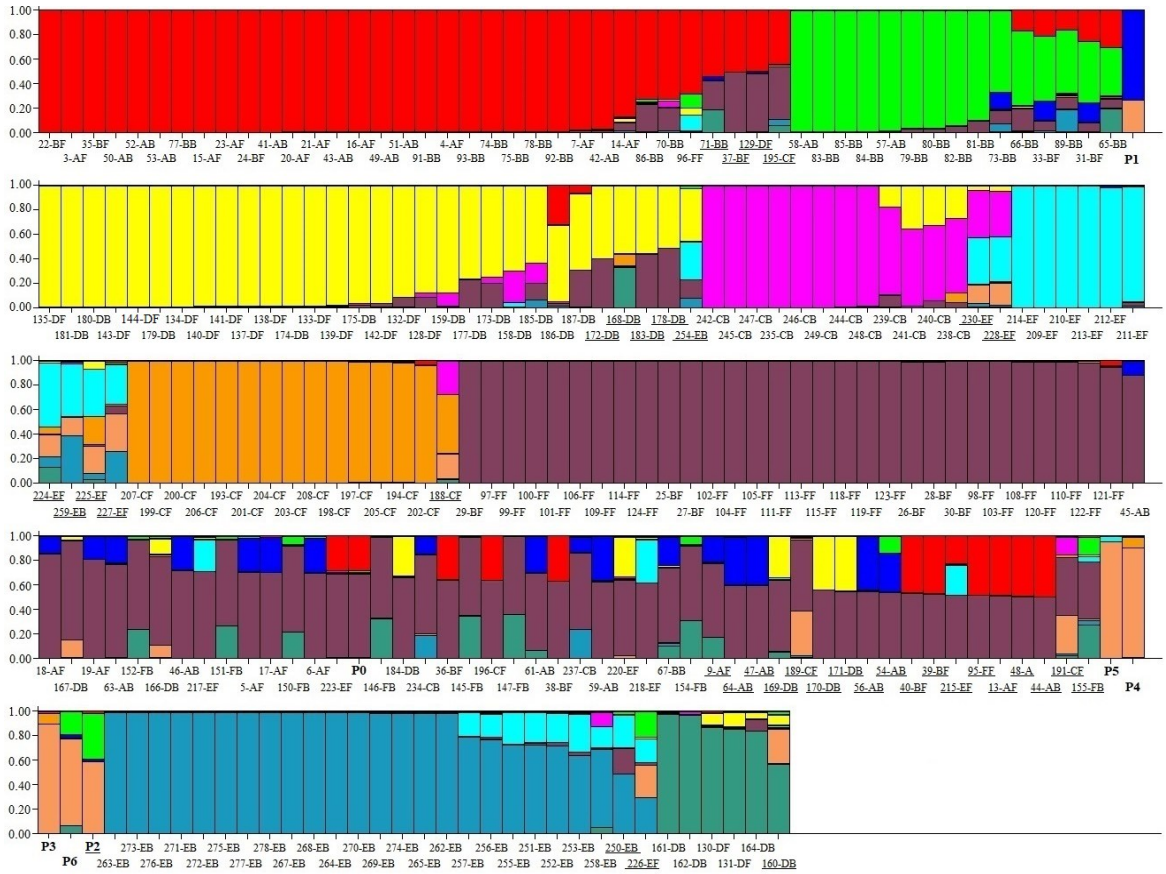


Fig. 2.2 Population structure of the population of 227 *Brassica napus* inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses and following two breeding methods and their parents based on 340 SSR alleles of *B. napus* and *B. oleracea* and using STRUCTURE at *K* = 11 (G = group). The inbred lines are represented by vertical bar on the x-axis, and the groups are differentiated by color. G1 = red, G2 = green, G3 = dark-blue, G4 = yellow, G5 = pink, G6 = blue, G7 = dark goldenrod, G8 = hot pink, G9 = light salmon, G10 = deep sky blue and G11 = aquamarine. Y-axis shows the probability membership (Q) in the group.

Parents: P0 = *B. napus* parent (A04-73NA); P1 = *B. oleracea* var. *alboglabra* line NRC-PBI; P2 = *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; P3 = *B. oleracea* var. *capitata* cv. Badger Shipper; P4 = *B. oleracea* var. *capitata* cv. Balbro; P5 = *B. oleracea* var. *capitata* cv. Bindsachsener; P6 = *B. oleracea* var. *italica* cv. Premium Crop.

Cross: A = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI (*n* = 36); B = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1 (*n* = 39); C = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper (*n* = 33); D = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro (*n* = 42); E = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener (*n* = 43); F = A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop (*n* = 34).

Inbred line description: The first three numbers (before hyphen) indicate the inbred line number (detailed pedigree of the lines presented in Supplemental Table 2.1). After hyphen, the first alphabet (A to F) indicate the cross from where the line was developed, and the last alphabet (F or B) indicate which breeding method (F = F_2-derived population; B = BC_1 (F_1 × *B. napus*)-derived population) was followed to develop these lines. The underlined lines are clustered in mixed group; they could not be strongly assigned to any of the groups due to their probability membership (Q) value less than 60%.

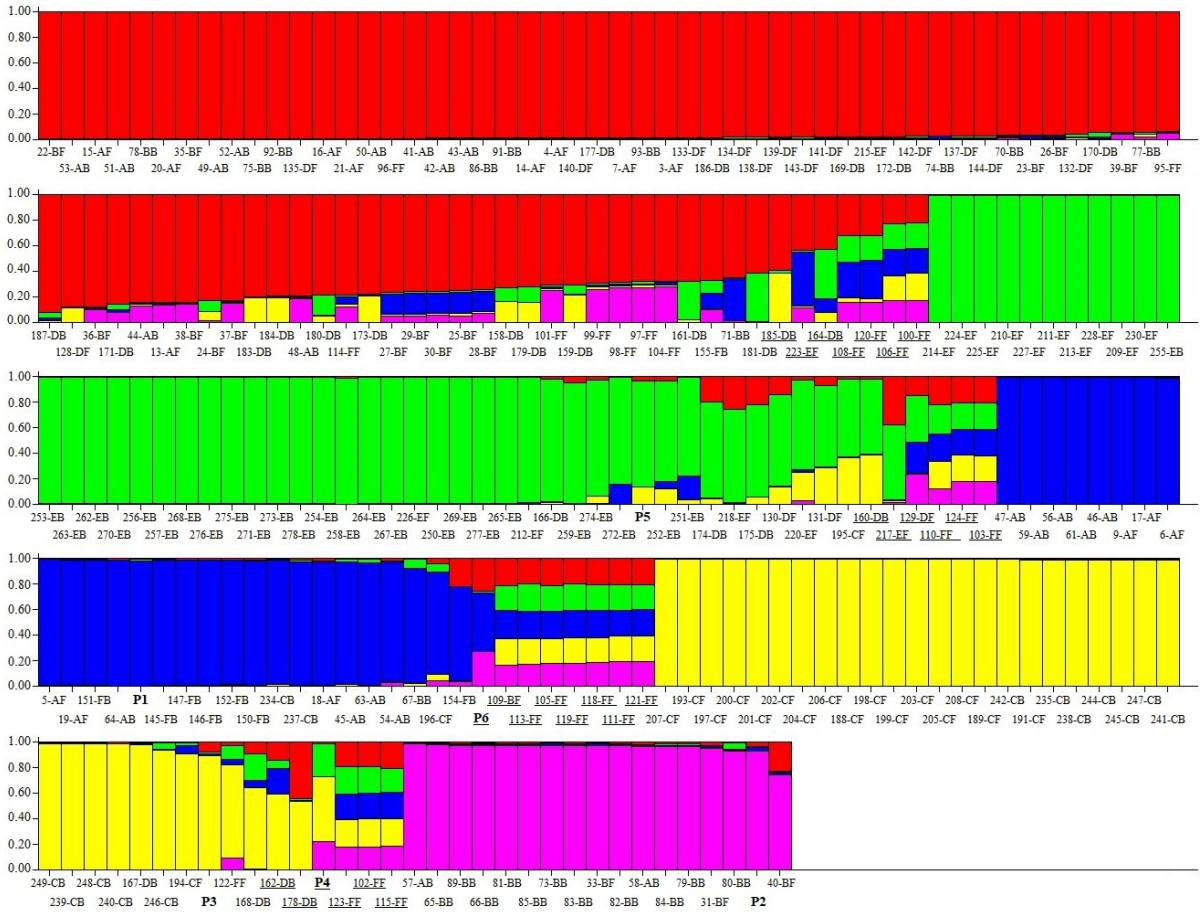


Fig. 2.3 Population structure of the population of 227 *Brassica napus* inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses and following two breeding methods and their parents based on 197 SSR alleles of *B. oleracea* and using STRUCTURE at $K = 5$ (G = group). The inbred lines are represented by vertical bar on the x-axis and the groups are differentiated by color. G1 = red, G2 = green, G3 = dark-blue, G4 = yellow, and G5 = pink. Y-axis shows the probability membership (Q) in the group.
Parents: P1 = *B. oleracea* var. *alboglabra* line NRC-PBI; P2 = *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; P3 = *B. oleracea* var. *capitata* cv. Badger Shipper; P4 = *B. oleracea* var. *capitata* cv. Balbro; P5 = *B. oleracea* var. *capitata* cv. Bindsachsener; P6 = *B. oleracea* var. *italica* cv. Premium Crop.

Description of the crosses and inbred lines is the same as described in Fig. 2.2 legend.

2.7 Supplemental materials

Supplemental Table 2.1 The list of the 227 inbred *Brassica napus* lines derived from F_2 and BC_1 of six *Brassica napus* × *B. oleracea* interspecific crosses

Inbred line number	Inbred line code	Cross	Breeding method[1]
1300-353	3	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-355	4	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-360	5	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-363	6	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-368	7	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-375	9	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-398	13	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-401	14	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-404	15	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-410	16	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-412	17	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-413	18	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-416	19	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-419	20	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-420	21	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1343-320	22	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-321	23	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-323	24	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-327	25	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-329	26	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-330	27	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-333	28	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-336	29	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-339	30	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-343	31	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-349	33	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-353	35	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-357	36	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-360	37	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-362	38	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-367	39	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-368	40	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1676-361	41	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-363	42	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-365	43	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-377	44	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-380	45	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-389	46	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-393	47	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-402	48	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-405	49	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-407	50	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-409	51	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-412	52	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-413	53	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-416	54	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-422	56	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-423	57	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-427	58	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-429	59	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-438	61	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-442	63	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-446	64	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1677-326	65	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC

Inbred line number	Inbred line code	Cross	Breeding method
1677-328	66	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-330	67	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-342	70	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-344	71	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-351	73	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-352	74	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-355	75	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-360	77	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-363	78	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-375	79	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-376	80	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-379	81	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-383	82	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-386	83	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-387	84	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-390	85	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-394	86	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-405	89	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-411	91	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-414	92	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-418	93	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1358-594	95	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-609	96	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-615	97	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-616	98	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-620	99	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-623	100	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-624	101	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-634	102	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-635	103	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-640	104	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-652	105	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-656	106	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-667	108	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-679	109	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-685	110	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-688	111	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-701	113	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-703	114	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-705	115	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-719	118	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-720	119	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-727	120	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-731	121	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-739	122	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-747	123	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-752	124	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1392-300	128	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-303	129	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-305	130	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-306	131	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-312	132	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-313	133	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-319	134	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-320	135	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-324	137	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-325	138	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-327	139	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-329	140	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-337	141	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-339	142	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F

Inbred line number	Inbred line code	Cross	Breeding method
1392-342	143	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-345	144	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1678-263	145	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-264	146	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-265	147	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-277	150	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-281	151	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-285	152	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-291	154	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-309	155	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1679-354	158	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-357	159	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-369	160	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-377	161	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-378	162	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-382	164	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-399	166	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-405	167	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-420	168	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-430	169	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-437	170	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-440	171	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-442	172	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-460	173	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-465	174	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-470	175	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-474	177	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-483	178	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-486	179	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-497	180	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-502	181	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-506	183	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-511	184	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-535	185	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-541	186	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-543	187	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1362-149	188	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-152	189	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-156	191	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-161	193	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-162	194	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-164	195	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-165	196	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-166	197	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-167	198	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-169	199	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-170	200	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-171	201	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-173	202	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-174	203	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-175	204	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-176	205	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-177	206	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-179	207	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-180	208	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1363-164	209	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-165	210	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-168	211	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-170	212	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-171	213	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F

Inbred line number	Inbred line code	Cross	Breeding method
1363-173	214	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-177	215	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-180	217	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-181	218	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-183	220	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-190	223	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-194	224	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-195	225	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-197	226	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-202	227	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-205	228	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-207	230	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1681-084	235	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-083	234	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-086	237	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-090	238	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-091	239	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-092	240	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-096	241	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-097	242	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-100	244	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-101	245	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-102	246	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-103	247	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-104	248	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-105	249	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1682-099	250	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-100	251	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-101	252	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-102	253	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-103	254	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-104	255	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-105	256	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-108	257	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-113	258	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-120	259	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-128	262	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-130	263	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-131	264	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-133	265	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-138	267	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-140	268	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-143	269	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-145	270	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-147	271	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-149	272	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-150	273	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-152	274	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-154	275	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-155	276	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-156	277	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-158	278	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC

[1] F = F_2-derived lines; BC = BC_1-derived lines

Supplemental Table 2.2 List of 95 SSR markers used for genotyping 110 F_{10} and 118 BC_1F_9 inbred *Brassica napus* lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses.

Source	Primer #	Primer name	Linkage group	Allele size (bp)	Motif	Forward primer sequence 5`-3`	Reverse primer sequence 5`-3`
AAFC[1]	2278	sN2087	C1	475	TC	GAACCTCGAAAACGGTTGAA	CTCCCCCGATCTATACCCAT
AAFC	2279	sN3734	C1	273	CT	CCCCTTCCGGTTAAACAAAT	AAAACAGACTTTGCCCGTTG
AAFC	2286	sN0691	C1	375	GT	GCAAATCTTGTTTTTGTGAGTACA	GTCTTGGAAGCAGCCTAACG
AAFC	2299	sR1078	C1	402	TGA/TGG	GGCGTGGGAGTAGGTGTAGA	GACTGATACCATCACGGGCT
AAFC	2300	sNRF94	C1	310	CT	GATGACTGTGCCTGCTAAACC	GCATCTCGATTCAATCCTCC
AAFC	2301	sN3569F	C1	189	CT	TGTACGTGCACCACGTTTTT	CTTCGATTACTCGGTGGCAT
AAFC	2310	sN11675	C1	263	GA	ATATTGGGGGTCCTGGAGTC	TCCTTGCTTGAGCCTTTCAT
AAFC	2309	sN1834	C1, C3, C9	277	GA	CCGTGAACGTCATTGATCTG	CCTTCTCATACCTCTCCCCC
AAFC	2297	sN11657	C1, C4	248	GA	CAGGTTGGTTTGACATGGTG	GCACACAGAGTGACGTTTGG
AAFC	624	sN0758	C1, C5, C7	346		ATTCAGCGTCTGATGCAGTG	ATGGGGTAATGCACCAAAAA
AAFC	2311	sN0842	C1, C9	438	GA	AAGCCTGACAATCCAAAACG	CGATTTCATGGCAAATTCCT
AAFC	262	sR10417	C2	247		CGGAGAAGAAACGAGCATTC	TAGGGTTTCTGACCCGATTG
AAFC	315	sN3761	C2	173		CGACAGAGGGTTCAAATGGT	CGGTGTGTAGGTCTGCTCAA
AAFC	2059	sNRE74	C2	158		CAATCATGAATATCGGCAACA	CGTCATTCCAAACTTTAGGTCA
AAFC	2069	sR2028	C2	251		ATGCCCCATGTGGATTGTAG	TTTGGTGGAAACCGATGAAT
AAFC	2072	sS2206	C2	120		TTTCATCATTTCGACTCACCC	TTATCTTCTCTCATTTCGCCG
AAFC	2082	sR1863	C2	257	$(ACA)_8$	TTTGATGGGTCTTCATCTTC	GAGGTTAAGGGTTTGGAGTT
Cheng *et al*. (2009)	2222	BnGMS633	C2	342	$(AT)_8$	CCAGTTCCATTCTCAATCAG	TATTTGTGTTCTCACGATGG
AAFC	2062	sN1825	C2, C3	185		CCACTGAGCGGTAGAGAAGG	CGGACTTTTACGGTGTTCGT
AAFC	2065	sS2268	C2, C3	185		CTTCTGCTCTGGCTGAAACA	TGATGTCTTCGCTGCTGTCT
AAFC	2366	sN3815	C2, C4, C6, C7	482	AG	TTCAAGCTATGCAGTGTGGC	GGTCTGGAAATCGCTGCTT
AAFC	2075	sORE66	C2, C5, C8	322		CGAGGTGGGAGAGATGAGAG	ATGGAACGCCAAAACAAAAA
AAFC	2063	sN1937	C2, C6	281		CCCGCACTTTCTTCCTATTG	GGTGATGGTAACGAGCGATT
AAFC	110	sN2316	C3	275		GAGTCGTCAGCGTCTTCCTC	TTTGATTCCCTCTGCATTCC
Piquemal *et al*. (2005)	435	CB10036	C3	179-185		ATTCATCTCCTGCTCGCTTAG	AAACCCAAACCAAAGTAAGAA
Piquemal *et al*. (2005)	439	CB10057	C3	190-220		CTAGGCTAAGGAAGATTGTCA	TAGTTTCTTCCTCCTGCTATC
Li *et al*. (2011)	1082	BoGMS0819	C3	114		AGGGAGATGGACACATTTAG	GAGAGAGGGCAAAGAAGATAG
Li *et al*. (2011)	1085	BoGMS0767	C3	114		AAACAAGTCAGATTCACCAAA	CTCTTCACCACTACCACAGTC
Li *et al*. (2011)	1092	BoGMS0570	C3	214		TACAATCTTCTTCGCTGCT	AAACCTGAAACTCCCTCAA
Li *et al*. (2011)	1104	BoGMS1360	C3	318		GAGACCAGAGAAGGAGGAAC	CACTCACTATCACACACACTCA
Li *et al*. (2011)	1107	BoGMS0953	C3	133		CCTCGTAAGTAACCGAATCA	AAACAGAAGATGGAGAAGGAG
Li *et al*. (2011)	1123	BoGMS0081	C3	293		AGTCCTAATGGTGCTCTTTGT	CTGTTGAGGTGTTGTCCTTT
AAFC	302	sS2277	C4	223		GATCTGCGGTAGGAATCGAA	CGTGCTACATAATAGGGAAAAACC

Source	Primer #	Primer Name	Linkage group	Allele size (bp)	Motif	Forward primer sequence 5`-3`	Reverse primer sequence 5`-3`
Piquemal *et al*. (2005)	731	CB10109	C4	281		GTGTAGCCAGCTTGATCCT	CTTCTTCTGATGCAGCAGTG
BBSRC[2]	764	Ra2-F11A	C4	211		TGAAACTAGGGTTTCCAGCC	CTTCACCATGGTTTTGTCCC
BBSRC	982	MR140	C4	143		CCCATATTCTAATCGTTCCA	TTCACTCATTCTTTGCTCATT
BBSRC	989	Ol11H02a	C4	203	AAC	TCTTCAGGGTTTCCAACGAC	AGGCTCCTTCATTTGATCCC
Piquemal *et al*. (2005)	990	BRAS061	C4	210-246		GCAGCCTTCAACTCCCATAGA	TGGGTTCGAGCAGGGTTC
Piquemal *et al*. (2005)	994	CB10493	C4	184-222		TGACGTGTGAGCAACAGA	CTGAGTCACAAGCCGAGT
AAFC	2099	sR0357	C4	376		CCGGCTCTTGTTTTATGGTT	AACACCGTTTCATCTTTGGC
AAFC	2113	sN3685R	C4	285		CCGCAAGCTCTTAACTCCAC	AACTGCATTCGTCCAGCTCT
AAFC	2115	sN3817	C4	169		CCTGCCGTAACGTTCTTGTT	ATCTTCGAAGCAATCTCGGA
Cheng *et al*. (2009)	2200	BnGMS347	C4	273	$(AT)_{14}$	TCACACAAATCTCCTCCTCT	AGGTATCAGCCAATGACTTC
Cheng *et al*. (2009)	2225	BnGMS681	C4	131	$(GT)_{8}$	GTCGAAGATTGTTGTCAGGT	TTCACGAAGAACCCTAGAAA
Li *et al*. 2011	2233	BoGMS0836	C4	146	$(AT)_{16}$	CATAAACACACCGAACAAGAC	ACGCAATGACACACATACAC
AAFC	2379	sN12743J	C4, C6, C8, C9	370	CT	CTAGCCACCATGAAAGGAGC	AAACCAAGCAAACCCATCAG
AAFC	2102	sNRG34	C4, C9	294		TCTCATTTTTCCTCAAGCTCC	CCACCAGCCATAGTCATCCT
AAFC	54	Snrco3	C5	192		AACTCATCGGGTCAAATTGC	GAAGAACAGAAGCAGCACCC
AAFC	616	sORA84	C5	178		CAAGAAACACCATCATTTCTCAA	GGCCCATTGATATGGAGATG
AAFC	621	sN2052	C5	416		GCTCCCAAGAGCAACAC	TCACAGTTGATCCCTGTTAAT
AAFC	721	sN0761	C5	298		CGGAATTAGTGGAGTGGGAA	TATCACTGTTGTCTGCCCCA
Li *et al*. (2011)	1056	BoGMS0590	C5	399		TGGTTTATCTTCATTCTTTGG	TATTGAGTTGTCGCACTTGA
AAFC	2445	sORB17	C5	414	CA	ACCATTGAGGTTTGTCGGAG	AAAGCTTCGGCAATAATGGA
AAFC	2448	sN12153I	C5	181	TCC/GCC	CCTCTCCCTTGGCTCTTCTT	CTGAGGAGAGGGTTTAGCGG
AAFC	2452	sN11661	C5	341	CT	CAGTCAATACTCGCCGAACA	AATCGGAGGGGCCATTATAG
AAFC	2453	sN12503	C5	290	AGG	CACGGAGGAACAGAGGAGAG	TCCCACTGGCCATAGTTAGG
AAFC	2477	sR0622	C5	377	AGG	CTTGGGAAGTTCAGGAGCAG	CCGGAACAAGCATAAGAGGA
AAFC	607	sN7410	C5, C8	155		CAGATGGGAAGAGCAAAAGC	ATGCCCTGGAGTCAATGTTC
Piquemal *et al*. (2005)	733	CB10211	C6	150		CAGCAGAGATCGATGGAG	ATAGAAGGCTGCCCCTC
BBSRC	756	Na10-C06	C6	223		TGGATGAAAGCATCAACGAG	ATCAATCAACACAAGCTGCG
Suwabe *et al*. (2002)	991	BRMS-015	C6	263	$(TG)_{4}$, $(GA)_{20}$	TCGCCAATAGAACCCAAAACTT	CATCTCCATTGCTGCATCTGCT
Cheng *et al*. (2009)	2213	BnGMS491	C6	161	$(AT)_{10}$	AAGTGTGTATTAGGGACGAGT	TCCCGTACTTCAAGCTGTAT
Li *et al*. (2011)	2236	BoGMS0632	C6	201	$(AG)_{20}$	CATCATCGTCCTCTTCTTCTTC	TATCATCCTTATTGGGTCTC
AAFC	2365	sN11904	C6	239	CTT	CAATGGATCGGATGGAGATT	GTCTTGTCTTCATGGTCGGG
AAFC	2373	sS2352	C6	183	GA	TGAGAAGGGGAACAGTCGAT	TGTGTTGTTTTGGATTTTGG
AAFC	2374	sN11862	C6	290	AC	AGGGACAACGAGCATACCAC	AGGCGCCTTCAATCCTATTT
AAFC	2122	sNRD41	C7	241	GT/GA	AAAGGGCGGTCTAGCATCTT	CGTCAATGCTCAAATCCCTT
Cheng *et al*. (2009)	2205	BnGMS386	C7	220	$(AT)_{12}$	TTGGCTCATCAATGACAATA	ACAATGTGGTAAACACGAAA

Source	Primer #	Primer Name	Linkage group	Allele size (bp)	Motif	Forward primer sequence 5`-3`	Reverse primer sequence 5`-3`
Li *et al*. (2011)	2242	BoGMS1065	C7	209		GGGTTGATTGGGAAGTGT	CTTAGCACCATTTGTTTGTATT
AAFC	2391	sN2564	C7	348	AAC/AAG	GAATTCCTTCTGGGCTTTCC	CTAAATGAGGATGGGAGCGA
AAFC	2393	sORF37	C7	100	GT	GAAGGCTCAACAAAAATGGG	AAGCCCAAAGGTAAGGAAGG
AAFC	2410	sN1975	C7	140	AG	TCCCTTGCCTTCTCTTCTTG	TCGGCCAAGCATCTCTAACT
AAFC	2428	sN3825J	C7	307	GA	CTGCGTCGTCGAAGTTCATA	TCTCCTTGAAAAACACAGCG
AAFC	2431	sN0706	C7	401	GA	TCCGACGGTCAAGATTAAGG	GGCTGTGGTGGATCTAGGAA
AAFC	240	sN2557	C8	456		GCATCACTCTAGGGTTTCCG	CAAAGCAACCGACAAGAACA
Piquemal *et al*. (2005)	489	CB10139	C8	170-180		TCTCAAAAGGATATGCGTGAA	CAAAACTCATCAGGGTTGTAG
Piquemal *et al*. (2005)	992	CB10028	C8	150-199		CTGCACATTTGAAATTGGTC	AAATCAACGCTTACCCACT
AAFC	2087	sN11670	C8	100		AGTCGGGCTCGTATATCTCG	GTTTCGTGGCGGAAATTAGA
Cheng *et al*. (2009)	2179	BnGMS3	C8	359	$(CTT)_{15}$	AAAGAGCCCACATGAAAGTA	TGAACTAGGCACCAAGAACT
Cheng *et al*. (2009)	2180	BnGMS4	C8	370	$(GAA)_{15}$	AAAGCTGCAGAAAGAAGATG	ATCCGTTCTATACTGCTCCA
Cheng *et al*. (2009)	2184	BnGMS83	C8	325	$(TCT)_{7}$	CCACTTGCAGCGTTATTATT	CGAGGAAATAGACAAAGTGG
Cheng *et al*. (2009)	2199	BnGMS336	C8	345	$(AT)_{15}$	ACCGAATAACAAGTCGAACA	TTGAAACACACCCATTTACA
Cheng *et al*. (2009)	2202	BnGMS352	C8	257	$(AT)_{14}$	AGTCCTGAAGCCTGAACATA	AGTTTGCCATCTCGTAGAAA
Li *et al*. 2011	2244	BoGMS0468	C8	259	$(AT)_{25}$	TGACAGCAACCAATGATG	CTCTCTGGAACCTTTGAACT
Li *et al*. 2011	2246	BoGMS0741	C8	286	$(TC)_{17}$	CTCAAACTCCGTCGCTCT	TCCTCCTCACTACTTTCTTCA
Li *et al*. 2011	2248	BoGMS0868	C8	226	$(CT)11(TC)_{5}$	AAATCCCAACGAGATAGGTAG	AGAAAGAAAGGAAGAAAGTGG
AAFC	225	sN0653	C9	194		TTCTGAATCTCCGCCGTATC	CTTTGGGGGCATCTTCAA
Iniguez-Luy *et al*. (2008)	751	FITO095	C9	233		AGATTTCATCCACAGCCTC	TTTGATTCTTGCGTTCTCTC
Cheng *et al*. (2009)	2182	BnGMS43	C9	234	$(ACA)_{8}$	TTTGATGGGTCTTCATCTTC	GAGGTTAAGGGTTTGGAGTT
Cheng *et al*. (2009)	2193	BnGMS213	C9	134	$(AAAGA)_{4}$	GTAGTACGGAGATGCGTGAT	AAAGAACGAGTTGACTTTCG
Cheng *et al*. (2009)	2204	BnGMS385	C9	197	$(AT)_{12}$	TTTCATGACTTAGCCACCTT	CCAAGTATTCAATTTCTGGC
Li *et al*. (2011)	2257	BoGMS0845	C9	199	$(AG)_{16}$	CCTTTGTCTTCTTCACTCTCC	ACCAGGCTCTTTCTTTCTCT
Li *et al*. (2011)	2258	BoGMS1283	C9	248	$(TCA)_{9}$	TTGTCATCATCCTCTTCACTC	TGCTATCCACTCTTCTTCTCA
	3040	3040	C9	239	$(AT)_{12}$	TCAAACTTTTGACTTTGAATATCCC	AAACAATTTTCAAGTTTTGGTCA
	3098	3098	C9	319	$(A)_{10}$	TGTGGTGGTCACTGACGATT	TCTATGGTCCTCCATGCACA

[1] AAFC = Agriculture and Agri-Food Canada.

[2] BBSRC = Biotechnology and Biological Science research council.

Supplemental Table 2.3 List of 340 alleles of different size (upper row) amplified by 95 SSR markers and the frequency of occurrence (lower row) of these alleles in 227 inbred *Brassica napus* lines derived from six *B. napus* × *B. oleracea* interspecific crosses

Locus	Allele size (bp) / Frequency of occurrence					
sN2087	458	467	472	491		
	0.526	0.047	0.045	0.382		
sN3734	282	291	298	301	307	
	0.061	0.116	0.344	0.126	0.353	
sN0691	358	365	396			
	0.058	0.341	0.601			
sR1078	401	426	433			
	0.375	0.152	0.473			
sNRF94	305	330				
	0.639	0.361				
sN3569F	178	181	214			
	0.121	0.566	0.313			
sN11675	221	236	245	248	250	284
	0.180	0.288	0.044	0.048	0.056	0.384
sN1834	242	274	285	292		
	0.078	0.112	0.759	0.051		
sN11657	251	261	266			
	0.210	0.065	0.725			
sN0758	333	343	360			
	0.159	0.431	0.409			
sN0842	420	430				
	0.892	0.108				
sR10417	248	271	274			
	0.496	0.116	0.388			
sN3761	191	197				
	0.691	0.309				
sNRE74	153	158	174	179		
	0.632	0.154	0.083	0.130		
sR2028	276	281	285			
	0.486	0.155	0.359			
sS2206	100	108	117	126	131	139
	0.026	0.205	0.227	0.027	0.337	0.178
sR1863	238	249	264	270	281	
	0.077	0.0359	0.079	0.052	0.642	
BnGMS633	332	359	368	373		
	0.674	0.167	0.086	0.074		
sN1825	175	202	206			
	0.338	0.546	0.115			
sS2268	134	138	145	170		
	0.149	0.628	0.060	0.162		
sN3815	483	494	499			
	0.172	0.150	0.678			
sORE66	290	298	348	354		
	0.162	0.611	0.093	0.134		
sN1937	284	298	316			
	0.393	0.556	0.051			
sN2316	281	289	292			
	0.094	0.551	0.355			
CB10036	150	155	162	183	187	195
	0.124	0.184	0.207	0.159	0.300	0.026
CB10057	187	208	214	246		
	0.165	0.046	0.357	0.432		
BoGMS0819	115	119	123	133		
	0.758	0.055	0.053	0.134		
BoGMS0767	111	130	137			
	0.097	0.292	0.610			
BoGMS0570	207	211	216	234	242	
	0.162	0.503	0.173	0.061	0.100	

Locus	Allele size (bp) / Frequency of occurrence					
BoGMS1360	325	330	339			
	0.156	0.695	0.149			
BoGMS0953	141	145	151			
	0.162	0.122	0.716			
BoGMS0081	265	306	312	317		
	0.275	0.373	0.174	0.178		
sS2277	236	244				
	0.116	0.884				
CB10109	277	280	297			
	0.214	0.370	0.416			
Ra2-F11A	211	226	230			
	0.317	0.236	0.447			
MR140	134	138	147	152		
	0.166	0.712	0.066	0.055		
Ol11H02	190	207	213			
	0.216	0.712	0.072			
BRAS061	202	210	247			
	0.425	0.112	0.464			
CB10493	174	195	217	223	229	
	0.445	0.101	0.093	0.294	0.068	
sR0357	356	374	380	391		
	0.055	0.108	0.776	0.061		
sN3685R	292	298	308			
	0.055	0.181	0.764			
sN3817	177	180	186			
	0.414	0.175	0.411			
BnGMS347	292	294	296	300	305	
	0.501	0.099	0.270	0.061	0.069	
BnGMS681	152	162				
	0.865	0.135				
BoGMS0836	129	141	145	150	164	
	0.098	0.095	0.656	0.050	0.100	
sN12743J	350	375	384			
	0.474	0.355	0.171			
sNRG34	252	294	299	311	316	
	0.179	0.083	0.068	0.100	0.569	
Snrco3	193	197	210			
	0.161	0.377	0.462			
sORA84	144	175	247	253		
	0.478	0.313	0.101	0.109		
sN2052	409	413	419	431		
	0.174	0.122	0.058	0.646		
sN0761	292	296	302	311	320	326
	0.221	0.083	0.073	0.064	0.513	0.046
BoGMS0590	399	421	428			
	0.724	0.069	0.207			
sORB17	395	418	423	430	434	
	0.408	0.046	0.055	0.425	0.066	
sN12153I	200	209				
	0.786	0.214				
sN11661	328	334	340	348	360	364
	0.404	0.040	0.118	0.072	0.300	0.066
sN12503	235	243	260	269	306	
	0.211	0.493	0.095	0.114	0.087	
sR0622	346	352	357	396		
	0.044	0.048	0.454	0.454		
sN7410	166	176	179			
	0.375	0.116	0.509			
CB10211	150	161	165			
	0.654	0.219	0.127			
Na10-C06	217	226	278			
	0.166	0.410	0.424			

Locus	Allele size (bp) / Frequency of occurrence						
BRMS-015	252	260					
	0.772	0.228					
BnGMS491	171	180	186				
	0.179	0.420	0.401				
BoGMS0632	191	198	210	220	224		
	0.069	0.585	0.178	0.068	0.100		
sN11904	257	263	280				
	0.524	0.111	0.365				
sS2352	202	207	212				
	0.668	0.129	0.203				
sN11862	285	296	304				
	0.262	0.453	0.284				
sNRD41	252	264	305				
	0.087	0.647	0.266				
BnGMS386	179	221	225	235	239	243	255
	0.328	0.085	0.028	0.339	0.155	0.042	0.023
BoGMS1065	221	230	238				
	0.532	0.195	0.274				
sN2564	368	380	388				
	0.598	0.175	0.227				
sORF37	109	113	117	121			
	0.175	0.628	0.141	0.056			
sN1975	149	155	167				
	0.154	0.489	0.357				
sN3825J	294	304	328	336			
	0.300	0.064	0.079	0.558			
sN0706	359	380	389	397	402	408	416
	0.147	0.122	0.100	0.112	0.055	0.375	0.090
sN2557	455	472					
	0.097	0.903					
CB10139	166	170	176				
	0.055	0.148	0.797				
CB10028	137	150	178	184			
	0.083	0.746	0.095	0.076			
sN11670	100	110	116				
	0.126	0.450	0.423				
BnGMS3	351	356					
	0.209	0.791					
BnGMS4	349	354	362	387			
	0.407	0.128	0.420	0.045			
BnGMS83	333	343	347				
	0.159	0.752	0.089				
BnGMS336	348	352					
	0.310	0.690					
BnGMS352	258	273	279				
	0.458	0.492	0.050				
BoGMS0468	234	241	262	269			
	0.105	0.623	0.090	0.182			
BoGMS0741	291	296	306				
	0.778	0.055	0.168				
BoGMS0868	245	256	265				
	0.186	0.592	0.222				
sN0653	207	213	217				
	0.076	0.417	0.506				
FITO095	250	288	291				
	0.444	0.111	0.445				
BnGMS43	248	253					
	0.506	0.494					
BnGMS213	138	153					
	0.665	0.335					
BnGMS385	219	238					
	0.823	0.177					

Locus	Allele size (bp) / Frequency of occurrence		
BoGMS0845	194	204	216
	0.067	0.102	0.831
BoGMS1283	254	269	
	0.072	0.928	
3040	249	257	
	0.162	0.838	
3098	340	342	
	0.657	0.343	

Supplemental Table 2.4 Genetic information statistics calculated for 95 SSR markers in 227 inbred *Brassica napus* lines (F_{10} and BC_1F_9) derived from *B. napus* × *B. oleracea* interspecific crosses

Locus	Expected allele size (bp)	PIC_P	PIC_{POP}	*Na*	*Ne*	*I*
sN2087	475	0.72	0.60	4	1.39	0.46
sN3734	273	0.77	0.73	5	1.47	0.50
sN0691	375	0.86	0.77	3	1.50	0.52
sR1078	402	0.71	0.55	3	1.67	0.59
sNRF94	310	0.48	0.66	2	1.45	0.49
sN3569F	189	0.73	0.47	3	1.56	0.54
sN11675	263	0.89	0.76	6	1.32	0.40
sN1834	277	0.79	0.76	4	1.06	0.13
sN11657	248	0.73	0.68	3	1.32	0.41
sN0758	346	0.78	0.78	3	1.85	0.65
sN0842	438	0.67	0.56	2	1.06	0.13
sR10417	247	0.76	0.52	3	1.15	0.25
sN3761	173	0.79	0.74	2	1.62	0.57
sNRE74	158	0.84	0.76	4	1.29	0.38
sR2028	251	0.86	0.52	3	1.29	0.38
sS2206	120	0.91	0.80	6	3.16	0.97
sR1863	257	0.88	0.80	5	1.15	0.26
BnGMS633	342	0.92	0.75	4	1.29	0.39
sN1825	185	0.77	0.60	3	1.88	0.66
sS2268	185	0.85	0.83	4	1.29	0.39
sN3815	482	0.88	0.73	3	1.30	0.40
sORE66	322	0.90	0.86	4	1.55	0.54
sN1937	281	0.73	0.54	3	1.79	0.63
sN2316	275	0.78	0.83	3	1.57	0.55
CB10036	179-185	0.89	0.79	6	3.15	0.95
CB10057	190-220	0.77	0.56	4	1.17	0.28
BoGMS0819	114	0.87	0.81	4	1.20	0.31
BoGMS0767	114	0.66	0.79	3	1.42	0.47
BoGMS0570	214	0.93	0.87	5	1.48	0.51
BoGMS1360	318	0.86	0.79	3	1.37	0.44
BoGMS0953	133	0.73	0.74	3	1.14	0.24
BoGMS0081	293	0.90	0.99	4	1.51	0.52
sS2277	223	0.75	0.66	2	1.15	0.25
CB10109	281	0.75	0.45	3	1.25	0.35
Ra2-F11A	211	0.58	0.36	3	2.57	0.55
MR140	143	0.92	0.81	4	1.33	0.41
Ol11H02a	203	0.83	0.68	3	1.26	0.36
BRAS061	210-246	0.79	0.64	3	1.29	0.38
CB10493	184-222	0.90	0.68	5	1.29	0.38
sR0357	376	0.92	0.86	5	1.50	0.52
sN3685R	285	0.82	0.79	3	1.28	0.38
sN3817	169	0.76	0.41	3	1.18	0.29
BnGMS347	273	0.92	0.90	5	1.84	0.65
BnGMS681	131	0.55	0.51	2	1.03	0.07
BoGMS0836	146	0.92	0.84	5	1.28	0.38
sN12743J	370	0.46	0.66	3	2.64	0.58
sNRG34	294	0.95	0.91	5	1.88	0.66
Snrco3	192	0.79	0.45	3	1.21	0.32
sORA84	178	0.90	0.60	4	1.23	0.33
sN2052	416	0.95	0.85	4	1.48	0.50
sN0761	298	0.88	0.81	6	1.87	0.66
BoGMS0590	399	0.89	0.77	3	1.37	0.44
sORB17	414	0.85	0.69	5	1.18	0.29
sN12153I	181	0.54	0.49	2	1.35	0.43
sN11661	341	0.92	0.75	6	1.24	0.35

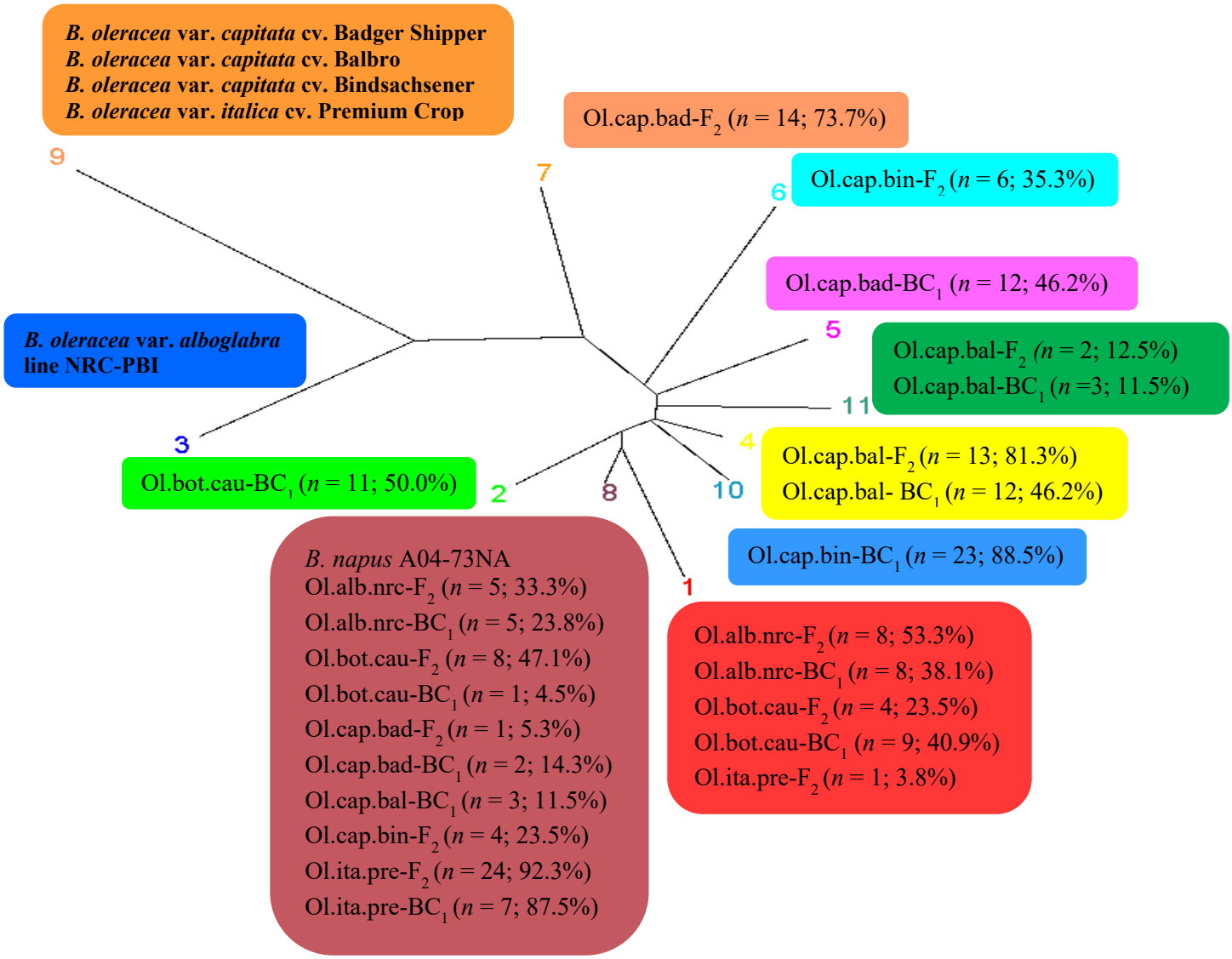
Locus	Allele size range (bp)	*PIC*$_P$	*PIC*$_{POP}$	*Na*	*Ne*	*I*
sN12503	290	0.88	0.81	5	1.06	0.14
sR0622	377	0.85	0.50	4	1.00	0.00
sN7410	155	0.67	0.44	3	1.10	0.19
CB10211	150	0.80	0.66	3	1.29	0.39
Na10-C06	223	0.68	0.81	3	1.74	0.62
BRMS-015	263	0.79	0.60	2	1.17	0.27
BnGMS491	161	0.78	0.51	3	1.18	0.29
BoGMS0632	201	0.94	0.87	5	1.49	0.51
sN11904	239	0.75	0.54	3	1.25	0.35
sS2352	183	0.93	0.81	3	1.47	0.50
sN11862	290	0.60	0.66	3	1.98	0.69
sNRD41	241	0.85	0.79	3	1.48	0.50
BnGMS386	220	0.85	0.88	7	1.98	0.69
BoGMS1065	209	0.86	0.80	3	1.61	0.57
sN2564	348	0.56	0.74	3	1.31	0.40
sORF37	100	0.91	0.83	4	1.38	0.44
sN1975	140	0.82	0.84	3	1.91	0.67
sN3825J	307	0.92	0.86	4	1.63	0.58
sN0706	401	0.96	0.93	7	1.86	0.66
sN2557	456	0.75	0.52	2	1.02	0.06
CB10139	170-180	0.63	0.76	3	1.39	0.45
CB10028	150-199	0.89	0.83	4	1.18	0.29
sN11670	100	0.76	0.38	3	1.09	0.17
BnGMS3	359	0.75	0.68	2	1.27	0.37
BnGMS4	370	0.84	0.61	4	1.17	0.28
BnGMS83	325	0.80	0.76	3	1.34	0.42
BnGMS336	345	0.68	0.70	2	1.43	0.48
BnGMS352	257	0.63	0.55	3	1.11	0.20
BoGMS0468	259	0.90	0.81	4	1.34	0.42
BoGMS0741	286	0.95	0.85	3	1.14	0.24
BoGMS0868	226	0.89	0.80	3	1.47	0.50
sN0653	194	0.61	0.38	3	1.06	0.13
FITO095	233	0.65	0.58	3	1.35	0.43
BnGMS43	234	0.30	0.24	2	1.55	0.54
BnGMS213	134	0.62	0.70	2	1.73	0.61
BnGMS385	197	0.75	0.69	2	1.07	0.15
BoGMS0845	199	0.88	0.78	3	1.15	0.26
BoGMS1283	248	0.80	0.59	2	1.03	0.08
3040	239	0.72	0.60	2	1.27	0.36
3098	319	0.72	0.66	2	1.40	0.46

PIC$_P$ = polymorphic information content in the parents; *PIC*$_{POP}$ = polymorphic information content in the inbred population; *Na* = number of alleles per locus; *Ne* = number of effective alleles per locus; *I* = Shannon's Information index

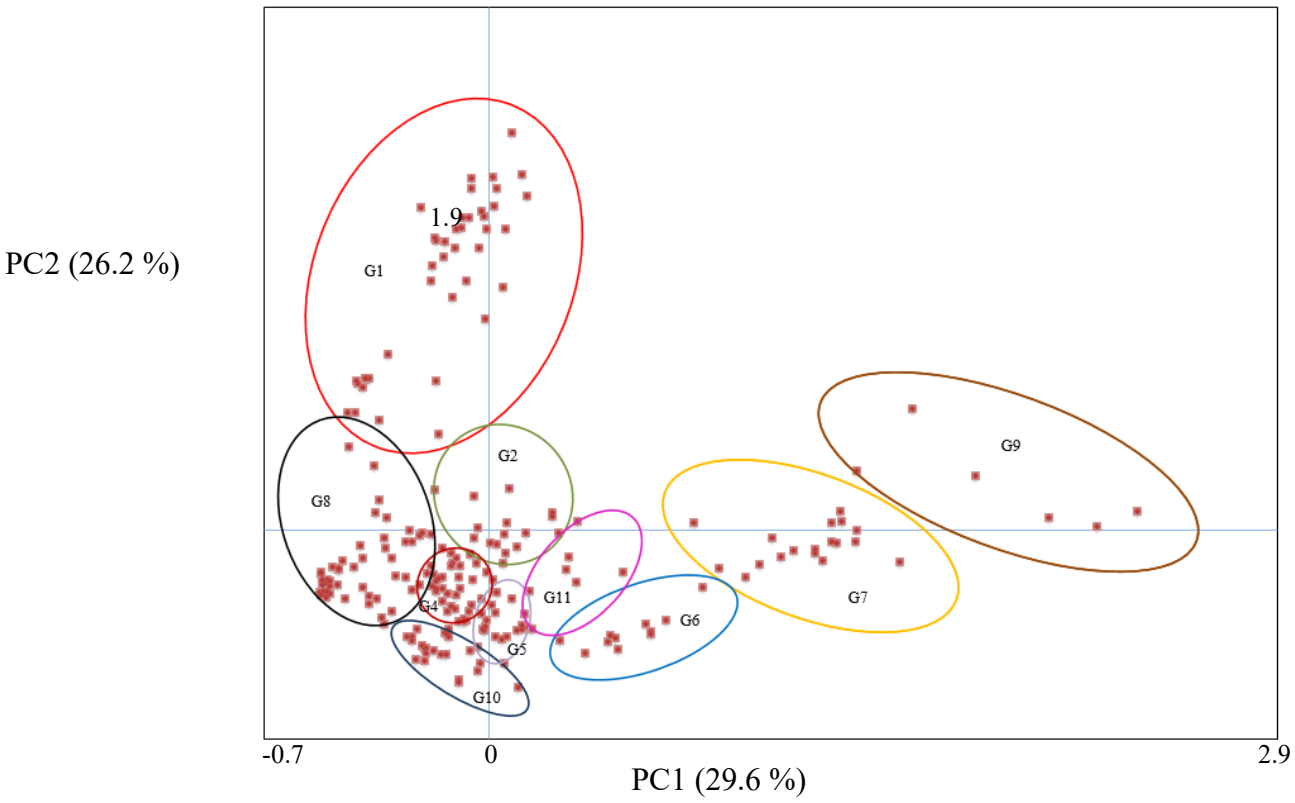
Supplemental Table 2.5 Analysis of molecular variance among and within the 227 inbred *Brassica napus* lines derived from six *B. napus* × *B. oleracea* interspecific crosses

Source of variation	Degree of freedom	Sum of squares	Mean squares	Estimated variance	Variation (%)	P-value
Among populations	P-1 = 11	1742.0	158.4	3.7	17%	<0.001
Among individuals (within populations)	N – P = 215	4129.7	19.2	1.6	8%	<0.001
Within individuals	N = 227	3656.0	16.1	16.1	75%	<0.001
Total	2N-1 = 453	9527.7		21.4	100%	

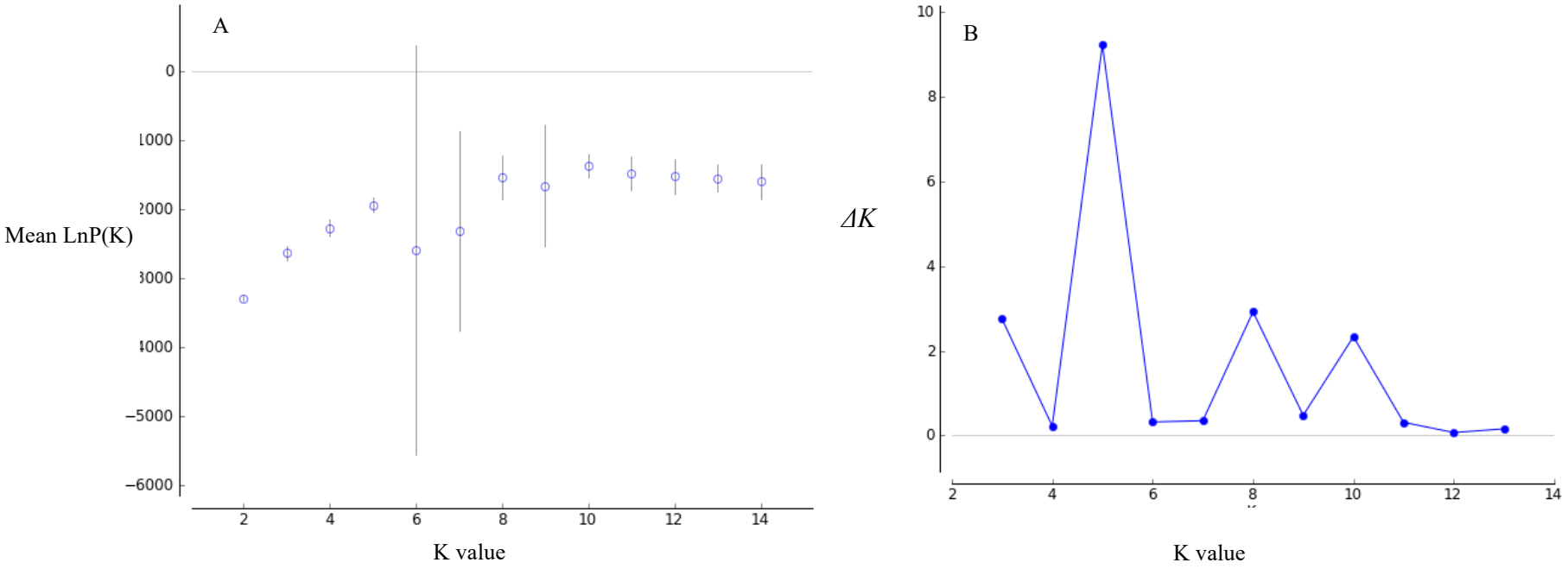
P = populations = 12; N= number of inbred lines = 227



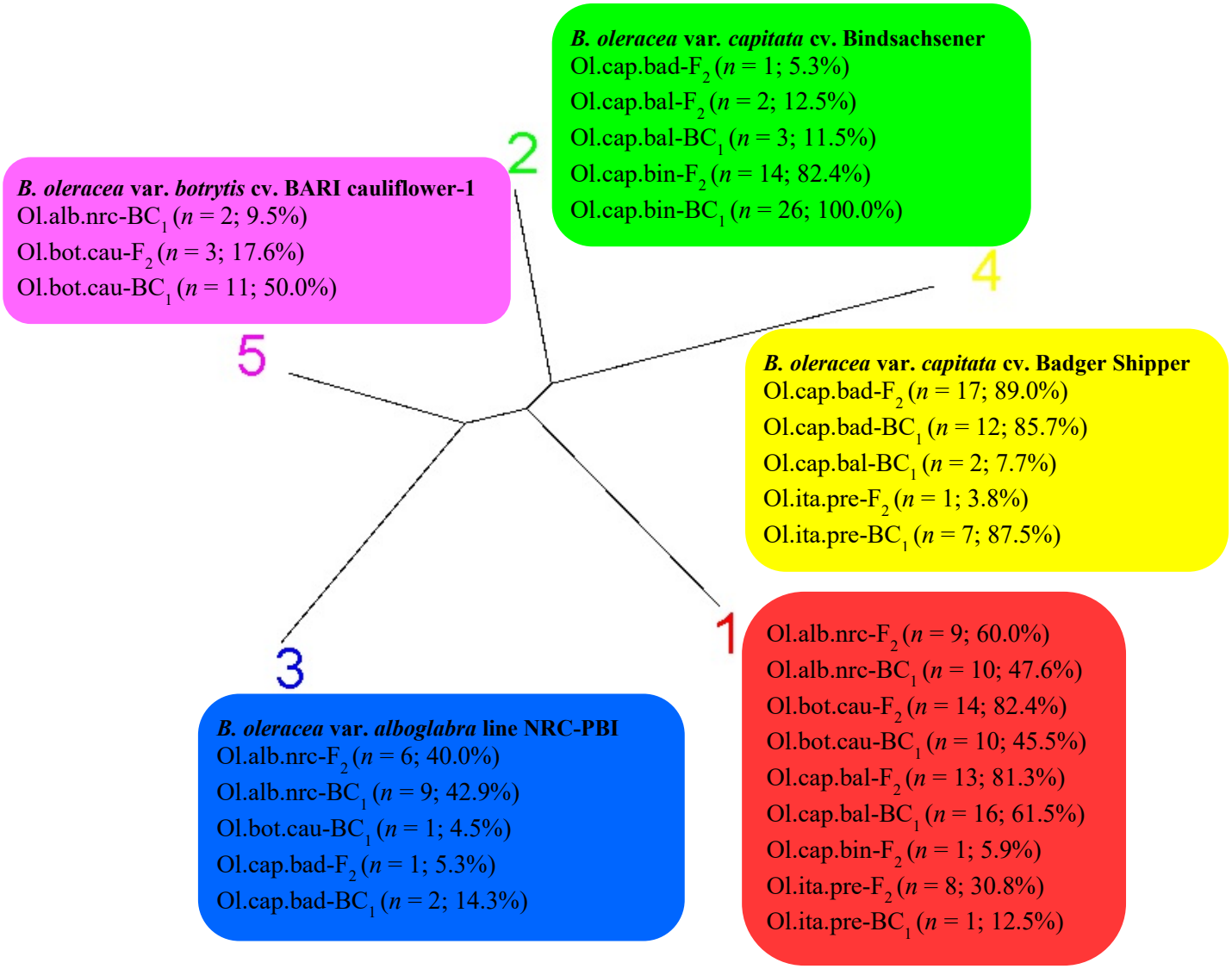
Supplemental Fig. 2.1 Phylogenetic tree of 227 *Brassica napus* lines derived from six *B. napus* × *B. oleracea* interspecific crosses and following two breeding methods. Tree was constructed through neighbor-joining method based on Nei's genetic distance estimated using 340 SSR alleles amplified by 95 markers with $K = 11$ groups implemented in STRUCTURE. The model with admixture and correlated allele frequencies with 10 replicates was carried in each run (K between 2 to 14) using a burn-in period of 10,000 followed by 100,000 MCMC repetitions in the STRUCTURE software. The number of lines (n) belonging to the 12 populations of the six crosses and the proportion (%) of the lines of a given population is presented in brackets. The *B. oleracea* var. *botrytis* cv. BARI cauliflower-1 and a total of 41 inbred lines were included in different mixed groups; they are not shown in this Figure.
Ol.alb.nrc = *B. napus* × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* × *B. oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = *B. oleracea* var. *italica* cv. Premium Crop; -F_2 = F_2-derived population; -BC_1 = BC_1-derived population; n = number of individuals. The parent cv. BARI cauliflower-1 tended to include into G9 and G2 with a probability of membership 58% and 38%, respectively.



Supplemental Fig. 2.2 Principal coordinate analysis (PCoA) of 227 *B. napus* inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses and their parents based on genotypic data of 340 SSR alleles amplified by 95 markers. The inbred lines are assigned to 10 groups, and the groups are indicated by circles. All *B. oleracea* parents fell into G9. Of the 227 inbred lines, 18 included in G7 which positioned closest to the *B. oleracea* parents; this group included 14 F_2-derived lines the cross involving cabbage cv. Badger Shipper.



Supplemental Fig. 2.3 The estimated mean log-likelihood of *K* values with standard deviation (A) and *ΔK* values (B) for the 227 inbred *Brassica napus* line population derived from *B. napus* × *B. oleracea* interspecific crosses and estimated using 197 SSR alleles of *B. oleracea*. Analysis was done following Evanno et al. (2005) to examine the rate of change of the slope of the log probability curve over the range of *K* values.



Supplemental Fig. 2.4 Phylogenetic tree of 227 inbred *Brassica napus* lines derived from six *B. napus* × *B. oleracea* interspecific crosses and following two breeding methods. Tree was constructed through neighbor-joining method based on Nei's genetic distance estimated using 197 SSR alleles of *B. oleracea* amplified by 94 markers with $K = 5$ groups implemented in STRUCTURE. The model with admixture and correlated allele frequencies with 10 replicates was carried in each run (K between 2 to 14) using a burn-in period of 10,000 followed by 100,000 MCMC repetitions in the STRUCTURE software. The number of lines (*n*) belonging to the 12 populations of the six crosses and the proportion (%) of the lines of a given population is presented in brackets. A total of 25 inbred lines and the *B. oleracea* parents' var. *capitata* cv. Balbro and var. *italica* cv. Premium Crop are included in mixed groups; they are not shown in this Figure.

Ol.alb.nrc = *B. napus* × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* × *B. oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = *B. napus* × *B. oleracea* var. *italica* cv. Premium Crop; -F_2 = F_2-derived population; -BC_1 = BC_1-derived population; *n* = number of lines.

Chapter 3

Potential of the C genome of different variants of *Brassica oleracea* for the improvement of agronomic and seed quality traits of *B. napus* canola[2]

3.1 Introduction

Brassica napus L. is an amphidiploid species (AC genomes, $n = 19$) evolved from the diploid species *B. oleracea* L. (C genome, $n = 9$) and *B. rapa* L. (A genome, $n = 10$) through interspecific hybridization (Nagaharu 1935). The genetic base of spring *B. napus* canola is known to be narrow (Hasan et al. 2006; Bus et al. 2011); therefore, there is a need to broaden the genetic base of this crop to make continued progress in breeding (for review, see Rahman 2013). Molecular marker analysis showed that the genomes of *B. oleracea* and *B. rapa* are genetically distinct from each other, as well as from the corresponding genomes of the amphidiploid species of *B. napus* (Thormann et al. 1994; Abel et al. 2005; Wu et al. 2014; Thakur et al. 2018). Wide diversity also exists within these two diploid species; this includes cabbage, broccoli, cauliflower, brussels sprouts, Chinese kale, and kohlrabi of *B. oleracea*, and turnip, Chinese cabbage, pak choi, and oilseed type of *B. rapa* (Warwick 2010; Izzah et al. 2013). The variants of these progenitor species can be a potential source of genetic variation for use in the breeding of *B. napus* canola to broaden the genetic base of this oilseed crop.

Exotic alleles of the parental species can be introduced into *B. napus* through *B. napus* × *B. rapa* (Brown and Brown 1996; Qian et al. 2006; Xiao et al. 2010; Mei et al. 2011; Li et al. 2013; Attri and Rahman 2018) and *B. napus* × *B. oleracea* (Quazi 1988; Bennett et al. 2008,

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2012; Rahman et al. 2011, 2015, 2017; Li, Zhou et al. 2014; Iftikhar et al. 2018) interspecific crosses, as well as through resynthesis of *B. napus* by crossing of *B. rapa* with *B. oleracea* (Akbar 1989; Rahman 2001, 2005; Girke et al. 2012a, 2012b). Allelic diversity of the allied *Brassica* species has also been introduced into *B. napus* through the development of a *Brassica* allohexaploid (AABBCC) followed by crossing this to *B. napus* (Rahman 2001; Li et al. 2004), as well as through the development of a digenomic hexaploid (AACCCC) followed by crossing with *B. rapa* (Li et al. 2013). Development of a euploid *B. napus* line from the progeny of *Brassica* interspecific cross is a challenging task due to meiotic anomaly and sterility. Furthermore, most of the *B. rapa* and *B. oleracea* variants are non-canola quality type (i.e., seed oil contains a high level of erucic acid [>40%] and seed meal contain a high level of glucosinolates [>80 μmol g^{-1} seed]). Therefore, these non-canola quality traits are introduced into the progeny of the interspecific crosses, which imposes a challenge for the development of a canola quality *B. napus* line. Despite these challenges, exotic alleles of the allied *Brassica* species have been introduced into *B. napus* by different researchers (Qian et al. 2006; Xiao et al. 2010; Rahman et al. 2011, 2015, 2017; Mei et al. 2011; Bennett et al. 2012; Li et al. 2013; Li, Zhou et al. 2014; Attri and Rahman 2018; Iftikhar et al. 2018) to broaden the genetic base of this crop, and a few *B. napus* cultivars were developed in China and Japan using the lines derived from the *B. napus* × *B. rapa* interspecific cross (Liu 2000; cited by Li, Zhou et al. 2014).

According to Bus et al. (2011) and Wu et al. (2014), genetic diversity in the C genome of *B. napus* is low compared with its A genome. Analysis of 25 wild *B. oleracea* and 248 spring, winter, and semi-winter types of *B. napus* by simple sequence repeat (SSR) markers revealed the existence of greater allelic diversity in the C genome of *B. oleracea* compared with the C genome of *B. napus* (Wu et al. 2014). This indicates the potential of using the *B. oleracea* gene pool to broaden the genetic base of the C genome of *B. napus*. Indeed, spring or semi-

winter *B. napus* lines carrying *B. oleracea* alleles have been developed by different researchers (Bennett et al. 2012; Li, Zhou et al. 2014; Rahman et al. 2015; Iftikhar et al. 2018). Some of the advanced generation inbred lines derived from *B. napus* × *B. oleracea* interspecific cross carried ~50% of the expected number of *B. oleracea* alleles (Rahman et al. 2015). However, the exotic alleles introgressed into the elite *B. napus* gene pool can disrupt the favorable allele combinations of the elite lines, and this can exert deleterious effect on some traits (Falk 2010). To minimize the level of this disruption, limited backcrossing of the F_1 to the elite lines or cultivars has been proposed by different researchers (Falk 2010; Holland 2014); however, this approach is expected to reduce the contribution of the exotic alleles in the resultant population.

Most of the studies with *B. oleracea* (Bennett et al. 2012; Li, Zhou et al. 2014; Rahman et al. 2015), as reviewed above, are based on the use of a single *B. oleracea* accession and mostly focused on introgression of the exotic alleles into *B. napus*. As mentioned above, wide diversity exists in this diploid progenitor species (dos Santos et al. 1994; Lázaro and Aguinagalde 1998; Simonsen and Heneen 1999; Izzah et al. 2013); however, to our knowledge, no study has so far been conducted to compare the value of the different variants of *B. oleracea* for use in the breeding of improved *B. napus* canola lines. Iftikhar et al. (2018) demonstrated the potential of developing canola quality euploid *B. napus* lines from *B. napus* × *B. oleracea* interspecific crosses involving different variants of *B. oleracea*. In this study, we used the inbred lines developed by Iftikhar et al. (2018), as well as inbred lines developed from additional crosses. These lines were evaluated in replicated field trials to identify the *B. oleracea* variants contributing desirable alleles in *B. napus* canola for improved agronomic and seed quality traits. We also investigated the impact of the application of two breeding techniques for the development of the inbred lines (viz., developed from F_2 or BC_1 [F_1 × *B. napus*]) on the performance of these inbred lines.

3.2 Materials and methods

3.2.1 Parent material

One zero-erucic-acid and low-glucosinolate (<15 μmol g^{-1} seed) spring *B. napus* true breeding line, A04-73NA, and six high-erucic (~40% erucic acid), high-glucosinolate (>60 μmol g^{-1} seed) *B. oleracea* cultivars and lines (var. *alboglabra* line NRC-PBI; var. *botrytis* cv. BARI Cauliflower-1; var. *capitata* cv. Badger Shipper, Bindsachsener, and Balbro; and var. *italica* cv. Premium Crop) were used as parents. The *B. napus* line A04-73NA was developed by the Canola Program of the University of Alberta, and seed of the *B. oleracea* var. *alboglabra* line NRC-PBI was collected from the National Research Council, Saskatoon, SK, Canada. Seeds of *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1 were collected from the Bangladesh Agricultural Research Institute, Bangladesh; *B. oleracea* var. *capitata* cv. Badger Shipper and Bindsachsener were from the Canola Program of the University of Alberta (Hasan et al. 2012), *B. oleracea* var. *capitata* cv. Balbro was from Hazera Seeds of Growth, Netherlands, and *B. oleracea* var. *italica* cv. Premium Crop was collected from Dr. Ron Howard, Alberta Agriculture and Forestry, Brooks, AB, Canada.

