Broadening of genetic diversity in spring canola (*Brassica napus* L.) by use of C genome of *B. oleracea* var. *capitata*, and the effect of this on the performance of the inbred lines and their test-hybrids

by

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Abstract

Broadening the genetic base of spring *B. napus* canola is important for continued progress in breeding of hybrid cultivars. The objectives of this study were to (i) assess the potential of the C genome of *B. oleracea* var. *capitata* (cabbage) for broadening the genetic base of the C genome of *B. napus* through *B. napus* × *B. oleracea* interspecific cross, (ii) investigate the prospect of developing euploid 'canola' lines from the F_2 and BC_1 (F_1 × *B. napus*) plants, (iii) study the inheritance of *B. oleracea* SSR alleles in the interspecific-cross-derived inbred lines, and (iv) investigate the heterotic potential of these inbred lines by crossing to the spring *B. napus* parent as tester. Several canola quality inbred *B. napus* lines carrying allelic diversity introgressed from *B. oleracea* were developed through self-pollination of the interspecific hybrids. Analysis of the inbred lines with the C genome SSR markers indicated that the F_2-derived lines carried a greater number of *B. oleracea* alleles as compared to the BC_1-derived lines; however, the BC_1-derived population retained a greater proportion of the theoretically expected number of alleles as compared to the F_2-derived population. The mid-parent heterosis (MPH) for seed yield ranged from -22.5 to 43.3 % (mean 9.3 %) where the best performing hybrid showed 29 % improvement over the *B. napus* tester. Significant correlation was observed between the performance of the inbred lines and the test-hybrids; however, no correlation was found between the performance of the inbred lines and MPH. In general, the F_2-derived population exhibited greater heterosis for seed yield as compared to the BC_1-derived population. Correlation between genetic distance of the parents and MPH for seed yield was 0.31. Results from this study, thus, extend our knowledge of the value of the C genome of cabbage (*B. oleracea*) for exploitation in hybrid canola breeding.

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List of Symbols and Abbreviations

±	Plus/minus
≤	Less than or equal to
<	Less than
>	Greater than
$	Dollar currency
µmol/g	seed Micromoles per gram *per se*ed
ng/µl	Nanogram per microliter
°C	Degrees Celsius
χ^2	Chi-square test statistics
2*n*	Diploid number of chromosomes
♂	Symbol for male gender
♀	Symbol for female gender
AAFC	Agriculture and Agri-Food Canada
ABI	Applied biosystem
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
BC_1	First backcross generation
BC_1F_1	First filial generation after first backcross
BC_1F_x	x^{th} generation of BC_1-derived population
BD	Bindsachsener
BS	Badger Shipper
cm	Centimeter

cv.	Cultivar
df	Degree of freedom
DH	Doubled haploid
DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleotide triphosphate
F_1	First filial generation
F_x	x^{th} generation of F_2-derived population
FAME	fatty acid methyl esters
FAO	Food and Agriculture Organization of the United Nations
Fig.	Figure
FISH	Fluorescent in situ hybridization
g	Gram
GSL	Glucosinolate
HOLL	High oleic acid-low linolenic acid
lm	linear model
LSmeans	least-squares means
m	Meter
Mbp	Mega base pair
mM	Millimole
µM	Micromole
mm	Millimeter
min	Minute
MPH	Mid-parent heterosis
MS	Mean squares
n	Haploid number of chromosomes

n	Number of observation
NP	Non-parental
NIRS	Near infrared reflectance spectroscopy
P-value	Probability value
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
r	Pearson's correlation coefficient
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
RPM	rotation per minute
SE	Standard Error
SS	Sum of squares
SSR	Simple sequence repeat
t-test	Test statistic for t-test
Taq polymerase	*Thermus aquaticus* polymerase
UPGMA	Unweighted pair-group method with arithmetic mean
USDA	United States Department of Agriculture
var.	Variety
vs.	Versus

Chapter 1

Literature review

1.1 Introduction

Brassica napus (AACC, 2*n* = 38) canola, also known as oilseed rape, is the second most important oilseed crop in the world after soybean (USDA ERS, 2015). World production of canola was 67.5 million tonnes in 2015 constituting about 12.8 % of the world oilseed supply (USDA ERS, 2015). The usage of its oil, depending on its fatty acid composition, ranges from salad oil to deep frying. Therefore, the fatty acid composition of this seed oil is equally or more important than the quantity of seed being harvested, as this determines the usefulness and the value of this oilseed crop. The seed meal is used as protein supplement in livestock feed.

The global demand of canola oil is increasing day-by-day because of its excellent fatty acid profile for edible purposes; therefore, production of this oilseed crop needs to be increased. There are several ways to increase production, such as through improved agronomic practices and/or through the development of high yielding cultivars. Currently, most of the *B. napus* canola cultivars are F_1 hybrid. In *B. napus*, the F_1 hybrids generally produce higher yield than the parent lines (Grant and Beversdorf, 1985). Therefore, there is an increasing interest among the canola breeders to exploit the phenomenon heterosis (superiority of the hybrid over its parents), for which existence of genetic diversity among the hybrid parental lines is critical (for review, see Rahman, 2013). Commercial plant breeding, which often uses only locally adapted cultivars and breeding lines in crossing for the development of new cultivars, has narrowed down the genetic base of *B. napus* canola. Therefore, it is crucial to broaden the genetic base of this crop using exotic alleles of the genetically distinct types of *B. napus* and its allied species, such as *B. rapa* (AA, 2*n* = 20) and *B. oleracea* (CC, 2*n* = 18). However, all introgressed alleles does not

necessarily contribute to the performance of the inbred lines, or in hybrid performance and heterosis. Therefore, it is important to investigate the potential value of these alleles for use in breeding.

1.2 The impact of canola on Canadian and the world economy

The *Brassica* oilseed crop is the second most important crop in the word after soybean in regards to the production of oilseed (**Figure 1-1**) and is the third most important after palm and soybean in regards to vegetable oil supply in the world (Rahman et al., 2013). In Canada, cultivation of this oilseed crop started by an immigrant farmer in Saskatchewan in 1930s. Currently, this country produces about 18 million tonnes of seed annually, and has become the largest producer of canola in the world followed by China and India (McVetty and Duncan, 2015)(Canola Council of Canada, 2015) (**Figure 1-2**). Canola contributes more than $19 billion annually to the Canadian economy, and has created about 249,000 jobs in Canada (Canola Council of Canada, 2013). Canola seed contains about 45 % oil; it is estimated that one percent increase of oil in this seed can add about $90 million to the industry (Canola Council of Canada, 2016a). Canola meal, a by-product of seed after extraction of the oil, contains about 36% protein; this meal is a good source of protein for use in animal feed. (Canola Council of Canada, 2016b).

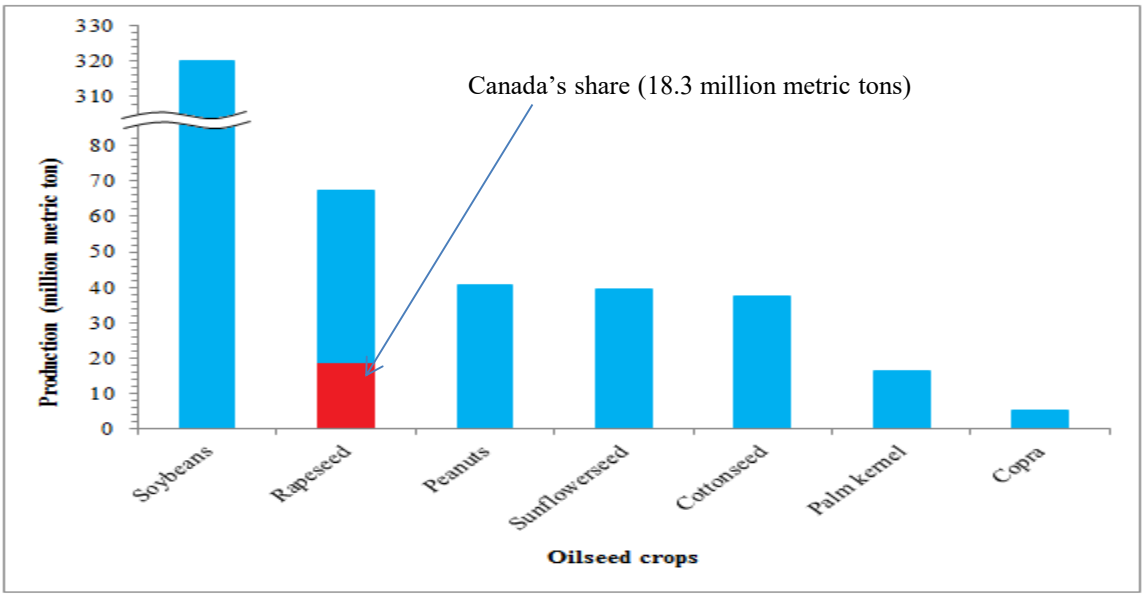

Figure 1-1. World oilseed production in 2015-16 ("USDA ERS," 2015)

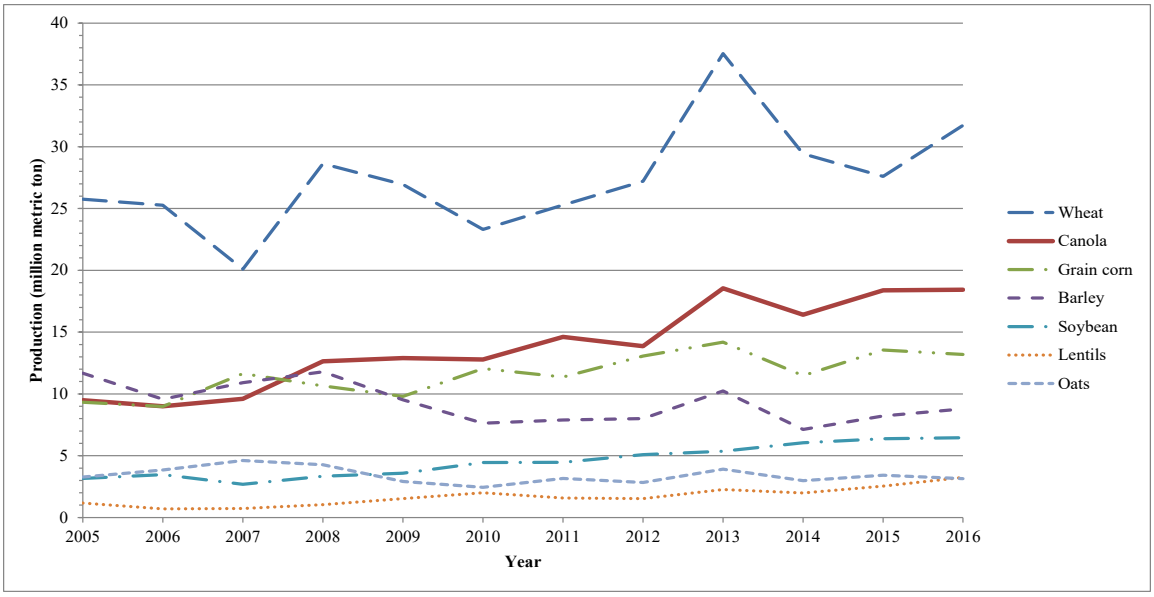

Figure 1-2. Production of *Brassica napus* canola as compared to major crops in Canada during the last decade

1.3 Polyploidy and genome evolution

Polyploidization has played an important role in the evolution of many eukaryotes including plants and vertebrates (Wendel, 2000). Studies have showed that polyploid species have evolved through hybridization between different populations of the parental species over a period of time

suggesting polyphyletic origin of most polyploid species (Soltis and Soltis, 1999). The newly emerged polyploid species generally undergo different genome reconstruction events, such as inter-genomic chromosomal exchanges, elimination of duplicated genes, gene silencing and transposon activation (Wendel, 2000; Chen and Ni, 2006). Interestingly, *Arabidopsis* genome evolution studies have showed that elimination of the duplicated genes is not a random process (Levy and Feldman, 2004), some of these genes retain in the genome due to their diversified functions (Blanc and Wolfe, 2004; Seoighe and Gehring, 2004).

1.3.1 *Brassica napus*: origin and subtypes

Based on study of the cytoplasmic genomes, two main routes were identified for the evolution of major diploid *Brassica* species: one route gave rise to *B. nigra* (BB), while the other route gave *B. oleracea* (CC) and *B. rapa* (AA). A *Diplotaxis erucoides* (n = 7) is believed to be the initial ancestor of *B. oleracea* and *B. rapa*, while *Sinapis* is the ancestor of *B. nigra* (Song et al., 1990; Warwick and Black, 1991). *B. napus* (AACC, 2n = 38) is an amphidiploid species, believed to have originated through hybridization between two diploid species *B. rapa* (AA, 2n = 20) and *B. oleracea* (CC, 2n = 18) (U, 1935, cited by Kimber and McGregor, 1995) (**Figure 1-3**). Unlike other oilseed crops, *B. napus* is a new crop which evolved about 7500 years ago (Chalhoub et al., 2014); however, it was domesticated only about 300–400 years ago (Go´mez-Campo and Prakash, 1999). Like other amphidiploid species, *B. napus* also known to have evolved in multiple locations involving independent hybridization between *B. rapa* and *B. oleracea* (Song and Osborn, 1992). One of the locations where the hybridization between *B. rapa* and *B. oleracea* is agreed to have occurred is the Mediterranean region, where the two parental species were found to co-exist. A study by Lagercrantz (1998), based on molecular markers, has identified high homoeology between the genomes of *B. napus* and its progenitor

species *B. rapa* and *B. oleracea* confirming that *B. napus* originated from these two diploid species. Based on fluorescent *in situ* hybridization (FISH), Snowdon et al. (2002) confirmed the presence of homologues of the A and C genome chromosomes, respectively, of *B. rapa* and *B. oleracea*, in *B. napus.*

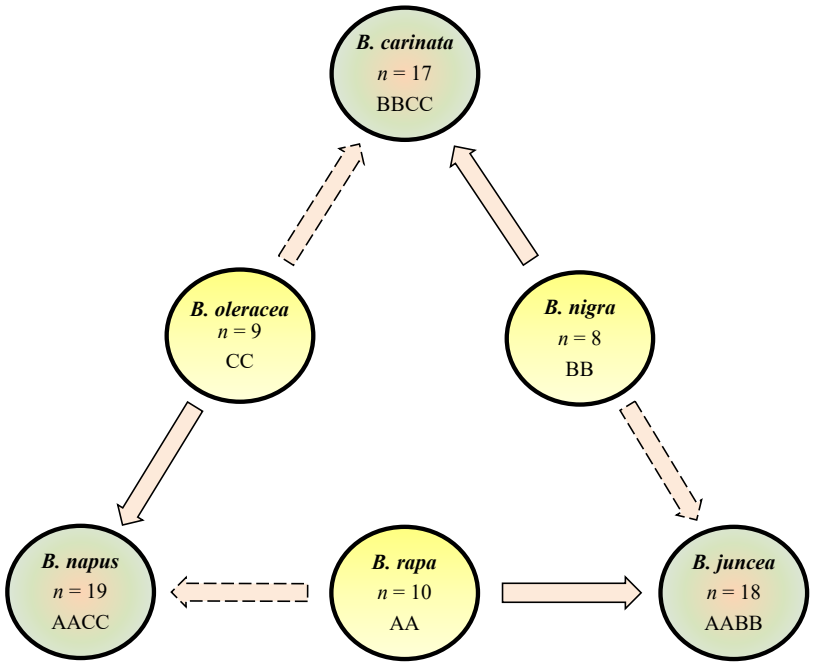

Figure 1-3. Cytogenetic relationship between amphidiploid and diploid species of the genus *Brassica*. Solid and broken lines represent male and female parents, respectively, of the amphidiploids (U, 1935, cited by Prakash et al., 2011)

1.3.2 Types of oilseed *B. napus*

B. napus is grown primarily as an oilseed crop, and to some extent as an edible vegetable and fodder crop; its cultivation has spread throughout the temperate and sub-tropical regions of the world. Based on the differences in growth habit, the oilseed *B. napus* is mainly classified into three groups: winter type, which is grown in Europe and requires vernalization to induce flowering; semi-winter type grown in China, which needs only a short period of vernalization; and spring type, which does not require vernalization for flowering and mainly grown in Canada,

Northern Europe, Australia, and in parts of Asia where it is grown as spring season crop (Friedt and Snowdon, 2009). Based on genetic diversity analysis by use of Restriction Fragment Length Polymorphism (RFLP) markers, Diers and Osborn (1994) found that these three growth habit types constitute three distinct gene pools.

1.3.3 Importance of *Arabidopsis* genome for understanding the genetics of *Brassica*

The knowledge of conservation of the gene sequences and their order among different species can be gained through comparative analysis of the genomes. Thus, the knowledge of genome sequence information of one species can be used to locate the genes in its related species. For example, *B. napus* has close phylogenic relationship with the model plant *Arabidopsis*; therefore, the genome sequence information of *Arabidopsis* can help to identify the candidate genes in *Brassica* through comparative analysis of the genomes of these two species. Study at whole chromosome level has disclosed extensive segmental rearrangement in the *Brassica* genome when compared with the genome of *Arabidopsis* (Lagercrantz, 1998); however, when the microstructure of the genome segments were compared, extensive collinearity of the conserved genes, particularly in the centromeric region, was found between the genomes of these two species (O'Neill and Bancroft, 2000; Rana et al., 2004). The sequencing of the *Arabidopsis* genome and identification of candidate genes has opened the possibility of identification of candidate genes in *Brassica* (Parkin et al., 2005; Kim et al., 2007). As the genome size of *Arabidopsis* (157 Mbp) (Bennett et al., 2003) is much smaller than that of *B. napus* (1130 Mbp) (*Brassica*.info, 2017), it is easier to study different traits in *Arabidopsis* and apply the results in *B. napus*. Niu et al. (2009) found that the genes involved in fatty acid biosynthesis and regulation of carbon flux were conserved between *B. napus* and *Arabidopsis*. Recently, the genome sequence of *B. napus* has been published (Chalhoub et al., 2014); this has opened a new avenue

for genetic improvement of this crop through the development of high density molecular markers, mapping of the traits and identifying candidate genes.

1.4 *Brassica* seed quality traits

The usefulness of vegetable oil for edible or industrial purpose is determined by its fatty acid composition. Traditional *Brassica* oilseed contained a high content of erucic acid in oil and glucosinolates in seed meal; these two seed constituents were the major limitations for use of this oil in edible purposes and the seed meal in animal feed. Seed oil from cruciferous (mustard family) plants contains a large amount of erucic acid, a monounsaturated C_{22} fatty acid; this fatty acid is not present in other vegetable oil. Traditional rapeseed contains 40-45 % erucic fatty acid in oil (Rakow, 2011) and 100-150 μmol glucosinolates (GSLs) per gram of dry matter in seed meal. The high level of erucic acid is not preferred in edible oil as this fatty acid is considered to be harmful for human health (Food Standards Australia New Zealand, 2003). Similarly, feeding monogastric animals with seed meal containing a high level of GSLs adversely affects their growth (Mawson et al., 1993; Friedt and Snowdon, 2009). *Brassica* oilseed cultivars with a low level of erucic acid (2 %) low glucosinolate content (< 30 μmol/g seed) were developed in the 1970s (Möllers, 2004). All canola cultivars available today are virtually free from erucic acid (< 1 %) and contain less than 30 μmol of total aliphatic glucosinolates per gram of air-dried, oil-free meal (Raymer, 2002). Presently, other types of oilseed *B. napus* cultivars with a modified fatty acid composition are available for different applications (Möllers, 2004). For instance, the *B. napus* oil with high content of erucic acid is used as lubricant and for production of polymers and biodegradable plastic, and oil with high oleic low linolenic acid (HOLL) contents is used as a frying oil because of higher oxidative stability (for review, see Scarth and Tang, 2006).

1.4.1 Genetic control of erucic acid

It is well understood from Harvey and Downey's (1964) experiment that two major gene loci are involved in the control of erucic acid synthesis in *B. napus* seed oil, and the genes act in additive manner. Ecke et al. (1995) mapped three QTL affecting seed oil content in *B. napus*, where two of these loci were associated with the two loci controlling erucic acid content; they also found a direct effect of the erucic acid genes on oil content. Li et al. (2014a) conducted an association mapping study using 472 accessions of *B. napus* and found that the two loci associated with erucic acid content in seed oil are located on the chromosome A8 and C3. These two erucic acid loci carry homoeologous *FAE1* (*fatty acid elongase 1*) genes involved in the control of erucic acid biosynthesis (Fourmann et al. 1998; Li et al. 2014a). Wu et al. (2008) found that a 4 bp deletion in the *FAE1* gene of the C genome results blockage of erucic acid synthesis, and thus confirmed that the zero erucic acid phenotype is due to mutation in the *FAE1* genes. In addition to the two major loci of A8 and C3, Qiu et al. (2006) also reported two minor loci on A1 and A2 involved in the control of erucic acid content in *B. napus* seed oil; the proportion of phenotypic variance explained by these four loci found to be 45.1, 30.4, 5.8 and 6.6%, respectively. Xu et al. (2015) identified an additional QTL, located on A7, involved in the control of erucic acid. Thus, the results reported above showed that the genes from both A and C genomes are involved in the control of erucic acid content in *B. napus* seed oil, and the zero erucic acid phenotype is due to recessive alleles in homozygous state at all loci.

1.4.2 Genetic control of glucosinolates

Glucosinolates are sulfur- and nitrogen-containing plant secondary metabolites mainly present in plants of the family *Brassicaceae* and related plant families. These compounds serve as defense for plants against insect and pest (Hopkins et al., 2009). The level of glucosinolates in seed and

plant parts is affected by various abiotic factors, such as drought, and the levels of sulphur and nitrogen in the soil (Hopkins et al., 2009). The inheritance of glucosinolate is complex as compared to erucic acid; this trait is controlled by many loci and also significantly influenced by environment (Basunanda et al., 2007). According to Kondra and Stefansson (1970), glucosinolate content in *B. napus* seed meal is controlled by at least four loci and the trait is under maternal genotype control. Uzunova et al. (1995) identified four QTL controlling glucosinolate content; whereas Toroser et al. (1995) detected two major and three minor QTL on five linkage groups of *B. napus.* Li et al. (2014a) performed association mapping using 472 *B. napus* accessions and detected four QTL located on A9, C2, C7 and C9 controlling this trait. Xu et al. (2015) mapped nine QTL controlling glucosinolate content on A3, A4, A8, A9, C1, C2, C7 and C9; these QTL collectively explained 83.8 % of the total phenotypic variance for this trait. Additional loci involved in the control of this trait in *B. napus* cannot be ruled out given that Rahman et al. (2014) detected QTL on A2 and A7, in addition to the QTL on A9, while working with the progenitor species *B. rapa*.

1.5 Genetic diversity

1.5.1 Genetic diversity in crop species

Genetic diversity present in a species represents the total number of alleles and the frequency of their occurrence (El-Esawi, 2017). Different plant species have various level of genetic diversity depending on their reproduction mechanism (self- or cross- pollinated), the means of seed dispersal and geographic spread (Brown, 1979; Hamrick, 1979). For example, plant populations of a cross-pollinated species are genetically more variable as compared to a self-pollinated species (Gottlieb, 1977). Essentially, a wide range of allelic diversity is required for a plant population to adapt to the changing environment and to increase fitness (Willi et al., 2006). A

low genetic diversity in a population or plant species implies that limited allelic variation is available in the gene pool; this is a bottleneck in crop breeding. The Irish famine is a classic example of how devastating consequences could be for this type of genetic bottleneck. Therefore, it is important to conserve and, wherever possible, introduce new alleles into crop germplasm to enlarge the pool of alleles for various traits (Reed and Frankham, 2003) including disease and pest resistance (O'Brien and Evermann, 1988). The knowledge of genetic relationships among the inbred lines can be useful in selecting parents in a crossing program, in assigning breeding materials to heterotic groups, and for identification of cultivars for plant variety protection (Hallauer and Miranda Fo, 1988).

1.5.2 Genetic diversity in *B. napus*

Despite a very short history of cultivation, *B. napus* has become one of the most important oilseed crops in the world. Wild form of *B. napus* does not exist, and land race of this species cannot be found (Iniguez-Luy and Federico, 2011); this indicates that genetic diversity in this species is narrow. A high level of homoeology exists between the A and the C genomes of *B. napus* (Cheung et al., 1997), and a high collinearity exists between the two genomes of *B. napus* and its two diploid progenitor species, *B. rapa* and *B. oleracea* (Chalhoub et al., 2014). Based on simple sequence repeat (SSR) marker analysis, Seyis et al. (2003) reported that a low genetic diversity exist in oilseed *B. napus*. Several reasons including restricted geographical distribution of a specific type and introgression of canola quality traits from a limited germplasm sources can be accounted as some of the reasons for the narrow genetic base of this species. Indeed, Bus et al., (2011) found low genetic diversity in *B. napus* germplasm, and proposed that strong selection for canola quality type might be one of the reasons for this. Currently, canola quality cultivars captured the major share of the total production of this crop; however, the alleles for low erucic

acid and low glucosinolate in majority of these cultivars were transferred from only two cultivars Liho and Bronowski, respectively (Friedt and Snowdon, 2009).