The following interspecific crosses were made using A04-73NA as female:

- A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI (Ol.alb.nrc, Chinese kale)
- A04-73NA × *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1 (Ol.bot.cau, cauliflower)
- A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper (Ol.cap.bad, cabbage)
- A04-73NA × *B. oleracea* var. *capitata* cv. Balbro (Ol.cap.bal, cabbage)
- A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener (Ol.cap.bin, cabbage)
- A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop (Ol.ita.pre, broccoli)

The F_1 plants were self-pollinated for F_2 seeds and backcrossed to the *B. napus* parent for BC_1 seeds. A flow diagram of the development of inbred *B. napus* lines from the *B. napus* × *B. oleracea* interspecific crosses is presented in Fig. 3.1. The F_2 and BC_1 population were subjected to pedigree breeding with selection for the two canola quality traits (zero erucic acid in oil and low glucosinolate in meal). The details of the development of canola quality advanced generation *B. napus* lines from these crosses are described in Iftikhar et al. (2018).

3.2.2 Field trials

A total of 279 advanced-generation canola quality (zero erucic acid and low glucosinolate) lines ($\geq F_9$ and BC_1F_8), which included 135 lines derived from F_2 and 144 from BC_1, of the abovementioned six *B. napus* × *B. oleracea* interspecific crosses (Supplemental Table 3.1) and their spring canola parent were evaluated in 10 field trials in 2016, 2017, and 2018 for different agronomic and seed quality traits. The six *B. oleracea* parents could not be tested in field trials, as most of them required vernalization for flowering and longer time to flower and mature. For the field trials, ~85 g seed of each line was needed, which was not possible to produce in a greenhouse by bag isolation. Therefore, open-pollinated seeds were used, which were produced by growing self-pollinated seeds of these advanced generation lines in field and harvesting seeds from the middle of the plots. Field trials in summer 2016 were grown at ERS-Michener, ERS-West 240, and St. Albert research farms of the University of Alberta, and in a grower's field at Killam, AB, Canada. In 2017, trials were grown at ERS-Michener, ERS-West 240, and at St. Albert, and the trial at St. Albert was seeded on two different dates (St. Albert1 and St. Albert2). In 2018, trials were grown at ERS-Michener and ERS-West 240. Seeding of all trials was done in six-row plots of 5.0- by 1.8-m size, except at Killam in 2016, which had five-row plots of 5.0- by 1.7-m size. Field plots were laid out in randomized block design with two replications, where each replication was divided into

sixteen blocks and each block included 23 entries and two check plots of A04-73NA. This subdivision of the replications into multiple blocks was needed to accommodate the large number of entries in a best possible piece of the land. Randomization of the experimental units was done using CropStat7.2 and Microsoft Excel 2007. Seeding of the trials was done with a plot seeder using 8.5 g seed per plot.

3.2.3 Agronomic and seed quality traits

The following agronomic and seed quality traits were recorded: days to flowering, plant height (cm), days to maturity, seed yield (kg ha^{-1}), and seed oil (%) and protein (%) contents. Days to flowering data were collected when ~50% of plants in a plot had at least one open flower, and the end of flowering data were collected when ~90% of plants in a plot did not have flowers; these two data were used to estimate the duration of flowering. Plant height (cm) was recorded at the end of flowering on a whole-plot basis by measuring height of the plants from soil surface. Days to maturity was recorded when silique color changed from green to light yellow or brown and seed color of silique on the main branch changed to brown or black. The end of flowering and days to maturity data were used to estimate the duration of grain-filling period. Plots were harvested with a plot combine, and plot yield data were converted to kilograms per hectare. Seed oil and protein contents were estimated using near-infrared reflectance spectroscopy (NIRS, Model 6500, Foss North America) in the Analytical Laboratory of the Canola Program of the University of Alberta. For this, 5 to 8 g of bulk open-pollinated seeds harvested from the field plots was used. A calibration equation available in this laboratory was used for quantification of oil and protein contents using the software WinISI II (Infrasoft International) and reported as the percentage of whole seed at 8.5% moisture.

3.2.4 SSR marker analysis

Genomic DNA was extracted from 228 F_{10} and BC_1F_9 plants and their seven parents (*B. napus* and *B. oleracea* cultivars or lines) using SIGMA DNA extraction kit (Sigma-Aldrich) following manufacture's instruction. A total of 96 polymorphic SSR markers (Supplemental Table 3.2) were selected from a total of 418 markers from the nine C genome linkage groups. This included 9 to 15 markers per linkage group. The polymorphic markers were used to genotype the 110 F_8 and 118 BC_1F_7 inbred lines. The forward primer of each SSR marker was labeled with a M13 tail at its 5′ end (5′-CACGACGTTGTAAAACGAC-3′) as described by Schuelke (2000), and they were labeled with the universal fluorescent dyes FAM, VIC, NED, and PET (Applied Biosystems). Polymerase chain reaction (PCR) for amplification of the genomic DNA was performed in a total volume of 15.5 μL, which included 20 ng genomic DNA, 5× PCR reaction buffer, 25 mM $MgCl_2$, 0.6 unit *Taq* DNA polymerase (Promega Corporation), 10 mM of each dNTP (Invitrogene Life Technologies), 5 μM of each forward and reverse primer, and 5 μM tag F (fluorescent dyes FAM, VIC, NED, and PET). The PCR was performed in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) with the following program: 1 cycle of 5 min at 95°C for initial denaturation; 35 cycles where each cycle consisted of 1 min at 95°C for denaturation, 1 min at 58°C for annealing, and 1.5 min at 72°C for extension; and the final extension time was 15 min at 72°C. Size-based separation of the amplified DNA fragments was done using a capillary electrophoresis AB Genetic Analyzer no. 3730 (Applied Biosystems).

3.2.5 Statistical analysis

3.2.5.1 ANOVA

A general linear mixed-effects model was fitted for each of the traits and the analysis was performed using PROC MIXED of the statistical software program SAS (SAS Institute), following the option METHOD = Type 3 sums of squares. In this analysis, environment consisted of 10 field trials conducted in up to four locations in each of the 3 yr (2016, 2017, and 2018) as detailed in the field trials section. The inbred lines used in this study were developed from six crosses—Ol.alb.nrc, Ol.bot.cau, Ol.cap.bad, Ol.cap.bin, Ol.cap.bal, and Ol.ita.pre—and following two breeding techniques (F_2– and BC_1–derived); therefore, the six crosses, two breeding techniques, and their interaction (cross × technique) were considered as fixed effects. Environment, replicate nested in environment, block nested in replicate × environment, and genotype nested in cross × technique were considered as random effects. The following linear model summarizes the sources of variation:

$$Y_{ijklmp} = \mu + C_i + T_j + \mathrm{CT}_{ij} + E_k + R_{l(k)} + B_{m(lk)} + G_{p(ij)} + \mathrm{GE}_{kp(ij)} + \varepsilon_{ijklmp}$$

where Y_{ijklmp} is the trait value observed for the pth genotype (inbred line) from the ith (i = 1, …, 6) cross and the jth (j = 1, 2) breeding technique grown in the mth (m = 1, …, 8) block nested within the lth (l = 1, 2) replication and the kth (k = 1, …, 10) environment; μ, C_i, T_j, CT_{ij}, E_k, $R_{l(k)}$, $B_{m(lk)}$, $G_{p(ij)}$, and $\mathrm{GE}_{kp(ij)}$ are the overall mean and effects due to the ith cross, the jth breeding technique, the ijth cross × breeding technique, the kth environment, the lth replication within the kth environment, the mth block within the lth replication and the kth environment, and the pth genotype within the ijth cross × breeding technique, and the kpth genotype × environment interaction, respectively; and ε_{ijklmp} is the random residual for the $ijklmp$th observation. All random effects [E_k, $R_{l(k)}$, $B_{m(lk)}$, $G_{p(ij)}$, and $\mathrm{GE}_{kp(ij)}$] and random residual are assumed to be independently and identically distributed, with mean zero and

variances being σ_E^2, σ_R^2, σ_B^2, σ_G^2, σ_{GE}^2, and σ_ε^2, respectively, where $E_k \approx N(0, \sigma_E^2)$; $R_{l(k)} \approx N(0, \sigma_R^2)$; $B_{m(lk)} \approx N(0, \sigma_B^2)$; $G_{p(ij)} \approx N(0, \sigma_G^2)$ and $G_{kp(ij)} \approx N(0, \sigma_{GE}^2)$, and $\varepsilon_{ijklmp} \approx N(0, \sigma_\varepsilon^2)$.

3.2.5.2 Least square means

Least square means (LSmeans) of the fixed effects were calculated with SAS, and the test for significant difference ($p < 0.05$) between the LSmeans was done using Tukey's multiple comparison adjustment for the *p* values.

3.2.5.3 Correlation

Phenotypic and genotypic Pearson correlation coefficient (*r*) values between the different agronomic and seed quality traits were calculated using multivariate REML (Residual Maximum Likelihood) analysis using SAS PROC MIXED for multiple-environment trials based on Holland (2006).

3.2.5.4 Broad-sense heritability

Broad sense heritability (*H*) was calculated as the ratio of genotypic to phenotypic variance based on the formula $H = \frac{s^2_G}{s^2_G + \frac{s^2_{G\times E}}{N_E} + \frac{s^2_e}{N_E N_R}}$ using SAS (http://improvestats.github.io/ImproveSAS_en/). In this formula, σ^2_G is the genotypic variance, $\sigma^2_{G\times E}$ is the genotype × environment variance, σ^2_ε is the residual variance, and N_E and N_R are the number of environments and replications, respectively (Zou, Zhao et al. 2016).

3.2.5.5 Multivariate analysis

Principal component analysis (PCA) was used to facilitate the identification of the inbred lines with differentiated performance when accounting for differences and interrelationships among the multiple agronomic and seed quality traits. The LSmeans values of the different

agronomic and seed quality traits of the inbred lines (obtained above) across the environments and replicates were standardized prior to analysis. Variable standardization and PCA were performed using the R (http://www.R-project.org/) package vegan (Oksanen et al. 2019) according to Borcard et al. (2018).

3.2.6 Genetic diversity analysis

The fragment analysis results from Applied Biosystems® ABI were scored for presence or absence of the alleles using the software program GeneMarker version 2.4.0 (SoftGenetics); however, all genotyping results were also confirmed manually. The absence (2) or presence (1) of the polymorphic amplification products were scored based on fragment length, and data were recorded in a 2/1 matrix for absence or presence of the marker amplicons. The 2/1 matrix was used to calculate Nei's genetic distance of the inbred lines from the common *B. napus* parent A04-73NA using the software GENALEX 6 (Peakall and Smouse 2006). Pearson's correlation (r) between genetic distance of the inbred lines from A04-73NA and different agronomic and seed quality traits were calculated using cor.test function and chart.correlation of the Performance Analytics package through R (Peterson et al. 2015).

3.3 Results

3.3.1 Agronomic and seed quality traits

Analysis of variance showed the presence of significant variation among the populations derived from six *B. napus* × *B. oleracea* interspecific crosses for all traits (viz., days to flowering, duration of flowering, days to maturity, plant height, seed yield, and seed oil and protein contents; Supplemental Table 3.3). In case of the two breeding techniques (F_2– and BC_1–derived populations), significant differences ($P < 0.05$) were found for all traits except duration of flowering and seed protein content. Interaction between the crosses and breeding

techniques was significant ($P < 0.05$) for all traits, indicating that the populations derived from the different crosses and following two breeding techniques performed differently. Broad-sense heritability for all traits, except duration of flowering, was moderately high (70%) to very high (>90%) in both the F_2– and BC_1–derived populations; however, a very low to low heritability (9–41%) was found for duration of flowering (Table 3.1).

The populations of the crosses involving Chinese kale, cauliflower, and broccoli, on average, flowered earlier than the populations derived from the crosses involving the three cabbage accessions (Fig. 3.2, Supplemental Fig. 3.1, Supplemental Tables 3.4 and 3.6). This difference was less evident in the case of duration of flowering, grain-filling period, and days to maturity. However, the population derived from the cross involving broccoli had significantly ($P < 0.05$) shorter duration of flowering than most of the other populations (Fig. 3.1, Supplemental Tables 3.4 and 3.6). Comparing the populations developed through the two breeding techniques, the BC_1–derived population, on average, flowered and matured only ~0.4 d earlier than the F_2–derived population.

Wide variation was found in all six populations for seed yield where inbred lines with seed yield statistically similar to or greater than the *B. napus* parent could be found in several populations (Fig. 3.2, Supplemental Table 3.5). Comparing the six populations, the population derived from the cross involving broccoli gave the greatest yield (3393.0 ± 167.2 kg ha^{-1}), followed by the population derived from the cross involving the cabbage cv. Balbro and the cauliflower cv. BARI Cauliflower-1. Seed yield of the populations derived from the crosses involving the other two cabbage cv. Badger Shipper and Bindsachsener was significantly ($P < 0.05$) lower than the populations derived from most of the other crosses. When comparing the F_2– and BC_1–derived populations of the six crosses, the F_2–derived

population yielded only 83 kg ha^{-1} more than the BC_1–derived population (Fig. 3.2, Supplemental Tables 3.4 and 3.5).

In the case of the seed quality traits, the F_2–derived populations, on average, had ~0.3% higher seed oil content than the BC_1–derived populations. Among the six populations, the populations derived from the crosses involving cauliflower and broccoli had significantly ($P < 0.05$) higher seed oil than the populations derived from the crosses involving the three cabbage cultivars (Fig. 3.2). In the case of seed protein content, populations derived from the crosses involving cabbage cv. Badger Shipper and Bindsachsener had significantly ($P < 0.05$) more seed protein than the other populations (Supplemental Table 3.5).

3.3.2 Correlation

Days to flowering showed a significant ($P < 0.001$) positive phenotypic correlation (r_p) and genotypic correlation (r_g)with days to maturity ($r_p = 0.73$, $r_g = 0.93$), and plant height ($r_p = 0.27$, $r_g = 0.84$), whereas negative correlation of this trait with duration of the grain-filling period ($r_p = -0.24$, $r_g = -0.57$), seed oil content ($r_p = -0.30$, $r_g = -0.36$), and seed yield ($r_p = -0.10$, $r_g = -0.12$) was observed. Seed yield also showed a significant ($P < 0.001$) positive correlation with duration of grain-filling period ($r_p = 0.14$, $r_g = 0.33$) and oil content ($r_p = 0.15$, $r_g = 0.55$) (Table 3.2). Thus, it is evident that the earlier flowering lines with longer duration of grain-filling period tended to produce greater seed yield with higher seed oil content. Correlation between seed oil and protein content was negative ($r_p = -0.83$, $r_g = -0.65$) in this population.

3.3.3 Genetic distance and correlation with traits

Mean genetic distance of the six populations from the *B. napus* canola parent A04-73NA varied from 0.26 to 0.57 (Supplemental Table 3.7). The population derived from *B. napus* ×

B. oleracea var. *italica* had the lowest genetic distance (i.e., carried the fewest *B. oleracea* alleles), whereas the population derived from *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper received the most *B. oleracea* alleles. When comparing the F_2– and BC_1–derived populations, no significant difference was found between these two populations (0.47 vs. 0.49). Genetic distance of the inbred lines from the common *B. napus* parent A04-73NA showed significant ($P < 0.001$) positive correlation with all traits except duration of the grain-filling period, seed yield, and oil content, where the correlations were negative (Fig. 3.3). The greatest positive correlation of genetic distance of the inbred lines was found with days to flowering and seed protein content ($r = 0.41$ and 0.40), and the greatest negative correlation was found with seed oil content ($r = -0.42$).

3.3.4 Multivariate analysis

Based on the agronomic and seed quality traits, PCA explained 65.5% of the total variation by the first two components (PC1 = 39.7%, PC2 = 25.8%, PC3 = 12.7%). The first PC mostly explained a gradient of days to flowering, plant height, seed oil content, days to maturity, and duration of the grain-filling period (Table 3.3). For example, early-flowering inbred lines grouped together on the left half of the ordination plot, and late-flowering inbred lines grouped together on the right half of the plot. Similarly, PC2 mostly explained a gradient of seed yield, seed protein content, and days to maturity (Table 3.3). For example, high-yielding inbred lines grouped together on the lower half of the ordination plot, and lower-yielding lines grouped together on the upper half (Fig. 3.4). The lower left part of the biplot showed that seed yield, oil content, and duration of the grain-filling period were positively correlated, and these three variables were negatively correlated with seed protein content and duration of flowering. The strong positive correlation found between days to flowering, maturity, and plant height from the estimates of correlation coefficient (Table 3.2) is also reflected in

multivariate analysis from the close association of the vectors for these traits in the PCA plot (Fig. 3.4). Duration of flowering displayed the shortest vector in the ordination plot; however, this trait was more important for the distribution of the inbred lines along PC3 (Table 3.3).

A large number of the inbred lines (e.g., 102, 117, 122; Fig. 3.4; Supplemental Tables 3.1 and 3.8) derived from the cross with broccoli displayed higher seed yield, higher seed oil content, longer duration of the grain-filling period, fewer days to flowering, fewer days to maturity, lower protein content, and shorter plant height than inbred lines derived from other crosses and the *B. napus* parent. Inbred lines derived from crosses with cauliflower and Chinese kale tended to cluster close to inbred lines derived from the cross with broccoli, with slight differences for all traits. Likewise, most of the inbred lines derived from the cross with cabbage cv. Badger Shipper clustered on the opposite side of the inbred lines derived from the cross with broccoli, showing the highest values for seed protein content, days to flowering and maturity, and plant height and the lowest values for seed yield and seed oil content.

Inbred lines derived from the cross with cabbage cv. Balbro tended to be distributed at the center of the ordination but showed higher values for days to flowering, days to maturity, and plant height, whereas the inbred lines derived from the cross with the cabbage cv. Bindsachsener spread out in the upper right part of the biplot, showing higher values for seed protein content but lower values for seed oil content and seed yield (Fig. 3.4).

No subtle difference was observed between the F_2– and BC_1–derived inbred lines for most of the traits. However, the F_2–derived inbred lines tended to be slightly distributed at the lower part of the biplot, showing higher values for seed yield, seed oil content, days to flowering, days to maturity, and plant height than the BC_1–derived inbred lines (Supplemental Fig. 3.2).

Based on seed yield of the inbred lines, which also carried acceptable agronomic and seed quality traits, the top 5% of lines were selected (Supplemental Table 3.8). Seed yield of these

lines varied from 3349.7 ± 185.0 to 3738.8 ± 185.0 kg ha^{-1} with a mean of 3540.5 ± 185.3 kg ha^{-1}, which is approximately 212.6 ± 37.6 kg ha^{-1} greater than seed yield of the *B. napus* parent A04-73NA (3327.9 ± 222.9 kg ha^{-1}). Mean days to flowering of these lines was 49.6 ± 1.8 d (A04-73NA, 51.4 ± 2.1 d), duration of flowering period was 18.4 ± 4.0 d (A04-73NA, 20.4 ± 3.9 d), days to maturity was 102.0 ± 2.9 d (A04-73NA, 103.8 ± 3.0 d), duration of the grain-filling period was 32.1 ± 1.8 d (A04-73NA, 30.7 ± 1.9 d), plant height was 113.4 ± 5.6 cm (A04-73NA, 120.6 ± 6.8 cm), seed oil content was 47.8 ± 0.6% (A04-73NA, 47.0 ± 0.9%), and protein content was 25.0 ± 0.8% (A04-73NA, 25.5 ± 0.9%). Genetic diversity of these lines from the *B. napus* parent varied from 0.15 to 0.55 (Supplemental Table 3.8), which showed almost no correlation (r = 0.092, df = 10) with seed yield; this further supports the result of the lack of strong correlation between genetic diversity and seed yield in this population (Fig. 3.3).

3.4 Discussion

Among the different gene pools available for broadening the genetic base of *B. napus* canola, the secondary gene pool *B. oleracea* is important, especially when taking the available knowledge into account that the genetic base of the C genome of *B. napus* is narrow relative to its A genome (Bus et al. 2011; Wu et al. 2014). This diploid vegetable crop species exhibits wide morphological diversity, such as plants with enlarged single apical bud (cabbages, var. *capitata*) or multiple axillary buds (brussels sprout, var. *gemmifera*), or enlarged head of inflorescence meristem (cauliflower, var. *botrytis*) or with head of crowded flower buds (broccoli, var. *italica*) or leafy plants (Chinese kale and kale, var. *alboglabra* and var. *acephala*) (Babula et al. 2007; reviewed in Hong et al. 2007; reviewed in Ciancaleoni et al. 2014; El-Esawi, 2017). Molecular marker analysis has also demonstrated the existence of wide genetic diversity in *B. oleracea* (Louarn et al. 2007; Izzah et al. 2013; Pelc et al. 2015;

El-Esawi et al. 2016; Tortosa et al. 2017), where cabbage, broccoli, cauliflower, and kale are found to be genetically quite distinct from each other (Pelc et al. 2015).

In this study, we used lines derived from crosses of six *B. oleracea* accessions belonging to the abovementioned variants of this species with a single *B. napus* canola line to introgress alleles from these variants into the C genome of *B. napus* (Iftikhar et al. 2018). This crossing design allowed us to make a direct comparison of the effect of the alleles of these *B. oleracea* variants on different agronomic and seed quality traits in *B. napus*. Indeed, significant difference was found between the six populations for different traits, and wide variation was also found within these populations for these traits including seed yield. Among the six populations, the population derived from the cross involving broccoli gave the greatest seed yield and had higher oil content than the other populations. Multivariate analysis also confirmed that a large number of the inbred lines derived from the cross with broccoli clustered together, and these lines had higher seed yield and oil content and flowered and matured earlier. This population, in fact, had the least genetic distance from the *B. napus* canola parent. In contrast, the population derived from the cross involving the cabbage cv. Badger Shipper had the greatest genetic distance (0.57) from the *B. napus* parent; however, it had low seed yield, had the lowest seed oil content, and was the latest flowering. This indicates that a large number of alleles introgressed from *B. oleracea* into the *B. napus* inbred lines apparently exerted a negative effect on the agronomic and seed quality traits; therefore, molecular tagging of the favorable alleles of *B. oleracea* will benefit the use of these alleles in a knowledge-based molecular breeding program. According to Falk (2010) and Iniguez-Luy and Federico (2011), the poor performance often observed of the inbred lines derived from Elite × Exotic crosses can result from disruption of favorable combinations of the beneficial alleles of the elite lines; this might also be a reason for the poor performance of some of the lines derived from the *B. napus* × *B. oleracea* interspecific crosses. Linkage of

favorable and unfavorable alleles in exotic germplasm (Holland 2014) and introgression of large chromosomal segments carrying unfavorable alleles (Primard-Brisset et al. 2005) might also have contributed to the poor performance of the lines. Poor performance of the inbred lines derived from wide crosses has also been reported by different researchers, such as in *B. napus* × *B. oleracea* var. *alboglabra* (Rahman et al. 2016) and *B. napus* × *B. rapa* and (*B. napus* × *B. rapa*) × *B. rapa* interspecific crosses (Qian et al. 2005).

A reduction in plant height is important in canola breeding, especially for the development hybrid cultivars, which tend to be taller (Cuthbert et al. 2009; Rahman et al. 2016); reduced plant height can also improve lodging resistance in *Brassica* (Wang, Chen, Chu et al. 2016; Miller et al. 2018). Several lines, especially those derived from the crosses involving Chinese kale and cauliflower, were shorter than the *B. napus* parent, suggesting that alleles for reduced plant height can also be found in *B. oleracea*. In the present study, plant height did not show a significant genotypic correlation with seed yield; however, this trait showed a significant positive correlation with days to flowering and maturity (Table 3.2). Earliness of flowering and maturity are important traits for spring canola (Rahman et al. 2017). In this regard, the *B. oleracea* alleles contributing to earliness and reduced plant height can enrich the genetic base of this crop with favorable alleles for the development of short-stature canola cultivars without any negative effect on seed yield.

Several inbred lines derived from these interspecific crosses flowered earlier than the common *B. napus* parent, indicating that the alleles of the C genome of *B. oleracea* can contribute to earliness in *B. napus*. Rahman et al. (2011) developed a *B. napus* line from the *B. napus* × *B. oleracea* var. *alboglabra* cross, which flowered 5 d earlier than the *B. napus* parent, and mapped the *alboglabra* allele contributing to earliness without being influenced by photoperiod on the chromosome C1 (Rahman et al. 2017, 2018). The occurrence of early-

flowering lines from the crosses involving other variants of *B. oleracea*, such as *botrytis*, *capitata*, and *italica*, indicates that these variants also carry early-flowering alleles. This trait showed high heritability in this population, which agrees with the reports by several other researchers (Schranz et al. 2002; Kebede et al. 2010; Raman et al. 2013). The significant positive correlation ($r > 0.41$, $P < 0.001$) of genetic distance of the inbred lines with days to flowering observed in the present study (Fig. 3.3) indicates that *B. oleracea* also carry alleles for lateness of flowering and maturity. In this regard, identification of the early-flowering alleles of the abovementioned variants of *B. oleracea* and use in breeding would enrich the gene pool of *B. napus* canola for the improvement of earliness in this crop.

In case of seed oil content, the three populations derived from the crosses involving cabbages had lower seed oil content than the populations derived from the crosses involving broccoli and cauliflower. According to Zhao et al. (2005), positive and negative alleles for seed oil content can be dispersed in different germplasm, whereas accumulation of the positive alleles from different parents can result transgressive segregation for this trait. Indeed, this was found to the greatest extent in the population derived from the cross involving cauliflower. This suggests that *B. oleracea* can also contribute high-oil alleles for the improvement of this trait in *B. napus* canola despite the fact that this diploid progenitor species has never been subjected to breeding for the improvement of seed oil content. In this study, seed oil showed a significant negative correlation with seed protein content, as has been reported by several researchers in *B. napus* (for review, see Rahman et al. 2013).

The elite canola lines and cultivars carry favorable allele combinations, which have been established over several cycles of breeding. In the present study, inbred *B napus* lines were developed through recurrent self-pollination of both F_2 and BC_1 (F_1 × *B. napus*). Theoretically, it was expected that, in absence of any selection, inbred lines derived from BC_1

will carry fewer *B. oleracea* alleles than the F_2–derived lines. However, genetic distance of the F_2– and BC_1–derived populations used in the present study was quite similar (0.47 vs. 0.49), which suggests that the loss of a greater number of *B. oleracea* alleles occurred in the F_2–derived population as compared with the BC_1–derived population. All inbred lines used in this study were canola quality (i.e., contained zero erucic acid in oil and low glucosinolate in seed meal). Selection for these two canola quality traits was apparently more intensive in the F_2–derived population than in the BC_1–derived population, and this might have contributed to the loss of *B. oleracea* alleles to a greater extent in the F_2–derived population. Rahman et al. (2015) also found ~2.7-fold more *B. oleracea* alleles in non-canola-quality-type inbred lines than in the canola-quality inbred lines while working with a single accession of this species. Such loss of alleles might have occurred in early generations, as has been found in case of a *B. napus* × *B. rapa* interspecific cross (Attri and Rahman 2018).

In conclusion, this study provided evidence of the potential value of the *B. oleracea* gene pool for the improvement of agronomic and seed quality traits of *B. napus* canola. Among the different variants of this gene pool, broccoli was found to have the greatest potential for increasing seed yield and cauliflower for both seed yield and oil content. The low to moderate negative correlation of genetic distance of the inbred lines from the *B. napus* parent with seed yield and oil content found in this study apparently resulted from introgression of unwanted alleles from the *B. oleracea* gene pool. However, lines with superior performance to the *B. napus* parent can also be selected from this population, suggesting that some of the *B. oleracea* alleles in combination with the *B. napus* alleles can improve the performance of this oilseed crop. Thus, the use of *B. oleracea* alleles in a knowledge-based breeding program can substantially improve the performance of oilseed *B. napus* canola.

3.5 Tables

Table 3.1 Estimates of variance components and broad-sense heritability for different agronomic and seed quality traits of the 279 inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses, involving a single *B. napus* line and six *B. oleracea* cultivars or lines belonging to four variants of this species, and following two breeding techniques (F_2– and BC_1–derived).

Variance components†	**Days to flowering**	**Duration of flowering**	**Duration of the grain-filling period**	**Plant height**	**Seed yield**	**Seed oil**	**Seed protein**
F_2–derived population							
Genotypic variance (σ^2_G)	2.3	0.05	0.3	13.8	31497.0	0.7	0.6
Genotype × environment variance ($\sigma^2_{G\times E}$)	1.6	2.1	0.5	13.8	20761.0	0.4	0.4
Residual variance (σ^2_E)	1.7	1.4	1.1	78.4	103462.0	1.1	1.0
No. of environments (N_E)	10	6	6	10	10	10	10
Heritability (H)	90.4	9.0	59.7	72.2	81.3	88.1	86.3
BC_1–derived population							
Genotypic variance (σ^2_G)	1.7	0.3	0.4	22.6	26019.0	0.8	0.4
Genotype × environment variance	1.8	1.5	0.3	15.1	27298.0	0.5	0.6
Residual variance (σ^2_E)	1.3	1.4	1.3	73.0	119442.0	1.2	1.1
No. of environments (N_E)	10	6	6	10	10	10	10
Heritability (H)	87.0	41.4	74.1	81.4	74.9	87.4	77.8

† σ^2_V (phenotypic variance of a quantitative trait) = $\sigma^2_G + \sigma^2_E + \sigma^2_{G\times E}$. Number of replications $N_R = 2$.

Table 3.2 Phenotypic and genotypic correlations between different agronomic and seed quality traits in 279 inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses involving a single *B. napus* line and six *B. oleracea* cultivars or lines, belonging to four variants of this species, and following two breeding techniques (F_2– and BC_1–derived).

Traits	Duration of flowering	Days to maturity	Duration of the grain-filling period	Plant height	Seed yield	Seed oil	Seed protein
Phenotypic correlation coefficients (r_p)							
Days to flowering	−0.06NS†	0.73***	−0.24***	0.27***	−0.10***	−0.30***	0.24***
Duration of flowering		0.45***	−0.48***	0.15***	−0.01NS	−0.21***	0.23***
Days to maturity			0.04NS	0.16***	0.02NS	−0.27***	0.24***
Duration of the grain-filling period				−0.13***	0.14***	0.07NS	−0.01NS
Plant height					0.11***	−0.06NS	0.05NS
Seed yield						0.15***	−0.13***
Seed oil content							−0.83***
Genotypic correlation coefficients (r_g)							
Days to flowering	0.19NS	0.93***	−0.57***	0.84***	−0.12***	−0.36***	0.18**
Duration of flowering		0.24*	−0.40***	0.31**	−0.77***	−0.36***	0.36**
Days to maturity			−0.46***	0.74***	0.13NS	−0.22*	−0.30**
Duration of the grain-filling period				−0.52***	0.33***	0.45***	−0.18*
Plant height					0.06NS	−0.25***	0.12NS
Seed yield						0.55***	−0.75***
Seed oil content							−0.65***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† NS, nonsignificant.

Table 3.3 Scores for different agronomic and seed quality traits after principal component analysis (PCA) for the first three components.

Traits	PC1	PC2	PC3
Days to flowering	1.995	−1.074	0.409
Duration of flowering	1.086	0.803	−1.658
Days to maturity	1.590	−1.510	0.324
Duration of the grain-filling period	−1.578	−0.061	1.290
Plant height	1.685	−1.273	0.002
Seed yield	−1.104	−1.686	−0.114
Seed oil	−1.664	−1.068	−0.763
Seed protein	1.313	1.585	0.842

3.6 Figures

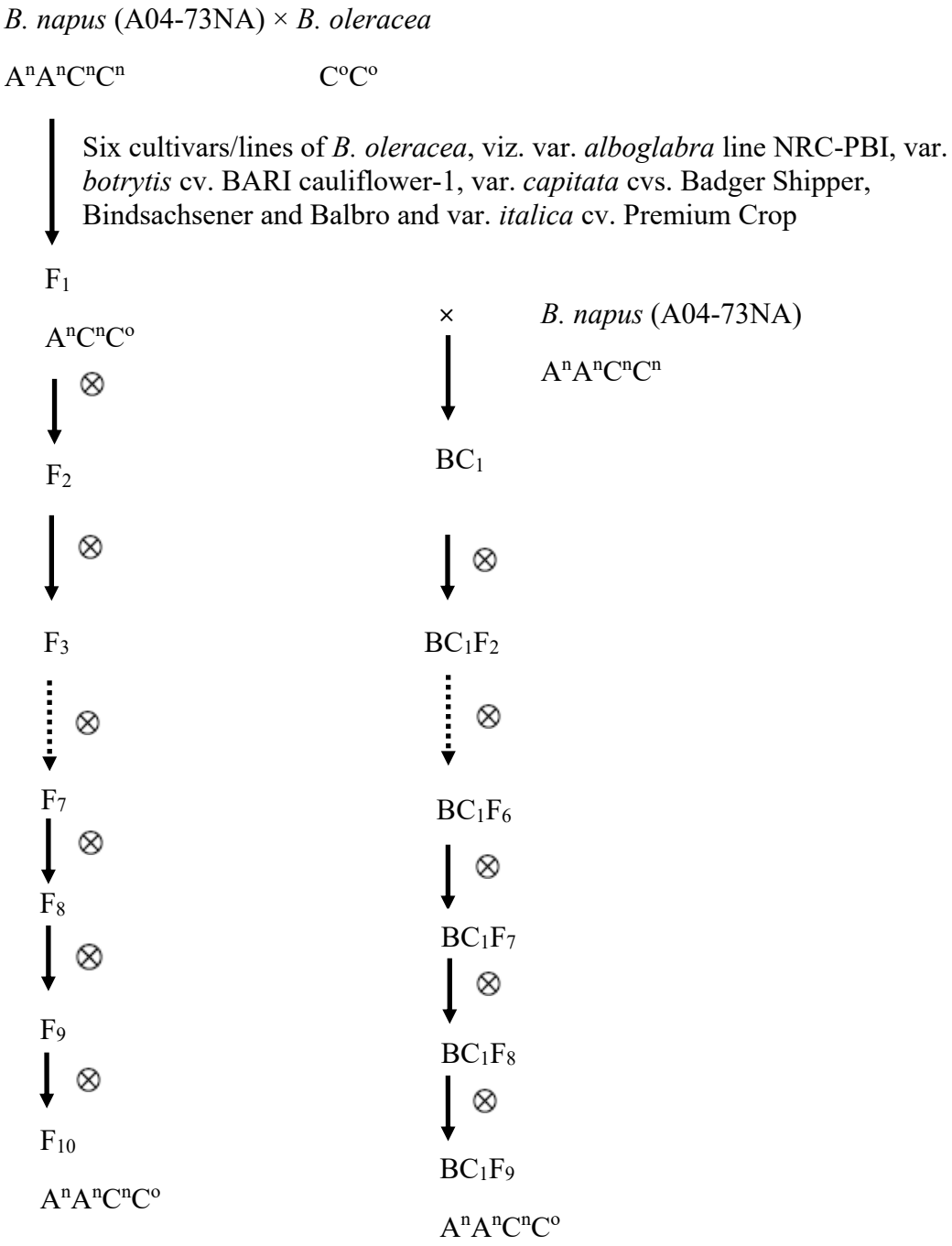


Fig. 3.1 A flow diagram illustrating the development of canola quality *Brassica napus* lines from *B. napus* × *B. oleracea* interspecific crosses. The F_1 plants were subjected to two breeding techniques: self-pollinations (⊗) of the F_1 plants for several generations and backcrossing (BC) the F_1 to the *B. napus* (A04-73NA) parent followed by self-pollination for several generations.

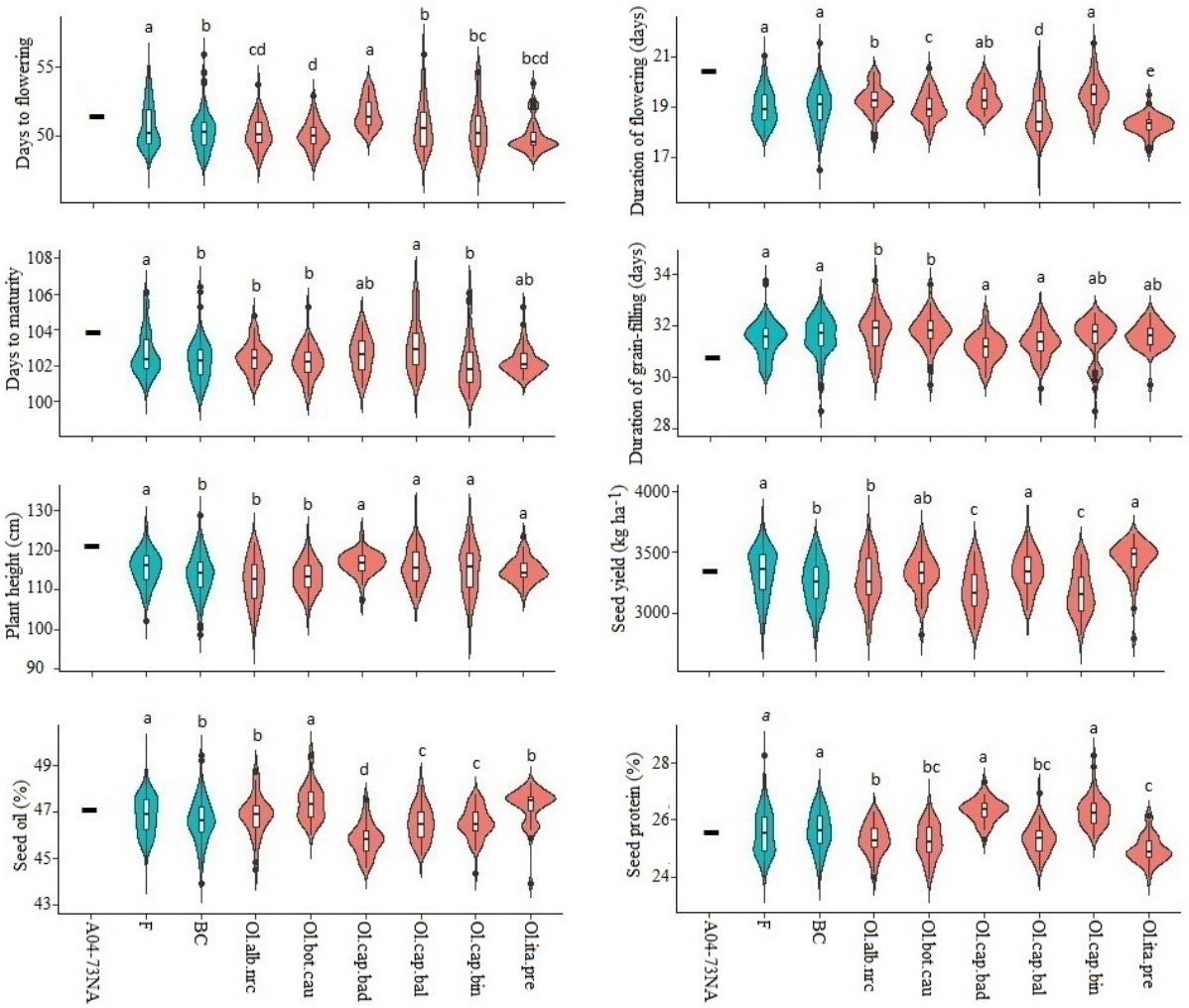


Fig. 3.2 Violin plot of six populations derived from six *Brassica napus* × *B. oleracea* interspecific crosses and following two breeding techniques (F_2– and BC_1–derived), and the common *B. napus* parent A04-73NA for different agronomic and seed quality traits. Blue color plots represent the two breeding techniques and red color plots represent the six crosses. Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI (n = 45); Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1 (n = 48); Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper (n = 38); Ol.cap.bal = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Balbro (n = 51); Ol.cap.bin = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Bindsachsener (n = 55); Ol.ita.pre = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop (n = 42). F = F_2–derived population (n = 135); BC = BC_1 (F_1 × *B. napus*)-derived population (n = 144). Least square mean values of the violin plots with the same letter are not significantly different at $P < 0.05$.

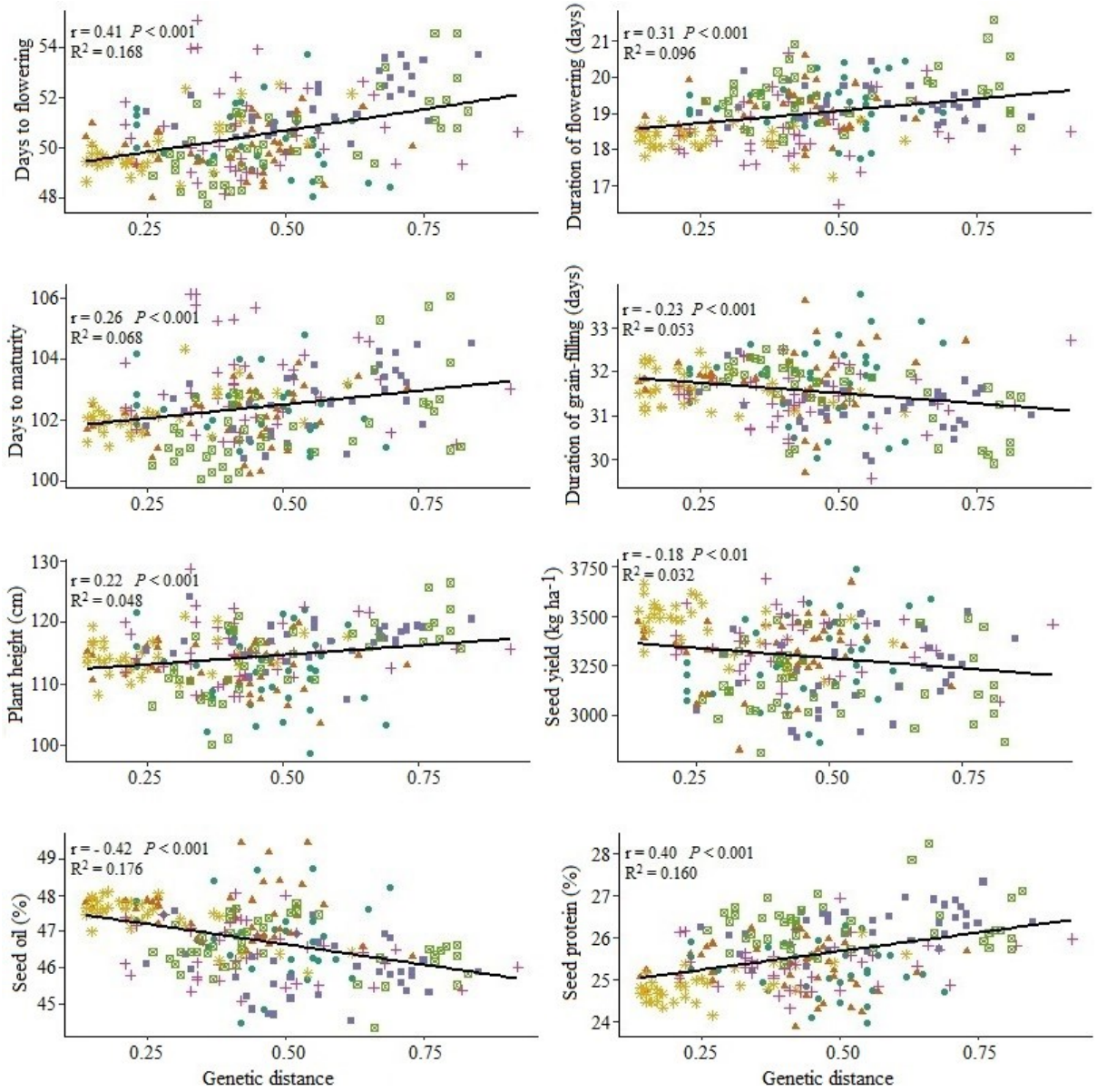


Fig. 3.3 Correlation between genetic distance of the *Brassica napus* inbred lines (n = 228), derived from six interspecific crosses of *B. napus* × *B. oleracea* (Ol.alb.nrc [circle, sea foam color], Ol.bot.cau [triangle, chestnut color], Ol.cap.bad [solid square, violet color], Ol.cap.bal [plus sign, purple color], Ol.cap.bin [square, green color], and Ol.ita.pre [asterisk, yellow color]) and following two breeding techniques (F_2– and BC_1–derived), and different agronomic and seed quality traits. Genetic distance of the inbred lines is the distance from the common *B. napus* parent estimated by simple sequence repeat (SSR) marker analysis.

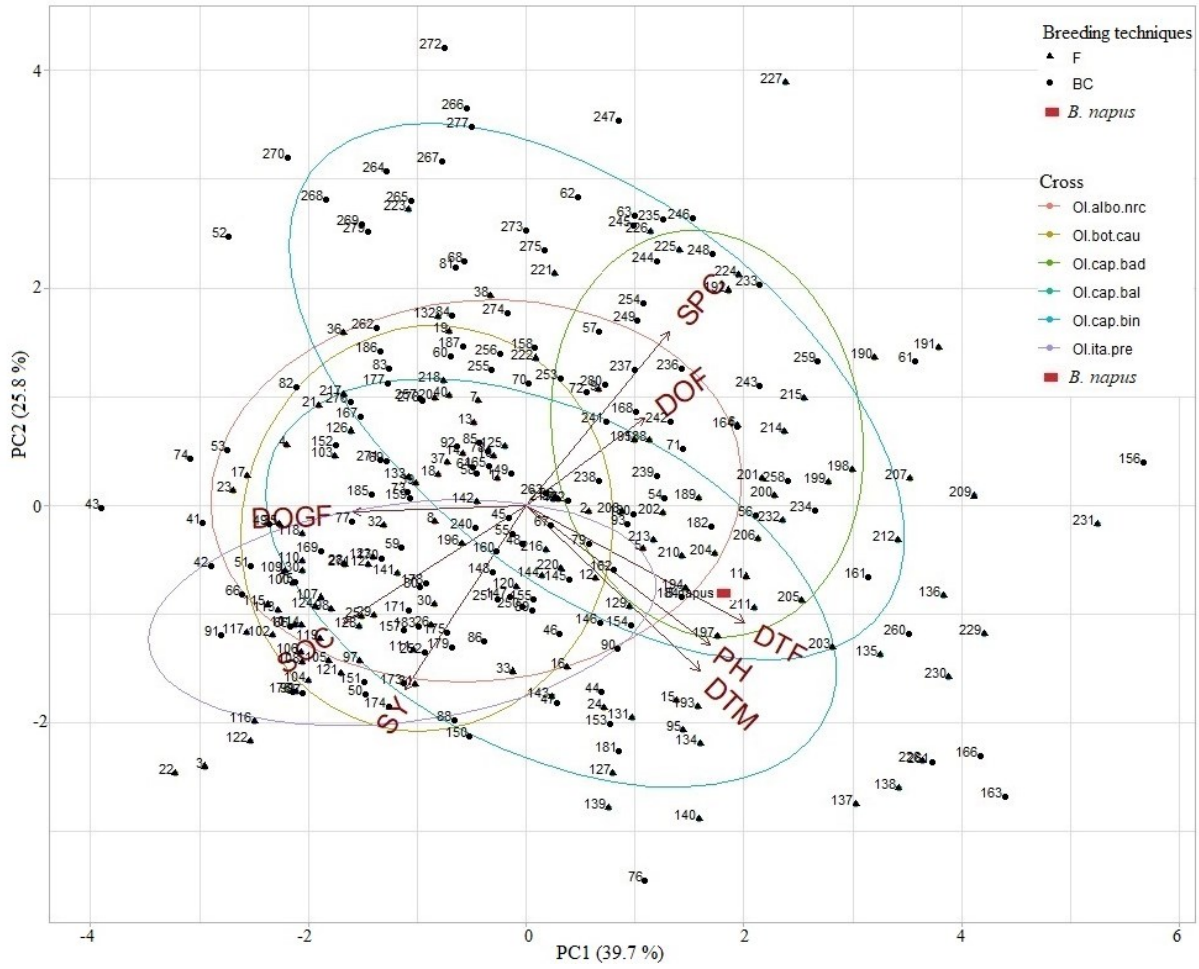


Fig. 3.4 Principal component analysis biplot of inbred lines (n = 279) derived from six *Brassica napus* × *B. oleracea* interspecific crosses and the common *B. napus* parent A04-73NA, illustrating the distribution of inbred lines characterized by different agronomic and seed quality traits in the space of the two principal components (PCs). The names of the inbred lines were shown in Supplemental Table 3-1. DTF = days to flowering; DOF = duration of flowering; DTM = days to maturity; DOGF = duration of the grain-filling period; PH = plant height; SY = seed yield; SOC = seed oil content; SPC = seed protein content. Ol.alb.nrc (red color) = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI (n = 45); Ol.bot.cau (mustard) = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1 (n = 48); Ol.cap.bad (green) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper (n = 38); Ol.cap.bal (teal) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro (n = 51); Ol.cap.bin (blue) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener (n = 55); Ol.ita.pre (violet) = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop (n = 42); *B. napus* parent (red rectangle). F (solid triangle) = F_2–derived population (n = 135); BC (solid circle) = BC_1 (F_1 × *B. napus*)-derived population (n = 144).

3.7 Supplemental materials

Supplemental Table 3.1 List of the F_2- and BC_1-derived inbred lines of six *Brassica napus* × *B. oleracea* interspecific crosses used in this study and their codes.

Inbred line number	Inbred line code	Cross	Breeding technique
1300-343	1	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-348	2	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-353	3	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-355	4	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-360	5	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-363	6	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-368	7	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-373	8	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-375	9	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-381	10	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-390	11	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-395	12	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-398	13	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-401	14	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-404	15	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-410	16	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-412	17	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-413	18	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-416	19	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-419	20	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-420	21	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1343-320	22	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-321	23	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-323	24	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-327	25	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-329	26	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-330	27	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-333	28	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-336	29	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-339	30	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-343	31	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-348	32	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-349	33	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-351	34	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-353	35	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-357	36	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-360	37	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-362	38	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-367	39	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-368	40	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1676-361	41	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-363	42	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-365	43	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-377	44	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-380	45	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-389	46	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-393	47	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-402	48	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-405	49	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-407	50	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-409	51	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-412	52	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC

Inbred line number	Inbred line code	Cross	Breeding technique
1676-413	53	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-416	54	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-421	55	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-422	56	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-423	57	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-427	58	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-429	59	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-432	60	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-438	61	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-440	62	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-442	63	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-446	64	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1677-326	65	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-328	66	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-330	67	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-334	68	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-340	69	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-342	70	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-344	71	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-348	72	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-351	73	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-352	74	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-355	75	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-358	76	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-360	77	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-363	78	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-375	79	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-376	80	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-379	81	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-383	82	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-386	83	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-387	84	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-390	85	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-394	86	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-395	87	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-401	88	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-405	89	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-409	90	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-411	91	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-414	92	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-418	93	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1358-594	95	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-609	96	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-615	97	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-616	98	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-620	99	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-623	100	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-624	101	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-634	102	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-635	103	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-640	104	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-652	105	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-656	106	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-660	107	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-667	108	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-679	109	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-685	110	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-688	111	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F

Inbred line number	Inbred line code	Cross	Breeding technique
1358-699	112	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-701	113	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-703	114	A04-73NA × B. oleracea var. italica cv. Premium Crop	F
1358-705	115	A04-73NA × B. oleracea var. italica cv. Premium Crop	F
1358-708	116	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-711	117	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-719	118	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-720	119	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-727	120	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-731	121	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-739	122	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-747	123	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-752	124	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1392-295	125	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-296	126	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-297	127	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-300	128	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-303	129	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-305	130	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-306	131	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-312	132	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-313	133	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-319	134	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-320	135	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-321	136	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-324	137	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-325	138	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-327	139	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-329	140	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-337	141	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-339	142	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-342	143	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-345	144	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1678-263	145	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-264	146	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-265	147	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-268	148	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-272	149	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-277	150	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-281	151	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-285	152	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-290	153	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-291	154	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-309	155	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-316	156	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1679-348	157	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-354	158	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-357	159	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-369	160	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-377	161	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-378	162	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-380	163	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-382	164	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-388	165	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-399	166	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-405	167	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-420	168	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-430	169	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC

Inbred line number	Inbred line code	Cross	Breeding technique
1679-437	170	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-440	171	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-442	172	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-460	173	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-465	174	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-470	175	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-473	176	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-474	177	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-483	178	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-486	179	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-497	180	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-502	181	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-503	182	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-506	183	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-511	184	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-535	185	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-541	186	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-543	187	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1362-149	188	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-152	189	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-155	190	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-156	191	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-158	192	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-161	193	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-162	194	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-164	195	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-165	196	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-166	197	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-167	198	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-169	199	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-170	200	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-171	201	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-173	202	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-174	203	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-175	204	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-176	205	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-177	206	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-179	207	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-180	208	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1363-164	209	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-165	210	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-168	211	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-170	212	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-171	213	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-173	214	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-177	215	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-178	216	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-180	217	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-181	218	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-182	219	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-183	220	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-185	221	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-186	222	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-190	223	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-194	224	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-195	225	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-197	226	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F

Inbred line number	Inbred line code	Cross	Breeding technique
1363-202	227	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-205	228	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-206	229	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-207	230	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-208	231	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-211	232	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1681-082	233	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-083	234	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-084	235	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-085	236	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-086	237	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-090	238	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-091	239	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-092	240	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-096	241	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-097	242	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-098	243	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-100	244	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-101	245	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-102	246	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-103	247	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-104	248	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-105	249	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1682-099	250	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-100	251	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-101	252	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-102	253	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-103	254	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-104	255	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-105	256	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-108	257	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-113	258	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-120	259	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-124	260	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-125	261	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-128	262	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-130	263	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-131	264	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-133	265	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-137	266	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-138	267	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-140	268	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-143	269	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-145	270	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-147	271	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-149	272	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-150	273	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-152	274	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-154	275	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-155	276	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-156	277	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-158	278	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-159	279	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-164	280	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC

Please note that Inbred line code 94 was given to the *Brassica napus* parent A04-73NA; thus, the total number of inbred lines = 280 – 1 = 279.

Supplemental Table 3.2 List of 96 SSR markers used for genotyping 110 F_{10} and 118 BC_1F_9 inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses and following two breeding techniques (F_2- and BC_1-derived).

Source	Primer #	Primer Name	Linkage group	Allele size (bp)	Motif	Forward primer sequence 5`-3`	Reverse primer sequence 5`-3`
AAFC[1]	2278	sN2087	C1	475	TC	GAACCTCGAAAACGGTTGAA	CTCCCCCGATCTATACCCAT
AAFC	2279	sN3734	C1	273	CT	CCCCTTCCGGTTAAACAAAT	AAAACAGACTTTGCCCGTTG
AAFC	2286	sN0691	C1	375	GT	GCAAATCTTGTTTTTGTGAGTACA	GTCTTGGAAGCAGCCTAACG
AAFC	2299	sR1078	C1	402	TGA/TGG	GGCGTGGGAGTAGGTGTAGA	GACTGATACCATCACGGGCT
AAFC	2300	sNRF94	C1	310	CT	GATGACTGTGCCTGCTAAACC	GCATCTCGATTCAATCCTCC
AAFC	2301	sN3569F	C1	189	CT	TGTACGTGCACCACGTTTTT	CTTCGATTACTCGGTGGCAT
AAFC	2310	sN11675	C1	263	GA	ATATTGGGGGTCCTGGAGTC	TCCTTGCTTGAGCCTTTCAT
AAFC	2309	sN1834	C1, C3, C9	277	GA	CCGTGAACGTCATTGATCTG	CCTTCTCATACCTCTCCCCC
AAFC	2297	sN11657	C1, C4	248	GA	CAGGTTGGTTTGACATGGTG	GCACACAGAGTGACGTTTGG
AAFC	624	sN0758	C1, C5, C7	346		ATTCAGCGTCTGATGCAGTG	ATGGGGTAATGCACCAAAAA
AAFC	2311	sN0842	C1, C9	438	GA	AAGCCTGACAATCCAAAACG	CGATTTCATGGCAAATTCCT
AAFC	262	sR10417	C2	247		CGGAGAAGAAACGAGCATTC	TAGGGTTTCTGACCCGATTG
AAFC	315	sN3761	C2	173		CGACAGAGGGTTCAAATGGT	CGGTGTGTAGGTCTGCTCAA
AAFC	2059	sNRE74	C2	158		CAATCATGAATATCGGCAACA	CGTCATTCCAAACTTTAGGTCA
AAFC	2069	sR2028	C2	251		ATGCCCCATGTGGATTGTAG	TTTGGTGGAAACCGATGAAT
AAFC	2072	sS2206	C2	120		TTTCATCATTTCGACTCACCC	TTATCTTCTCTCATTTCGCCG
AAFC	2082	sR1863	C2	257	$(ACA)_8$	TTTGATGGGTCTTCATCTTC	GAGGTTAAGGGTTTGGAGTT
Cheng *et al*. (2009)	2222	BnGMS633	C2	342	$(AT)_8$	CCAGTTCCATTCTCAATCAG	TATTTGTGTTCTCACGATGG
AAFC	2062	sN1825	C2, C3	185		CCACTGAGCGGTAGAGAAGG	CGGACTTTTACGGTGTTCGT
AAFC	2065	sS2268	C2, C3	185		CTTCTGCTCTGGCTGAAACA	TGATGTCTTCGCTGCTGTCT
AAFC	2366	sN3815	C2, C4, C6, C7	482	AG	TTCAAGCTATGCAGTGTGGC	GGTCTGGAAATCGCTGCTT
AAFC	2075	sORE66	C2, C5, C8	322		CGAGGTGGGAGAGATGAGAG	ATGGAACGCCAAAACAAAAA
AAFC	2063	sN1937	C2, C6	281		CCCGCACTTTCTTCCTATTG	GGTGATGGTAACGAGCGATT
AAFC	110	sN2316	C3	275		GAGTCGTCAGCGTCTTCCTC	TTTGATTCCCTCTGCATTCC
Piquemal *et al*. (2005)	435	CB10036	C3	179-185		ATTCATCTCCTGCTCGCTTAG	AAACCCAAACCAAAGTAAGAA
Piquemal *et al*. (2005)	439	CB10057	C3	190-220		CTAGGCTAAGGAAGATTGTCA	TAGTTTCTTCCTCCTGCTATC
Li *et al*. (2011)	1082	BoGMS0819	C3	114		AGGGAGATGGACACATTTAG	GAGAGAGGGCAAAGAAGATAG
Li *et al*. (2011)	1085	BoGMS0767	C3	114		AAACAAGTCAGATTCACCAAA	CTCTTCACCACTACCACAGTC
Li *et al*. (2011)	1092	BoGMS0570	C3	214		TACAATCTTCTTCGCTGCT	AAACCTGAAACTCCCTCAA
Li *et al*. (2011)	1104	BoGMS1360	C3	318		GAGACCAGAGAAGGAGGAAC	CACTCACTATCACACACACTCA
Li *et al*. (2011)	1107	BoGMS0953	C3	133		CCTCGTAAGTAACCGAATCA	AAACAGAAGATGGAGAAGGAG
Li *et al*. (2011)	1123	BoGMS0081	C3	293		AGTCCTAATGGTGCTCTTTGT	CTGTTGAGGTGTTGTCCTTT
Source	Primer #	Primer Name	Linkage group	Allele	Motif	Forward primer sequence 5`-3`	Reverse primer sequence 5`-3`

				size (bp)			
AAFC	302	sS2277	C4	223		GATCTGCGGTAGGAATCGAA	CGTGCTACATAATAGGGAAAA ACC
Piquemal *et al*. (2005)	731	CB10109	C4	281		GTGTAGCCAGCTTGATCCT	CTTCTTCTGATGCAGCAGTG
BBSRC[2]	764	Ra2-F11A	C4	211		TGAAACTAGGGTTTCCAGCC	CTTCACCATGGTTTTGTCCC
BBSRC	982	MR140	C4	143		CCCATATTCTAATCGTTCCA	TTCACTCATTCTTTGCTCATT
BBSRC	989	Ol11H02a	C4	203	AAC	TCTTCAGGGTTTCCAACGAC	AGGCTCCTTCATTTGATCCC
Piquemal *et al*. (2005)	990	BRAS061	C4	210-246		GCAGCCTTCAACTCCCATAGA	TGGGTTCGAGCAGGGTTC
Piquemal *et al*. (2005)	994	CB10493	C4	184-222		TGACGTGTGAGCAACAGA	CTGAGTCACAAGCCGAGT
AAFC	2099	sR0357	C4	376		CCGGCTCTTGTTTTATGGTT	AACACCGTTTCATCTTTGGC
AAFC	2113	sN3685R	C4	285		CCGCAAGCTCTTAACTCCAC	AACTGCATTCGTCCAGCTCT
AAFC	2115	sN3817	C4	169		CCTGCCGTAACGTTCTTGTT	ATCTTCGAAGCAATCTCGGA
Cheng *et al*. (2009)	2200	BnGMS347	C4	273	$(AT)_{14}$	TCACACAAATCTCCTCCTCT	AGGTATCAGCCAATGACTTC
Cheng *et al*. (2009)	2225	BnGMS681	C4	131	$(GT)_8$	GTCGAAGATTGTTGTCAGGT	TTCACGAAGAACCCTAGAAA
Li *et al*. 2011	2233	BoGMS0836	C4	146	$(AT)_{16}$	CATAAACACACCGAACAAGAC	ACGCAATGACACACATACAC
AAFC	2379	sN12743J	C4, C6, C8, C9	370	CT	CTAGCCACCATGAAAGGAGC	AAACCAAGCAAACCCATCAG
AAFC	2102	sNRG34	C4, C9	294		TCTCATTTTTCCTCAAGCTCC	CCACCAGCCATAGTCATCCT
AAFC	54	Snrco3	C5	192		AACTCATCGGGTCAAATTGC	GAAGAACAGAAGCAGCACCC
AAFC	616	sORA84	C5	178		CAAGAAACACCATCATTTCTCAA	GGCCCATTGATATGGAGATG
AAFC	621	sN2052	C5	416		GCTCCCAAGAGCAACAC	TCACAGTTGATCCCTGTTAAT
AAFC	721	sN0761	C5	298		CGGAATTAGTGGAGTGGGAA	TATCACTGTTGTCTGCCCCA
Li *et al*. (2011)	1056	BoGMS0590	C5	399		TGGTTTATCTTCATTCTTTGG	TATTGAGTTGTCGCACTTGA
AAFC	2445	sORB17	C5	414	CA	ACCATTGAGGTTTGTCGGAG	AAAGCTTCGGCAATAATGGA
AAFC	2448	sN12153I	C5	181	TCC/GCC	CCTCTCCCTTGGCTCTTCTT	CTGAGGAGAGGGTTTAGCGG
AAFC	2452	sN11661	C5	341	CT	CAGTCAATACTCGCCGAACA	AATCGGAGGGGCCATTATAG
AAFC	2453	sN12503	C5	290	AGG	CACGGAGGAACAGAGGAGAG	TCCCACTGGCCATAGTTAGG
AAFC	2477	sR0622	C5	377	AGG	CTTGGGAAGTTCAGGAGCAG	CCGGAACAAGCATAAGAGGA
AAFC	607	sN7410	C5, C8	155		CAGATGGGAAGAGCAAAAGC	ATGCCCTGGAGTCAATGTTC
Piquemal *et al*. (2005)	733	CB10211	C6	150		CAGCAGAGATCGATGGAG	ATAGAAGGCTGCCCCTC
BBSRC	756	Na10-C06	C6	223		TGGATGAAAGCATCAACGAG	ATCAATCAACACAAGCTGCG
Suwabe *et al*. (2002)	991	BRMS-015	C6	263	$(TG)_4$, $(GA)_{20}$	TCGCCAATAGAACCCAAAACTT	CATCTCCATTGCTGCATCTGCT
Cheng *et al*. (2009)	2213	BnGMS491	C6	161	$(AT)_{10}$	AAGTGTGTATTAGGGACGAGT	TCCCGTACTTCAAGCTGTAT
Li *et al*. (2011)	2236	BoGMS0632	C6	201	$(AG)_{20}$	CATCATCGTCCTCTTCTTCTTC	TATCATCCTTATTGGGTCTC
AAFC	2365	sN11904	C6	239	CTT	CAATGGATCGGATGGAGATT	GTCTTGTCTTCATGGTCGGG
AAFC	2373	sS2352	C6	183	GA	TGAGAAGGGGAACAGTCGAT	TGTGTTGTTTTGGATTTTGG
AAFC	2374	sN11862	C6	290	AC	AGGGACAACGAGCATACCAC	AGGCGCCTTCAATCCTATTT
AAFC	2122	sNRD41	C7	241	GT/GA	AAAGGGCGGTCTAGCATCTT	CGTCAATGCTCAAATCCCTT
Source	Primer #	Primer Name	Linkage group	Allele	Motif	Forward primer sequence 5`-3`	Reverse primer sequence 5`-3`

				size (bp)			
Cheng *et al*. (2009)	2205	BnGMS386	C7	220	$(AT)_{12}$	TTGGCTCATCAATGACAATA	ACAATGTGGTAAACACGAAA
Li *et al*. (2011)	2242	BoGMS1065	C7	209		GGGTTGATTGGGAAGTGT	CTTAGCACCATTTGTTTGTATT
AAFC	2391	sN2564	C7	348	AAC/AAG	GAATTCCTTCTGGGCTTTCC	CTAAATGAGGATGGGAGCGA
AAFC	2393	sORF37	C7	100	GT	GAAGGCTCAACAAAAATGGG	AAGCCCAAAGGTAAGGAAGG
AAFC	2410	sN1975	C7	140	AG	TCCCTTGCCTTCTCTTCTTG	TCGGCCAAGCATCTCTAACT
AAFC	2420	sNRD41	C7	241	GT/GA	AAAGGGCGGTCTAGCATCTT	CGTCAATGCTCAAATCCCTT
AAFC	2428	sN3825J	C7	307	GA	CTGCGTCGTCGAAGTTCATA	TCTCCTTGAAAAACACAGCG
AAFC	2431	sN0706	C7	401	GA	TCCGACGGTCAAGATTAAGG	GGCTGTGGTGGATCTAGGAA
AAFC	240	sN2557	C8	456		GCATCACTCTAGGGTTTCCG	CAAAGCAACCGACAAGAACA
Piquemal *et al*. (2005)	489	CB10139	C8	170-180		TCTCAAAAGGATATGCGTGAA	CAAAACTCATCAGGGTTGTAG
Piquemal *et al*. (2005)	992	CB10028	C8	150-199		CTGCACATTTGAAATTGGTC	AAATCAACGCTTACCCACT
AAFC	2087	sN11670	C8	100		AGTCGGGCTCGTATATCTCG	GTTTCGTGGCGGAAATTAGA
Cheng *et al*. (2009)	2179	BnGMS3	C8	359	$(CTT)_{15}$	AAAGAGCCCACATGAAAGTA	TGAACTAGGCACCAAGAACT
Cheng *et al*. (2009)	2180	BnGMS4	C8	370	$(GAA)_{15}$	AAAGCTGCAGAAAGAAGATG	ATCCGTTCTATACTGCTCCA
Cheng *et al*. (2009)	2184	BnGMS83	C8	325	$(TCT)_{7}$	CCACTTGCAGCGTTATTATT	CGAGGAAATAGACAAAGTGG
Cheng *et al*. (2009)	2199	BnGMS336	C8	345	$(AT)_{15}$	ACCGAATAACAAGTCGAACA	TTGAAACACACCCATTTACA
Cheng *et al*. (2009)	2202	BnGMS352	C8	257	$(AT)_{14}$	AGTCCTGAAGCCTGAACATA	AGTTTGCCATCTCGTAGAAA
Li *et al*. 2011	2244	BoGMS0468	C8	259	$(AT)_{25}$	TGACAGCAACCAATGATG	CTCTCTGGAACCTTTGAACT
Li *et al*. 2011	2246	BoGMS0741	C8	286	$(TC)_{17}$	CTCAAACTCCGTCGCTCT	TCCTCCTCACTACTTTCTTCA
Li *et al*. 2011	2248	BoGMS0868	C8	226	$(CT)11(TC)_{5}$	AAATCCCAACGAGATAGGTAG	AGAAAGAAAGGAAGAAAGTGG
AAFC	225	sN0653	C9	194		TTCTGAATCTCCGCCGTATC	CTTTGGGGGCATCTTCAA
Iniguez-Luy *et al*. (2008)	751	FITO095	C9	233		AGATTTCATCCACAGCCTC	TTTGATTCTTGCGTTCTCTC
Cheng *et al*. (2009)	2182	BnGMS43	C9	234	$(ACA)_{8}$	TTTGATGGGTCTTCATCTTC	GAGGTTAAGGGTTTGGAGTT
Cheng *et al*. (2009)	2193	BnGMS213	C9	134	$(AAAGA)_{4}$	GTAGTACGGAGATGCGTGAT	AAAGAACGAGTTGACTTTCG
Cheng *et al*. (2009)	2204	BnGMS385	C9	197	$(AT)_{12}$	TTTCATGACTTAGCCACCTT	CCAAGTATTCAATTTCTGGC
Li *et al*. (2011)	2257	BoGMS0845	C9	199	$(AG)_{16}$	CCTTTGTCTTCTTCACTCTCC	ACCAGGCTCTTTCTTTCTCT
Li *et al*. (2011)	2258	BoGMS1283	C9	248	$(TCA)_{9}$	TTGTCATCATCCTCTTCACTC	TGCTATCCACTCTTCTTCTCA
	3040	3040	C9	239	$(AT)_{12}$	TCAAACTTTTGACTTTGAATATCCC	AAACAATTTTCAAGTTTTGGTCA
	3098	3098	C9	319	$(A)_{10}$	TGTGGTGGTCACTGACGATT	TCTATGGTCCTCCATGCACA

[1] AAFC = Agriculture and Agri-Food Canada.

[2] BBSRC = Biotechnology and Biological Science research council.

Supplemental Table 3.3 Analysis of variance for different agronomic and seed quality traits of 279 inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses and following two breeding techniques (F_2- and BC_1-derived).

Source	df	Error df	MS	F value	P value
Days to flowering					
Cross	5	267.1	232.6	6.8	<.0001***
Technique	1	267.2	183.7	5.4	0.0210*
Cross*Technique	5	267.2	263.9	7.7	<.0001***
Residual	5149		3.1		
Duration of flowering (days)					
Cross	5	266.7	111.7	24.1	<.0001***
Technique	1	266.9	2.2	0.5	0.4950NS
Cross*Technique	5	267.1	13.4	2.9	0.0146*
Residual	3021		3.03		
Days to maturity					
Cross	5	284.1	6895.5	293.6	<.0001***
Technique	1	272.1	160.6	7.0	0.0089**
Cross*Technique	5	270.6	148.4	6.4	<.0001***
Residual	4441		87.5		
Duration of grain-filling (days)					
Cross	5	266.9	32.0	5.5	<.0001***
Technique	1	267.0	4.3	0.7	0.4 NS
Cross*Technique	5	267.1	10.5	1.8	0.1NS
Residual	3020		1.6		

Source	df	Error df	MS	F value	P value
Plant height (cm)					
Cross	5	267.2	2340.6	5.8	<.0001***
Technique	1	267.4	2820.3	7.0	0.0089**
Cross*Technique	5	267.4	1589.2	4.0	0.0019**
Residual	5150		90.0		
Seed yield (kg ha^{-1})					
Cross	5	267.2	7166802.0	12.8	<.0001***
Technique	1	267.5	8316386.0	14.9	0.0001***
Cross*Technique	5	267.4	1757905.0	3.2	0.0089**
Residual	5148		134158.0		
Seed oil (%)					
Cross	5	267.2	261.3	25.1	<.0001***
Technique	1	267.3	95.5	9.2	0.0027**
Cross*Technique	5	267.2	68.6	6.6	<.0001***
Residual	5144		1.6		
Seed protein (%)					
Cross	5	267.2	282.5	51.0	<.0001***
Technique	1	267.5	1.4	0.3	0.6133NS
Cross*Technique	5	267.4	20.6	3.7	0.0029**
Residual	5144		1.5		

Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.00$, NS Not significant.
Data for duration of flowering and duration of grain-filling was available for six environment and data for days to maturity was available for nine environments.

Supplemental Table 3.4 Least square means ± SE of the F_2- and BC_1-derived populations of six *Brassica napus* × *B. oleracea* interspecific crosses for different agronomic and seed quality traits.

Cross[1]	Breeding technique[2]	Days to flowering	Duration of flowering (days)	Days to maturity	Duration of grain-filling (days)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
Cross and Breeding technique									
A04-73NA		51.4 ± 2.1^{abc}	20.4 ± 3.9^{a}	103.8 ± 3.0^{abcd}	30.7 ± 1.9^{ab}	120.6 ± 6.8^{a}	3327.9 ± 222.9^{abc}	47.0 ± 0.9^{abc}	25.5 ± 0.9^{ab}
Ol.alb.nrc	F_2	50.5 ± 1.8^{c}	19.2 ± 3.9^{bcd}	102.5 ± 2.9^{abcd}	31.7 ± 1.8^{ab}	112.9 ± 5.5^{abc}	3264.5 ± 168.8^{c}	46.9 ± 0.5^{bc}	25.4 ± 0.7^{b}
	BC_1	50.1 ± 1.8^{c}	19.3 ± 3.9^{bcd}	102.4 ± 2.9^{abcd}	31.8 ± 1.8^{b}	111.6 ± 5.5 ^{a}	3291.4 ± 168.3^{bc}	46.8 ± 0.5^{bc}	25.3 ± 0.7^{b}
Ol.bot.cau	F_2	50.2 ± 1.8^{c}	18.9 ± 3.9^{e}	102.5 ± 2.9^{bcd}	31.9 ± 1.8^{b}	113.3 ± 5.5^{abc}	3323.8 ± 169.2^{bc}	47.7 ± 0.5^{a}	25.3 ± 0.7^{b}
	BC_1	50.0 ± 1.8^{c}	19.1 ± 3.9^{cde}	102.2 ± 2.9^{bcd}	31.7 ± 1.8^{b}	113.2 ± 5.5^{abc}	3312.4 ± 167.7^{bc}	47.2 ± 0.5^{ab}	25.3 ± 0.7^{b}
Ol.cap.bad	F_2	52.3 ± 1.8^{a}	19.3 ± 3.9^{bcd}	103.2 ± 2.9^{ab}	31.0 ± 1.8^{a}	117.8 ± 5.5^{a}	3269.5 ± 168.8^{c}	46.1 ± 0.5^{c}	26.4 ± 0.7^{a}
	BC_1	50.9 ± 1.8^{bc}	19.4 ± 3.9^{bc}	101.9 ± 2.9^{cd}	31.3 ± 1.8^{ab}	115.4 ± 5.5^{a}	3088.0 ± 169.7^{c}	45.5 ± 0.6^{d}	26.2 ± 0.7^{a}
Ol.cap.bal	F_2	51.1 ± 1.8^{bc}	19.0 ± 3.9^{de}	103.5 ± 2.9^{a}	31.2 ± 1.8^{ab}	116.5 ± 5.5^{a}	3401.4 ± 169.0^{ab}	46.5 ± 0.5^{c}	25.1 ± 0.7^{b}
	BC_1	50.6 ± 1.8^{c}	18.4 ± 3.9^{f}	102.8 ± 2.8^{abc}	31.5 ± 1.8^{ab}	115.4 ± 5.5^{a}	3304.2 ± 167.5^{bc}	46.5 ± 0.5^{c}	25.4 ± 0.7^{b}
Ol.cap.bin	F_2	51.5 ± 1.8^{ab}	19.6 ± 3.9^{b}	102.9 ± 2.9^{abc}	31.3 ± 1.8^{ab}	118.3 ± 5.5^{a}	3182.0 ± 168.3^{c}	46.2 ± 0.5^{c}	26.5 ± 0.7^{a}
	BC_1	49.9 ± 1.8^{c}	19.5 ± 3.9^{b}	101.5 ± 2.8^{d}	31.6 ± 1.8^{ab}	112.2 ± 5.5^{bc}	3159.4 ± 167.5^{c}	46.8 ± 0.5^{c}	26.2 ± 0.7^{a}
Ol.ita.pre	F_2	49.5 ± 1.8^{c}	18.4 ± 3.9^{f}	101.9 ± 2.9^{cd}	31.7 ± 1.8^{ab}	114.4 ± 5.5^{ab}	3498.6 ± 167.6^{a}	47.5 ± 0.5^{a}	24.8 ± 0.7^{b}
	BC_1	51.3 ± 1.8^{bc}	18.2 ± 3.9^{f}	103.1 ± 2.9^{abc}	31.4 ± 1.8^{ab}	116.3 ± 5.6^{a}	3287.5 ± 171.8^{bc}	46.5 ± 0.6^{c}	25.3 ± 0.8^{b}
Cross:									
Ol.alb.nrc		50.3 ± 1.8^{cd}	19.3 ± 3.9^{b}	102.5 ± 2.8^{b}	31.8 ± 1.8^{b}	112.3 ± 5.4^{b}	3277.9 ± 166.6^{b}	46.8 ± 0.5^{b}	25.3 ± 0.7^{b}
Ol.bot.cau		50.1 ± 1.8^{d}	19.0 ± 3.9^{c}	102.2 ± 2.8^{b}	31.8 ± 1.8^{b}	113.3 ± 5.4^{b}	3318.1 ± 166.6^{ab}	47.4 ± 0.5^{a}	25.3 ± 0.7^{bc}
Ol.cap.bad		51.6 ± 1.8^{a}	19.4 ± 3.9^{ab}	102.6 ± 2.8^{ab}	31.2 ± 1.8^{a}	116.6 ± 5.5^{a}	3178.8 ± 167.0^{c}	45.8 ± 0.5^{d}	26.3 ± 0.7^{a}
Ol.cap.bal		50.9 ± 1.8^{b}	18.7 ± 3.9^{d}	103.2 ± 2.8^{a}	31.3 ± 1.8^{a}	116.0 ± 5.4^{a}	3352.8 ± 166.5^{a}	46.5 ± 0.5^{c}	25.3 ± 0.7^{bc}
Ol.cap.bin		50.7 ± 1.7^{bc}	19.5 ± 3.9^{a}	102.2 ± 2.8^{b}	31.5 ± 1.8^{ab}	115.2 ± 5.4^{a}	3170.7 ± 166.3^{c}	46.5 ± 0.5^{c}	26.3 ± 0.7^{a}
Ol.ita.pre		50.4 ± 1.8^{bcd}	18.3 ± 3.9^{e}	102.5 ± 2.9^{ab}	31.5 ± 1.8^{ab}	115.3 ± 5.5^{a}	3393.0 ± 167.2^{a}	47.0 ± 0.5^{b}	25.1 ± 0.7^{c}
Breeding technique									
F_2		50.9 ± 1.7^{a}	19.0 ± 3.9^{a}	102.7 ± 2.8^{a}	31.5 ± 1.8^{a}	115.5 ± 5.4^{a}	3323.3 ± 165.3^{a}	46.8 ± 0.5^{a}	25.6 ± 0.7^{a}
BC_1		50.5 ± 1.7^{b}	19.0 ± 3.9^{a}	102.3 ± 2.8^{b}	31.6 ± 1.8^{a}	114.0 ± 5.4^{b}	3240.5 ± 165.4^{b}	46.5 ± 0.5^{b}	25.6 ± 0.7^{a}

[1] Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop.

[2] F_2 = F_2-derived population; BC_1 = BC_1 (F_1 × *B. napus*)-derived population.

Each section (Cross and Breeding technique, Cross, Breeding technique) has grouped separately.
LS mean values with the same letter are not significantly different at $P < 0.05$.

Supplemental Table 3.5 Least square means ± SE. differences among the inbred population derived from six *Brassica napus* × *B. oleracea* interspecific crosses and following two breeding techniques F_2 and BC_1 for different agronomic and seed quality traits.

Cross[1]	BT[2]	Cross	BT	Days to flowering	Duration of flowering (days)	Days to maturity	Duration of grain-filling (days)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
A04-73NA		Ol.albo.nrc		1.1 ± 1.4^{NS}	1.1 ± 0.4^{NS}	1.3 ± 1.0^{NS}	-1.0 ± 0.6^{NS}	8.4 ± 4.3^{NS}	48.9 ± 158.7^{NS}	0.1 ± 0.7^{NS}	0.2 ± 0.5^{NS}
		Ol.bot.cau		1.3 ± 1.4^{NS}	1.4 ± 0.4*	1.6 ± 1.0^{NS}	-1.1 ± 0.6^{NS}	7.4 ± 4.3^{NS}	11.0 ± 158.6^{NS}	-0.4 ± 0.7^{NS}	0.3 ± 0.5^{NS}
		Ol.cap.bad		-0.3 ± 1.4^{NS}	1.1 ± 0.4^{NS}	1.1 ± 1.0^{NS}	-0.4 ± 0.6^{NS}	3.9 ± 4.3^{NS}	139.7 ± 159.1^{NS}	1.2 ± 0.7^{NS}	-0.8 ± 0.5^{NS}
		Ol.cap.bal		0.5 ± 1.4^{NS}	1.8 ± 0.4***	0.6 ± 1.0^{NS}	-0.6 ± 0.6^{NS}	4.8 ± 4.3^{NS}	-14.9 ± 158.4^{NS}	0.5 ± 0.7^{NS}	0.2 ± 0.5^{NS}
		Ol.cap.bin		0.7 ± 1.4^{NS}	0.9 ± 0.4^{NS}	1.6 ± 1.0^{NS}	-0.7 ± 0.6^{NS}	5.8 ± 4.3^{NS}	158.6 ± 158.3^{NS}	0.5 ± 0.7^{NS}	-0.8 ± 0.5^{NS}
		Ol.ita.pre		1.3 ± 1.4^{NS}	2.1 ± 0.4***	1.5 ± 1.0^{NS}	-0.9 ± 0.6^{NS}	5.7 ± 4.3^{NS}	-110.1 ± 158.8^{NS}	-0.3 ± 0.7^{NS}	0.6 ± 0.5^{NS}
Ol.albo.nrc		Ol.bot.cau		0.2 ± 0.3^{NS}	0.3 ± 0.1^{NS}	0.3 ± 0.2^{NS}	-0.02 ± 0.1^{NS}	-1.0 ± 1.0^{NS}	-40.2 ± 35.6^{NS}	-0.6 ± 0.2**	0.1 ± 0.1^{NS}
		Ol.cap.bad		-1.3 ± 0.3***	-0.1 ± 0.1^{NS}	-0.1 ± 0.2^{NS}	0.6 ± 0.2**	-4.3 ± 1.0***	99.2 ± 37.4^{NS}	1.1 ± 0.2***	-1.0 ± 0.1***
		Ol.cap.bal		-0.6 ± 0.3^{NS}	0.6 ± 0.1***	-0.7 ± 0.2*	0.4 ± 0.1^{NS}	-3.7 ± 0.9**	-74.9 ± 35.1^{NS}	0.4 ± 0.2^{NS}	0.0 ± 0.1^{NS}
		Ol.cap.bin		-0.4 ± 0.3^{NS}	-0.3 ± 0.1^{NS}	0.3 ± 0.2^{NS}	0.3 ± 0.1^{NS}	-3.0 ± 0.9*	107.3 ± 34.2*	0.4 ± 0.1^{NS}	-1.0 ± 0.1***
		Ol.ita.pre		-0.1 ± 0.3^{NS}	1.0 ± 0.1***	-0.1 ± 0.3^{NS}	0.2 ± 0.2^{NS}	-3.1 ± 1.0*	-115.1 ± 38.4*	-0.2 ± 0.2^{NS}	0.3 ± 0.1^{NS}
Ol.bot.cau		Ol.cap.bad		-1.5 ± 0.3***	-0.4 ± 0.1^{NS}	-0.4 ± 0.2^{NS}	0.6 ± 0.2***	-3.3 ± 1.0*	139.4 ± 37.2**	1.7 ± 0.2***	-1.1 ± 0.1***
		Ol.cap.bal		-0.8 ± 0.3^{NS}	0.3 ± 0.1^{NS}	-1.0 ± 0.2***	0.4 ± 0.1*	-2.7 ± 0.9^{NS}	-34.7 ± 34.9^{NS}	1.0 ± 0.2***	0.0 ± 0.1^{NS}
		Ol.cap.bin		-0.6 ± 0.3^{NS}	-0.5 ± 0.1***	-0.1 ± 0.2^{NS}	0.3 ± 0.1^{NS}	-1.9 ± 0.9^{NS}	147.4 ± 33.9***	1.0 ± 0.1***	-1.1 ± 0.1***
		Ol.ita.pre		-0.3 ± 0.3^{NS}	0.7 ± 0.1***	-0.3 ± 0.3^{NS}	0.2 ± 0.1^{NS}	-2.0 ± 1.0^{NS}	-74.9 ± 38.2^{NS}	0.4 ± 0.2^{NS}	0.2 ± 0.1^{NS}
Ol.cap.bad		Ol.cap.bal		0.7 ± 0.3^{NS}	0.7 ± 0.1***	-0.6 ± 0.2^{NS}	-0.2 ± 0.2^{NS}	0.6 ± 1.0^{NS}	-174.1 ± 36.8***	-0.7 ± 0.2***	1.0 ± 0.1***
		Ol.cap.bin		0.9 ± 0.3*	-0.2 ± 0.1^{NS}	0.4 ± 0.2^{NS}	-0.3 ± 0.1^{NS}	1.4 ± 1.0^{NS}	8.1 ± 35.9^{NS}	-0.7 ± 0.2***	0.0 ± 0.1^{NS}
		Ol.ita.pre		1.2 ± 0.3**	1.1 ± 0.1***	0.1 ± 0.3^{NS}	-0.4 ± 0.2^{NS}	1.3 ± 1.1^{NS}	-214.3 ± 40.0***	-1.2 ± 0.2***	1.3 ± 0.1***
Ol.cap.bal		Ol.cap.bin		0.2 ± 0.3^{NS}	-0.9 ± 0.1***	1.0 ± 0.2***	-0.1 ± 0.1^{NS}	0.7 ± 0.9^{NS}	182.2 ± 33.5***	0.0 ± 0.1^{NS}	-1.0 ± 0.1***
		Ol.ita.pre		0.5 ± 0.3^{NS}	0.4 ± 0.1^{NS}	0.7 ± 0.2^{NS}	-0.2 ± 0.1^{NS}	0.6 ± 1.0^{NS}	-40.2 ± 37.8^{NS}	-0.5 ± 0.2*	0.2 ± 0.1^{NS}
Ol.cap.bin		Ol.ita.pre		0.3 ± 0.3^{NS}	1.3 ± 0.1***	0.3 ± 0.2^{NS}	-0.1 ± 0.1^{NS}	-0.1 ± 1.0^{NS}	-222.4 ± 36.9***	-0.5 ± 0.2**	1.3 ± 0.1***
BC		F		-0.4 ± 0.2*	-0.1 ± 0.1^{NS}	0.4 ± 0.1**	0.1 ± 0.1^{NS}	-1.5 ± 0.6**	-82.9 ± 21.0***	-0.3 ± 0.1**	0.0 ± 0.1^{NS}
Ol.albo.nrc	BC	Ol.albo.nrc	F	-0.4 ± 0.4^{NS}	0.1 ± 0.2^{NS}	-0.1 ± 0.3^{NS}	0.6 ± 0.2^{NS}	-1.3 ± 1.4^{NS}	26.9 ± 50.6^{NS}	-0.1 ± 0.2^{NS}	-0.2 ± 0.2^{NS}
		Ol.bot.cau	BC	0.1 ± 0.4^{NS}	0.1 ± 0.2^{NS}	0.2 ± 0.3^{NS}	0.1 ± 0.2^{NS}	-1.6 ± 1.3^{NS}	-21.0 ± 46.7^{NS}	-0.4 ± 0.2^{NS}	0.0 ± 0.1^{NS}
		Ol.bot.cau	F	-0.1 ± 0.4^{NS}	0.5 ± 0.2^{NS}	0.2 ± 0.3^{NS}	-0.1 ± 0.0^{NS}	-1.7 ± 1.4^{NS}	-32.5 ± 52.1^{NS}	-1.0 ± 0.2**	0.0 ± 0.2^{NS}
		Ol.cap.bad	BC	-0.8 ± 0.4^{NS}	-0.1 ± 0.2^{NS}	0.5 ± 0.3^{NS}	0.5 ± 0.2^{NS}	-3.8 ± 1.4^{NS}	203.4 ± 53.6**	1.3 ± 0.2***	-1.0 ± 0.2***
		Ol.cap.bad	F	-2.2 ± 0.4***	0.0 ± 0.2^{NS}	-0.8 ± 0.3^{NS}	0.8 ± 0.2*	-6.2 ± 1.4***	21.9 ± 50.6^{NS}	0.7 ± 0.2^{NS}	-1.2 ± 0.2***
		Ol.cap.bal	BC	-0.5 ± 0.4^{NS}	0.9 ± 0.2***	-0.4 ± 0.3^{NS}	0.3 ± 0.2^{NS}	-3.8 ± 1.2^{NS}	-12.8 ± 46.1^{NS}	0.3 ± 0.2^{NS}	-0.2 ± 0.1^{NS}
		Ol.cap.bal	F	-1.0 ± 0.4^{NS}	0.3 ± 0.2^{NS}	-1.2 ± 0.3^{NS}	0.6 ± 0.2^{NS}	-4.9 ± 1.4*	-110.1 ± 51.3^{NS}	0.3 ± 0.2^{NS}	0.1 ± 0.2^{NS}
		Ol.cap.bin	BC	0.2 ± 0.4^{NS}	-0.2 ± 0.2^{NS}	0.9 ± 0.3^{NS}	0.2 ± 0.2^{NS}	-0.6 ± 1.2^{NS}	132.0 ± 46.0^{NS}	0.0 ± 0.2^{NS}	-0.9 ± 0.1***
		Ol.cap.bin	F	-1.4 ± 0.4*	-0.3 ± 0.2^{NS}	-0.5 ± 0.3^{NS}	0.5 ± 0.2^{NS}	-6.6 ± 1.3***	109.4 ± 48.8^{NS}	0.6 ± 0.2^{NS}	-1.2 ± 0.2***
		Ol.ita.pre	BC	-1.2 ± 0.5^{NS}	1.1 ± 0.2***	-0.7 ± 0.4^{NS}	0.4 ± 0.2^{NS}	-4.7 ± 1.6^{NS}	3.9 ± 59.9^{NS}	0.3 ± 0.3^{NS}	-0.1 ± 0.2^{NS}
Cross[1]	BT[2]	Cross	BT	Days to	Duration of	Days to	Duration of	Plant height	Seed yield (kg	Seed oil	Seed

				flowering	flowering (days)	maturity	grain-filling (days)	(cm)	ha^{-1})	(%)	protein (%)
		Ol.ita.pre	F	0.6 ± 0.4^{NS}	$0.9 \pm 0.2^{***}$	0.5 ± 0.3^{NS}	0.1 ± 0.2^{NS}	-2.7 ± 1.3^{NS}	$-207.2 \pm 46.4^{***}$	$-0.8 \pm 0.2^{**}$	0.5 ± 0.1^{NS}
Ol.albo.nrc	F	Ol.bot.cau	BC	0.4 ± 0.4^{NS}	0.1 ± 0.2^{NS}	-0.3 ± 0.3^{NS}	0.01 ± 0.2^{NS}	-0.4 ± 1.3^{NS}	-47.9 ± 48.5^{NS}	-0.3 ± 0.2^{NS}	0.2 ± 0.2^{NS}
Ol.albo.nrc	F	Ol.bot.cau	F	0.3 ± 0.4^{NS}	0.4 ± 0.2^{NS}	0.4 ± 0.4^{NS}	-0.2 ± 0.2^{NS}	-0.5 ± 1.4^{NS}	-59.3 ± 53.6^{NS}	$-0.8 \pm 0.2^{*}$	0.2 ± 0.2^{NS}
		Ol.cap.bad	BC	-0.4 ± 0.4^{NS}	-0.2 ± 0.2^{NS}	-0.6 ± 0.4^{NS}	0.4 ± 0.2^{NS}	-2.5 ± 1.5^{NS}	176.5 ± 55.2^{NS}	$1.4 \pm 0.2^{***}$	$-0.8 \pm 0.2^{***}$
		Ol.cap.bad	F	$-1.9 \pm 0.4^{***}$	-0.1 ± 0.2^{NS}	-0.7 ± 0.3^{NS}	0.7 ± 0.2^{NS}	$-4.9 \pm 1.4^{*}$	-5.0 ± 52.2^{NS}	$0.8 \pm 0.2^{*}$	$-1.0 \pm 0.2^{***}$
		Ol.cap.bal	BC	-0.2 ± 0.4^{NS}	$0.9 \pm 0.2^{***}$	0.3 ± 0.3^{NS}	0.3 ± 0.2^{NS}	-2.5 ± 1.3^{NS}	-39.7 ± 47.9^{NS}	0.4 ± 0.2^{NS}	0.0 ± 0.2^{NS}
		Ol.cap.bal	F	-0.7 ± 0.4^{NS}	0.3 ± 0.2^{NS}	$\_1.0 \pm 0.3^{NS}$	0.5 ± 0.2^{NS}	-3.6 ± 1.4^{NS}	-136.9 ± 52.9^{NS}	0.4 ± 0.2^{NS}	0.3 ± 0.2^{NS}
		Ol.cap.bin	BC	0.5 ± 0.4^{NS}	-0.3 ± 0.2^{NS}	$\_1.0 \pm 0.3^{NS}$	0.1 ± 0.2^{NS}	0.7 ± 1.3^{NS}	105.2 ± 47.8^{NS}	0.1 ± 0.2^{NS}	$-0.8 \pm 0.2^{***}$
		Ol.cap.bin	F	-1.0 ± 0.4^{NS}	-0.3 ± 0.2^{NS}	$\_0.3 \pm 0.3^{NS}$	0.5 ± 0.2^{NS}	$-5.4 \pm 1.4^{**}$	82.5 ± 50.5^{NS}	0.7 ± 0.2^{NS}	$-1.0 \pm 0.2^{***}$
		Ol.ita.pre	BC	-0.8 ± 0.5^{NS}	$1.0 \pm 0.2^{***}$	0.6 ± 0.4^{NS}	0.4 ± 0.3^{NS}	-3.4 ± 1.7^{NS}	-23.0 ± 61.3^{NS}	0.4 ± 0.3^{NS}	0.1 ± 0.2^{NS}
		Ol.ita.pre	F	0.9 ± 0.4^{NS}	$0.9 \pm 0.2^{***}$	0.6 ± 0.3^{NS}	0.05 ± 0.2^{NS}	-1.5 ± 1.3^{NS}	$-234.1 \pm 48.2^{***}$	-0.6 ± 0.2^{NS}	$0.6 \pm 0.2^{**}$
Ol.bot.cau	BC	Ol.bot.cau	F	-0.1 ± 0.4^{NS}	0.3 ± 0.2^{NS}	0.1 ± 0.3^{NS}	-0.2 ± 0.2^{NS}	-0.1 ± 1.3^{NS}	-11.5 ± 50.0^{NS}	-0.6 ± 0.2^{NS}	0.0 ± 0.2^{NS}
		Ol.cap.bad	BC	-0.8 ± 0.4^{NS}	-0.2 ± 0.2^{NS}	0.3 ± 0.3^{NS}	0.4 ± 0.2^{NS}	-2.1 ± 1.4^{NS}	$224.4 \pm 51.7^{**}$	$1.7 \pm 0.2^{***}$	$-1.0 \pm 0.2^{***}$
		Ol.cap.bad	F	$-2.3 \pm 0.4^{***}$	-0.2 ± 0.2^{NS}	$\_1.0 \pm 0.3^{NS}$	$0.7 \pm 0.2^{*}$	$-4.6 \pm 1.3^{*}$	42.9 ± 48.4^{NS}	$1.1 \pm 0.2^{***}$	$-1.2 \pm 0.2^{***}$
		Ol.cap.bal	BC	-0.6 ± 0.3^{NS}	$0.8 \pm 0.2^{***}$	$\_0.6\pm 0.3^{NS}$	0.2 ± 0.2^{NS}	-2.1 ± 1.2^{NS}	8.2 ± 43.7^{NS}	$0.7 \pm 0.2^{*}$	-0.2 ± 0.1^{NS}
		Ol.cap.bal	F	-1.1 ± 0.4^{NS}	0.2 ± 0.2^{NS}	$\_1.3 \pm 0.3^{**}$	0.5 ± 0.2^{NS}	-3.3 ± 1.3^{NS}	-89.1 ± 49.3^{NS}	$0.7 \pm 0.2^{*}$	0.1 ± 0.2^{NS}
		Ol.cap.bin	BC	0.1 ± 0.3^{NS}	-0.4 ± 0.2^{NS}	0.8 ± 0.3^{NS}	0.1 ± 0.2^{NS}	1.0 ± 1.2^{NS}	$153.0 \pm 43.7^{*}$	0.4 ± 0.2^{NS}	$-0.9 \pm 0.1^{***}$
		Ol.cap.bin	F	$-1.4 \pm 0.4^{**}$	-0.4 ± 0.2^{NS}	$\_0.7 \pm 0.3^{NS}$	0.4 ± 0.2^{NS}	$-5.0 \pm 1.3^{**}$	130.4 ± 46.7^{NS}	$1.0 \pm 0.2^{**}$*	$-1.2 \pm 0.1^{***}$
		Ol.ita.pre	BC	-1.2 ± 0.5^{NS}	$0.9 \pm 0.2^{**}$	$\_0.9 \pm 0.4^{NS}$	0.4 ± 0.2^{NS}	-3.0 ± 1.6^{NS}	24.9 ± 58.1^{NS}	0.7 ± 0.3^{NS}	-0.1 ± 0.2^{NS}
		Ol.ita.pre	F	0.5 ± 0.3^{NS}	$0.8 \pm 0.2^{***}$	0.3 ± 0.3^{NS}	0.03 ± 0.2^{NS}	-1.1 ± 1.2^{NS}	$-186.3 \pm 44.1^{**}$	-0.4 ± 0.2^{NS}	$0.5 \pm 0.1^{*}$
Ol.bot.cau	F	Ol.cap.bad	BC	-0.7 ± 0.4^{NS}	-0.6 ± 0.2^{NS}	$\_0.3 \pm 0.4^{NS}$	0.6 ± 0.2^{NS}	-2.0 ± 1.5^{NS}	$235.8 \pm 56.5^{**}$	$2.2 \pm 0.2^{***}$	$-1.0 \pm 0.2^{***}$
		Ol.cap.bad	F	$-2.1 \pm 0.4^{***}$	-0.5 ± 0.2^{NS}	$\_1.0 \pm 0.3^{NS}$	$0.9 \pm 0.2^{**}$	-4.5 ± 1.4^{NS}	54.3 ± 53.6^{NS}	$1.7 \pm 0.2^{***}$	$-1.1 \pm 0.2^{***}$
		Ol.cap.bal	BC	-0.4 ± 0.4^{NS}	0.5 ± 0.2^{NS}	0.8 ± 0.3^{NS}	0.4 ± 0.2^{NS}	-2.1 ± 1.3^{NS}	19.6 ± 49.4^{NS}	$1.2 \pm 0.2^{***}$	-0.2 ± 0.2^{NS}
		Ol.cap.bal	F	-1.0 ± 0.4^{NS}	-0.1 ± 0.2^{NS}	$\_1.4 \pm 0.4^{*}$	0.7 ± 0.2^{NS}	-3.2 ± 1.5^{NS}	-77.6 ± 54.3^{NS}	$1.3 \pm 0.2^{***}$	0.1 ± 0.2^{NS}
		Ol.cap.bin	BC	0.3 ± 0.4^{NS}	$-0.7 \pm 0.2^{*}$	$\_0.6 \pm 0.3^{NS}$	0.3 ± 0.2^{NS}	1.1 ± 1.3^{NS}	$164.5 \pm 49.3^{*}$	$1.0 \pm 0.2^{***}$	$-0.9 \pm 0.2^{***}$
		Ol.cap.bin	F	-1.3 ± 0.4^{NS}	$-0.7 \pm 0.2^{*}$	$\_0.7 \pm 0.4^{NS}$	0.6 ± 0.2^{NS}	$-4.9 \pm 1.4^{*}$	141.9 ± 52^{NS}	$1.5 \pm 0.2^{***}$	$-1.2 \pm 0.2^{***}$
		Ol.ita.pre	BC	-1.1 ± 0.5^{NS}	0.6 ± 0.2^{NS}	0.9 ± 0.4^{NS}	0.6 ± 0.6^{NS}	-3.0 ± 1.7^{NS}	36.4 ± 62.5^{NS}	$1.2 \pm 0.3^{***}$	-0.1 ± 0.2^{NS}
		Ol.ita.pre	F	0.7 ± 0.4^{NS}	0.5 ± 0.2^{NS}	0.2 ± 0.3^{NS}	0.2 ± 0.2^{NS}	-1.0 ± 1.3^{NS}	$-174.8 \pm 49.7^{*}$	0.2 ± 0.2^{NS}	0.5 ± 0.2^{NS}
Ol.cap.bad	BC	Ol.cap.bad	F	-1.4 ± 0.4^{NS}	0.1 ± 0.2^{NS}	$\_1.3 \pm 0.4^{*}$	0.3 ± 0.2^{NS}	-2.4 ± 1.5^{NS}	-181.5 ± 55.2^{NS}	-0.6 ± 0.2^{NS}	-0.2 ± 0.2^{NS}
		Ol.cap.bal	BC	0.3 ± 0.4^{NS}	$1.0 \pm 0.2^{***}$	$\_0.9 \pm 0.3^{NS}$	-0.2 ± 0.2^{NS}	0.0 ± 1.4^{NS}	$-216.2 \pm 51.1^{**}$	$-1.0 \pm 0.2^{***}$	$0.8 \pm 0.2^{***}$
		Ol.cap.bal	F	-0.2 ± 0.4^{NS}	0.4 ± 0.2^{NS}	$\_1.6 \pm 0.4^{**}$	0.1 ± 0.2^{NS}	-1.1 ± 1.5^{NS}	$-313.4 \pm 55.9^{***}$	$-0.9 \pm 0.2^{**}$	$1.1 \pm 0.2^{***}$
		Ol.cap.bin	BC	1.0 ± 0.4^{NS}	-0.1 ± 0.2^{NS}	0.4 ± 0.3^{NS}	-0.3 ± 0.2^{NS}	3.2 ± 1.4^{NS}	-71.3 ± 51.0^{NS}	$-1.2 \pm 0.2^{***}$	0.0 ± 0.2^{NS}
		Ol.cap.bin	F	-0.6 ± 0.4^{NS}	-0.2 ± 0.2^{NS}	$\_1.0 \pm 0.3^{NS}$	0.02 ± 0.2^{NS}	-2.9 ± 1.4^{NS}	-94.0 ± 53.6^{NS}	-0.7 ± 0.2^{NS}	-0.2 ± 0.2^{NS}
		Ol.ita.pre	BC	-0.4 ± 0.5^{NS}	$1.2 \pm 0.2^{***}$	$\_1.2 \pm 0.4^{NS}$	-0.1 ± 0.3^{NS}	-0.9 ± 1.7^{NS}	-199.5 ± 63.8^{NS}	$-1.0 \pm 0.3^{*}$	$0.9 \pm 0.2^{***}$
		Ol.ita.pre	F	$1.4 \pm 0.4^{*}$	$1.0 \pm 0.2^{***}$	$\_0.1 \pm 0.3^{NS}$	-0.4 ± 0.2^{NS}	1.0 ± 1.4^{NS}	$-410.6 \pm 51.4^{***}$	$-2.0 \pm 0.2^{***}$	$1.4 \pm 0.2^{***}$
Cross[1]	BT[2]	Cross	BT	Days to	Duration of	Days to	Duration of	Plant height	Seed yield (kg	Seed oil	Seed

				flowering	flowering (days)	maturity	grain-filling (days)	(cm)	ha^{-1})	(%)	protein (%)
Ol.cap.bad	F	Ol.cap.bal	BC	$1.7 \pm 0.4^{***}$	$1.0 \pm 0.2^{***}$	$\_0.4 \pm 0.3^{NS}$	-0.5 ± 0.2^{NS}	2.4 ± 1.3^{NS}	-34.7 ± 47.8^{NS}	-0.5 ± 0.2^{NS}	$1.0 \pm 0.2^{***}$
		Ol.cap.bal	F	1.2 ± 0.4^{NS}	0.4 ± 0.2^{NS}	$\_0.3 \pm 0.3^{NS}$	-0.2 ± 0.2^{NS}	1.3 ± 1.4^{NS}	-131.9 ± 52.9^{NS}	-0.4 ± 0.2^{NS}	$1.3 \pm 0.2^{***}$
		Ol.cap.bin	BC	$2.4 \pm 0.4^{***}$	-0.2 ± 0.2^{NS}	$\_1.7 \pm 0.3^{***}$	-0.6 ± 0.2^{NS}	$5.6 \pm 1.3^{**}$	110.2 ± 47.8^{NS}	$-0.7 \pm 0.2^{*}$	0.2 ± 0.2^{NS}
		Ol.cap.bin	F	0.8 ± 0.4^{NS}	-0.2 ± 0.2^{NS}	0.3 ± 0.3^{NS}	-0.2 ± 0.2^{NS}	-0.4 ± 1.4^{NS}	87.5 ± 50.5^{NS}	-0.1 ± 0.2^{NS}	-0.1 ± 0.2^{NS}
		Ol.ita.pre	BC	1.1 ± 0.5^{NS}	$1.1 \pm 0.2^{***}$	$\_0.1 \pm 0.4^{NS}$	-0.3 ± 0.3^{NS}	1.5 ± 1.7^{NS}	-18 ± 61.3^{NS}	-0.4 ± 0.3^{NS}	$1.1 \pm 0.2^{***}$
Ol.cap.bad	F	Ol.ita.pre	F	$2.8 \pm 0.4^{***}$	$1 \pm 0.2^{***}$	$1.3 \pm 0.3^{*}$	$-0.6 \pm 0.2^{*}$	3.4 ± 1.3^{NS}	$-229.1 \pm 48.2^{***}$	$-1.5 \pm 0.2^{***}$	$1.6 \pm 0.2^{***}$
Ol.cap.bal	BC	Ol.cap.bal	F	-0.5 ± 0.4^{NS}	$-0.6 \pm 0.2^{*}$	$\_0.7 \pm 0.3^{NS}$	0.3 ± 0.2^{NS}	-1.1 ± 1.3^{NS}	-97.2 ± 48.6^{NS}	0.1 ± 0.2^{NS}	0.3 ± 0.2^{NS}
		Ol.cap.bin	BC	0.7 ± 0.3^{NS}	$-1.2 \pm 0.2^{***}$	$1.3 \pm 0.3^{***}$	-0.2 ± 0.2^{NS}	3.2 ± 1.2^{NS}	$144.9 \pm 43^{*}$	-0.2 ± 0.2^{NS}	$-0.7 \pm 0.1^{***}$
		Ol.cap.bin	F	-0.9 ± 0.4^{NS}	$-1.2 \pm 0.2^{***}$	$\_0.1 \pm 0.3^{NS}$	0.2 ± 0.2^{NS}	-2.9 ± 1.2^{NS}	122.2 ± 46^{NS}	0.3 ± 0.2^{NS}	$-1 \pm 0.1^{***}$
		Ol.ita.pre	BC	-0.7 ± 0.5^{NS}	0.1 ± 0.2^{NS}	$\_0.3 \pm 0.4^{NS}$	0.1 ± 0.2^{NS}	-0.9 ± 1.6^{NS}	16.7 ± 57.6^{NS}	0 ± 0.2^{NS}	0.1 ± 0.2^{NS}
		Ol.ita.pre	F	1.1 ± 0.3^{NS}	0 ± 0.2^{NS}	0.9 ± 0.3^{NS}	-0.2 ± 0.2^{NS}	1 ± 1.2^{NS}	$-194.4 \pm 43.4^{***}$	$-1 \pm 0.2^{***}$	$0.7 \pm 0.1^{***}$
Ol.cap.bal	F	Ol.cap.bin	BC	1.2 ± 0.4^{NS}	-0.5 ± 0.2^{NS}	$\_2.0 \pm 0.3^{***}$	-0.4 ± 0.2^{NS}	4.3 ± 1.3^{NS}	$242.1 \pm 48.6^{***}$	-0.3 ± 0.2^{NS}	$-1.1 \pm 0.2^{***}$
		Ol.cap.bin	F	-0.3 ± 0.4^{NS}	-0.6 ± 0.2^{NS}	0.7 ± 0.3^{NS}	-0.1 ± 0.2^{NS}	-1.7 ± 1.4^{NS}	$219.5 \pm 51.3^{**}$	0.3 ± 0.2^{NS}	$-1.3 \pm 0.2^{***}$
		Ol.ita.pre	BC	-0.1 ± 0.5^{NS}	0.7 ± 0.2^{NS}	$\_0.5 \pm 0.4^{NS}$	-0.2 ± 0.3^{NS}	0.2 ± 1.7^{NS}	114 ± 61.9^{NS}	0 ± 0.3^{NS}	-0.2 ± 0.2^{NS}
		Ol.ita.pre	F	$1.6 \pm 0.4^{**}$	$0.6 \pm 0.2^{*}$	$1.6 \pm 0.3^{***}$	-0.5 ± 0.2^{NS}	2.1 ± 1.3^{NS}	-97.2 ± 49^{NS}	$-1.1 \pm 0.2^{***}$	0.3 ± 0.2^{NS}
Ol.cap.bin	BC	Ol.cap.bin	F	$-1.6 \pm 0.4^{**}$	0 ± 0.2^{NS}	$\_1.3 \pm 0.3^{**}$	0.4 ± 0.2^{NS}	$-6.1 \pm 1.2^{***}$	-22.6 ± 46^{NS}	0.6 ± 0.2^{NS}	-0.3 ± 0.1^{NS}
		Ol.ita.pre	BC	-1.3 ± 0.5^{NS}	$1.3 \pm 0.2^{***}$	$\_1.6 \pm 0.4^{**}$	0.3 ± 0.2^{NS}	-4.1 ± 1.6^{NS}	-128.1 ± 57.6^{NS}	0.3 ± 0.2^{NS}	$0.9 \pm 0.2^{***}$
		Ol.ita.pre	F	0.4 ± 0.3^{NS}	$1.2 \pm 0.2^{***}$	$\_0.4 \pm 0.3^{NS}$	-0.05 ± 0.2^{NS}	-2.2 ± 1.2^{NS}	$-339.3 \pm 43.3^{***}$	$-0.8 \pm 0.2^{**}$	$1.4 \pm 0.1^{***}$
Ol.cap.bin	F	Ol.ita.pre	BC	0.2 ± 0.5^{NS}	$1.3 \pm 0.2^{***}$	0.2 ± 0.4^{NS}	-0.1 ± 0.2^{NS}	2 ± 1.6^{NS}	-105.5 ± 59.8^{NS}	-0.3 ± 0.3^{NS}	$1.1 \pm 0.2^{***}$
		Ol.ita.pre	F	$2 \pm 0.4^{***}$	$1.2 \pm 0.2^{***}$	0.9 ± 0.3^{NS}	-0.4 ± 0.2^{NS}	3.9 ± 1.3^{NS}	$-316.6 \pm 46.4^{***}$	$-1.4 \pm 0.2^{***}$	$1.7 \pm 0.1^{***}$
Ol.ita.pre	BC	Ol.ita.pre	F	$1.7 \pm 0.5^{**}$	-0.1 ± 0.2^{NS}	1.2 ± 0.4^{NS}	-0.3 ± 0.2^{NS}	1.9 ± 1.6^{NS}	$-211.1 \pm 57.9^{*}$	$-1.1 \pm 0.2^{**}$	0.5 ± 0.2^{NS}

* Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, ^{NS} Not significant.

[1] Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop.

[2] BT = Breeding technique: F = F_2-derived population; BC = BC_1 $9F_1$ × *B. napus*)-derived population.

Supplemental Table 3.6 Descriptive statistics of the F_2- and BC_1-derived lines of six *Brassica napus* × *B. oleracea* interspecific crosses for different agronomic and seed quality traits.

Cross[1]		A04-73NA	Ol.alb.nrc		Ol.bot.cau		Ol.cap.bad		Ol.cap.bal		Ol.cap.bin		Ol.ita.pre	
			F[2]	BC[3]	F	BC	F	BC	F	BC	F	BC	F	BC
Days to flowering	Mean	51.4	50.4	50.0	50.1	50.0	52.3	50.9	51.1	50.6	51.5	49.9	49.5	51.2
	Sd[4]	5.2	5.7	5.5	5.6	5.7	5.6	5.3	6.2	5.7	5.7	5.6	5.4	5.9
	Min	37.0	39.0	38.0	38.0	38.0	39.0	39.0	36.0	35.0	37.0	36.0	38.0	37.0
	Max	67.0	67.0	66.0	66.0	67.0	69.0	66.0	87.0	67.0	70.0	68.0	65.0	65.0
Duration of flowering (days)	Mean	20.4	19.3	19.3	18.8	19.1	19.3	19.4	19.0	18.4	19.6	19.5	18.4	18.2
	Sd	10.1	8.7	9.1	8.4	8.6	9.8	9.5	8.9	8.5	9.9	9.4	7.7	7.7
	Min	9.0	9.0	9.0	8.0	9.0	7.0	7.0	8.0	7.0	8.0	8.0	10.0	9.0
	Max	45.0	40.0	42.0	40.0	40.0	45.0	42.0	44.0	42.0	47.0	45.0	40.0	41.0
Days to maturity	Mean	101.8	103.6	103.3	103.1	103.2	103.2	101.9	97.1	96.5	102.9	101.5	95.6	96.8
	Sd	12.3	8.3	8.3	8.5	8.6	7.5	7.2	16.4	16.3	7.6	7.3	16.4	16.2
	Min	54.0	90.0	90.0	90.0	90.0	90.0	90.0	53.0	52.0	90.0	89.0	52.0	54.0
	Max	128.0	123.0	123.0	123.0	123.0	120.0	118.0	130.0	129.0	122.0	119.0	128.0	129.0
Duration of grain-filling (days)	Mean	30.7	31.7	31.8	31.9	31.7	31.0	31.3	31.2	31.5	31.3	31.7	31.7	31.4
	Sd	4.3	4.0	4.3	3.8	4.0	4.4	4.4	4.4	4.2	4.6	4.4	3.9	3.9
	Min	19.0	20.0	19.0	22.0	20.0	19.0	19.0	16.0	19.0	18.0	18.0	20.0	19.0
	Max	37.0	39.0	40.0	39.0	39.0	40.0	38.0	37.0	38.0	38.0	38.0	38.0	38.0
Plant height (cm)	Mean	120.6	112.8	111.2	113.0	113.2	117.9	115.3	116.1	115.0	118.3	112.2	114.1	116.1
	Sd	20.4	18.5	18.2	19.2	17.8	20.4	18.1	20.8	20.2	20.3	19.0	18.9	20.1
	Min	70.0	78.0	70.0	72.0	72.0	72.0	80.0	60.0	72.0	70.0	70.0	78.0	72.0
	Max	168.0	166.0	154.0	162.0	162.0	160.0	160.0	162.0	162.0	168.0	164.0	160.0	160.0
Seed yield (kg ha^{-1})	Mean	3327.1	3263.7	3292.9	3327.8	3315.1	3275.3	3085.2	3403.0	3299.0	3184.8	3158.5	3497.2	3285.8
	Sd	644.8	681.7	686.9	662.7	643.9	552.7	595.5	661.1	632.1	587.3	680.1	682.9	662.0
	Min	1386.5	1796.7	1732.8	1988.7	1754.0	1869.9	1422.7	1834.1	1212.8	1506.0	1150.8	1780.4	1739.6
	Max	5001.9	5584.7	5479.2	5164.8	5494.9	5068.8	4582.1	4972.3	5002.3	4845.3	5059.3	5096.2	5167.1
Seed oil (%)	Mean	47.0	46.9	46.8	47.7	47.2	46.0	45.5	46.5	46.5	46.2	46.8	47.6	46.5
	Sd	1.9	2.2	2.3	2.1	2.3	2.1	1.9	2.1	2.1	2.0	2.0	2.2	2.7
	Min	43.3	41.8	41.2	43.1	42.9	42.0	41.9	41.9	40.7	39.9	41.7	42.8	41.4
	Max	51.8	54.1	53.2	53.0	54.5	52.6	50.6	54.1	53.3	53.6	53.2	53.3	53.9
Seed protein (%)	Mean	25.5	25.4	25.3	25.3	25.3	26.4	26.2	25.1	25.4	26.5	26.2	24.8	25.3
	Sd	2.4	2.9	2.8	2.8	2.9	2.4	2.3	2.6	2.5	2.4	2.3	2.6	3.0
	Min	19.5	16.3	17.1	17.4	17.3	19.0	19.4	18.0	18.7	19.8	19.0	17.7	18.8
	Max	29.5	31.2	30.1	30.3	30.3	30.6	30.5	29.6	30.9	32.9	31.6	29.7	31.0

[1] Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop.

[2] F = F_2-derived population.

[3] BC = BC_1 F_1 × *B. napus*.-derived population.

[4] Sd = Standard deviation.

Supplemental Table 3.7 Nei's mean genetic distance of inbred population derived from six *Brassica napus* × *B. oleracea* interspecific crosses, and following two breeding techniques (F_2- and BC_1-derived) from the common *B. napus* parent.

Cross[1]	Breeding technique[2]	Mean genetic distance
Ol.alb.nrc	F	0.44
	BC	0.48
Ol.bot.cau	F	0.33
	BC	0.47
Ol.cap.bad	F	0.65
	BC	0.45
Ol.cap.bal	F	0.43
	BC	0.45
Ol.cap.bin	F	0.64
	BC	0.42
Ol.ita.pre	F	0.21
	BC	0.41
Ol.alb.nrc		0.46
Ol.bot.cau		0.41
Ol.cap.bad		0.57
Ol.cap.bal		0.45
Ol.cap.bin		0.50
Ol.ita.pre		0.26
F_2		0.47
BC_1		0.49

[1] Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop.

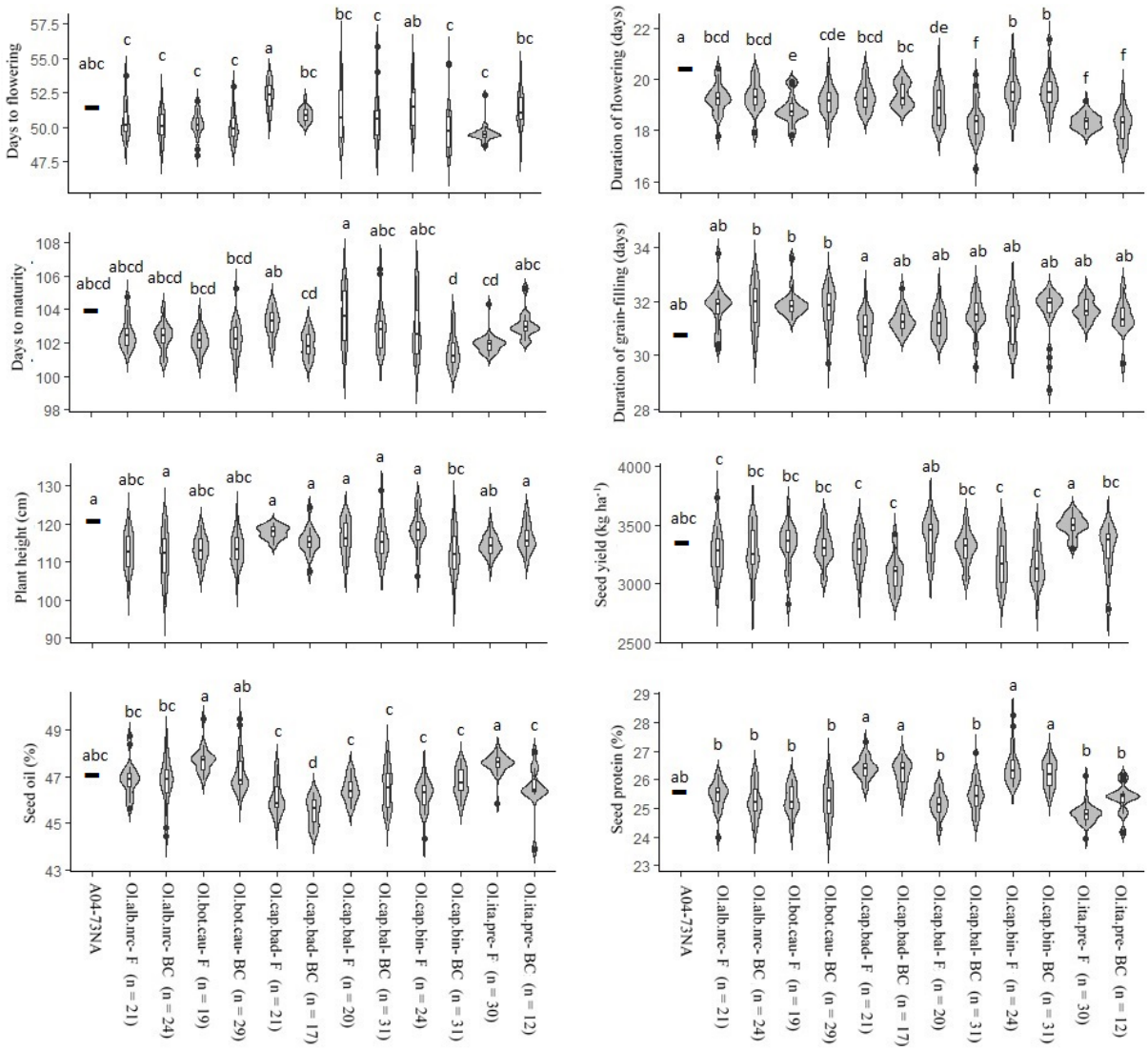
[2] F = F_2-derived population; BC = BC_1 (F_1 × *B. napus*)-derived population.

Supplemental Table 3.8 Least square means ± SE of 14 lines, selected from 279 advanced generation lines based on different agronomic and seed quality traits, of six *Brassica napus* × *B. oleracea* interspecific crosses.

Cross[1]	Breeding technique[2]	Inbred line code	Genetic diversity from A04-73NA	Inbred line number	Days to flowering	Duration of flowering (days)	Days to maturity	Duration of grain-filling (days)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
A04-73NA					51.4 ± 2.1	20.4 ± 3.9	103.8 ± 3.0	30.7 ± 1.9	120.6 ± 6.8	3327.9 ± 222.9	47.0 ± 0.9	25.5 ± 0.9
Ol.alb.nrc	F	3	0.55	1300.353	50.1 ± 1.8	18.6 ± 4.0	101.7 ± 2.9	31.9 ± 1.8	112.6 ± 5.6	3738.8 ± 185.0	48.8 ± 0.6	24.0 ± 0.8
Ol.bot.cau	F	22	0.54	1343.320	50.1 ± 1.8	18.8 ± 4.0	103.1 ± 2.9	32.6 ± 1.8	108.9 ± 5.6	3671.2 ± 185.0	49.5 ± 0.6	24.2 ± 0.8
Ol.bot.cau	F	35	0.44	1343.353	50.1 ± 1.8	17.8 ± 4.0	102.9 ± 2.9	33.6 ± 1.8	106.7 ± 5.6	3463.4 ± 185.0	47.1 ± 0.6	26.1 ± 0.8
Ol.cap.bal	BC	169	0.33	1679.430	49.0 ± 1.8	17.6 ± 4.0	102.3 ± 2.9	32.4 ± 1.8	113.2 ± 5.6	3415.0 ± 185.0	47.1 ± 0.6	25.5 ± 0.8
Ol.cap.bin	BC	251	0.52	1682.100	51.2 ± 1.8	19.1 ± 4.0	101.8 ± 2.9	31.4 ± 1.8	118.5 ± 5.6	3468.1 ± 185.0	47.8 ± 0.6	25.7 ± 0.8
Ol.cap.bin	BC	271	0.46	1682.147	49.4 ± 1.8	19.5 ± 4.0	101.1 ± 2.9	31.8 ± 1.8	112.3 ± 5.6	3459.4 ± 185.0	47.1 ± 0.6	25.5 ± 0.8
Ol.ita.pre	F	99	0.15	1358.620	49.4 ± 1.8	18.5 ± 4.0	102.0 ± 2.9	32.2 ± 1.8	116.9 ± 5.6	3616.4 ± 185.0	47.9 ± 0.6	25.0 ± 0.8
Ol.ita.pre	F	102	0.25	1358.634	49.4 ± 1.8	18.1 ± 4.0	102.3 ± 2.9	32.5 ± 1.8	112.4 ± 5.6	3604.8 ± 185.0	47.5 ± 0.6	25.1 ± 0.8
Ol.ita.pre	F	103	0.22	1358.635	48.8 ± 1.8	18.5 ± 4.0	101.6 ± 2.9	31.7 ± 1.8	110.0 ± 5.6	3349.7 ± 185.0	47.1 ± 0.6	25.4 ± 0.8
Ol.ita.pre	F	108	0.24	1358.667	49.1 ± 1.8	18.5 ± 4.0	101.7 ± 2.9	31.4 ± 1.8	115.2 ± 5.6	3615.2 ± 185.0	47.8 ± 0.6	24.5 ± 0.8
Ol.ita.pre	F	112	-	1358.699	48.7 ± 1.8	18.7 ± 4.0	101.8 ± 2.9	31.7 ± 1.8	116.4 ± 5.6	3471.8 ± 185.0	47.2 ± 0.6	25.1 ± 0.8
Ol.ita.pre	F	117	-	1358.711	49.3 ± 1.8	18.0 ± 4.0	101.3 ± 2.9	32.2 ± 1.8	113.9 ± 5.6	3607.2 ± 189.5	47.6 ± 0.6	25.0 ± 0.8
Ol.ita.pre	F	122	0.15	1358.739	49.5 ± 1.8	17.8 ± 4.0	102.1 ± 2.9	32.3 ± 1.8	116.3 ± 5.6	3660.5 ± 185.0	48.0 ± 0.6	24.5 ± 0.8
Ol.ita.pre	BC	151	0.27	1678.281	50.3 ± 1.8	18.4 ± 4.0	102.1 ± 2.9	31.1 ± 1.8	114.4 ± 5.6	3425.5 ± 185.0	48.1 ± 0.6	24.2 ± 0.8

[1] Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop.

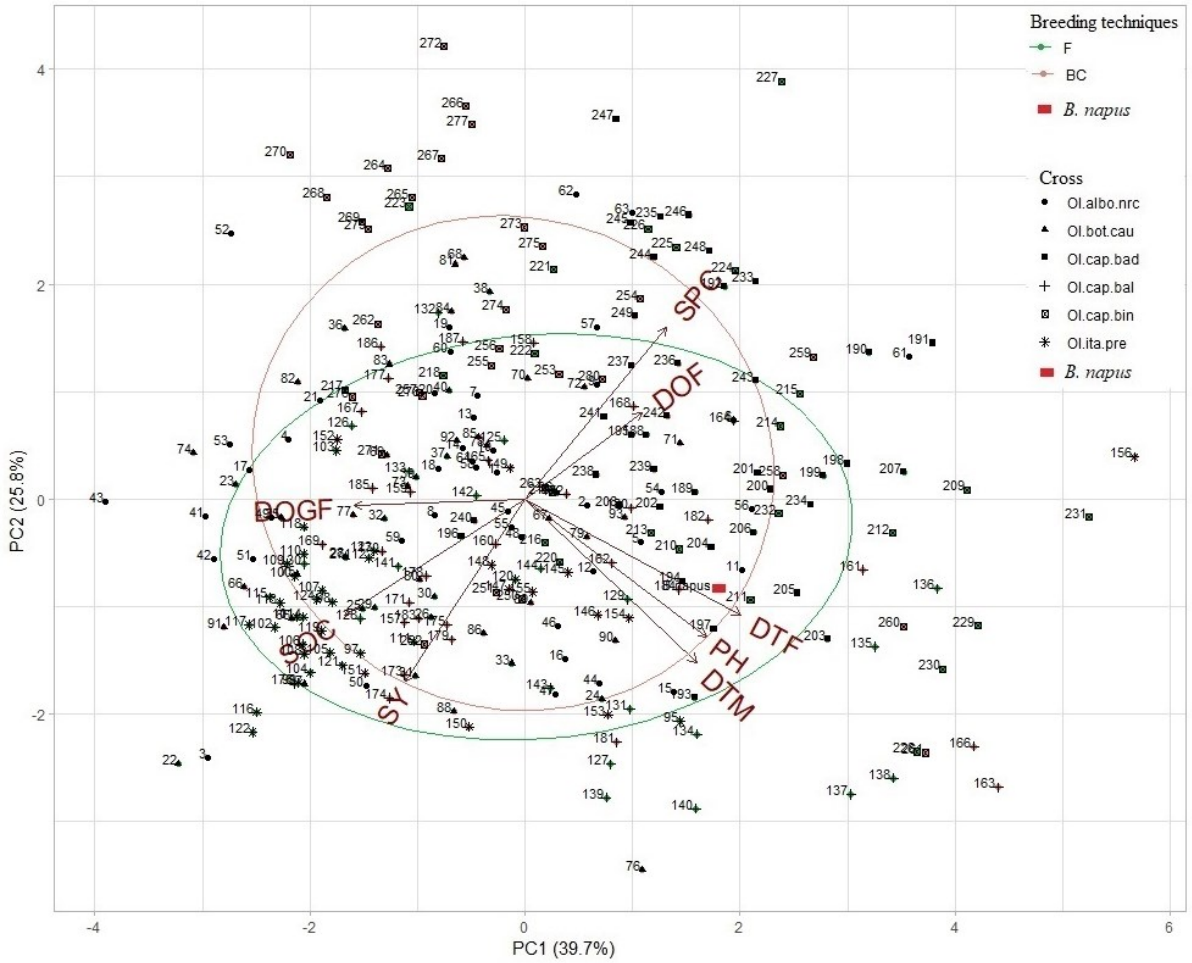
[2] F = F_2-derived lines; BC = BC_1 (F_1 × *B. napus*)-derived lines.



Supplemental Fig. 3.1 Violin plot of 12 populations derived from six *Brassica napus* × *B. oleracea* interspecific crosses and following two breeding techniques (F_2- and BC_1-derived) and the common *B. napus* parent A04-73NA.
Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1; Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bal = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Balbro; Ol.cap.bin = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Bindsachsener; Ol.ita.pre = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop.

F = F_2-derived population; BC = BC_1 (F_1 × *B. napus*)-derived population.

LSmean values of the violin plots with the same letter are not significantly different at $P < 0.05$.



Supplemental Fig. 3.2 Principal components analysis biplot of inbred lines (n = 279) derived from two breeding techniques (F_2- and BC_1-derived) and the common *B. napus* parent A04-73NA, illustrating the distribution of inbred lines characterized by different agronomic and seed quality traits in the space of the two principal components. The names of the inbred lines were shown in Supplemental Table 3-1.

DTF = Days to flowering; DOF = Duration of flowering; DTM = Duration of maturity; DOGF = Duration of grain-filling; PH = Plant height; SY = Seed yield; SOC = Seed oil; SPC = Seed protein.

Ol.alb.nrc (solid circle) = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI (*n* = 45); Ol.bot.cau (solid triangle) = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1 (*n* = 48); Ol.cap.bad (solid square) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper (*n* = 38); Ol.cap.bal (plus sign) = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Balbro (*n* = 51); Ol.cap.bin (empty square) = *B. napus* (A04-73NA) × B. *oleracea* var. *capitata* cv. Bindsachsener (*n* = 55); Ol.ita.pre (star) = *B. napus* (A04-73NA) × *B. oleracea* var. *italica* cv. Premium Crop (*n* = 42); *B. napus* parent (red rectangle).

F (red color) = F_2-derived population (*n* = 135); BC (green) = BC_1 (F_1 × *B. napus*)-derived population (*n* = 144).

Chapter 4

Potential of the C genome of the different variants of *Brassica oleracea* for heterosis in spring *B. napus* canola [3]

4.1 Introduction

Brassica napus L. (AACC, 2*n* = 38) canola, also known as oilseed rape, is one of the most important oilseed crops in the world. It is an amphidiploid species as a result of hybridization between *B. oleracea* (C genome, *n* = 9) and *B. rapa* (A genome, *n* = 10) (U 1935). *B. napus* is a young agricultural crop species – originated about 7500 years ago (Chalhoub et al. 2014). Spring, winter and semi-winter growth habit types exist in this crop species; however, genetic diversity within the germplasm of a given growth habit type is quite narrow (Bus et al. 2011). Currently, hybrid canola cultivars have taken the majority of the market share; for example, in Canada more than 90% of the canola acreage is captured by this type of cultivars (Morrison et al. 2016). To develop high yielding hybrid cultivars, presence of genetic diversity between the hybrid-parent lines is needed (for review, see Rahman 2013). The narrow genetic base of the canola crop germplasm is one of the limitations for increasing seed yield in hybrid cultivars through exploitation of heterosis or hybrid vigor (reviewed in Zou, Zhu et al. 2010); therefore, introgression of exotic heterotic alleles into *B. napus* canola is needed. The need of broadening the genetic base of *B. napus* canola through introgression of allelic diversity from *B. rapa* and *B. oleracea* has been suggested by several researchers (Jesske et al. 2013; Rahman 2013; Zhang et al. 2015; for review, see Wang, Xian et al. 2017).