Apparently, genetic diversity in the A genome of *B. napus* is higher than in its C genome (Bus et al., 2011). Based on fixation index F_{ST} from SNP data analysis, Wang et al. (2014) reported that only 6-10 % of the genomic regions of *B. napus* contain most genes for important agronomic traits, and these regions might have been under strong selection pressure for the improvement of these traits in breeding programs. Interestingly, they found higher number of F_{ST} signals from the C genome than the A genome indicating that the C genome might have contributed greater number of valuable alleles for important traits in the elite *B. napus* lines and cultivars. Intensive selection pressure for agronomic and seed quality traits on this genome might be one of the reasons for low genetic diversity found in the C genome by Bus et al. (2011).

1.5.3 Sources of genetic diversity in *B. napus*

1.5.3.1 Within the species

Diers and Osborn (1994) reported that *B. napus* of different growth habit types, such as the European winter, Chinese semi-winter and spring type are genetically distinct from each other. Bus et al. (2011) reported that rutabagas are genetically distinct from these three types of *B. napus*. In addition to breeding history, adaptation of germplasm in distinct environment also played an important role in creating genetic diversity among the different types of *B. napus* (Bus et al., 2011). Genetic diversity in spring *B. napus* canola can be enriched by using its primary genepool, such as winter and Chinese semi-winter type, and rutabaga in breeding. Efforts have been made by different researchers, such as Butruille et al. (1999), Quijada et al. 2004), Kebede et al. (2010) and Rahman and Kebede (2012) to utilize genetic diversity of the winter type *B. napus,* and by Qian et al. (2007) to utilize genetic diversity of the semi-winter type *B. napus* to

broaden the genetic base of spring canola and to increase the level of heterosis in hybrid cultivars. Some of the researchers, for example Rahman and Kebede (2012), reported that the spring growth habit inbred lines derived from winter × spring canola cross often exhibit late flowering and maturity; therefore, an additional cycle of breeding is required for the improvement of these traits.

1.5.3.2 Introgression of genetic diversity from allied species

Even though the A and C genomes of *B. napus* are found to be colinear with the A and C genomes of its progenitor species *B. rapa* and *B. oleracea*, respectively (Cai et al., 2014b), considerable variation exists between the genomes of this amphidiploid species and the two allied species (Thormann et al., 1994; Abel et al., 2005). Therefore, greater allelic diversity in *B. napus* canola can be achieved by using the progenitor species *B. rapa* and *B. oleracea* in the breeding of *B. napus.* These progenitor species can be crossed directly to *B. napus* for the development of genetically distinct *B. napus* lines. *B. rapa* naturally hybridizes with *B. napus* (Hansen et al., 2001), therefore it is easier to produce *B. napus* × *B. rapa* interspecific hybrids; several researchers introduced alleles from *B. rapa* into *B. napus* (Qian et al., 2006; Mei et al., 2011; Attri and Rahman, 2017). Alternatively, these two progenitor species can be crossed to resynthesize *B. napus*; however, performance of this new type of *B. napus* is generally poor for seed yield (Kraling, 1987). Therefore, the resynthesized *B. napus* is generally crossed to natural *B. napus* to increase allelic diversity in the natural type (Becker et al., 1995; Morgan et al., 1998; Fujii and Ohmido, 2011; Karim et al., 2014). In the past, alleles for specific traits, such as self-incompatibility and early flowering were introgressed from *B. oleracea* by different researchers through *B. napus* × *B. oleracea* interspecific cross (Ripley and Beversdorf, 2003; Rahman et al., 2011). *B. napus* was also resynthesized from *B. oleracea* and *B. rapa* with primary objective of

introducing alleles for important traits, such as self-incompatibility (Rahman, 2005), yellow seed color (Rahman, 2001; Wen et al., 2008) and resistance to silique shattering (Morgan et al., 1998).

Wide morphological and genetic diversity is present in *B. oleracea* (Izzah et al., 2013; El-Esawi et al., 2016); however, this species has not been utilized in the breeding of *B. napus*. Recently, Bennett et al. (2012) and Rahman et al. (2015) demonstrated the prospect of broadening the genetic base of spring *B. napus* canola by using *B. oleracea* var. *alboglabra*, and Li et al. (2014b) used a *B. oleracea* var. *acephala* line in the breeding of semi-winter *B. napus*. Therefore, other genetically distinct types of this diploid species can be utilized to broaden the genetic base of spring *B. napus*.

1.6 Hybrid breeding and heterosis

Shull (1948) proposed the term heterosis while working with corn in 1910's to portray the phenomenon whereby two genetically diverse parents produce superior hybrid progeny. Several hypotheses have been proposed to explain the phenomenon heterosis or hybrid vigor, such as dominance hypothesis (Keeble and Pellew, 1910; Bruce, 1910), over-dominance hypothesis (Hull, 1945), and epistasis hypothesis (Fasoulas and Allard, 1962). The dominance hypothesis considers that hybrid vigor is an outcome of a combination of different dominant alleles from two genetically different parents; while the over-dominance hypothesis suggests that performance of heterozygous genotype is superior over either of the dominant or recessive homozygous conditions (Bradshaw, 2016). The epistasis hypothesis describes heterosis results from non-allelic interaction among different loci. Among the field crops, heterosis was firstly utilized in maize; afterwards, hybrid cultivars have been developed in many crops like cotton, canola, sorghum and sunflower, and exploited the phenomenon heterosis for different traits including seed yield. However, the extent of increased yield in hybrid cultivars varies depending

on crop species (for review, see Fu et al., 2014). Because of higher yield in F_1 hybrids, this type of cultivars has been widely accepted by growers. With the availability of pollination control mechanism in different crops for production of commercial hybrid seeds in cost effective manner, the development of hybrid cultivars has gained increasing interest among the plant breeders (Duvick, 2005).

In a hybrid breeding program, the breeding lines need to be evaluated to assess their potential as hybrid parents. For this, they should first be tested for *per se* performance, general combining ability (GCA) and then for specific combining ability (SCA). The estimates of GCA and SCA help to understand the gene actions (additive or non-additive) responsible for heterosis for the specific trait. Different methods, such as test cross and diallel cross, can be applied for these purposes. Diallel cross is the best method for estimating GCA and SCA, but it is very time consuming and expensive to produce many hybrid combinations with large number of parents especially when pollination control system is not available for the newly developed breeding lines. Furthermore, it is also important to investigate the feasibility of predicting performance of the hybrid based on performance of the parental lines. Ertiro et al. (2013) and Miedaner et al. (2014) while working with maize and rye, respectively, reported a positive correlation between the performance of inbred and hybrid; however, the degree of correlation varied depending on traits and environmental conditions. This suggests the importance of evaluation of as many lines as possible for their hybrid performance based on availability of the resources. Test-cross method is generally used for screening of a large number of inbred lines for their performance in hybrid. If the number of lines is very large, it becomes difficult to use several testers for evaluating the inbred lines for their potential as hybrid parents.

1.6.1 Hybrid breeding and heterosis in *B. napus*

B. napus is a predominantly self-pollinating crop with an outcrossing of about 20 % (Rakow and Woods, 1987); however, outcrossing can be as much as 36 % (Persson, 1956 cited by Rakow and Woods, 1987). This indicates the possibility of utilizing the phenomenon heterosis, which is more prominent in cross-pollinated cross than in self-pollinating crops like wheat. In *B. napus*, the level of heterosis can vary from trait to trait. For example, Grant and Beversdorf (1985) reported positive heterosis of up to 72 % for seed yield, but a negative value for heterosis up to 18% for protein content over the better parent in spring *B. napus* and demonstrated the feasibility of utilization of the seed yield heterosis at commercial level.

Several researchers produced test-hybrids using a single tester to illustrate the importance of allelic diversity introgressed from winter (Butruille et al., 1999; Quijada et al., 2004) or semi-winter (Udall et al., 2006) type to increase seed yield in spring *B. napus* hybrid canola. Riaz et al. (2001) observed mid-parent heterosis for seed yield ranging from 26 % to 169 % in test-hybrids produced by use of genetically distinct *B. napus* inbred lines. Gehringer et al. (2007) examined test-hybrids generated by crossing of 190 winter type doubled haploid (DH) lines, carrying genome content from resynthesized *B. napus,* to a male sterile *B. napus* line as tester, and reported up to 43% mid-parent heterosis for seed yield. Similarly, Qian et al. (2007) also found positive mid-parent heterosis for seed yield in hybrids produced by crossing of spring type *B. napus* to Chinese semi-winter type carrying alleles introgressed from *B. rapa*. Radoev et al. (2008) and Basunanda et al. (2010) witnessed 30 % and 16.3 % heterosis, respectively, for seed yield in winter *B. napus* test-hybrids produced using doubled-haploid lines carrying alleles from resynthesized *B. napus*; they reported that dominance and epistasis effects primarily contribute to heterosis. Girke et al. (2012) reported –3.5 to 47.2 % mid-parent heterosis, and up

to 16.6 % heterosis over *B. napus* tester for seed yield in the hybrids produced from resynthesized *B. napus* and natural winter *B. napus* testers. Li et al. (2014) evaluated test-hybrids produced by crossing of semi-winter *B. napus* lines carrying genome content of *B. oleracea* var. *acephala* and a semi-winter elite *B. napus* line, and found heterosis for seed yield, plant height, number of branches per plant and main inflorescence length. Rahman et al. (2016) compared the effect of allelic diversity introgressed into spring *B. napus* canola from various sources, such as genetically distinct spring *B. napus* canola, winter *B. napus* canola and *B. oleracea*, for heterosis in spring *B. napus* canola hybrid. They found the highest mid-parent heterosis for the inbred population carrying the genome content of *B. oleracea* and the highest hybrid yield for the population carrying genome content of winter canola. The correlation between mid-parent heterosis and inbred line yield was negative, but slightly positive between hybrid yield and inbred yield in all three populations. This suggests that the alleles exerting non-additive effect in the genetic control of heterosis can be found frequently in *B. oleracea*, and the additive effect of the genes for greater seed yield in hybrids or GCA can be found in winter canola.

The availability of different pollination control systems, such as *Ogu*-INRA and Male Sterility Lembke system (MSL-system), the floral morphology of this crop, and the involvement of private seed companies in *B. napus* canola breeding has changed the cultivar type of this crop from open-pollinated to F_1 hybrids (Renard et al., 1997; Bradshaw, 2016; reviewed in, El-Mezawy et al., 2016).

1.6.2 Genetic diversity and heterosis

Genetically distinct parent lines are essential for the development of hybrid cultivars and utilization of heterosis; therefore, understanding the relationship between genetic diversity of the parent lines and heterosis is important in hybrid breeding. Ali et al. (1995) reported that genetic

diversity between the parents positively correlates with heterosis for seed yield, number of silique per plant and number of seeds per silique in winter canola. Li et al. (2014) also found significant positive correlation between mid-parent heterosis for seed yield and genome content introgressed from *B. oleracea* while working with Chinese semi-winter *B. napus*. On the contrary, Diers et al. (1996) found that genetic distance between the parents alone is not sufficient to predict heterosis in spring *B. napus* canola. However, they found that combination of GCA and genetic distance of the parents give greater estimate of heterosis. Similarly, Qian et al. (2007) also detected lower correlation between genetic distance of the inbred lines and hybrid performance than correlation between GCA of the parents and hybrid performance; this indicates that additive effect of the genes or GCA play an important role in hybrid performance. Luo et al. (2016) also found that genetic distance between the parents, estimated by SNP markers, does not correlate with heterosis for harvest index (HI), but slightly positively correlate with heterosis for seed yield. Jesske et al. (2013) evaluated the test-hybrids produced from crossing of resynthesized *B. napus* and natural winter *B. napus* (tester) and found slightly negative correlation between genetic distance of the parents and hybrid yield. This might be due to the extreme genetic divergence between the parents resulting from the use of wild *B. oleracea* to produce the resynthesized *B. napus* lines.

1.7 Research objectives

1.7.1 Long term objectives

The long-term objective of this research is to broaden the genetic base of the Canadian spring *B. napus* canola through introgression of allelic diversity from its allied species for higher heterosis in hybrid canola cultivars.

1.7.2 Short term objectives

The following short-term objectives were laid out for this M.Sc. thesis research:

- Estimate the genetic diversity of the spring *B. napus* lines derived from F_2 of *B. napus* × *B. oleracea* var. *capitata* and BC_1 of (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus* crosses using SSR (simple sequence repeats) markers.
- Compare the F_2- and BC_1-derived inbred lines for allelic diversity, agronomic and seed quality traits.
- Estimate the heterotic potential of the C genome alleles introgressed from *B. oleracea* into newly developed spring *B. napus* canola lines in test-hybrids.

In addition to these studies, data available from canola program of the University of Alberta on the development of interspecific hybrids from *B. napus* × *B. oleracea* crosses, and the development of *B. napus* type canola quality inbred lines from these interspecific crosses were also analyzed, and the results are included in this thesis.

1.8 Research hypotheses

The following research hypotheses were tested in this M.Sc. thesis research

- The inbred lines derived from F_2 will exhibit greater diversity than the inbred lines derived from BC_1.
- Inbred lines carrying allelic diversity introgressed from *B. oleracea* will exhibit heterosis in test-hybrids with elite canola line.

Chapter 2

Development of canola quality inbred lines from *B. napus* × *B. oleracea* var. *capitata* interspecific crosses

2.1 Introduction

Brassica napus (AACC, 2*n* = 18) canola is an important oilseed crop in the family *Brassicaceae*. This crop is mainly grown for its edible oil, and plays an important role in the Canadian and world economy. It contributed more than $19 billion to the Canadian economy and created about 249,000 jobs in Canada in 2013 (Canola Council of Canada, 2013).

This is an amphidiploid species carrying two genomes, A and C, contributed by its two progenitor species *B. rapa* and *B. oleracea*, respectively (Parkin et al., 1995). The natural *B. napus* contains a high content of erucic acid in seed oil (> 40%) and glucosinolate in seed meal (> 60 μmol/g seed), which are anti-nutritional for human and animal health, respectively. The two major modifications made through plant breeding that gave rise to canola were reduced contents of erucic acid (< 2 %) in seed oil and glucosinolate (< 30 μmol/g seed meal) in seed meal. In most commercial cultivars, the canola quality genes were transferred from only a small number of donors: low erucic acid from the German cv. Liho and low glucosinolate from the Polish cv. Bronowski (Stefansson and Downey, 1995). The use of canola quality germplasm with limited genetic variation in repeated cycles of breeding has contributed to the narrow genetic diversity observed today in this crop (for review, see Rahman, 2013).

Canola quality line cultivars or hybrids are currently grown in most of the canola-growing countries. Growing of cultivars with narrow diversity is comparable to monoculture, and this can be disastrous for several reasons, such as change in climate and evolution of new insect pests and diseases (Smith et al., 2015). Currently, hybrid canola cultivars have captured

most canola acreage in most countries. To increase the yield potential of hybrid cultivars, it is critical to have genetic diversity among the parent lines (Riaz et al., 2001). Therefore, there is a need to diversify the canola-quality germplasm in breeding programs.

Some efforts have been made to broaden the genetic base of spring *B. napus* canola by introgressing diverse alleles from its primary genepool, such as winter and semi-winter *B. napus,* and the secondary gene pool, such as *B. rapa* and *B. oleracea*. Among these, very limited effort has been made towards the utilization of *B. oleracea* in the breeding of spring *B. napus* canola (for review, see Rahman, 2013). The primary genepool can provide some allelic diversity as genetic diversity in this gene pool is known to be low; in contrast, the secondary genepool, which includes progenitor and allied species, possesses wide allelic diversity (Seyis et al., 2003) which can be used for the improvement of spring *B. napus* canola. It is well understood that the A and C genomes of *B. napus* are genetically distinct from the A and C genomes of *B. rapa* and *B. oleracea,* respectively (Thormann et al., 1994); genetic exchange between these two genomes does not occur while staying together in this amphidiploid species (Howell et al., 2008). Therefore, genetic enrichment of these two sub-genomes of *B. napus* canola is needed. Apparently, genetic diversity in the A genome of *B. napus* is higher than in its C genome (Bus et al., 2011). This might be due to the reason that *B. rapa* can hybridize with *B. napus* naturally (Hansen et al., 2001) and many researchers have also used *B. rapa* (Chen et al., 2010; Mei et al., 2011) to enrich the A genome of *B. napus*. In contrast, very few attempts have been made to diversify the C genome of *B. napus* by using *B. oleracea* despite wide genetic diversity exists in this progenitor species (Song et al., 1988; Louarn et al., 2007; Izzah et al., 2013).

Bennett et al. (2012) and Rahman et al. (2016) investigated the potential of the use of *B. oleracea* to diversify the C genome of spring *B. napus* canola by using the *B. oleracea* variant

alboglabra. Similarly, Li et al. (2014) demonstrated the feasibility of using *B. oleracea* var. *acephala* to diversify the C genome of Chinese semi-winter *B. napus*. The objective of this research was to explore *B. oleracea* var. *capitata* (cabbage) as a source of C genome alleles to diversify the C genome of spring *B. napus* canola and to investigate the potential of these genetically diversified canola lines for use in hybrid breeding.

2.2 Materials and methods

2.2.1 Parental lines and populations

The parental materials of the crosses used in this study were a spring *B. napus* (AACC, 2*n* = 38) line A04-73NA and two *B. oleracea* var. *capitata* (CC, 2*n* = 18) cabbage cultivars, Badger Shipper (BS) and Bindsachsener (BD). The *B. napus* line A04-73NA was developed by the Canola Program of the University of Alberta, and seeds of the two *B. oleracea* var. *capitata* cultivars were collected from Green Gene International, UK. Both the *B. oleracea* parents contained a high content of erucic fatty acid (> 40 %) in seed oil, and a high content of glucosinolate (> 60 µmol/g seed) in seed meal; while A04-73NA is a canola quality breeding line, i.e. contains < 2 % erucic acid in seed oil and < 30 µmol glucosinolate per gram seed meal.

2.2.2 Development of interspecific hybrids (F_1) and backcrosses (BC_1) plants

The *B. napus* line A04-73NA was crossed as female to the two *B. oleracea* cultivars to retain the cytoplasm of *B. napus* in the hybrid progenies:

1) A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper (Cross ID: 1362). Different generation populations derived from F_2 of this cross are designated as BS-F_x, where 'x' indicates the generation.

2) A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener (Cross ID: 1363). Different generation populations derived from F_2 of this cross are designated as BD-F_x, where 'x' indicates the generation.

It is difficult to produce F_1 plants of *B. napus* × *B. oleracea* interspecific crosses through conventional crossing followed by harvest of F_1 seeds (Downey et al., 1980). Therefore, *in vitro* ovule culture technique, as described by Bennett et al. (2008), was applied to rescue the interspecific hybrid embryos and generate F_1 plants. The F_1 plants were backcrossed to the *B. napus* parent using the F_1's as female and the following BC_1 plants were produced:

1) (A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper) × A04-73NA (Cross ID: 1681). Different generation populations derived from BC_1 of this cross are designated as BS-BC_1F_x, where 'x' indicates the generation.
2) (A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener) × A04-73NA (Cross ID: 1682). Different generation populations derived from BC_1 of this cross are designated as BD-BC_1F_x, where 'x' indicates the generation.

All these interspecific crosses were made by the Canola Program of the University of Alberta, and I received F_6 and BC_1F_5 generation populations planted in field in 2014.

2.2.3 Development of F_2- and BC_1-derived RILs

The F_1 plants were self-pollinated manually to produce F_2 population (**Figure 2-1**). The F_2 and BC_1 populations were grown in a greenhouse and self-pollinated by bag isolation with transparent and micro perforated plastic bags. The F_3 and BC_1F_2, F_4 and BC_1F_3, F_5 and BC_1F_4, and F_7 and BC_1F_6 populations were grown in a greenhouse (21°/18° ± 2 °C day/night) in spring 2012, winter 2012-13, winter 2013-14 and 2014-15, respectively, located at the Agriculture-Forestry building of the University of Alberta. The F_6 and BC_1F_5 populations were grown in field

in summer 2014 at the Edmonton Research Station of the University of Alberta in two-meter-long single row plots with 50 cm space between the rows. The F_8 and BC_1F_7 populations were grown in field in summer 2015 along with their test-hybrids. These populations were also grown in greenhouse in winter 2015-16 to produce F_9 and BC_1F_8 generations. In all cases, self-pollinated seeds obtained through bag isolation of individual plants were used to grow the next generation population. In all these generations, selection primarily focused on plant fertility, zero erucic acid content in seed oil and low glucosinolate content in seed meal.

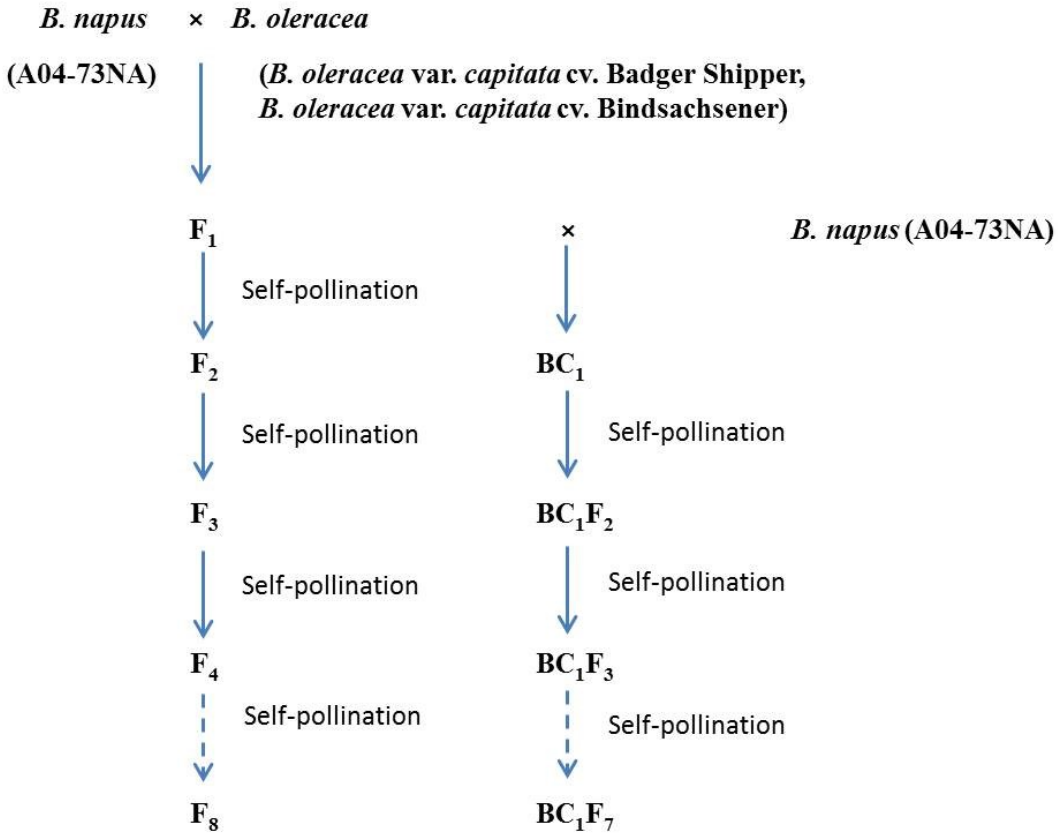

Figure 2-1. Flow diagram showing the development of recombinant inbred lines (RILs) from *B. napus* × *B. oleracea* interspecific crosses.

2.2.4 Estimation of plant fertility

Plant fertility in F_2 and BC_1, F_3 and BC_1F_2, F_6 and BC_1F_5, and F_7 and BC_1F_6 generation populations was estimated based on the ability of the plants to produce seed under bag isolation.

In addition to this, silique length (mm) and number of seeds per silique was also recorded for F_3, F_4 and BC_1F_3, and F_6 and BC_1F_5 populations as a measure of estimate of their fertility. For this, length of five siliques from the middle of the main branch was measured and the number of seeds produced in these siliques was counted. The mean values of silique length and number of seeds per silique were calculated and used for statistical analysis.

2.2.5 Estimation of relative nuclear DNA content by flow cytometer

Relative nuclear DNA content in F_7, BC_1F_6, F_8 and BC_1F_7 generation plants and their parents, grown in a greenhouse, were estimated using a Partec Cyflow® Space flow cytometer. For this, leaf samples of about 5 mm^2 were collected in petri dishes from two-week old plants and 0.4 ml nuclei extraction buffer was added to each sample. The samples were chopped by a sharp razor blade and incubated for 2 minutes. After that, the samples were filtered through 50 µm CellTrics® filter into sample tubes and 1.6 ml staining buffer was added, and the samples were incubated for 60 seconds before being analyzed by the Partec flow cytometer.

2.2.6 Fatty acid analysis

Self-pollinated seeds harvested from F_2, F_3, F_4, and BC_1 and BC_1F_3 generation plants were analyzed for erucic acid content by a gas chromatograph. For this, 0.10 to 0.25 g seed from individual plants were crushed in N-pentane in 50 ml conical tube and the samples were centrifuged at 1500 rpm for 15-20 minutes. The supernatant was transferred to 10 × 75 mm glass tube and left overnight for evaporation of N-pentane and recovery of the extracted oils. The extracted oil was methylated to produce fatty acid methyl esters (FAME) and analyzed by a Hewlett-Packard chromatograph (model 6890 N) equipped with a flame ionization detector to determine the fatty acid profile of the oils (for detail, see Bennett et al. 2008).

2.2.7 Glucosinolate analysis

Glucosinolate (GSL) analysis was performed on self-pollinated seeds of F_3, F_4 and BC_1F_3 generation populations, and open-pollinated seeds of F_6, F_8, and BC_1F_5 and BC_1F_7 generation populations by use of a Near Infrared Reflectance Spectroscopy (NIRS) (Foss North America, Eden Prairie, MN). NIRS is an important technique for analysis of glucosinolate, oil and protein contents in seeds in a non-destructive manner, and allows analysis of a large number of samples in a short period of time (Möllers, 2004). For NIR analysis, 2.5 to 4.0 g self-pollinated seeds harvested from individual plants grown in greenhouse or 5 to 8 g bulk open-pollinated seeds harvested from field plots were used. Glucosinolate content was calculated on whole-seed basis at 8.5 % moisture content and reported as μmol/g seed.

2.2.8 Statistical analysis

Data collected from different populations grown in different growth conditions were analyzed separately. In most cases, unless specified in a particular section or data table, four populations, two derived from F_2, namely BS-F_x and BD-F_x, and two derived from BC_1, namely BS-BC_1F_x and BD-BC_1F_x, (subscripted 'x' indicates filial generation) were grown along with the spring *B. napus* canola parent, A04-73NA. Different statistical parameters were calculated for all populations separately, as well as based on pooled-data of the two crosses to examine the relationship between F_2- and BC_1-derived populations.

All the data were analyzed using R 3.3.2 statistical computing and graphics software (R Core Team, 2016). Instead of normal arithmetic mean, LSmeans were calculated because of unequal number of observations in different populations. For this, the LSmeans package of 'R' (Lenth, 2016) was used for calculating mean, standard error (SE), confidence intervals, and pair-wise comparison of various populations. Tukey's multiple comparison tests was used for

pairwise comparison of the population means; this test is very efficient when sample sizes are unequal. The whole and the selected population means were compared using Student's t-test.

2.3 Results

2.3.1 Development of interspecific hybrids, and F_2 and BC_1 populations

A total of 34 buds of *B. napus* were pollinated with pollen from the two *B. oleracea* parents; this yielded 30 fertilized (developed to normal size) ovules **(Table 2-1)**. Thus, the average number of ovules per cross was 2.26. Culture of these ovules in liquid media resulted a total of 34 embryos – 11 embryos from A04-73NA × *B. oleracea*-BS and 23 embryos from A04-73NA × *B. oleracea*-BD crosses. Thus, the number of embryos obtained from crossing of the two *B. oleracea* parents, Badger Shipper and Bindsachsener, to *B. napus* was 0.73 and 1.21 per pollinated bud, respectively. Finally, a total of 29 F_1 plants from the two crosses were transferred to soil. Apparently, the cross A04-73NA × *B. oleracea*-BD produced greater number of F_1 per cross (1.05 F_1/cross) as compared to A04-73NA × *B. oleracea*-BS (0.60 F_1/cross).

Two hundred six and 191 buds of the F_1 plants of A04-73NA × *B. oleracea*-BS and A04-73NA × *B. oleracea*-BD crosses were self-pollinated manually from where 62 (0.30 seeds/bud-pollination) and 85 (0.45 seeds/bud-pollination) F_2 seeds were harvested, respectively (**Table 2-2**). Thus, the average number of F_2 seeds per pollinated bud was 0.37.

The F_1 plants of the two crosses were also crossed to the *B. napus* parent A04-73NA to produce backcross (BC_1) seeds, viz. BS-BC_1 and BD-BC_1. A total of 507 backcrosses (250 BS-BC_1 and 257 BD-BC_1) were made, which gave 40 BC_1 seeds (23 BS-BC_1 and 17 BD-BC_1). Thus, the efficiency of production of BC_1 seed was 0.08 per cross (0.09 for BS-BC_1 and 0.07 for BD-BC_1) (**Table 2-3**). While comparing the efficiency of production of F_2 and BC_1 seeds,

surprisingly, the success was higher with the production of F_2 (0.37 seeds/pollination) than BC_1 (0.08 seeds/pollination).

Table 2-1. Development of interspecific hybrids of *B. napus* × *B. oleracea* var. *capitata* crosses through application of *in vitro* ovule culture technique

Pedigree[1]	No. crosses	No. ovules cultured	No. ovules/ cross	No. embryos to growth media	No. embryos/ cross	No. F_1 plantlets grown	No. F_1 produced/ cross
B. nap × *B. ole.cap.*BS	15	30	2.00	11	0.73	9	0.60
B. nap × *B. ole.cap.*BD	19	47	2.47	23	1.21	20	1.05
Total	34	77	2.26	34	1.00	29	0.85

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

Table 2-2. Development of F_2 populations of *B. napus* × *B. oleracea* var. *capitata* interspecific crosses through bud-pollination of the F_1 plants

Pedigree[1]	Cross ID	No. bud-pollinations of F_1 plants	No. F_2 seeds harvested	No. F_2 seeds /bud-pollination	No. F_2 plants grown	No. F_2 plants produced seeds	% fertile F_2 plants
B. nap × *B. ole.cap.*BS	BS-F_2	206	62	0.30	60	18	30.0
B. nap × *B. ole.cap.*BD	BD-F_2	191	85	0.45	60	20	33.3
Total		397	147	0.37	120	38	31.7

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

Table 2-3. Development of BC_1 populations of (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus* interspecific crosses through backcrossing of the F_1 plants with pollen from the *B. napus* parent

Pedigree[1]	Cross ID	No. crosses	No. BC_1 seeds harvested	No. BC_1 seeds/ pollination	No. BC_1 plants grown	No. BC_1 plants produced seeds	% fertile BC_1 plants
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1	250	23	0.09	20	12	60.0
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1	257	17	0.07	15	10	66.7
Total		507	40	0.08	35	22	62.9

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

2.3.2 Plant fertility in F_2- and BC_1-derived populations

A total of 120 F_2 plants (60 plants for each population) were grown in a greenhouse where 38 plants (31.7 %) produced viable seeds under self-pollination by bag isolation, i.e. were fertile (**Table 2-2**). In case of BC_1, a total of 35 plants were grown in greenhouse where 22 plants (62.9 %) produced seeds under bag isolation (**Table 2-3**). Thus, fertility of the F_2 and BC_1 population was significantly different; the proportion of fertile plants in BC_1 (62.9 %) was almost twice as compared to F_2 (31.7 %).

In F_3 and BC_1F_2 generation, a total of 111 plants belonging to 31 families and 131 plants belonging to 17 families, respectively, of the two crosses were grown in a greenhouse (**Table 2-4**). About 77 % (85/111) of the F_3 plants were fertile, while only 25 % (33/131) of the BC_1F_2 plants were fertile. In case of F_4 and BC_1F_3, a total of 245 plants (43 families) and 151 plants (33 families) were grown of which, respectively, 73.1 % and 67.6 % plants were fertile. Interestingly, percent fertile plants in F_4 was almost similar to that observed in F_3 (73.1 % vs. 76.6 %); in contrast, percent fertile plants in BC_1F_3 was almost three-times greater than that observed in BC_1F_2 (67.6 % vs. 25.2 %).

In case of F_6 and BC_1F_5 generations, 190 and 222 plants were self-pollinated in field of which, respectively, 71.1 % and 75.7 % plants were fertile, i.e. produced seeds under self-pollination. In F_7 and BC_1F_6 generation, respectively, 98.8 % and 97.1 % of the total number of plants were fertile.

In summary, plant fertility, in general, increased with the advancement of generation (**Table 2-4**, **Figure 2-2**); in advanced generation, fertility of the F_2- and BC_1-derived populations was quite comparable. In F_7 and BC_1F_6, almost 100% of the plants were fertile. No significant difference was observed between the populations derived from the two crosses involving the two *B. oleracea* parents for the recovery of fertile plants.

Table 2-4. Plant fertility in F_2- and BC_1-derived populations of *B. napus* × *B. oleracea* var. *capitata* interspecific crosses

Cross[1]	Population ID	Gen.[2]	No. plants grown (families)	No. fertile plants (families)	% fertile plants
B. nap × *B. ole.cap.*BS	BS-F_3	F_3	51 (15)	39 (12)	76.5
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1F_2	BC_1F_2	79 (10)	18 (8)	22.8
B. nap × *B. ole.cap.*BD	BD-F_3	F_3	60 (16)	47 (15)	78.3
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1F_2	BC_1F_2	52 (7)	15 (5)	28.8
Pooled	F_3	F_3	111 (31)	85 (27)	76.6
Pooled	BC_1F_2	BC_1F_2	131 (17)	33 (13)	25.2
B. nap × *B. ole.cap.*BS	BS-F_4	F_4	104 (15)	70 (13)	67.3
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1F_3	BC_1F_3	50 (18)	39 (16)	78.0
B. nap × *B. ole.cap.*BD	BD-F_4	F_4	141 (27)	109 (25)	77.3
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1F_3	BC_1F_3	101 (15)	63 (12)	62.4
Pooled	F_4	F_4	245 (42)	179 (38)	73.1
Pooled	BC_1F_3	BC_1F_3	151 (33)	102 (28)	67.6
B. nap × *B. ole.cap.*BS	BS-F_6	F_6	76 (27)	42 (21)	55.3
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1F_5	BC_1F_5	84 (33)	63 (26)	75.0
B. nap × *B. ole.cap.*BD	BD-F_6	F_6	114 (41)	93 (39)	81.6
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1F_5	BC_1F_5	138 (47)	105 (45)	76.1
Pooled	F_6	F_6	190 (68)	135 (60)	71.1
Pooled	BC_1F_5	BC_1F_5	222 (80)	168 (71)	75.7
B. nap × *B. ole.cap.*BS	BS-F_7	F_7	32 (32)	32 (32)	100.0
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1F_6	BC_1F_6	30 (30)	29 (29)	96.7
B. nap × *B. ole.cap.*BD	BD-F_7	F_7	48 (48)	47 (47)	97.9
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1F_6	BC_1F_6	73 (73)	71 (71)	97.3
Pooled	F_7	F_7	80 (80)	79 (79)	98.8
Pooled	BC_1F_6	BC_1F_6	103 (103)	100 (100)	97.1

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] F_6 and BC_1F_5 generation populations were grown in field; all other populations were grown in greenhouse

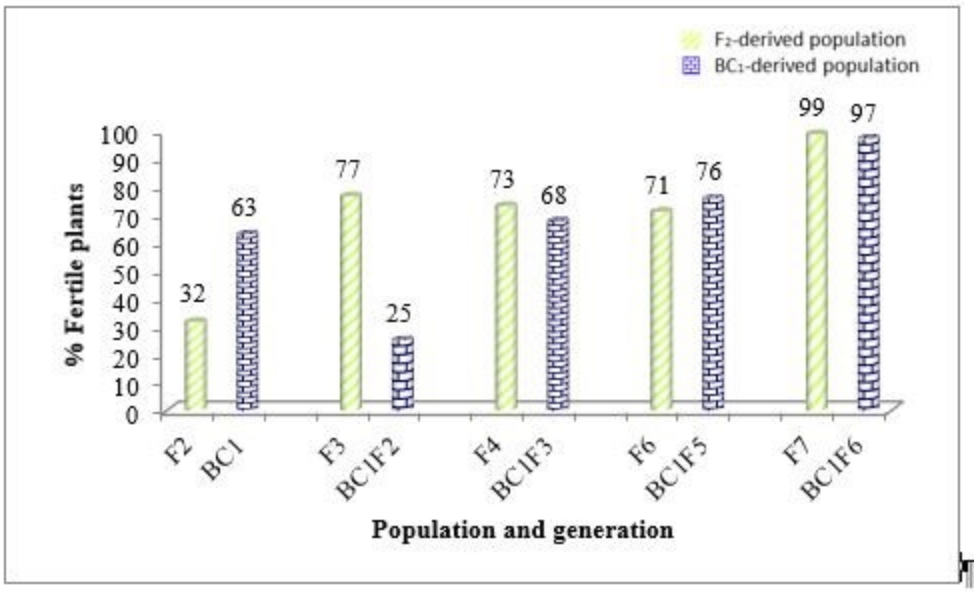

Figure 2-2. Fertility in different generation populations of *B. napus* × *B. oleracea* interspecific crosses. The F_2-derived populations included pooled data of *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper and *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener crosses. The BC_1-derived populations included pooled data of (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* crosses. All populations were grown in greenhouse except F_6 and BC_1F_5, which were grown in field.

2.3.3 Silique length and number of seeds per silique

2.3.3.1 Silique length (mm)

Silique length was measured in F_3, F_4 and BC_1F_3 generation populations. In F_3, a total of 85 plants, 38 of BS-F_3 and 47 of BD-F_3, were measured (**Table 2-5**) where the length of silique ranged from 18.0 to 68.4 mm with a mean of 38.0 ± 1.68 SE mm and 10.2 to 59.2 mm with a mean of 28.2 ± 1.51 SE mm, respectively. The mean length of silique of these populations were statistically different from each other ($p < 0.001$), as well as from A04-73NA (51.3 ± 5.18 SE mm). In this generation, mean silique length of pooled data of the two crosses (32.6 ± 1.23 SE mm) was statistically similar to that of pooled data of the selected population of these two crosses (30.7 ± 1.55 SE mm) ($p > 0.05$).

In F_4, the mean length of silique increased significantly compared to the F_3 population (44.0 ± 0.90 SE vs. 32.6 ± 1.23 SE mm) (**Table 2-5**). In case of BC_1F_3, the mean length of silique (43.3 ± 1.36 SE mm) was not significantly different from F_4, but it was significantly lower than that of the *B. napus* parent (54.5 ± 3.12 SE mm). In F_4 and BC_1F_3, the mean lengths of silique of the selected population were not significantly different from that of the whole population.

In case of F_6 and BC_1F_5, silique length varied from 33.9 to 73.2 mm with a mean of 53.0 ± 0.86 SE mm and 36.3 to 72.9 mm with a mean of 52.9 ± 0.75 SE mm, respectively. Silique length of these populations was still significantly lower than that of A04-73NA (61.3 ± 1.56 SE mm); however, the length of silique of these populations increased with the advancement of generation.

2.3.3.2 Number of seeds per silique

The number of seeds per silique varied from 0.0 to 29.6 with a mean of 5.9 ± 0.81 SE in BS-F_3 population and from 0.0 to 20.0 with a mean of 4.3 ± 0.73 SE in BD-F_3 population (**Table 2-5**). The mean number of seeds per silique in the selected population of the two crosses was 4.8 ± 0.65 SE which was statistically similar to the mean number of seeds per silique of the whole population (5.0 ± 0.55 SE) ($p > 0.05$).

In BS-F_4 and BD-F_4, the number of seeds per silique varied from 1.4 to 16.7 with a mean of 8.6 ± 0.66 SE and from 1.8 to 14.2 with a mean of 6.4 ± 0.63 SE, respectively; in case of BS-BC_1F_3 and BD-BC_1F_3, it varied from 2.5 to 19.5 with a mean of 9.0 ± 0.79 SE and from 2.3 to 11.2 with a mean of 7.3 ± 1.40 SE, respectively. The mean number of seeds per silique for pooled-F_4 and pooled-BC_1F_3 populations were 7.4 ± 0.47 SE and 8.6 ± 0.70 SE, respectively,

which was statistically similar. The number of seeds per silique in all populations was significantly lower than that of the *B. napus* parent, A04-73NA (20.1 ± 1.66 SE) ($p < 0.05$).

The number of seeds per silique in F_6 (22.7 ± 0.65 SE) and BC_1F_5 (23.5 ± 0.59 SE) population increased as compared to the previous generations; however, it was still significantly lower as compared to A04-73NA (32.8 ± 1.31 SE).

2.3.3.3 Correlation between silique length (mm) and number of seeds per silique

Pearson's correlation coefficient between silique length and number of seeds per silique was calculated for F_3, F_4 and BC_1F_3, and F_6 and BC_1F_5 generation populations (**Figure 2-3**). In all cases, significant positive correlation was found between these two traits, i.e. the number of seeds per silique increased with the increase in the length of silique. The coefficient of correlation (r) ranged between 0.33 and 0.75.

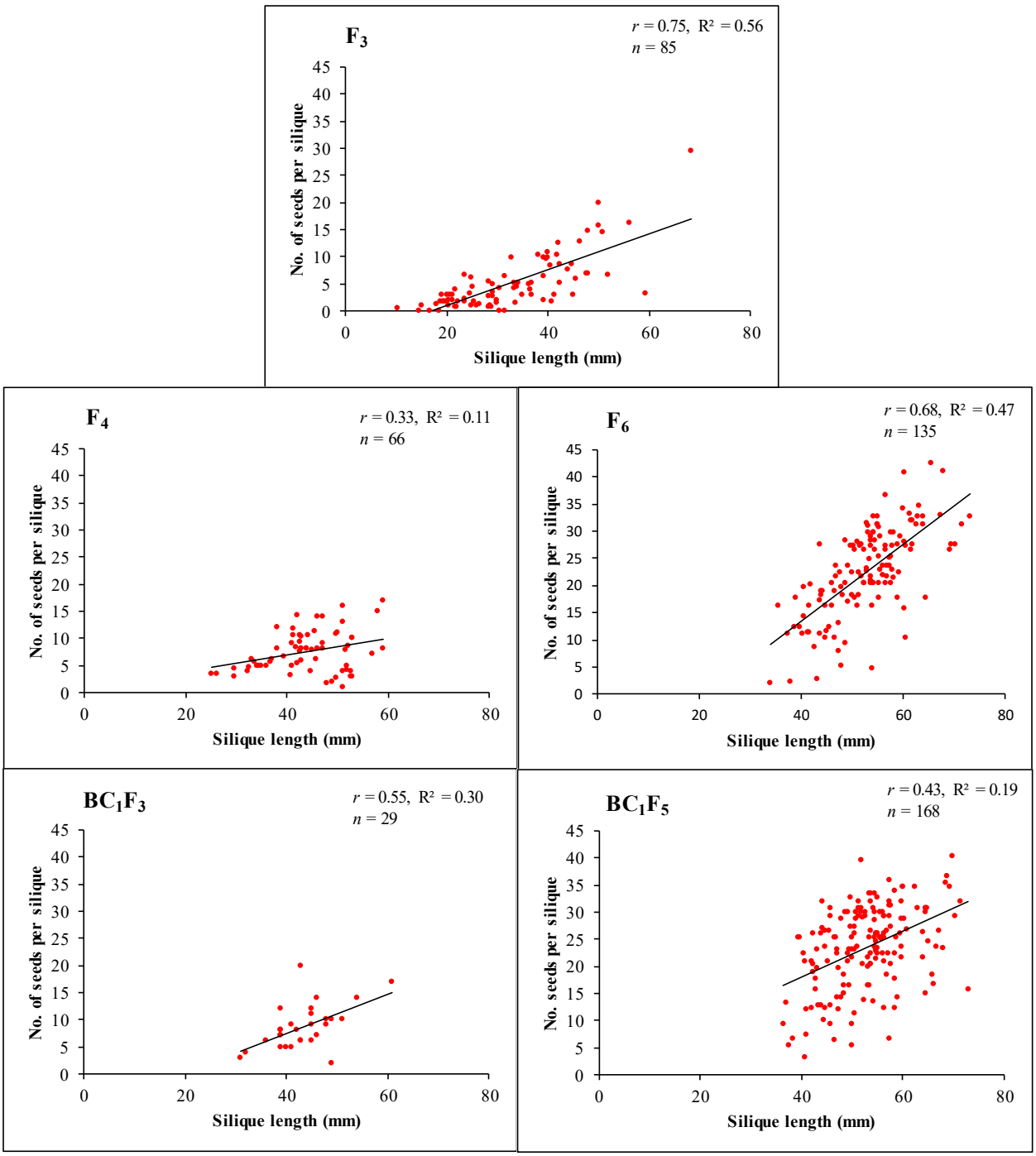

Figure 2-3. Scatter plot for correlation between silique length and number of seeds per silique in F_3, F_4 and BC_1F_3, and F_6 and BC_1F_5 generation populations, The F_3, F_4 and F_6 populations included pooled data of *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper and *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener crosses, and the BC_1F_3 and BC_1F_5 populations included pooled data of (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* crosses. All these generations except F_6 and BC_1F_5 were grown in greenhouse.

Table 2-5. Silique length and number of seeds per silique in F_3, F_4 and BC_1F_3, and F_6 and BC_1F_5 generation populations of two *B. napus* × *B. oleracea* interspecific crosses.

Cross[1]	Popn. ID	Gen.[2]	Whole population					Selected population				
			No. plants	Silique length (mm)		No. seeds /silique		No. plants	Silique length (mm)		No. seeds /silique	
				Range	Mean ± SE	Range	Mean ± SE[3]		Range	Mean ± SE	Range	Mean ± SE[3]
B. nap × *B. ole.cap.*BS	BS-F_3	F_3	38	18.0 – 68.4	38.0 ± 1.68 b	0.0 – 29.6	5.9 ± 0.81 b	16	21.2 – 56.2	36.2 ± 2.35 j	1.0 – 16.2	5.6 ± 1.06 j
B. nap × *B. ole.cap.*BD	BD-F_3	F_3	47	10.2 – 59.2	28.2 ± 1.51 c	0.0 – 20.0	4.3 ± 0.73 b	27	15.0 – 50.0	27.4 ± 1.81 k	0.6 – 20.0	4.3 ± 0.82 j
Pooled	F_3	F_3	85	10.2 – 68.4	32.6 ± 1.23 y	0.0 – 29.6	5.0 ± 0.55 y	43	15.0 – 56.2	30.7 ± 1.55 v	0.6 – 20.0	4.8 ± 0.65 v
B. napus (A04-73NA)			4	45.4 – 62.3	51.3 ± 5.18 ax	17.2 – 24.3	19.8 ± 2.50 ax		45.4 – 62.3	51.3 ± 5.18 iu	17.2 – 24.3	19.8 ± 2.50 iu
B. nap × *B. ole.cap.*BS	BS-F_4	F_4	31	33.3 – 58.7	46.9 ± 1.25 ab	1.4 – 16.7	8.6 ± 0.66 b	11	37.7 – 50.8	44.9 ± 1.48 j	7.7 – 13.7	10.0 ± 1.02 j
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1F_3	BC_1F_3	22	31.3 – 61.0	42.4 ± 1.49 bc	2.5 – 19.5	9.0 ± 0.79 b	6	38.5 – 45.0	41.6 ± 2.01 j	4.6 – 19.5	10.9 ± 1.39 j
B. nap × *B. ole.cap.*BD	BD-F_4	F_4	35	25.2 – 52.8	41.3 ± 1.18 c	1.8 – 14.2	6.4 ± 0.63 b	18	32.5 – 52.8	43.7 ± 1.16 j	1.8 – 14.2	7.3 ± 0.80 j
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1F_3	BC_1F_3	7	39.4 – 50.6	45.9 ± 2.64 abc	2.3 – 11.2	7.3 ± 1.40 b	4	43.0 – 49.3	45.5 ± 2.46 j	2.3 – 11.2	6.4 ± 1.70 j
Pooled	F_4	F_4	66	25.2 – 58.7	44.0 ± 0.90 y	1.4 – 16.7	7.4 ± 0.47 y	29	32.5 – 52.8	44.2 ± 0.88 v	1.8 – 14.2	8.4 ± 0.68 v
Pooled	BC_1F_3	BC_1F_3	29	31.3 – 61.0	43.3 ± 1.36 y	2.3 – 19.5	8.6 ± 0.70 y	10	38.5 – 49.3	43.2 ± 1.49 v	2.3 – 19.5	9.1 ± 1.15 v
B. napus (A04-73NA)			5	46.3 – 62.0	54.5 ± 3.12 ax	16.0 – 23.7	20.1 ± 1.66 ax		46.3 – 62.0	54.5 ± 3.12 iu	16.0 – 23.7	20.1 ± 1.66 iu
B. nap × *B. ole.cap.*BS	BS-F_6	F_6	42	34.9 – 73.2	54.3 ± 1.26 b	2.7 – 42.5	24.5 ± 1.08 b	32	38.9 – 72.1	55.4 ± 1.22 j	5.3 – 41.5	26.0 ± 1.16 j
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1F_5	BC_1F_5	63	36.3 – 69.7	51.6 ± 1.10 b	3.1 – 39.6	23.3 ± 0.95 b	30	38.2 – 65.0	52.0 ± 1.40 j	3.9 – 39.6	24.4 ± 1.30 j
B. nap × *B. ole.cap.*BD	BD-F_6	F_6	93	33.9 – 70.2	52.1 ± 1.09 b	1.5 – 38.9	21.6 ± 0.93 b	48	36.4 – 69.6	54.2 ± 1. 41 j	4.5 – 38.0	25.8 ± 1.34 j
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1F_5	BC_1F_5	105	37.0 – 72.9	53.9 ± 0.96 b	3.9 – 40.3	23.7 ± 0.79 b	73	42.0 – 72.9	55.3 ± 1.06 j	8.9 – 40.3	25.3 ± 0.98 j
Pooled	F_6	F_6	135	33.9 – 73.2	53.0 ± 0.86 y	1.5 – 42.5	22.7 ± 0.65 y	80	36.4 – 72.1	54.7 ± 0.95 v	4.5 – 41.5	25.9 ± 1.01 v
Pooled	BC_1F_5	BC_1F_5	168	36.3 – 72.9	52.9 ± 0.75 y	3.1 – 40.3	23.5 ± 0.59 y	103	38.2 – 72.9	54.3 ± 0.81 v	3.9 – 40.3	25.0 ± 0.91 v
B. napus (A04-73NA)			21	49.2 –77.9	61.3 ± 1.56 ax	25.3 – 38.4	32.8 ± 1.31 ax		49.2 –77.9	61.3 ± 1.56 iu	25.3 – 38.4	32.8 ± 1.31 iu

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2]All populations except F_6 and BC_1F_5 were grown in greenhouse

[3]The letters indicate comparison among the populations grown under same environment. In case of the whole population , the trio 'abc' indicates comparison among the BS-F_x, BS-BC_1F_x, BD-F_x, BD-BC_1F_x and *B. napus*, the trio 'xyz' indicates comparison among the pooled-F_x, pooled-BC_1F_x and *B. napus*; in case of the selected population, the trio 'ijk' indicates comparison among the BS-F_x, BS-BC_1F_x, BD-F_x, BD-BC_1F_x and *B. napus,* and the trio 'uvw' indicates comparison among the pooled-F_x, pooled-BC_1F_x and *B. napus*

2.3.4 Relative nuclear DNA content

Relative nuclear DNA content in different generation populations derived from *B. napus* × *B. oleracea* interspecific crosses were compared with the *B. napus* parent A04-73NA based on partec reading. The output of this is a form of graph, where the x-axis represents the relative quantity of DNA in a cell and the y-axis represents the number of cells counted (**Figure 2-4**).