[3] A version of this Chapter has been published as: Nikzad A, Kebede B, Pinzon J, Bhavikkumar J, Wang X, Yanga R-C, and Rahman H (2020) Potential of the C Genome of Different Variants of *Brassica oleracea* for heterosis in spring *B. napus* Canola. Front. Plant Sci. 10:1691. doi: 10.3389/fpls.2019.01691

The association of hybrid vigour with genetic diversity as well as with general combining ability (GCA) of the parents has been investigated by different researchers in oilseed *B. napus*. Genetic divergence of the parental lines is thought to be related to the superior performance of the F_1 hybrid (Sernyk and Stefansson 1983; Lefort-Buson et al. 1987; Ali et al. 1995; Diers et al. 1996; Basunanda et al. 2007; Tan et al. 2007; Sang et al. 2015); however, this relationship has been found not to be always strong in this crop (Yu et al. 2005; Qian et al. 2007, 2009; Girke et al. 2012b; Luo et al. 2016). GCA of the parents also seems to play an important role in this association (Diers et al. 1996; Qian et al. 2009; Tian et al. 2017).

Several studies have been conducted to evaluate the effect of the alleles of the primary and secondary gene pools of *Brassica* on heterosis in oilseed *B. napus*. For example, in case of the primary gene pool, Udall et al. (2004) found heterosis for seed yield in F_1 hybrids developed by crossing of spring *B. napus* lines, carrying alleles introgressed from Chinese semi-winter type, with natural spring *B. napus*. Similarly, Quijada et al. (2004) found that introgression of alleles from winter *B. napus* can improve seed yield in spring *B. napus* canola hybrids. In case of the secondary gene pool, Qian et al. (2003) showed that Chinese *B. rapa* can be a valuable gene pool for alleles for increasing biomass yield in *B. napus*, and greater biomass at vegetative and maturity stage was found to be associated with higher seed yield in spring canola hybrids (Zhang and Flottmann 2016). Introgression of alleles from *B. rapa* into *B. napus* has also been found to improve seed yield in *B. napus* hybrids (Qian et al. 2005). Introgression of allelic diversity from *B. rapa* into Chinese semi-winter type, in fact, make this type genetically distinct from the European and Canadian spring *B. napus* (Qian et al. 2005, 2007), and alleles of the Chinese semi-winter type found to contribute to seed yield heterosis in spring or winter *B. napus* (Qian et al. 2007, 2009). To date, very few studies have been conducted (Li, Zhou et al. 2014; Rahman et al. 2015) to understand the effect of the *B.*

oleracea alleles on heterosis in *B. napus* – despite wide morphological and genetic diversity exist in this diploid species (Lázaro and Aguinagalde 1998; Faltusová et al. 2011; Izzah et al. 2013; El-Esawi et al. 2016; Yousef et al. 2018). This suggests the need of understanding the *B. oleracea* gene pool for seed yield heterosis in *B. napus* canola hybrid. It has also been reported that genetic diversity in the C genome is lower than the A genome of *B. napus* (Bus et al. 2011; Delourme et al. 2013; Thakur et al. 2018). This underlines the need to increase genetic diversity in the C genome of *B. napus* by introducing allelic diversity from *B. oleracea* – not only for increasing the level of heterosis in *B. napus* hybrid canola but also for continued improvement of the germplasm of this crop through breeding.

As reviewed above, wide diversity exists in *B. oleracea*; therefore, it can be hypothesized that the alleles of the different variants of *B. oleracea* would exhibit different levels of heterosis in *B. napus* hybrids. To our knowledge, no study has so far been conducted to understand the value of the different variants of *B. oleracea* for heterosis of agronomic and seed quality traits including seed yield in *B. napus* canola. In this study, we compared six *B. napus* canola populations, derived from *B. napus* × *B. oleracea* interspecific crosses involving a spring type *B. napus* canola line and six *B. oleracea* accessions belonging to four variants of the species for the level of heterosis in spring *B. napus* canola hybrids. Furthermore, the effect of limited backcrossing of the interspecific hybrid to the *B. napus* parent on the re-constituted *B. napus* canola lines for the level of heterosis has also been investigated.

4.2 Materials and Methods

4.2.1 Plant materials

A total of 110 F_7 lines derived from crossing of a single spring *B. napus* line A04-73NA to six *B. oleracea* accessions belonging to four variants of this species, viz. var. *alboglabra* line NRC-PBI, var. *botrytis* cv. BARI cauliflower, var. *capitata* cvs. Badger Shipper,

Bindsachsener, and Balbro, and var. *italica* cv. Premium Crop, and 118 BC_1F_6 lines derived from crossing of the above-mentioned F_1's to the *B. napus* parent A04-73NA were used.

The spring *B. napus* parent A04-73NA is a canola quality line (zero erucic acid in oil and < 15 µmol glucosinolate g^{-1} seed) developed by the Canola Program of the University of Alberta. All *B. oleracea* accessions were high-erucic (~40% erucic acid) and high glucosinolate (> 60 µmol glucosinolate g^{-1} seed) type. Seeds of the *B. oleracea* accessions var. *alboglabra* line-NRC (PBI) was obtained from the National Research Council, Saskatoon, Canada; var. *botrytis* cv. BARI cauliflower from the Bangladesh Agricultural Research Institute, Bangladesh; var. *italica* cv. Premium Crop from Dr. Ron Howard, Alberta Agriculture and Forestry, Brooks, Canada; var. *capitata* cv. Balbro from Hazera Seeds of Growth, Netherlands; and var. *capitata* cvs. Badger Shipper and Bindsachsener from germplasm collection of the Canola Program of the University of Alberta (Hasan et al. 2012). The interspecific crosses from where the above-mentioned inbred lines were developed are listed below (Supplemental Table 4.1):

- A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI (Ol.alb.nrc, Chinese kale)
- A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower (Ol.bot.cau, cauliflower)
- A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper (Ol.cap.bad, cabbage)
- A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener (Ol.cap.bin, cabbage)
- A04-73NA × *B. oleracea* var. *capitata* cv. Balbro (Ol.cap.bal, cabbage)
- A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop (Ol.ita.pre, broccoli)

The F_1 plants were self -pollinated to produce the F_2 seeds and backcrossed to the *B. napus* parent to produce BC_1 seeds. The F_2 and BC_1 population were subjected to pedigree breeding with selection for spring growth habit, plant fertility and the two canola quality traits, zero erucic acid in seed oil and low glucosinolate in seed meal. All 228 (110 + 118) inbred lines were confirmed to be spring type euploid *B. napus* ($2n = 38$) possessing the canola quality

properties (Iftikhar et al. 2018). Theoretically, the C genome of this inbred population ($A^nA^nC^{n/o}C^{n/o}$) was expected to be composed of the C genome of *B. napus* ($A^nA^nC^nC^n$) and the C genome of *B. oleracea* (C^oC^o), where the proportion of *B. oleracea* alleles was expected to be 50% in case of the F_2-derived population and 25% in case of the BC_1-derived population.

4.2.2 Production of test hybrids

Test hybrid seeds were produced by manual crossing of the 228 inbred lines as male and the *B. napus* line A04-73NA as female. For this, a total of 110 F_7 plants of A04-73NA × *B. oleracea* and 118 BC_1F_6 plants of (A04-73NA × *B. oleracea*) × A04-73NA of the six crosses were grown in a greenhouse (21/18˚C ± 2˚C day/night, 16h photoperiod) of the Department of Agricultural, Food, and Nutritional Science, University of Alberta in 2014-15 winter, and test hybrid seeds as well as self-pollinated F_8 and BC_1F_7 generation seeds were produced for field trial in 2015. Two to three plants of each of the F_8 and BC_1F_7 lines were grown in greenhouse in 2015-16 winter and their test hybrid seeds were produced for field trial in 2016. In the same way, test hybrid seeds were produced in greenhouse for 2017 and 2018 field trials.

4.2.3 Field trial

The test hybrids, their self-pollinated male parent lines, and the common *B. napus* parent A04-73NA were grown in field plots in the summer of 2015, 2016, 2017, and 2018. In 2015, the trial was grown at Edmonton Research Station (ERS), and in the remaining years, the trials were grown at St. Albert Research Station of the University of Alberta.

In 2015, seeding was done manually in 3-row plots of 1.0 × 1.2 m (length × width) size where 66 seeds were seeded in 22 spots in the middle row with 5 cm spacing between the

spots, while 44 seeds were seeded in each of the two guard rows. After crop establishment (2 leaf stage), thinning was done in the middle row where 22 ± 2 plants were retained. Plot size in 2016 was 2.0 m × 1.3 m (length × width) and in 2017 and 2018 was 3.0 m × 1.3 m; however, the number rows per plot in 2016 and 2017 was four while in 2018 it was two. In all these three years, seeding was done by a plot seeder. Amount seed used per plot was 1.5 g in 2016, and 2.0 g in 2017 and 2018. The difference in the size of the plots in different years was due to the availability of the test hybrid seeds.

Field plots were laid out in a way that the test hybrids were bordered by their respective parents, where the hybrid and its two parents constituted one experimental unit. This design enabled direct comparison of the test hybrids with their parents and precise estimation of mid-parent heterosis (MPH); however, this design had also increased the total number of plots in a replication to 513. To accommodate this large number of plots in a best uniform piece of a land, field plots were laid out in an incomplete randomized block design, where each replication was divided into multiple blocks. Number of replications in all year was two, and randomization of the experimental units (hybrid and parents) was done using Cropstat version 7.2 and Microsoft Excel 2007.

4.2.4 Agronomic and seed quality traits

The following agronomic and seed quality traits were collected from the middle rows on plot basis: Days to flowering, plant height (cm), days to maturity, seed yield (kg ha^{-1}), and seed oil (%) and protein (%) contents. In addition to this, the duration of flowering time and grain-filling period data was collected in 2017 and 2018. Days to flowering data were collected when about 50% plants in a plot had at least one open flower. The end of flowering data was collected when about 90% plants in a plot did not have flower. Days to flowering and end of flowering data was collected two times in a week. Duration of flowering time was calculated

by subtracting days to flower from the end of flowering date. Plant height (cm) data was collected at the end of flowering on a whole plot basis by measuring height of the plants from soil surface. Days to maturity data was collected from the middle rows when silique color changed from green to light yellow or brown and seed color of the silique on the main branch (examined by opening the silique) changed to brown or black. Grain-filling period was calculated by subtracting the end of flowering date from the maturity date. Plots were harvested with a plot combine and plot yield data was converted to kg/ha.

Seed oil and protein contents were estimated using near-infrared spectroscopy (NIRS, Model 6500, Foss North America, Eden Prairie, MN) in the Analytical Laboratory of the Canola Program of the University of Alberta. For this, five to eight gram bulk open-pollinated seeds harvested from the field plots were used. A calibration equation available in this laboratory was used for quantification of oil and protein contents using the software WinISI II (Infrasoft International, LLC.). Oil and protein contents were calculated on whole seed basis at 8.5% moisture and reported as percent of the whole seed.

4.2.5 Simple sequence repeat (SSR) marker analysis

Genomic DNA of the above-mentioned 227 (110 + 117) F_2- and BC_1-derived inbred lines and their seven parents (*B. napus* A04-73NA and six *B. oleracea* cultivars/lines) was extracted using SIGMA DNA extraction kit (Sigma-Aldrich, St. Louis, MO, USA) following manufacture's instruction. A total of 95 polymorphic SSR markers (Nikzad et al. 2019) from nine C-genome linkage groups (LG) were selected from 418 markers for genotyping the populations. Polymerase chain reactions (PCR) for amplification of the genomic DNA was performed in a total volume of 15.5 µl, which included 20 ng genomic DNA, 5× PCR reaction buffer, 25 mM $MgCl_2$, 0.6 unit *Taq* DNA polymerase (Promega Corporation, Madison, WI), 10 mM each dNTP (Invitrogene Life Technologies Inc., Burlington, ON), 5

μM of each forward and reverse primer, and 5 μM tag F (fluorescent dyes FAM, VIC, NED, and PET). PCR was carried out in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA) with the following program: 1 cycle of 5 min at 95 °C for initial denaturation, 35 cycles where each cycle consisted of 1 min at 95 °C for denaturation, 1 min at 58°C for annealing and 1.5 min at 72 °C for extension, and the final extension time was 15 min at 72 °C. Size-based separation of the amplified DNA fragments was done using a capillary electrophoresis AB Genetic Analyzer No. 3730 (Applied Biosystems, Foster City, CA).

4.2.6 Statistical analysis

Best linear unbiased prediction (BLUP) was used to estimate genotypic value of the inbred lines and their test hybrids for different agronomic and seed quality traits. In this analysis, replication, block nested in replication and genotype were considered as random effects, and the analysis was done using the statistical software program of SAS (SAS Institute, 2010).

4.2.6.1 Heterosis

MPH (mid-parent heterosis) and NPH (heterosis over the *B. napus* parent) was calculated for different agronomic and seed quality traits using the estimates from BLUP and using the following formulas:

$$\text{Mid-parent value} = \frac{\text{Parent 1} + \text{Parent 2}}{2}$$

$$\text{MPH} = \frac{\text{Test hybrid value - Mid-parent value}}{\text{Mid-parent value}} \times 100$$

$$\text{NPH} = \frac{\text{Test hybrid value – A04-73NA value}}{\text{A04-73NA value}} \times 100$$

4.2.6.2 Analysis of variance (ANOVA)

ANOVA for different traits was performed using data of the inbred lines and test hybrids, and MPH and NPH values with the statistical software program of SAS using PROC MIXED through the option METHOD = Type 3 sums of squares. In this analysis, environment consisted of four field trials conducted over four years (2015, 2016, 2017 and 2018) as detailed in the Field trial section. The inbred lines used in this study to produce the test hybrids were developed from six crosses (Ol.alb.nrc, Ol.bot.cau, Ol.cap.bad, Ol.cap.bin, Ol.cap.bal and Ol.ita.pre) and following two breeding methods (F_2- and BC_1-derived). The six crosses, the two breeding methods and their interaction (cross × method) were considered as fixed effect; while the environment and genotype nested in cross × method were considered as random effect. The following linear model summarize the sources of variation:

$$Y_{ijkp} = \mu + C_i + T_j + (CT)_{ij} + E_k + G_{p(ij)} + GE_{kp(ij)} + \varepsilon_{ijkp}$$

where Y_{ijkp} is the trait value observed for the p^{th} genotype (inbred line) from the i^{th} (i = 1, …, 6) cross and the j^{th} (j = 1, 2) breeding method grown in the k^{th} (k = 1, …, 4) environment; μ, C_i, T_j , $(CT)_{ij}$, E_k, $G_{p(ij)}$ and $GE_{kp(ij)}$ are the overall mean and effects due to the i^{th} cross, the j^{th} breeding method, the ij^{th} cross × breeding method, the k^{th} environment, the p^{th} genotype within the ij^{th} cross × breeding method and the kp^{th} genotype × environment interaction, respectively; ε_{ijkp} is the random residual for the $ijkp^{th}$ observation. All random effects (E_k, $G_{p(ij)}$ and $GE_{kp(ij)}$) and random residual are assumed to be independently and identically distributed with mean zero and variances being σ_E^2, σ_G^2 , σ_{GE}^2 and σ_ε^2, respectively, i.e, $E_k \sim N(0, \sigma_E^2)$; $G_{p(ij)} \sim N(0, \sigma_G^2)$ and $G_{kp(ij)} \sim N(0, \sigma_{GE}^2)$ and $\varepsilon_{ijkp} \sim N(0, \sigma_\varepsilon^2)$.

4.2.6.3 Least square means

LSmeans of the fixed effects were calculated with SAS based on the estimates from BLUP, and test for significant difference ($\alpha \leq 0.05$) between the LSmeans was done following

Tukey's test. Pearson's correlation coefficient (*r*) values were calculated using cor.test function and chart.correlation of the PerformanceAnalytics package (Peterson *et al*. 2015).

4.2.6.4 Multivariate analyses

Principal component analysis (PCA) was used to facilitate the identification of the test hybrids with differentiated performance when accounting for differences and interrelationships among the multiple agronomic and seed quality traits. Lsmean values of the test hybrids (obtained above) across the environments for different agronomic and seed quality traits were used for the PCA and data standardized (mean = 0, variance =1) prior to this analysis. Data standardization and PCA were performed using R package vegan (Oksanen et al. 2019) according to Borcard et al. (2018).

4.2.6.5 Genetic diversity analysis

The fragment analysis results from ABI were scored for presence or absence of marker alleles using the software program GeneMarker® version 2.4.0 (SoftGenet- ics LLC, State College, PA); however, all genotyping results were confirmed manually as well. The absence (0) or presence (1) of the amplification products were scored based on fragment length, and data recorded in a 0/1 matrix for the presence/absence of the marker amplicons. The 0/1 matrix was used to calculate Nei's genetic distance using the software GENALEX 6 (Peakall and Smouse 2006), and Pearson's correlation (*r*) between genetic distance of the inbred lines from the common *B. napus* parent and performance of the inbred lines and test hybrids was calculated for different agronomic and seed quality traits using cor.test function and chart.correlation in R statistical computing program (Peterson et al. 2015).

4.3 Results

4.3.1 Agronomic and seed quality traits

Among the six inbred populations, populations derived from the crosses involving broccoli gave the greatest seed yield, while the population derived from the cross involving cabbage cv. Badger Shipper gave the lowest yield (Fig. 4.1; Supplemental Table 4.2); seed yield of the population derived from the cross involving cauliflower was comparable to the population derived from the cross involving broccoli. Test hybrid populations gave significantly (P <0.001) higher yield than their corresponding inbred populations (Fig. 4.1; Supplemental Fig. 4.1; Supplemental Table 4.2). Among the different populations, test hybrids of the populations derived from the crosses involving cauliflower, Chinese kale and two of cabbages cvs. Badger Shipper and Bindsachsener gave higher yield than the population derived from the crosses involving the other *B. oleracea* accessions including broccoli as well as the common *B. napus* parent A04-73NA. About 4.0 to 11.1% MPH was found for yield in the six populations — the highest MPH was found in the population derived from the cross involving Chinese kale and the lowest in the population derived from the cross involving broccoli (Fig. 4.2; Supplemental Tables 4.2 and 4.4). About 2.5 fold greater magnitude of heterosis was observed in the test hybrid population derived from the cross involving Chinese kale (11.1 ± 2.2) as compared to test hybrid population derived from the cross involving broccoli (4.0 ± 2.2) (Supplemental Table 4.2). Wide variation was found for MPH within the test hybrid populations where individual hybrid producing up to 82.7 % MPH was identified (data not shown). In all cases, MPH was higher than NPH (Supplemental Table 4.2). The highest NPH for seed yield was observed in the population derived from the cross involving Chinese kale (4.8%) while the lowest in the population derived from the cross involving broccoli (0.2%) (Fig. 4.2); individual test hybrid exhibiting with up to 63.8 % NPH was

identified within the population derived from the cross involving cauliflower (data not shown). While comparing the two breeding methods (F_2- and BC_1-derived) for seed yield, no significant difference was found between these methods for the development of the inbred lines as well as in their test hybrid populations (Fig. 4.1; Supplemental Table 4.5). Average MPH of the BC_1-derived population was 8.8% while it was 8.0% for the F_2-derived population (Fig. 4.2; Supplemental Table 4.3).

As compared to seed yield, much less contrasting difference was found between the inbred and test hybrid (Fig. 4.1) and between MPH and NPH (Fig. 4.2) for days to flowering and maturity. In general, the test hybrid populations flowered significantly ($P < 0.001$) earlier, had shorter duration of flowering and took longer grain-filling period than the inbred populations — these factors might have contributed to the greater seed yield in the test hybrid populations (Fig. 4.1; Supplemental Tables 4.2 and 4.3). MPH for these flowering and maturity traits was very low — in most cases less than 2.0% (Fig. 4.2; Supplemental Table 4.2).

While comparing the populations developed following two breeding methods, the BC_1-derived inbred population flowered (47.9 ± 0.67) and matured (106.0 ± 1.3) significantly ($P < 0.05$) earlier than the F_2-derived population. Test hybrid populations of the BC_1-derived lines still flowered earlier (47.1 ± 0.6, $P < 0.05$) and had longer grain-filling period (29.5 ± 5.4) than test hybrid population of the F_2-derived lines (Supplemental Tables 4.3 and 4.5).

In contrast to the above-mentioned flowering and maturity traits, test hybrid populations of all crosses were significantly ($P < 0.05$) taller than the inbred populations (Fig. 4.1) and exhibited significantly greater MPH than NPH (Fig. 4.2); however, the extent of MPH (1.0%) and NPH (-1.0 to -2.8%) was negligible (Supplemental Table 4.2).

Among the six populations, inbred and test hybrids of the crosses involving cauliflower had the greatest seed oil content (Fig. 4.1; Supplemental Table 4.2). Almost no MPH was found for seed oil (-0.1 to -0.6%) and protein (0.0 to 0.7%) contents, suggesting the importance of additive effect of the genes in the genetic control of these two seed quality traits (Supplemental Table 4.2).

4.3.2 Correlation

Days to flowering showed a positive correlation ($P < 0.001$) with duration of flowering and days to maturity, while it showed a negative correlation ($P < 0.001$) with grain-filling period in both inbred and test hybrid populations (Fig. 4.3). No significant correlation of this trait was found with seed yield; however, seed yield showed a significant ($P < 0.001$) negative correlation with duration of flowering, and a positive correlation with grain-filling period and seed oil content in both inbred and test hybrid populations. This suggests that high yielding lines or test hybrids with high oil content and earliness of flowering and maturity and longer grain-filling period can be obtained from this population.

Correlation between the performance of inbred lines and their test hybrids was studied to investigate the extent of the effect of the inbred lines on the performance of the hybrids for different agronomic and seed quality traits including seed yield. The inbred population showed significant positive correlation ($P < 0.001$) with the test hybrid population for all agronomic and seed quality traits (Fig. 4.3). This suggests that additive genes play an important role in the genetic control of these traits; therefore, improvement of these traits in the inbred lines will be needed for the improvement of these traits in the hybrids. However, in the cases of seed yield, duration of flowering and grain-filling period, the r values of ≤ 0.40 suggests that significant amount of non-additive effect of the genes are also involved in the genetic control of these traits. For majority of the traits, performance of the inbred lines

showed significant ($P < 0.05$) negative correlation with MPH; however, correlation between the performance of the inbred lines and NPH was significant ($P < 0.001$) and positive for all traits, including seed yield ($r = 0.30$) (Fig. 4.4).

4.3.3 Genetic distance and molecular marker analysis

To understand the effect of genetic distance of the inbred lines from the *B. napus* parent on the level of heterosis in test hybrids, correlation between genetic distance and the performance of inbred lines, test hybrids as well as MPH and NPH was calculated. Genetic distance of the inbred lines from the *B. napus* parent showed a weak negative correlation ($r = -0.14$) with seed yield in the inbred population; however, this correlation was positive ($r = 0.26$) in the test hybrid population as well as with MPH ($r = 0.31$) and NPH ($r = 0.24$) (Fig. 4.5). A moderate to weak positive correlation of genetic distance was found with days to flowering ($r = 0.30$), duration of flowering ($r = 0.35$), and days to maturity ($r = 0.29$) in the inbred population; however, this correlation was negligible in the test hybrid population. A positive correlation of the genetic distance of the inbred lines with days to flowering, duration of flowering and days to maturity indicate that *B. oleracea* alleles delayed flowering and maturity in the inbred lines; however, the negative effect of these *B. oleracea* alleles has been repressed to some extent by the alleles of *B. napus* in the test hybrids. Genetic distance showed a negative correlation ($r = -0.29$) with grain-filling period in the inbred population; however, this correlation was positive ($r = 0.32$) for MPH. A moderate positive correlation of genetic distance was found with plant height in the inbred ($r = 0.30$) and test hybrid population ($r = 0.47$) as well as for MPH and NPH. Almost no correlation was found between

genetic distance and seed oil or protein content in both inbred and hybrid population (Fig. 4.5).

It was expected that the test hybrid population to be heterozygous at different loci for the alleles originating from the *B. oleracea* and *B. napus* parents. The position of the 95 SSR markers used in this study together with heterozygosity of the markers in the test hybrid population, deduced from marker genotype of the inbred lines and the common *B. napus* parent A04-73NA, is presented in Supplemental Fig. 4.2. Of the 95 SSR markers, heterozygous loci could be deduced in the test hybrid population for 89 (93.7%) markers. For a given marker, the proportion (%) of loci to be heterozygous in the test hybrid population varied from 0.5 to 55.4% with being only six markers showing heterozygosity 78.6 to 100%; the average heterozygosity of the 89 markers in the entire population was 19.6%. Among the different chromosomes, markers from the chromosome C7 (28.9%) showed the greatest and the markers from C9 (12.1%) showed the least heterozygosity.

4.3.4 Performance of the top, medium and poor inbred lines in test hybrids

The performance of the top 5%, poorest 5% and 5% medium yielding inbred lines were compared with their hybrids as well as for the level of MPH. Among these three groups, greatest MPH was found in the hybrids of the poorest inbred lines. However, test hybrids of the top 5% inbred lines gave significantly greater seed yield than test hybrids of the other two groups indicating the importance of both additive and non-additive genes of the hybrid parents for increased seed yield in F_1 hybrids (Fig. 4.6).

In case of the other agronomic and seed quality traits, the top performing inbred lines also resulted the best performing hybrids indicating the importance of the additive effect genes in the control of these traits. Among these traits, least difference between the performance of the hybrids of the top and poorest inbred lines was found for duration of flowering and grain-

filling period indicating the importance of non-additive effect of the genes in the genetic control of these traits; this was also evident from the occurrence of about 5% MPH for these traits.

4.3.5 Multivariate analysis

4.3.5.1 Test hybrid populations

The first three PCs explained 74.3% of the total variation (PC1: 36.0%, PC2: 23.9%, PC3: 14.5%) for different agronomic and seed quality traits. PC1 showed high correlation with various traits (Supplemental Table 4.6). The test hybrids with short duration of flowering but having long grain-filling period, and the test hybrids with high seed yield and high oil but low protein content were grouped together on right half of the plot (Fig. 4.7), and were mostly derived from the crosses with Chinese kale and cauliflower. PC2 explained mostly a gradient of days to flowering and maturity, and seed protein content (Supplemental Table 4.6). The early flowering and maturing test hybrids, and the test hybrids with high seed protein content were grouped together on the lower half of the ordination plot (Fig. 4.7-A). The upper right part of the biplot (Fig. 4.7-A) showed that seed yield, seed oil content and grain-filling period were positively correlated and these three variables were negatively correlated with duration of flowering and seed protein content. A strong positive correlation between days to flowering and maturity was also reflected from close association of the vectors for these traits at the upper left part of the biplot (Fig. 4.7-A). PC3 explained a gradient of plant height and seed yield (Supplemental Table 4.6, Fig. 4.7-B). High yielding test hybrids with long stature, particularly those derived from the crosses with cabbages cvs. Badger Shipper and Bindsachsener, grouped together on the lower half of the ordination plot compared to the test hybrids derived from the crosses involving cabbage cv. Balbro and broccoli which tended to be distributed at the upper side of the biplot.

4.3.5.2 Mid-Parent heterosis

In case of MPH, the first three PC explained 67.4% of the total variation (PC1: 38.7%, PC2: 17.2%, PC3: 11.5%) for the agronomic and seed quality traits. PC1 showed high correlation with all traits except plant height (Supplemental Table 4.6). PC2 explained mostly a gradient of the duration of flowering, and seed oil and protein contents; whereas, PC3 explained a gradient of plant height (Supplemental Table 4.6). Individuals showing high MPH for seed yield, oil, protein, grain-filling period, and early flowering and maturity grouped together on the left half of the plot (Fig. 4.8-A), while, individuals showing high MPH for seed protein content grouped together on the upper right half of the ordination plot (Fig. 4.8-A). Plant height was more important trait for distribution of the individuals along the PC3 (Fig. 4.8-B); therefore, individuals showing high MPH for this trait grouped together on the upper half of the ordination plot (Fig. 4.8-B). No striking differences were observed between the F_2- and BC_1-derived individuals for most of the traits as was found based on LSmeans data (Fig. 4.8).

4.4 Discussion

Since identification of the phenomenon heterosis or hybrid vigour in maize, the development of F_1 hybrid cultivars has received much attention to the breeders. Some field crops, such as maize, sunflower and canola, and vegetable crops, such as cabbage and cauliflower grown today are predominantly hybrid cultivars. Currently, hybrid cultivars of *B. napus* canola captured more than 90 percent of the total canola planted area in Canada (Morrison et al. 2016). However, the narrow genetic base of *B. napus* resulted from intensive selection by breeders is one of the bottlenecks for continual improvement of this type of cultivars for seed yield and other agronomic and seed quality traits (Jesske et al. 2013; Rahman et al. 2016; Zhao et al. 2016). Therefore, broadening the genetic base of the spring *B. napus* canola, especially its C genome which genetic base is known to be narrow as compared to its A

genome (Bus et al. 2011; Delourme et al. 2013; Wu et al. 2014), is needed for exploitation of heterosis in this crop from a long-term perspective. In this study, we compared the performance of the test hybrids of the inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses involving four variants of *B. oleracea* and a single *B. napus* line. The design of the production of test hybrids laid out in this research, i.e. the inbred lines were crossed to the *B. napus* parent, allowed us to estimate the effect of the alleles of the different variants of *B. oleracea* for heterosis in *B. napus* canola. We found that seed yield in hybrids in most cases was significantly greater than the *B. napus* parent suggesting that *B. oleracea* alleles contributed to increased seed yield in spring *B. napus* canola hybrids. Of the six populations studied, the inbred population derived from the cross involving broccoli gave higher yield than the inbred populations derived from the other five crosses. While evaluating only the inbred lines in larger plot (5.0 m × 1.7-1.8 m) trials, we also found similar results (Nikzad et al. 2019). However, in this study, we found that the test hybrids of the inbred population of broccoli yielded less than the other test hybrid populations; this inbred population, in fact, had the least genetic distance from the *B. napus* parent (data not shown). This indicate that this variant of *B. oleracea* might carry less heterotic alleles for seed yield, or depletion of favorable heterotic alleles might have occurred during the development of this population.

In the present study, we used 95 SSR markers from nine C-genome linkage groups (average 10.7 SSR markers per linkage group). This is not a large number of markers when compared with SNPs; however, limited number (e.g., 18 to 55) of SSRs can provide good information of genetic diversity, as has been reported by several researchers (Wang et al. 2009; Chen et al. 2007; Chen et al. 2017). Genetic distance of the inbred lines, estimated based on the above-mentioned 95 SSR markers, showed a weak or negative (r = -0.14) correlation with seed yield in the inbred population; however, this correlation was positive in the test hybrid

population as well as with MPH. This indicates that several alleles of *B. oleracea* in homozygous condition gave poor yield in the inbred population; however, at least, some of the alleles were capable of contributing to heterosis through non-additive genetic effect. Involvement of non-additive gene effect for high seed yield in hybrids was also evident from a weak correlation between the performance of the inbred lines and the hybrids, as well as from a weak correlation of the performance of the inbred lines with MPH and NPH. Involvement of both additive and non-additive genes in the genetic control of seed yield in *B. napus* hybrids has also been reported by several researchers (Radoev et al. 2008; Qian et al. 2009). While working with a single *B. oleracea* accession, Rahman et al. (2016) also found the evidence that the alleles of *B. oleracea* contributing to heterosis may not necessarily contribute to seed yield in the inbred lines. The effect of *B. oleracea* alleles on lateness of flowering and maturity and longer duration of flowering is also evident from the positive correlation of genetic distance with these traits in the inbred population. However, several lines flowered and matured earlier than the *B. napus* parent (Fig. 4.1) suggesting that, at least, some of the *B. oleracea* alleles can exhibit favorable effect on these traits; identification of these alleles using high-density markers and molecular mapping approach will be needed for use in a molecular breeding program.

Interspecific hybridization in *Brassica* can induce a number of genetic changes in the genome through homoeologous recombination between the chromosomes (Udall et al. 2005; Leflon et al. 2006; Zou et al. 2011) and this can create new genetic variation and exert significant effect on seed yield (Zou et al. 2011; Fu et al. 2012). While working with *B. napus* × *B. rapa* interspecific cross, Fu et al. (2012) found that the novel alleles generated in the progeny of this interspecific cross can contribute to heterosis for seed yield in *B. napus* through allelic and non-allelic interactions. Zou, Zhu et al. (2010) found improved agronomic performance and strong heterosis for seed yield in hybrids of natural *B. napus* and *B. napus* lines carrying

A and C genome contents introgressed from *B. rapa* and *B. carinata*, respectively. Intersubgenomic heterosis in *Brassica* for seed yield in *B. napus* has also been reported by Qian et al. (2005) and Wei, Wang et al. (2016). In the present study, the average MPH for seed yield in the six test hybrid populations was 8.5%, and about 67% of test hybrids yielded higher than the common *B. napus* parent. Of the six populations we used in this study, greater proportion of the test hybrids of the inbred lines derived from the crosses involving cabbage cv. Balbro and broccoli cv. Premium Crop gave lower yield than the common *B. napus* parent. Multivariate analysis showed that the best hybrid gave about 30% MPH for seed yield and this hybrid originated from the inbred line 74 (Supplemental Table 4.1) derived from the cross involving var. *botrytis* cv. BARI Cauliflower. However, high MPH in individual hybrid was also observed in the populations derived from crosses with Chinese kale and cabbage cv. Badger Shipper. Thus, the wide variation for heterosis observed between the six test hybrid populations might have resulted from the effect of variable alleles from these *B. oleracea* variants. It is also probable that novel genetic variation arose in the progeny of these interspecific crosses might have also contributed to the observed heterosis; further study will be needed to confirm this.

In the present study, backcrossing of the F_1 to the *B. napus* parent, theoretically, would have diluted the exotic genome content in the BC_1-derived inbred population, while the F_2-derived inbred population was expected to have a greater proportion of the genome content of *B. oleracea* and consequently would have resulted greater genetic variation and stronger heterosis in the test hybrids. However, in practice, no significant difference for seed yield was found between the test hybrid populations developed following these two breeding methods. It was also expected that, the BC_1-derived inbred population will be closer to the common *B. napus* parent than the F_2 derived population in regards to SSR allele diversity; however, these two populations were genetically quite similar (distance from the *B. napus* parent was 0.47

and 0.49 for the F_2- and BC_1-derived populations, respectively). Stronger selection on the F_2-derived population as compared to the BC_1-derived population for spring growth habit and the two canola quality traits (zero erucic acid and low glucosinolate) might be one of the reasons for this genetic similarity as well as similar seed yield of the populations developed following these two breeding methods. In contrast, Schelfhout *et al.* (2008) identified greater number of lines with high seed yield in BC_1-derived population as compared to F_2-derived population of *B. napus* × *B. juncea* interspecific cross.

Almost no heterosis was found for seed oil and protein contents in the test hybrid populations of the inbred lines derived from the six interspecific crosses. These two traits are mainly controlled by additive genes (Shen et al. 2005; Variath et al. 2009; for review, see Rahman et al. 2013; Cheng et al. 2016; Chao et al. 2017) which could be the reason for the lack of significant heterosis for these two seed traits as has been found in other studies as well (Grant and Beversdorf 1985; Rahman et al. 2016). The occurrence of strong positive correlation between the performance of the inbred lines and test hybrids for seed oil and protein contents and weak correlation of these traits with MPH suggests that these two traits are largely controlled by additive genes. Therefore, improvement of the hybrid parent lines will be needed to achieve high oil and protein contents in the hybrid cultivars. However, positive heterosis for seed oil content has also been reported by Shen et al. (2005).

Several inbred lines of the *B. napus* × *B. oleracea* crosses flowered significantly earlier than the common *B. napus* parent indicating that the alleles of the C genome of *B. oleracea* can contribute to earliness in *B. napus*. Test hybrid populations also flowered and matured slightly earlier than their inbred populations. The results of the present study agree with the results reported by Long et al. (2007) and Rahman et al. (2016). The earlier flowering and maturing phenotype apparently resulted from partial to complete dominance of some of the genes governing these two quantitative traits (Sernyk and Stefansson 1983; Cuthbert et al.

2009). Days to flowering in the inbred and test hybrid populations used in the present study didn't show significant correlation with seed yield. This trait has been reported to exhibit a significant negative (Udall et al. 2004; Raman et al. 2016) or a non-significant correlation (Butruille et al. 1999) in spring *B. napus* depending on the types of materials used and test environmental condition. In contrast, the duration of flowering and grain-filling period, respectively, exhibited a significant negative and positive correlation with seed yield. The negative correlation between the duration of flowering and seed yield might have resulted from the failure of the late flowering lines and hybrids to reach physiological maturity at the time of harvest as all plots were desiccated at the same time, and this might had penalized the late flowering ones for seed yield. On the other hand, the longer grain-filling period might had resulted fully developed seeds and, thus, contributed to the positive correlation of this trait with seed yield. Gan et al. (2016) also reported a negative correlation between the duration of flowering and seed yield while a positive correlation between the duration of grain–filling period and seed yield under Canadian environment.

In conclusion, results from this study showed that the *B. oleracea* alleles introgressed into spring *B. napus* canola inbred lines can exhibit heterosis for seed yield in *B. napus* hybrids. Among the different variants of *B. oleracea* used in this study, cauliflower, Chinese kale and some of the cabbages showed great potential to increase seed yield in spring canola hybrids. However, improvement of the seed yield of the hybrid parent lines will also be needed to increase seed yield in the hybrids as evident from positive correlation of the performance of inbred lines with hybrid yield as well as with NPH. In this regard, alleles introgressed from broccoli can also contribute to hybrid breeding. Thus, introgression of genome content of *B. oleracea* can broaden the genetic base of the C genome of *B. napus* for the development of improved spring *B. napus* canola hybrid cultivars.

4.5 Figures

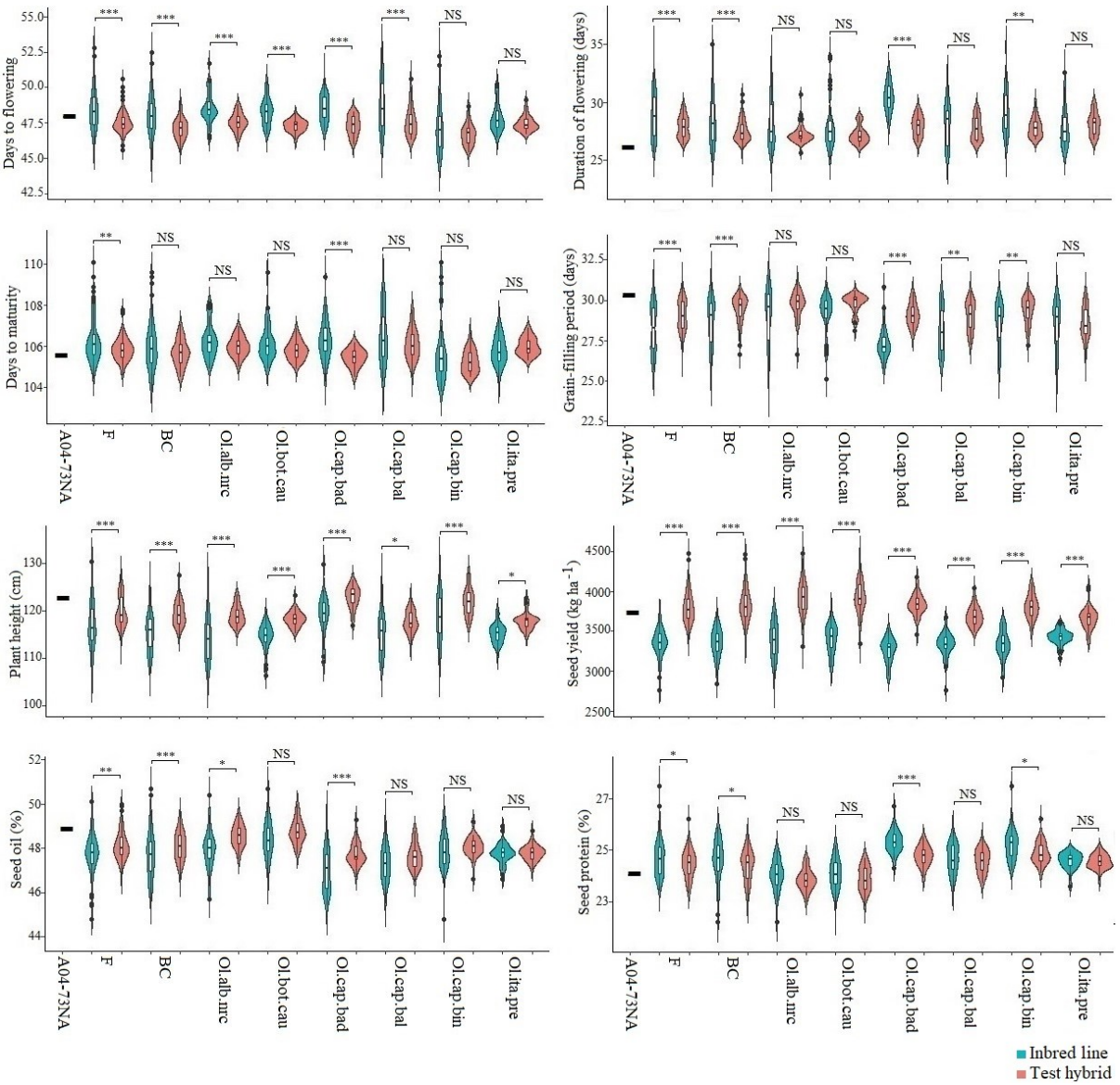


Fig. 4.1 Violin plot of six inbred populations derived from six *Brassica napus* × *B. oleracea* interspecific crosses and following two breeding methods (F_2- and BC_1-derived) and their test hybrids. Data of the common *B. napus* parent A04-73NA is also included. Teal bars represent inbred lines and blush bars represent the test hybrids.
Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI (*n* = 36); Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1 (*n* = 40); Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper (*n* = 33); Ol.cap.bal = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro (*n* = 42); Ol.cap.bin = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener (*n* = 43); Ol.ita.pre = A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop (*n* = 34).

F = F_2-derived population (*n* = 110); BC = BC_1 (F_1 × *B. napus*)-derived population (*n* = 118).

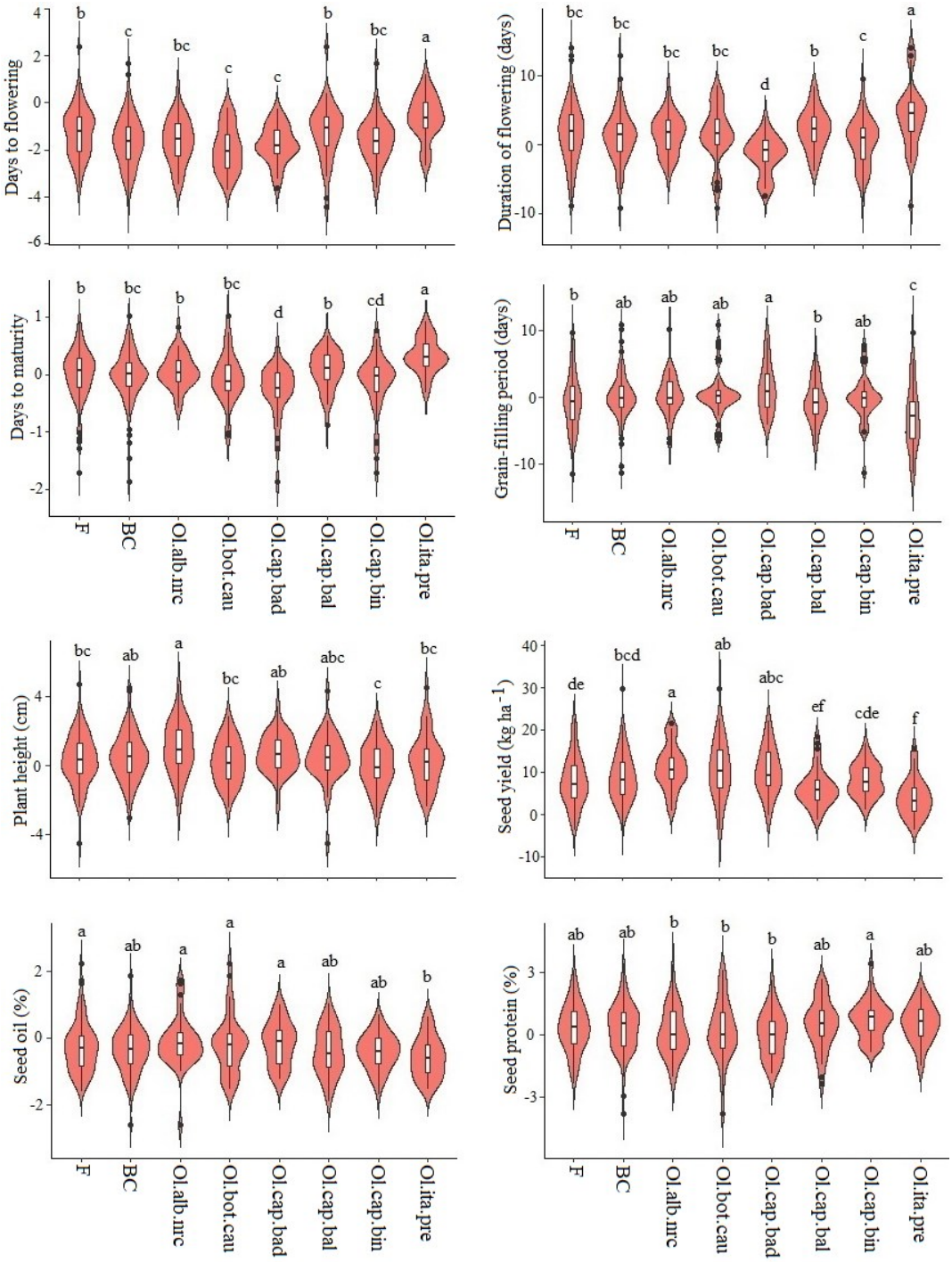


Fig. 4.2 Violin plot of six test hybrid populations developed using six inbred populations derived from six *Brassica napus* × *B. oleracea* interspecific crosses and following two breeding methods (F_2- and BC_1-derived) for mid-parent heterosis (MPH).
Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI (*n* = 36); Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1 (*n* = 40); Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper (*n* = 33); Ol.cap.bal = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro (*n* = 42); Ol.cap.bin = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener (*n* = 43); Ol.ita.pre = A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop (*n* = 34).

F = F_2-derived population (*n* = 110); BC = BC_1 (F_1 × *B. napus*)-derived population (*n* = 118).

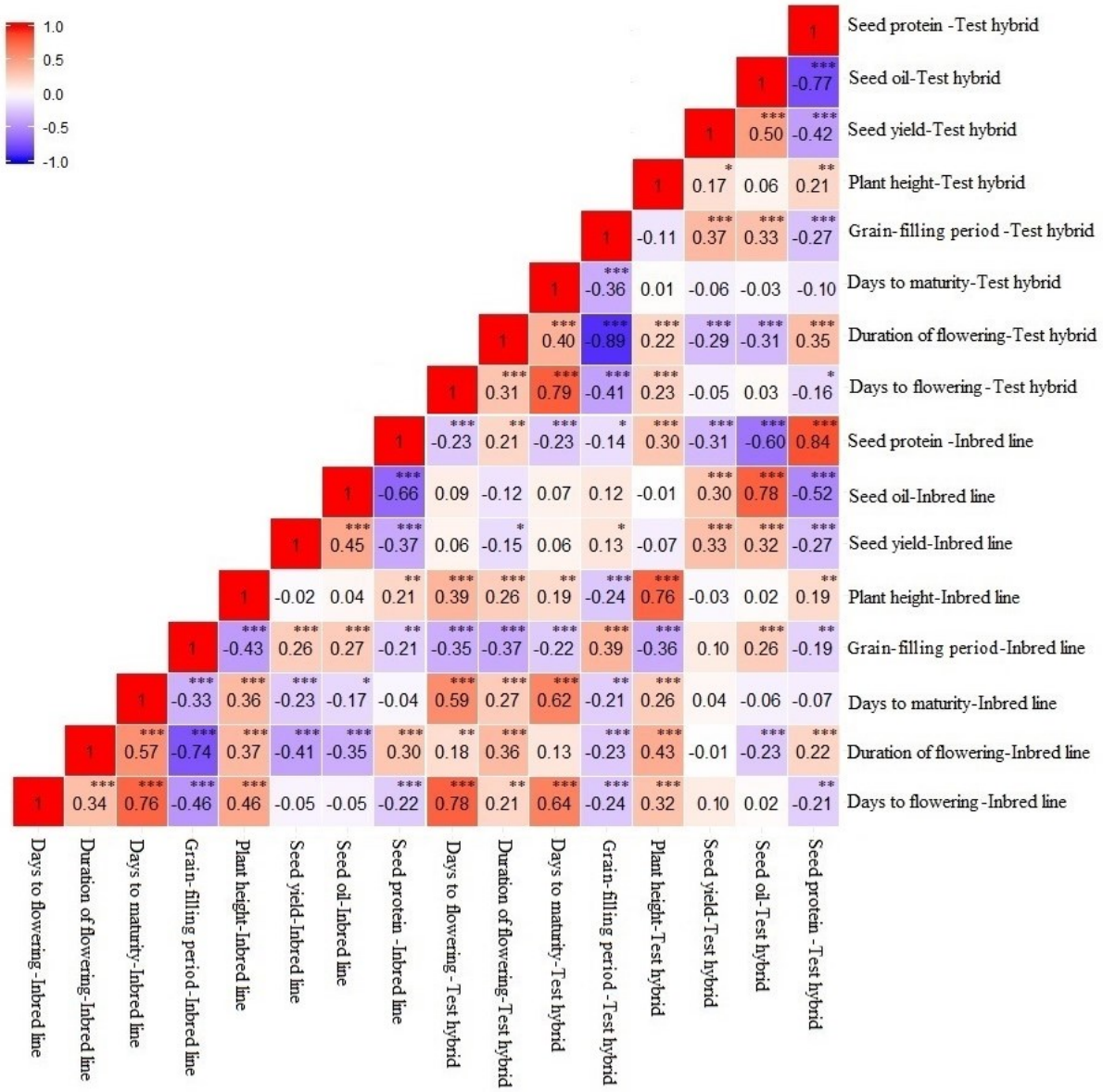


Fig. 4.3 Correlation between different agronomic and seed quality traits in an inbred population of 228 lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses and in their test hybrids. The strength and direction of the correlation are indicated by the color: Red represents the positive correlation while blue represents the negative correlation; the intensity of the color indicates the strength of the correlation.

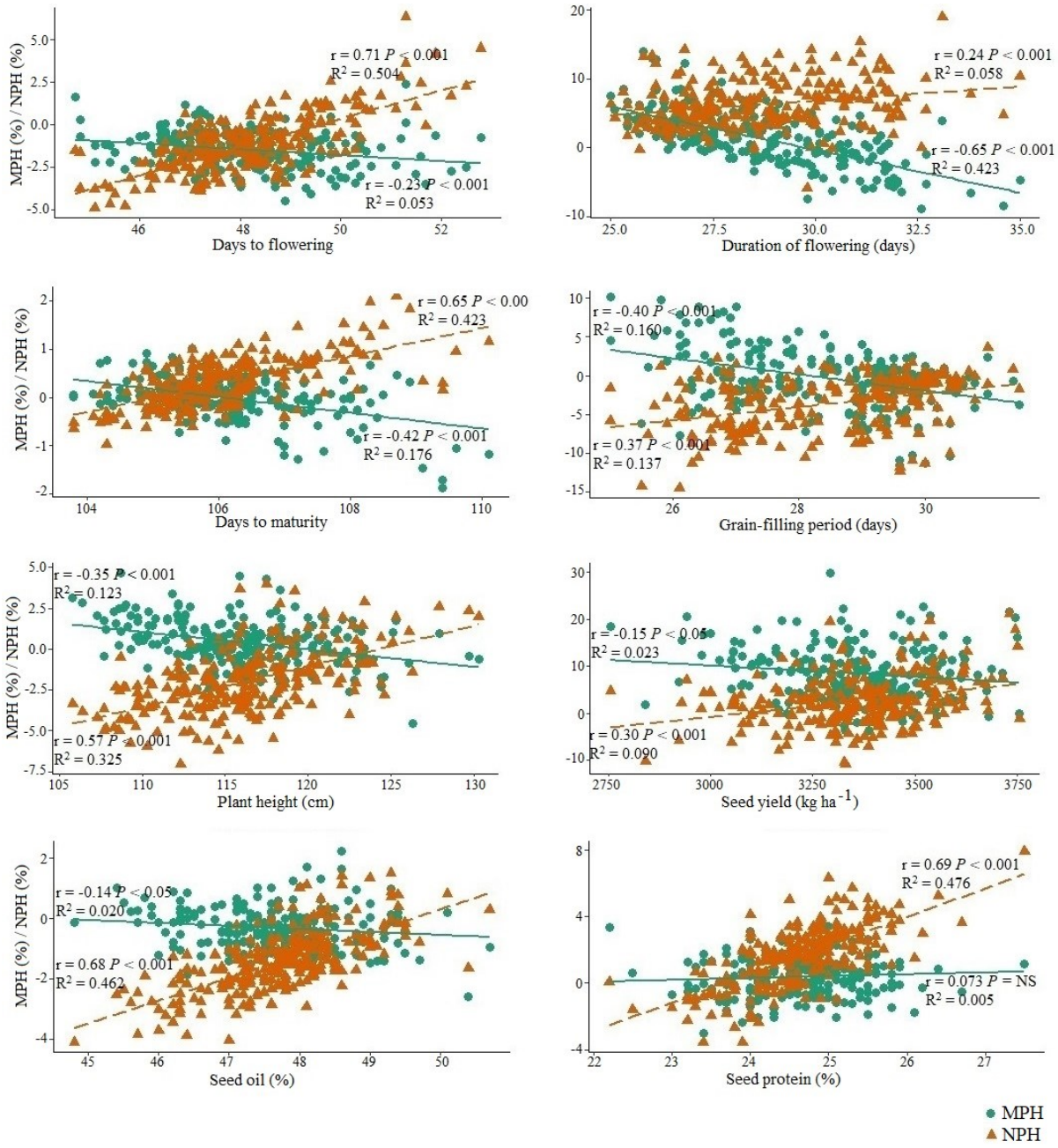


Fig. 4.4 The relationship of the performance of the inbred lines (n = 228) derived from six *Brassica napus* × *B. oleracea* interspecific crosses with mid-parent heterosis (MPH) and heterosis over the common *B. napus* parent (NPH) for different agronomic and seed quality traits. Green circles and green solid lines represent MPH and orange triangles and orange broken line represents NPH.

Fig. 4.5 The relationship of the genetic distance (GD) of the inbred lines (n = 228), derived from six *Brassica napus* × *B. oleracea* interspecific crosses and developed following two breeding methods (F_2- and BC_1-derived populations), with the performance of the inbred lines (IN), their test hybrids (TC), and with mid-parent heterosis (MPH) and heterosis over the common *B. napus* parent (NPH) for different agronomic and seed quality traits. Orange dots represents the F_2-derived population and green dots represents the BC_1-derived population

DTF = Days to flowering; DOF = Duration of flowering; DTM = Days to maturity; GFP = Grain-filling period; PH = Plant height; SY = Seed yield; SOC = Seed oil content; SPC = Seed protein content.

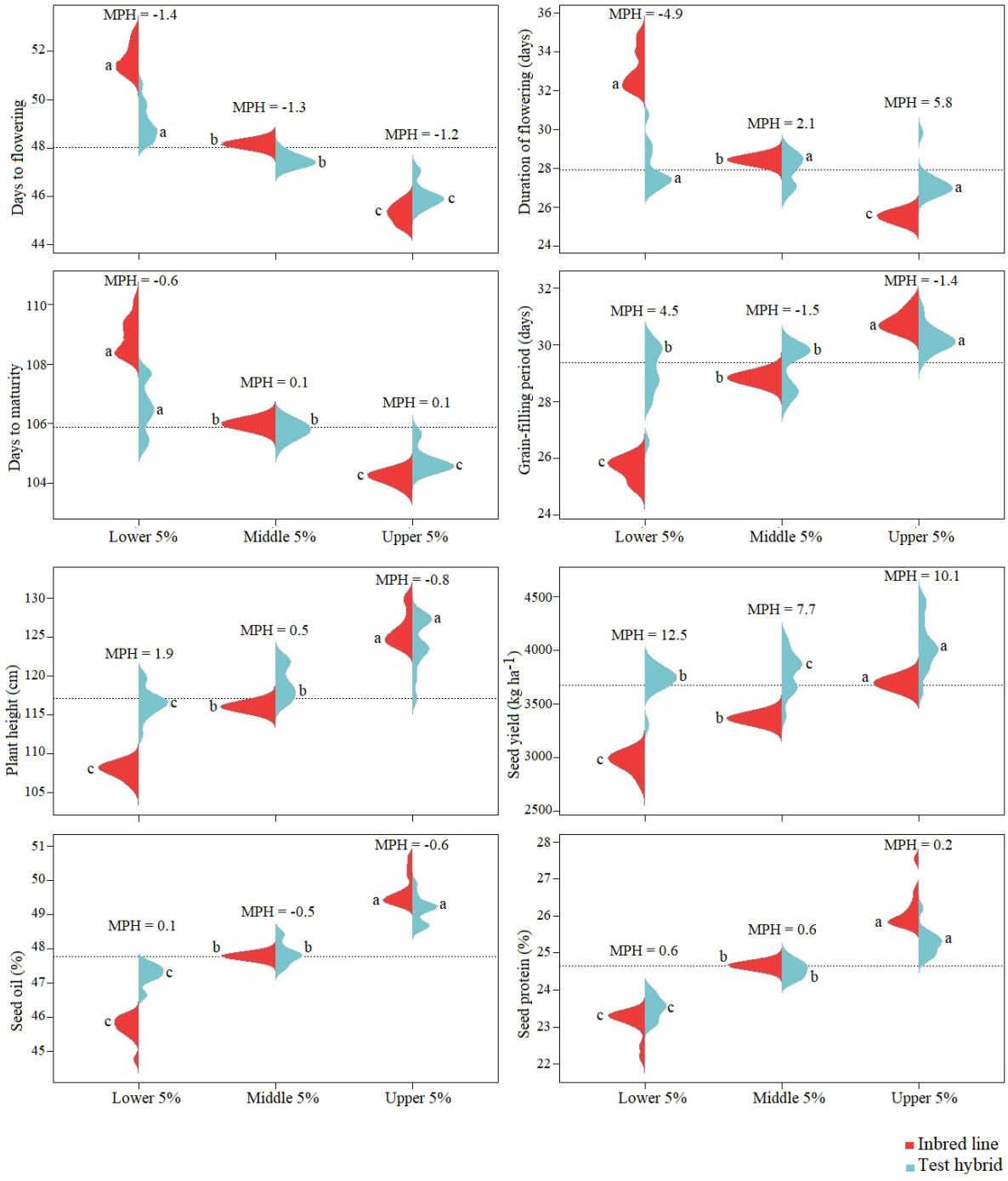


Fig. 4.6 Beanplot of the 5% top, medium and poorest performing inbred lines, derived from *Brassica napus* × *B. oleracea* interspecific crosses, and the performance of their test hybrids. Red color represents the inbred lines and blue color represents the test hybrids. MPH = percent mid-parent heterosis.
Inbred lines and test hybrids are compared separately; the same letter for the inbred or hybrid indicates the values are not significantly different at $P < 0.05$.

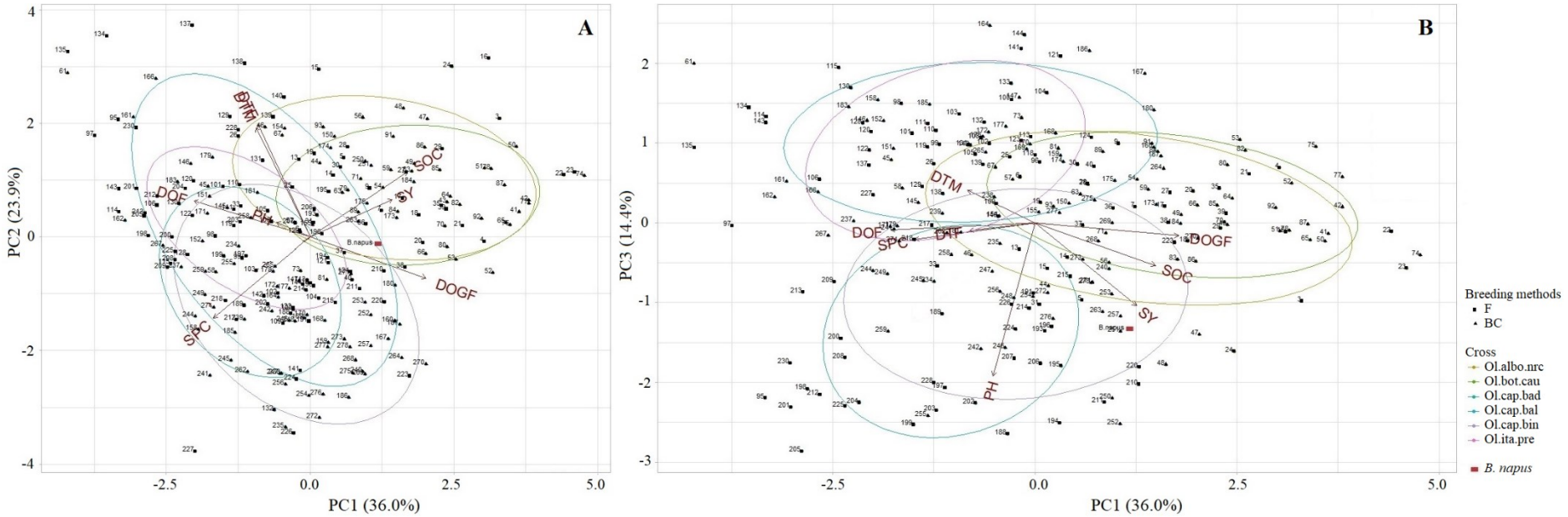


Fig. 4.7 Principal component analysis biplot of the test hybrids (n = 228) of the inbred lines, derived from six *Brassica napus* × *B. oleracea* interspecific crosses and following two breeding methods (F_2- and BC_1-derived), and the common *B. napus* parent A04-73NA, illustrating the distribution of the test hybrids characterized by different agronomic and seed quality traits in the space of the first principal component (PC1) versus PC2 (A), and PC1 versus PC3 (B). The name of the inbred lines of the test hybrids are shown in Supplemental Table 4.1.
DTF = Days to flowering; DOF = Duration of flowering; DTM = Days to maturity; DOGF = Duration of grain-filling period; PH = Plant height; SY = Seed yield; SOC = Seed oil content; SPC = Seed protein content; Ol.alb.nrc (mustard color) = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI (n = 36); Ol.bot.cau (green) = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1 (n = 40); Ol.cap.bad (teal) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper (n = 33); Ol.cap.bal (blue) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro (n = 42); Ol.cap.bin (violet) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener (n = 43); Ol.ita.pre (pink) = A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop (n = 34); *B. napus* parent (red rectangle); F (solid square) = F_2-derived population (n = 110); BC (solid triangle) = BC_1 (F_1 × *B. napus*)-derived population (n = 118).

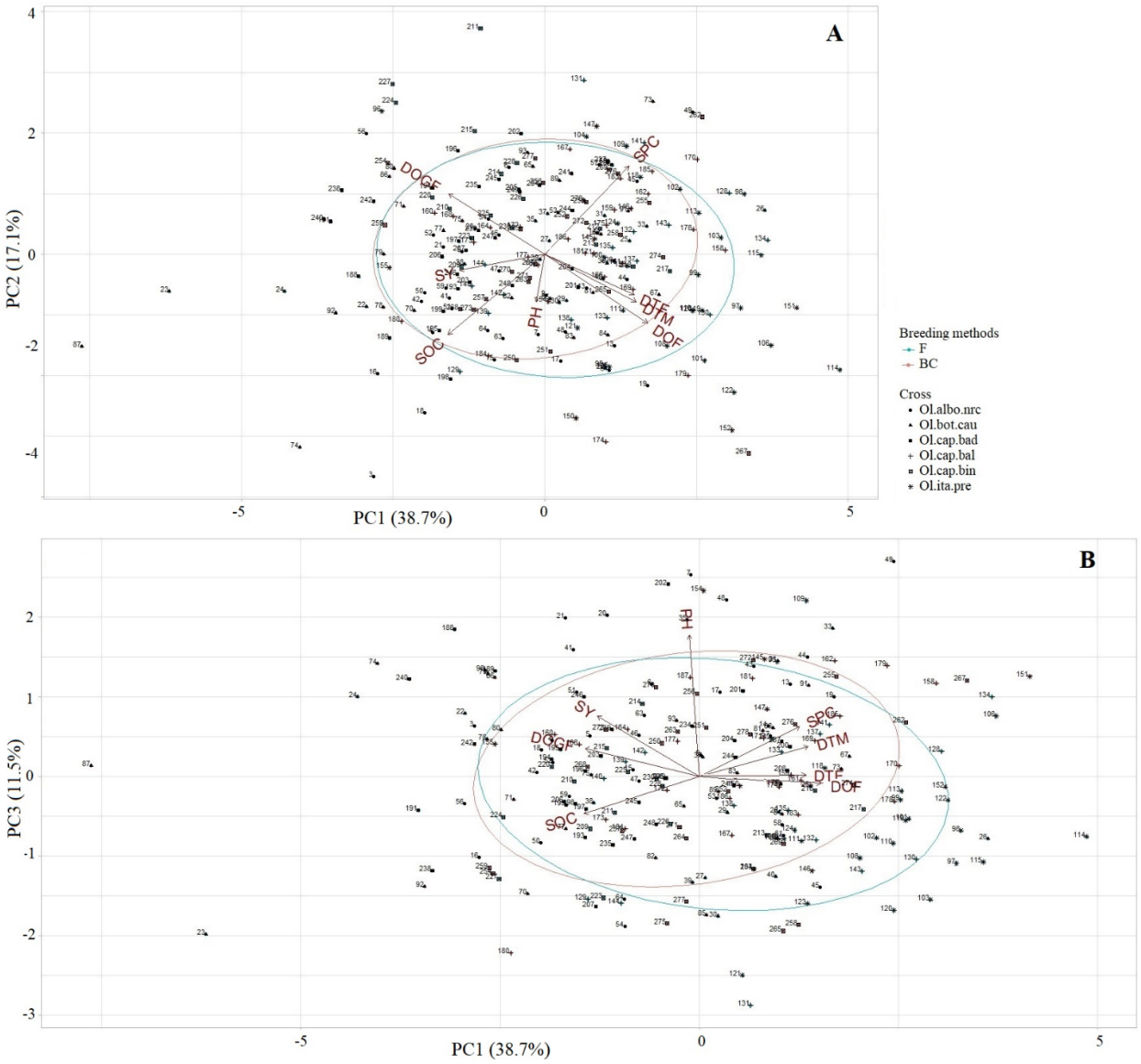


Fig. 4.8 Principal component analysis biplot of mid-parent heterosis (MPH; *n* = 228) of the test hybrids of the inbred lines, derived from six *Brassica napus* × *B. oleracea* interspecific crosses and following two breeding methods (F_2- and BC_1-derived), illustrating the distribution of test hybrids characterized by different agronomic and seed quality traits in the space of the first principal component (PC1) versus PC2 (A), and PC1 versus PC3 (B). The name of the inbred lines of the test hybrids exhibiting mid-parent heterosis (MPH) are shown in Supplemental Table 4.1.