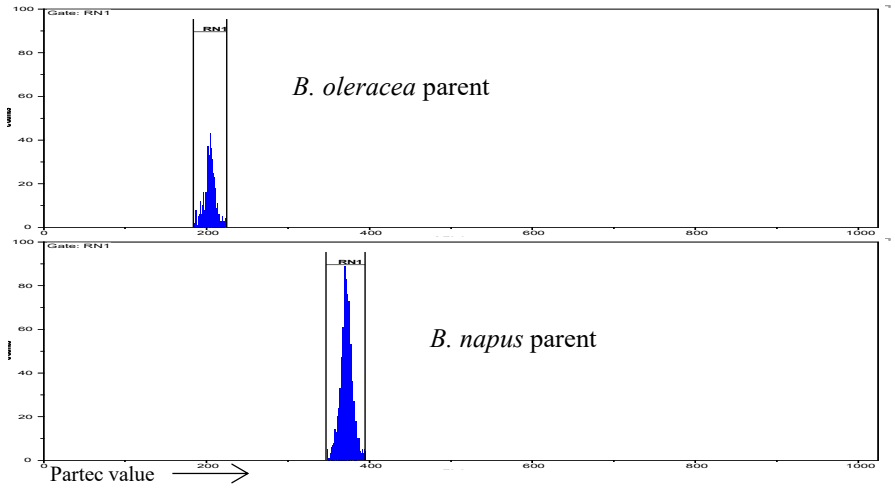

Figure 2-4. The flow cytometer graph showing relative nuclear DNA content in *B. napus* and one of the *B. oleracea* var. *capitata* parents

Mean relative nuclear DNA content of the F_7 population was 355.6 ± 1.62 SE; this was significantly different from that of the BC_1F_6 population (365.1 ± 1.46 SE) ($p < 0.001$) as well as from the *B. napus* parent A04-73NA (370.3 ± 2.62 SE) ($p < 0.001$). On the other hand, the relative nuclear DNA content of the BC_1F_6 population was statistically similar to that of the *B. napus* parent **(Table 2-6, Figure 2-5)**. The confidence interval ($p < 0.05$) for partec value of A04-73NA was 365.12 to 375.47; based on this, the interspecific cross derived plants having partec value within this confidence interval were considered as *B. napus* type. The proportion of the BS-F_7, BD-F_7 and pooled F_7 plants falling within this confidence interval was 32.2 %, 33.3 % and 32.9 %, respectively, while in case of BS-BC_1F_6, BD-BC_1F_6 and pooled-BC_1F_6, the proportion was 30.0 %, 30.9 % and 30.6 %, respectively. The mean partec values of the whole and the

selected F_7 populations (355.6 ± 1.62 SE vs. 360.4 ± 1.16 SE) were significantly different ($p < 0.05$); whereas, the whole and the selected BC_1F_6 populations (365.1 ± 1.46 SE vs. 365.2 ± 1.04 SE) were statistically similar.

The mean relative nuclear DNA content of the F_8 and BC_1F_7 populations were 354.0 ± 1.00 SE and 352.2 ± 0.97 SE, respectively. These values were statistically similar to that of the *B. napus* parent (353.9 ± 1.48 SE) ($p > 0.05$) **(Table 2-6)**. The confidence interval ($p < 0.05$) of the *B. napus* parent was 351.0 to 356.8. Based on this, 76.2 %, 33.3 % and 53.3 % plants, respectively, of the BS-F_7, BD-F_7, and pooled-F_7 populations were considered as *B. napus* type plants; in contrast, 29.4 %, 41.9 % and 37.5 % plants, respectively, of the BS-BC_1F_6, BD-BC_1F_6 and pooled-BC_1F_6 populations were considered as *B. napus* type.

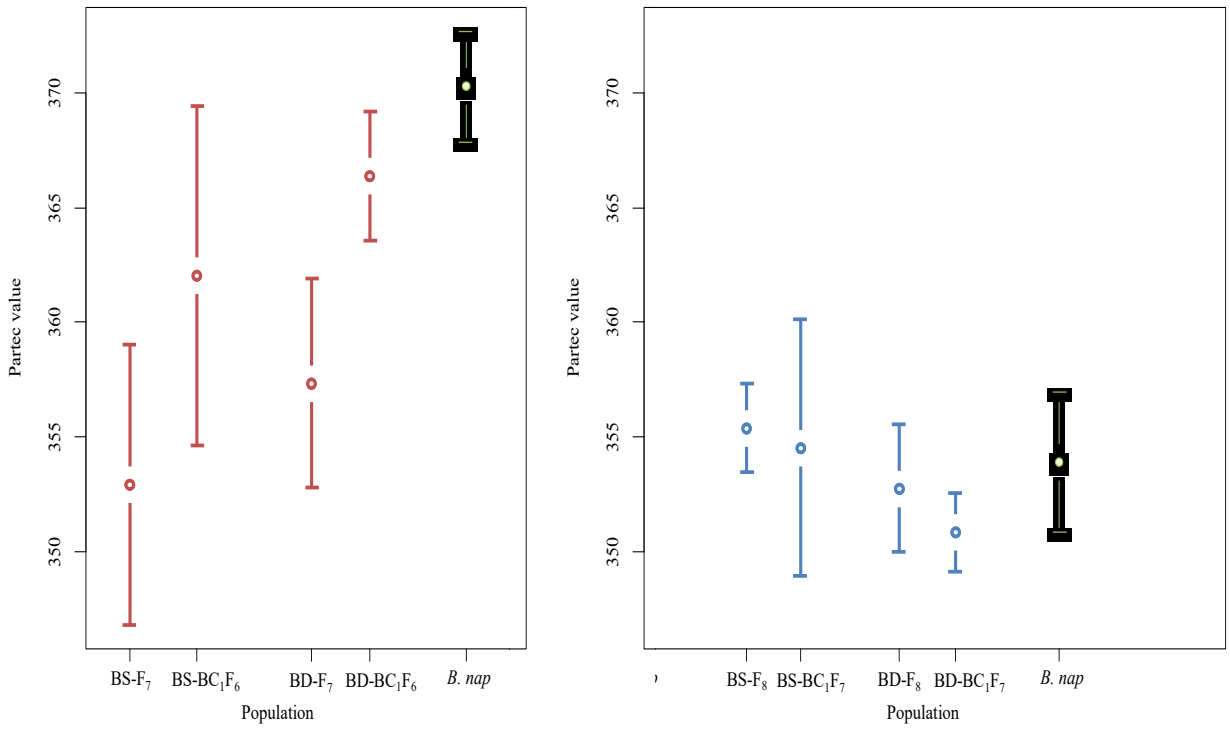

Figure 2-5. The mean (o) and standard error bars showing comparison of partec values for relative nuclear DNA content in different generation populations of *B. napus* × *B. oleracea* interspecific crosses

Table 2-6. Partec values for relative nuclear DNA content in F_7, F_8, BC_1F_6 and BC_1F_7 generation populations of *B. napus* × *B. oleracea* interspecific crosses.

Cross[1]	Popn. ID	Gen.[2]	Whole population				Selected population			
			No. plants	Partec value		No. *B. napus* type (%)	No. plants	Partec value		No. *B. napus* type (%)
				Range	Mean ± SE[3]			Range	Mean ± SE[3]	
B. nap × *B. ole.cap.*BS	BS-F_7	F_7	31	298.2 – 375.3	352.9 ± 2.58 b	10 (32.2)	24	342.6 – 375.3	359.9 ± 1.87 j*	10 (32.2)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1F_6	BC_1F_6	30	277.5 – 387.5	362.0 ± 2.62 ab	9 (30.0)	21	345.6 – 387.5	365.5 ± 2.00 ij	7 (23.3)
B. nap × *B. ole.cap.*BD	BD-F_7	F_7	48	311.8 – 388.8	357.6 ± 2.07 b	16 (33.3)	37	340.7 – 378.7	360.7 ± 1.50 j	15 (31.3)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1F_6	BC_1F_6	68	345.3 – 415.6	366.4 ± 1.74 ab	21 (30.9)	55	345.3 – 395.0	365.1 ± 1.23 ij	18 (26.5)
Pooled	F_7	F_7	79	298.2 – 388.8	355.6 ± 1.62 y	26 (32.9)	61	340.7 – 378.7	360.4 ± 1.16 w*	25 (31.6)
Pooled	BC_1F_6	BC_1F_6	98	277.5 – 415.6	365.1 ± 1.46 x	30 (30.6)	76	345.3 – 395.0	365.2 ± 1.04 v	25 (25.5)
B. napus (A04-73NA)	parent	parent	30	357.7 – 384.2	370.3 ± 2.62 ax				370.3 ± 2.62 iu	
B. oleracea var. *capitata*	parent	parent	7	197.3 – 216.8	208.1 ± 2.93				208.1 ± 2.93	
B. nap × *B. ole.cap.*BS	BS-F_8	F_8	21	348.0 – 370.2	355.4 ± 1.45 a	16 (76.2)	No selection; all to the next generation			
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1F_7	BC_1F_7	17	337.8 – 379.9	354.5 ± 1.61 a	5 (29.4)				
B. nap × *B. ole.cap.*BD	BD-F_8	F_8	24	341.4 – 367.6	352.8 ± 1.36 a	8 (33.3)				
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1F_7	BC_1F_7	31	337.0 – 360.5	350.84 ± 1.19 a	13 (41.9)				
Pooled	F_8	F_8	45	341.4 – 370.2	354.0 ± 1.00 x	24 (53.3)				
Pooled	BC_1F_7	BC_1F_7	48	337.0 – 379.9	352.2 ± 0.97 x	18 (37.5)				
B. napus (A04-73NA)	parent	parent	20	340.8 – 366.9	353.9 ± 1.48 ax					
B. oleracea var. *capitata*	parent	parent	5	189.6 – 207.2	198.9 ± 2.07) by					

[1]*B. nap* = *B. napus* line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2]All populations were grown in greenhouse

[3]The letters indicate comparison among the populations grown under same environment. In case of the whole population, the trio 'abc' indicates comparison among the BS-F_x, BS-BC_1F_x, BD-F_x, BD-BC_1F_x and *B. napus*, the trio 'xyz' indicates comparison among the pooled-F_x, pooled-BC_1F_x and *B. napus*; in case of the selected population, the trio 'ijk' indicates comparison among the BS-F_x, BS-BC_1F_x, BD-F_x, BD-BC_1F_x and *B. napus,* and the trio 'uvw' indicates comparison among the pooled-F_x, pooled-BC_1F_x and *B. napus*

[4]Asterisks indicate significant of difference between the selected and the whole populations; significance codes for p value of 0.001 = ***, 0.01 = ** and 0.05 = *

2.3.5 Seed quality traits: Erucic acid

Erucic acid content in seed oil of the F_2 plants of the two crosses ranged from 0.10 to 25.07 % with a mean of 12.47 ± 1.62 SE % **(Table 2-8)**. The mean erucic acid content of the BS-F_2 and BD-F_2 populations, were 14.26 ± 1.79 SE % and 7.45 ± 3.00 SE %, respectively; while the mean erucic acid content of the BS-BC_1 and BD-BC_1 populations were 9.03 ± 2.12 SE % and 4.47 ± 2.53 SE %. The mean erucic acid content of the BS-F_2 and BD-F_2 populations, and of the BS-BC_1 and BD-BC_1 populations were statistically similar ($p > 0.05$). There was no significant difference between BS-F_2 and BS-BC_1, as well as between BD-F_2 and BD-BC_1 populations; however, while comparing pooled data, mean erucic acid content in F_2 (12.47 ± 1.62 SE %) was significantly higher than that of BC_1 (7.15 ± 1.71 SE %).

Frequency distribution of F_2 and BC_1 populations for erucic acid content is presented in **Figure 2-6**. The F_2 and BC_1 populations fall into distinct groups where the zero erucic acid (< 2 %) plants could easily be identified. The ratio for the presence vs. absence of erucic acid in F_2 population (15 vs. 4) fitted well with the segregation ratio of 3:1 ($\chi^2 = 0.21$) suggesting that a single Mendelian gene is involved on the control of this trait in this population. In contrast, deviation from simple Mendelian 1:1 segregation was found in the BC_1 population (12 vs. 5) ($\chi^2 = 17.64$, $p < 0.01$) (**Table 2-7**); this distorted segregation might have resulted from small population size.

Erucic acid content in BS-F_3 population ranged from 0.03 % to 28.67 % and in BD-F_3 population, it ranged from 0.05 % to 26.99 % with a mean of 13.82 ± 1.49 SE % and 8.90 ± 1.47 SE %, respectively; this difference was statistically significant ($p < 0.05$). The selected populations of these two crosses (BS-F_3 = 6.01 ± 1.24 SE % and BD-F_3 = 4.06 ± 1.17 SE %) were significantly different from the original populations ($p < 0.001$ and < 0.01).

Table 2-7. Possible genotypes and their expected frequencies, and segregation of erucic acid alleles in F_2 and BC_1 of *B. napus* × *B. oleracea* interspecific crosses (Rahman et al., 2015), where the *B. oleracea* parent was high (>40 %) in erucic acid

Genotype	Expected frequency in F_2[1]	Expected frequency in BC_1[1]	Frequency of "+" alleles[2]	Expected Erucic content (%)	
A^0 A^0C^0 C^0 A^0C^0 C^0	4/16 (25.00)	2/4 (50.0)	0	0	Zero erucic acid
A^0 A^0 C^0 C^+	2/16 (12.50)	¼ (25.0)	0.25	10	"+" erucic acid
A^0 C^+ C^0	4/16 (25.00)	¼ (25.0)	0.33	13.2	
A^0 A^0C^+ C^+ C^+ C^0	3/16 (18.75)	–	0.50	20	
A^0C^+ C^+	2/16 (12.50)	–	0.66	26	
C^+ C^+	1/16 (6.25)	–	1	40	

[1] In bracket, percentage
[2] "+" indicates the high erucic acid allele

The mean erucic acid content of the BS-F_4 and BD-F_4 populations were 6.90 ± 0.94 SE % and 4.65 ± 0.84 SE %, respectively, where 54.8 % and 64.1 % plants were zero-erucic acid type. Mean erucic acid content in BS-BC_1F_3 and BD-BC_1F_3 populations were higher (9.99 ± 1.22 SE % and 6.10 ± 0.93 SE %) than that of the corresponding F_4 populations of the two crosses – this apparently resulted from no selection being performed for zero erucic acid in BC_1F_2 generation. Selection in F_4 and BC_1F_3 populations resulted significantly lower content of erucic acid in the selected populations (0.99 ± 0.43 SE % and 1.80 ± 0.57 SE %) (**Table 2-8**). About 94 % and 87 % plants, respectively, of the selected F_4 and BC_1F_3 populations were zero-erucic acid type as compared to 60.0 % and 43.0 % plants of the original populations. Analysis of parent vs. offspring generation showed that, other than zero-erucic acid plants, plants with ≤ 10 % erucic acid often yielded zero-erucic acid progenies **(Appendix: 2-1)**.

Table 2-8. Erucic acid content (%) and occurrence of zero-erucic acid plants in different generation populations of two *B. napus* × *B. oleracea* interspecific crosses

Cross[1]	Popn. ID	Gen.[2]	Whole population				Selected population			
			No. plants (families)	Range	Mean ± SE[3]	No. zero-erucic acid plants (%)[5]	No. plants (families)	Range	Mean ± SE[3]	No. zero-erucic acid plants (%)[5]
B. nap × *B. ole.cap.*BS	BS-F_2	F_2	14 (1)	0.48 – 25.07	14.26 ± 1.79 a	2 (14.3)	No selection; all to next generation			
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1	BC_1	10 (1)	0.24 – 16.51	9.03 ± 2.12 ab	2 (20.0)	No selection; all to next generation			
B. nap × *B. ole.cap.*BD	BD-F_2	F_2	5 (1)	0.10 – 15.18	7.45 ± 3.00 ab	2 (40.0)	No selection; all to next generation			
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1	BC_1	7 (1)	0.34 – 8.95	4.47 ± 2.53 b	3 (42.9)	No selection; all to next generation			
Pooled	F_2	F_2	19 (2)	0.10 – 25.07	12.47 ± 1.62 x	4 (21.1)	No selection; all to next generation			
Pooled	BC_1	BC_1	17 (2)	0.24 – 16.51	7.15 ± 1.71 y	5 (29.4)	No selection; all to next generation			
B. nap × *B. ole.cap.*BS	BS-F_3	F_3	34 (11)	0.03 – 28.67	13.82 ± 1.49 a	6 (17.6)	16 (6)	0.03 – 13.70	6.01 ± 1.24 i ***	6 (37.5)
B. nap × *B. ole.cap.*BD	BD-F_3	F_3	35 (14)	0.05 – 26.99	8.90 ± 1.47 b	10 (28.6)	18 (11)	0.05 – 12.80	4.06 ± 1.17 i **	9 (50.0)
Pooled	F_3	F_3	69 (25)	0.03 – 28.67	11.32 ± 1.05	16 (23.2)	34 (17)	0.03 – 13.70	4.98 ± 0.85 ***	15 (44.1)
B. nap	–	–	1	0.06 – 0.06	0.06	–	–	–	–	–
B. nap × *B. ole.cap.*BS	BS-F_4	F_4	62 (13)	0.03 – 24.69	6.90 ± 0.94 ab	34 (54.8)	24 (8)	0.03 – 12.11	1.73 ± 0.67 ij ***	21 (87.5)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BS-BC_1F_3	BC_1F_3	37 (14)	0.05 – 29.39	9.99 ± 1.22 a	11 (29.7)	14 (8)	0.05 – 16.85	3.22 ± 0.88 I ***	11 (78.6)
B. nap × *B. ole.cap.*BD	BD-F_4	F_4	78 (21)	0.04 – 25.28	4.65 ± 0.84 b	50 (64.1)	39 (17)	0.04 – 11.98	0.53 ± 0.53 j **	38 (97.4)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BD-BC_1F_3	BC_1F_3	63 (12)	0.05 – 21.52	6.10 ± 0.93 ab	32 (50.8)	21 (8)	0.34 – 8.95	0.85 ± 0.72 ij ***	20 (95.2)
Pooled	F_4	F_4	140 (34)	0.03 – 25.28	5.65 ± 0.64 x	84 (60.0)	63 (25)	0.03 – 12.11	0.99 ± 0.43 u ***	59 (93.7)
Pooled	BC_1F_3	BC_1F_3	100 (26)	0.05 – 29.39	7.54 ± 0.75 x	43 (43.0)	35 (16)	0.05 – 16.85	1.80 ± 0.57 u ***	31 (88.6)

[1]*B. nap* = *B. napus* line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2]All populations were grown in greenhouse

[3]The letters indicate comparison among the populations grown under same environment. In case of the whole population, the trio 'abc' indicates comparison among the BS-F_x, BS-BC_1F_x, BD-F_x, BD-BC_1F_x and *B. napus*, the trio 'xyz' indicates comparison among the pooled-F_x, pooled-BC_1F_x and *B. napus*; in case of the selected population, the trio 'ijk' indicates comparison among the BS-F_x, BS-BC_1F_x, BD-F_x, BD-BC_1F_x and *B. napus,* and the trio 'uvw' indicates comparison among the pooled-F_x, pooled-BC_1F_x and *B. napus*

[4]Asterisks indicate significant of difference between the selected and the whole populations; significance codes for p value of 0.001 = ***, 0.01 = ** and 0.05 = *

[5] Plants with erucic acid content < 2.0 % in seed oil were considered as zero-erucic acid type

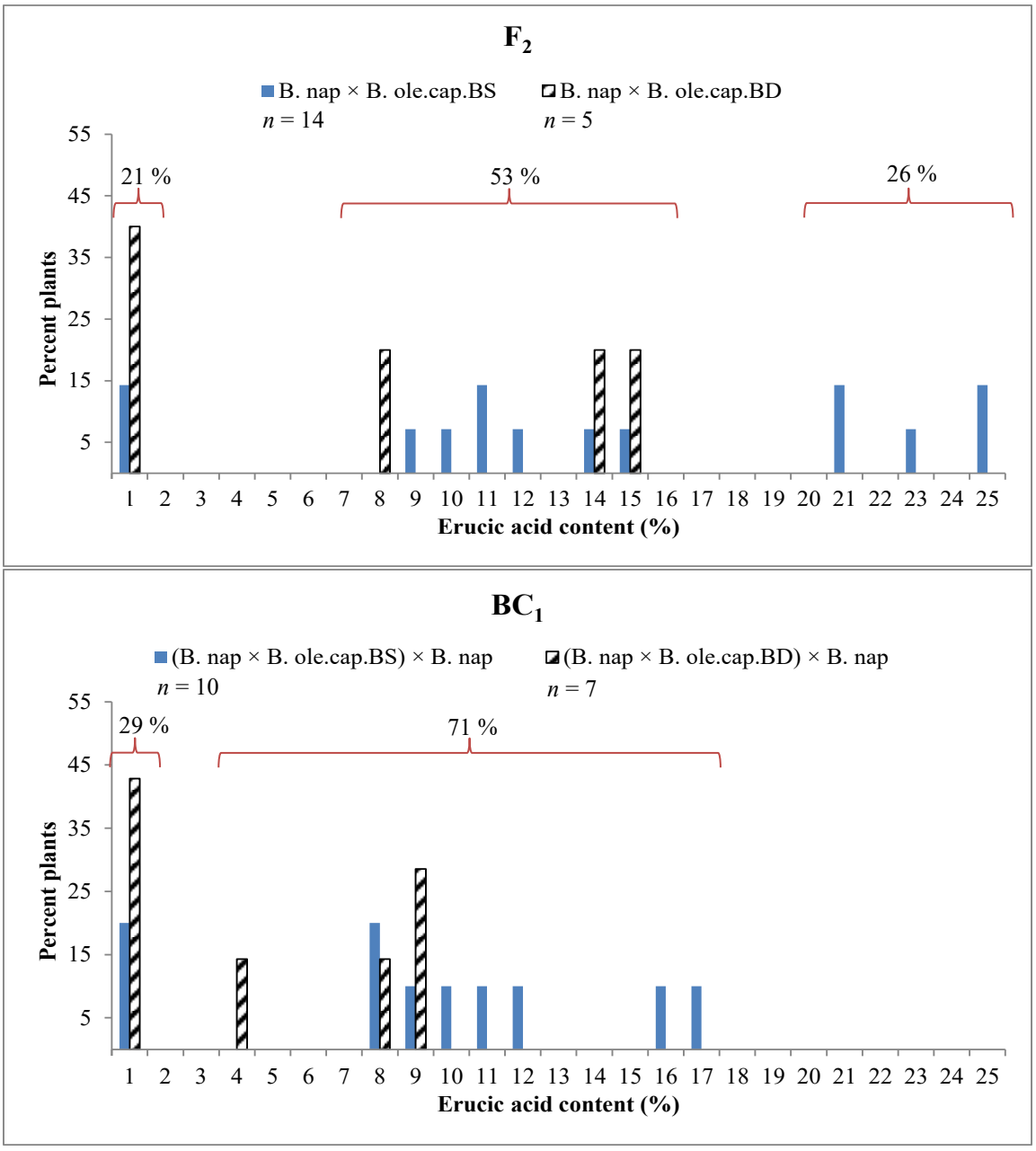

Figure 2-6. Frequency distribution for erucic acid content in F_2 plants of *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper (BS) and *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener (BD) interspecific crosses, and BC_1 plants of (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* interspecific crosses. The percentages are given for pooled data of the two F_2 and the two BC_1 populations.

2.3.6 Seed quality traits: Glucosinolates (GSL)

GSL content in the BS-F_3 population varied between 12.2 and 58.0 µmol/g seed with a mean of 35.9 ± 2.22 SE; 14.8 % plants of this population was low GSL type. In case of BD-F_3, GSL content ranged from 5.6 to 49.0 with a mean of 24.2 ± 2.31 SE; a higher proportion of plants (28 %) of this population was low GSL type (**Table 2-9**). In case of pooled-data of the F_3 population, mean GSL content was 30.3 ± 1.77 SE, which was significantly higher than that of the *B. napus* parent A04-73NA (8.1 ± 4.72 SE).

Mean GSL content of the BS-F_4, BD-F_4, BS-BC_1F_3 and BD-BC_1F_3 populations was 21.0 ± 1.58 SE, 20.2 ± 1.35 SE, 21.2 ± 2.80 SE and 13.0 ± 3.02 SE µmol/g seed, respectively. All these four populations were statistically similar ($p > 0.05$), and none of the selected populations was significantly different from the whole population. GSL content of the pooled F_4 population (20.6 ± 1.03 SE) was significantly lower than that of the F_3 population (30.3 ± 1.77 SE). About 43 % and 62 % plants, respectively, of the F_4 and BC_1F_3 population had low content of GSL in seed.

GSL content in F_6 and BC_1F_5 populations ranged from 14.5 to 59.6 and 13.1 to 59.4 µmol/g seed, respectively, with mean of 29.1 ± 1.21 SE and 29.4 ± 1.19 SE; these values were significantly greater than that of A04-73NA (20.24 ± 2.52 SE) ($p < 0.001$). Surprisingly, mean GSL content of the F_6 and BC_1F_5 populations was higher than that of the F_4 and BC_1F_3 populations (20.6 ± 1.06 SE and 17.4 ± 2.12 SE). This might be due to the effect of growth conditions – the former populations were grown in field while the later populations in greenhouse. Furthermore, in the earlier generation of BC_1F_5, i.e. in BC_1F_3, GSL analysis was done on a few plants due to lack of the required quantity of seeds needed to perform this analysis; therefore, most plants of this generation were not selected for GSL content previously,

and this also added to the increased content of GSL in BC_1F_5 as compared to BC_1F_3. Strong selection for low GSL content was exercised in F_6 and BC_1F_5; only the plants having GSL content of less than 30 µmol/g seed were selected, and this resulted significant difference between the whole and the selected populations.

Mean GSL content in F_8 and BC_1F_7 was lower than that of the previous generations (F_6 and BC_1F_5) – an effect of selection for this trait was clearly evident (**Table 2-9**) **(Figure 2-7)**. The mean GSL content of the whole F_8 and BC_1F_7 populations was 21.6 ± 0.84 SE and 21.2 ± 0.76 SE µmol/g seed, respectively. These values were statistically similar to the check, *B. napus* A04-73NA, (23.4 ± 1.21 SE) ($p < 0.05$). More than 85 % of the F_8 and BC_1F_7 plants were low GSL type; selection at this stage further decreased GSL content (19.4 ± 0.49 SE and 18.0 ± 0.47 SE) in these two populations ($p < 0.05$ and 0.01).

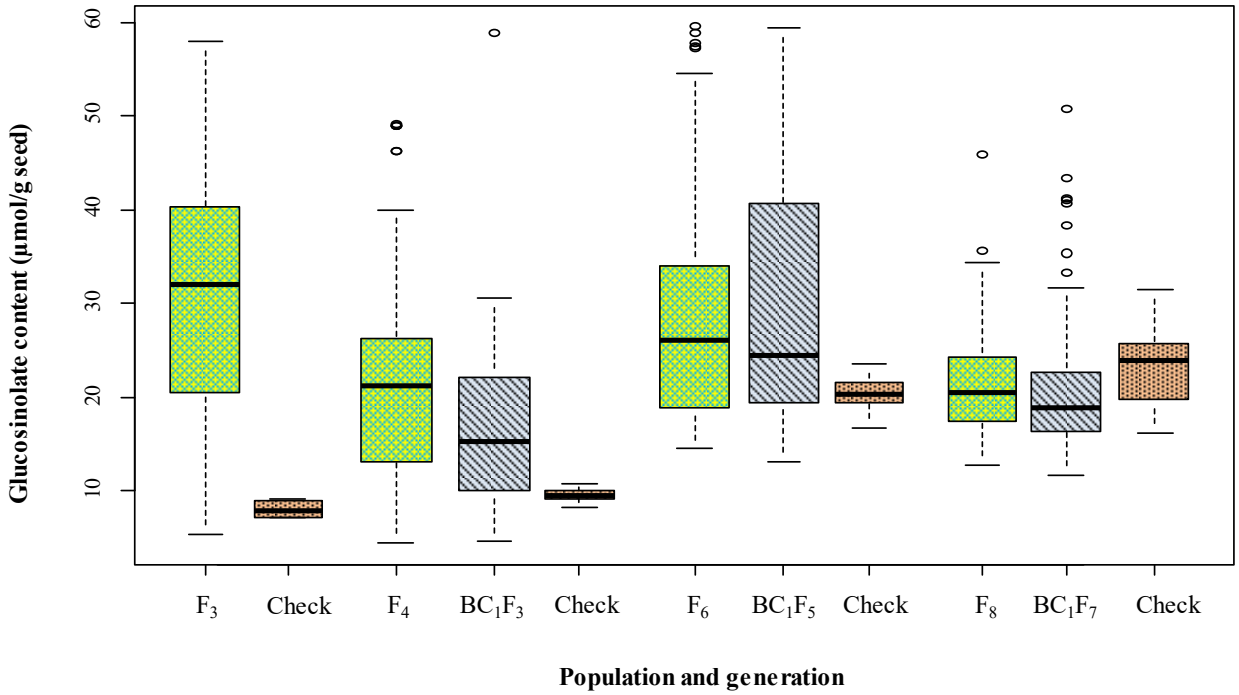

Figure 2-7. The box plot diagram showing comparison of glucosinolate contents between different generation populations of *B. napus* × *B. oleracea* interspecific crosses and the *B. napus* parent. The F_2-derived populations included pooled data of *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper and *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener crosses; whereas, the BC_1-derived populations included pooled data of (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* crosses. The F_3, F_4 and BC_1F_3 populations were grown in greenhouse, while the F_6, BC_1F_5, F_8 and BC_1F_7 populations were grown in field. The *B. napus* A04-73NA parent was used as check.

Table 2-9. Glucosinolate (GSL) content (µmol/g seed) and occurrence of low GSL plants in different generation populations of two *B. napus* × *B. oleracea* interspecific crosses

Cross[1]	Gen.[2]	Whole population				Selected population			
		No. plants (families)	Range	Mean ± SE[3]	No. ≤ *B. nap* plants (%)[5]	No. plants (families)	Range	Mean ± SE[3]	No. ≤ *B. nap* plants (%)[5]
B. nap × *B. ole.cap.*BS	F_3	27 (11)	12.2 – 58.0	35.9 ± 2.22 a	4 (14.8)	11 (6)	12.2 – 58.0	30.3 ± 3.76 i	3 (27.3)
B. nap × *B. ole.cap.*BD	F_3	25 (11)	5.6 – 49.0	24.2 ± 2.31 b	7 (28)	13 (9)	5.6 – 49.0	22.5 ± 3.45 ij	4 (30.8)
Pooled	F_3	52 (22)	5.6 – 58.0	30.3 ± 1.77 x	11 (21.2)	24 (15)	5.6 – 58.0	26.1 ± 2.6 u	7 (29.2)
B. nap		6	7.2 – 9.1	8.1 ± 4.72 cy		6	7.2 – 9.1	8.1 ± 4.72 jv	
B. nap × *B. ole.cap.*BS	F_4	44 (11)	8.7 – 46.1	21.0 ± 1.58 a	20 (45.5)	16 (6)	12.0 – 46.1	22.2 ± 2.34 i	8 (50.0)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1F_3	14 (8)	8.5 – 58.9	21.2 ± 2.80 a	6 (42.9)	6 (5)	8.5 – 28.7	15.7 ± 3.83 i	4 (66.7)
B. nap × *B. ole.cap.*BD	F_4	60 (16)	4.5 – 49.1	20.2 ± 1.35 a	25 (41.7)	29 (11)	5.6 – 32.3	16.6 ± 1.74 i	16 (55.2)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1F_3	12 (5)	4.6 – 29.6	13.0 ± 3.02 a	10 (83.3)	1 (1)	16.8 – 16.8	–	1 (100.0)
Pooled	F_4	104 (27)	4.5 – 49.1	20.6 ± 1.03 x	45 (43.3)	45 (17)	5.6 – 46.1	18.5 ± 1.42 u	24 (53.3)
Pooled	BC_1F_3	26 (13)	4.6 – 58.9	17.4 ± 2.07 xy	16 (61.5)	7 (6)	8.5 – 28.7	15.9 ± 3.60 u	5 (71.4)
B. nap		7	8.4 – 10.9	9.61 ± 3.96 ay		7	8.4 – 10.9	9.61 ± 3.96 iu	
B. nap × *B. ole.cap.*BS	F_6	34 (34)	15.2 – 57.4	31.3 ± 1.98 a	9 (26.5)	16 (16)	22.8 – 29.1	26.2 ± 0.86 i **	6 (37.5)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1F_5	39 (39)	13.8 – 49.6	28.1 ± 1.87 ab	23 (59.0)	13 (13)	18.1 – 27.8	23.2 ± 0.96 ij *	11 (84.6)
B. nap × *B. ole.cap.*BD	F_6	58 (58)	14.5 – 59.6	27.9 ± 1.52 ab	30 (51.7)	19 (19)	14.5 – 26.1	18.3 ± 0.79 k ***	17 (89.5)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1F_5	55 (55)	13.1 – 59.4	30.3 ± 1.54 a	27 (49.1)	31 (31)	13.1 – 29.5	21.0 ± 0.62 jk ***	24 (77.4)
Pooled	F_6	92 (92)	14.5 – 59.6	29.1 ± 1.21 y	39 (42.4)	80 (35)	14.5 – 29.1	21.9 ± 0.71 u ***	23 (65.7)
Pooled	BC_1F_5	94 (94)	13.1 – 59.4	29.4 ± 1.19 y	50 (53.2)	103 (44)	13.1 – 29.5	21.7 ± 0.63 u ***	35 (79.5)
B. nap		21	16.8 – 23.6	20.24 ± 2.52 bx		21	16.8 – 23.6	20.24 ± 2.52 jku	
B. nap × *B. ole.cap.*BS	F_8	25 (25)	14.8 – 35.6	22.3 ± 1.33 a	22 (88.0)	21 (21)	14.8 – 24.6	20.9 ± 0.69 j	21 (100.0)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1F_7	21 (21)	13.7 – 41.1	20.3 ± 1.45 a	20 (95.2)	17 (17)	13.7 – 25.0	19.4 ± 0.76 jk	17 (100.0)
B. nap × *B. ole.cap.*BD	F_8	37 (37)	12.7 – 45.9	21.2 ± 1.09 a	29 (78.4)	24 (24)	12.7 – 25.0	18.1 ± 0.64 k *	24 (100.0)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1F_7	55 (55)	11.8 – 50.7	21.6 ± 0.90 a	44 (80.0)	31 (31)	11.8 – 24.2	17.2 ± 0.56 k **	31 (100.0)
Pooled	F_8	62 (62)	12.7 – 45.9	21.6 ± 0.84 x	51 (82.3)	45 (45)	12.7 – 25.0	19.4 ± 0.49 v *	45 (100.0)
Pooled	BC_1F_7	76 (76)	11.8 – 50.7	21.2 ± 0.76 x	64 (84.2)	48 (48)	11.8 – 25.0	18.0 ± 0.47 v **	48 (100.0)
B. nap		30	16.3 – 31.5	23.4 ± 1.21 ax		30	16.3 – 31.5	23.4 ± 1.21 iu	

[1] *B. nap* = *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] The F_3, F_4 and BC_1F_3 generations were grown in greenhouse; all other populations were grown under field condition

[3] The letters indicate comparison among the populations grown under same environment. In case of the whole population, the trio 'abc' indicates comparison among the BS-F_x, BS-BC_1F_x, BD-F_x, BD-BC_1F_x and *B. napus*, the trio 'xyz' indicates comparison among the pooled-F_x, pooled-BC_1F_x and *B. napus*; in case of the selected population, the trio 'ijk' indicates comparison among the BS-F_x, BS-BC_1F_x, BD-F_x, BD-BC_1F_x and *B. napus,* and the trio 'uvw' indicates comparison among the pooled-F_x, pooled-BC_1F_x and *B. napus*

[4] Asterisks indicate significant of difference between the selected and the whole populations; significance codes for p value of 0.001 = ***, 0.01 = ** and 0.05 = *

[5] Confidence intervals ($p < 0.05$) of *B. napus* A04-73NA parent for GSL content were 0.0 – 17.5 (grown with F_3), 1.8 – 17.4 (grown with F_4 and BC_1F_3), 15.5 – 25.2 (grown with F_6 and BC_1F_5) and 21.0 – 25.8 µmol/g seed grown with (F_8 and BC_1F_7)

2.4 Discussion

Interspecific hybridization has played an important role in plant evolution. *B. napus* evolved in nature from hybridization between *B. oleracea* and *B. rapa*. However, this hybridization event has occurred involving few variants of these two diploid progenitor species; this is one of the reasons for the narrow genetic diversity being detected in this crop (e.g Hasan et al., 2006; Bus et al., 2011). Intensive breeding for canola quality traits has further narrowed down the genetic base of this crop germplasm (Friedt and Snowdon, 2009). Of the A and C genomes of *B. napus*, the A genome seems to possess greater genetic diversity compared to the C genome (Bus et al., 2011); this might have resulted from a natural cross between *B. napus* and *B. rapa* (Hansen et al., 2001) and active efforts made by different researchers in crossing these two species (Qian et al., 2006; Mei et al., 2011; Attri and Rahman, 2017). However, this fortune did not happen in case of the C genome; this is primarily due to poor crossability between these two species (Downey et al., 1980) as well as the difficulty of producing this interspecific hybrid in laboratory. Optimization of the embryo rescue technique has made it easier to produce hybrids of *B. oleracea* and *B. napus* (Quazi, 1988; Bennett et al., 2008) and consequently transfer of traits/alleles from *B. oleracea* to *B. napus*.

In this study two different *B. oleracea* var. *capitata* cultivars, namely, Badger Shipper (BS) and Bindsachsener (BD), were crossed to a spring *B. napus* canola line A04-73NA. The *B. napus* parent was used as female to retain the cytoplasm of *B. napus* in the progenies derived from these interspecific crosses. It is also well established that using tetraploid (here amphidiploid) as female in crossing with diploid species increases the chance of getting interspecific hybrid plants (Downey et al., 1980; Quazi, 1988). Of the two crosses made in this study, the cross with the *B. oleracea* parent Bindsachsener yielded greater number of

interspecific F_1 plants as compared to the cross involving the other *B. oleracea* parent (**Table 2-1**). This is in agreement with the results reported by Chiang et al. (1977) and Rahman (2004) that genotypic difference between the parents can affect the crossability and the production of F_1 plants. The success of production of the *B. napus* × *B. oleracea* interspecific F_1 was higher in this study (0.85 plant/pollination) as compared to the success reported by Quazi (1988) (0.15 plant/ pollination). This might have resulted not only from genotypic difference between the parents used in crossing, but also from the technique applied in these studies. In the present study, *in vitro* ovule culture technique was applied, while Quazi (1988) applied embryo culture technique to produce *B. napus* × *B. oleracea* interspecific hybrid. Takeshita et al. (1980) also reported higher success of ovule culture as compared to embryo culture in production of distant hybrids.

While comparing the efficiency of production of F_1 plants through application of ovule culture technique and BC_1 plants through conventional crossing, the success was higher in production of F_1 (0.85 F_1 plant/cross) than BC_1 (0.08 BC_1 plant/cross). This indicates the usefulness of ovule culture technique of embryo rescue in achieving higher number of interspecific hybrids. Also, meiotic anomalies in F_1 plants (ACC) might have contributed to the low success in the production of BC_1 seeds.

Most of the traits in the amphidiploid *B. napus* are controlled by at least two sets of genes, one set from the A genome and another from the C genome. Considering that erucic acid content is controlled by two major homoeologous loci located on the A and C genome chromosomes, the A genome of the F_1 ACC plants carried the zero erucic acid alleles; therefore, F_2 segregation for erucic acid will reflect the presence or absence of the high erucic acid allele of the C genome (**Table 2-7**). Segregation for this trait in F_2 followed the expected 3:1 ratio, while this deviated significantly in BC_1 population. Segregation distortion for erucic acid content in the

progenies of *B. napus* × *B. oleracea* interspecific cross have also been reported by Bennett et al. (2008, 2012) and Rahman et al. (2015).

In early generations, selection for *B. napus* type plant was made mainly based on plant fertility which also reflected the length of silique and the number of seeds per silique. Significant positive correlation was found between silique length and number of seeds per silique in different generation populations (r = 0.33 to 0.75). Similar strength of correlation between these two traits have also been reported by Zhang et al. (2011) (r = 0.73) and Cai et al. (2014) (r = 0.47); however, Qi et al. (2014) reported low correlation (r = 0.10) between these two traits.

Flow cytometric analysis of the advanced generation populations showed that the BC_1-derived population was closer to the *B. napus* parent for nuclear DNA content than the F_2-derived populations – an effect of backcrossing was evident. However, the majority of the F_2- and BC_1-derived population had nuclear DNA content similar to that of the *B. napus*. In other words, these plants stabilized as euploid *B. napus* type.

GSL content did not respond to selection as much as erucic acid content did. This is primarily due to lower heritability (Zhang and Zhou, 2006) and the involvement of a greater number of loci controlling this trait (Basunanda et al., 2007; Xu et al., 2015). However, selection was quite effective for this trait; more than 80 % of F_8 and BC_1F_7 plants were low GSL type

Chapter 3

Study of allelic diversity in F_2- and BC_1-derived populations of *B. napus* × *B. oleracea* by use of SSR molecular markers

3.1 Introduction

Genetic diversity within a population can be defined as the number of different alleles of all genes and the frequency of their occurrence within the population. The level of overall and trait specific allelic diversity varies from species to species, but it is important for continued improvement of our crop plants and better adaptability to the changing environment (reviewed by El-Esawi 2016). In case of spring *B. napus* (AACC, 2*n* = 38) canola, where most of the cultivars are hybrid, presence of adequate genetic diversity in a breeding program is crucial for the development of competitive cultivars through exploitation of the phenomenon heterosis. Unfortunately, the diversity in spring *B. napus* canola has decreased over the years for several reasons, such as breeding often carried out in a close population (Cowling, 2007; Fu and Gugel, 2010), intensive breeding for canola quality traits (Bus et al., 2011) and the genetic bottleneck occurred during the evolution of this species. Fu and Gugel (2010) reported that, the number of SSR alleles and mean heterozygosity in spring *B. napus* cultivars and lines have declined over the years of breeding (1940s to 2000s) in a Canadian breeding program. Therefore, it is important to broaden the genetic base of this crop through introduction of useful alleles from exotic germplasm including its allied species.

Genetic diversity in spring *B. napus* canola can be broadened through introgression of allelic diversity from various sources, such as winter and semi-winter types of *B. napus*, its progenitor species, such as *B. oleracea* (CC, 2*n* = 18) and *B. rapa* (AA, 2*n* = 20), and its allied species, such as *B. carinata* (BBCC, 2*n* = 34) and *B. juncea* (AABB, 2*n* = 36) (for review, see

Rahman, 2013). While introgressing exotic alleles into *B. napus* canola, it is important to assess the value of these alleles in the newly developed lines for their effect on various agronomic and quality traits including seed yield in open-pollinated and hybrid cultivars. The knowledge of the genetic distance among the breeding lines is important for selection of parents in a hybrid breeding program for exploitation of the phenomenon heterosis or hybrid vigor.

Genetic diversity and relatedness among the individuals or populations can be determined by use of different morphological or biochemical traits, or molecular markers (Mohammadi and Prasanna, 2003). Yu et al. (2005) and Hu et al. (2007) assessed genetic diversity in *B. napus* germplasm from China and Europe based on various morphological and agronomic traits. Similarly, the Manhattan dissimilarity coefficients, estimated by use of morphological traits, were used by Sheikh et al. (2011) to estimate the extant of diversity among 24 *B. carinata* lines carrying the alleles of *B. napus* and *B. juncea*. However, assessment of genetic diversity based on morphological traits is labor intensive and time consuming. Furthermore, all morphological traits cannot be recorded until harvest, and these traits can be affected by environment which can influence the result. The protein markers, isozymes, have been used by different researchers to evaluate genetic diversity in *B. napus* and *B. rapa* (Zhao and Becker 1998) and *B. oleracea* (Làzaro and Aguinagalde, 1998); however, their abundance and polymorphism is low as compared to DNA markers (reviewed by, Kumar et al., 2009), and this is one of the constraints for use of this marker in genetic diversity analysis.

The use of DNA-based molecular markers, which are not influenced by the environment, has increased over the last 2-3 decades; this is a powerful tool for assessment of genetic diversity in crop germplasm as well as for identification of genetically distinct parents for use in hybrid breeding (Lee, 1995). Prior to the invention of polymerase chain reaction (PCR) technique for

amplification of genomic DNA, restriction fragment length polymorphism (RFLP) markers were extensively used for assessment of genetic diversity (Halldén et al., 1994; Diers and Osborn, 1994). While studying genetic diversity in *B. napus* by use of RFLP and allozyme markers, Becker et al. (1995) found that comparable results can be obtained by use of these two marker types. The PCR technique has allowed researchers for using a range of DNA markers, such as random amplified polymorphic DNA (RAPD) (Shengwu et al., 2003; Mohammadi et al., 2009), amplified fragment length polymorphism (AFLP) (Lombard et al., 2000; Seyis et al., 2003; Qian et al., 2006) and simple sequence repeats (SSR) (Hasan et al., 2006; Wang et al., 2009; Gyawali et al., 2013; Guo et al., 2016), to estimate genetic diversity in *B. napus*.

Condit and Hubbell (1991) reported the abundance of microsatellites in plant genome. Microsatellites or SSRs are DNA fragments of various length, consisting of tandemly repeated nucleotide units (mono, di, tri, tetra, penta and so on) present in both coding and non-coding regions of the genomes of most eukaryotic organisms (Powell et al., 1996). This type of marker has several advantages, such as co-dominance of the alleles, high abundance and random distribution throughout the genome, and high reproducibility over many other type of markers (reviwed by, Park et al., 2009). Recently, the availability of *Brassica* genome sequences has made it cost-effective to develop a large number of SSR markers (Iniguez-Luy et al., 2008; Hobson and Rahman, 2016).

By use of SSR markers, Bus et al. (2011) and Wu et al. (2014) reported that the C genome of *B. napus* has lower allele diversity than the A genome; therefore, it is critical to broaden the genetic base of this genome of this crop. *B. oleracea* is the donor of the C genome of *B. napus*; however, the C genome of this diploid species is very diverse and distinct from the C genome of *B. napus* (Thormann et al., 1994). Therefore, the allelic diversity of *B. oleracea* can

be utilized to broaden genetic base of the amphidiploid species *B. napus* (Li et al., 2014b; Rahman et al., 2015). Rahman et al. (2015) assessed several inbred lines derived from *B. napus* × *B. olearcea* var. *alboglabra* interspecific cross by SSR markers, and identified *B. napus* plants carrying up to 54% alleles of the C-genome of *B. oleracea*. This suggests that the progenitor species *B. oleracea* can be used as a valuable reservoir of alleles to diversify the genetic base of spring *B. napus* canola. *B. oleracea* can be grouped into a number of variants based on their morphological traits and allelic diversity (Quiros and Farnham, 2011). Among the different variants of *B. oleracea*, the var. *capitata* found to be genetically distinct from the other variants of this species (Song et al., 1988; Louarn et al., 2007; Izzah et al., 2013). In this study, I investigated the extent of allelic diversity introgressed from *B. oleracea* var. *capitata* into spring *B. napus* canola lines derived from interspecific cross between these two species. SSR markers have proved to be effective for estimation of genetic diversity; therefore, I have chosen to use this type of marker in this study.