DTF = Days to flowering; DOF = Duration of flowering; DTM = Days to maturity; DOGF = Duration of grain-filling period; PH = Plant height; SY = Seed yield; SOC = Seed oil content; SPC = Seed protein content; Ol.alb.nrc (soild circle) = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI (*n* = 36); Ol.bot.cau (solid triangle) = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower-1 (*n* = 40); Ol.cap.bad (solid square) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper (*n* = 33); Ol.cap.bal (plus sign) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro (*n* = 42); Ol.cap.bin (empty square) = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener (*n* = 43); Ol.ita.pre (star) = A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop (*n* = 34); *B. napus* parent (red rectangle); F (green circle) = F_2-derived population (*n* = 110); BC (brown circle) = BC_1 (F_1 × *B. napus*)-derived population (*n* = 118).

4.6 Supplemental materials

Supplemental Table 4.1 Codes of the F_2- and BC_1-derived inbred lines of six *Brassica napus* × *B. oleracea* interspecific crosses

Inbred line number	Inbred line code	Cross	Breeding method
1300-353	3	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-355	4	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-360	5	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-363	6	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-368	7	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-375	9	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-398	13	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-401	14	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-404	15	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-410	16	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-412	17	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-413	18	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-416	19	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-419	20	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1300-420	21	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	F
1343-320	22	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-321	23	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-323	24	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-327	25	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-329	26	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-330	27	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-333	28	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-336	29	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-339	30	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-343	31	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-349	33	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-353	35	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-357	36	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-360	37	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-362	38	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-367	39	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1343-368	40	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	F
1676-361	41	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-363	42	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-365	43	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-377	44	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-380	45	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-389	46	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-393	47	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-402	48	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-405	49	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-407	50	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-409	51	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-412	52	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-413	53	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-416	54	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-422	56	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-423	57	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-427	58	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-429	59	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-438	61	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-442	63	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1676-446	64	A04-73NA × *B. oleracea* var. *alboglabra* line NRC-PBI	BC
1677-326	65	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC

Inbred line number	Inbred line code	Cross	Breeding method
1677-328	66	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-330	67	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-342	70	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-344	71	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-351	73	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-352	74	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-355	75	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-360	77	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-363	78	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-375	79	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-376	80	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-379	81	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-383	82	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-386	83	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-387	84	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-390	85	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-394	86	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-395	87	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-405	89	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-411	91	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-414	92	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1677-418	93	A04-73NA × *B. oleracea* var. *botrytis* cv. BARI cauliflower	BC
1358-594	95	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-609	96	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-615	97	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-616	98	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-620	99	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-623	100	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-624	101	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-634	102	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-635	103	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-640	104	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-652	105	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-656	106	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-667	108	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-679	109	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-685	110	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-688	111	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-701	113	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-703	114	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-705	115	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-719	118	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-720	119	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-727	120	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-731	121	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-739	122	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-747	123	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1358-752	124	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	F
1392-300	128	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-303	129	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-305	130	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-306	131	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-312	132	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-313	133	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-319	134	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-320	135	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-324	137	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-325	138	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-327	139	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-329	140	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-337	141	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F

Inbred line number	Inbred line code	Cross	Breeding method
1392-339	142	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-342	143	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1392-345	144	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	F
1678-263	145	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-264	146	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-265	147	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-277	150	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-281	151	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-285	152	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-291	154	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1678-309	155	A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop	BC
1679-354	158	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-357	159	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-369	160	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-377	161	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-378	162	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-382	164	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-399	166	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-405	167	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-420	168	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-430	169	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-437	170	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-440	171	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-442	172	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-460	173	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-465	174	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-470	175	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-474	177	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-483	178	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-486	179	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-497	180	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-502	181	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-506	183	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-511	184	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-535	185	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-541	186	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1679-543	187	A04-73NA × *B. oleracea* var. *capitata* cv. Balbro	BC
1362-149	188	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-152	189	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-156	191	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-161	193	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-162	194	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-164	195	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-165	196	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-166	197	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-167	198	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-169	199	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-170	200	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-171	201	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-173	202	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-174	203	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-175	204	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-176	205	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-177	206	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-179	207	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1362-180	208	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	F
1363-164	209	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-165	210	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-168	211	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-170	212	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F

Inbred line number	Inbred line code	Cross	Breeding method
1363-171	213	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-173	214	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-177	215	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-180	217	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-181	218	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-183	220	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-190	223	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-194	224	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-195	225	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-197	226	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-202	227	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-205	228	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1363-207	230	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	F
1681-084	235	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-083	234	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-086	237	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-090	238	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-091	239	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-092	240	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-096	241	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-097	242	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-100	244	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-101	245	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-102	246	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-103	247	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-104	248	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1681-105	249	A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper	BC
1682-099	250	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-100	251	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-101	252	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-102	253	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-103	254	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-104	255	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-105	256	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-108	257	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-113	258	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-120	259	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-128	262	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-130	263	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-131	264	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-133	265	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-138	267	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-140	268	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-143	269	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-145	270	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-147	271	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-149	272	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-150	273	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-152	274	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-154	275	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-155	276	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-156	277	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC
1682-158	278	A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener	BC

Supplemental Table 4.2 Least square means ± SE of the inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses, their test-hybrids, mid-parent heterosis (MPH), and heterosis over the common *B. napus* parent (NPH) for different agronomic and seed quality traits.

Cross[1]	Pop. type	Days to flowering	Duration of flowering (day)	Days to maturity	Grain-filling period (day)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
Ol.alb.nrc	Inbred	48.6 ± 0.6	28.2 ± 2.8	106.3 ± 1.3	29.1 ± 5.4	114.1 ± 7.7	3387.2 ± 495.6	48.0 ± 1.3	24.0 ± 1.2
	Hybrid	47.6 ± 0.6	27.3 ± 2.8	106.0 ± 1.3	29.7 ± 5.4	119.1 ± 7.7	3932.1 ± 495.6	48.6 ± 1.3	23.9 ± 1.2
	t test[2]	***	NS	NS	NS	***	***	*	NS
	MPH	-1.6 ± 0.9	1.5 ± 3.8	0.1 ± 0.4	0.3 ± 2.6	1.0 ± 1.1	11.1 ± 2.2	-0.1 ± 0.3	0.1 ± 0.6
	NPH	-1.3 ± 0.9	6.3 ± 3.8	0.4 ± 0.4	-2.2 ± 2.6	-2.1 ± 1.1	4.8 ± 2.2	-1.5 ± 0.3	0.9 ± 0.6
	t test[3]	NS	***	NS	NS	***	***	***	NS
Ol.bot.cau	Inbred	48.3 ± 0.6	28.1 ± 2.8	106.2 ± 1.3	29.1 ± 5.4	114.4 ± 7.7	3402.0 ± 495.6	48.4 ± 1.3	24.1 ± 1.2
	Hybrid	47.3 ± 0.6	27.2 ± 2.8	105.8 ± 1.3	29.8 ± 5.4	118.3 ± 7.7	3936.2 ± 495.6	48.8 ± 1.3	23.9 ± 1.2
	t test	***	NS	NS	NS	***	***	NS	NS
	MPH	-2.0 ± 0.9	1.4 ± 3.8	-0.1 ± 0.4	0.4 ± 2.6	0.2 ± 1.1	10.7 ± 2.1	-0.2 ± 0.3	0.2 ± 0.6
	NPH	-1.9 ± 0.9	6.0 ± 3.8	0.2 ± 0.4	-2.0 ± 2.6	-2.8 ± 1.1	4.3 ± 2.1	-1.1 ± 0.3	1.1 ± 0.6
	t test	NS	***	NS	NS	***	***	***	NS
Ol.cap.bad	Inbred	48.6 ± 0.6	30.4 ± 2.8	106.3 ± 1.3	27.4 ± 5.4	118.9 ± 7.7	3260.2 ± 495.7	47.0 ± 1.3	25.3 ± 1.2
	Hybrid	47.3 ± 0.6	28.0 ± 2.8	105.5 ± 1.3	29.1 ± 5.4	122.8 ± 7.7	3834.7 ± 495.7	47.7 ± 1.3	24.8 ± 1.2
	t test	***	***	***	***	***	***	***	***
	MPH	-1.8 ± 0.9	-1.5 ± 3.8	-0.3 ± 0.5	1.3 ± 2.6	0.7 ± 1.1	10.3 ± 2.2	-0.2 ± 0.3	0.0 ± 0.7
	NPH	-0.9 ± 0.9	5.6 ± 3.8	0.3 ± 0.5	-4.1 ± 2.6	-1.6 ± 1.1	2.9 ± 2.2	-1.9 ± 0.3	2.4 ± 0.7
	t test	NS	***	***	***	***	***	***	***
Ol.cap.bal	Inbred	48.8 ± 0.6	28.1 ± 2.8	106.5 ± 1.3	28.1 ± 5.4	115.4 ± 7.7	3339.0 ± 495.5	47.3 ± 1.3	24.7 ± 1.2
	Hybrid	47.7 ± 0.6	27.8 ± 2.8	106.1 ± 1.3	29.0 ± 5.4	117.8 ± 7.7	3693.8 ± 495.6	47.6 ± 1.3	24.6 ± 1.2
	t test	***	NS	NS	**	*	***	NS	NS
	MPH	-1.2 ± 0.9	2.2 ± 3.8	0.1 ± 0.4	-0.8 ± 2.6	0.5 ± 1.1	6.3 ± 2.1	-0.4 ± 0.3	0.5 ± 0.6
	NPH	-0.1 ± 0.9	6.1 ± 3.8	0.5 ± 0.4	-4.5 ± 2.6	-1.0 ± 1.1	1.5 ± 2.1	-1.5 ± 0.3	1.3 ± 0.6
	t test	**	***	**	***	**	**	***	NS
Ol.cap.bin	Inbred	47.1 ± 0.6	29.3 ± 2.8	105.6 ± 1.3	28.5 ± 5.4	118.7 ± 7.7	3333.2 ± 495.5	47.8 ± 1.3	25.2 ± 1.2
	Hybrid	46.7 ± 0.6	27.9 ± 2.8	105.3 ± 1.3	29.4 ± 5.4	122.0 ± 7.7	3809.6 ± 495.5	48.1 ± 1.3	24.9 ± 1.2
	t test	NS	**	NS	**	***	***	NS	*
	MPH	-1.6 ± 0.9	0.4 ± 3.8	-0.2 ± 0.4	-0.2 ± 2.6	0.0 ± 1.1	8.3 ± 2.1	-0.4 ± 0.3	0.7 ± 0.6
	NPH	-2.1 ± 0.9	5.8 ± 3.8	0.1 ± 0.4	-3.3 ± 2.6	-2.5 ± 1.1	1.6 ± 2.1	-1.3 ± 0.3	2.9 ± 0.6
	t test	NS	***	NS	**	***	***	***	***
Ol.ita.pre	Inbred	47.9 ± 0.6	27.9 ± 2.8	105.8 ± 1.3	28.4 ± 5.4	115.2 ± 7.7	3426.5 ± 495.7	47.8 ± 1.3	24.6 ± 1.2
	Hybrid	47.5 ± 0.6	28.2 ± 2.8	106.0 ± 1.3	28.6 ± 5.4	117.9 ± 7.7	3668.1 ± 495.7	47.8 ± 1.3	24.5 ± 1.2
	t test	NS	NS	NS	NS	*	***	NS	NS
	MPH	-0.6 ± 0.9	4.1 ± 3.8	0.3 ± 0.5	-2.9 ± 2.6	0.3 ± 1.1	4.0 ± 2.2	-0.6 ± 0.3	0.5 ± 0.7
	NPH	-0.3 ± 0.9	7.4 ± 3.8	0.4 ± 0.5	-6.1 ± 2.6	-1.8 ± 1.1	0.2 ± 2.2	-1.2 ± 0.3	1.2 ± 0.7
	t test	NS	*	NS	*	***	NS	NS	NS
A04-73NA		47.9 ± 0.6	26 ± 2.8	105.5 ± 1.3	30.2 ± 5.4	122.2 ± 7.7	3716.6 ± 495.1	48.8 ± 1.3	24 ± 1.2

[1] Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower; Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bin = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener; Ol.cap.bal = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro; Ol.ita.pre = A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop; [2] *t test*: Inbred vs. hybrid; [3] *t test*: MPH vs. NPH.

Supplemental Table 4.3 Least square means ± SE of the inbred populations derived from two breading methods (F_2- and BC_1-derived), their test-hybrids, mid-parent heterosis (MPH), and heterosis over the common *B. napus* parent (NPH) for different agronomic and seed quality traits

Breeding method[1]	Pop. type[2]	Days to flowering	Duration of flowering (day)	Days to maturity	Grain-filling period (day)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
F	Inbred	48.5 ± 0.6	28.9 ± 2.8	106.3 ± 1.3	28.2 ± 5.4	116.9 ± 7.7	3359.1 ± 495.3	47.8 ± 1.3	24.7 ± 1.2
	Hybrid	47.5 ± 0.6	27.9 ± 2.8	105.9 ± 1.3	29.1 ± 5.4	119.9 ± 7.7	3795.1 ± 495.3	48.1 ± 1.3	24.5 ± 1.2
	t test[3]	***	***	**	***	***	***	**	*
	MPH	-1.3 ± 0.8	0.0 ± 0.4	0.0 ± 0.4	-0.7 ± 2.6	0.3 ± 1.1	8.0 ± 2.1	-0.3 ± 0.3	0.3 ± 0.6
	NPH	-0.6 ± 0.8	6.8 ± 3.7	0.4 ± 0.4	-4.4 ± 2.6	-1.7 ± 1.1	2.5 ± 2.1	-1.3 ± 0.3	1.6 ± 0.6
	t test[4]	**	***	***	***	***	***	***	***
BC	Inbred	47.9 ± 0.6	28.4 ± 2.8	106.0 ± 1.3	28.7 ± 5.4	115.5 ± 7.7	3357.6 ± 495.2	47.7 ± 1.3	24.6 ± 1.2
	Hybrid	47.1 ± 0.6	27.5 ± 2.8	105.7 ± 1.3	29.5 ± 5.4	119.3 ± 7.7	3828.5 ± 495.2	48.1 ± 1.3	24.4 ± 1.2
	t test	***	***	NS	***	***	***	***	*
	MPH	-1.7 ± 0.8	1.2 ± 3.7	0.0 ± 0.4	0.1 ± 2.6	0.5 ± 1.1	8.8 ± 2	-0.3 ± 0.3	0.4 ± 0.6
	NPH	-1.6 ± 0.8	5.6 ± 3.7	0.2 ± 0.4	-2.9 ± 2.6	-2.2 ± 1.1	2.7 ± 2	-1.5 ± 0.3	1.7 ± 0.6
	t test	NS	***	***	***	***	***	***	***
CV (%)	Inbred	5.2	16.1	2.6	22.3	12.2	28.1	5.4	9.7
	Hybrid	4.0	12.2	2.4	19.9	12.2	24.7	4.6	9.1

[1] F = F_2-derived populations of the cross; BC = BC_1 (F_1 × *B. napus*)-derived population of the cross.

[2] Pop. type = population type: Inbred = inbred line population; Hybrid = test hybrid population; MPV = mid-parent value; MPH = mid-parent heterosis; NPH = heterosis over common parent *B. napus* canola.

[3] *t test*: Inbred vs. hybrid.

[4] *t test*: MPH vs. NPH.

Supplemental Table 4.4 Comparisons of the least square mean (± SE) values of the inbred lines, derived from six *Brassica napus* × *B. oleracea* interspecific crosses, their common *B. napus* parent (A04-73NA), test-hybrids, mid-parent heterosis (MPH), and heterosis over the common *B. napus* parent (NPH) for different agronomic and seed quality traits

Comparisons		Days to flowering	Duration of flowering (day)	Days to maturity	Grain-filling period (day)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
A04-73NA	Ol.alb.nrc-Inbred	-0.7 ± 0.2**	-2.2 ± 0.3***	-0.8 ± 0.1***	1.2 ± 0.2***	8.1 ± 0.6***	329.5 ± 27.0***	0.8 ± 0.1***	0.0 ± 0.1NS
A04-73NA	Ol.alb.nrc-Hybrid	0.3 ± 0.2NS	-1.3 ± 0.3***	-0.5 ± 0.1*	0.5 ± 0.2NS	3.1 ± 0.6***	-215.5 ± 27.0***	0.2 ± 0.1NS	0.2 ± 0.1NS
A04-73NA	Ol.bot.cau-Inbred	-0.5 ± 0.2NS	-2.1 ± 0.3***	-0.7 ± 0.1***	1.1 ± 0.2***	7.8 ± 0.5***	314.6 ± 25.8***	0.4 ± 0.1*	0.0 ± 0.1NS
A04-73NA	Ol.bot.cau-Hybrid	0.6 ± 0.2*	-1.2 ± 0.3***	-0.3 ± 0.1NS	0.4 ± 0.2NS	3.9 ± 0.5***	-219.6 ± 25.8***	0.0 ± 0.1NS	0.1 ± 0.1NS
A04-73NA	Ol.cap.bad-Inbred	-0.7 ± 0.2**	-4.4 ± 0.3***	-0.9 ± 0.1***	2.8 ± 0.2***	3.4 ± 0.6***	456.4 ± 28.1***	1.8 ± 0.1***	-1.3 ± 0.1***
A04-73NA	Ol.cap.bad-Hybrid	0.6 ± 0.2*	-2.0 ± 0.3***	0.0 ± 0.1NS	1.1 ± 0.2***	-0.6 ± 0.6NS	-118.0 ± 28.1**	1.1 ± 0.1***	-0.7 ± 0.1***
A04-73NA	Ol.cap.bal-Inbred	-1.0 ± 0.2***	-2.1 ± 0.2***	-1.0 ± 0.1***	2.1 ± 0.2***	6.8 ± 0.5***	377.6 ± 25.2***	1.5 ± 0.1***	-0.6 ± 0.1***
A04-73NA	Ol.cap.bal-Hybrid	0.2 ± 0.2NS	-1.7 ± 0.2***	-0.7 ± 0.1***	1.2 ± 0.2***	4.5 ± 0.5***	22.9 ± 25.3NS	1.2 ± 0.1***	-0.5 ± 0.1***
A04-73NA	Ol.cap.bin-Inbred	0.7 ± 0.2***	-3.2 ± 0.2***	-0.2 ± 0.1NS	1.7 ± 0.2***	3.5 ± 0.5***	383.4 ± 25.0***	1.0 ± 0.1***	-1.2 ± 0.1***
A04-73NA	Ol.cap.bin-Hybrid	1.1 ± 0.2***	-1.8 ± 0.2***	0.2 ± 0.1NS	0.9 ± 0.2***	0.3 ± 0.5NS	-92.9 ± 25.1*	0.7 ± 0.1***	-0.8 ± 0.1***
A04-73NA	Ol.ita.pre-Inbred	0.0 ± 0.2NS	-1.9 ± 0.3***	-0.3 ± 0.1NS	1.8 ± 0.2***	7.0 ± 0.6***	290.2 ± 27.6***	1.0 ± 0.1***	-0.5 ± 0.1***
A04-73NA	Ol.ita.pre-Hybrid	0.4 ± 0.2NS	-2.2 ± 0.3***	-0.6 ± 0.1**	1.6 ± 0.2***	4.3 ± 0.6***	48.5 ± 27.6NS	1.0 ± 0.1***	-0.5 ± 0.1***
Ol.alb.nrc-Inbred	Ol.alb.nrc-Hybrid	1.0 ± 0.2***	0.9 ± 0.3NS	0.3 ± 0.2NS	-0.7 ± 0.2NS	-5.0 ± 0.7***	-544.9 ± 35.4***	-0.6 ± 0.1**	0.2 ± 0.1NS
Ol.alb.nrc-Inbred	Ol.bot.cau-Inbred	0.2 ± 0.2NS	0.1 ± 0.3NS	0.1 ± 0.2NS	0.0 ± 0.2NS	-0.3 ± 0.7NS	-14.9 ± 34.5NS	-0.4 ± 0.1NS	-0.1 ± 0.1NS
Ol.alb.nrc-Inbred	Ol.bot.cau-Hybrid	1.3 ± 0.2***	1.0 ± 0.3NS	0.5 ± 0.2NS	-0.7 ± 0.2NS	-4.2 ± 0.7***	-549.0 ± 34.5***	-0.8 ± 0.1***	0.1 ± 0.1NS
Ol.alb.nrc-Inbred	Ol.cap.bad-Inbred	0.0 ± 0.2NS	-2.2 ± 0.4***	0.0 ± 0.2NS	1.7 ± 0.2***	-4.7 ± 0.8***	127.0 ± 36.3*	1.0 ± 0.1***	-1.3 ± 0.1***
Ol.alb.nrc-Inbred	Ol.cap.bad-Hybrid	1.3 ± 0.2***	0.2 ± 0.4NS	0.8 ± 0.2**	0.0 ± 0.2NS	-8.7 ± 0.8***	-447.5 ± 36.3***	0.2 ± 0.1NS	-0.8 ± 0.1***
Ol.alb.nrc-Inbred	Ol.cap.bal-Inbred	-0.3 ± 0.2NS	0.1 ± 0.3NS	-0.2 ± 0.2NS	0.9 ± 0.2**	-1.3 ± 0.7NS	48.2 ± 34.1NS	0.7 ± 0.1***	-0.7 ± 0.1***
Ol.alb.nrc-Inbred	Ol.cap.bal-Hybrid	0.9 ± 0.2***	0.5 ± 0.3NS	0.2 ± 0.2NS	0.1 ± 0.2NS	-3.6 ± 0.7***	-306.6 ± 34.2***	0.3 ± 0.1NS	-0.6 ± 0.1***
Ol.alb.nrc-Inbred	Ol.cap.bin-Inbred	1.4 ± 0.2***	-1.0 ± 0.3NS	0.7 ± 0.2*	0.5 ± 0.2NS	-4.6 ± 0.7***	54.0 ± 34.0NS	0.2 ± 0.1NS	-1.2 ± 0.1***
Ol.alb.nrc-Inbred	Ol.cap.bin-Hybrid	1.8 ± 0.2***	0.4 ± 0.3NS	1.0 ± 0.2***	-0.3 ± 0.2NS	-7.8 ± 0.7***	-422.4 ± 34.0***	-0.1 ± 0.1NS	-0.9 ± 0.1***
Ol.alb.nrc-Inbred	Ol.ita.pre-Inbred	0.7 ± 0.2NS	0.3 ± 0.4NS	0.5 ± 0.2NS	0.6 ± 0.2NS	-1.1 ± 0.7NS	-39.3 ± 35.9NS	0.2 ± 0.1NS	-0.6 ± 0.1***
Ol.alb.nrc-Inbred	Ol.ita.pre-Hybrid	1.1 ± 0.2***	0.0 ± 0.4NS	0.3 ± 0.2NS	0.5 ± 0.2NS	-3.8 ± 0.7***	-280.9 ± 35.9***	0.2 ± 0.1NS	-0.5 ± 0.1***
Ol.alb.nrc-Hybrid	Ol.bot.cau-Inbred	-0.7 ± 0.2*	-0.8 ± 0.3NS	-0.2 ± 0.2NS	0.6 ± 0.2NS	4.7 ± 0.7***	530.1 ± 34.5***	0.1 ± 0.1NS	-0.2 ± 0.1NS
Ol.alb.nrc-Hybrid	Ol.bot.cau-Hybrid	0.3 ± 0.2NS	0.1 ± 0.3NS	0.2 ± 0.2NS	-0.1 ± 0.2NS	0.8 ± 0.7NS	-4.1 ± 34.5NS	-0.2 ± 0.1NS	0.0 ± 0.1NS
Ol.alb.nrc-Hybrid	Ol.cap.bad-Inbred	-1.0 ± 0.2***	-3.1 ± 0.4***	-0.3 ± 0.2NS	2.4 ± 0.2***	0.2 ± 0.8NS	671.9 ± 36.3***	1.6 ± 0.1***	-1.5 ± 0.1***
Ol.alb.nrc-Hybrid	Ol.cap.bad-Hybrid	0.3 ± 0.2NS	-0.6 ± 0.4NS	0.5 ± 0.2NS	0.6 ± 0.2NS	-3.7 ± 0.8***	97.4 ± 36.3NS	0.8 ± 0.1***	-0.9 ± 0.1***
Ol.alb.nrc-Hybrid	Ol.cap.bal-Inbred	-1.2 ± 0.2***	-0.8 ± 0.3NS	-0.5 ± 0.2NS	1.6 ± 0.2***	3.7 ± 0.7***	593.1 ± 34.1***	1.3 ± 0.1***	-0.8 ± 0.1***
Ol.alb.nrc-Hybrid	Ol.cap.bal-Hybrid	-0.1 ± 0.2NS	-0.4 ± 0.3NS	-0.1 ± 0.2NS	0.7 ± 0.2NS	1.3 ± 0.7NS	238.3 ± 34.2***	0.9 ± 0.1***	-0.7 ± 0.1***
Ol.alb.nrc-Hybrid	Ol.cap.bin-Inbred	0.4 ± 0.2NS	-1.9 ± 0.3***	0.4 ± 0.2NS	1.2 ± 0.2***	0.4 ± 0.7NS	598.9 ± 34.0***	0.7 ± 0.1***	-1.4 ± 0.1***
Ol.alb.nrc-Hybrid	Ol.cap.bin-Hybrid	0.9 ± 0.2**	-0.5 ± 0.3NS	0.7 ± 0.2**	0.4 ± 0.2NS	-2.9 ± 0.7**	122.5 ± 34.0*	0.5 ± 0.1*	-1.0 ± 0.1***
Ol.alb.nrc-Hybrid	Ol.ita.pre-Inbred	-0.3 ± 0.2NS	-0.6 ± 0.4NS	0.2 ± 0.2NS	1.3 ± 0.2***	3.9 ± 0.7***	505.6 ± 35.9***	0.8 ± 0.1***	-0.7 ± 0.1***
Ol.alb.nrc-Hybrid	Ol.ita.pre-Hybrid	0.1 ± 0.2NS	-0.8 ± 0.4NS	0.0 ± 0.2NS	1.1 ± 0.2***	1.2 ± 0.7NS	264 ± 35.9***	0.8 ± 0.1***	-0.7 ± 0.1***
Ol.bot.cau-Inbred	Ol.bot.cau-Hybrid	1.0 ± 0.2***	0.9 ± 0.3NS	0.4 ± 0.2NS	-0.7 ± 0.2NS	-3.9 ± 0.7***	-534.2 ± 33.6***	-0.3 ± 0.1NS	0.2 ± 0.1NS
Ol.bot.cau-Inbred	Ol.cap.bad-Inbred	-0.2 ± 0.2NS	-2.3 ± 0.3***	-0.2 ± 0.2NS	1.7 ± 0.2***	-4.5 ± 0.7***	141.8 ± 35.4**	1.5 ± 0.1***	-1.3 ± 0.1***
Ol.bot.cau-Inbred	Ol.cap.bad-Hybrid	1.0 ± 0.2***	0.1 ± 0.3NS	0.7 ± 0.2*	0.0 ± 0.2NS	-8.4 ± 0.7***	-432.7 ± 35.4***	0.7 ± 0.1***	-0.7 ± 0.1***
Ol.bot.cau-Inbred	Ol.cap.bal-Inbred	-0.5 ± 0.2NS	0.0 ± 0.3NS	-0.3 ± 0.2NS	1.0 ± 0.2***	-1.0 ± 0.7NS	63.0 ± 33.2NS	1.1 ± 0.1***	-0.6 ± 0.1***
Ol.bot.cau-Inbred	Ol.cap.bal-Hybrid	0.7 ± 0.2*	0.3 ± 0.3NS	0.1 ± 0.2NS	0.1 ± 0.2NS	-3.4 ± 0.7***	-291.7 ± 33.3***	0.8 ± 0.1***	-0.5 ± 0.1***
Ol.bot.cau-Inbred	Ol.cap.bin-Inbred	1.2 ± 0.2***	-1.2 ± 0.3*	0.5 ± 0.2NS	0.6 ± 0.2NS	-4.3 ± 0.7***	68.8 ± 33.0NS	0.6 ± 0.1***	-1.1 ± 0.1***
Ol.bot.cau-Inbred	Ol.cap.bin-Hybrid	1.6 ± 0.2***	0.2 ± 0.3NS	0.9 ± 0.2***	-0.3 ± 0.2NS	-7.6 ± 0.7***	-407.5 ± 33.1***	0.4 ± 0.1NS	-0.8 ± 0.1***
Ol.bot.cau-Inbred	Ol.ita.pre-Inbred	0.4 ± 0.2NS	0.2 ± 0.3NS	0.4 ± 0.2NS	0.7 ± 0.2NS	-0.8 ± 0.7NS	-24.4 ± 35.1NS	0.7 ± 0.1***	-0.5 ± 0.1***

Comparisons		Days to flowering	Duration of flowering (day)	Days to maturity	Grain-filling period (day)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
Ol.bot.cau-Inbred	Ol.ita.pre-Hybrid	0.9 ± 0.2**	-0.1 ± 0.3NS	0.2 ± 0.2NS	0.5 ± 0.2NS	-3.5 ± 0.7***	-266.1 ± 35.1***	0.6 ± 0.1**	-0.5 ± 0.1***
Ol.bot.cau-Hybrid	Ol.cap.bad-Inbred	-1.3 ± 0.2***	-3.2 ± 0.3***	-0.5 ± 0.2NS	2.4 ± 0.2***	-0.5 ± 0.7NS	676.0 ± 35.4***	1.8 ± 0.1***	-1.4 ± 0.1***
Ol.bot.cau-Hybrid	Ol.cap.bad-Hybrid	0.0 ± 0.2NS	-0.8 ± 0.3NS	0.3 ± 0.2NS	0.7 ± 0.2NS	-4.5 ± 0.7***	101.5 ± 35.4NS	1.0 ± 0.1***	-0.9 ± 0.1***
Ol.bot.cau-Hybrid	Ol.cap.bal-Inbred	-1.5 ± 0.2***	-0.9 ± 0.3NS	-0.7 ± 0.2*	1.7 ± 0.2***	2.9 ± 0.7**	597.2 ± 33.2***	1.5 ± 0.1***	-0.8 ± 0.1***
Ol.bot.cau-Hybrid	Ol.cap.bal-Hybrid	-0.4 ± 0.2NS	-0.5 ± 0.3NS	-0.3 ± 0.2NS	0.8 ± 0.2*	0.6 ± 0.7NS	242.5 ± 33.3***	1.1 ± 0.1***	-0.7 ± 0.1***
Ol.bot.cau-Hybrid	Ol.cap.bin-Inbred	0.1 ± 0.2NS	-2.0 ± 0.3***	0.2 ± 0.2NS	1.3 ± 0.2***	-0.4 ± 0.7NS	603.0 ± 33.0***	0.9 ± 0.1***	-1.3 ± 0.1***
Ol.bot.cau-Hybrid	Ol.cap.bin-Hybrid	0.6 ± 0.2NS	-0.6 ± 0.3NS	0.5 ± 0.2NS	0.4 ± 0.2NS	-3.6 ± 0.7***	126.7 ± 33.1**	0.7 ± 0.1***	-1.0 ± 0.1***
Ol.bot.cau-Hybrid	Ol.ita.pre-Inbred	-0.6 ± 0.2NS	-0.7 ± 0.3NS	0.0 ± 0.2NS	1.4 ± 0.2***	3.1 ± 0.7**	509.8 ± 35.1***	1.0 ± 0.1***	-0.7 ± 0.1***
Ol.bot.cau-Hybrid	Ol.ita.pre-Hybrid	-0.2 ± 0.2NS	-1.0 ± 0.3NS	-0.2 ± 0.2NS	1.2 ± 0.2***	0.4 ± 0.7NS	268.1 ± 35.1***	1.0 ± 0.1***	-0.7 ± 0.1***
Ol.cap.bad-Inbred	Ol.cap.bad-Hybrid	1.3 ± 0.2***	2.4 ± 0.4***	0.9 ± 0.2***	-1.7 ± 0.2***	-3.9 ± 0.8***	-574.5 ± 37.1***	-0.8 ± 0.2***	0.6 ± 0.1***
Ol.cap.bad-Inbred	Ol.cap.bal-Inbred	-0.3 ± 0.2NS	2.3 ± 0.3***	-0.1 ± 0.2NS	-0.8 ± 0.2NS	3.4 ± 0.7***	-78.8 ± 35.0NS	-0.3 ± 0.1NS	0.7 ± 0.1***
Ol.cap.bad-Inbred	Ol.cap.bal-Hybrid	0.9 ± 0.2**	2.7 ± 0.3***	0.2 ± 0.2NS	-1.6 ± 0.2***	1.1 ± 0.7NS	-433.5 ± 35.1***	-0.7 ± 0.1***	0.7 ± 0.1***
Ol.cap.bad-Inbred	Ol.cap.bin-Inbred	1.4 ± 0.2***	1.2 ± 0.3*	0.7 ± 0.2**	-1.2 ± 0.2***	0.2 ± 0.7NS	-73.0 ± 34.9NS	-0.9 ± 0.1***	0.1 ± 0.1NS
Ol.cap.bad-Inbred	Ol.cap.bin-Hybrid	1.8 ± 0.2***	2.6 ± 0.3***	1.1 ± 0.2***	-2 ± 0.2***	-3.1 ± 0.7**	-549.4 ± 34.9***	-1.1 ± 0.1***	0.5 ± 0.1**
Ol.cap.bad-Inbred	Ol.ita.pre-Inbred	0.7 ± 0.2NS	2.5 ± 0.4***	0.6 ± 0.2NS	-1.1 ± 0.2***	3.6 ± 0.8***	-166.2 ± 36.8***	-0.8 ± 0.2***	0.8 ± 0.1***
Ol.cap.bad-Inbred	Ol.ita.pre-Hybrid	1.1 ± 0.2***	2.2 ± 0.4***	0.3 ± 0.2NS	-1.2 ± 0.2***	0.9 ± 0.8NS	-407.9 ± 36.8***	-0.8 ± 0.2***	0.8 ± 0.1***
Ol.cap.bad-Hybrid	Ol.cap.bal-Inbred	-1.5 ± 0.2***	-0.1 ± 0.3NS	-1.0 ± 0.2***	1.0 ± 0.2**	7.4 ± 0.7***	495.7 ± 35.0***	0.4 ± 0.1NS	0.1 ± 0.1NS
Ol.cap.bad-Hybrid	Ol.cap.bal-Hybrid	-0.4 ± 0.2NS	0.2 ± 0.3NS	-0.6 ± 0.2*	0.1 ± 0.2NS	5.0 ± 0.7***	140.9 ± 35.1**	0.1 ± 0.1NS	0.2 ± 0.1NS
Ol.cap.bad-Hybrid	Ol.cap.bin-Inbred	0.1 ± 0.2NS	-1.3 ± 0.3*	-0.2 ± 0.2NS	0.6 ± 0.2NS	4.1 ± 0.7***	501.5 ± 34.9***	-0.1 ± 0.1NS	-0.5 ± 0.1***
Ol.cap.bad-Hybrid	Ol.cap.bin-Hybrid	0.6 ± 0.2NS	0.1 ± 0.3NS	0.2 ± 0.2NS	-0.3 ± 0.2NS	0.8 ± 0.7NS	25.1 ± 34.9NS	-0.3 ± 0.1NS	-0.1 ± 0.1NS
Ol.cap.bad-Hybrid	Ol.ita.pre-Inbred	-0.6 ± 0.2NS	0.1 ± 0.4NS	-0.3 ± 0.2NS	0.7 ± 0.2NS	7.6 ± 0.8***	408.2 ± 36.8***	0.0 ± 0.2NS	0.2 ± 0.1NS
Ol.cap.bad-Hybrid	Ol.ita.pre-Hybrid	-0.2 ± 0.2NS	-0.2 ± 0.4NS	-0.6 ± 0.2NS	0.5 ± 0.2NS	4.9 ± 0.8***	166.6 ± 36.8***	-0.1 ± 0.2NS	0.2 ± 0.1NS
Ol.cap.bal-Inbred	Ol.cap.bal-Hybrid	1.2 ± 0.2***	0.4 ± 0.3NS	0.4 ± 0.2NS	-0.9 ± 0.2**	-2.3 ± 0.7*	-354.8 ± 32.8***	-0.3 ± 0.1NS	0.1 ± 0.1NS
Ol.cap.bal-Inbred	Ol.cap.bin-Inbred	1.7 ± 0.2***	-1.1 ± 0.3*	0.8 ± 0.2***	-0.4 ± 0.2NS	-3.3 ± 0.7***	5.8 ± 32.6NS	-0.5 ± 0.1**	-0.5 ± 0.1***
Ol.cap.bal-Inbred	Ol.cap.bin-Hybrid	2.1 ± 0.2***	0.3 ± 0.3NS	1.2 ± 0.2***	-1.2 ± 0.2***	-6.5 ± 0.7***	-470.6 ± 32.7***	-0.8 ± 0.1***	-0.2 ± 0.1NS
Ol.cap.bal-Inbred	Ol.ita.pre-Inbred	0.9 ± 0.2***	0.2 ± 0.3NS	0.7 ± 0.2*	-0.3 ± 0.2NS	0.2 ± 0.7NS	-87.5 ± 34.7NS	-0.5 ± 0.1NS	0.1 ± 0.1NS
Ol.cap.bal-Inbred	Ol.ita.pre-Hybrid	1.3 ± 0.2***	0.0 ± 0.3NS	0.5 ± 0.2NS	-0.5 ± 0.2NS	-2.5 ± 0.7*	-329.1 ± 34.7***	-0.5 ± 0.1*	0.1 ± 0.1NS
Ol.cap.bal-Hybrid	Ol.cap.bin-Inbred	0.5 ± 0.2NS	-1.5 ± 0.3***	0.5 ± 0.2NS	0.5 ± 0.2NS	-0.9 ± 0.7NS	360.5 ± 32.7***	-0.2 ± 0.1NS	-0.6 ± 0.1***
Ol.cap.bal-Hybrid	Ol.cap.bin-Hybrid	0.9 ± 0.2***	-0.1 ± 0.3NS	0.8 ± 0.2***	-0.4 ± 0.2NS	-4.2 ± 0.7***	-115.8 ± 32.7*	-0.4 ± 0.1NS	-0.3 ± 0.1NS
Ol.cap.bal-Hybrid	Ol.ita.pre-Inbred	-0.2 ± 0.2NS	-0.2 ± 0.3NS	0.3 ± 0.2NS	0.6 ± 0.2NS	2.5 ± 0.7*	267.3 ± 34.7***	-0.1 ± 0.1NS	0.0 ± 0.1NS
Ol.cap.bal-Hybrid	Ol.ita.pre-Hybrid	0.2 ± 0.2NS	-0.4 ± 0.3NS	0.1 ± 0.2NS	0.4 ± 0.2NS	-0.2 ± 0.7NS	25.6 ± 34.7NS	-0.2 ± 0.1NS	0.0 ± 0.1NS
Ol.cap.bin-Inbred	Ol.cap.bin-Hybrid	0.4 ± 0.2NS	1.4 ± 0.3**	0.4 ± 0.2NS	-0.8 ± 0.2**	-3.3 ± 0.7***	-476.4 ± 32.5***	-0.3 ± 0.1NS	0.3 ± 0.1*
Ol.cap.bin-Inbred	Ol.ita.pre-Inbred	-0.7 ± 0.2*	1.3 ± 0.3**	-0.2 ± 0.2NS	0.1 ± 0.2NS	3.5 ± 0.7***	-93.2 ± 34.5NS	0.0 ± 0.1NS	0.6 ± 0.1***
Ol.cap.bin-Inbred	Ol.ita.pre-Hybrid	-0.3 ± 0.2NS	1.1 ± 0.3NS	-0.4 ± 0.2NS	-0.1 ± 0.2NS	0.8 ± 0.7NS	-334.9 ± 34.5***	0.0 ± 0.1NS	0.7 ± 0.1***
Ol.cap.bin-Hybrid	Ol.ita.pre-Inbred	-1.2 ± 0.2***	-0.1 ± 0.3NS	-0.5 ± 0.2NS	0.9 ± 0.2**	6.7 ± 0.7***	383.1 ± 34.5***	0.3 ± 0.1NS	0.3 ± 0.1NS
Ol.cap.bin-Hybrid	Ol.ita.pre-Hybrid	-0.7 ± 0.2*	-0.3 ± 0.3NS	-0.7 ± 0.2**	0.8 ± 0.2*	4.0 ± 0.7***	141.5 ± 34.5**	0.3 ± 0.1NS	0.3 ± 0.1NS
Ol.ita.pre-Inbred	Ol.ita.pre-Hybrid	0.4 ± 0.2NS	-0.3 ± 0.4NS	-0.2 ± 0.2NS	-0.2 ± 0.2NS	-2.7 ± 0.8*	-241.6 ± 36.5***	0.0 ± 0.2NS	0.0 ± 0.1NS
Ol.alb.nrc-MPH	Ol.alb.nrc-NPH	-0.3 ± 0.3NS	-4.8 ± 0.9***	-0.4 ± 0.1NS	3.1 ± 0.4***	3.1 ± 0.4***	6.2 ± 1.2***	1.3 ± 0.2***	-0.8 ± 0.3NS
Ol.alb.nrc-MPH	Ol.bot.cau-MPH	0.3 ± 0.3NS	0.1 ± 0.9NS	0.1 ± 0.1NS	-0.1 ± 0.8NS	0.8 ± 0.4NS	0.4 ± 1.2NS	0.1 ± 0.2NS	0.0 ± 0.3NS
Ol.alb.nrc-MPH	Ol.bot.cau-NPH	0.2 ± 0.3NS	-4.5 ± 0.9***	-0.2 ± 0.1NS	2.3 ± 0.8NS	3.9 ± 0.4***	6.8 ± 1.2***	1.0 ± 0.2***	-1.0 ± 0.3NS
Ol.alb.nrc-MPH	Ol.cap.bad-MPH	0.1 ± 0.3NS	3.0 ± 0.9NS	0.4 ± 0.1NS	-1.0 ± 0.9NS	0.4 ± 0.4NS	0.8 ± 1.3NS	0.0 ± 0.2NS	0.1 ± 0.4NS
Ol.alb.nrc-MPH	Ol.cap.bad-NPH	-0.7 ± 0.3NS	-4.1 ± 0.9**	-0.2 ± 0.1NS	4.3 ± 0.9***	2.6 ± 0.4***	8.2 ± 1.3***	1.8 ± 0.2***	-2.3 ± 0.4***
Ol.alb.nrc-MPH	Ol.cap.bal-MPH	-0.4 ± 0.3NS	-0.7 ± 0.9NS	0.0 ± 0.1NS	1.0 ± 0.8NS	0.6 ± 0.4NS	4.8 ± 1.2**	0.3 ± 0.2NS	-0.4 ± 0.3NS
Ol.alb.nrc-MPH	Ol.cap.bal-NPH	-1.6 ± 0.3***	-4.6 ± 0.9***	-0.4 ± 0.1**	4.8 ± 0.8***	2.0 ± 0.4***	9.6 ± 1.2***	1.4 ± 0.2***	-1.2 ± 0.3*
Ol.alb.nrc-MPH	Ol.cap.bin-MPH	-0.1 ± 0.3NS	1.1 ± 0.9NS	0.2 ± 0.1NS	0.5 ± 0.8NS	1.1 ± 0.4NS	2.8 ± 1.2NS	0.3 ± 0.2NS	-0.6 ± 0.3NS
Ol.alb.nrc-MPH	Ol.cap.bin-NPH	0.5 ± 0.3NS	-4.3 ± 0.9***	0.0 ± 0.1NS	3.6 ± 0.8***	3.5 ± 0.4***	9.4 ± 1.2***	1.2 ± 0.2***	-2.8 ± 0.3***

Comparisons		Days to flowering	Duration of flowering (day)	Days to maturity	Grain-filling period (day)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
Ol.alb.nrc-MPH	Ol.ita.pre-MPH	-1.0 ± 0.3NS	-2.6 ± 0.9NS	-0.3 ± 0.1NS	3.2 ± 0.9*	0.7 ± 0.4NS	7.1 ± 1.3***	0.5 ± 0.2NS	-0.4 ± 0.3NS
Ol.alb.nrc-MPH	Ol.ita.pre-NPH	-1.3 ± 0.3**	-5.9 ± 0.9***	-0.4 ± 0.1*	6.4 ± 0.9***	2.8 ± 0.4***	10.9 ± 1.3***	1.1 ± 0.2***	-1.1 ± 0.3NS
Ol.alb.nrc-NPH	Ol.bot.cau-MPH	0.7 ± 0.3NS	4.9 ± 0.9***	0.5 ± 0.1***	-2.6 ± 0.8NS	-2.2 ± 0.4***	-5.9 ± 1.2***	-1.3 ± 0.2***	0.7 ± 0.3NS
Ol.alb.nrc-NPH	Ol.bot.cau-NPH	0.6 ± 0.3NS	0.3 ± 0.9NS	0.2 ± 0.1NS	-0.2 ± 0.8NS	0.8 ± 0.4NS	0.5 ± 1.2NS	-0.4 ± 0.2NS	-0.2 ± 0.3NS
Ol.alb.nrc-NPH	Ol.cap.bad-MPH	0.5 ± 0.3NS	7.8 ± 0.9***	0.7 ± 0.1***	-3.5 ± 0.9**	-2.7 ± 0.4***	-5.4 ± 1.3**	-1.3 ± 0.2***	0.9 ± 0.4NS
Ol.alb.nrc-NPH	Ol.cap.bad-NPH	-0.4 ± 0.3NS	0.7 ± 0.9NS	0.2 ± 0.1NS	1.9 ± 0.9NS	-0.5 ± 0.4NS	1.9 ± 1.3NS	0.5 ± 0.2NS	-1.5 ± 0.4**
Ol.alb.nrc-NPH	Ol.cap.bal-MPH	-0.1 ± 0.3NS	4.1 ± 0.9***	0.3 ± 0.1NS	-1.4 ± 0.8NS	-2.5 ± 0.4***	-1.5 ± 1.2NS	-1.1 ± 0.2***	0.4 ± 0.3NS
Ol.alb.nrc-NPH	Ol.cap.bal-NPH	-1.2 ± 0.3**	0.3 ± 0.9NS	-0.1 ± 0.1NS	2.4 ± 0.8NS	-1.0 ± 0.4NS	3.3 ± 1.2NS	0.0 ± 0.2NS	-0.4 ± 0.3NS
Ol.alb.nrc-NPH	Ol.cap.bin-MPH	0.3 ± 0.3NS	5.9 ± 0.9***	0.6 ± 0.1***	-2.0 ± 0.8NS	-2.0 ± 0.4***	-3.4 ± 1.2NS	-1.1 ± 0.2***	0.2 ± 0.3NS
Ol.alb.nrc-NPH	Ol.cap.bin-NPH	0.8 ± 0.3NS	0.5 ± 0.9NS	0.3 ± 0.1NS	1.2 ± 0.8NS	0.5 ± 0.4NS	3.2 ± 1.2NS	-0.1 ± 0.2NS	-2.0 ± 0.3***
Ol.alb.nrc-NPH	Ol.ita.pre-MPH	-0.7 ± 0.3NS	2.3 ± 0.9NS	0.1 ± 0.1NS	0.7 ± 0.9NS	-2.3 ± 0.4***	0.8 ± 1.3NS	-0.9 ± 0.2**	0.4 ± 0.3NS
Ol.alb.nrc-NPH	Ol.ita.pre-NPH	-1.0 ± 0.3NS	-1.1 ± 0.9NS	0.0 ± 0.1NS	3.9 ± 0.9***	-0.3 ± 0.4NS	4.6 ± 1.3*	-0.2 ± 0.2NS	-0.3 ± 0.3NS
Ol.bot.cau-MPH	Ol.bot.cau-NPH	-0.1 ± 0.3NS	-4.6 ± 0.9***	-0.3 ± 0.1NS	2.4 ± 0.8NS	3.0 ± 0.4***	6.4 ± 1.2***	0.9 ± 0.2***	-0.9 ± 0.3NS
Ol.bot.cau-MPH	Ol.cap.bad-MPH	-0.2 ± 0.3NS	2.9 ± 0.9NS	0.2 ± 0.1NS	-0.9 ± 0.9NS	-0.5 ± 0.4NS	0.4 ± 1.2NS	0.0 ± 0.2NS	0.1 ± 0.3NS
Ol.bot.cau-MPH	Ol.cap.bad-NPH	-1.1 ± 0.3*	-4.2 ± 0.9***	-0.3 ± 0.1NS	4.5 ± 0.9***	1.8 ± 0.4***	7.8 ± 1.2***	1.7 ± 0.2***	-2.3 ± 0.3***
Ol.bot.cau-MPH	Ol.cap.bal-MPH	-0.7 ± 0.3NS	-0.8 ± 0.9NS	-0.1 ± 0.1NS	1.2 ± 0.8NS	-0.3 ± 0.4NS	4.4 ± 1.2**	0.2 ± 0.2NS	-0.3 ± 0.3NS
Ol.bot.cau-MPH	Ol.cap.bal-NPH	-1.9 ± 0.3***	-4.7 ± 0.9***	-0.6 ± 0.1***	4.9 ± 0.8***	1.2 ± 0.4NS	9.2 ± 1.2***	1.3 ± 0.2***	-1.2 ± 0.3*
Ol.bot.cau-MPH	Ol.cap.bin-MPH	-0.4 ± 0.3NS	1.0 ± 0.9NS	0.1 ± 0.1NS	0.6 ± 0.8NS	0.2 ± 0.4NS	2.5 ± 1.2NS	0.2 ± 0.2NS	-0.5 ± 0.3NS
Ol.bot.cau-MPH	Ol.cap.bin-NPH	0.2 ± 0.3NS	-4.4 ± 0.9***	-0.1 ± 0.1NS	3.7 ± 0.8***	2.7 ± 0.4***	9.1 ± 1.2***	1.1 ± 0.2***	-2.7 ± 0.3***
Ol.bot.cau-MPH	Ol.ita.pre-MPH	-1.4 ± 0.3***	-2.7 ± 0.9NS	-0.4 ± 0.1*	3.3 ± 0.9**	-0.1 ± 0.4NS	6.7 ± 1.2***	0.4 ± 0.2NS	-0.4 ± 0.3NS
Ol.bot.cau-MPH	Ol.ita.pre-NPH	-1.6 ± 0.3***	-6.0 ± 0.9***	-0.5 ± 0.1***	6.5 ± 0.9***	1.9 ± 0.4***	10.5 ± 1.2***	1.0 ± 0.2***	-1.0 ± 0.3NS
Ol.bot.cau-NPH	Ol.cap.bad-MPH	-0.1 ± 0.3NS	7.5 ± 0.9***	0.5 ± 0.1***	-3.3 ± 0.9**	-3.5 ± 0.4***	-6.0 ± 1.2***	-0.9 ± 0.2***	1.1 ± 0.3NS
Ol.bot.cau-NPH	Ol.cap.bad-NPH	-1.0 ± 0.3NS	0.4 ± 0.9NS	0.0 ± 0.1NS	2.1 ± 0.9NS	-1.3 ± 0.4NS	1.4 ± 1.2NS	0.8 ± 0.2**	-1.3 ± 0.3**
Ol.bot.cau-NPH	Ol.cap.bal-MPH	-0.6 ± 0.3NS	3.8 ± 0.9**	0.1 ± 0.1NS	-1.2 ± 0.8NS	-3.3 ± 0.4***	-2.0 ± 1.2NS	-0.7 ± 0.2*	0.6 ± 0.3NS
Ol.bot.cau-NPH	Ol.cap.bal-NPH	-1.8 ± 0.3***	-0.1 ± 0.9NS	-0.3 ± 0.1NS	2.5 ± 0.8NS	-1.8 ± 0.4***	2.8 ± 1.2NS	0.4 ± 0.2NS	-0.2 ± 0.3NS
Ol.bot.cau-NPH	Ol.cap.bin-MPH	-0.3 ± 0.3NS	5.6 ± 0.9***	0.4 ± 0.1*	-1.8 ± 0.8NS	-2.8 ± 0.4***	-3.9 ± 1.2*	-0.7 ± 0.2*	0.4 ± 0.3NS
Ol.bot.cau-NPH	Ol.cap.bin-NPH	0.3 ± 0.3NS	0.2 ± 0.9NS	0.1 ± 0.1NS	1.3 ± 0.8NS	-0.3 ± 0.4NS	2.7 ± 1.2NS	0.2 ± 0.2NS	-1.8 ± 0.3***
Ol.bot.cau-NPH	Ol.ita.pre-MPH	-1.3 ± 0.3**	1.9 ± 0.9NS	-0.1 ± 0.1NS	0.9 ± 0.9NS	-3.1 ± 0.4***	0.3 ± 1.2NS	-0.5 ± 0.2NS	0.6 ± 0.3NS
Ol.bot.cau-NPH	Ol.ita.pre-NPH	-1.5 ± 0.3***	-1.4 ± 0.9NS	-0.2 ± 0.1NS	4.1 ± 0.9***	-1.1 ± 0.4NS	4.1 ± 1.2*	0.1 ± 0.2NS	-0.1 ± 0.3NS
Ol.cap.bad-MPH	Ol.cap.bad-NPH	-0.9 ± 0.3NS	-7.1 ± 1.0***	-0.6 ± 0.1***	5.4 ± 0.9***	2.2 ± 0.4***	7.4 ± 1.3***	1.8 ± 0.2***	-2.4 ± 0.4***
Ol.cap.bad-MPH	Ol.cap.bal-MPH	-0.5 ± 0.3NS	-3.7 ± 0.9**	-0.4 ± 0.1*	2.1 ± 0.8NS	0.2 ± 0.4NS	4.0 ± 1.2NS	0.2 ± 0.2NS	-0.5 ± 0.3NS
Ol.cap.bad-MPH	Ol.cap.bal-NPH	-1.7 ± 0.3***	-7.6 ± 0.9***	-0.8 ± 0.1***	5.9 ± 0.8***	1.7 ± 0.4**	8.8 ± 1.2***	1.3 ± 0.2***	-1.3 ± 0.3**
Ol.cap.bad-MPH	Ol.cap.bin-MPH	-0.2 ± 0.3NS	-1.9 ± 0.9NS	-0.1 ± 0.1NS	1.5 ± 0.8NS	0.7 ± 0.4NS	2.0 ± 1.2NS	0.2 ± 0.2NS	-0.7 ± 0.3NS
Ol.cap.bad-MPH	Ol.cap.bin-NPH	0.4 ± 0.3NS	-7.3 ± 0.9***	-0.4 ± 0.1*	4.7 ± 0.8***	3.2 ± 0.4***	8.6 ± 1.2***	1.2 ± 0.2***	-2.9 ± 0.3***
Ol.cap.bad-MPH	Ol.ita.pre-MPH	-1.2 ± 0.3*	-5.6 ± 1.0***	-0.6 ± 0.1***	4.2 ± 0.9***	0.4 ± 0.4NS	6.3 ± 1.3***	0.4 ± 0.2NS	-0.5 ± 0.4NS
Ol.cap.bad-MPH	Ol.ita.pre-NPH	-1.4 ± 0.3***	-8.9 ± 1.0***	-0.7 ± 0.1***	7.5 ± 0.9***	2.4 ± 0.4***	10.1 ± 1.3***	1.1 ± 0.2***	-1.2 ± 0.4NS
Ol.cap.bad-NPH	Ol.cap.bal-MPH	0.3 ± 0.3NS	3.4 ± 0.9*	0.2 ± 0.1NS	-3.3 ± 0.8**	-2.0 ± 0.4***	-3.4 ± 1.2NS	-1.5 ± 0.2***	1.9 ± 0.3***
Ol.cap.bad-NPH	Ol.cap.bal-NPH	-0.8 ± 0.3NS	-0.5 ± 0.9NS	-0.2 ± 0.1NS	0.5 ± 0.8NS	-0.6 ± 0.4NS	1.4 ± 1.2NS	-0.5 ± 0.2NS	1.1 ± 0.3NS
Ol.cap.bad-NPH	Ol.cap.bin-MPH	0.7 ± 0.3NS	5.2 ± 0.9***	0.4 ± 0.1**	-3.9 ± 0.8***	-1.5 ± 0.4**	-5.4 ± 1.2***	-1.5 ± 0.2***	1.7 ± 0.3***
Ol.cap.bad-NPH	Ol.cap.bin-NPH	1.2 ± 0.3**	-0.2 ± 0.9NS	0.2 ± 0.1NS	-0.7 ± 0.8NS	0.9 ± 0.4NS	1.3 ± 1.2NS	-0.6 ± 0.2NS	-0.5 ± 0.3NS
Ol.cap.bad-NPH	Ol.ita.pre-MPH	-0.3 ± 0.3NS	1.5 ± 1.0NS	-0.1 ± 0.1NS	-1.1 ± 0.9NS	-1.9 ± 0.4***	-1.1 ± 1.3NS	-1.4 ± 0.2***	1.9 ± 0.4***
Ol.cap.bad-NPH	Ol.ita.pre-NPH	-0.6 ± 0.3NS	-1.8 ± .01NS	-0.2 ± 0.1NS	2.1 ± 0.9NS	0.2 ± 0.4NS	2.7 ± 1.3NS	-0.7 ± 0.2NS	1.2 ± 0.4*
Ol.cap.bal-MPH	Ol.cap.bal-NPH	-1.2 ± 0.3**	-3.9 ± 0.9***	-0.4 ± 0.1**	3.8 ± 0.8***	1.5 ± 0.4**	4.8 ± 1.2**	1.1 ± 0.2***	-0.9 ± 0.3NS
Ol.cap.bal-MPH	Ol.cap.bin-MPH	0.3 ± 0.3NS	1.8 ± 0.9NS	0.2 ± 0.1NS	-0.6 ± 0.8NS	0.5 ± 0.4NS	-2.0 ± 1.1NS	0.0 ± 0.2NS	-0.2 ± 0.3NS
Ol.cap.bal-MPH	Ol.cap.bin-NPH	0.9 ± 0.3NS	-3.6 ± 0.9**	0.0 ± 0.1NS	2.6 ± 0.8NS	3.0 ± 0.4***	4.7 ± 1.1**	0.9 ± 0.2***	-2.4 ± 0.3***
Ol.cap.bal-MPH	Ol.ita.pre-MPH	-0.6 ± 0.3NS	-1.9 ± 0.9NS	-0.3 ± 0.1NS	2.2 ± 0.8NS	0.2 ± 0.4NS	2.3 ± 1.2NS	0.2 ± 0.2NS	-0.1 ± 0.3NS

Comparisons		Days to flowering	Duration of flowering (day)	Days to maturity	Grain-filling period (day)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
Ol.cap.bal-MPH	Ol.ita.pre-NPH	-0.9 ± 0.3NS	-5.2 ± 0.9***	-0.4 ± 0.1*	5.4 ± 0.8***	2.2 ± 0.4***	6.1 ± 1.2***	0.8 ± 0.2**	-0.7 ± 0.3NS
Ol.cap.bal-NPH	Ol.cap.bin-MPH	1.5 ± 0.3***	5.7 ± 0.9***	0.7 ± 0.1***	-4.4 ± 0.8***	-1.0 ± 0.4NS	-6.8 ± 1.1***	-1.1 ± 0.2***	0.6 ± 0.3NS
Ol.cap.bal-NPH	Ol.cap.bin-NPH	2.1 ± 0.3***	0.2 ± 0.9NS	0.4 ± 0.1**	-1.2 ± 0.8NS	1.5 ± 0.4**	-0.1 ± 1.1NS	-0.1 ± 0.2NS	-1.6 ± 0.3***
Ol.cap.bal-NPH	Ol.ita.pre-MPH	0.6 ± 0.3NS	2.0 ± 0.9NS	0.2 ± 0.1NS	-1.6 ± 0.8NS	-1.3 ± 0.4*	-2.5 ± 1.2NS	-0.9 ± 0.2***	0.8 ± 0.3NS
Ol.cap.bal-NPH	Ol.ita.pre-NPH	0.3 ± 0.3NS	-1.4 ± 0.9NS	0.1 ± 0.1NS	1.6 ± 0.8NS	0.7 ± 0.4NS	1.3 ± 1.2NS	-0.3 ± 0.2NS	0.1 ± 0.3NS
Ol.cap.bin-MPH	Ol.cap.bin-NPH	0.6 ± 0.3NS	-5.4 ± 0.8***	-0.2 ± 0.1NS	3.2 ± 0.8**	2.5 ± 0.4***	6.6 ± 1.1***	0.9 ± 0.2***	-2.2 ± 0.3***
Ol.cap.bin-MPH	Ol.ita.pre-MPH	-1.0 ± 0.3NS	-3.7 ± 0.9**	-0.5 ± 0.1***	2.8 ± 0.8NS	-0.3 ± 0.4NS	4.3 ± 1.2*	0.2 ± 0.2NS	0.2 ± 0.3NS
Ol.cap.bin-MPH	Ol.ita.pre-NPH	-1.2 ± 0.3**	-7.0 ± 0.9***	-0.6 ± 0.1***	6.0 ± 0.8***	1.7 ± 0.4***	8.1 ± 1.2***	0.8 ± 0.2**	-0.5 ± 0.3NS
Ol.cap.bin-NPH	Ol.ita.pre-MPH	-1.5 ± 0.3***	1.8 ± 0.9NS	-0.2 ± 0.1NS	-0.4 ± 0.8NS	-2.8 ± 0.4***	-2.4 ± 1.2NS	-0.8 ± 0.2*	2.4 ± 0.3***
Ol.cap.bin-NPH	Ol.ita.pre-NPH	-1.8 ± 0.3***	-1.6 ± 0.9NS	-0.4 ± 0.1*	2.8 ± 0.8*	-0.8 ± 0.4NS	1.4 ± 1.2NS	-0.1 ± 0.2NS	1.7 ± 0.3***
Ol.ita.pre-MPH	Ol.ita.pre-NPH	-0.3 ± 0.3NS	-3.4 ± 1.0*	-0.1 ± 0.1NS	3.2 ± 0.9*	2.0 ± 0.4***	3.8 ± 1.3NS	0.6 ± 0.2NS	-0.7 ± 0.4NS

* Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, NS Not significant.

[1] Ol.alb.nrc = *B. napus* (A04-73NA) × *B. oleracea* var. *alboglabra* line NRC-PBI; Ol.bot.cau = *B. napus* (A04-73NA) × *B. oleracea* var. *botrytis* cv. BARI cauliflower; Ol.cap.bad = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper; Ol.cap.bin = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener; Ol.cap.bal = *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* cv. Balbro; Ol.ita.pre = A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop; Inbred = inbred line population; Hybrid = test hybrid population; MPH = mid-parent heterosis; NPH = heterosis over common *B. napus* parent.