3.2 Materials and methods

3.2.1 Plant material

Two interspecific crosses were made by using a spring type *B. napus* canola line A04-73NA and two *B. oleracea* var. *capitata* cvs. Badger Shipper and Bindsachsener: *B. napus* A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper and *B. napus* A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener.

The F_1 plants were subjected to self-pollination for several generations from where several F_2-derived spring *B. napus* canola lines were developed (F_8 generation). The F_1 plants were also backcrossed to the *B. napus* parent A04-73NA to produce BC_1 plants; these plants were self-pollinated for several generations from where several spring *B. napus* canola lines

(BC_1F_7 generation) were developed. The detail of this is described in Chapter 2. From these F_8 and BC_1F_7 populations, a total of 45 F_8 lines consisting of 21from *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper (BS-F_8) cross and 24 from *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener (BD-F_8) cross, and a total of 48 BC_1F_7 lines consisting of 17 from (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* (BS-BC_1F_7) cross and 31from (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* (BD-BC_1F_7) cross, thus, adding up to a combined total of 93 recombinant inbred lines (RILs) were used for SSR marker analysis (**Appendix: 3-1**).

3.2.2 Screening of the parents for identification of polymorphic markers

A total of 418 SSR primer pairs from the nine C genome linkage groups of *B. napus* were used for screening the two *B. oleracea* var. *capitata* parents, cvs. Badger Shipper and Bindsachsener, and the spring *B. napus* canola line A04-73NA to identify the polymorphic markers. These markers were obtained from Agriculture and Agri. Food Canada (AAFC), through a material transfer agreement, and markers developed by different researchers, such as Suwabe et al. (2002), Celera AgGen consortium (reported in Piquemal et al. (2005)), Iniguez-Luy et al. (2008), Cheng et al. (2009), and Li et al. (2011). The forward primer of each SSR marker was appended with the universal M13 primer sequence 5′-CACGACGTTGTAAAACGAC-3′ labeled with fluorescent dyes FAM, VIC, NED and PET (Applied Bio-systems, Foster City, CA). Markers producing clear polymorphic bands in the parents were selected to analyze the F_2- and BC_1-derived populations for genetic diversity.

3.2.3 Genotyping of RILs with polymorphic SSR markers.

3.2.3.1 DNA extraction

Genomic DNA was extracted from leaf tissue of the RILs. For this, leaf samples were collected from three-week old plants grown in a greenhouse, and wrapped with aluminum foil and stored at -80 ^{o}C until use. The DNA was extracted using Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI) as per guidelines of manufacturer with slight modification as described in **Figure 3-1**.

The quality and concentration of the stock DNA was measured using NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The stock DNA samples were diluted to 20 - 30 ng/µl using double distilled water. These working DNA samples were used as template for PCR amplification.

3.2.3.2 Polymerase chain reaction (PCR) amplification

Axygen^{TM} 96-well Half-skirt PCR microplates were used for amplification of the genomic DNA through PCR technique. The PCR plates included 93 RILs and 3 parents. The 93 RILs were coded 1 to 93, and the three parents *B. napus* , *B. oleracea* var. *capitata* cv. Badger Shipper (BS) and *B. oleracea* var. *capitata* cv. Bindsachsener (BD) were coded 94, 95 and 96, respectively (**Figure 3-2**).

Each PCR reaction was carried out with a total of 13.15 µl reaction volume in each well, comprising of 1.25 µl template DNA (concentration 20-30 ng/ µl), 2.5 µl PCR buffer, 0.30 µl of 10 mM dNTPs, 1 µl of 25 mM $MgCl_2$, 0.25 µl of each forward and reverse primer at a concentration of 10 µM, 0.3 µl dye (FAM, VIC, NED or PET), 7.3 µl double distilled water and 0.125 unit of Taq polymerase enzyme. Amplification of the genomic DNA fragments was done using a Verity^{TM} Dx 96-well Thermal Cycler and Proflex^{TM} PCR System.

The PCR amplification included an initial denaturation of 5 min at 95 ºC, then 35 cycles where each cycle consisted of denaturation for 1 min at 95 ºC, annealing for 1 min at 58 ºC and extension for 1 min 30 sec at 72 ºC, and a final extension of 30 min at 72 ºC to add poly-A tail to the 3′- ends of the PCR products.

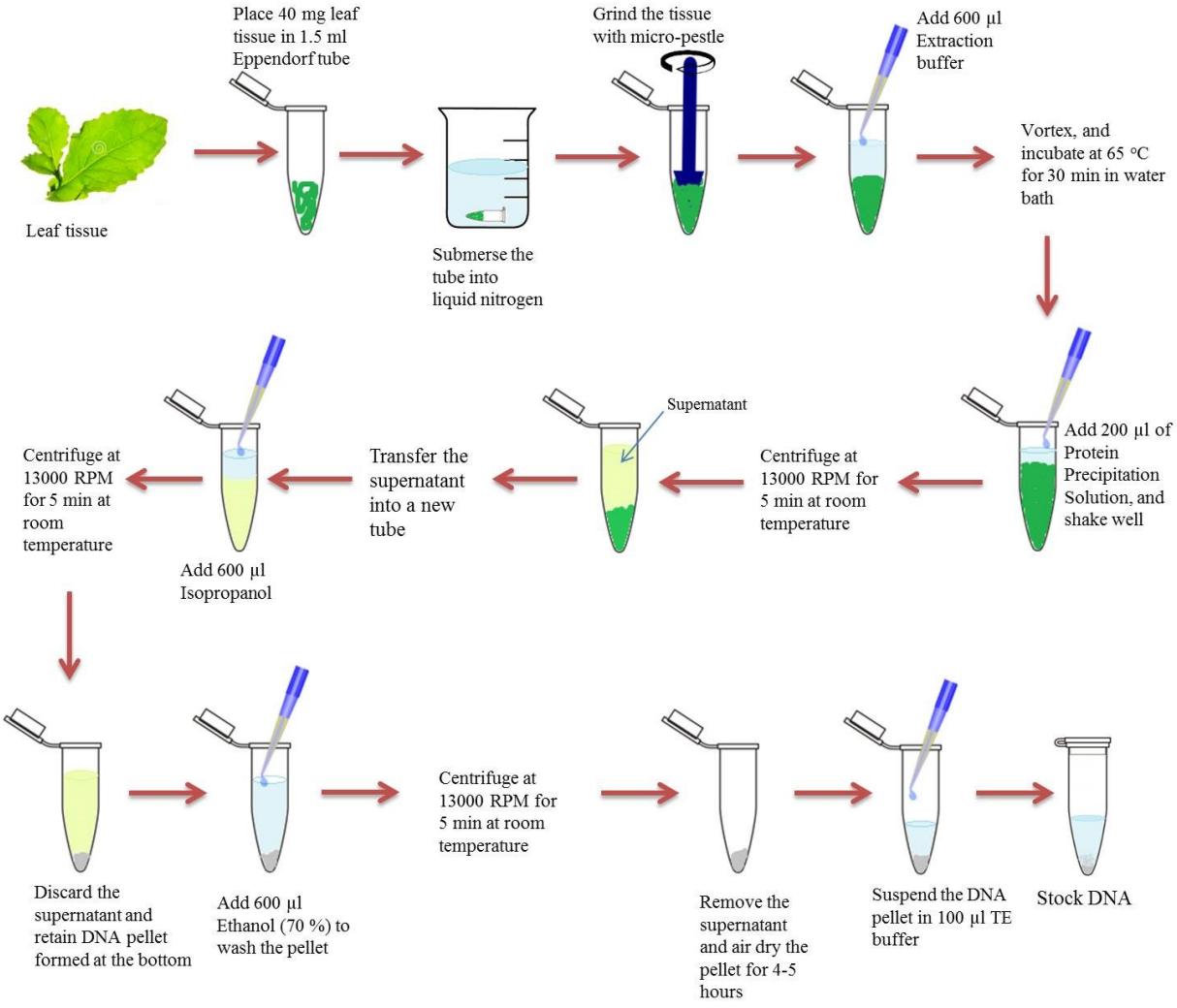

Figure 3-1. Protocol for isolation of genomic DNA from leaf tissue.

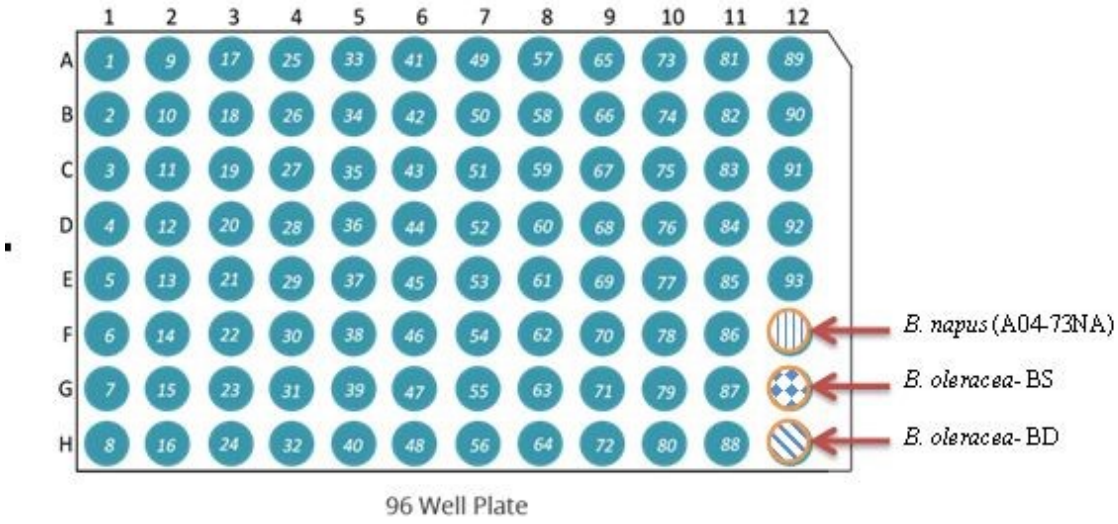

Figure 3-2. PCR plate design followed for amplification of genomic DNA of the inbred lines and their parents.

3.2.3.3 Fragment analysis run using Applied Biosystems

The samples were numbered 1 to 96 and arranged in an ABI plate in the same order as in PCR amplification. The ABI plates were prepared using Hi-Di™ Formamide as size standard buffer and PCR products from four plates having four different fluorescent dyes. In 1000 µl of Hi-Di, 6 µl size standard (GeneScan™ 500 LIZ®) was mixed; 8 µl of this mixture was poured into each well of the ABI plate. 1 µl PCR product of each sample from all four plates was pipetted into the respective wells of the ABI plate making 12 µl reaction volume in each well. The loaded ABI plate was incubated at 95 ºC for 2 minutes to denature the samples, and this followed cooling on ice block for 2 minutes. After that, size-based separation of the amplified DNA fragments was done using a capillary electrophoresis AB Genetic Analyzer No. 3730 (Life technologies, Foster City, CA).

3.2.4 Statistical analysis

The ABI results were scored for presence or absence of the alleles using GeneMarker® version 2.4.0 developed by SoftGenetics®. Binary code was used for scroring the presence and absence of the peak (fragment); presence was noted as "1" and absence as "0".

AMOVA, to estimate the genetic variation within and among the populations, was performed using GenAIEx 6.5 software (Peakall and Smouse, 2006). The criteria proposed by Wright (1978) were used to categorize the genetic differentiation, where Fst value of 0 – 0.05, 0.05 – 0.15, 0.15 – 0.25 and > 0.25 indicate little, moderate, high and very high genetic difference, respectively. (reviewed by Lopes et al. 2007).

Dice genetic similarity coefficient between pairs of RILs was calculated using the Numerical Taxonomy and Multivariate Analysis System software (NTSYSpc 2.2 Rohlf, 2000) following Nei and Li (1979). The GD between different pairs of populations was estimated using Nei's method (Nei, 1978).

Dendrogram and principal coordinate analysis showing genetic relationship between the different F_2- and BC_1-derived inbred lines were constructed following Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical clustering method with NTSYSpc 2.2 program ver. 2.2 (Rohlf, 2000).

To study the inheritance and occurrence of *B. oleracea* alleles in the RILs, the total number of SSR loci/alleles and the observed number of *B. oleracea* alleles was calculated. The total number of SSR loci in a population was calculated using the following formula: Number polymorphic loci × No. plants in the population, and total number of possible alleles in the population was calculated by multiplying the total no. of SSR loci by two. The observed number of *B. oleracea* alleles in the population was calculated using the following formula: (No. loci

homozygous for *B. oleracea* allele × 2) + No. loci heterozygous for *B. oleracea* and *B. napus* alleles + No. loci heterozygous for *B. oleracea* and non-parental (allele not present in the parents, but appeared in segregating population) alleles. The expected number of *B. oleracea* alleles in F_2- or BC_1-derived population was calculated by multiplying the total number of alleles in the population by 0.5 or 0.25, respectively. The occurrence of *B. oleracea* alleles is reported as proportion of the total analyzed alleles in a population or in a plant or in a linkage group.

To compare the observed number of *B. oleracea* alleles with the expected number, χ^2 test was done using two classes for each linkage group: Occurrence of *B. oleracea* alleles vs. occurrence of the other alleles (*B. napus* + non-parental alleles + lost alleles) (**Table 3-6** and **Table 3-7**).

3.3 Results

3.3.1 Genotyping of F_8 and BC_1F_7 RILs with polymorphic SSR markers

A total of 418 markers from the nine C genome linkage groups were tested of which 116 markers were found to be polymorphic between the *B. napus* and *B. oleracea* var. *capitata* parents. Thus, average proportion of polymorphic marker was 27.8 %; this ranged from 19.2 % in case of linkage group C3 to 35.2 % in case of linkage group C4 (**Table 3-1**). For genotyping of the F_8 and BC_1F_7 populations, 93 polymorphic markers producing clear and reproducible fragments, as showed in **Figure 3-3**, were selected covering all nine C genome linkage groups; the number of markers per linkage group varied from 8 to 14. List of the markers used for genotyping is presented in **Appendix 3-2**.

A total of 307 alleles were amplified by the 93 polymorphic markers, and this translates to 3.3 alleles per marker **(Table 3-2)**. Of the total 307 alleles, 277 were polymorphic between the *B. napus* and *B. oleracea* var. *capitata* parents, where 150 alleles were specific to *B. oleracea*. Thus, the average number of alleles specific to *B. oleracea* was 1.6 per marker. During genotyping of the F_8 and BC_1F_7 plants, few new alleles which were not present in either of the parents were also detected from all linkage groups; these alleles were designated as 'non-parental alleles'. The number of this type of allele per SSR marker varied from 0.27 for linkage group C5 to 1.0 in case of linkage group C7 with a mean of 0.54 (**Table 3-2**).

Table 3-1. SSR markers screened from the nine C genome linkage groups for identification of markers polymorphic between the *B. napus* and *B. oleracea* var. *capitata* parents

Linkage group	No. markers screened	No. polymorphic markers	No. monomorphic markers	% polymorphic markers	No. markers used for genotyping
C1	43	13	30	30.2	12
C2	39	10	29	25.6	10
C3	78	15	63	19.2	12
C4	54	19	35	35.2	14
C5	38	13	25	34.2	11
C6	35	9	26	25.7	8
C7	38	13	25	34.2	8
C8	33	11	22	33.3	9
C9	60	13	47	21.7	9
Total	418	116	302	27.8	93

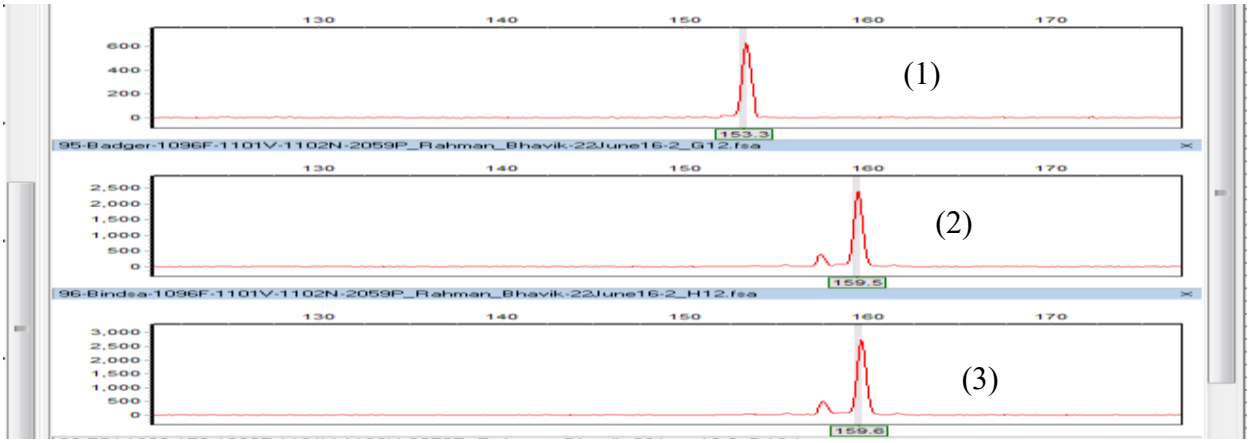

Figure 3-3. The capillary electrophorogram showing allelic variation between *B. napus* (1) and *B. oleracea* var. *capitata* cvs. Badger Shipper (2) and Bindsachsener (3) detected by the primer pair sNRE74 (Marker no. 2059)

Table 3-2. Number of alleles amplified by 93 polymorphic SSR markers from the nine C genome linkage groups in the *B. napus* and *B. oleracea* var. *capitata* parents, and non-parental alleles amplified in F_8 and BC_1F_7 populations derived from *B. napus* × *B. oleracea* var. *capitata* interspecific crosses.

LG	No. SSR markers	No. alleles	No. mono-morphic alleles	No. polymorphic alleles	No. polymorphic alleles/SSR	No. *B. napus* specific alleles	No. *B. oleracea* specific alleles	No. *B. oleracea* alleles/SSR	No. non-parental alleles[1]	No. non-parental alleles/SSR [1]
C1	12	45	3	42	3.5	20	22	1.8	4	0.33
C2	10	34	3	31	3.1	14	17	1.7	4	0.40
C3	12	37	5	32	2.7	13	19	1.6	9	0.75
C4	14	50	2	48	3.4	27	21	1.5	6	0.43
C5	11	39	4	35	3.2	18	17	1.5	3	0.27
C6	8	31	7	24	3.0	10	14	1.8	6	0.75
C7	8	21	0	21	2.6	8	13	1.6	8	1.00
C8	9	25	2	23	2.6	8	15	1.7	5	0.56
C9	9	25	4	21	2.3	9	12	1.3	5	0.56
Total	93	307	30	277	3.0	127	150	1.6	50	0.54

[1] SSR marker allele that could not be detected in the parents, but appeared in F_8/ BC_1F_7 population.

3.3.2 Genetic relationship among and within the F_8 and BC_1F_7 populations

A binary data matrix of DNA fragments amplified by 93 polymorphic SSR markers was used for AMOVA, and cluster and principal coordinate analysis. The AMOVA was performed to compare genetic variation among and within the four populations: BS-F_8, BD-F_8, BS-BC_1F_7 and BD-BC_1F_7. The proportion of variation among and within the populations was 32 % and 68 %, respectively, with F_{ST} value of 0.47 indicating the existence of high genetic difference among the four populations as well as within the populations (**Table 3-3**). To further study the difference between the populations, genetic distance between the pairs of populations was estimated using Nei's formula (Nei, 1978) (**Table 3-4**) The highest genetic distance of 0.17 was found between BS-F_8 and BD-BC_1F_7, while the lowest genetic distance of 0.06 was found between BS-BC_1F_7 and BD-BC_1F_7 populations.

Table 3-3. Analysis of molecular variance (AMOVA) of the two F_8 and two BC_1F_7 populations derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses

Source of variation	df	SS	MS	Variance component	% variation	F_{ST}
Among populations	3	1242.1	310.53	15.54	32.0	0.47
Within populations	92	2965.6	32.95	32.95	68.0	
Total	95	4207.7		48.49	100.0	

Table 3-4. Genetic distance among the four inbred populations developed from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses

Populations	BS-F_8	BD-F_8	BS-BC_1F_7	BD-BC_1F_7
BS-F_8	0.00			
BD-F_8	0.15	0.00		
BS-BC_1F_7	0.16	0.08	0.00	
BD-BC_1F_7	0.17	0.06	0.09	0.00

Cluster analysis (**Figure 3-4**) and Principal Coordinate Analysis (**Figure 3-5**) were performed to group the F_8 and BC_1F_7 generation lines of the two crosses. Dice similarity coefficients of the *B. napus* parent A04-73NA with *B. oleracea*-BS and *B. oleracea*-BD were 0.15 and 0.14, respectively, while similarity coefficient between the two *B. oleracea* parents was

0.58 (**Appendix: 3.3**). In general, the inbred lines derived from the same interspecific cross tended to group together. The BC_1-derived lines of BS-BC_1F_7 and BD-BC_1F_7 formed one large cluster falling closer to the *B. napus* parent; in contrast, the F_2-derived lines formed multiple clusters where many of these lines were considerably different from the parents and some lines were closer to the *B. oleracea* parent (**Figure 3-4**).

In most cases, the advanced generation lines derived from a single F_2 or BC_1 plant tended to group together (**Figure 3-4**). The 93 inbred lines used in this study were descended from 14 F_2/BC_1 plants (**Appendix: 3-1**) and they could be grouped into 16 clusters; the progenies of 12 plants formed 12 clusters, while the progenies of one BS-F_2 and one BS-BC_1 plant each formed two clusters.

In case of principal coordinate analysis, the 1st, 2nd and 3rd coordinates explained 19.1 %, 10.7 % and 9.7 % variation, respectively, adding up to a total of 39.5 % variation. The first principal coordinate clearly separated the inbred lines of BS-F_8 from the rest of the lines, while the second and third principal coordinates distinguished the lines of BS-BC_1F_7 and BD-BC_1F_7 from each other (**Figure 3-5**).

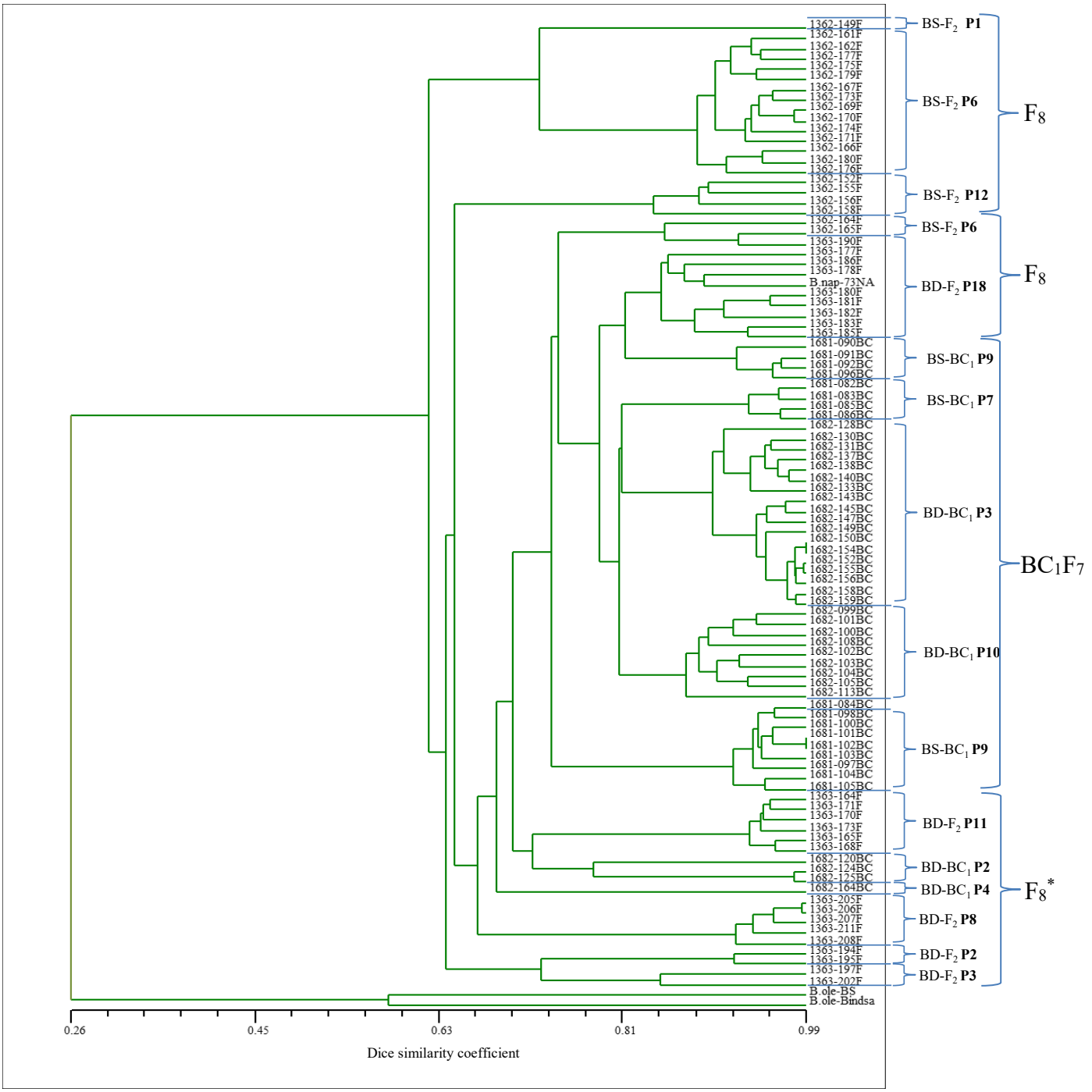

Figure 3-4. Cluster analysis showing genetic relationship among 21 BS-F_8 (starting with 1362), 17 BS-BC_1F_7 (starting with 1681), 24 BD-F_8 (starting with 1363) and 31 BD-BC_1F_7 (starting with 1682) lines, derived from *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper (BS-F_8), (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* (BS-BC_1F_7), *B. napus* × *B. oleracea* var. *capitata,* cv. Bindsachsener (BD-F_8) and (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* (BD-BC_1F_7) interspecific crosses, respectively, based on 93 polymorphic SSR markers. * This cluster includes a few BD-BC_1F_7 plants.

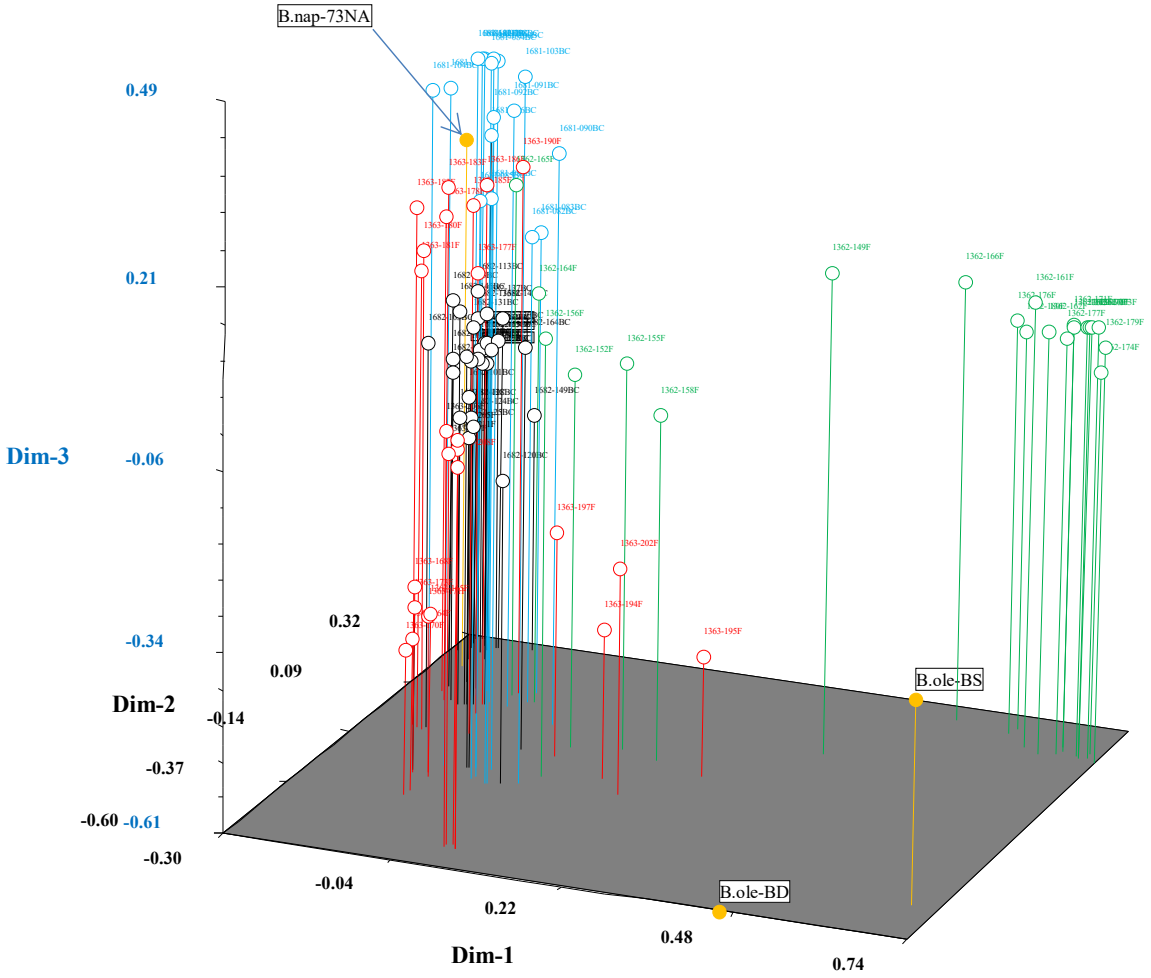

Figure 3-5. Principal coordinate plot of 21, 17, 24 and 31 inbred lines of BS-F_8 (-----), BS-BC_1F_7 (-----), BD-F_8 (-----), and BD-BC_1F_7 (-----) generation lines, respectively, derived from *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper (BS-F_8), (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* (BS-BC_1F_7), *B. napus* × *B. oleracea* var. *capitata,* cv. Bindsachsener (BD-F_8) and (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* (BD-BC_1F_7) interspecific crosses, respectively, by use of 93 SSR markers. The first, second and third coordinates explained 19.1, 10.7 and 9.7 % of the total variation, respectively.

3.3.3 Occurrence of *B. oleracea* alleles in F_8 and BC_1F_7 generation populations

Of the total 93 polymorphic SSR markers, a set of 65 and 67 markers which amplified clearly distinguishable alleles in the *B. oleracea* parents var. *capitata* cv. Badger Shipper and var. *capitata* cv. Bindsachsener, respectively, were selected to study the inheritance of *B. oleracea* alleles in BS-F_8 and BS-BC_1F_7, and BD-F_8 and BD-BC_1F_7 populations (**Appendix: 3-2**). Of the 65 and 67 SSR markers, 45 SSR primer pairs each amplified a single allele in *B. napus* and *B. oleracea* parents, and therefore, these markers could be used without ambiguity. On the other hand, 20 and 22 SSR primer pairs amplified more than one loci in the *B. napus* parent, probably amplified homoeologous loci from the A and C genomes. When more than one fragments were detected in *B. napus*, the alleles were assigned to the A or C genome based on the following criteria used by Chen et al. (2008): the *B. napus* allele that substitutes the C genome allele of *B. oleracea* in different inbred lines could be considered belonging to the same locus and therefore could be assigned to the C genome of *B. napus*.

Of the total number of SSR alleles detected in the advanced generation populations, 33.8 ± 2.25 % and 24.9 ± 2.47 % of the alleles were derived from *B. oleracea*, respectively in BS-F_8 and BD-F_8; in case of the two BC_1 derived populations, BS-BC_1F_7 and BD-BC_1F_7, the proportion of *B. oleracea* alleles was 20.2 ± 2.28 % and 15.8 ± 0.94 %, respectively (**Table 3-5**). Theoretically, in absence of any selection, of the total number of SSR alleles present in the advanced generation lines derived from the F_2 of *B. napus* × *B. oleracea* var. *capitata* and BC_1 of (*B. napus* × *B. oleracea* var. *capitata*) × *B. napus*, 50 % and 25 % of the alleles, respectively, were expected to be derived from *B. oleracea*. However, the observed proportion of the *B. oleracea* alleles in the two F_2-derived populations BS-F_8 and BD-F_8 was 67.9 % (922/1358) and 49.9 % (800/1602) of the expected, respectively (**Table 3-6**); in case of the two BC_1-derived

populations BS-BC_1F_7 and BD-BC_1F_7, the observed proportion was 80.9 % (446/551) and 63.4 % (656/1035) of the expected, respectively (**Table 3-7**). Thus, the number of *B. oleracea* alleles observed in the four populations was significantly lower than the expected number (see **Table 3-6** and **Table 3-7** for χ^2 values).

Variation between the plants for the proportion of *B. oleracea* allele was also found in these populations (**Figure 3-6A**). *Brassica oleracea* alleles constituting about 42 % of the total number of alleles was found in a BS-F_8 plant, while a plant lacking *B. oleracea* allele was found in the BD-F_8 population. While comparing the populations developed based on the two *B. oleracea* parents, population derived from the cross involving Badger Shipper carried greater proportion of *B. oleracea* alleles.

The extent of introgression of *B. oleracea* alleles in the four populations also varied depending on the linkage group; however, no consistent pattern could be established taking all four populations together. For most linkage groups, the observed proportion of *B. oleracea* alleles was lower than the expected; however, for a few linkage groups, the observed proportion was equal or higher than the expected (**Figure 3-7, A and B**). For example, the C5 and C8 linkage groups of BS-F_8, the C5 of BS-BC_1F_7, and the C5 and C7 linkage groups of BD-BC_1F_7 carried the expected proportion of *B. oleracea* alleles ($p > 0.05$) (**Table 3-6** and **Table 3-7**); however, higher proportion compared to expected was found in C1, C3 and C7 of BS-BC_1F_7 population. For most of the linkage groups, the occurrence of *B. oleracea* allele was lower in the BC_1-derived populations as compared to F_2-derived populations (**Figure 3-7, A and B**), as expected.

Interestingly, non-parental alleles comprising up to 12.7 % of the total number of SSR alleles was found in the individual plants of the two populations, BD-F_8 (mean 6.7 ± 0.68 SE %)

and BD-BC_1F_7 (mean 5.4 ± 0.21 SE %), derived from *B. napus* × *B. oleracea* cv. Bindsachsener cross, while up to 6.2 % non-parental alleles was found in the plants of the two populations, BS-F_8 (mean 2.5 ± 0.40 SE %) and BS-BC_1F_7 (mean 2.1 ± 0.53 SE %), derived from *B. napus* × *B. oleracea* cv. Badger Shipper crosses (**Figure 3-6B**, and **Figure 3-7CD**; **Table 3-5**). Thus, on average, the proportion of non-parental alleles was higher in the population derived from the cross involving the *B. oleracea* parent Bindsachsener than the cross involving Badger Shipper. Furthermore, the proportion of non-parental alleles also varied among the linkage groups – this type of alleles constituted up to 23.2 % of the total alleles in C6 of BD-BC_1F_7; in contrast, *B. oleracea* alleles in this linkage group was 14.8 % of the total.

While comparing the potential of the two *B. oleracea* parents as a source of allelic diversity, the *B. oleracea* parent Badger Ship*per se*emed to have contributed greater number of alleles as compared to Bindsachsener. In contrast, greater number of non-parental alleles were generated in case of the cross involving Bindsachsener. When both *B. oleracea* and non-parental alleles were taken into account, the two F_2-derived populations developed by use of the two *B. oleracea* parents, i.e. BS-F_8 and BD-F_8 (36.3 % vs. 31.6 %) found to carry similar extent of allelic diversity; similar scenario was also found in the case of the two BC_1-derived populations BS-BC_1F_7 and BD-BC_1F_7 (22.3 % vs. 21.2 %). (**Table 3-5**).

Table 3-5. Summary of the occurrence of the proportion of *B. oleracea* (B. ole) and non-parental (NP) alleles in F_8 and BC_1F_7 generation populations of *B. napus* × *B. oleracea* var. *capitata* interspecific crosses.

Cross	Gen.	No. SSR loci	No. plants	% loci with *B. ole* alleles/ plant[1]		% loci with non-parental alleles/ plant[1]	
				Range	Mean ± SE	Range	Mean ± SE
B. nap × *B. ole-BS*	F_8	65	21	1.5 – 41.5	33.8 ± 2.25	0.0 – 5.4	2.5 ± 0.40
(*B. nap* × *B. ole-BS*) × *B. nap*	BC_1F_7	65	17	7.7 – 33.1	20.2 ± 2.28	0.0 – 6.2	2.1 ± 0.53
B. nap × *B. ole-BD*	F_8	67	24	0.0 – 39.6	24.9 ± 2.47	0.7 – 12.7	6.7 ± 0.68
(*B. nap* × *B. ole-BD*) × *B. nap*	BC_1F_7	67	31	9.7 – 33.6	15.8 ± 0.94	3.7 – 9.0	5.4 ± 0.21
Pooled F_2-derived	F_8		45	0.0 – 41.5	29.0 ± 1.80	0.0 – 12.7	4.7 ± 0.51
Pooled BC_1-derived	BC_1F_7		45	7.7 – 33.6	17.3 ± 1.04	0.0 – 9.0	4.3 ± 0.33
Pooled-all			93	0.0 – 41.5	23.0 ± 1.18	0.0 – 12.7	4.5 ± 0.30

[1]Calculated based on the proportion of this type of loci of the total number of SSR loci
Non-parental allele = alleles could not be detected in either *B. napus* or *B. oleracea* parents, but appeared in the advanced generation lines derived from *B. napus* × *B. oleracea* interspecific crosses.

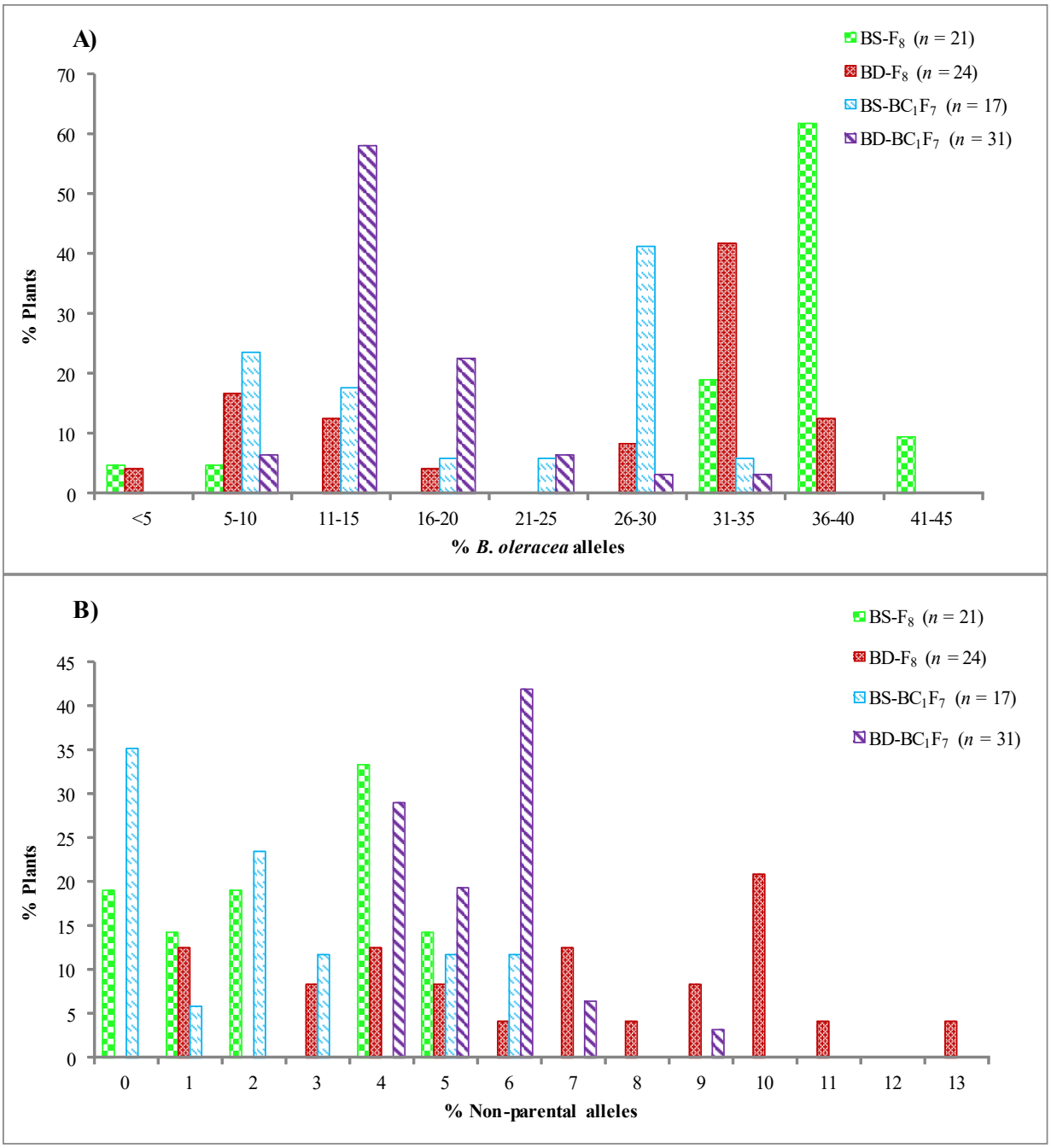

Figure 3-6. Frequency distribution of **A)** BS-F_8 and BS-BC_1F_7 populations derived, respectively, from *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper and (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* interspecific crosses, and BD-F_8 and BD-BC_1F_7 populations derived respectively from *B. napus* × *B. oleracea* var. *capitata,* cv. Bindsachsener and (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* interspecific crosses for occurrence of *B. oleracea* alleles, and **B)** BS-F_8 and BS-BC_1F_7 populations derived, respectively, from *B. napus* × *B. oleracea* var. *capitata* cv. BS and (*B. napus* × *B. oleracea* var. *capitata* cv. BS) × *B. napus* interspecific crosses, and BD-F_8 and BD-BC_1F_7 populations derived respectively from *B. napus* × *B. oleracea* var. *capitata,* cv. BD and (*B. napus* × *B. oleracea* var. *capitata* cv. BD) × *B. napus* interspecific crosses for the occurrence of non-parental alleles.

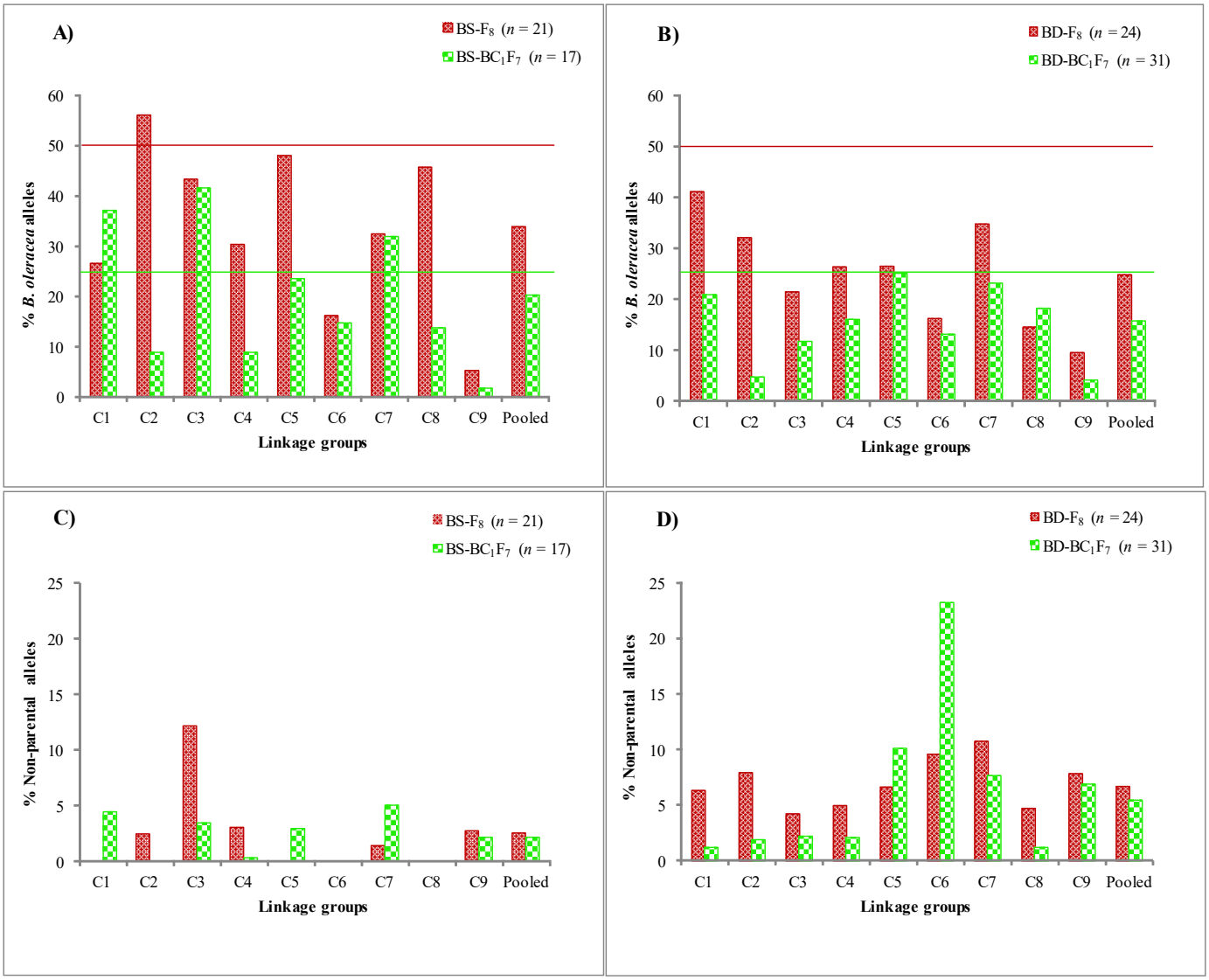

Figure 3-7. Frequency distribution of **A)** BS-F_8 and BS-BC_1F_7 populations derived, respectively, from *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper and (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* interspecific crosses and **B)** BD-F_8 and BD-BC_1F_7 populations derived, respectively, from *B. napus* × *B. oleracea* var. *capitata,* cv. Bindsachsener and (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* interspecific crosses for the occurrence of *B. oleracea* alleles, and **C)** BS-F_8 and BS-BC_1F_7 and **D)** BD-F_8 and BD-BC_1F_7 populations for the occurrence of non-parental alleles in nine C-genome linkage groups. The 50 % and 25 % are the thresholds for the expected proportion of *B. oleracea* alleles in F_2- and BC_1-derived populations, respectively, in absence of selection for any of these alleles.

Table 3-6. Inheritance of SSR marker alleles in F_8 populations of *B. napus* (B.n.) × *B. oleracea* var. *capitata* (B.o.) interspecific crosses.