Supplemental Table 4.5 Comparison of the least square mean (± SE) values of the F_2- and BC_1-derived inbred lines, their test-hybrids, mid-parent heterosis (MPH), and heterosis over the common *B. napus* parent (NPH) for different agronomic and seed quality traits

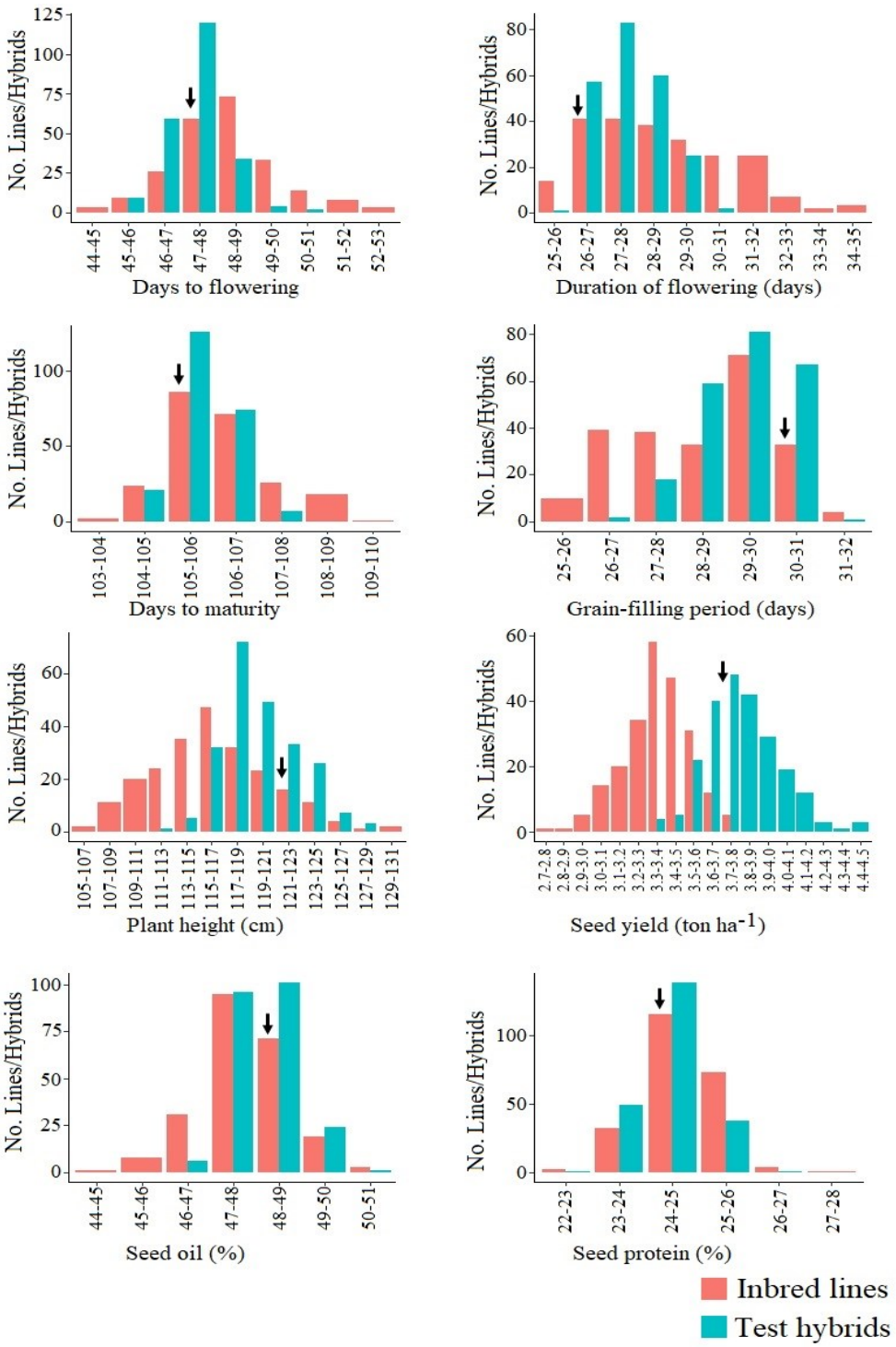
Comparisons		Days to flowering	Duration of flowering (day)	Days to maturity	Grain-filling period (day)	Plant height (cm)	Seed yield (kg ha^{-1})	Seed oil (%)	Seed protein (%)
BC-Hybrid	BC-Inbred	-0.8 ± 0.1***	-0.9 ± 0.2***	-0.3 ± 0.1NS	0.8 ± 0.1***	3.9 ± 0.4***	471.0 ± 21.3***	0.4 ± 0.1***	-0.2 ± 0.1*
BC-Hybrid	F-Inbred	-1.4 ± 0.1***	-1.3 ± 0.2***	-0.6 ± 0.1***	1.2 ± 0.1***	2.5 ± 0.5***	469.4 ± 21.7***	0.3 ± 0.1**	-0.3 ± 0.1**
BC-Hybrid	F-Hybrid	-0.4 ± 0.1**	-0.4 ± 0.2NS	-0.2 ± 0.1NS	0.4 ± 0.1*	-0.6 ± 0.5NS	33.4 ± 21.7NS	0.0 ± 0.1NS	-0.1 ± 0.1NS
BC-Inbred	F-Inbred	-0.5 ± 0.1***	-0.4 ± 0.2NS	-0.3 ± 0.1*	0.4 ± 0.1*	-1.4 ± 0.5*	-1.5 ± 21.7NS	-0.1 ± 0.1NS	0.0 ± 0.1NS
BC-Inbred	F-Hybrid	0.4 ± 0.1*	0.5 ± 0.2*	0.1 ± 0.1NS	-0.4 ± 0.1*	-4.5 ± 0.5***	-437.5 ± 21.7***	-0.4 ± 0.1***	0.2 ± 0.1NS
F-Inbred	F-Hybrid	-0.9 ± 0.1***	-1.0 ± 0.2***	-0.4 ± 0.1**	0.8 ± 0.1***	3.1 ± 0.5***	436.0 ± 22.1***	0.3 ± 0.1**	-0.2 ± 0.1*
BC-NPH	BC-MPH	0.0 ± 0.2NS	-4.5 ± 0.5***	-0.3 ± 0.1***	3.0 ± 0.5***	2.7 ± 0.2***	6.2 ± 0.7***	1.2 ± 0.1***	-1.3 ± 0.2***
BC-NPH	F-MPH	-0.3 ± 0.2NS	4.0 ± 0.5***	0.2 ± 0.1**	-2.2 ± 0.5***	-2.5 ± 0.2***	-5.3 ± 0.7***	-1.2 ± 0.1***	1.4 ± 0.2***
BC-NPH	F-NPH	-1.0 ± 0.2***	-1.2 ± 0.5NS	-0.2 ± 0.1*	1.5 ± 0.5**	-0.5 ± 0.2NS	0.2 ± 0.7NS	-0.3 ± 0.1NS	0.1 ± 0.2NS
BC-MPH	F-MPH	-0.4 ± 0.2NS	-0.4 ± 0.5NS	0.0 ± 0.1NS	0.8 ± 0.5NS	0.1 ± 0.2NS	0.8 ± 0.7NS	-0.1 ± 0.1NS	0.0 ± 0.2NS
BC-MPH	F-NPH	-1.0 ± 0.2***	-5.6 ± 0.5***	-0.4 ± 0.1***	4.5 ± 0.5***	2.2 ± 0.2***	6.4 ± 0.7***	0.9 ± 0.1***	-1.2 ± 0.2***
F-MPH	F-NPH	-0.7 ± 0.2**	-5.2 ± 0.5***	-0.4 ± 0.1***	3.7 ± 0.5***	2.1 ± 0.2***	5.5 ± 0.8***	1.0 ± 0.1***	-1.3 ± 0.2***

* Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$, ^{NS} Not significant.

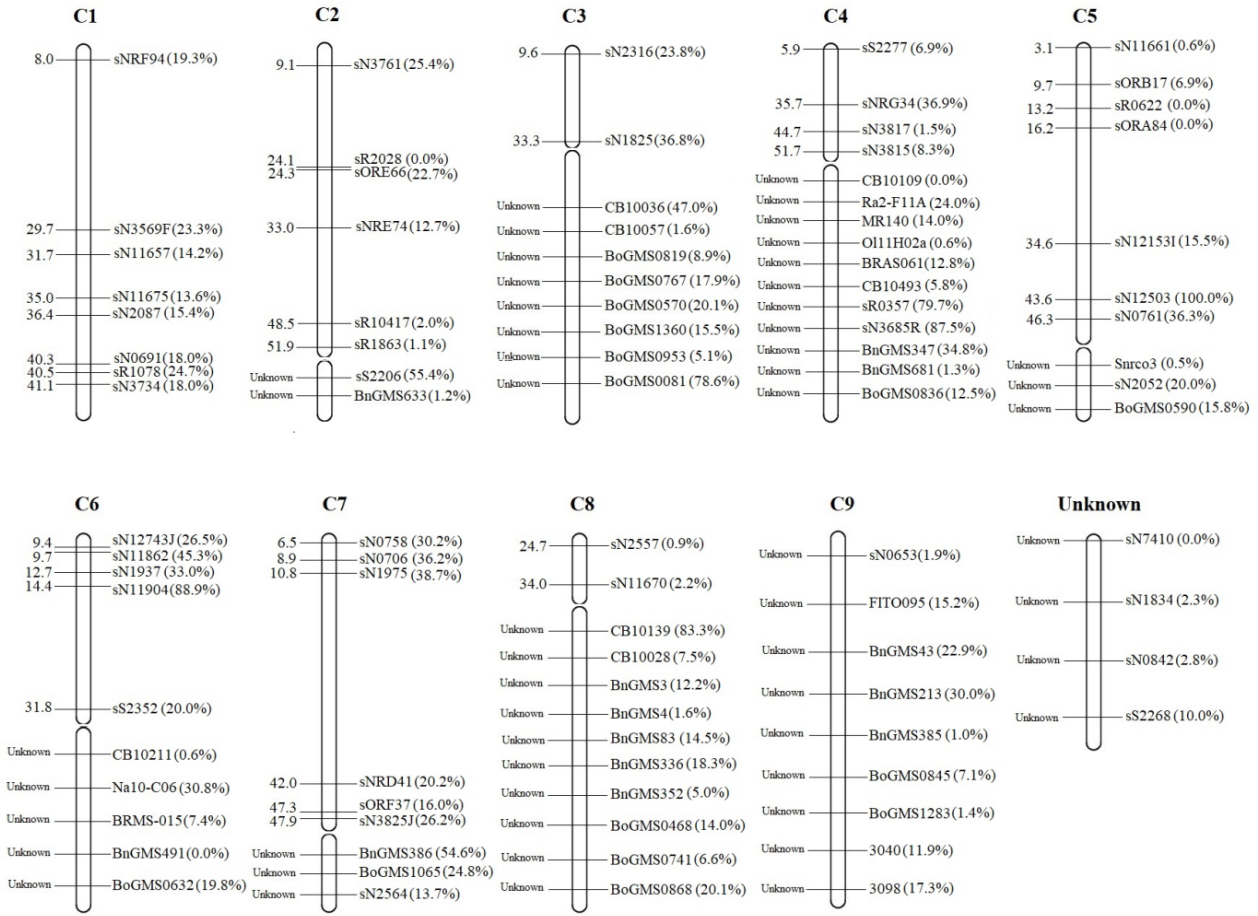
Note: F-inbred = Inbred lines derived from F_2; BC-inbred = Inbred lines derived from BC_1; F-hybrid = Hybrids developed based on F_2-derived inbred lines; BC-hybrid = Hybrids developed based on BC_1-derived inbred lines; F-MPH: mid-parent heterosis for population derived from F_2; BC-MPH: mid-parent heterosis for population derived from BC_1; F-NPH: heterosis over the common *B. napus* parent for the population derived from F_2; BC-NPH: heterosis over the common *B. napus* parent for the population derived from BC_1.

Supplemental Table 4.6 Scores for different agronomic and seed quality traits after Principal Component Analysis (PCA) for the first three components

Traits	PC1	PC2	PC3
Test hybrids:			
Days to flowering = DTF	-0.8217	1.7976	-0.0992
Duration of flowering = DOF	-1.8268	0.5785	-0.09448
Days to maturity = DTM	-0.8524	1.7437	0.41602
Grain-filling period = DOGF	1.8246	-0.6553	-0.16712
Plant height = PH	-0.5313	0.2309	-1.96986
Seed yield = SY	1.2934	0.597	-1.0654
Seed oil content = SOC	1.5302	1.034	-0.54861
Seed protein content = SPC	-1.5214	-1.2921	-0.21204
Mid-parent heterosis (MPH):			
Days to flowering = DTF	1.4242	-0.6382	0.01531
Duration of flowering = DOF	1.6349	-1.0947	-0.09407
Days to maturity = DTM	1.4573	-0.7593	0.39634
Grain-filling period = DOGF	-1.5248	0.9603	0.37388
Plant height = PH	-0.1356	-0.7594	1.88871
Seed yield = SY	-1.3601	-0.25	0.80121
Seed oil content = SOC	-1.5416	-1.2738	-0.49495
Seed protein content = SPC	1.3412	1.4029	0.66308



Supplemental Fig. 4.1 Bar plots of the distribution of the inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses and their test hybrids for different agronomic and seed quality traits. Vertical arrows indicate the values of the *B. napus* parent. Blush colour bars represent inbred lines and teal bars represent the test hybrids.



Supplemental Fig. 4.2 Distribution of the 95 SSR markers used in the present study on different chromosomes of the C genome of *Brassica napus*. Map position of the markers showed on the left side of the chromosomes are in million bp. Markers from the C genome chromosomes which could not be positioned in *Brassica napus* (Chalhoub et al. 2014) or in *B. oleracea* (Parkin et al. 2014) reference genome are indicated by 'unknown' and drawn as a separate segment of the chromosome. The extent of heterozygosity of a marker in the test hybrid population deduced based on marker genotype of the 227 inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses and the common *B. napus* parent A04-73NA are shown in brackets after marker name.

Chapter 5

Association mapping of agronomic and seed quality traits in a *Brassica napus* population derived from six *B. napus* × *B. oleracea* interspecific crosses[4]

5.1 Introduction

Brassica napus canola (AACC, 2*n* = 38) is the second largest oil crop in the world after soybean. The current annual production of *Brassica* oilseeds in the world is about 71 million metric tons (Statista 2019); however, the global demand for this seed oil is increasing. To meet this growing demand, an increased production of this crop is needed, and this can largely be achieved through the development of high-yielding cultivars with good agronomic and seed quality traits. Some of the important agronomic traits of this oilseed crop are seedling and early season vigour, earliness of flowering and maturity, duration of flowering and grain-filling period, plant height, lodging resistance and shattering resistance (Buzza 1995). Breeding for increased seed oil and protein content are also needed to meet the future demand of oil and protein for the growing world population (for review, see Rahman et al. 2013 and Gacek et al. 2018).

A knowledge of the genetic basis of the agronomic and seed quality traits including seed yield is important for the improvement of this oilseed crop through breeding. Most of these traits are under complex quantitative genetic control and are also influenced by environment (Si et al. 2003; Long et al. 2007; Mei et al. 2009; Chen, Geng et al. 2010; Rahman et al. 2018). Several genomic regions affecting seed yield (Quijada et al. 2006; Udall et al. 2006; Shi et al. 2009; Chen, Geng et al. 2010; Raman et al. 2016; Rahman et al. 2017), plant height (Mei et al. 2009;

[4] Nikzad A, Kebede B, Miles Buchwaldt, Isobel A. P. Parkin and Rahman H (2020) Association mapping of agronomic and seed quality traits in a *Brassica napus* population derived from six *B. napus* × *B. oleracea* interspecific crosses. Mol. Breeding.

Shi et al. 2009; Würschum et al. 2011; Wang et al. 2015), flowering time (Osborn et al. 1997; Long et al. 2007; Chen, Geng et al. 2010; Luo et al. 2014; Wei et al. 2014; Raman et al. 2016; Rahman et al. 2017), maturity (Shi et al. 2009), seed oil (Burns et al. 2003; Delourme et al. 2006; Qiu et al. 2006; Teh and Möllers 2016) and protein content (Zhao et al. 2006; Würschum et al. 2011; Schatzki et al. 2014; Teh and Möllers 2016) have been detected in *B. napus* using genetic linkage maps and following QTL mapping approaches. These QTLs, in many cases, were detected in a large interval between the flanking markers (reviewed in Körber et al. 2016 and Zheng et al. 2017) which is an impediment for reliable use of the markers in molecular breeding.

To overcome this limitation, association mapping using a diverse set of cultivars and lines and high-density markers, such as single nucleotide polymorphism (SNP) markers (Ott et al. 2017) has been employed for identification of QTL and tightly linked markers. Following this approach, QTL for different traits in *B. napus*, such as seed yield (Luo et al. 2015; Schiessl et al. 2015; Körber et al. 2016; Luo et al. 2017), seed oil and protein content (Li, Chen et al. 2014; Körber et al. 2016; Liu, Fan et al. 2016), plant height (Luo et al. 2015; Schiessl et al. 2015; Körber et al. 2016; Li et al. 2016; Sun et al. 2016; Zheng et al. 2017), flowering (Schiessl et al. 2015; Wang, Chen, Xu et al. 2016; Xu et al. 2016; Wei et al. 2017; Zhou et al. 2018) and maturity time (Körber et al. 2016) have been detected; most of these studies usied a natural *B. napus* lines.

It is well documented that genetic diversity in *B. napus*, particulary in canola types, is narrow compared to the extent of diversity that exists in its two parental species *B. rapa* and *B. oleracea* (e.g., Thakur et al. 2018). The evolution of *B. napus* from a limited number of variants of its parental species, as well as the intensive breeding conducted over the last few decades within the closed gene pool (Fu and Gugel 2010) are some of the reasons for this narrow genetic diversity

in this crop. This narrow diversity is not only an impediment for further improvement of this crop through breeding (for review, see Rahman 2013), but also for mapping and identification of loci and alleles for any trait that might be found in the *Brassica* A and C genomes. Therefore, introgression of new alleles from the allied species will not only diversify the genetic base of *B. napus* canola, but will extend our knowledge of the genes controlling such traits, as well as identify marker for use in a molecular breeding program. The utility of the genes and alleles of the related species for the improvement of *B. napus* was demonstrated by Rahman et al. (2011, 2017, 2018) through introgression of an early flowering allele from *B. oleracea* var. *alboglabra* into *B. napus*; creating a novel flowering time locus in the C genome which could not be detected using natural *B. napus* populations.

Previously, we reported the potential value of an inbred *B. napus* population derived from six interspecific crosses of *B. napus* × *B. oleracea* for different agronomic and seed quality traits (Nikzad et al. 2019). Using simple sequence repeat (SSR) markers, we also demonstrated the extent of *B. oleracea* alleles introgressed into these lines (Nikzad et al. 2020) and the impact of these alleles on agronomic and seed quality traits (Nikzad et al. 2019). This population, thus, offers a valuable genetic resource for identification of new loci and alleles for multiple traits. The objective of the current research was to perform a genome-wide association study (GWAS) using high-density SNP markers and the above-mentioned *B. napus* population derived from *B. napus* × *B. oleracea* interspecific crosses, to identify loci controlling different agronomic and seed quality traits. Additionally, the mapping of these traits was confirmed using SSR marker data.

5.2 Materials and Methods

5.2.1 Plant Material, Field Trials and Phenotyping

The plant material used in this study was developed using one spring *B. napus* canola line A04-73NA (zero erucic acid, low glucosinolate (< 15 µmol g^{-1} seed)) and six high-erucic (>40% erucic acid), high glucosinolate (> 60 µmol g^{-1} seed) *B. oleracea* cultivars and lines, viz. var. *alboglabra* line-NRC (PBI), var. *botrytis* cv. BARI cauliflower-1, var. *capitata* cvs. Badger Shipper, Bindsachsener and Balbro and var. *italica* cv. Premium Crop (Fig. 3.1). A total of 184 *B. napus* inbred lines, which included 90 F_{10} and 94 BC_1F_9 lines, developed from F_2 and BC_1 (F_1 × *B. napus* parent) of six *B. napus* × *B. oleracea* interspecific crosses involving the single *B. napus* line and the six *B. oleracea* lines and cultivars were used in this study. The detail of the development of these *B. napus* inbred lines was described previously (Nikzad et al. 2019). The C genome of this population ($A^nA^nC^{n/o}C^{n/o}$) was theoretically expected to be composed of the C genome of *B. napus* ($A^nA^nC^nC^n$) and the C genome of *B. oleracea* (C^oC^o), while the A genome was expected to be similar to the *B. napus* parent with the assumption that very little homoeologous pairing between the A and C genomes occurred during the development of these lines. Under this scenario, the inbred lines of this population will segregate for a part of the C genome; therefore, the use of a population comprising about 180 lines in an association study can be justified.

As described by Nikzad et al. (2019), the above mentioned 184 *B. napus* inbred lines and their spring *B. napus* parent A04-73NA were grown in 10 field trials conducted over three years; this included four trials in each of 2016 and 2017 and two trials in 2018. Field plots were laid out in randomized block design with two replications. The following agronomic and seed quality traits

were recorded: days to flowering, end of flowering (days), plant height (cm), days to maturity, duration of flowering time and grain-filling period (days), seed yield (kg ha^{-1}), seed oil (%), protein (%) and glucosinolate (µmol/g seed) content (Nikzad et al. 2019).

5.2.2 SNP discovery using genotype by sequencing

Young leaves of the 184 inbred lines and their seven parents were collected from seedlings grown in a greenhouse. About 200 mg bulk leaf sample from three plants of a line was placed in 2 ml safe-lock Eppendorf tube and stored in –80 ˚C for one night prior to crushing using a Mixer Mill (TissueLyser II, Qiagen, Germany). Genomic DNA was extracted using SIGMA DNA extraction kit (Sigma-Aldrich, St. Louis, MO, USA) following manufacture's instruction. DNA concentration and purity of the samples was assessed using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The samples were processed and sequenced using tunable genotyping-by-sequencing (tGBS®) method by Data2Bio (Ames, IW, USA). Genomic DNA was digested using two restriction enzymes NSpI (5′-RCATG^Y-3′) and BfuCI/Sau3AI (5′-^GATC-3′) which created 3´and 5´overhangs, respectively. Two single-strand oligos, one containing a sample-specific internal barcode and the other a universal oligo, were ligated to the complementary 3´ and 5´ overhangs, respectively. All 191 treated DNA was pooled for the construction of tGBS libraries and sequencing. The raw sequence data was demultiplexed by barcode, which was subsequently removed bioinformatically from each sequence. The barcode-trimmed sequence reads of genotype were further trimmed using the trimming software, Lucy (Chou and Holmes 2001; Li and Chou 2004) to remove low-quality reads based on Phred quality scores of Q15.

The quality trimmed reads were aligned to the reference *B. napus* GCA_000751015.1 (Chalhoub et al. 2014) with bowtie2 version 2.2.0 using the –local, --sensitive, -k 50 and --score-min L,0,0.8 parameters. SNP calling was carried out based on the reads that align to a single location in the reference genome using the Genome Analysis Toolkit (GATK) version 3.2.0 UnifiedGenotyper tool with parameters -glm BOTH and -ploidy 2. SNPs with minor allele frequency (MAF) less than 5% and heterozygous calls (heterozygous loci) were considered as missing data, and the inbred lines with more than 24% missing data were eliminated from the analysis. Based on this, a total of 3,131 SNP markers were retained and used for association mapping.

In addition to SNP marker data, genotypic data of the 184 inbred lines based on 96 SSR markers were also used in this study for identification of QTLs controlling these traits. The details of the SSR markers and genotyping of the lines was described previously (Nikzad et al. 2019).

5.2.3 Statistical Analyses

5.2.3.1 Phenotypic Data Analysis

Best linear unbiased prediction (BLUP) was used to estimate genotypic value of the inbred lines for different agronomic and seed quality traits in each environment (i.e., each of 10 combination of locations and years) using the MIXED procedure of SAS (SAS Institute, 2010) to remove the replication and block variation from the data. In this analysis, replication, block nested in replication and genotype were considered as random effects. Least square means (LSmeans) for each trait were calculated with the LSmeans statement in the MIXED procedure of SAS.

5.2.3.2 Association Analysis

Population structure of the 184 inbred lines was studied with 3,131 SNP markers through principal component analysis (PCA) (Price et al. 2006) and a kinship analysis was done using GAPIT (Genome Association and Prediction Integrated Tool) package (Lipka et al. 2012) in R v3.6.0 to reduce the spurious association between markers and traits that arise from population structure (type I error) and to account for the relationship between inbred lines, respectively. The optimal number of principal components (PCs) was determined based on the Bayesian information criteria (BIC) using GAPIT, and a kinship matrix was used to create a cluster heatmap of the mapping population according to VanRaden algorithm in GAPIT. Association mapping was performed using mixed linear model (MLM) implemented in FarmCPU (Fixed and random model Circulating Probability Unification) in R v3.6.0 (Liu, Huang et al. 2016), taking population structure (PCs) and kinship coefficients (genetic relatedness) into account. FarmCPU method assumes that quantitative trait nucleotides are evenly distributed on the genome and can effectively eliminate confounding effects and improves the statistical power of MLM by implementing the fixed effect model and random effect model iteratively into the final model.

Significance of association between the markers and the traits was tested using the Bonferroni-corrected threshold of p-value = 0.000001 ($-\log_{10}(p) = 6.0$) using FarmCPU. To adjust this threshold, the phenotypes were permuted 2000 times in order to break the relationship with the genotypes and to control the experiment-wise type I error. The Manhattan plots were drawn in R using FarmCPU based on P ($-\log_{10}P$) values for all SNP markers. In addition to this, single marker analysis (SMA) using the SNP as well as SSR marker data was done in R with $-\log_{10}(p) = 4.5$. Phenotypic variance (R^2) for each haplotype block of the trait was calculated using regression analysis in R; for this haplotype block of 2 cM left and right of the SNPs with LOD

(Logarithm of odds) score higher than 4.5 was used. Multiple SNP across the genome could be associated with the traits of interest with different LOD score; however, a single SNP with greatest score was intended to identify from a genomic region allowing a maximum of 1-2% recombination between the marker and the trait. Considering 1 cM equals to about 1 Mb genomic region, regression analysis was run to scan 1-2 Mb region for identification of QTNs (quantitative trait nucleotides) associated with the trait.

To further confirm the effect of one QTL at a time, the inbred population was partitioned based on alternative alleles of each SNP marker from the QTL region found to be associated with the trait, and a *t*-test was done for significant difference between these two groups using the TTEST procedure of SAS.

5.3 Results

Frequency distribution of the 184 inbred lines for different agronomic and seed quality traits is presented in Supplemental Fig. 5.1; in all cases, a continuous variation was found suggesting that all these traits are under quantitative genetic control. Broad-sense heritability (*H*) for different traits ranged from 34.0% for duration of flowering to 90.9% for seed glucosinolate content (Supplemental Table 5.2).

Distribution of the 3,131 SNP markers on the A and C genomes is shown in Fig. 5.1. As expected from the pedigree of the inbred population as derived from six *B. napus* × *B. oleracea* interspecific hybrids ($A^nC^{n/o}C^{n/o}$), the A genome chromosomes carried very few SNP markers while the majority of the markers were located in the C genome. For the A genome, the number of markers ranged from 23 in A8 to 98 in A3 with a mean of 61.5 ± 28.3; while for the C genome, the number of markers ranged from 228 in C6 to 320 in C7 with a mean of 269.3 ±

31.1. Thus, of the total number of the SNP markers, only 19.64% markers were located in the A genome while 77.42% of the markers located in the C genome; location of 2.93% of the markers could not be determined (Fig. 5.1).

Of the 184 inbred lines genotyped by SNP markers, 10 lines had greater than 24% missing marker data were considered not suitable for use in association mapping. Thus, a total of 175 lines were used in GWAS and further analyses.

Bayesian information criterion (BIC)-based model selection conducted in GAPIT suggested the optimal number of principal components (PCs) for GWAS analysis to be three. PCA analysis based on 3,131 SNP markers showed the existence of two small and one large group in the population where the three PCs accounted for 47.2, 35.6 and 17.7% of the genetic variance (Supplemental Fig. 5.2A). Kinship analysis of the inbred lines showed that 67.4% of the kinship coefficients were equal to 0 and 32.6% were less than 0.1 (Supplemental Fig. 5.2B). Cluster analysis using the kinship data showed considerable genetic differentiation and lack of any strong clustering in the population, suggesting the suitability of the population for use in association mapping (Supplemental Fig. 5.2C).

Association mapping of days to flowering using SNP markers and LSmean data from all 10 trials identified at least three QTLs on chromosomes C2, C5 and C6 with a probability greater than the Bonferroni corrected significance threshold of $-\log_{10}(p) = 5.7$ (Fig. 5.2). Among these, the C2 (10.7 Mb) and C5 (28.7 and 31.3 Mb) QTLs could be detected in the majority of the individual field trials conducted over three years; significant SNP markers from these QTLs explained about 30% and 20% of the total phenotypic variance, respectively (Table 5.1). On the other hand, the C6 QTL (32.3 Mb) only explained about a half to one-third amount (11%) of the total

phenotypic variance as compared to the C2 and C5 QTLs. Of the three QTLs, the C2 QTL also was detected using the SSR marker sN3761 located at 9.1 Mb on C2 in the *B. napus* genome (Supplemental Table 5.1). An additional QTL from C3 (18.9 Mb) affecting days to flowering could be detected with the Bonferroni significance threshold of $-\log_{10}(p) = 4.5$ (determined using 2000-times permutation test) (Fig. 5.2 and Table 5.1). However, this minor QTL was detected in only a few trials and explained <10% of the total phenotypic variance. Partitioning the population based on alternative alleles of the SNP markers from these QTLs and applying a *t*-test for determining significant differences ($p < 0.001$) further confirmed the effect of these genomic regions on days to flowering in this *B. napus* population (Fig. 5.5).

Four major QTLs located at C1 (15.1 Mb), C5 (47.3 Mb) and C8 (7.5 and 11.9 Mb) were detected for seed oil content based on LSmeans data of all trials; these QTLs also were detected in the majority of the individual trials (Fig. 5.3 and Table 5.1). QTL from these chromosomes could also be detected using SSR marker data (Supplemental Table 5.1). These QTL explained about 18-30% of the total phenotypic variance. Additional QTLs from C1, C3 and C7 were detected based on LSmeans data; however, these QTLs could be detected in only a few trials (Fig. 5.3; Table 5.1).

Association mapping identified SNP markers from chromosomes C1 (32.8 Mb), C2 (43.0 Mb), C3 (25.4) and C5 (41.8 Mb) with probability greater than the Bonferroni significance threshold of $-\log_{10}(p) = 5.7$, and from C6 (4.2 Mb) with probability $-\log_{10}(p) = 5.1$ associated with seed glucosinolate content. These markers were also identified through single marker analysis using SNP marker data (Fig. 5.3 and Table 5.1). Among these, the C2, C5 and C6 QTLs were detected in 10, six and nine trials, respectively (Fig. 5.4) and the individual QTL explained 34-50% of the total phenotypic variance (Table 5.1). Single marker analysis also detected SSR marker from C1

(sN2087; 36.4 Mb), C2 (sN1825; 33.3 Mb) and C5 (sN0761; 46.3 Mb); however, these markers are located at about 4-10 Mb away from the SNP markers; the difference between the position of the SNP and SSR markers might be due to poor genome coverage with the limited number of SSR markers. Thus, the results provide evidence for the presence of QTLs on C1, C2, C3, C5 and C6 affecting seed glucosinolate content in *B. napus*. The effect of these QTLs on seed glucosinolate content could also be observed through partitioning the phenotypic data based on the alternative alleles of the SNP markers (Fig. 5.5).

No QTL for other traits, such as duration of flowering and grain-filling period, days to maturity, plant height, seed yield and seed protein content could be detected consistently in all trials as well as based in LSmeans data. However, association mapping and single marker analysis identified a SNP marker from C3 (37.5 Mb) affecting the duration of flowering in two locations, and this QTL could also be detected based on LSmeans data (Supplemental Fig. 5.3). Similarly, one QTL from C1 (33.4 Mb) affecting the grain-filling period in two locations (Supplemental Fig. 5.4), and one QTL from C7 (16.3 Mb) affecting seed protein content in one location (Supplemental Fig. 5.5) could also be detected in addition to detecting these QTLs based on LSmeans data.

5.4 Discussion

It has been well documented that the A and C genomes of *B. napus* evolved from a common ancestor through polyploidization (Parkin et al. 2003). Therefore, multiple copies of a gene can be found in each of the two genomes (Parkin et al. 2014). This is one of the impediments for identification of the majority of QTLs controlling a complex trait in the A and C genomes when using a bi-parental mapping approach with limited parental lines necessarily studied. In this

study, we used an inbred population derived from six *B. napus* × *B. oleracea* interspecific crosses involving six *B. oleracea* accessions belonging to four different variants of this species and a *B. napus* canola line. Given that wide diversity exists in *B. oleracea* (Thakur et al. 2018), this population was expected to be highly diverse and carry alleles which are not present in natural *B. napus*. Assuming that there was no or a little homoeologous recombination occurred between the A and C genome chromosomes in the progeny of the six *B. napus* × *B. oleracea* interspecific hybrids (ACC), the A genome of the inbred lines was expected to be the same as the A genome of *B. napus* canola line, while the C genome was expected to be a blend of the C genome of the *B. oleracea* accessions and the *B. napus* line. Thus, the population was expected to be segregating for only a part of the C genome, and this would allow us to focus on the contribution of the C genome in contrast to working with the whole complex A and C genomes.

It is also well established that strong homoeology exists between the A and C genomes which can result in homoeologous recombination between the chromosomes of these two genomes in the plants where at least one of the two genomes occur in a haploid state, such as in a digenomic haploid (AC) (Attia and Röbbelen 1986a, 1986b) or in a trigenomic haploid (ABC) (Attia et al. 1987) or in AABC, BBAC and CCAB interspecific hybrids (Mason et al. 2010, 2017). Occurrence of homoeologous recombination between the *Brassica* A and C genomes has also been reported by Higgins et al. (2018) using te *Brassica* Infinium 60K SNP array. Our results showed that about 19.6% (615/3,131) of the SNP markers were mapped to the A genome while 77.4% (2,424/3,131) to the C genome of the *B. napus* inbred lines derived from the *B. napus* × *B. oleracea* interspecific crosses. It is possible that the SNP markers detected in the A genome resulted from homoeologous recombination between the A and C genome chromosomes of *B. napus* and *B. oleracea*, although inaccurate mapping of reads between the closely related

genomes could contribute to this finding. Autosyndetic pairing of the A genome chromosomes can also occur in the haploid genome condition (Armstrong and Keller 1980) and might have contributed to the observed genetic variation in the A genome. However, in the present study, we did not detect any QTL for any of the traits in the A genome. In contrast, Rahman (2001) reported a transfer of yellow seed color genes from the A genome of yellow sarson (*B. rapa*, AA) into the C genome of *B. napus* in a progeny derived from ((*B. carinata* (BBCC) × yellow sarson) × *B. napus* interspecific cross. In this regard, it might be expected that transfer of some genes/QTLs occurred in the inbred population used in this study from the C to the A genome. However, in previous studies it has been noted that there is a bias in genome exchange resulting from homoeologous recombination with the C genome in the majority of instances replaced by the A genome (Higgins et al. 2018; Samans et al. 2017). In the current study this would result in duplication of the A genome and loss of the C genome, but with no variation in the A genome these regions would be impossible to detect with SNP genotyping.

The genome coverage of the SNPs markers in the mapping population used in this study was one SNP per 0.8 cM based on a total length of about 2500 cM for the *B. napus* genome (Delourme et al. 2013). While considering only the C genome, where QTL mapping has been focused, the density of the markers was even greater. Körber et al. (2016) also conducted a GWAS for QTL mapping of seed quality traits in *B. napus* using SNP markers with a density of one SNP per 0.7 cM. In this regard, the number of SNP markers used in this study would be suitable for use in GWAS for identification of QTLs.

Earliness of flowering and maturity are important traits in the breeding of spring *B. napus* canola for cultivation in a geographical region where crop growing season is short, such as on the Canadian prairies. Days to flowering generally correlates well with days to maturity (Shiranifar

et al. 2020); therefore, selection for earliness of flowering is generally performed to develop an early-maturing cultivar. In this study, we identified QTLs from the C genome chromosomes C2 (10.8 Mb), C3 (18.9 Mb), C5 (28.7 Mb and 31.3 Mb) and C6 (32.3 Mb) associated with days to flowering; among these C2 and C5 QTLs could be detected in the majority of the trials. Previously, Rahman et al. (2017) reported an environmentally stable QTL on C1 in a spring *B. napus* population carrying a C genome introgression from *B. oleracea* var. *alboglabra*. This QTL was found not to be influenced by photoperiod (Rahman et al. 2018). However, in this study, we were not able to detect this C1 QTL, likely due to the use of different *B. oleracea* var. *alboglabra* lines, in contrast, we detected novel QTLs on C2 and C5 that affected flowering time in majority of the field trials. Using a spring *B. napus* mapping population, Luo et al. (2014) reported two QTLs on C8 and C9, and using a diverse *B. napus* population, Körber et al. (2016) reported a QTL at 23.0 Mb position of C2; however, the SNP marker of the C2 QTL detected in our study is located at more distal at 10.8 Mb. QTLs on the top of C2 of *B. napus* have also been reported by Quijada et al. (2006), Wei et al. (2014) (7.8-9.0 cM), Wang, Chen et al. (2016) (7.0-13.7 Mb), Xu et al. (2016) (26.5 and 41.6 Mb), Rahman et al. (2017) (32.9-45.1 and 6.9-16.3 cM) and Jian et al. (2019) (42.4 and 50.1 cM), as well as on C2 chromosome of *B. oleracea* by Bohuon et al. (1998), Rae et al. (1999) (78 cM) and Okazaki et al. (2007). In case of C5, QTLs affecting flowering time has been reported by Wei et al. (2014) (5.1-9.3 and 9.3-17.3 cM), Xu et al. (2016) (0.2, 3.7 and 42.8 Mb) and He et al. (2018) (23.9-27.1 and 27.1-37.3 cM). Physical position (Mb) of the C2 and C5 QTLs, which we detected in majority of the field trials, could be compared with only few of the above-mentioned reports. Based on this, the C2 QTL detected in our study might be the same as the one reported by Wang, Chen et al. (2016). However, the physical position of the C5 QTL that we detected is located at a different position as compared to

the position of the QTLs reported by Xu et al.; while the C5 QTLs reported by Wei et al. and He et al. were detected only in one of their field trials. Thus, it is apparent that the C5 QTL that we detected in the present study has not been reported previously; for this QTL, the alternative allele derived from *B. oleracea* var. *capitata* cv. Bindsachsener.

For seed quality traits, we detected SNP markers from C1 (15.1 and 23.3 Mb), C3 (12.6 and 25.4 Mb), C5 (47.3 Mb), C7 (33.9 Mb) and C8 (7.5 and 11.9 Mb) affecting seed oil, and markers from C1 (32.8 Mb), C2 (43.0 Mb), C3 (25.4 Mb), C5 (41.8 Mb) and C6 (4.2 Mb) affecting seed glucosinolate contents. In case of seed oil content, the QTLs of C1, C5 and C8 were detected in the majority of the trials. QTLs in the C genome of *B. napus* affecting seed oil content have been reported by several researchers. For example, QTLs for seed oil content were detected on chromosomes C1 (e.g., 5.0 cM and 191.0 cM), C2 (e.g., 4.5 cM, 7.0 cM, 13.6 cM and 18.1 cM), C3 (e.g., 88.9 cM, 89.7 cM and 0.5 Mb), C4, C5 (e.g., 36.3 cM, 52.6 cM, 70.0 cM, 5.9 Mb and 9.5 Mb), C6, C7 (e.g., 77.8 cM, 78.9 cM and 37.1 Mb), C8 (e.g., 10.0 cM and 59.0 cM) and C9 (Delourme et al. 2006; Qiu et al. 2006; Chen, Geng et al. 2010; Zou, Jiang et al 2010; Wang et al. 2013; Huang et al. 2016; Körber et al. 2016; Chao et al. 2017; Xiao et al. 2019). Physical position of the C3, C5 and C7 QTLs we detected could be compared with the QTLs reported by Körber et al. (2016) and Xiao et al. (2019); however, none of these were found to locate to the same position.

Seed oil and protein content generally show significant negative correlation (for review, see Rahman et al., 2013) and co-localized QTLs for these seed quality traits have been reported by several researchers (e.g. Huang et al. 2016; Chao et al. 2017). QTLs independently affecting seed oil and protein content have also been reported (e.g. Mahmood et al. 2006; Zhao et al. 2006). In the present study we did not find a co-localized QTL for protein content which could be detected

in majority of the trials as well as based on LSmean data. QTLs independently affecting seed oil and protein content have important implications in breeding for increasing these two seed constituents simultaneously.

For seed glucosinolate content, QTLs in the C genome has been reported on C1 (6.4 Mb), C2 (93.5-94.7 cM, 30.9 cM, 36.7 cM, 54.6 cM, 67.8 cM and 72.9 cM), C3 (22.8-27.6 cM), C5 (12.1 Mb), C7 (24.0 cM), C8 (0.0-10.4 cM) and C9 (1.7 Mb and 1.8 Mb) (Zhao and Meng 2003; Feng et al. 2011; Körber et al. 2016; He et al. 2018; Liu et al. 2020). Of the five QTLs that we detected in this study, the C2 and C6 QTLs explained the greatest amount of phenotypic variance; however, their physical position could not be compared with the QTLs reported previously; the position of the C5 QTL detected in our study located about 30 Mb away from the QTL reported by Körber et al. (2016).

In conclusion, results from the present study demonstrated the utility of *B. napus* lines carrying *B. oleracea* introgressions for the identification of QTLs in the C genome, as well as for detection of beneficial alleles for the C genome of this diploid progenitor species for the improvement of *B. napus* canola. This study also identified a number of QTLs which seem to be quite stable over the environments; this has important implications for the improvement of canola for growing in a diverse range of growing conditions. Thus, the knowledge gained and molecular markers identified from this study are expected to facilitate molecular breeding programs for the improvement of *B. napus* canola for flowering time, and seed oil and glucosinolate content.

5.5 Tables

Table 5.1 List of SNP markers significantly associated with days to flowering (DTF), and seed oil (SO), and seed glucosinolate (SG) contents identified through both association mapping and single marker analysis using LSmeans data of 175 *B. napus* inbred lines, derived from six *B. napus* × *B. oleracea* interspecific crosses, from 10 trials and 3,131 single nucleotide polymorphism (SNP) markers.

Trait	SNP	Alleles	LG[1]	Position (Mb)	*p*-value	-LOG$_{10}$(*P*)[2]	PVE[3] (%)
DTF	SNP260	A/G	C2	10 775 899	8.15×10^{-7}	6.09	31
DTF	SNP320	A/G	C3	18 867 152	8.71×10^{-6}	5.06	7
DTF	SNP2030	G/A	C5	28 650 014	4.78×10^{-8}	7.32	20
DTF	SNP1223	C/A	C5	31 336 141	5.99×10^{-9}	8.22	22
DTF	SNP2898	T/C	C6	32 339 867	5.11×10^{-8}	7.29	11
SO	SNP210	A/G	C1	15 118 624	2.06×10^{-8}	7.69	30
SO	SNP2570	T/A	C1	23 267 743	2.09×10^{-8}	7.68	21
SO	SNP2666	T/C	C3	12 578 943	7.32×10^{-10}	9.14	21
SO	SNP324	A/T	C3	25 417 288	5.50×10^{-9}	8.26	20
SO	SNP509	A/G	C5	47 333 516	7.0×10^{-11}	10.16	27
SO	SNP615	A/C	C7	33 939 501	1.71×10^{-6}	5.77	16
SO	SNP1390	C/G	C8	7 487 928	6.37×10^{-7}	6.20	18
SO	SNP2238	G/T	C8	11 861 028	1.20×10^{-8}	7.92	23
SG	SNP997	C/T	C1	32 758 890	2.20×10^{-6}	5.66	18
SG	SNP2650	T/C	C2	43 013 522	2.88×10^{-11}	10.54	50
SG	SNP1889	G/C	C3	25 417 297	1.10×10^{-7}	6.96	14
SG	SNP1251	C/T	C5	41 822 504	2.07×10^{-8}	7.68	34
SG	SNP2868	T/C	C6	4 195 275	7.51×10^{-6}	5.12	49

[1] Linkage group
[2] Bonferroni significance threshold and corrected threshold determined using permutation test
[3] Phenotypic variation explained by SNP

5.6 Figures

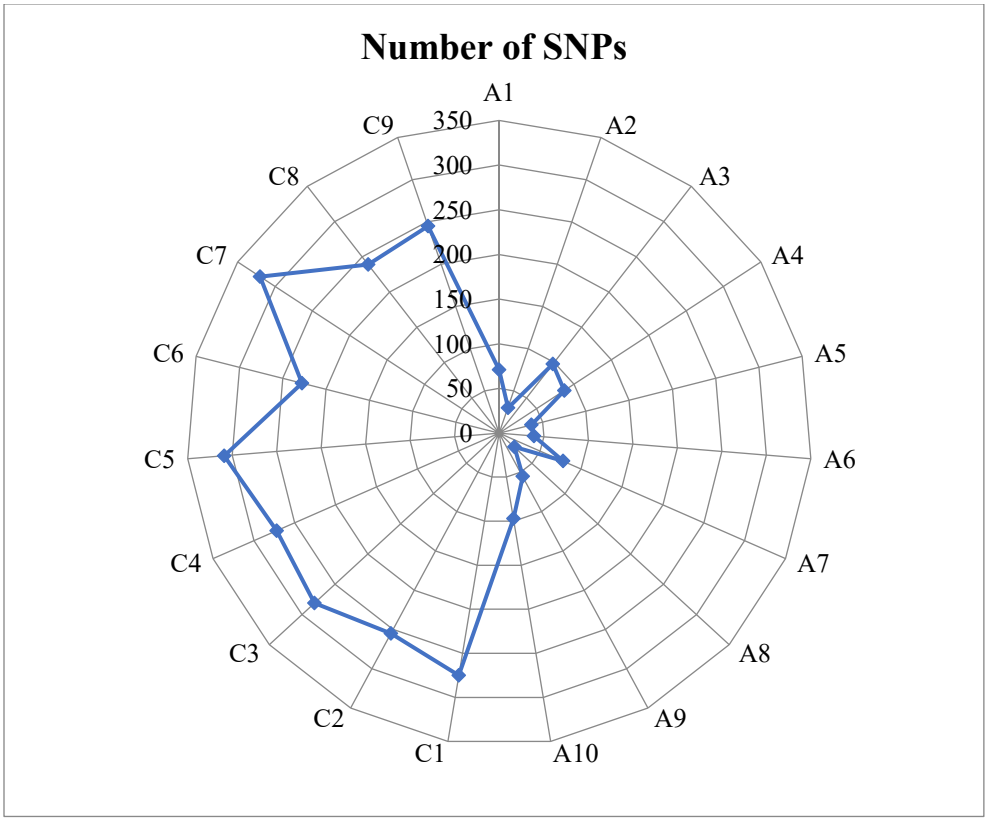


Fig. 5.1 Distribution of 3,131 SNP markers in the A and C genome chromosomes of the *Brassica napus* inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses. A1-10 represent A genome and C1-9 represent C genome chromosomes.

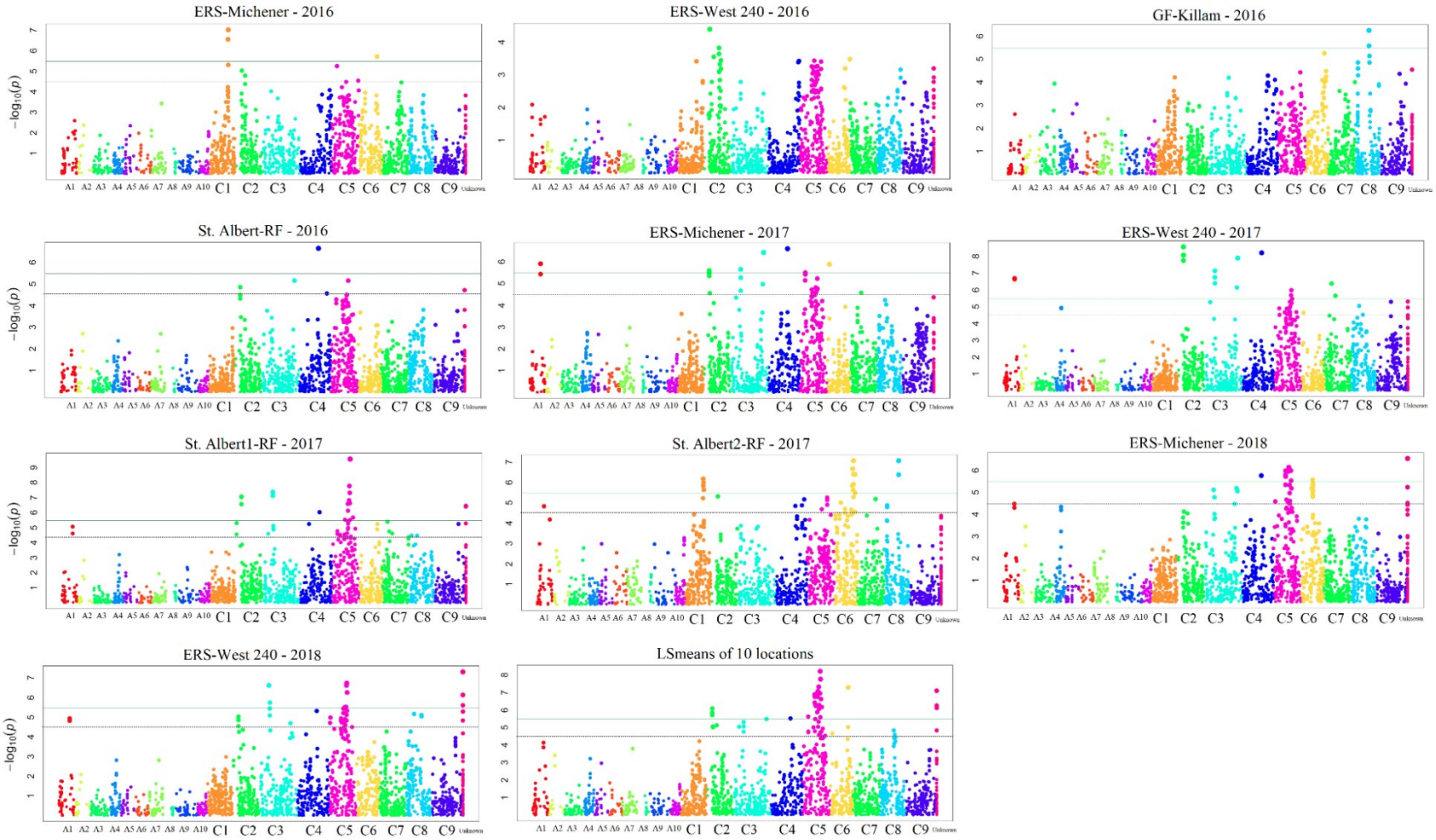


Fig. 5.2 Manhattan plots of genome-wide association analysis for days to flowering using 175 *B. napus* inbred lines, derived from six *B. napus* × *B. oleracea* interspecific crosses, and 3,131 SNP markers; results from LSmeans of all 10 field trials as well as individual trials are presented. ERS = Edmonton Research Station of the University of Alberta; GF-Killam = grower's Field at Killam; St. Albert-RF = St. Albert Research Farm of the University of Alberta. The solid horizontal line represents the Bonferroni significance threshold of $-\log_{10}(p) = 5.7$ and the broken horizontal line represents the corrected threshold $-\log_{10}(p) = 4.5$ determined using permutation test.

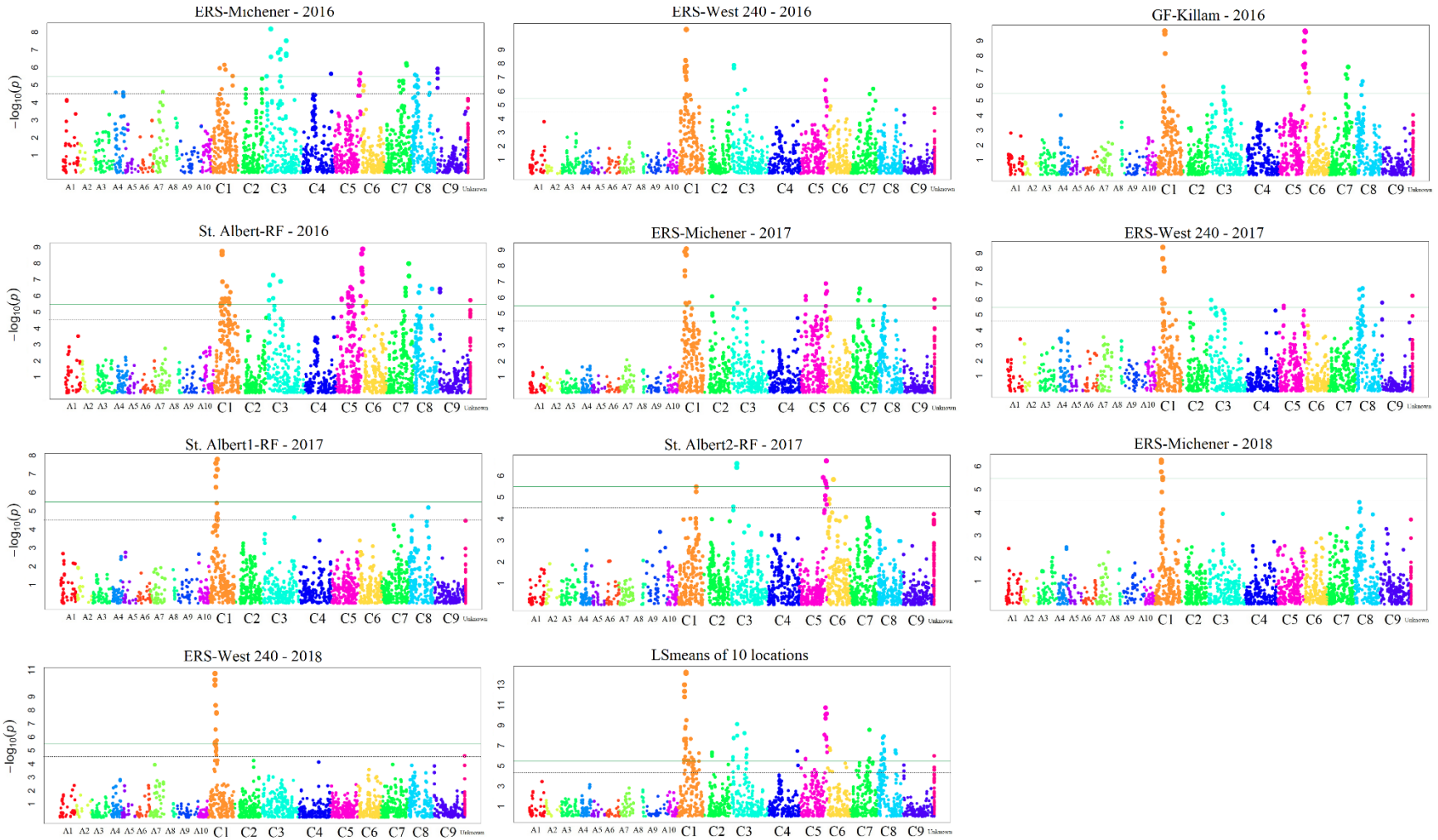


Fig. 5.3 Manhattan plots of genome-wide association analysis for seed oil content (%) using 175 *B. napus* inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses, and 3,131 SNP markers; results from LSmeans of all 10 field trials as well as individual trials are presented. ERS = Edmonton Research Station of the University of Alberta; GF-Killam = grower's Field at Killam; St. Albert-RF = St. Albert Research Farm of the University of Alberta. The solid horizontal line represents the Bonferroni significance threshold of -$\log_{10}$ (p) = 5.7 and the broken horizontal line represents the corrected threshold –$\log_{10}$ (p) = 4.5 determined using permutation test.

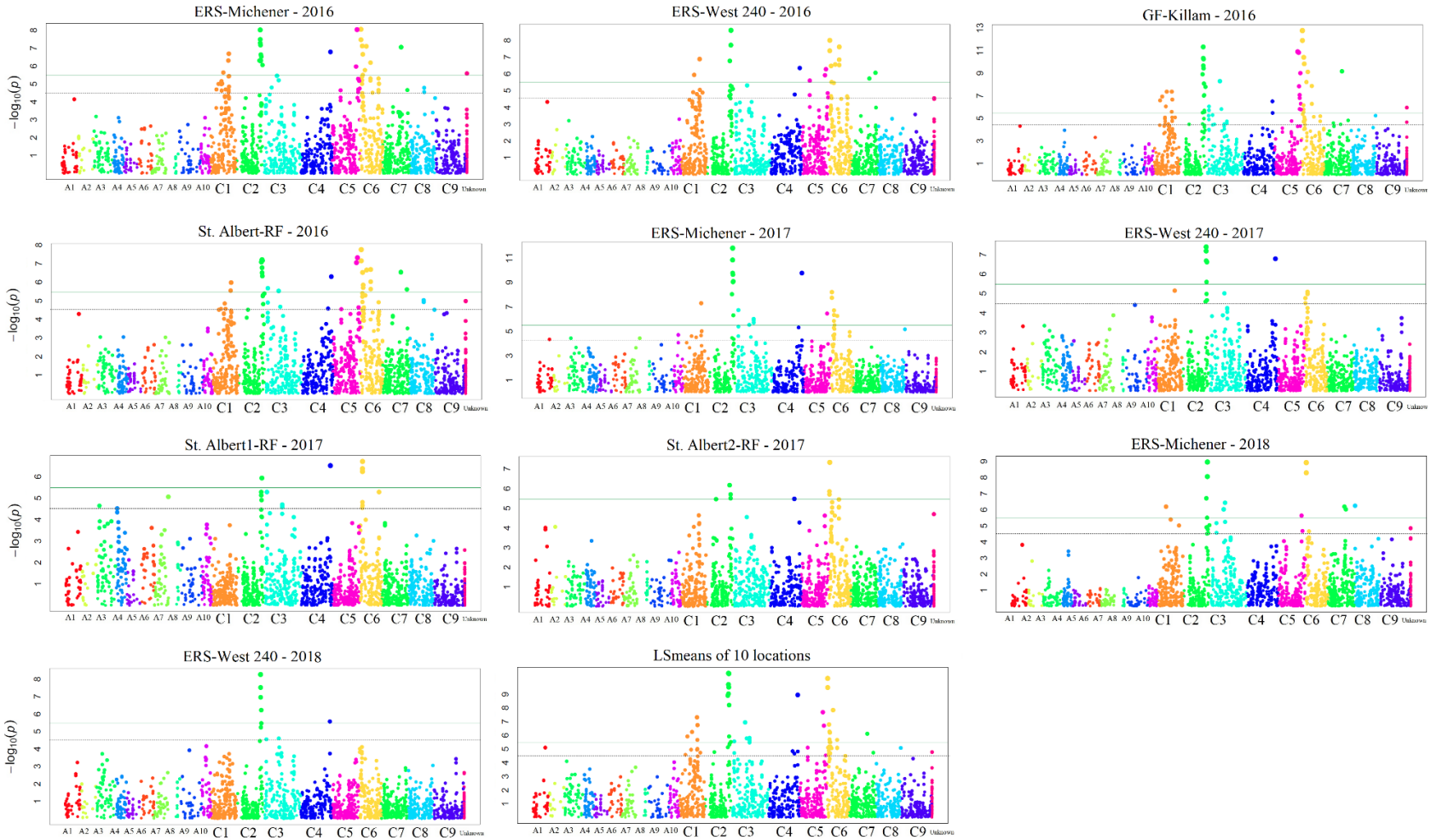


Fig. 5.4 Manhattan plots of genome-wide association analysis for seed glucosinolate content (µmol g^{-1} seed) using 175 *B. napus* inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses, and 3,131 SNP markers; results from LSmeans of all field trials as well individual trials are presented. ERS = Edmonton Research Station of the University of Alberta; GF-Killam = grower's Field at Killam; St. Albert-RF = St. Albert Research Farm of the University of Alberta. The solid horizontal line represents the Bonferroni significance threshold of -$\log_{10}$ (p) = 5.7 and the broken horizontal line represents the corrected threshold –$\log_{10}$ (p) = 4.5 determined using permutation test.

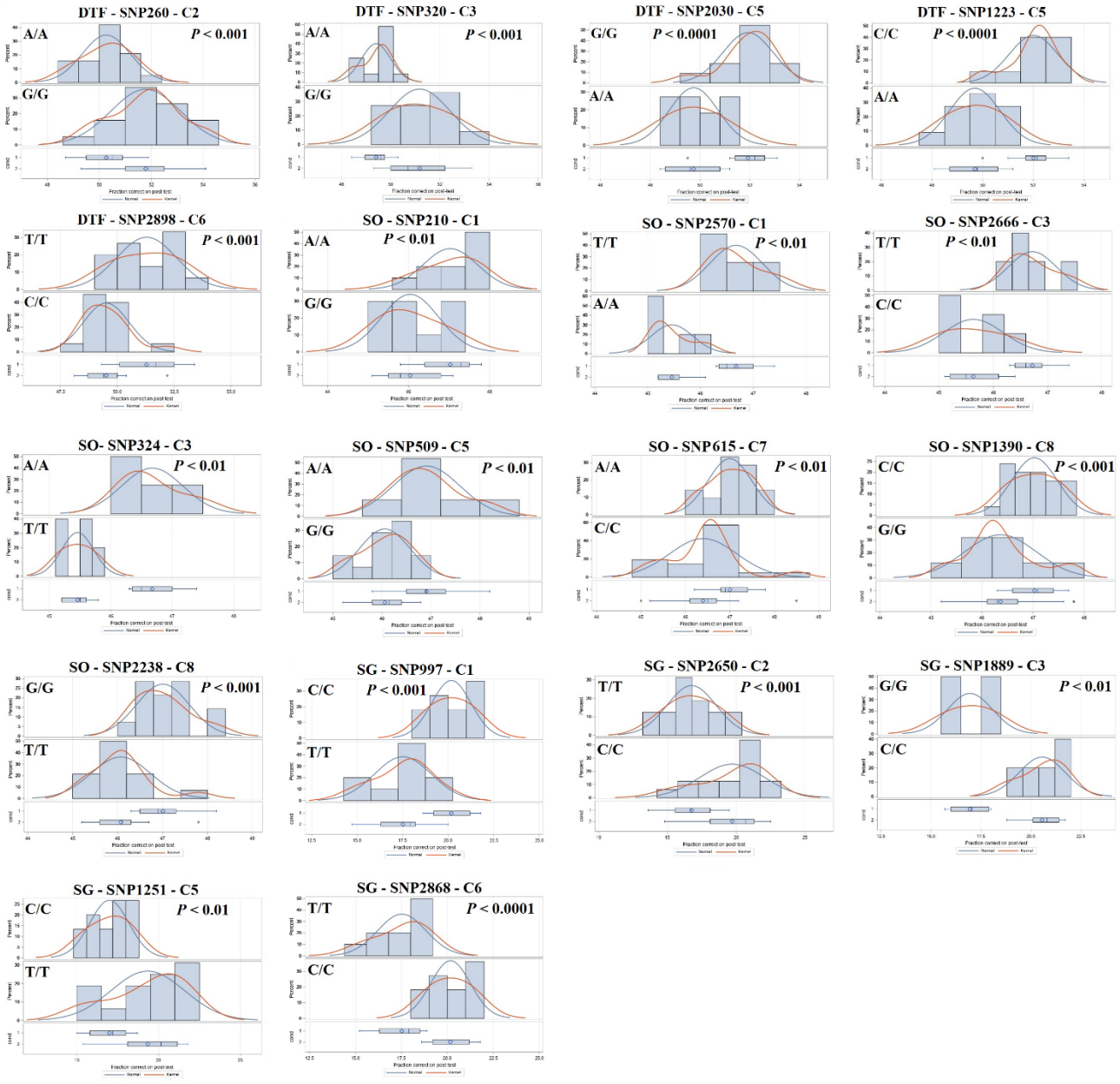


Fig. 5.5 Comparison of the *Brassica napus* inbred population, derived from six *B. napus* × *B. oleracea* interspecific crosses, partitioned based on reference and alternative alleles of the SNP markers significantly associated with days to flowering (DTF), seed oil (SO) and seed glucosinolate (SG) content; *p*-value <0.01 indicates the two groups are significantly different. The upper figure represents the distribution of the population for the reference allele while the lower figure for the alternative allele.

5.7 Supplemental material

Supplemental Table 5.1 Identification of quantitative trait loci (QTL) for days to flowering (DTF), seed oil (SO) and seed glucosinolate (SG) content through single marker analysis and using LSmeans data of 175 *Brassica napus* inbred lines, derived from six *B. napus* × *B. oleracea* interspecific crosses, and 96 simple sequence repeat (SSR) markers.

Trait	SSR	LG[1]	Position (Mb)	*p*-value	-LOG$_{10}$(*P*)[2]	SNP effect[3]	PVE[4] (%)
DTF	sN3761	C2	9 074 097	2.04×10^{-03}	2.69	0.43	18
DTF	BoGMS0468	C8	Unknown	3.20×10^{-04}	3.49	0.44	10
DTF	BoGMS0868	C8	Unknown	1.03×10^{-04}	3.99	0.47	25
SO	sN11657	C1	31 685 513	4.37×10^{-04}	3.36	-0.36	15
SO	sNRF94	C1	7 985 883	2.87×10^{-04}	3.54	-0.32	14
SO	sN2316	C3	9 581 482	1.07×10^{-04}	3.97	-0.24	15
SO	BoGMS1065	C7	Unknown	3.36×10^{-03}	2.47	-0.18	23
SO	BoGMS0868	C8	Unknown	6.84×10^{-04}	3.16	-0.23	15
SG	sN2087	C1	36 426 396	1.27×10^{-03}	2.90	-0.93	5
SG	sN1825	C2	33 253 390	8.15×10^{-03}	2.09	0.55	18
SG	sN0761	C5	46 315 526	1.37×10^{-03}	2.86	0.85	33

[1] Linkage group

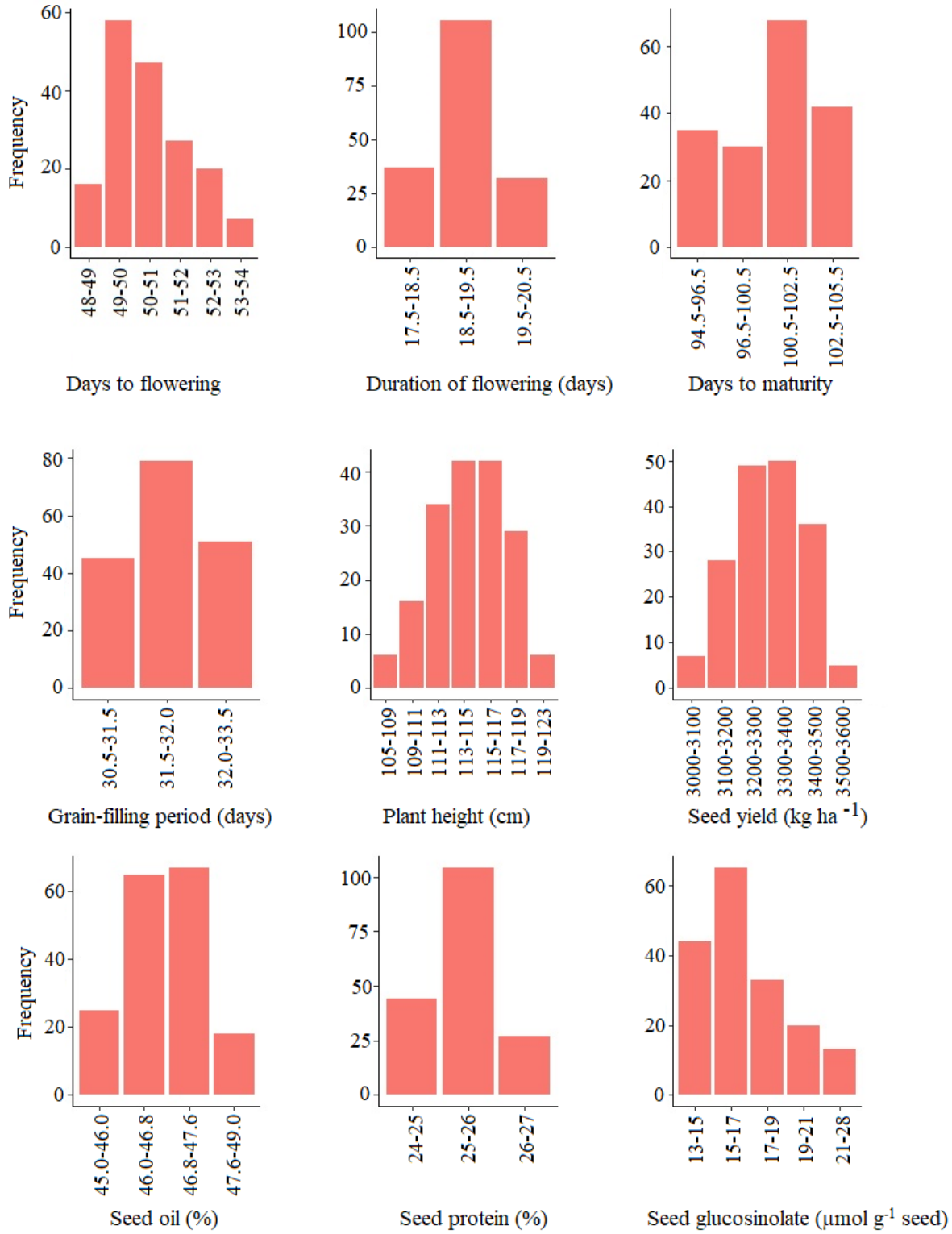
[2] Bonferroni significance threshold and corrected threshold determined using permutation test

[3] Estimated effects of the alleles obtained from mixed linear model; negative sign indicate the effect of the *B. oleracea* allele over the *B. napus* allele

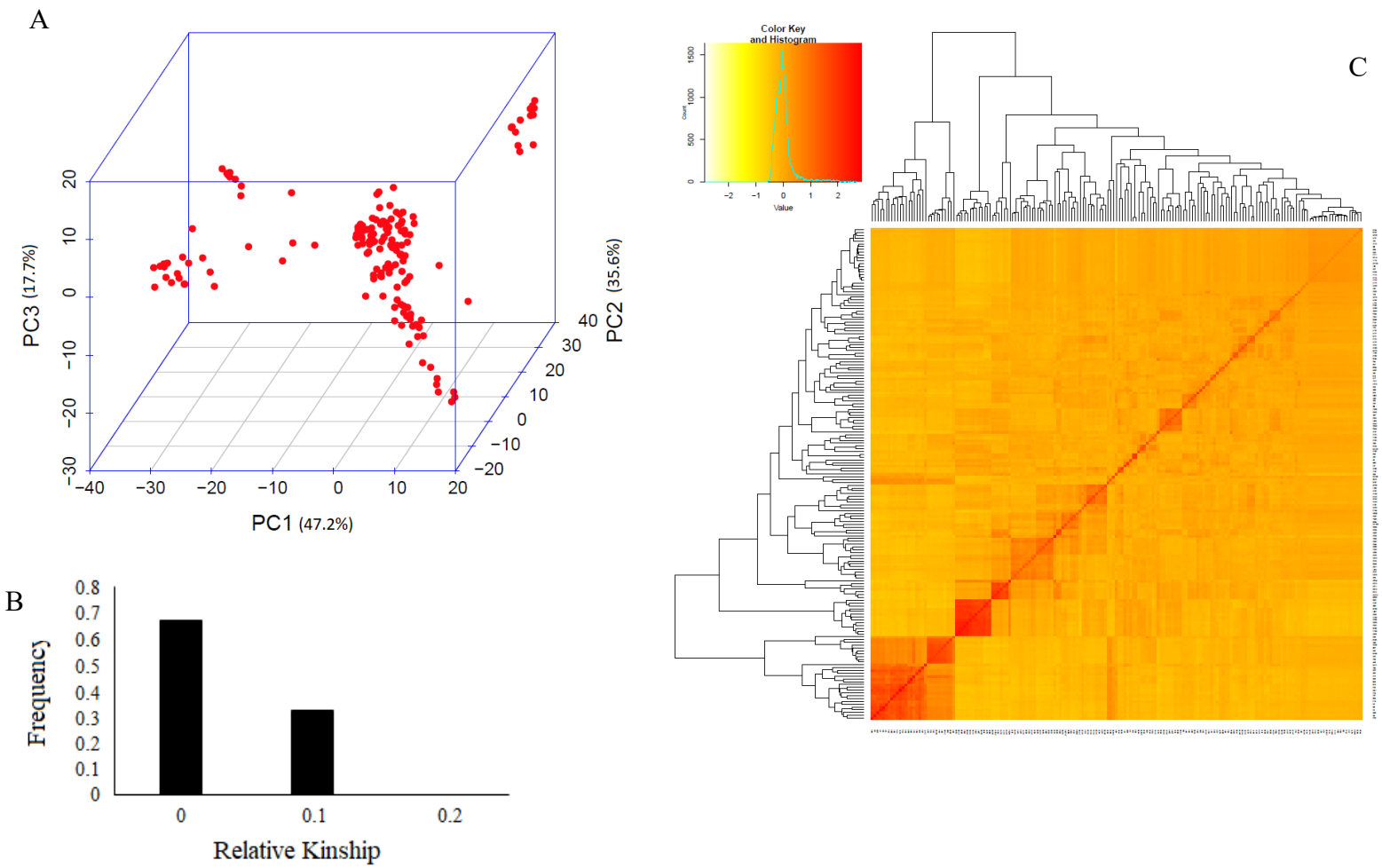
[4] Phenotypic variation explained by SNP

Supplemental Table 5.2 Broad sense heritability for different agronomic and seed quality traits estimated in a *Brassica napus* inbred population derived from six *B. napus* ×*B. oleracea* interspecific crosses and following two breeding methods (F_2- and BC_1-derived).

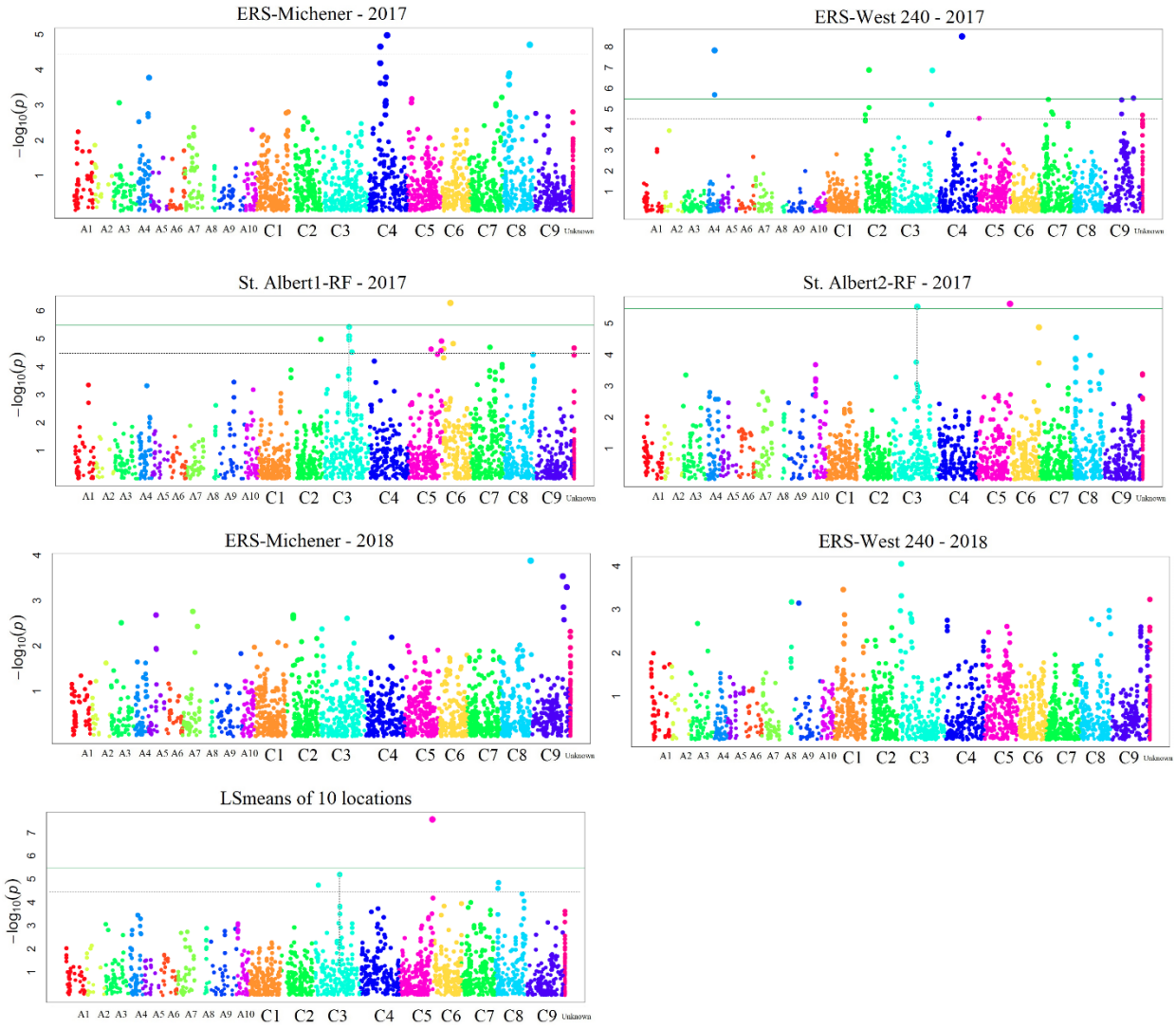
Traits	Heritability
Days to flower	89.87
Duration flowering (day)	34.01
Duration of grain-filling (day)	69.34
Plant height (cm)	79.92
Seed yield (%)	77.91
Seed oil (%)	89.87
Seed protein (%)	83.47
Seed glucosinolate (μmol g^{-1})	97.89



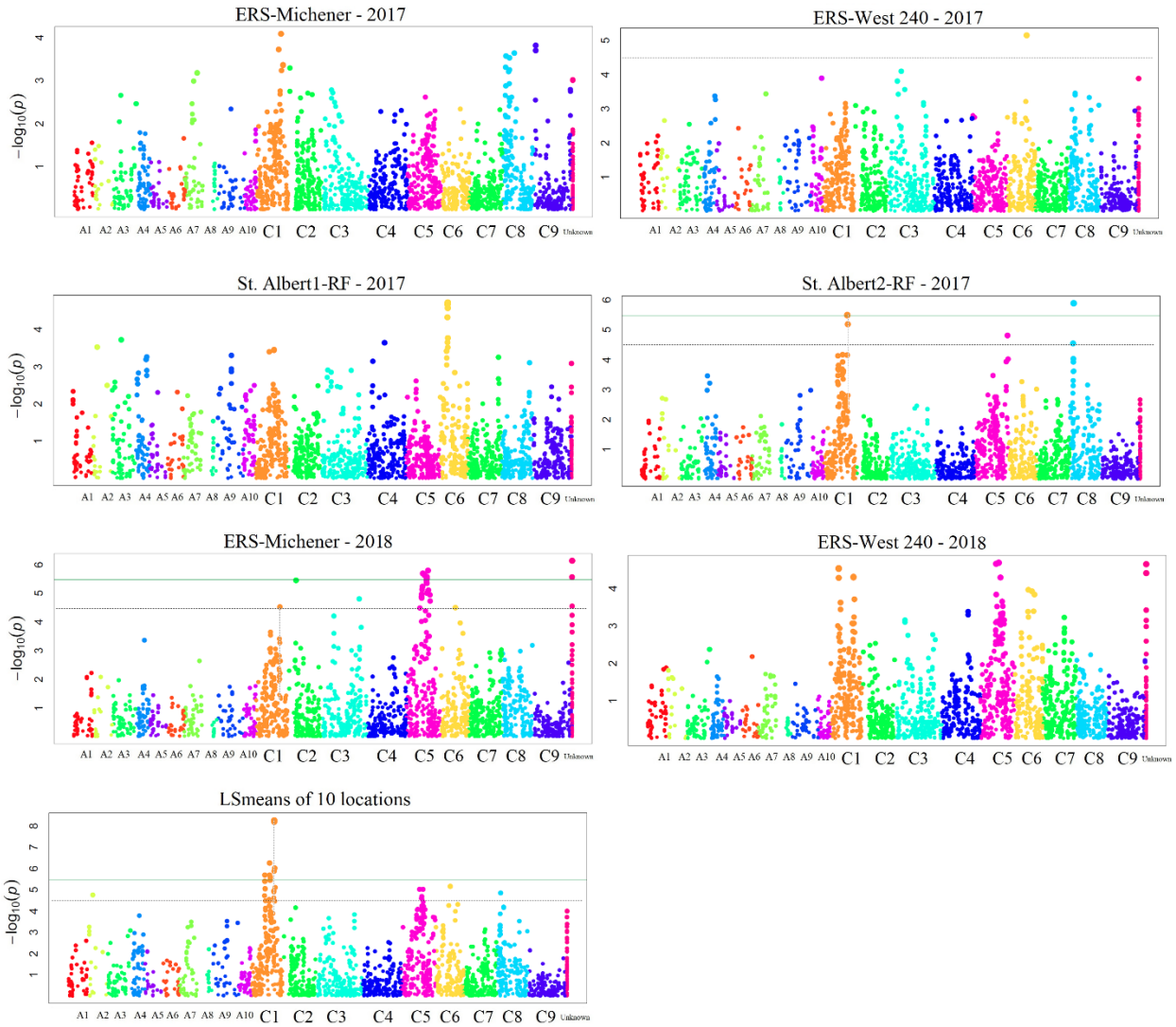
Supplemental Fig. 5.1 Frequency distribution of 175 *B. napus* inbred lines derived from six *B. napus* × *B. oleracea* interspecific crosses for different agronomic and seed quality traits. LSmeans data used for these histograms.



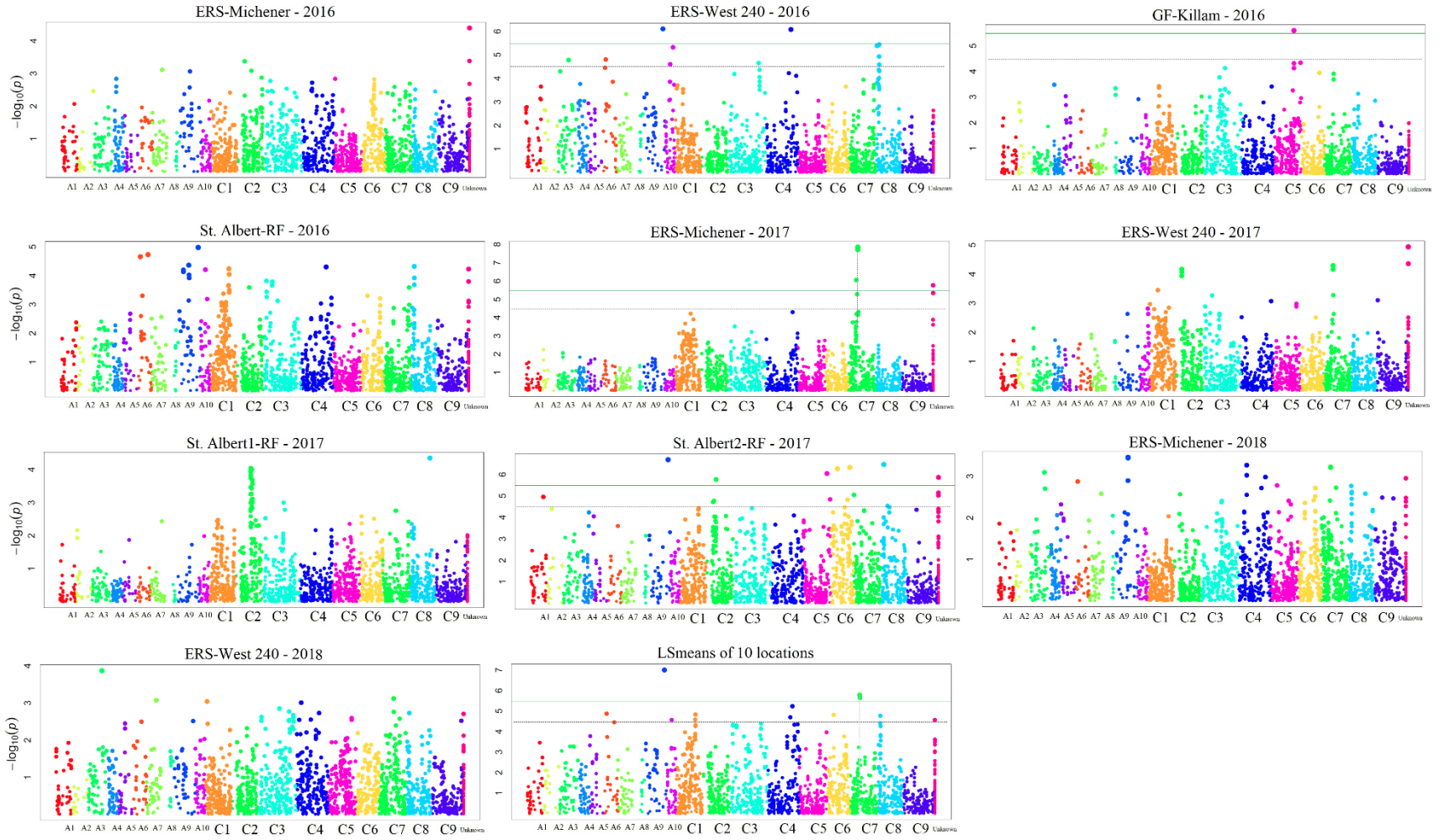
Supplemental Fig. 5.2 Analysis of population structure and kinship for 175 inbred lines derived from six *Brassica napus* × *B. oleracea* interspecific crosses based on 3,131 SNP markers. (A) Plot of the first three principal components; (B) frequency distribution of the relative kinship values; and (C) heatmap of pairwise kinship matrix values according to VanRaden algorithm; the dark red color represents the inbred lines more related to each other.



Supplemental Fig. 5.3 Manhattan plots of genome-wide association analysis for duration of flowering (days) using 175 *B. napus* inbred lines, derived from six *B. napus* × *B. oleracea* interspecific crosses, and 3,131 SNP markers; results from six individual field trials as well as LSmeans data from all trials are presented. ERS = Edmonton Research Station of the University of Alberta; GF-Killam = grower's Field at Killam; St. Albert-RF = St. Albert Research Farm of the University of Alberta. The solid horizontal line represents the Bonferroni significance threshold of $-\log_{10}(p) = 5.7$ and the broken horizontal line represents the corrected threshold $-\log_{10}(p) = 4.5$ determined using permutation test.



Supplemental Fig. 5.4 Manhattan plots of genome-wide association analysis for grain-filling period (days) using 175 *B. napus* inbred lines, derived from six *B. napus* × *B. oleracea* interspecific crosses, and 3,131 SNP markers; results from six individual field trials as well as LSmeans data from all trials are presented. ERS = Edmonton Research Station of the University of Alberta; GF-Killam = grower's Field at Killam; St. Albert-RF = St. Albert Research Farm of the University of Alberta. The solid horizontal line represents the Bonferroni significance threshold of $-\log_{10}(p) = 5.7$ and the broken horizontal line represents the corrected threshold $-\log_{10}(p) = 4.5$ determined using permutation test.



Supplemental Fig. 5.5 Manhattan plots of genome-wide association analysis for seed protein content (%) using 175 *B. napus* inbred lines, derived from six *B. napus* × *B. oleracea* interspecific crosses, and 3,131 SNP markers; results from 10 individual field trials as well as LSmeans data from all trials are presented. ERS = Edmonton Research Station of the University of Alberta; GF-Killam = grower's Field at Killam; St. Albert-RF = St. Albert Research Farm of the University of Alberta. The solid horizontal line represents the Bonferroni significance threshold of $-\log_{10}(p) = 5.7$ and the broken horizontal line represents the corrected threshold $-\log_{10}(p) = 4.5$ determined using permutation test.

Chapter 6

6.1 General discussion

Canola (*Brassica napus*; AACC, 2*n* = 38) is the second largest oil crop in the world after soybean (Statista 2019). This amphidiploid species evolved from hybridization between turnip rape (*B. rapa*; AA, 2*n* = 20) and cabbage (*B. oleracea*; CC, 2*n* = 18) (Iniguez-Luy and Federico 2011) about 7500 years ago (Chalhoub et al. 2014). Evolution of this amphidiploid species from a limited number of variants of its progenitor species might be one of the reasons for the narrow genetic diversity currently seen in this crop (for review see Rahman 2013). Selection bottleneck in the last few decades for the canola quality traits (zero erucic acid and low glucosinolate) might be another cause of this narrow genetic diversity in *B. napus* (Cowling 2007; Bus et al. 2011), and this is an impediment for continued improvement of this crop ─ whether open-pollinated or hybrid cultivars (for review, see Rahman 2013; Jesske et al. 2013; Zhao et al. 2016). The development of high yielding canola cultivars with good agronomic and seed quality traits will help to meet the growing global demand for edible vegetable oil (Statista 2019). To achieve this, there is a need to enhance the genetic base of this crop through introduction of beneficial alleles from exotic germplasm including its parental species *B. rapa* and *B. oleracea* (Qian et al. 2006; Jesske et al. 2013; for review, see Rahman 2013; Zhang et al. 2015). While crossing a crop species to its exotic germplasm including allied species, introduction of undesirable alleles as well as disturbance in desired combinations of the alleles in crop germplasm can occur; this needs be taken into account while using any unadapted germplasm in breeding (Falk 2010). On the other hand, interspecific hybridization can also result a change in the genome through homoeologous recombination between the chromosomes (Udall et al. 2005; Leflon et al. 2006;

Zou et al. 2011) and this can create new genetic variation for different traits (Zou et al. 2011; Fu et al. 2012).

Exotic alleles of the allied species has been introgressed into *B. napus* by several research groups (Qian et al. 2006; Xiao et al. 2010; Rahman et al. 2011, 2015, 2017; Mei et al. 2011; Li et al. 2013; Li, Zhou et al. 2014; Attri and Rahman 2018; Iftikhar et al. 2018) which has broadened the genetic base of this crop to some extent. However, most researchers have focused on introducing the alleles from *B. rapa* (Qian et al. 2005, 2006; Xiao et al. 2010; Mei et al. 2011; Li et al. 2013; Attri and Rahman 2018), and this apparently has broadened the genetic base of the A genome of this crop relative to the C genome (Bus et al. 2011). Indeed, most of the Chinese semi-winter *B. napus* has *B. rapa* in their parentage (Qian et al. 2005; Zou, Zhu et al. 2010; Mei et al. 2011; Li et al. 2013). This makes this gene pool genetically distinct from other types of *B. napus*, such as the spring and winter types (Diers and Osborn 1994; Bus et al. 2011). In case of the C genome, wide morphological (reviewed in Ciancaleoni et al. 2014; El-Esawi, 2017) and genetic diversity (Pelc et al. 2015; Tortosa et al. 2017) exists in *B. oleracea* which make it a potential source of genetic variation for broadening the genetic base of the C genome of *B. napus* canola.

In addition to broadening the genetic base of our crop plants, an understanding of the genetic basis of agronomic and seed quality traits including seed yield is needed for efficient improvement of the crop through breeding. Today, with the availability of genome sequence information and high-density molecular markers, fine mapping of the genomic regions or QTLs can be done for different traits (Li, Chen et al. 2014; Luo et al. 2015; Schiessl et al. 2015; Körber et al. 2016; Sun et al. 2016; Wang, Chen, Xu et al. 2016; Luo et al. 2017), and this can help breeders to design molecular markers for use in knowledge-based breeding for the development of improved canola cultivars. Furthermore, using populations derived from wide crosses, novel

QTLs and beneficial alleles can be identified for use in breeding. For example, Rahman et al. (2017, 2018) identified a novel QTL on chromosome C1 associated with earliness of flowering and photo-insensitivity; this allele has been introgressed from *B. oleracea* var. *alboglabra* into *B. napus*.

To understand the effect of the C genome of different variants of *B. oleracea* in *B. napus*, I used six spring *B. napus* canola populations derived from crossing a single *B. napus* line A04-73NA to six *B. oleracea* accessions belonging var. *alboglabra* (line NRC-PBI), var. *botrytis* (cv. BARI Cauliflower-1), var. *capitata* (cvs. Badger Shipper, Bindsachsener, and Balbro), and var. *italica* (cv. Premium Crop) and studied the following: (i) genetic structure of the *B. napus* canola population; (ii) effect of the alleles introgressed from the C genome of *B. oleracea* on different agronomic and seed quality traits in *B. napus*; (iii) impact of the allelic diversity of these *B. oleracea* accessions on heterosis in *B. napus*; and (iv) identify the loci affecting different agronomic and seed quality traits.

Results from this study revealed a wide genetic variation within the inbred populations developed from the six *B. napus* × *B. oleracea* interspecific crosses and following use of two breeding techniques (F_2- and BC_1-derived). Unique alleles introgressed from *B. oleracea* into *B. napus* canola have been identified in our study. Consequently, the use of these inbred populations in canola breeding is expected to broaden the genetic base of Canadian spring *B. napus* canola, especially its C genome which is known to have a narrow genetic base (Bus et al. 2011). Results from this study also reveled that 75% of the unique SSR alleles of the *B. oleracea* parents introgressed into the *B. napus* population, where the greatest introgression occurred in the population derived from the cross involving var. *capitata* cv. Bindsachsener. The effect of these exotic alleles also reflected on the agronomic and seed quality traits; for example, the

population derived from the cross involving var. *alboglabra* exhibited a significant shorter plant height than the *B. napus* parent. Plant height showed a positive genotypic and phenotypic correlation with days to flower and maturity; however, no genotypic correlation of this trait was found with seed yield. This indicated that, favorable alleles for the development of early flowering and short-stature *B. napus* inbred lines can be introgressed from the *B. oleracea* gene pool. Favorable alleles of *B. oleracea* var. *alboglabra* contributing to earliness without any negative effect on seed yield has also been introgressed into *B. napus* by Rahman et al. (2017, 2018). In case of test hybrid population, days to flower do not show significant correlation with seed yield. However, several researches reported a significant negative correlation (Udall et al. 2004; Raman et al. 2016) or a non-significant correlation (Butruille et al. 1999) between these two traits in spring *B. napus*. This difference in the results might be due to the types of materials used and the field environmental condition under which these studies were being conducted.

The duration of flowering showed a significant negative correlation with seed yield in both inbred and test hybrid populations. This negative correlation might have resulted from the failure of the late flowering lines and test hybrids to reach physiological maturity at the time of harvest, as all plots were desiccated at the same time and this might have penalized the late-flowering and maturing ones. Negative correlation between the duration of flowering and seed yield has also been reported by Gan et al. (2016) under Canadian field conditions.

The inbred population derived from the cross involving var. *botrytis* had the highest seed oil content with seed yield comparable to the population derived from the cross involving var. *italica*. This suggests that this variant of *B. oleracea* carry novel alleles not only for high-oil but also for high seed yield for the improvement of *B. napus* canola ─ despite this variant of *B.*

oleracea has never been subjected to breeding for the improvement of seed oil content and seed yield.

The inbred population derived from the cross involving var. *capitata* cv. Badger shipper showed the greatest genetic distance from the *B. napus* parent and carried the greatest number of *B. oleracea* alleles as revealed using SSR markers. However, this population had low seed yield and oil content, and also flowered latest. Low oil content was also found in the inbred population derived from the cross involving var. *capitata* cv. Bindsachsener and Balbro. This indicate that several *B. oleracea* alleles may exert deleterious effect on the performance of *B. napus* inbred lines; therefore, a knowledge-based use of the *B. oleracea* alleles will be needed in a breeding program. The poor performance of the inbred lines derived from Elite (well adapted genotypes with desirable charateristics) × Exotic (non-adapted genotype carring desirable and underirable alleles) crosses can also result from disturbance in favorable combination of alleles of the elite lines (Falk 2010), linkage between favorable and unfavorable alleles in exotic germplasm (Holland 2014) or introgression of large genomic region carrying unfavorable alleles (reviewed in Primard-Brisset et al. 2005).

Almost no heterosis was found for seed oil and protein contents in test hybrid populations of the inbred lines derived from the six *B. napus* × *B. oleracea* interspecific crosses. Grant and Beversdorf (1985) and Rahman et al. (2016) also found no heterosis for these two seed quality traits; however, Shen et al. (2005) reported up to 7% mid-parent heterosis in hybrids of spring and semi-winter *B. napus* canola, and Cuthbert et al. (2011) reported up to 9% high-parent heterosis in spring high-erucic acid *B. napus* hybrids for seed oil content. Seed oil and protein contents are generally controlled by the genes exerting additive effect (for review, see Rahman et al. 2013); therefore, no heterosis for these traits would, theoretically, be expected. However, the

occurrence of heterosis for oil content by the above-mentioned researchers might be due to high oil content in the hybrid parent lines. As the oil and protein contents are mainly under additive genetic control, general combining ability (GCA) of both parents should be considered to improve these traits in a hybrid breeding program (Shen et al. 2005).

The inbred population derived from the cross involving var. *italica,* which was most similar to the *B. napus* parent in respect to SSR alleles, had the greatest seed yield and shortest duration of flowering; seed oil content of this population was comparable to the population derived from the cross involving var. *botrytis*. However, test hybrid of the inbred population derived from the cross involving *italica* gave the lowest yield. This might be due to the depletion of favorable heterotic alleles during the development of this population; it is also probable that the var. *italica* carry fewer heterotic alleles for seed yield as compared to the other three variants of *B. oleracea*.

While comparing the inbred lines derived from F_2- and BC_1 populations, SSR markers analysis showed a similar genetic distance of these two populations from the common *B. napus* parent. Theoretically, it was expected that backcrossing the F_1 plants to the canola parent would dilute the genome content of *B. oleracea* in the BC_1-derived population. However, the occurrence of a similar number of *B. oleracea* alleles in both F_2- and BC_1-derived lines indicate that, a greater loss of *B. oleracea* alleles occurred in the F_2-derived population as compared to the BC_1-derived population. This might have resulted from a stronger selection pressure for the canola quality traits in the F_2-derived population as compared to the BC_1-derived population. Attri and Rahman (2018) also reported the loss of *B. rapa* alleles in inbred lines derived from *B. napus* × *B. rapa* interspecific cross, with this loss found to occur by the F_4 generation. Despite these two populations carried a similar number of *B. oleracea* alleles, some phenotypic difference between them was found. For example, the F_2-derived population gave greater seed yield than the BC_1-

derived population, while the BC_1–derived population flowered and matured earlier than the F_2-derived population. In contrast, Schelfhout *et al.* (2008) found a greater number of lines with high seed yield in BC_1-derived population as compared to F_2-derived population of *B. napus* × *B. juncea* interspecific cross. However, when the test hybrid populations developed using these two types of inbreds were compared, no significant difference could be found between these two types of test hybrids for seed yield.

Genetic distance of the inbred lines from the *B. napus* parent, estimated using SSR markers, showed a weak and negative correlation with seed yield; however, a positive correlation was found in test hybrid population as well as for MPH. The performance of the inbred lines showed a weak correlation with the performance of the test hybrids, as well as with the level of MPH and NPH. This indicated that several alleles of *B. oleracea* in homozygous condition gave poor yield in the inbred population; however, at least, some of the alleles were capable of contributing to heterosis through a non-additive effect of the genes in the test hybrids. Rahman et al. (2016) also found that the alleles of *B. oleracea* var. *alboglabra* introgressed into *B. napus* can exert a non-additive effect in the genetic control of heterosis. Genetic distance of the inbred lines showed a positive correlation with days to flower, days to maturity and duration of flowering; but this correlation was weak in test hybrid population and was negative with MPH. This indicates that several *B. oleracea* alleles are not favorable in *B. napus* for these traits; however, the occurrence of several early flowering and maturing inbred lines in the population derived from the *B. napus* × *B. oleracea* crosses suggests that some of the *B. oleracea* alleles can contribute to earliness in *B. napus* canola. This further strengthen the results reported by Rahman et al. (2011, 2017, 2018) that favorable alleles for this as well as other traits can be found in *B. oleracea* for use in the breeding of *B. napus* canola.

As mentioned above, the population derived from *B. napus* × *B. oleracea* interspecific cross offers a valuable genetic resource for identification of new loci and alleles in the C genome for different agronomic and seed quality traits. A genome-wide association study using the above-mentioned inbred population and 3,131 SNP markers detected several QTLs for days to flowering, and seed oil and glucosinolate contents; some of the QTLs/alleles has not been reported previously. For example, using natural *B. napus* populations, several researches (Quijada et al. 2006; Luo et al. 2014; Wei et al. 2014; Körber et al. 2016; Wang, Chen, Xu et al. 2016; Rahman et al. 2017; He et al. 2018; Jian et al. 2019) detected QTLs for days to flowering on different C-genome chromosomes including C5. The genome sequence information of *B. napus* (Chalhoub et al. 2014) gave the opportunity to compare the physical positions of the QTLs from different study. Based on this, the QTLs on C5 identified at 28.7 Mb and 31.3 Mb positions seems to be novel, with these QTLs having not been reported previously. Alleles within these QTLs contributed to the earliness of flowering derived from *B. oleracea* var. *capitata* cv. Bindsachsener. QTLs on C1, C3, C5, C7 and C8 affecting seed oil content, and QTLs on C1, C2, C3, C5 and C6 affecting seed glucosinolate content were also identified in this study. Previously, several researchers detected QTLs on all nine C genome chromosomes affecting seed oil content (Delourme et al. 2006; Qiu et al. 2006; Chen, Geng et al. 2010; Zou, Jiang et al 2010; Wang et al. 2013; Huang et al. 2016; Körber et al. 2016; Chao et al. 2017; Xiao et al. 2019) and QTLs on C1, C2, C3, C5, C7, C8 and C9 affecting seed glucosinolate content (Zhao and Meng 2003; Feng et al. 2011; Körber et al. 2016; He et al. 2018; Liu et al. 2020). Comparison of the physical position of the C3, C5 and C7 QTLs for oil content detected in this study with the QTLs reported by Körber et al. (2016) and Xiao et al. (2019) showed that these QTLs are located at different positions. Similarly, comparison of the physical position of the C5 QTL for seed glucosinolate

content detected in this study with the C5 QTL reported by Körber et al. (2016) showed that these two QTLs are located about 30 Mb away from each other. Thus, the results from this Ph.D. thesis research provided evidence that the *B. oleracea* gene pool can be used for broadening the genetic base of *B. napus* canola and in the breeding of *B. napus* canola to improve seed yield and agronomic and seed quality traits in open-pollinated and hybrid cultivars, as well as to identify novel QTLs and alleles for different traits.

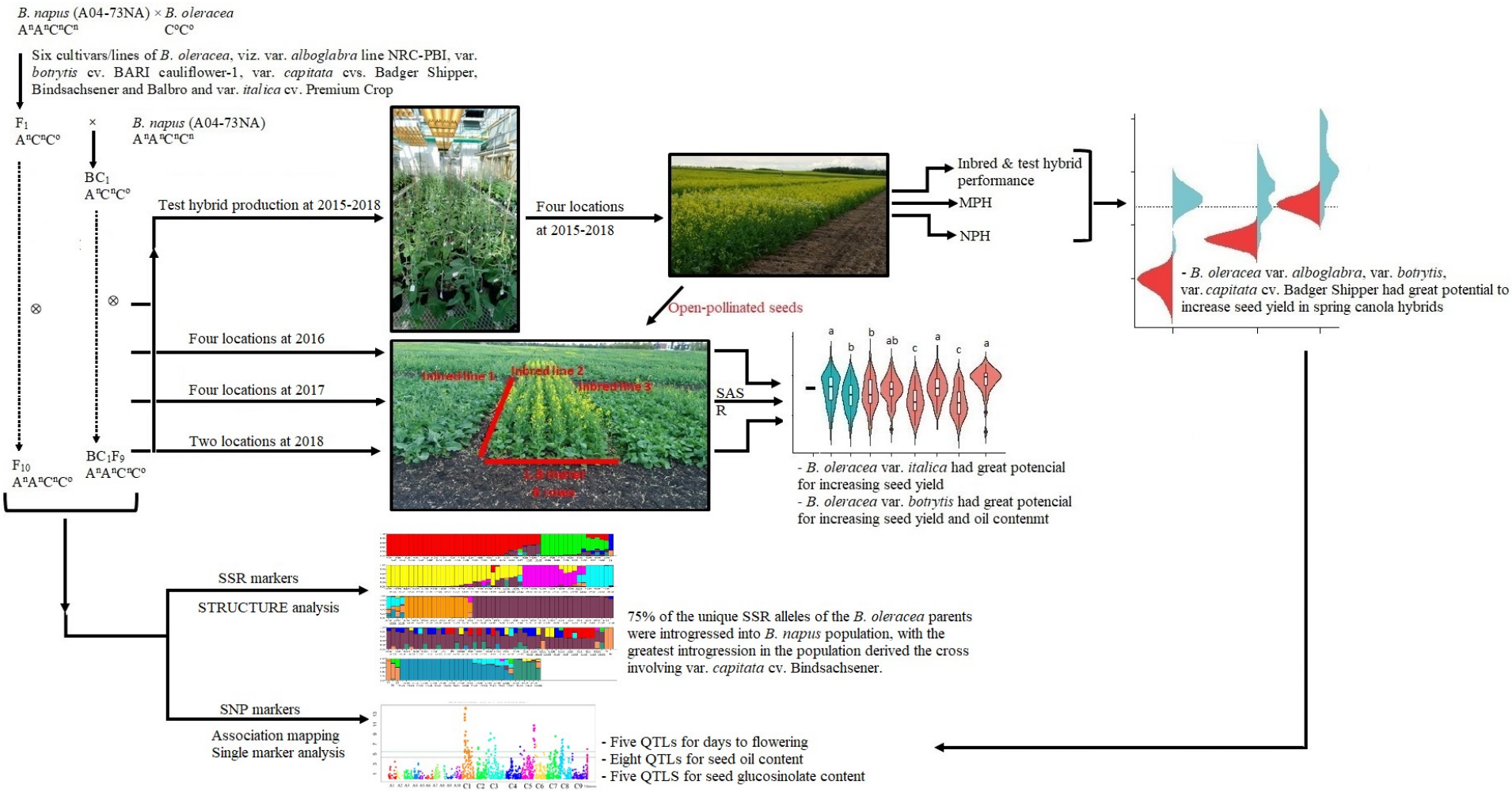


Fig. 6.1 An overview of the PhD thesis research and the results obtained from this study.
MPH = mid-parent heterosis; NPH = heterosis over the common *B. napus* parent; SAS = the statistical software program of SAS; R = R project for statistical computing; SSR = simple sequence repeat; SNP = single nucleotide polymorphism; QTL = Quatitative trait loci.

6.2 General conclusion, impact and future study

In conclusion, genetic diversity analysis of the *B. napus* inbred lines derived from *B. napus* × *B. oleracea* interspecific crosses as well as GWAS with this population showed that novel alleles from the *B. oleracea* gene pool can be introgressed into *B. napus* canola. However, loss of several alleles can occur in the developed *B. napus* lines in advanced generation suggesting the need for selection in early generation for retention of a greater number of *B. oleracea* alleles during the development of canola quality inbred lines. Performance of the *B. napus* inbred lines demonstrated that, among the different variants of *B. oleracea* gene pool, var. *italica* carries the greatest potential for increasing seed yield in *B. napus*, while var. *botrytis* holds promise for increasing both seed yield and oil content. Despite introgression of unwanted alleles of *B. oleracea* has been observed in the *B. napus* inbred population, several lines with high seed yield (139, 22, 3, 99 and 102), earliness of flowering (264, 52, 268, 36 and 265) and high oil content (65, 22, 49, 66 and 3) can be selected from this population (Supplemental Table 2.1). While studying the impact of the *B. oleracea* gene pool on heterosis in *B. napus*, the var. *botrytis*, var. *alboglabra* and some of the accessions of the var. *capitata* showed great potential for the improvement of the *B. napus* hybrids cultivars. Finally, the value of the *B. oleracea* gene pool for use in the breeding of *B. napus* canola has been demonstrated by QTL mapping of flowering time and seed oil and glucosinolate contents, and through identification of novel alleles, e.g. flowering time allele of *B. oleracea* contributing to the earliness in *B. napus*. Thus, the use of *B. oleracea* in the breeding of *B. napus* canola is expected to improve the open-pollinated as well as hybrid cultivars of this crop.

The following additional studies using the inbred and test hybrid populations expected to further extend our knowledge of the utility of the *B. oleracea* gene pool for the improvement of *B. napus* canola cultivars:

1. The genomic regions contributing to heterosis for different agronomic and seed quality traits need to be identified using the genotypic and phenotypic data of the inbred and test hybrid populations by the use of association mapping approach.

2. The candidate genes affecting flowering time, seed oil and glucosinolate contents need to be identified through fine mapping of the QTLs reported in this study.

3. Additive and epistatic gene effects for different agronomic and seed quality traits need to be identified through analysis of the inbred lines with the minimal and maximal expression of the traits using quantile method.

4. The inbred lines of Supplemental Table 3.8 can be crossed in a diallel mating design to estimate general and specific combining ability.

5. The very early-flowering inbred lines identified in this study can be used to understand the molecular basis of flowering time and their effect on the physiology of the plants.

6. Funcational SNPs affecting the level of gene expression can be identified for flowering time, seed oil and glucosinolate content by the exclusion of nonfunctional SNPs and the orthologous and paralogous genes.

References

Abbadi A and Leckband G (2011) Rapeseed breeding for oil content, quality, and sustainability. Eur. J. Lipid Sci. Technol. 113:1198–1206. doi:10.1002/ejlt.201100063

Abel S, Möllers C and Becker HC (2005) Development of synthetic *Brassica napus* lines for the analysis of "fixed heterosis" in allopolyploid plants. Euphytica 146:157–163. doi:10.1007/s10681-005-3364-7

Agrama H, Eizenga G and Yan W (2007) Association mapping of yield and its components in rice cultivars. Mol. Breed. 19(4):341–56. doi:10.1007/s11032-006-9066-6

Akbar MA (1989) Resynthesis of *Brassica napus* aiming for improved earliness and carried out by different approaches. Hereditas 111:239–246. doi:10.1111/j.1601-5223.1990.tb00402.x

Ali M, Copeland L, Elias S and Kelly J (1995) Relationship between genetic distance and heterosis for yield and morphological traits in dry edible bean and faba bean. Theor. Appl. Genet. 91:118–121. doi:10.1007/BF00220867

Allender CJ and King GJ (2010) Origins of the amphiploid species *Brassica napus* L. investigated by chloroplast and nuclear molecular markers. BMC Plant Biol. 10:54-62. doi:10.1186/1471-2229-10-54

Anio A (2001) Genetic variation, development and availability of useful germplasm plant breeding. Plant breed. Seed Sci. 45:33-43.

Annisa, Chen S and Cowling W (2013) Global genetic diversity in oilseed *Brassica rapa*. Crop Pasture Sci. 64:993-1007. doi:10.1071/CP13206

Armstrong KC and Keller WA (1980) Chromosome pairing in haploids of *Brassica oleracea*. Genome 24:735-739. doi:10.1139/g82-079

Attia T and Robbelen G (1986a) Cytogenetic relationship within cultivated Brassica analyzed in amphihaploids from the three diploid ancestors. Can. J. Genet. Cytol. 28:323–329.

Attia T and Robbelen G (1986b) Meiotic pairing in haploid and amphidiploids of spontaneous versus synthetic origin in *rape*, *Brassica napus* L. Can. J. Genet. Cytol. 28:330–334.

Attia T, Busso C and Robbelen G (1987) Digenomic triploids for an assessment of chromosome relationships in the cultivated diploid *Brassica* species. Genome 29:326–330. doi:10.1139/g87-053

Attri R and Rahman H (2018) Introgression of allelic diversity from genetically distinct variants of *Brassica rapa* into *Brassica napus* canola and inheritance of the *B. rapa* alleles. Crop Pasture Sci. 69:94-106. doi:10.1071/CP17193

Babula D, Kaczmarek M, Ziolkowski PA and Sadowski J (2007) *Brassica oleracea*. In Kole C (Ed.), Genome Mapping and Molecular Breeding in Plants. Springer, Berlin. p. 227–285.

Bansal P, Banga S and Banga SS (2012) Heterosis as investigated in terms of polyploidy and

genetic diversity usig designed *Brassica juncea* amphiploid and its progenitor diploid species. PLoS One 7:e29607. doi:10.1371/journal.pone.0029607

Basunanda P, Radoev M, Ecke W, Friedt W, Becker H and Snowdon RJ (2010) Comparative mapping of quantitative trait loci involved in heterosis for seedling and yield traits in oilseed rape (*Brassica napus* L.). Theor. Appl. Genet. 120:271-281. doi:10.1007/s00122-009-1133-z

Basunanda P, Spiller TH, Hasan M, Gehringer A, Schondelmaier J, Lühs W, Friedt W and Snowdon RJ (2007) Marker-assisted increase of genetic diversity in a double-low seed quality winter oilseed rape genetic background. Plant Breed. 126:581-587. doi:10.1111/j.1439-0523.2007.01404.x

Batley J, Hopkins CJ, Cogan NOI, Hand M, Jewell E, Kaur J, Kaur S, Li X, Ling AE, Love C, Mountford H, Todorovic M, Vardy M, Walkiewicz M, Spangenberg GC and Edwards D (2007) Identification and characterisation of simple sequence repeat (SSR) markers from *Brassica napus* expressed sequences. Mol. Ecol. Notes 7:886-889. doi:10.1111/j.1471-8286.2007.01738.x

Becker HC, Engqvist GM and Karlsson B (1995) Comparison of rapeseed cultivars and resynthesized lines based on allozyme and RFLP markers. Theor. Appl. Genet. 91:62–67. doi:10.1007/BF00220859

Bell JM (1984) Nutrient and toxicants in rapeseed meal: a review. J. Anim. Sci. 58:996-1010. doi:10.2527/jas1984.584996x

Bennett RA, Séguin-Swartz G and Rahman H (2012) Broadening genetic diversity in canola using C-genome species *Brassica oleracea* L. Crop Sci. 52:2030–2039. doi:10.2135/cropsci2011.11.0580

Bennett RA, Thiagarajah MR, King JR and Rahman MH (2008) Interspecific cross of *Brassica oleracea* var. *alboglabra* and *B. napus*: effects of growth condition and silique age on the efficiency of hybrid production, and inheritance of erucic acid in the self-pollinated backcross generation. Euphytica 164:593-601. doi:10.1007/s10681-008-9788-0

Bohuon EJR, Ramsay LD, Craft JA, Arthur AE, Marshall DF, Lydiate DJ and Kearsey MJ (1998) The association of flowering time quantitative trait loci with duplicated regions and candidate loci in *Brassica oleracea*. Genetics 150:393-401. PMID:9725855

Boopathi NM (2013) Genetic mapping and marker assisted selection: basics, practice and benefits. Springer India. pp 45-60. doi:10.1007/978-81-322-0958-4_1

Borcard D, Gillet F and Legendre P (2018) Numerical ecology with R. Springer Int. Publ., Cham, Switzerland. p. 151–174. doi:10.1007/978-3-319-71404-2_5

Branca F (2008) Cauliflower and broccoli. In Prohens J and Nuez F (Eds.) Vegetables I. Springer, New York, NY, pp 147–182

Branca F and Cartea E (2011) *Brassica*. In Kole C (Ed.) Wild crop relatives: genomic and breeding resources oilseeds. Springer Heidelberg Dordrecht London New York. pp 17-36

Brandle JE and McVetty PBE (1989) Hetrosis and combining ability in hybrids derived from

oilseed rape cultivars and inbred lines. Crop Sci. 29:1191-1195.

Brichler JA, Auger DL and Riddle NC (2003) In search of the molecular basis of heterosis. Plant Cell 15:2236–2239. doi:10.1105/tpc.151030

Brown AF, Yousef GG, Chebrolu KK, Byrd RW, Everhart KW, Thomas A, Reid RW, Parkin IAP, Sharpe AG, Oliver R, Guzman I and Jackson EW (2014) High density single nucleotide polymorphism (SNP) array mapping in *Brassica oleracea*: identification of QTL associated with carotenoid variation in broccoli florets. Theor. Appl. Genet. 127:2051- 2064. doi:10.1007/s00122-014-2360-5

Brown J and Brown AP (1996) Gene transfer between canola (*Brassica napus* L. and *B. campestris* L.) and related weed species. Ann. Appl. Biol. 129:513–522. doi:10.1111/j.1744-7348.1996.tb05773.x

Bruce A (1910) The Mendelian theory of heredity and the augmentation of vigor. Science 32:627-628. doi:10.1126/science.32.827.627-a

Burns MJ, Barnes SR, Bowman JC, Clarke MHE, Werner CP and Kearsey MJ (2003) QTL analysis of an intervarietal set of substitution lines in *Brassica napus*: (I) Seed oil content and fatty acid composition. Heredity 90:39-48. doi:10.1038/sj.hdy.6800176

Bus A, Hecht J, Huettel B, Reinhardt R and Stich B (2012) High-throughput polymorphism detection and genotyping in *Brassica napus* using next-generation RAD sequencing. BMC genomics 13:281. doi:10.1186/1471-2164-13-281

Bus A, Körber N, Parkin IAP, Samans B, Snowdon RJ, Li J and Stich B (2014) Species- and genome-wide dissection of the shoot ionome in *Brassica napus* and its relationship to seedling development. Front. Plant Sci. 5:485-497. doi:10.3389/fpls.2014.00485

Bus A, Körber N, Snowdon RJ and Stich B (2011) Patterns of molecular variation in a species-wide germplasm set of *Brassica napus*. Theor. Appl. Genet. 123:1413–1423. doi:10.1007/s00122-011-1676-7

Butruille DV, Guries RP and Osborn TC (1999) Increasing yield of spring oilseed rape hybrids through introgression of winter germplasm. Crop Sci. 39:1491-1496. doi:10.2135/cropsci1999.3951491x

Buzza GC (1995) Plant breeding. In Kimber DS and McGregor DI (Eds.) *Brassica* Oilseeds: production and utilization. CAB International, pp.153-175.

Cai D, Xiao Y, Yang W, Ye W, Wang B, Younas M, Wu J and Liu K (2014) Association mapping of six yield-related traits in rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 127:85-96. doi:10.1007/s00122-013-2203-9

Canola Council of Canada (1999) Low erucic acid rapeseed oil derived from canola-quality (low erucic acid, low glucosinolate) *Brassica juncea* (L.) Czern. Winnipeg, Manitoba, Canada. Retrieved from https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-foods-gen/documents/document/ucm265975.pdf.

Canola Council of Canada (2016) Industry Overview. [ONLINE] Available at: http://www.canolacouncil.org/markets-stats/industry-overview/ (Accessed 22 May 2016)

Canola Council of Canada (2018) Health benefits of canola oil. [ONLINE] Available at: http://www.canolacouncil.org/oil-and-meal/canola-oil/health-benefits-of-canola-oil/ (Accessed 28 May 2018).

Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. J. Hered. 99:616-623. doi:10.1093/jhered/esn048

Chalhoub B, Denoeud F, Liu S, Parkin IAP, Tang H, Wang X, Chiquet J, Belcram H, Tong C, Samans B, Correa M, Da Silva C, Just J, Falentin C, Koh CS, Clainche IL. Bernard M and et al. (2014) Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science 345:950-953. doi:10.1126/science.1253435

Channa SA, Tian H, Mohammed MI, Zhang R, faisal S, Guo Y, Klima M, Stamm M and Hu S (2018) Heterosis and combining ability analysis in Chinese semi-winter × exotic accessions of rapeseed (*Brassica napus* L.). Euphytica 214:134. doi:10.1007/s10681-018-2216-1

Chao H, Wang H, Wang X, Guo L, Gu J, Zhao W, Li B, Chen D, Raboanatahiry N and Li M (2017) Genetic dissection of seed oil and protein content and identification of networks associated with oil content in *Brassica napus*. Sci. Rep. 7:46295. doi:10.1038/srep46295

Chen G, Geng J, Rahman M, Liu X, Tu J, Fu T, Li G, McVetty PBE and Tahir M (2010) Identification of QTL for oil content, seed yield, and flowering time in oilseed rape (*Brassica napus*). Euphytica 175:161-174. doi:10.1007/s10681-010-0144-9

Chen L, Wan H, Qian J, Guo J, Sun C, Wen J, Yi B, Ma C, Tu J, Song L, Fu T and Shen J (2018) Genome-wide association study of cadmium accumulation at the seedling stage in rapeseed (*Brassica napus* L.). Front. Plant Sci. 9. doi:10.3389/fpls.2018.00375

Chen R, Hara T, Ohsawa R and Yoshioka Y (2017) Analysis of genetic diversity of rapeseed genetic resources in Japan and core collection construction. Breed. Sci. 67:239-247. doi:10.1270/jsbbs.16192

Chen S, Nelson M, Ghamkhar K and Cowling W (2011) Genetic diversity and distinctiveness revealed by SSR markers among rapeseed (*Brassica napus* L.) genotypes from Australia, China and India. In: Proceedings of the 12th International Rapeseed Congress 2:248-252.

Chen S, Nelson MN, Ghamkhar K, Fu T and Cowling W (2007) Divergent patterns of allelic diversity from similar origins: the case of oilseed rape (*Brassica napus* L.) in China and Australia. Genome 51:1-10. doi:10.1139/G07-095

Chen S, Nelson MN, Ghamkhar K, Fu T and Cowling W (2008) Divergent patterns of allelic diversity from similar origins: the case of oilseed rape (*Brassica napus* L.) in China and Australia. Genome 51:1–10. doi:10.1139/g07-095

Chen S, Zou J, Cowling W and Meng J (2010) Allelic diversity in a novel gene pool of canola-quality *Brassica napus* enriched with alleles from *B. rapa* and *B. carinata*. Crop Pasture Sci. 61:483–492. doi:10.1071/CP09327

Chen ZJ (2010) Molecular mechanisms of polyploidy and hybrid vigor. Trends Plant Sci. 15:57-85. doi:10.1016/j.tplants.2009.12.003

Chen ZJ (2013) Genomic and epigenetic insights into the molecular bases of heterosis. Nat. Rev. Genet. 14:471–482. doi:10.1038/nrg3503

Cheng F, Mandakova T, Wu J, Lysak MA and Wang X (2013) Deciphering the diploid ancestral genome of the mesohexaploid *Brassica rapa*. Plant Cell 25:1541-1554. doi:10.1105/tpc.113.110486

Cheng X, Xia S, Zeng X, Gu J, Yang Y, Xu J, Liu C, Liu K and Wu J (2016) Identification of quantitative trait loci associated with oil content and development of near isogenic lines for stable *qOC-A10* in *Brassica napus* L. Can. J. Plant Sci. 96:423-432. doi:10.1139/cjps-2014-0442

Cheng X, Xu J, Xia S, Gu J, Yang Y, Fu J, Qian X, Zhang S, Wu J and Liu K (2009) Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor. Appl. Genet. 118:1121-1131. doi:10.1007/s00122-009-0967-8

Cheung F, Trick M, Drou N, Lim YP, Park JY, Kwon SJ, Kim JA, Scott R, Pires JC, Paterson AH, Town C and Bancroft I (2009) Comparative analysis between homoeologous genome segments of *Brassica napus* and its progenitor species reveals extensive sequence-level divergence. Plant Cell 21:1912-1928. doi:10.1105/tpc.108.060376

Chou H-H and Holmes M (2001) DNA sequence quality trimming and vector removal. Bioinformatics 17:1093-1104. doi:10.1093/bioinformatics/17.12.1093

Christensen S, Bothmer R, Poulsen G, Maggioni L, Phillip M, Andersen BA and Jørgensen RB (2011) AFLP analysis of genetic diversity in leafy kale (*Brassica oleracea* L. convar. *acephala* (DC.) Alef.) landraces, cultivars and wild populations in Europe. Genet. Resour. Crop Ev. 58:657–666. doi:10.1007/s10722-010-9607-z

Christophersen BO and Bremer J (1972) Erucic acid — an inhibitor of fatty acid oxidation in the heart. Biochim. Biophys. Acta, Lipids Lipid Metab. 280:506-514. doi:10.1016/0005-2760(72)90130-0

Ciancaleoni S, Chiarenza GL, Raggi L, Branca F and Negri V (2014) Diversity characterization of broccoli (*Brassica oleracea* L. var. *italica* Plenck) landraces for their on-farm (in situ) safeguard and use in breeding programs. Genet. Resour. Crop Evol. 61:451–464. doi:10.1007/s10722-013-0049-2

Clandinin MT and Yamashiro S (1982) Dietary factors affecting the incidence of dietary fat-induced myocardial lesions. J. Nutr. 112:825-828. doi:10.1093/jn/112.4.825

Clarke WE, Higgins EE, Plieske J, Wieseke R, Sidebottom Ch, Khedikar Y, Batley J, Edwards D, Meng J, Li R, Lawley CT, Pauquet J, Laga B, Cheung W, Iniguez-Luy F and et al. (2016) A high-density SNP genotyping array for *Brassica napus* and its ancestral diploid species based on optimised selection of single-locus markers in the allotetraploid genome. Theor. Appl. Genet. 129:1887-1899. doi:10.1007/s00122-016-2746-7

Cockerham CC and Zeng ZB (1996) Design III with marker loci. Genetics 143:1437-1456.

PMID:8807314

Collins GN (1921) Dominance and vigor of first generation hybrids. Am. Nat. 55:116-133. doi:10.1086/279800

Cowling W (2007) Genetic diversity in Australian canola and implications for crop breeding for changing future environments. Field Crop Res. 104:103–111. doi:10.1016/j.fcr.2006.12.014

Crow JF (1948) Alternative hypotheses of hybrid vigor. Genetics 33:477-487. PMID:17247292

Crum CW, Prescott JM and Christensen PJ (1993) Genetic approaches to increased nutritional value in oilseed meal. In Applewhite TH (Eds.). In: Proceeding of the World Conference on Oilseed Technology and Utilization. Budapest.

Cuthbert RD, Crow G and MCVetty PBE (2009) Assessment of agronomic performance and heterosis for agronomic traits in hybrid high erucic acid rapeseed (HEAR). Can. J. Plant Sci. 89:227–237. doi:10.4141/CJPS08160

Cuthbert RD, Crow G and McVetty PBE (2011) Assessment of seed quality performance and heterosis for seed quality traits in hybrid high erucic acid rapeseed (HEAR). Can. J. Plant Sci. 91:837-846. doi: /10.4141/cjps10205

Delourme R, Falentin C, Fomeiu BF, Boillot M, Lassalle G, André I, Duarte J, Gauthier V, Lucante N, Marty A, Pauchon M, Pichon JP, Ribiére N, Trotoux G, Blanchard P, Ribiére N, Martinant JP and Pauquet J (2013) High-density SNP-based genetic map development and linkage disequilibrium assessment in *Brassica napus* L. BMC Genomics 14:120-138. doi:10.1186/1471-2164-14-120

Delourme R, Falentin C, Huteau V, Clouet V, Horvais R, Gandon B, Specel S, Hanneton L, Dheu JE, Deschamps M, Margale E, Vincourt P and Renard M (2006) Genetic control of oil content in rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 113:1331–1345. doi:10.1007/s00122-006-0386-z

Diers BW and Osborn TC (1994) Genetic diversity of oilseed *Brassica napus* germplasm based on restriction fragment length polymorphisms. Theor. Appl. Genet. 88:662-668. doi: 10.1007/BF01253968

Diers BW, Mcvetty PBE and Osborn TC (1996) Relationship between heterosis and genetic distance based on Restriction Fragment Length Polymorphism markers in oilseed rape (*Brassica napus* L.). Crop Sci. 36:79–83. doi:10.2135/cropsci1996.0011183X003600010014x

dos Santos JB, Nienhuis J, Skroch P and Tivang J (1994) Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes. Theor. Appl. Genet. 87:909–915. doi:10.1007/BF00225784

Downey RK (1964) A selection of *Brassica campestris* L. containing no erucic acid in its seed oil. Can. J. Plant Sci. 44:295. doi:10.4141/cjps64-057

Downey RK and Harvey BL (1963) Methods of breeding for oil quality in rape. Can. J. Plant Sci. 43:271-275. doi:10.4141/cjps63-054

Downey RK, Klassen AJ and Stringam GP (1980) Rapeseed and mustard. In: hybridization of Crop Plants. Am. Soc. Crop Sci. 495-509.

Duran C, Appleby N, Edwards D and Batley J (2009b) Molecular genetic markers: discovery, applications, data storage and visualization. Curr. Bioinform. 4:16-27. doi:10.2174/157489309787158198

Duran C, Edwards D and Batley J (2009a) Genetic maps and the use of synteny. In Somers DL, Langridge DJ and Gustafson P (Eds.) Plant genomics, methods and protocols. Humana Press, USA. pp 41-56.

Earl DA and VonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4:359-361. doi:10.1007/s12686-011-9548-7

East EM (1936) Heterosis. Genetics 21:375-397.

Edwards J and Hertel K (2011) Canola: growth and development. State of New South Wales Department of Primary Industries. pp 1–96.

El-Esawi MA (2017) Genetic diversity and evolution of *Brassica* genetic resources: from morphology to novel genomic technologies — a review. Plant Genet. Resour. 15:388–399. doi:10.1017/S1479262116000058

El-Esawi MA, Germaine K, Bourke P and Malone R (2016) Genetic diversity and population structure of *Brassica oleracea* germplasm in Ireland using SSR markers. C. R. Biol. 339:1– 8. doi:10.1016/j.crvi.2016.02.002

Ertiro BT, Semagn K, Das B, Olsen M, Labuschange M, Worku M, Wegary D, Azmach G, Ogugo V, Keno T, Abebe B, Chibsa T and Menkir A (2017) Genetic variation and population structure of maize inbred lines adapted to the mid-altitude sub-humid maiz agro-ecology of Ethiopia using single nucleotide polymorphic (SNP) markers. BMC Genomics 18:777- 788. doi:10.1186/s12864-017-4173-9

Evanno G, Regnaut S and Goudet J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14:2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Falk DE (2010) Generating and maintain diversity at the elite level in crop breeding. Genome 53:982–991. doi:10.1139/ G10-081

Faltusová Z, Kučera L and Ovesná J (2011) Genetic diversity of *Brassica oleracea* var. *capitata* gene bank accessions assessed by AFLP. Electron. J. Biotechn. 14:1-10. doi:10.2225/vol14-issue3-fulltext-4

Feng J, Long Y, Shi L, Shi J, Barker G and Meng J (2011) Characterization of metabolite quantitative trait loci and metabolic networks that control glucosinolate concentration in the seeds and leaves of *Brassica napus*. New Phytologist 193:96–108 doi:10.1111/j.1469-8137.2011.03890.x

Flint-Garcia S, Thornsberry J and Buckler E (2003) Structure of linkage disequilibrium in plants. Annu. Rev. Plant Biol. 54:357-374. doi:10.1146/annurev.arplant.54.031902.134907

Flint-Garcia SA, Buckler ES, Tiffin P, Ersoz E and Springer NM (2009) Heterosis is prevalent for multiple traits in diverse maize germplasm. PLoS One 4:e7433. doi:10.1371/journal.pone.0007433

Food and Agriculture Organization of the United Nations (FAO) (2018) Top 10 country production of rapeseed [ONLINE] Available at: http://www.fao.org/faostat/en/#rankings/countries_by_commodity (Accessed 16 April 2019).

Fopa-Fomeju B, Falentin C, Lassalle G, Manzanares Dauleux MJ and Delourme R (2014) Homoeologous duplicated regions are involved in quantitative resistance of *Brassica napus* to stem canker. BMC Genomics 15:498.

Fourmann M, Barret P, Renard M, Pelletier G, Delourme R and Brunel D (1998) The two genes homologous to *Arabidopsis* FAE1 cosegregate with the two loci governing erucic acid content in *Brassica napus*. Theor. Appl. Genet. 96:852–858. doi:10.1007/s001220050812

Frascaroli E, Cane MA, Landi P, Pea GG, Villa M, Morgante M and Me P (2007) Classical genetic and quantitative trait loci analyses of heterosis in a maize hybrid between two elite inbred lines. Genetics 176:625–644. doi:10.1534/genetics.106.064493

Fritsche S, Wang X, Li J, Stich B, Kopisch-Obuch FJ, Endrigkeit J, Leckband G, Dreyer F, Friedt W, Meng J and Jung C (2012) A candidate gene-based association study of tocopherol content and composition in rapeseed (*Brassica napus*). Front. Plant Sci. 3:129-143. doi:10.3389/fpls.2012.00129

Fu D, Qian W, Zou J and Meng J (2012) Genetic dissection of intersubgenomic heterosis in *Brassica napus* carrying genomic components of *B. rapa*. *Euphytica* 184:151-164. doi:10.1007/s10681-011-0533-8

Fu Y-B and Gugel RK (2009) Genetic variability of Canadian elite cultivars of summer turnip rape (*Brassica rapa* L.) revealed by simple sequence repeat markers. Can. J. Plant Sci. 89:865–874. doi:10.4141/CJPS09021

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

Fujimoto R, Uezono K, Ishikura S, Osabe K, Peacock WJ and Dennic ES (2018) Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems. Breed. Sci. 68:145-158. doi:10.1270/jsbbs.17155

Gacek K, Bartkowiak-Broda I and Batley J (2018) Genetic and molecular regulation of seed proteins (SSPs) to improve protein nutritional value of oilseed rape (*Brassica napus* L.) seeds. Front. Plant Sci. 9:890. doi:10.3389/fpls.2018.00890

Gan Y, Harker KN, Kutcher HR, Gulden RH, Irvine B, May WE and O'Donovan JT (2016) Canola seed yield and phenological responses to plant density. Can. J. Plant Sci. 96:151–159. doi:10.1139/cjps-2015-0093

Gao C, Yin J, Mason AS, Tang Z, Ren X, Li C, An Z, Fu D and Li J (2014) Regularities in simple sequence repeat variations induced by a cross of resynthesized *Brassica napus* and natural *Brassica napus*. Plant Omics 7:35-46. ISSN:1836-3644

Girke A, Schierholt A and Becker HC (2012a) Extending the rapeseed gene pool with resynthesized *Brassica napus* L. I: Genetic diversity. Genet. Resour. Crop Evol. 59:1441–1447. doi:10.1007/s10722-011-9772-8

Girke A, Schierholt A and Becker HC (2012b) Extending the rapeseed gene pool with resynthesized *Brassica napus* II: Heterosis. Theor. Appl. Genet. 124:1017–1026. doi:10.1007/s00122-011-1765-7

Grant I and Beversdorf WD (1985) Heterosis and combining ability estimates in spring-planted oilseed rape (*Brassica napus* L.). Can. J. Genet. Cytol. 27:472-478. doi:10.1139/g85-069

Guo M and Rafalski JA (2013) Gene expression and heterosis in maize hybrids. In Chen ZJ and Birchler JA (Eds.) Polyploid and hybrid genomics. John Wiley & Sons, Inc. pp 59–84. doi:10.1002/9781118552872.ch4

Guo S, Zhang X, Zeng D, Zhang M, Niu Y, Wang L and Liu Y (2016) genetic diversity of resynthesized *Brassica napus* lines from SW China assessed by main agronomic traits and SSR markers in comparison with common *B. napus* lines. Euphytica 207:95–108. doi:10.1007/s10681-015-1540-y

Gyawali S, Harrington M, Durkin J, Horner K, Parkin IAP, Hegedus DD, Bekkaoui D and Buchwaldt L (2016) Microsatellite markers used for genome-wide association mapping of partial resistance to *Sclerotinia sclerotiorum*in a world collection of *Brassica napus*. Mol. Breed. 36:72-85. DOI 10.1007/s11032-016-0496-5

Gyawali S, Hegedus DD, Parkin IAP, Poon J, Higgins EE, Horner K, Bekkaoui DR, Coutu C and Buchwaldt L (2013) Genetic diversity and population structure in a world collection of *Brassica napus* accessions with emphasis on those from South Korea, Japan and Pakistan. Crop Sci. 53:1537–1545. doi:10.2135/cropsci2012.10.0614

Hasan M, Seyis F, Badani AG, Pons-Kühnemann J, Friedt W, Lühs W and Snowdon RJ (2006) Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet. Resour. Crop Evol. 53:793-802. doi:10.1007/s10722-004-5541-2

Hasan MJ, Strelkov SE, Howard RJ and Rahman H (2012) Screening of *Brassica* germplasm for resistance to *Plasmodiophora brassica* pathotypes prevalent in Canada for broadening diversity in clubroot resistance. Can. J. Plant Sci. 92:501–515. doi:10.4141/cjps2010-006

He Y, Fu Y, Hu D, Wei D and Qian W (2018) QTL Mapping of Seed Glucosinolate Content Responsible for Environment in *Brassica napus*. Front. Plant Sci. 9:891. doi:10.3389/fpls.2018.00891

He Y, Wu D, Wei D, Fu Y, Cui Y, Dong H, Tan C and Qian W (2017) GWAS, QTL mapping and gene expression analyses in *Brassica napus* genetic control of branching morphogenesis. Sci. Rep. 7:15971. doi:10.1038/s41598-017-15976-4

Herve Y (2003) Choux. In Pitrat M and Foury C (Eds.) History de legumes, des origins a l'oree du XXI siecle. INRA, Paris, France

Higgins EE, Clarke WE, Howell EC, Armstrong SJ and Parkin IAP (2018). Detecting *de Novo* homoeologous recombination events in cultivated *Brassica napus* using a genome-wide SNP array. G3 8:2673-2683. doi.org/10.25387/ g3.6207530.

Hobson N and Rahman H (2016) Genome-wide identification of SSR markers in the *Brassica* A genome and their utility in breeding. Can. J. Plant Sci. 96:808-818. doi:10.1139/cjps-2015-0250

Hochholdinger F and Baldauf J (2018) heterosis in plants. Curr. Biol. 28:R1075-R1095.

Holland JB (2006) Breeding: plants, modern. In: N.K.V. Alfen, editor, Encyclopedia of agriculture and food systems. Vol. 2. Acad. Press, London. p. 187–200.

Holland JB (2014) Breeding: plants, modern. Enc. Agr. Food Systems 2:187-200. Enc. doi:10.1016/B978-0-444-52512-3.00226-6

Hollick JB and Chandler VL (1998) Epigenetic allelic states of a maize transcriptional regulatory locus exhibit overdominant gene action. Genetics 150:891-897. PMID:9755217

Hong CP, Kwon SJ, Kim JS, Yang TJ, Park BS and Lim YP (2007) Progress in understanding and sequencing the genome of *Brassica rapa*. Int. J. Plant Genomics 2008:582837. doi:10.1155/2008/582837

Hopkins CJ, Cogan NOI, Hand M, Jewell E, Kaur J, Li X, Lim GAC, Ling AE, Love C, Mountford H, Todorovic M, Vardy M, Spangenberg GC, Edwards D and Batley J (2007) Sixteen new simple sequence repeat markers from *Brassica juncea* expressed sequences and their cross-species amplification. Mol. Ecol. Notes 7:697-700. doi:10.1111/j.1471-8286.2007.01681.x

Hu SH, Yu Ch, Zhao H, Sun G, Zhao S, Vyvadilova M and Kucera V (2007) Genetic diversity of *Brassica napus* L. germplasm from China and Europe assessed by some ergonomically important characters. Euphytica 154:9-16. doi:10.1007/s10681-006-9263-8

Hua JP, Xing YZ, Xu CG, Sun XL, Yu SB and Zhang Q (2002) Genetic dissection of an elite rice hybrid revealed that heterozygotes are not always advantageous for performance. Genetics 162:1885–1895. PMID:12524357

Huang L, Yang Y, Zhang F and Cao J (2017) A genome-wide SNP-based genetic map and QTL mapping for agronomic traits in Chinese cabbage. Sci. Rep. 7:46305. doi:10.1038/srep46305

Huang S, Deng L, Guan M, Li J, Lu K, Wang H, Fu D, Mason AS, Liu Sh and Hua W (2013) Identification of genome-wide single nucleotide polymorphisms in allopolyploid crop *Brassica napus*. BMC Genomics 14:717-726. doi:10.1186/1471-2164-14-717

Huang X-Q, Huang T, Hou G-Z, Li L, Hou Y and Lu Y-H (2016) Identification of QTLs for seed quality traits in rapeseed (*Brassica napus* L.) using recombinant inbred lines (RILs). Euphytica 210:1-16. doi:10.1007/s10681-016-1675-5

Hull FH (1945) Recurrent selection for specific combining ability in corn. J. Am. Soc. Agron.

37:134-145.

Iftikhar R, Wang X and Rahman H (2018) Broadening the genetic base of *Brassica napus* canola by interspecific crosses with different variants of *B. oleracea*. Euphytica 214:133–149. doi:10.1007/s10681-018-2213-4

Iniguez-Luy FL and Federico ML (2011) The genetics of *Brassica napus* L. In Bancroft I and Schmidt R (Eds.) Genetics and genomics of the *Brassicacea*e. Springer, New York Dordrecht Heidelberg London, pp 291–322. doi:10.1007/978-1-4419-7118-0_1

Iniguez-Luy FL, Voort AV and Osborn TC (2008) Development of a set of public SSR markers derived from genomic sequence of a rapid cycling *Brassica oleracea* L. genotype. Theor. Appl. Genet. 117:977-985. doi:10.1007/s00122-008-0837-9

Izzah NK, Lee J, Perumal S, Park JY, Ahn K, Fu D, Kim G, Nam Y and Yang T (2013) Microsatellite-based analysis of genetic diversity in 91 commercial *Brassica oleracea* L. cultivars belonging to six varietal groups. *Genet. Resour.* Crop Evol. 60:1967-1986. doi:10.1007/s10722-013-9966-3

Jeong SY, Ahmed NU, Jung HJ, Kim HT, Park JI and Nou IS (2017) Discovery genes for heterosis breeding in *Brassica oleracea* L. Acta Physiol. Plant. 39:180. DOI 10.1007/s11738-017-2474-x

Jesske T, Olberg B, Schierholt A and Becker HC (2013) Resynthesized lines from domesticated and wild *Brassica* taxa and their hybrids with *B. napus* L.: genetic diversity and hybrid yield. Theor. Appl. Genet. 126:1053–1065. doi:10.1007/s00122-012-2036-y

Jestin C, Lode M, Vallee P, Domin C, Falentin C, Horvais´R, Coedel S, Manzanares-Dauleux MJ and Delourme R (2011) Association mapping of quantitative resistance for *Leptosphaeria maculans* in oilseed rape (*Brassica napus* L.). Mol. Breed. 27:271−287. doi:10.1007/s11032-010-9429-x

Jian H, Zhang A, Ma J, Wang T, Yang B, Shuang LS, Liu M, Li J, Xu X, Paterson AH and Liu L (2019) Joint QTL mapping and transcriptome sequencing analysis reveal candidate flowering time genes in *Brassica napus* L. BMC Genomics 20:21. doi:10.1186/s12864-018-5356-8

Jones DF (1917) Dominance of linked factors as a means of accounting for heterosis. Genetics 2:466-479. PMID:16586724

Jonsson R (1978) Breeding for improved oil and meal quality in rape (*Brassica napus* L.) and turnip rape (*Brassica campestris* L.). Hereditas 87:205-218. doi:10.1111/j.1601-5223.1978.tb01264.x

Kalia RK, Rai MK, Kalia S, singh R and Dhawan AK (2011) Microsatellite markers: an overview of the recent progress in plants. Euphytica 177:309-334. doi:10.1007/s10681-010-0286-9

Kapoor R, Banga SS and Banga SK (2009) A microsatellite (SSR) based linkage map of *Brassica rapa*. New Biotechnol. 26:239-243. doi:10.1016/j.nbt.2009.09.003

Kaur G, Bansal P, Kaur B and Banga S (2007) Genetic diversity and its association with heterosis in *Brassica rapa*. In: Proceeding of the 12th International Rapeseed Congress, 26-30 June 2007, Wuhan, China Volume I, pp 144-146

Kebede B and Rahman H (2014) Quantitative trait loci (QTL) mapping of silique length and petal color in *Brassica rapa*. Plant Breed. 133:609–614. doi:10.1111/pbr.2014.133.issue-5

Kebede B, Cheema K, Greenshields DL, Li C, Selvaraj G and Rahman H (2012) Construction of genetic linkage map and mapping of QTL for seed color in *Brassica rapa*. Genome, 55:813–823. doi:10.1139/g2012-066

Kebede B, Thiagarajah M, Zimmerli C and Rahman H (2010) Improvement of open-pollinated spring rapeseed (*Brassica napus* L.) through introgression of genetic diversity from winter rapeseed. Crop Sci. 50:1236–1243. doi:10.2135/cropsci2009.06.0352

Khajali F and Slominski BA (2012) Factors that affect the nutritive value of canola meal for poultry. Poultry Sci. 91:2564-2575. doi:10.3382/ps.2012-02332

Kimber DS and McGregor DI (1995) Physiology: crop development, growth and yield. In Kimber DS and McGregor DI (Eds.) *Brassica* Oilseeds: Production and Utilization. CABI, Wallingford, UK. pp 1–10. doi:10.1002/lipi.19960980908

Kirk JTO and Oram RN (1981) Isolation of erucic acid-free lines of *Brassica juncea*: Indian mustard now a potential oilseed crop in Australia. J. Aust. I. Agr. Sci. 47:51-52.

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA and Mayrose I (2015) CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15:1179–1191. doi:10.1111/1755-0998.12387

Körber N, Bus A, Li J, Higgins J, Bancroft I, Higgins EE, Parkin IAP, Salazar-Colqui B, Snowdon RJ and Stich B (2015) Seedling development traits in *Brassica napus* examined by gene expression analysis and association mapping. BMC Plant Biol. 15:136-157. doi:10.1186/s12870-015-0496-3

Körber N, Bus A, Li J, Parkin IAP, Wittkop B, Snowdon RJ and Stich B (2016) Agronomic and seed quality traits dissected by genome-wide association mapping in *Brassica napus*. Front. Plant Sci. 7:386-407. doi:10.3389/fpls.2016.00386

Koscielny CB, Gardner SW and Duncan RW (2018) Impact of high temperature on heterosis and general combining ability in spring canola (*Brassica napus* L.). Field Crop Res. 221:61–70. doi:10.1016/j.fcr.2018.02.014

Kumar A, Salisbury PA, Gurung AM and Barbetti MJ (2015) Impoertant and origin. In Kumar A., Banga SS, Meena PD and Kumar PR (Eds.) *Brassica* oilseeds, Breeding and management. CABI Publishing, Wallingford, UK. pp 1-10. doi:10.1079/9781780644837.0000

Kumar P, Gupta VK, Misra AK, Modi DR and Pandey BK (2009) Potential of molecular markers in plant biotechnology. Plant Omics 2:141–162.

Kutka F (2011) Open-pollinated vs. hybrid maize cultivars. Sustainability 3:1531-1554.

doi:10.3390/su3091531

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

Lammerink J and Morice IM (1971) Breeding biennial rapeseed (*Brassica napus* L.) with a low content of erucic acid. New Zeal. J. Agr. Res. 14:752-760. doi:10.1080/00288233.1971.10421669

Lázaro A and Aguinagalde I (1998) Genetic diversity in *Brassica oleracea* L. (Cruciferae) and wild relatives (2*n* = 18) using isozymes. Ann. Bot. 82:829–833. doi:10.1006/anbo.1998.0763

Leflon M, Eber F, Letanneur JC, Chelysheva L, Coriton O, Huteau V, Ryder CD, Barker G, Jenczewski E and Chevre AM (2006) Pairing and recombination at meiosis of *Brassica rapa* (AA) × *Brassica napus* (AACC) hybrids. Theor. Appl. Genet. 113:1467–1480. doi:10.1007/s00122-006-0393-0

Lefort-Buson M, Guillot-Lemoine B and Dattee Y (1987) Heterosis and genetic distance in rapeseed (*Brassica napus* L.): crosses between European and Asiatic selfed lines. Genome 29:413–418. doi:10.1139/g87-072

Leonte C and Arsene MC (2016) Assessment of genetic diversity of some *Brassica napus* L, cultivars revealed by molecular markers and phenotypic evaluation. Indian J. Biotechnol. 15:568-575.

Li F, Chen B, Xu K, Gao G, Yan G, Qiao J, Li J, Li H, Li L, Xiao X, Zhang T, Nishio T and Wu X (2016) A genome-wide association study of plant height and primary branch number in rapeseed (*Brassica napus*). Plant Sci. 242:169–177. doi:10.1016/j.plantsci.2015.05.012

Li F, Chen B, Xu K, Wu J, Song W, Bancroft I, Harper AL, Trick M, Liu S, Gao G, Wang N, Yan G, Qiao J, Li J, Xiao X, Zhang T and Wu X (2014) Genome-wide association study dissects the genetic architecture of seed weight and seed quality in rapeseed (*Brassica napus* L.). DNA Res. 21:355–367. doi:10.1093/dnares/dsu002

Li H, Chen X, Yang Y, Xu J, Gu J, Fu J, Qian X, Zhang S, Wu J and Liu K (2011) Development and genetic mapping of microsatellite markers from whole genome shotgun sequences in *Brassica oleracea*. Mol. Breed. 28:585-596. doi:10.1007/s11032-010-9509-y

Li H, Yang Q, Fan N, Zhang M, Zhai H, Ni Z and Zhang Y (2017) Quantitative trait locus analysis of heterosis for plant height and ear height in an elite maize hybrid zhengdan 958 by design III. BMC Genetics 18:36-46. doi:10.1186/s12863-017-0503-9

Li L, Lu K, Chen Z, Mu T, Hu Z and Li X (2008) Dominance, overdominance and epistasis condition the heterosis in two heterotic rice hybrids. Genetics 180:1725‑1742. doi:10.1534/genetics.108.091942

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

Li M, Qian W, Meng J and Li Z (2004) Construction of novel *Brassica napus* genotypes through chromosomal substitution and elimination using interploid species hybridization. Chromosome Res. 12:417–426. doi:10.1023/B:CHRO.0000034722.66981.94

Li Q, Mei J, Zhang Y, Li J, Ge X, Li Z and Qian W (2013) A large-scale introgression of genomic components of *Brassica rapa* into *B. napus* by the bridge of hexaploid derived from hybridization between *B. napus* and *B. oleracea*. Theor. Appl. Genet. 126:2073–2080. doi:10.1007/s00122-013-2119-4

Li Q, Zhou Q, Mei J, Zhang Y, Li J, Li Z, Ge X, Xiong Z, Huang Y and Qian W (2014) Improvement of *Brassica napus* via interspecific hybridization between *B. napus and B. oleracea*. Mol. Breed. 34:1955–1963. doi:10.1007/s11032-014-0153-9

Li R, Jeong K, Davis JT, Lee S, Michelmore RW, Kim S and Maloof JN (2018) Integrated QTL and eQTL mapping provides insights and candidate genes for fatty acid composition, flowering time, and growth traits in a F_2 population of a novel synthetic allopolyploid *Brassica napus*. Front. Plant Sci. 9:1632. doi:10.3389/fpls.2018.01632

Li S and Chou H-H (2004) LUCY2: an interactive DNA sequence quality trimming and vector removal tool. Bioinformatics 20:2865-2866. doi:10.1093/bioinformatics/bth302

Li Z, Coffey L, Garfin J, Miller ND, White MR, Spalding EP, de Leon N, kaeppler SM, Schnable PS, Springer NM and Hirsch CN (2018) Genotype-by-environment interactions affecting heterosis in maize. PLoS One 13:e0191321. doi:10.1371/journal.pone.0191321

Lin L, Allemekinders H, Dansby A, Campbell L, Durance-Tod S, Berger A and Jones PJ (2013) Evidence of health benefits of canola oil. Nutr. Rev. 71:370-385. doi:10.1111/nure.12033

Lipka AE, Tian F, Wang QS, Peiffer J, Li M, Bradbury PJ, Gore MA, Buckler ES and Zhang Z (2012) GAPIT: genome association and prediction integrated tool. Bioinformatics 28:2397–2399. doi: 10.1093/bioinformatics/bts444

Lippman ZB and Zamir D (2007) Heterosis: revisiting the magic. Trends Genet. 23:60-66. doi:10.1016/j.tig.2006.12.006

Liu HL (2000) Genetics and breeding in rapeseed. Chin. Agric. Univ. Press, Beijing.

Liu R, Qian W and Meng J (2002) Association of RFLP markers and biomass heterosis in trigenomic hybrids of oilseed rape (*Brassica napus* × *B. campestris*). Theor. Appl. Genet. 105:1050–1057. doi:10.1007/s00122-002-1050-x

Liu S, Fan C, Li J, Cai G, Yang Q, Wu J, Yi X, Zhang C and Zhou Y (2016) A genome-wide association study reveals novel elite allelic variations in seed oil content of *Brassica napus*. Theor. Appl. Genet. 129:1203–1215. doi:10.1007/s00122-016-2697-z

Liu S, Liu Y, Yang X, Tong C, Edwards D, Parkin IAP, Zhao M, Ma J, Yu J, Huang S, Wang X, Wang J, Lu K, Fang Z, Bancroft I, Yang TJ, HU Q, Wang X, Yue Z, Li H and et al. (2014) The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploid genoms. Nat. Commun. 5:3930. doi:10.1038/ncomms4930

Liu X, Huang M. Fan B, Buckle ES and Zhang Z (2016) Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. PLoS Genet 12: e1005767. doi:10.1371/journal.pgen.1005767

Liu Y, Zhou X, Yan M, Wang P, Wang H, Xin Q, Yang L, Hong D and Yang G (2020) Fine mapping and candidate gene analysis of a seed glucosinolate content QTL, qGSL-C2, in rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 133:479-490. doi:10.1007/s00122-019-03479-x

Lombard V, Baril CP, Dubreuil P, Blouet F and Zhang D (2000) Genetic relationships and fingerprinting of rapeseed cultivars by AFLP:consequences for varietal registration. Crop Sci. 40:1417–1425. doi:10.2135/cropsci2000.4051417x

Long Y, Shi J, Qiu D, Li R, Zhang C, Wang J, Hou J, Zhao J, Shi L, Park BS, Choi SR, Lim YP and Meng J (2007) Flowering time quantitative trait loci analysis of oilseed *Brassica* in multiple environments and genomewide alignment with *Arabidopsis*. Genetics 177:2433-2444. doi:10.1534/genetics.107.080705

Louarn S, Torp AM, Holme IB, Andersen SB and Jensen BD (2007) Database derived microsatellite markers (SSRs) for cultivar differentiation in *Brassica oleracea*. Genet. Resour. Crop Ev. 54:1717–1725. doi:10.1007/s10722-006-9181-6

Love HK (1988) Inheritance of seed aliphatic qlucosinolates in oilseed *Brassica* species. Ph.D. Thesis, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Canada.

Love HK, Rakow G, Raney JP and Downey RK (1990) Development of low glucosinolate mustard. Can. J. Plant Sci. 70:419-424. doi:10.4141/cjps90-049

Lu H, Romero-Severson J and Bernardo R (2003) Genetic basis of heterosis explored by simple sequence repeat markers in a random-mated maize population. Theor. Appl. Genet. 107:494-502. doi:10.1007/s00122-003-1271-7

Lu K, Peng L, Zhang Ch, Lu J, Yang B, Xiao Zh, Liang Y, Xu X, Qu C, Zhang K, Liu L, Zhu Q, Fu M, Yuan X and Li J (2017) Genome-wide association and transcriptome analyses reveal candidate genes underlying yield-determining traits in *Brassica napus*. Front. Plant Sci. 8:206-222. doi:10.3389/fpls.2017.00206

Lu Y, Yan J, Guimarães C, Taba S, Hao Z, Gao S, Chen S, Li J, Zhang S, Vivek BS, Magorokosho C, Mugo S, Makumbi D, Parentoni SN, Shah T, Rong T, Crouch JH and Xu Y (2009) Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. Theor. Appl. Genet. 120:93-115. doi:10.1007/s00122-009-1162-7

Luo X, Ding Y, Zhang L, Yue Y, Snyder JH, Ma Ch and Zhu J (2017) Genomic prediction of genotypic effects with epistasis and environment interactions for yield-related traits of rapeseed (*Brassica napus* L.). Front. Genet. 8:15. doi:10.3389/fgene.2017.00015

Luo X, Ma C, Yi B, Tu J, Shen J and Fu T (2016) Genetic distance revealed by genomic single

nucleotide polymorphisms and their relationships with harvest index heterotic traits in rapeseed (*Brassica napus* L.). Euphytica 209:41–47. doi:10.1007/s10681-015-1629-3

Luo X, Ma C, Yue Y, Hu K, Li Y, Duan Z, Wu M, Tu J, Shen J, Yi B and Fu T (2015) Unravelling the complex trait of harvest index in rapeseed (*Brassica napus* L.) with association mapping. BMC Genomics 16:379. doi:10.1186/s12864-015-1607-0

Luo YX, Luo CY, Du DZ, Fu Z, Yao YM, Xu CC and Zhang HS (2014) Quantitative trait analysis of flowering time in spring rapeseed (*B. napus* L.). Euphytica 200:321–335. doi:10.1007/s10681-014-1140-2

Lysak MA, Koch MA, Pecinka A and Schubert I (2005) Chromosome triplication found across the tribe *Brassiceae*. Genome Res. 15:516-525. doi:10.1101/gr.3531105

Maheshwari P and Kovalchuk I (2016) Genetic transformation of crops for oil production. In Mckeon TA, Hayes DG, Hildebrand DF and Weselake RJ (Eds.) Industrial oil crops. Elsevier, Amsterdam and AOCS Press, Urbana, pp. 379-412

Mahmood T, Rahman MH, Stringam GR, Yeh F and Good AG (2006) Identification of quantitative trait loci (QTL) for oil and protein contents and their relationships with other seed quality traits in *Brassica juncea*. Theor. Appl. Genet. 113:1211-1220. doi:10.1007/s00122-006-0376-1

Mason AS, Higgins EE, Snowdon RJ, Batley J, Stein A, Werner C and Parkin IAP (2017) A user guide to the *Brassica* 60K illumina infinium™ SNP genotyping array. Theor. Appl. Genet. 130:621-633. doi:10.1007/s00122-016-2849-1

Mason AS, Huteau V, Eber F, Coriton O, Yan G, Nelson MN, Cowling W and Chevre A-M (2010) Genome structure affects the rate of autosyndesis and allosyndesis in AABC, BBAC and CCAB *Brassica* interspecific hybrids. Chromosome Res. 18:655-666. doi:10.1007/s10577-010-9140-0

McVetty PBE, Dilantha Fernando WG, Li G, Tahir M and Zelmer C (2011) High-erucic acid, low-glucosinolate rapeseed (HEAR) cultivar development in Canada. In Hou CT and Shaw JF (Eds.) Biocatalysis and Agricultural Biotechnology. CRC Press, London, New York. pp 43-62.

McVetty PBE, Mietkiewska E, Omonov T, Curtis J, Taylor DC and Weselake RJ (2016) *Brassica* spp. oils. In McKeon TA, Hayes DG, Hildebrand DF and Weselake RJ (Eds.) Industrial oil crops. AOCS Press, London, UK. pp 113-146.

Mei DS, Wang HZ, Hu Q, Li YD, Xu YS and Li YC (2009) QTL analysis on plant height and flowering time in *Brassica napus*. Plant Breed. 128:458-465. doi:10.1111/j.1439-0523.2008.01528.x

Mei J, Fu Y, Qian L, Xu X, Li J and Qian W (2011) Effectively widening the gene pool of oilseed rape (*Brassica napus* L.) using Chinese *B. rapa* in a 'virtual allopolyploid' approach. Plant Breed. 130:333–337. doi:10.1111/j.1439-0523.2011.01850.x

Messing J and Dooner HK (2006) Organization and variability of the maize genome. Curr. Opin. Plant Biol. 9:157-163. doi:10.1016/j.pbi.2006.01.009

Meuwissen T and Goddard M. (2000) Fine mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. Genetics 155:421–30. PMID:10790414

Miller CN, Harper AL, Trick M, Wellner N, Werner P, Waldron KW and Bancroft I (2018) Dissecting the complex regulation of lodging resistance in *Brassica napus*. Mol. Breed. 38:30–48. doi:10.1007/s11032-018-0781-6

Miranda C, Urrestarazu J, Santesteban LG, Royo JB and Urbina V (2010) Genetic diversity and structure in a collection of ancient Spanish pear cultivars assessed by microsatellite markers. J. Am. Soc. Hortic. Sci. 135:428–437. doi:10.21273/JASHS.135.5.428

Morrison MJ, Harker KN, Blackshaw RE, Holzapfel CJ and O'Donovan JT (2016) Canola yield improvement on the Canadian prairies from 2000 to 2013. Crop Pasture Sci. 67:245-252. doi:10.1071/CP15348

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

Nath UK, Kim HT, Khatun Kh, Park JI, Kang KK and Nou IS (2016) Modification of fatty acid profiles of rapeseed (*Brassica napus* L.) oil for using as food, industrial feed-stock and biodiesel. Plant Breed. Biotech. 4:123-134. doi:10.9787/PBB.2016.4.2.123

Nei M and Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. PNAS, USA 76:5269‑5273. doi:10.1073/pnas.76.10.5269

Newkirk RW (2009) Canola meal, feed industry guide. Canadian International Grains Institute, Winnipeg, Manitoba.

Newkirk RW, Classen HL, Scott TA and Edney MJ (2003) The digestibility and content of amino acids in toasted and non-toasted canola meals. Can. J. Anim. Sci. 83:131-139.

Nikzad A, Kebede B, Bhavikkumar J and Rahman H (2020) Study of genetic structure of a *Brassica napus* canola population derived from six interspecific crosses of *B. napus* × *B. oleracea*. Can. J. Plant Sci. (Submitted)

Nikzad A, Kebede B, Pinzon J, Bhavikkumar J, Yang R-C and Rahman H (2019) Potential of the C-genome of different variants of *Brassica oleracea* for the improvement of agronomic and seed quality traits of *B. napus* canola. Crop Sci. 59:2608–2620. doi:10.2135/cropsci2019.05.0304

Okazaki K, Sakamoto K, Kikuchi R, Saito A, Togashi E, Kuginuki Y, Matsumoto S and Hirai M (2007) Mapping and characterization of FLC homologs and QTL analysis of flowering time in *Brassica oleracea*. Theor. Appl. Genet. 114:595-608. doi:10.1007/s00122-006-0460-6

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E and Wagner H (2019) Package 'vegan', community ecology package. Retrieved from https://cran.r-project.org/web/packages/vegan/vegan.pdf (Accessed 04 March 2019).

Oplinger ES, Hardman LL, Gritton ET, Doll JD and Kelling KA (1989) Canola (Rapeseed). Alternative Field Crops Manual. https://hort.purdue.edu/newcrop/afcm/canola.html.

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

Ott A, Liu S, Schnable JC, Yeh CTE, Wang KS and Schnable PS (2017) tGBS® genotyping-by-sequencing enables reliable genotyping of heterozygous loci. Nucleic Acids Res. 45:e178. doi:10.1093/nar/gkx853

Parkin IAP, Gulden SM, Sharpe AG, Lukens L, Trick M, Osborn TC and Lydiate DJ (2005) Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. Genetics 171:765–781. doi:10.1534/genetics.105.042093

Parkin IAP, Koh C, Tang H, Robinson SJ, Kagale S, Clarke WE, Town CD, Nixon J, Krishnakumar V, Bidwell SL, Denoeud F, Belcram H, Links MG, Just J, Clarke C, Bender T, Huebert T, Mason AS, Pires JC, etc. (2014) Transcriptome and methylome profiling reveals relics of genome dominance in the mesopolyploid *Brassica oleracea*. Genome Biology 15:R77. doi:10.1186/gb-2014-15-6-r77

Parkin IAP, Sharpe AG and Lydiate DJ (2003) Patterns of genome duplication within the *Brasssica napus* genome. Genome 46:291-303. doi:10.1139/G03-006

Patel R, Yadav RC, Avtar R, Jangra S, Boken GD, Singh B and Yadav R (2018) Genetic diversity analysis for salinity tolerance in Indian Mustard [*Brassica juncea* (L.)] using SSR markers. Int. J. Curr. Microbiol. Appl. Sci. 7:1776-1785. doi:10.20546/ijcmas.2018.701.215

Peakall R and Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288–295. doi:10.1111/j.1471-8286.2005.01155.x

Pelc SE, Couillard DM, Stansell ZJ and Farnham MW (2015) Genetic diversity and population structure of collected landraces and their relationship to other *Brassica oleracea* crops. Plant Genome 8:1–11. doi:10.3835/plantgenome2015.04.0023

Peterson BG, Carl P and Ulrich J (2015) Performance and risk analysis of financial time series, including packages PerformanceAnalytics and PortfolioAnalytic. Retrieved from http://www.r-forge.r-project.org/projects/returnanalytics (Accessed 04 June 2018).

Phillips RL (2010) Mobilizing science to break yield barriers. Crop Sci. 50:S-99-S-108. doi:10.2135/cropsci2009.09.0525

Piquemal J, Cinquin E, Couton F, Rondeau C, Seignoret E, Doucet I, Perret D, Villeger MJ, Vincourt P and Blanchard P (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor. Appl. Genet. 111:1514–1523. doi:10.1007/s00122-005-0080-6

Powell W, Machray GC and Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 1:215–222. doi:10.1016/S1360-1385(96)86898-0

Pradhan AK, Gupta V, Mukhopadhyay A, Arumugam N, Sodhi YS and Pental D (2003) A high-density linkage map in *Brassica juncea* (Indian mustard) using AFLP and RFLP markers. Theor. Appl. Genet. 106:607–614. doi:10.1007/s00122-002-1083-1

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA and Reich D (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics 38:904–909. doi:10.1038/ng1847

Primard-Brisset C, Poupard JP, Horvais R, Eber F, Pelletier G, Renard M and Delourme R (2005) A new recombind double low restorer line for the Ogu-INRA cms in rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 111:736–746. doi:10.1007/s00122-005-2059-8

Pritchard JK, Stephens M and Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959. PMID:10835412

Przybylski R, Mag T, Eskin N and McDonald B (2005) Canola oil. In Shahidi F (Ed.) Bailey's industrial oil and fat products, vol. 2. New York:Wiley. pp 61-121.

Pua E-C (1993) Regeneration of plants from protoplasts of *Brassica alboglabra* Bailey (Chinese kale). In Bajaj YPS (Ed.) Biotechnology in agriculture and forestry 22, Plant Protoplasts and Genetic Engineering II. Springer, Verlag Berlin Heidelberg, pp 22-30

Qian W, Chen X, Fu D, Zou J and Meng J (2005) Intersubgenomic heterosis in seed yield potential observed in a new type of *Brassica napus* introgressed with partial *Brassica rapa* genome. Theor. Appl. Genet. 110:1187–1194. doi:10.1007/s00122-005-1932-9

Qian W, Li Q, Noack J, Sass O, Meng J, Frauen M and Jung C (2009) Heterotic patterns in rapeseed (*Brassica napus* L.): II. crosses between European winter and Chinease semi-winter lines. Plant Breed. 128:466-470. doi:10.1111/j.1439-0523.2008.01597.x

Qian W, Liu R and Meng J (2003) Genetic effects on biomass yield in interspecific hybrids between *Brassica napus* and *B. rapa*. Euphytica 134:9-15. doi:10.1023/A:102618082

Qian W, Meng J, Li M, Frauen M, Sass O, Noack J and Jung C (2006) Introgression of genomic components from Chinese *Brassica rapa* contributes to widening the genetic diversity in rapeseed (*B. napus* L.), with emphasis on the evolution of Chinese rapeseed. Theor. Appl. Genet. 113:49–54. doi:10.1007/s00122-006-0269-3

Qian W, Sass O, Meng J, Li M, Frauen M and Jung C (2007) Heterotic patterns in rapeseed (*Brassica napus* L.): I. crosses between spring and Chinese semi-winter lines. Theor. Appl. Genet. 115:27-34. doi:10.1007/s00122-007-0537-x

Qiu D, Morgan C, Shi J, liu Y, Li R, Zhuang X, Wang Y, Tan X, Dietrich E, Weihmann T, Everett C, Vanstraelen S, Beckett P, Fraser F, Trick M, Barnes S, Wilmer J, Schmidt R, Li J, Li D, Meng J and Bancroft I (2006) A comparative linkage map of rapeseed and its use for QTL analysis of seed oil and erucic acid content. Theor. Appl. Genet. 114:67–80. doi:10.1007/s00122-006-0411-2

Qu C, Hasan M, Kun L, Liu L, Liu X, Xie J, Wang M, Lu J, Odat N, Wang R, Chen L, Zhanglin T and Jiana L (2012) genetic diversity and relationship analysis of the *Brassica napus* germplasm using simple sequence repeat (SSR) markers. Afr. J. Biotechnol. 11:6923-6933. doi:0.5897/AJB11.3475

Qu C, Jia L, Fu F, Zhao H, Lu K, Wei L, Xu X, Liang Y, Li Sh, Wang R and Li J (2017)

Genome-wide association mapping and identification of candidate genes for fatty acid composition in *Brassica napus* L. using SNP markers. BMC Genomics 18:232-249. doi:10.1186/s12864-017-3607-8

Qu C, Li S, Duan X, Fan J, Jia L, Zhao H, Lu K, Li J, Xu X and Wang R (2015) Identification of candidate genes for seed glucosinolate content using association mapping in *Brassica napus* L. Genes 6:1215-1229. doi:10.3390/genes6041215

Quazi MH (1988) Interspecific hybrids between *Brassica napus* L. and *B. oleracea* L. developed by embryo culture. Theor. Appl. Genet. 75:309–318. doi:10.1007/BF00303970

Quijada P, Udall J, Lambert B and Osborn T (2006) Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (*Brassica napus* L.): 1. Identification of genomic regions from winter germplasm. Theor. Appl. Genet. 113:549–561. doi:10.1007/s00122-006-0323-1

Quijada P, Udall JA, Polewicz H, Vogelzang RD and Osborn TC (2004) Phenotypic effects of introgressing French winter germplasm into hybrid spring canola (*Brassica napus* L). Crop Sci. 44, 1982–1989.

Quiros CF and Farnham MW (2011) The genetics of *Brassica oleracea*. In Schmidt R and Bancroft I (Eds.) Genetics and genomics of the *Brassicaceae*. Genetics and genomics: crops and models 9. Springer Science and Business Media, pp 261–290. doi:10.1007/978-1-4419-7118-0_2

Quiros CF and Paterson AH (2004) Genome mapping and analysis. In Pua EC and Douglas CJ (Eds.), Biotechnology in agriculture and forestry, Vol 54, *Brassica*. Springer, Berlin. pp 31–42.

Radoev M, Becker HC and Ecke W (2008) Genetic analysis of heterosis for yield and yield components in rapeseed (*Brassica napus* L.) by quantitative trait locus mapping. Genetic 179:1547–1558. doi:10.1534/genetics.108.089680

Rae AM, Howell EC and Kearsey MJ (1999) More QTL for flowering time revealed by substitution lines in *Brassica oleracea*. Heredity 83:586-596. doi:10.1038/sj.hdy.6886050

Rahman H (2001) Production of yellow-seeded *Brassica napus* through interspecific crosses. Plant Breed. 120:463-472. doi:10.1046/j.1439-0523.2001.00640.x

Rahman H (2013) Review: breeding spring canola (*Brassica napus* L.) by the use of exotic germplasm. Can. J. Plant Sci. 93:363–373. doi:10.4141/cjps2012-074

Rahman H, Bennett RA and Kebede B (2017) Mapping of days to flower and seed yield in spring oilseed *Brassica napus* carrying genome content introgressed from *Brassica oleracea*. Mol. Breed. 37:5. doi:10.1007/s11032-016-0608-2

Rahman H, Bennett RA and Kebede B (2018) Molecular mapping of QTL alleles of *Brassica oleracea* affecting days to flowering and photosensitivity in spring *Brassica napus*. PLoS One 13:e0189723. doi:10.1371/journal.pone.0189723

Rahman H, Bennett RA and Séguin-Swartz G (2015) Broadening genetic diversity in *Brassica napus* canola: development of canola-quality spring *B. napus* from *B. napus* × *B. oleracea* var.

alboglabra interspecific crosses. Can. J. Plant Sci. 95:29–41. doi:10.4141/cjps-2014-017

Rahman H, Bennett RA and Yang R-C (2016) Patterns of heterosis in three distinct inbred populations of spring *Brassica napus* canola. Crop Sci. 56:2536–2545. doi:10.2135/cropsci2016.01.0041

Rahman H, Harwood J and Weselake R (2013) Increasing seed oil content in *Brassica* species through breeding and biotechnology. Lipid Technol. 25:182–185. doi:10.1002/lite.201300291

Rahman H, Kebede B, Zimmerli C and Yang RC (2014) Genetic study and QTL mapping of seed glucosinolate content in *Brassica rapa* L. Crop Sci. 54:537-543. doi:10.2135/cropsci2013.06.0391

Rahman MH (2004) Optimum age of siliques for rescue of hybrid embryos from crosses between *Brassica oleracea*, *B. rapa* and *B. carinata*. Can. J. Plant Sci. 84:965-969. doi:10.4141/P04-003

Rahman MH (2005) Resynthesis of *Brassica napus* for self- incompatibility: Self-incompatibility reaction, inheritance and breeding potential. Plant Breed. 124:13–19. doi:10.1111/j.1439-0523.2004.01045.x

Rahman MH, Bennett RA, Yang R-C and Thiagarajah MR (2011) Exploitation of the late flowering species *Brassica oleracea* L. for the improvement of earliness in *B. napus* L. an untraditional approach. Euphytica 177:365–374. doi:10.1007/s10681-010-0253-5

Rahman MH, Joersbo M and Poulsen MH (2001) Development of yellow-seeded *Brassica napus* of double low quality. Plant Breed.120:473-478. doi:10.1046/j.1439-0523.2001.00639.x

Rakow G (2004) Species origin and economic importance of *Brassica*. In Pua EC and Douglas CJ (Eds.) Biotechnology in agriculture and forestry 54. Springer-Verlag Berlin Heidelberg, New York. pp 1-11.

Rakow G, Raney JP and Males D (1995) Field performance of canola quality *Brassica juncea*. In: Proceeding of the 9th International Rapeseed Congress, Cambridge, U.K. 2:428-430.

Raman H, Raman R, Eckermann P, Coombes N, Manoli S, Zou X, Edwards D, Meng J, Prangnell R, Stiller J, Batley J, Luckett D, Wratten N and Dennis E (2013) Genetic and physical mapping of flowering time loci in canola (*Brassica napus* L.). Theor. Appl. Genet. 126:119–132. doi:10.1007/s00122-012-1966-8

Raman H, Raman R, Kilian A, Detering F, Carling J, Coombes N, Diffey S, Kadkol G, Edwards D, McCully M, Ruperao P, Parkin IAP, Batley J, Luckett DJ and Wratten N (2014b) Genome-wide delineation of natural variation for pod shatter resistance in *Brassica napus*. PLoS One 9:e101673.

Raman H, Dalton-Morgan J, Diffey S, Raman R, Alamery S, Edwards D and Batley J (2014a) SNP markers-based map construction and genome-wide linkage analysis in *Brassica napus*. Plant Biotechnol J. 12:851-860. doi:10.1111/pbi.12186

Raman R, Diffey S, Carling J, Cowley RB, Kilian A, Luckett DJ and Raman H (2016) Quantitative genetic analysis of grain yield in an Australian *Brassica napus* doubled-haploid population. Crop & Pasture Science 67:298–307. http://dx.doi.org/10.1071/CP15283

Reif JC, hallauer AR and Melchinger AE (2005) Heterosis and heterotic patterns in maize. Maydica 50:215-223.

Rezaeizad A, Wittkop B, Snowdon R, Hasan M, Mohammadi V, Zali A and Friedt W (2011) Identification of QTLs for phenolic compounds in oilseed rape (*Brassica napus* L.) by association mapping using SSR markers. Euphytica 177:335−342. doi:10.1007/s10681-010-0231-y

Richey FD (1942) Mock-dominance and hybrid vigor. Science 96:280-281. doi:10.1126/science.96.2490.280

Rohlf FJ (2004) NTSYS-pc numerical taxonomy and multivariate analysis system, ver. 2.1. Exeter Software. Applied Biostatistics Inc., New York, USA.

Saha S, Molla MR, Chandra D and Rahman L (2008) Assessment of genetic variation and relationships within the varieties of four *Brassica* species by RAPD markers. Aust. J. Crop Sci. 2:105–114.

Samans B, Chalhoub B and Snowdon RJ (2017) Surviving a genome collision: genomic signatures of allopolyploidization in the recent crop species *Brassica napus*. Plant Genome 10:1-15. doi:10.3835/plantgenome2017.02.0013

Samayoa LF, Malvar RA and Butrón A (2017) QTL for Maize midparent heterosis in the heterotic pattern american Dent × European flint under corn borer pressure. Front. Plant Sci. 8:573. doi:10.3389/fpls.2017.00573

Sang SF, Wang H, Mei DS, Liu J, Fu L, Wang J, Wang WX and Hu Q (2015) Correlation analysis between heterosis and genetic distance evaluated by genome-wide SNP chip in *Brassica napus*. Scientia Agricultura Sinica 48:2469–2478. doi:10.3864/j.issn.0578-1752.2015.12.020

Sauer F and Kramer J (1983) The problems associated with high erucic acid rapeseed oils and some fish oils on experimental animals. In Kramer J, Sauer F and Pigden W (Eds.) High and low erucic acid rapeseed oils: Production, usage, chemistry and toxilogical evaluation. Academic Press, Canada.

Scarth R and Tang J (2006) Modification of oil using conventional and transgenic approaches. Crop Sci. 46:1225-1236. doi:10.2135/cropsci2005.08-0245

Schatzki J, Ecke W, Becker HC and Mollers C (2014) Mapping of QTL for the seed storage proteins cruciferin and napin in a winter oilseed rape doubled haploid population and their inheritance in relation to other seed traits. Theor. Appl. Genet. 127:1213-1222. doi:10.1007/s00122-014-2292-0

Schelfhout CJ, Wroth JM, Yan G and Cowling W (2008) Enhancement of genetic diversity in canola-quality Brassica napus and B. juncea by interspecific hybridization. Aust J Agric Res 59:918‑925. doi: 10.1071/AR07425

Schiessl S, Iniguez-Luy F, Qian W and Snowdon RJ (2015) Diverse regulatory factors associate with flowering time and yield response in winter-type *Brassica napus*. BMC Genomics 16:737. doi:10.1186/s12864-015-1950-1

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L and Graves TA (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112-1115. doi:10.1126/science.1178534

Schnell FW and Cockerham CC (1992) Multiplicative vs. arbitrary gene action in heterosis. Genetics 131:461-469. PMID:1644280

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

Schuelke M (2000) An economic method for the fluorescent labelling of PCR fragments. Nat. Biotechnol. 18:233–234. doi:10.1038/72708

Semel Y, Nissenbaum J, Menda N, Zinder M, Krieger U, issman N, Pleban T, Lippman Z, Gur A and Zamir D (2006) Overdominant quantitative trait loci for yield and fitness in tomato. PNAS USA 103 12981–12986.

Sernyk JL and Stefansson BR (1983) Heterosis in summer rape (*Brassica napus* L.). Can. J. Plant Sci. 63:407–413. doi:10.4141/cjps83-046

Sharafi Y, Majidi MM, Hossein Goli SA and Rashidi F (2015) Oil content and fatty acids composition in *Brassica* species. Int. J. Food Prop. 18:2145-2154. doi:10.1080/10942912.2014.968284

Shavorskaya OA (2004) Identification of genes affecting flowering time variation in *Brassica* species (Doctoral dissertation). Retrieved from Swedish University of Agricultural Science. pp 26-30.

Shen JX, Fu TD, Yang GS, MA CZ and Tu JX (2005) Genetic analysis of rapeseed self-incompatibility lines reveals significant heterosis of different patterns for yield and oil content traits. Plant Breed. 124:111-116. doi:10.1111/j.1439-0523.2004.01069.x

Shete S, Tiwari H and Elston RC (2000) On estimating the heterozygosity and polymorphism information content value. Theor. Popul. Biol. 57:265-271. doi:10.1006étpbi.2000.1452

Shi J, Li R, Qiu D, Jiang C, Long Y, Morgan C, Bancroft I, Zhao J and Meng J (2009) Unraveling the complex trait of crop yield with quantitative trait loci mapping in *Brassica napus*. Genetics 182:851–861. doi:10.1534/genetics.109.101642

Shi J, Li R, Zou J, Long Y and Meng J (2011) A dynamic and complex network regulates the heterosis of yield-correlated traits in rapeseed (*Brassica napus* L.). PLoS One 6:e21645. doi:10.1371/journal.pone.0021645

Shiranifar B, Hobson N, Kebede B, Yang R-C and Rahman H (2020) Potential of rutabaga (*Brassica napus* var. *napobrassica*) gene pool for se in the breeding of *B. napus* canola. Crop

Sci. 60:157-171. doi:10.1002/csc2.20074

Shull G H (1911) The genotypes of maize. Am. Nat. 45:234-252.

Shull GH (1908) The composition of a field of maize. American Breeding Association Report 4:296 - 301.

Si P, Mailer RJ, Galwey N and Turner DW (2003) Influence of genotype and environment on oil and protein concentrations of canola (*Brassica napus* L.) grown across southern Australia. Crop Pasture Sci 54:397. doi:10.1071/AR01203

Simonsen V and Heneen WK (1995) Genetic variation within and among different cultivars and landraces of *Brassica campestris* l. and *B. oleracea* L. based on isozymes. Theor. Appl. Genet. 91:346–352. doi:10.1007/BF00220898

Singh S, Singh VV, Ambawat S, Dubey M and Singh D (2017) screening and estimation of allelic differentiation in Indian mustard using SSR markers for background selection. Int. J. Curr. Microbiol. Appl. Sci. 6:2506-2516. doi:10.20546/ijcmas.2017.609.308

Snogerup S (1980) The wild forms of the *Brassica oleracea* group (2n = 18) and the possible relations to the cultivated ones. In Tsunoda S, Hinata K and Gomez- Campo C (Eds.) *Brassica* crops and wild allies. Japanese Scientific Society Press, Tokyo, pp 121–132.

Snowdon RJ and Friedt W (2004) Molecular markers in *Brassica* oilseed breeding: current status and future possibilities. Plant Breed.123:1-8. doi:10.1111/j.1439-0523.2003.00968.x

Song K and Osborn TC (1992) Polyphyletic origins of *Brassica napus*: new evidence based on organelle and nuclear RFLP analyses. Genome 35:992–1101. doi:10.1139/g92-152

Soto-Cerda BJ and Cloutier S (2012) association mapping in plant genomes. In Caliskan M (Ed.), Genetic diversity in plants. Rijeka: InTech. pp. 29–54. doi:10.5772/33005

Starmer KP, Brown J and Davis JB (1998) Heterosis in spring canola hybrids grown in northern Idaho. Crop Sci. 38:376–380. doi:10.2135/cropsci1998.0011183X003800020018x

Statista (2018a) Worldwide oilseed production in 2016/2017, by type (in million metric tons). [ONLINE] Available at: https://www.statista.com/statistics/267271/worldwide-oilseed-production-since-2008/ (Accessed 22 May 2018).

Statista (2018b) Area of canola harvested in Canada from 2008 to 2017 (in million hectares). [ONLINE] Available at: https://www.statista.com/statistics/454261/area-of-canola-harvested-in-canada/ (Accessed 16 April 2019).

Statista (2019) Worldwide oilseed production in 2018/2019, by type (in million metric tons). [ONLINE] Available at: https://www.statista.com/statistics/267271/worldwide-oilseed-production-since-2008/ (Accessed 20 September 2019).

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

Stefansson BR and Kondra ZP (1975) Tower summer rape. Can. J. Plant Sci. 55:343-344. Doi:10.4141/cjps75-053

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

Stuber CW, Lincoln SE, Wolff DW, Helentjaris T and Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823-839. PMID:1468633

Su T, Li P, Yang J, Sui G, Yu Y, Zhang D, Zhao X, Wang W, Wen C, Yu S and Zhang F (2018) Development of cost-effective single nucleotide polymorphism marker assays for genetic diversity analysis in *Brassica rapa*. Mol. Breed. 38:42-55. doi:10.1007/s11032-018-0795-0

Sun C, Wang B, Yan L, Hu K, Liu S, Zhou Y, Guan C, Zhang Z, Li J, Zhang J, Chen S, Wen J, Ma C, Tu J, Shen J, Fu T and Yi B (2016) Genome-Wide association study provides insight into the genetic control of plant height in rapeseed (*Brassica napus* L.). Front. Plant Sci. 7:1102. doi:10.3389/fpls.2016.01102

Sun Z, Wang Z, Tu J, Zhang J, Yu F, McVetty PBE and Li G (2007) An ultadense genetic recombination map for *Brassica napus*, consisting of 13551 SRAP markers. Theor. Appl. Genet. 114:1305-1317. doi:10.1007/s00122-006-0483-z

Suwabe K, Iketani H, Nunome T, Kage T and Hirai M (2002) Isolation and characterization of microsatellites in *Brassica rapa* L. Theor. Appl. Genet. 104:1092-1098. doi:10.1007/s00122-002-0875-7

Suwabe K, Tsukazaki H, Iketani H, Hatakeyama K, Kondo M, Fujimura M, Nunome T, Fukuoka H, Hirai M and Matsumoto S (2006) Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: the genetic origin of clubroot resistance. Genetics 173:309–319. doi:10.1534/genetics.104.038968

Tahir M, Zelmer CD and McVetty PBE (2012) Oliseed *Brassica*. In Kole C, Joshi CP and Shonnard DR (Eds.) Handbook of bioenergy crop plants. CRC Press, Taylor & Francis Group, U.S. pp 453-482.

Tan ZM, Li YC, Hu Q, Mei DS, Li YD and Xu YS (2007) Heterosis prediction based on genetic distance estimated by molecular markers in rapeseed. Chin. J. Oil Crop Sci. 29:20–26.

Teh L and Mollers C (2016) Genetic variation and inheritance of phytosterol and oil content in a doubled haploid population derived from the winter oilseed rape Sansibar × Oase cross. Theor. Appl. Genet. 129:181-199. doi:10.1007/s00122-015-2621-y

Thakur AK, Singh KH, Singh L, Nanjundan J, Khan YJ and Singh D (2018) SSR markers variations in *Brassica* species provide insight into the origin and evolution of *Brassica* amphidiploids. Hereditas 155:6–17. doi:10.1186/s41065-017-0041-5

Thakur AK, Singh KH, Singh L, Nanjundan J, Rana MK and Singh D (2015) Transferability of SSR markers of *Brassica* sp. to some Poplar varieties of *Brassica juncea*. PNAS, India, Sect. B. Biol. Sci. 85:1001-1010. doi:10.1007/s40011-014-0486-5

The Paleo Diet (2018) Vegetable oil fatty acid composition. [ONLION] Available at: http://thepaleodiet.com/%20vegetable-oil-fatty-acid-composition/ (Accessed 28 May 2018).

Thormann CE, Ferreira ME, Camargo LEA, Tivang JG and Osborn TC (1994) Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theor. Appl. Genet. 88:973-980. doi:10.1007/BF00220804

Tian H, Yan J, Channa SA, Zhang R, Guo Y and Hu S (2017) Analysis of the genome genetic diversity among *Brassica napus*, *B. rapa* and *B. juncea* accessions using specific simple sequence repeat markers. Pak. J. Bot. 49:125-132.

Tian HY, Channa SA and Hu SW (2017) Relationship between genetic distance, combining ability and heterosis in rapeseed (*Brassica napus* L.). Euphytica 213:1-12. doi:10.1007/s10681-016-1788-x

Tortosa M, Velasco P, Afonso D, Padilla G, Rios D and Soengas P (2017) Characterization of a Spanish *Brassica oleracea* collection using molecular and biochemical markers. Sci. Hortic. 219:344–350. doi:10.1016/j.scienta.2017.03.021

Tripathi MK and Mishra AS (2007) Glucosinolates in animal nutrition: a review. Anim. Feed Sci. Technol. 132:1-27. doi:10.1016/j.anifeedsci.2006.03.003

Udall JA, Quijada P and Osborn TC (2005) Detection of chromosomal rearrangements derived from homeologous recombination in four mapping populations of *Brassica napus* L. Genetics 169:967-979. doi:10.1534/genetics.104.033209

Udall JA, Quijada PA, Lambert B and Osborn TC (2006) Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (*Brassica napus* L.): 2. Identification of alleles from unadapted germplasm. Theor. Appl. Genet. 113:597–609. doi:10.1007/s00122-006-0323-1

Udall JA, Quijada PA, Polewicz H, Vogelzang R and Osborn TC (2004) Phenotypic efects of introgressing Chinese winter and resynthesized *Brassica napus* L. germplasm into hybrid spring canola. Crop Sci. 44:1990–1996. doi:10.2135/cropsci2004.1990

United States Department of Agriculture (USDA) (2016) Soybeans & Oil Crops, Canola. [ONLINE] Available at: http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx (Accessed 10 May 2016).

Unterseer S, Bauer E, Haberer G, Seidel M, Knaak C, Ouzunova M, Meitinger T, Strom TM, Fries R, Pausch H, Bertani C, Davassi A, Mayer KF and Schön C (2014) A powerful tool for genome analysis in maize: development and evaluation of the high density 600 K SNP genotyping array. BMC Genomics 15:823- 838. doi:10.1186/1471-2164-15-823

Van Inghelandt D, Melchinger AE, Martinant JP and Stich B (2012) Genome-wide association mapping of flowering time and northern corn leaf blight (*Setosphaeria turcica*) resistance in a vast commercial maize germplasm set. BMC Plant Biol. 12:56-71. doi:10.1186/1471-2229-12-56

Variath MT, Wu JG, Li YX, Chen GL and Shi CH (2009) Genetic analysis for oil and protein contents of rapeseed (*Brassica napus* L.) at different developmental times. Euphytica 166, 145-153. doi:10.1007/s10681-008-9851-x

Wang J, Kaur S, Cogan OI, Dobrowolski MP, Salisbury PA, Burton WA, Baillie R, Hand M, Hopkins C, Forster JW, Smith KF and Spangenberg G (2009) Assessment of genetic diversity in Australian canola (*Brassica napus* L.) cultivars using SSR markers. Crop Pasture Sci. 60:1193-1201. doi:10.1007/BF00220859

Wang J, Xian X, Xu X, Qu C, Lu K, Li J and Liu L (2017) Genome-wide association mapping of seed coat color in *Brassica napus*. J. Agr. Food Chem. 65:5229-5237. doi:10.1021/acs.jafc.7b01226

Wang L, Zhang F, Fu T and Shen J (2017) A review: status and prospect of heterosis prediction in rapeseed (*Brassica napus* L.) Oil Crop Sci. 2:184-194. doi:10.3969/j.issn.2096-2428.2017.03.006

Wang N, Chen B, Xu K, Gao G, Li F, Qiao J, Yan G, Li J, Li H and Wu X (2016) Association mapping of flowering time QTLs and insight into their contributions to rapeseed growth habits. Front. Plant Sci. 7:338. doi:10.3389/fpls.2016.00338

Wang X, Wang H, Long Y, Li D, Yin Y, Tian J, Chen L, Liu L, Zhao W, Zhao Y, Yu L and Li M (2013) Identification of QTLs associated with oil content in a high-oil *Brassica napus* cultivar and construction of a high-density consensus map for QTLs comparison in *B. napus*. PLOS One 8:e80569. doi:10.1371/journal.pone.0080569

Wang X, Wang H, Long Y, Liu L, Zhao Y, Tian J, Zhao W, Li B, Chen L, Chao H and Li M (2015) Dynamic and comparative QTL analysis for plant height in different developmental stages of *Brassica napus* L. Theor. Appl. Genet. 128, 1175–1192. doi:10.1007/s00122-015-2498-9

Wang X, Wang H, Wang J, Sun R, Wu J, Liu Sh, Bai Y, Mun JH, Bancroft I, Cheng F, Huang S, Li X, Hua W, Wang J, Wang X, Freeling M, Pires JC, Paterson AH and et al. (2011) The genome of the mesopolyploid crop species *Brassica rapa*. Nat. Genet. 43:1035-1039. doi:10.1038/ng.919

Wang Y, Chen W, Chu P, Wan S, Yang M, Wang M and Guan R (2016) Mapping a major QTL responsible for dwarf architecture in *Brassica napus* using a single-nucleotide polymorphism marker approach. BMC Plant Biol. 16:178–187. doi:10.1186/s12870-016-0865-6

Warburton ML and Hoisington D (2001) Application of molecular marker techniques to the use of international germplasm collections. In Henry RJ (Ed.) Plant genotyping: the DNA fingerprinting of plants. CABI Publishing, Wallingford, UK. pp 83-93.

Warwick SI (2010) Brassicaceae in agriculture. In Schmidt R and Bancroft I (Eds.) Genetics and Genomics of the *Brassicaceae*. Genetics and Genomics:Crops and Models 9. Springer Science and Business Media. pp 33–66. doi:10.1007/978-1-4419-7118-0_2

Wei D, Cui Y, He Y, Xiong Q, Qian L, Tong C, Lu G, Ding Y, Li J, Jung C and Qian W (2017)

A genome-wide survey with different rapeseed ecotypes uncovers footprints of domestication and breeding. J. Exp. Bot. 68:4791-4801. doi:10.1093/jxb/erx311

Wei D, Mei J, FuY, Disi JO, Li J and QianW (2014) Quantitative trait loci analyses for resistance to *Sclerotinia sclerotiorum* and flowering time in *Brassica napus*. Mol Breed 34:1797–1804. doi:10.1007/s11032-014-0139-7

Wei L, Jian H, Lu K, Filardo F, Yin N, Liu L, Qu C, Li W, Du H and Li J (2016) Genome-wide association analysis and differential expression analysis of resistance to *Sclerotinia* stem rot in *Brassica napus*. Plant Biotechnol. J. 14:1368−1380. doi:10.1111/pbi.12501

Wei Z, Wang M, Chang S, Wu C, Liu P, Meng J and Zou J (2016) Introgressing subgenome components from *Brassica rapa* and *B. carinata* to *B. juncea* for brodening its genetic base and exploring intersubgenomic heterosis. Front. Plant Sci. 7:1677. doi:10.3389/fpls.2016.01677

Westman AL and Kresovich S (1998) The potential for cross-taxa simple-sequence repeat (SSR) amplificaion between *Arabidopsis thaliana* L. and crop *Brassicas*. Theor. Appl. Genet. 96:272-281. doi:10.1007/s001220050736

Wickramasuriya SS, Yi YJ, Yoo J, Kang NK and Heo JM (2015) A review of canola meal as an alternative feed ingredient for ducks. J. Anim. Sci. Tech. 57:29-38. doi:10.1186/s40781-015-0062-4

Wu J, Li F, Xu K, Gao G, Chen B, Yan G, Wang N, Qiao J, Li J, Li H, Zhang T, Song W and Wu X (2014) Assessing and broadening genetic diversity of a rapeseed germplasm collection. Breed. Sci. 64:321–330. doi:10.1270/jsbbs.64.321

Würschum T, Liu W, Maurer HP, Abel S and Reif JC (2011) Dissecting the genetic architecture of agronomic traits in multiple segregating populations in rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 124:153-161. doi:10.1007/s00122-011-1694-5

Xiao Y, Chen L, Zou J, Tian E, Xia W and Meng J (2010) Development of a population for substantial new type *Brassica napus* diversified at both A/C genomes. Theor. Appl. Genet. 121:1141–1150. doi:10.1007/s00122-010-1378-6

Xiao Z, Zhang C, Tang F, Yang B, Zhang L, Liu J, Huo Q, Wang S, Li S, Wei L, Du H, Qu C, Lu K, Li J and Li N (2019) Oil content by combination of genome-wide association and transcriptome analysis in the oilseed crop *Brassica napus.* Biotechnol Biofuels 12:216. doi:10.1186/s13068-019-1557-x

Xu L, Hu K, Zhang Z, Guan C, Chen S, Hua W, Li J, Wen J, Yi B, Shen J, Ma C, Tu J and Fu T (2016) Genome-wide association study reveals the genetic architecture of flowering time in rapeseed (*Brassica napus* L.). DNA Res. 23:43–52. doi: 10.1093/dnares/dsv035

Xu Y (2010) Molecular Plant Breeding. CABI, Oxfordshire, Uk.

Yan J, Warburton M and Crouch J (2011) Association mapping for enhancing maize (L.) genetic improvement. Crop Sci. 51(2):433–449. doi:10.2135/cropsci2010.04.0233

Yeh FC, Yang RC and Boyle T (1999) POPGENE software package version 1.31 for population

genetic analysis. University of Alberta; Edmonton, AB, Canada.

Yousef EAA, Müller T, Börner A and Schmid KJ (2018) Comparative analysis of genetic diversity and differentiation of cauliflower (*Brassica oleracea* var. *botrytis*) accessions from two ex situ genebanks. PLOS One 13:e0192062. doi:10.5281/ zenodo.887955

Yu CY, Hu SW, Zhao HX, Guo AG and Sun GL (2005) Genetic distances revealed by morphological characters, isozymes, proteins and RAPD markers and their relationships with hybrid performance in oilseed rape (*Brassica napus* L.). Theor. Appl. Genet. 110:511–518. doi:10.1007/s00122-004-1858-7

Zhang H and Flottmann S (2016) Seed yield of canola (*Brassica napus* L.) is determind primarily by biomass in a high-yielding environment. Crop Pasture Sci. 67:369-380. doi:10.1071/CP15236

Zhang J, Li G, Li H, Pu X, Jiang J, Chai L, Zheng B, Cui C, Yang Z, Zhu Y and Jiang L (2015) Transcriptome analysis of interspecific hybrid between *Brassica napus* and *B. rapa* reveals heterosis for oil rape improvement. Int. J. Genomics 1–11. doi:10.1155/2015/230985

Zhang Y, Li Q, Cui Y, Liu Z, Chen Z, He Y, mei J, Xiong Q, Li X and Qian W (2018) Genetic characterization and fine mapping for multi-inflorescence in *Brassica napus* L. Theor. Appl. Genet. 131:2311-2319. doi:10.1007/s00122-018-3153-z

Zhao J, and Meng J (2003) Detection of loci controlling seed glucosinolate content and their association with *Sclerotinia* resistance in *Brassica napus*. Plant Breed. 122:19–23. doi: 10.1046/j.1439-0523.2003.00784.x

Zhao J, Becker HC, Zhang D, Zhang Y and Ecke W (2005) Oil content in a European × Chinese rapeseed population: QTL with additive and epistatic effects and their genotype-environment interactions. Crop Sci. 45:51–59. doi:10.2135/cropsci2005.0051

Zhao J, Becker HC, Zhang D, Zhang Y and Ecke W (2006) Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. Theor. Appl. Genet. 113:33-38. doi:10.1007/s00122-006-0267-5

Zhao X, Li B, Zhang K, Hu K, Yi B, Wen J, Ma C, Shen J, Fu T and Tu J (2016) Breeding signature of combining ability improvement revealed by a genomic variation map from recurrent selection population in *Brassica napus*. *Sci. Rep.* 6, 29553. doi: 10.1038/srep29553

Zheng M, Peng C, Liu H, Tang M, Yang H, Li X, Liu J, Sun X, Wang X, Xu J, Hua W and Wang H (2017) Genome-Wide association study reveals candidate genes for control of plant height, branch initiation height and branch number in rapeseed (*Brassica napus* L.). Front. Plant Sci. 8:1246. doi:10.3389/fpls.2017.01246

Zhou Q, Han D, Mason AS, Zhou C, Zheng W, Li Y, Wu C, Fu D and Huang Y (2018) Earliness traits in rapeseed (*Brassica napus*): SNP loci and candidate genes identified by genome-wide association analysis. DNA Res. 25:229-244. doi:10.1093/dnares/dsx052

Ziolkowski PA, Kaczmarek M, Babula-Skowronska D and Sadowski J (2012) *Brassica* genome evolution: dynamics and plasticity. In Edwards D, Batley J, Parkin IAP and Kole Ch (Eds.)

Genetics, genomics and breeding of oilseed *Brassicas*. CRC Press, USA. pp 14-46.

Zou J, Fu DH, Gong HH, Qian W, Xia W, Pires JC, Li RY, Long Y, Mason AS, Yang TJ, Lim YP, Park BS and Meng J (2011) De novo genetic variation associated with retrotransposon activation, genomic rearrangements and trait variation in a recombinant inbred line population of *Brassica napus* derived from interspecific hybridization with *Brassica rapa*. *Plant J.* 68, 212–224. doi:10.1111/j.1365-313X.2011.04679.x

Zou J, Hu D, Liu P, Raman H, Liu Z, Liu X, Parkin IAP, Chalhoub B and Meng J (2016) Co-linearity and divergence of the A subgenome of *Brassica juncea* compared with other *Brassica* species carring different A subgenomes. BMC Genomics 17:18. doi:10.1186/s12864-015-2343-1

Zou J, Jiang C, Cao Z, Li R, Long Y, Chen S and Meng J (2010) Association mapping of seed oil content in *Brassica napus* and comparison with quantitative traitloci identified from linkage mapping. Genome 53:908–916. doi:10.1139/G10-075

Zou J, Zhao Y, Liu P, Shi L, Wang X, Wang M, Meng J and Reif JC (2016) Seed quality traits can be predicted with high accuracy in *Brassica napus* using genomic data. PLoS One 11:e0166624. doi:10.1371/journal.pone.0166624

Zou J, Zhu J, Huang S, Tian E, Xiao Y, Fu D, Tu J, Fu T and Meng J (2010) Broadening the avenue of intersubgenomic heterosis in oilseed *Brassica*. Theor. Appl. Genet. 120:283–290. doi:10.1007/s00122- 009-1201-4