LG	Total SSR markers	No. polym. loci	Total no. of plants	Total no. of SSR loci[1]	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 het. for B.o. and NP alleles (%)	No. loci het. for B.n. and NP alleles (%)	No. loci homo. for NP alleles (%)	No. loci lacking B.o./ B.n. alleles (%)	No. loci missing ampli-fication (%)	Chi-square (Segr. for homo. / het. loci)[2]	Total obs. B.o. alleles[3]	Total exp. B.o. alleles[4]	% of the exp. no. of B.o. alleles obs.	Chi-square (Segr. for alleles)[5]
B. napus (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper																	
C1	8	8	21	168	39 (23.2)	11 (6.5)	110 (65.5)	0 (0.0)	0 (0.0)	0 (0.0)	8 (4.8)	0 (0.0)	108.6*	89	168	53.0	74.3*
C2	6	6	21	126	65 (51.6)	11 (8.7)	40 (31.7)	0 (0.0)	2 (1.6)	2 (1.6)	5 (4.0)	1 (0.8)	119.0*	141	125	112.8	4.1*
C3	7	7	21	147	62 (42.2)	3 (2.0)	39 (26.5)	0 (0.0)	0 (0.0)	18 (12.2)	24 (16.3)	1 (0.7)	11.1*	127	146	87.0	4.9*
C4	12	12	21	252	72 (28.6)	8 (3.2)	143 (56.7)	0 (0.0)	11 (4.4)	2 (0.8)	14 (5.6)	2 (0.8)	45.5*	152	250	60.8	76.8*
C5	8	8	21	168	80 (47.6)	1 (0.6)	54 (32.1)	0 (0.0)	0 (0.0)	0 (0.0)	32 (19.0)	1 (0.6)	5.0	161	167	96.4	0.4
C6	4	4	21	84	13 (15.5)	1 (1.2)	38 (45.2)	0 (0.0)	0 (0.0)	0 (0.0)	31 (36.9)	1 (1.2)	13.0*	27	83	32.5	75.6*
C7	7	7	21	147	46 (31.3)	0 (0.0)	74 (50.3)	3 (2.0)	1 (0.7)	0 (0.0)	23 (15.6)	0 (0.0)	7.5*	95	147	64.6	36.8*
C8	6	6	21	126	57 (45.2)	1 (0.8)	60 (47.6)	0 (0.0)	0 (0.0)	0 (0.0)	7 (5.6)	1 (0.8)	0.1	115	125	92.0	1.6
C9	7	7	21	147	7 (4.8)	1 (0.7)	129 (87.8)	0 (0.0)	0 (0.0)	4 (2.7)	6 (4.1)	0 (0.0)	109.5*	15	147	10.2	237.1*
All	**65**	**65**	**21**	**1365**	**441 (32.3)**	**37 (2.7)**	**687 (50.3)**	**3 (0.2)**	**14 (1.0)**	**26 (1.9)**	**150 (11.0)**	**7 (0.5)**	**138.8***	**922**	**1358**	**67.9**	**280.0***
B. napus (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener																	
C1	8	8	24	192	67 (34.9)	24 (12.5)	78 (40.6)	0 (0.0)	0 (0.0)	12 (6.3)	11 (5.7)	0 (0.0)	394.1*	158	192	82.3	12.0*
C2	5	5	24	120	36 (30.0)	5 (4.2)	54 (45.0)	0 (0.0)	5 (4.2)	7 (5.8)	13 (10.8)	0 (0.0)	28.1*	77	120	64.2	30.8*
C3	6	6	24	144	31 (21.5)	0 (0.0)	87 (60.4)	0 (0.0)	0 (0.0)	6 (4.2)	19 (13.2)	1 (0.7)	27.7*	62	143	43.4	91.8*
C4	13	13	24	312	75 (24.0)	15 (4.8)	196 (62.8)	0 (0.0)	1 (0.3)	15 (4.8)	9 (2.9)	1 (0.3)	125.3*	165	311	53.1	137.1*
C5	7	7	24	168	43 (25.6)	3 (1.8)	101 (60.1)	0 (0.0)	2 (1.2)	10 (6.0)	9 (5.4)	0 (0.0)	26.1*	89	168	53.0	74.3*
C6	5	5	24	120	15 (12.5)	9 (7.5)	53 (44.2)	0 (0.0)	3 (2.5)	10 (8.3)	30 (25.0)	0 (0.0)	137.3*	39	120	32.5	109.4*
C7	7	7	24	168	54 (32.1)	9 (5.4)	74 (44.0)	0 (0.0)	0 (0.0)	18 (10.7)	11 (6.5)	2 (1.2)	62.3*	117	166	70.5	28.9*
C8	8	8	24	192	27 (14.1)	2 (1.0)	149 (77.6)	0 (0.0)	2 (1.0)	8 (4.2)	2 (1.0)	2 (1.0)	84.5*	56	190	29.5	189.0*
C9	8	8	24	192	16 (8.3)	5 (2.6)	152 (79.2)	0 (0.0)	2 (1.0)	14 (7.3)	3 (1.6)	0 (0.0)	117.7*	37	192	19.3	250.3*
All	**67**	**67**	**24**	**1608**	**364 (22.6)**	**72 (4.5)**	**944 (58.7)**	**0 (0.0)**	**15 (0.9)**	**100 (6.2)**	**107 (6.7)**	**6 (0.4)**	**596.8***	**800**	**1602**	**49.9**	**803.0***

Note: Non-parental (NP) alleles are those detected in the populations derived from these interspecific crosses, but not detected in the parents.

[1] Product of the number of polymorphic loci and number of plants

[2] Chi-square test for goodness of fit was done based on observed and expected number loci (i) homozygous for *B. oleracea* allele, (ii) heterozygous for *B. oleracea* and *B. napus* alleles, and (iii) homozygous for *B. napus* allele. Expected proportion of these three marker genotypes would, respectively, be 49.61 %, 0.78 % and 49.61 % of the total number of loci of these three classes. Asterisk indicates p-value < 0.05.

[3] Calculated based on the following formula: (No. loci homozygous for B.o. alleles × 2) + No. loci heteterozygous for B.o. and B.n. alleles + No. loci heterozygous for B.o. and NP alleles

[4] Calculated based on the following formula: [(Total no. SSR loci in the population – no. loci missing amplification) × 2] × 0.5. In this case, the total number of loci in the population minus number loci missing amplification multiplied by 2 gives the maximum number of alleles expected in the population; multiplication of this number by 0.5 gives maximum expected number of *B. oleracea* alleles occurred during the development of these F_2-derived lines/families

[5] Chi-square test for goodness of fit was done based on observed and expected number of *B. oleracea* and other (*B. napus* alleles, NP alleles, and loci without *B. oleracea* or *B. napus* alleles) alleles. Expected proportion of alleles of these two classes would, respectively, be 50% and 50%. Asterisk indicates p-value < 0.05.

Table 3-7. Inheritance of SSR marker alleles in BC_1F_7 populations of *B. napus* (B.n.) × *B. oleracea* var. *capitata* (B.o.) interspecific crosses.

LG	Total SSR markers	No. polym. loci	Total no. of plants	Total no. of SSR loci[1]	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 het. for B.o. and NP alleles (%)	No. loci het. For B.n. and NP alleles (%)	No. loci homo. for NP alleles (%)	No. loci lacking B.o./ B.n. alleles (%)	No. loci missing ampli-fication (%)	Chi-square (Segr. for homo. / het. loci)[2]	Total obs. B.o. alleles[3]	Total exp. B.o. alleles[4]	% of the exp. no. of B.o. alleles obs.	Chi-square (Segr. for alleles)[5]
B. napus (A04-73NA) × *B. oleracea* var. *capitata* cv. Badger Shipper																	
C1	8	8	17	136	46 (33.8)	9 (6.6)	74 (54.4)	0 (0.0)	0 (0.0)	6 (4.4)	0 (0.0)	1 (0.7)	74.9*	101	67.5	149.6	22.2*
C2	6	6	17	102	8 (7.8)	2 (2.0)	87 (85.3)	0 (0.0)	0 (0.0)	0 (0.0)	5 (4.9)	0 (0.0)	15.5*	18	51.0	35.3	28.5*
C3	7	7	17	119	49 (41.2)	1 (0.8)	53 (44.5)	0 (0.0)	0 (0.0)	4 (3.4)	11 (9.2)	1 (0.8)	29.5*	99	59.0	167.8	36.2*
C4	12	12	17	204	17 (8.3)	2 (1.0)	173 (84.8)	0 (0.0)	1 (0.5)	0 (0.0)	11 (5.4)	0 (0.0)	25.7*	36	102.0	35.3	56.9*
C5	8	8	17	136	32 (23.5)	0 (0.0)	95 (69.9)	0 (0.0)	0 (0.0)	4 (2.9)	5 (3.7)	0 (0.0)	1.0	64	68.0	94.1	0.3
C6	4	4	17	68	9 (13.2)	2 (2.9)	30 (44.1)	0 (0.0)	0 (0.0)	0 (0.0)	27 (39.7)	0 (0.0)	8.9*	20	34.0	58.8	7.7*
C7	7	7	17	119	38 (31.9)	0 (0.0)	54 (45.4)	0 (0.0)	0 (0.0)	6 (5.0)	20 (16.8)	1 (0.8)	14.3*	76	59.0	128.8	6.5*
C8	6	6	17	102	14 (13.7)	0 (0.0)	88 (86.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7.6*	28	51.0	54.9	13.8*
C9	7	7	17	119	0 (0.0)	3 (2.5)	112 (94.1)	1 (0.8)	0 (0.0)	2 (1.7)	1 (0.8)	0 (0.0)	41.2*	4	59.5	6.7	69.0*
All	**65**	**65**	**17**	**1105**	**213 (19.3)**	**19 (1.7)**	**766 (69.3)**	**1 (0.1)**	**1 (0.1)**	**22 (2.0)**	**80 (7.2)**	**3 (0.3)**	**21.0***	**446**	**551.0**	**80.9**	**26.7***
B. napus (A04-73NA) × *B. oleracea* var. *capitata* cv. Bindsachsener																	
C1	8	8	31	248	47 (19.0)	10 (4.0)	185 (74.6)	0 (0.0)	0 (0.0)	3 (1.2)	2 (0.8)	1 (0.4)	37.5*	104	123.5	84.2	4.1*
C2	5	5	31	155	7 (4.5)	1 (0.6)	129 (83.2)	0 (0.0)	4 (2.6)	1 (0.6)	13 (8.4)	0 (0.0)	28.2*	15	77.5	19.4	67.2*
C3	6	6	31	186	22 (11.8)	0 (0.0)	146 (78.5)	0 (0.0)	0 (0.0)	4 (2.2)	14 (7.5)	0 (0.0)	13.8*	44	93.0	47.3	34.4*
C4	13	13	31	403	62 (15.4)	6 (1.5)	317 (78.7)	0 (0.0)	1 (0.2)	8 (2.0)	9 (2.2)	0 (0.0)	17.4*	130	201.5	64.5	33.8*
C5	7	7	31	217	54 (24.9)	1 (0.5)	138 (63.6)	0 (0.0)	0 (0.0)	22 (10.1)	2 (0.9)	0 (0.0)	1.3	109	108.5	100.5	0.003
C6	5	5	31	155	18 (11.6)	5 (3.2)	49 (31.6)	0 (0.0)	0 (0.0)	36 (23.2)	47 (30.3)	0 (0.0)	35.4*	41	77.5	52.9	22.9*
C7	7	7	31	217	50 (23.0)	1 (0.5)	128 (59.0)	0 (0.0)	1 (0.5)	16 (7.4)	16 (7.4)	5 (2.3)	1.1	101	106.0	95.3	0.3
C8	8	8	31	248	44 (17.7)	3 (1.2)	192 (77.4)	0 (0.0)	0 (0.0)	3 (1.2)	5 (2.0)	1 (0.4)	5.4	91	123.5	73.7	11.4*
C9	8	8	31	248	9 (3.6)	3 (1.2)	214 (86.3)	0 (0.0)	0 (0.0)	17 (6.9)	5 (2.0)	0 (0.0)	52.2*	21	124.0	16.9	114.1*
All	**67**	**67**	**31**	**2077**	**313 (15.1)**	**30 (1.4)**	**1498 (72.1)**	**0 (0.0)**	**6 (0.3)**	**110 (5.3)**	**113 (5.4)**	**7 (0.3)**	**71.5***	**656**	**1035.0**	**63.4**	**185.0***

Note: Non-parental (NP) alleles are those detected in the populations derived from these interspecific crosses, but not detected in the parents.

[1] Product of the number of polymorphic loci and number of plants

[2] Chi-square test for goodness of fit was done based on observed and expected number loci (i) homozygous for *B. oleracea* allele, (ii) heterozygous for *B. oleracea* and *B. napus* alleles, and (iii) homozygous for *B. napus* allele. Expected proportion of these three marker genotypes would, respectively, be 24.61 %, 0.78 % and 74.61 % of the total number of loci of these three classes. Asterisk indicates p-value < 0.05.

[3] Calculated based on the following formula: (No. loci homozygous for B.o. alleles × 2) + No. loci heteterozygous for B.o. and B.n. alleles + No. loci heterozygous for B.o. and NP alleles

[4] Calculated based on the following formula: [(Total no. SSR loci in the population – no. loci missing amplification) × 2] × 0.25. In this case, the total number of loci in the population minus number loci missing amplification multiplied by 2 gives the maximum number of alleles expected in the population; multiplication of this number by 0.25 gives maximum expected number of *B. oleracea* alleles occurred during the development of these BC_1-derived lines/families.

[5] Chi-square test for goodness of fit was done based on observed and expected number of *B. oleracea* and other (*B. napus* alleles, NP alleles, and loci without *B. oleracea* or *B. napus* alleles) alleles. Expected proportion of alleles of these two classes would, respectively, be 25% and 75%. Asterisk indicates p-value < 0.05.

3.4 Discussion

Genetic diversity in the C sub-genome of *B. napus* is lower than that in the A sub-genome (Bus et al., 2011; Zhao et al., 2016) rendering the need of improvement of this sub-genome. It is widely acknowledged that the C genome of *B. oleracea* is distinct from the C genome of *B. napus* (Thormann et al., 1994; Wu et al., 2014), and wide diversity exist in the gene pool of *B. oleracea* (Izzah et al., 2013). Therefore, this diploid species can be used to enrich the diversity of alleles in the C genome of *B. napus* (for review, see Rahman, 2013). Rahman et al. (2015) and Li et al. (2014) showed the usefulness of the two variants of *B. oleracea,* viz. *alboglabra* and *acephala,* for broadening the genetic base of *B. napus.* The species *B. oleracea* includes more than 14 variants, and these variants are very distinct both genetically and morphologically (Quiros and Farnham, 2011; Izzah et al., 2013); therefore, it is important to evaluate additional variants of this species for their potential of enriching the C genome of *B. napus*. In this study, two cultivars of *B. oleracea* var. *capitata*, viz. Badger Shipper and Bindsachsener*,* were used to understand the potential of this variant for broadening of allelic diversity in the C genome of spring *B. napus* canola.

In present study, a total of 307 alleles were detected in the *B. napus* and *B. oleracea* parents by use of 93 polymorphic SSR markers which gave an average of 3.3 alleles per marker; this result is comparable to 3.16 and 3.4 alleles per SSR marker detected, respectively, by Wu et al. (2014) and Xiao et al. (2012) in 248 and 192 inbred lines. The lower dice similarity coefficient (0.15) between the *B. napus* and *B. oleracea* parents in contrast to the higher coefficient (0.58) between the two *B. oleracea* parents suggests that significant difference existed between the C genomes of these two species, as well as between the two *B. oleracea* parents.

In this study, F_8 plants carrying up to 42 % *B. oleracea* alleles were found; this was slightly lower than that (up to 54 %) reported by Rahman et al. (2015) while working with *B. oleracea* var. *alboglabra*. Similarly, Li et al. (2014) found up to 50 % *B. oleracea* var. *acephala* alleles in F_4 plants (mean 29.9 %) of this interspecific cross. In case of BC_1F_7, plants carrying up to 33.6 % *B. oleracea* alleles were identified.

While comparing the F_2- and BC_1-derived populations for the expected proportion of *B. oleracea* alleles, the BC_1-derived populations carried a greater proportion of the expected number of alleles as compared to the F_2-derived populations. This indicates that a greater proportion of *B. oleracea* alleles were eliminated during the development of the F_2-derived population as compared to the BC_1-derived population, which might be due to greater meiotic anomalies in F_2 and its subsequent generation populations than in BC_1. This is also evident from higher fertility in the BC_1 population compared to the F_2 population (**Table 2-2** and **Table 2-3**).

The occurrence of *B. oleracea* alleles varied among the nine C-genome linkage groups; the least content of *B. oleracea* genome was detected in C9. This linkage group is known to carry a locus for glucosinolate content (Xu et al., 2015); selection for low glucosinolate content might have resulted elimination of greater portion of the *B. oleracea* genome from this linkage group in the advanced generation lines developed from this interspecific cross. The moderate correlation between the two F_2-derived ($r = 0.36$; $R^2 = 0.13$) as well as the two BC_1-derived ($r = 0.54$; $R^2 = 0.29$) populations for *B. oleracea* alleles for different linkage groups indicated the absence of preferential retention of *B. oleracea* alleles in these linkage groups.

In the four inbred populations developed in this study from two *B. napus* × *B. oleracea* interspecific crosses, non-parental alleles constituted about 4.5 % of the total alleles (**Table 3-5**). Zhang et al. (2005) observed about 14 non-parental alleles in 11 lines of synthetic hexaploid

wheat while working with 90 SSR markers. In case of *Brassica*, Qian et al. (2005) detected 6 % non-parental alleles for AFLP markers in the populations derived from *B. napus* × *B. rapa* crosses. Wu et al. (2014) reported 9.3 % non-parental alleles for SSR markers in the progenies derived from resynthesized *B. napus* × natural *B. napus* crosses. Dhaka et al. (2017) observed 2.8 % non-parental alleles in a doubled haploid population of *B. juncea* while working with 637 SSR markers. Our knowledge of how these non-parental alleles are being generated is limited; recombination between homoeologous loci, mutation in SSR regions or small-scale rearrangement of the genome by transposable elements could be the possible reasons for the occurrence of such alleles. The occurrence of non-parental alleles revealed that interspecific hybridization not only introduces allelic diversity from allied species, but also creates new alleles which can further broaden the genetic base of our crop species.

Cluster analysis showed that the advanced generation inbred lines derived from an early generation plant tended to group together. This suggests that selection of greater number of genetically diverse plants in early segregating generation population (F_2 and BC_1) would be needed to achieve greater genetic diversity among the advanced generation inbred lines derived from this interspecific cross.

Chapter 4

Evaluation of the recombinant inbred lines (RILs) derived from F_2 and backcross (F_1 × *B. napus*) populations of *B. napus* × *B. oleracea* interspecific crosses for *per se* performance and heterosis

4.1 Introduction

The term heterosis, coined by Shull (1948) in 1914, can simply be defined as superior performance of the F_1 as compared to its parents. Early in 1980s, Sernyk and Stefansson (1983) and Grant and Beversdorf (1985) reported heterosis for seed yield in spring *B. napus* suggesting the potential for the development of hybrid cultivars of this crop. Currently, most of the canola cultivars available for commercial production are hybrids; therefore, it is important to increase the level of heterosis in this crop. Lefort-Buson et al. (1987) observed higher heterosis in the hybrids developed based on inbred lines from European and Asiatic origin as compared to the hybrids developed based on the inbred lines from the same geographic origin. This indicates the importance of genetic distance between the parents for hybrid vigor; this was later supported by Riaz et al. (2001). In contrast, Qian et al. (2007) found that parental GCA, as compared to genetic distance, shows stronger correlation with hybrid performance.

The extent of heterosis in *B. napus* can vary for different traits; for instance, Radoev et al. (2008) observed 30% heterosis for seed yield and only 0.7% heterosis for seed weight in winter *B. napus* hybrids. Researchers have shown that alleles introgressed from winter (Rahman et al., 2016) and Chinese semi-winter (Qian et al., 2007) type of *B. napus* carrying *B. rapa* alleles can increase heterosis for seed yield in spring type *B. napus*. Similarly, genome contents introgressed from *B. rapa* and *B. carinata* have also been found to contribute to heterosis in *B. napus* (Qian et al., 2005; Zou et al., 2010). In the case of *B. oleracea*, Li et al. (2014) found a significant

positive correlation between the mid-parent heterosis and genome content introgressed from *B. oleracea* var. *acephala.* Rahman et al. (2016) reported that the alleles contributing to non-additive effect of heterosis can be found frequently in *B. oleracea* var. *alboglabra* as compared to winter and spring type *B. napus*. Thus, it is evident that only two variants of *B. oleracea*, viz. *alboglabra* and *acephala* have been investigated for heterotic potential despite wide morphological and genetic diversity exists in this diploid species (Làzaro and Aguinagalde, 1998). This indicates the possibility of utilization of the genome contents from other form of *B. oleracea* for heterosis in *B. napus.*

It is important to examine the relationship between the performance of the parental lines and their hybrids to explain the phenomenon heterosis and the importance of the inbred lines for high yield in hybrid cultivars. Smith (1986) observed a low correlation between the performance of the inbred lines and hybrids in a simulation study in maize. Similarly, Mihaljevic et al. (2005) and Flint-Garcia et al. (2009) also found a low correlation between hybrid and inbred for seed yield; however, they found a higher correlation for other traits in maize. In *B. napus*, limited information is available of the correlation between the performance of the hybrid and the inbred lines. Shen et al. (2005) reported a positive correlation between the performance of hybrid and inbred line for seed yield and oil content, and yield contributing traits, while Rahman et al. (2016) found a negative correlation between mid-parent heterosis and *per se* performance of the inbred lines, but a positive correlation between inbred performance and hybrid seed yield in spring *B. napus* canola based on populations derived from *B. napus* × *B. oleracea,* spring × spring and winter × spring *B. napus* crosses.

Plant growth environment often exerts a major influence on the expression of a quantitative trait including seed yield. Environment can differ over the years as well as over the

locations. Several researchers have reported that seed yield and yield-contributing traits, as well as other seed quality traits of *B. napus* are significantly affected by genotype, environment and genotype × environment interaction (Shafii et al., 1992; Si et al., 2003; Seyis et al., 2006; Shi et al., 2011; Nowosad et al., 2016). Therefore, it is important to evaluate the inbred lines and their hybrids in multiple trials for reliable data.

The spring *B. napus* inbred lines, carrying genome contents of *B. oleracea*, reported in Chapter 2 were used for this part of the study. The objective of this study was (i) to evaluate these inbred lines for different agronomic and seed quality traits including seed yield, plant height, days to flowering, and seed glucosinolate, oil and protein contents, (ii) to investigate the heterotic potential of the allelic diversity introgressed from *B. oleracea* into *B. napus* canola through evaluating the test-hybrids developed based on these inbred lines, and (iii) to investigate the relationship between the genetic diversity of the hybrid parents and heterosis. For the development of test-hybrids, I used the *B. napus* parent A04-73NA as a tester. The inbred lines derived from the *B. napus* × *B. oleracea* crosses and the tester *B. napus* parent differed primarily for the C-genome alleles introgressed from *B. oleracea*; therefore, heterosis in the test-hybrids would primarily be due to the effect of the genome content introgressed from *B. oleracea.*

4.2 Materials and methods

4.2.1 Parent material

Interspecific crosses were made between *B. napus* (line A04-73NA) and *B. oleracea* var. *capitata*, as described in Section 2.2.1 and 2.2.2, and advanced generation recombinant inbred lines (RILs) were developed through 7−8 generation of self-pollination as described in Section 2.2.3 of Chapter 2. From the total number of inbred lines developed from these crosses, 93 lines

comprising 21 F_2- and 17 BC_1-derived lines of A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper (BS), and 24 F_2- and 31 BC_1-derived lines of A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener (BD) were used for production of test-hybrids with the spring *B. napus* parent A04-73NA (**Appendix: 3-1**). The above-mentioned four inbred line populations, hereafter, will be referred to as BS-F, BS-BC, BD-F and BD-BC, respectively.

4.2.2 Test-hybrid trials

4.2.2.1 Production of test-hybrid and self-pollinated seeds

The 93 inbred lines were crossed manually as male to the spring *B. napus* parent A04-73NA as female in a greenhouse in winter 2014-15 and 2015-16. The inbred lines were grown in gallon pots with two plants per pot, while the A04-73NA plants were grown in 5 × 5 inches pots with one plant per pot. Test-hybrid seeds were produced through manual emasculation of the female followed by pollination with fresh pollen collected from the male. The pollinated buds were covered with paper bag to prevent contamination from any unwanted pollen. The paper bags were removed 4-5 days after pollination to ensure that siliques get enough room to grow. Siliques were harvested at about 45 days after pollination when they started to turn brown. Thus, a total of 93 test-hybrids were produced based on the 93 inbred lines. Along with the production of test-hybrids, self-pollinated seeds of the inbred lines were produced by bag isolation using transparent and micro perforated plastic bags.

4.2.2.2 Field evaluation of the test-hybrids

All 93 inbred lines and their 93 test-hybrids were evaluated in replicated field trials for heterosis for different agronomic and seed quality traits including seed yield. The field trials were conducted at Edmonton Research Station (south campus) of the University of Alberta in summer

2015 and at St. Albert Research Station of the University of Alberta in summer 2016. The test-hybrids were grown along with their respective parents, where the two parents were grown on the two sides of the test-hybrid (**Figure 4-1**). This layout allowed direct comparison of the hybrids with the parents.

In 2015, 1.2 m × 1.0 m plot consisting of three rows was seeded manually; number of replication was two. Of the three rows, the middle-row and each guard-row was seeded with 0.3 and 0.15 g seeds, respectively. Thinning was done at 3−4 leaf stage to retain 22 plants in the middle-row and 12-18 plants in the guard-rows. Randomization of the accessions was done using CropStat 7.2 (International Rice Research Institute, Los Banos, Philippines).

In 2016, plot size was 2.0 m × 1.3 m consisting of 4 rows, seeded with 1.3 g seeds per plot with a plot seeder, and number of replication was two. Randomization of the accessions was done using PBTools 1.4 (International Rice Research Institute, Los Banos, Philippines).

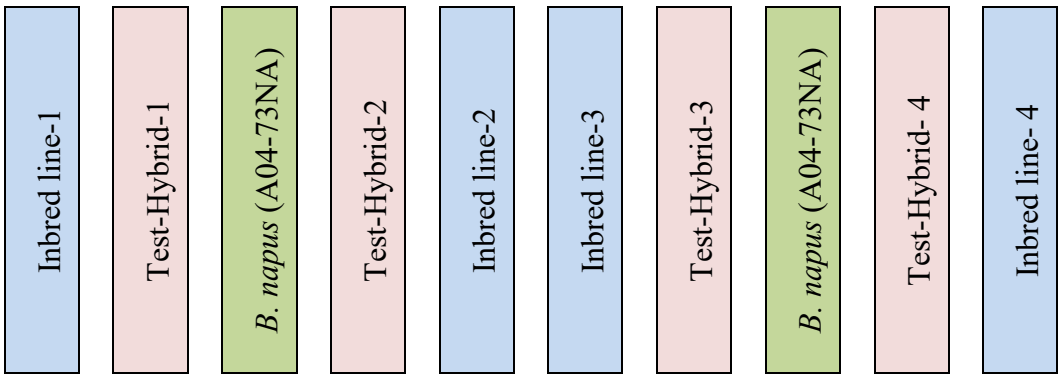

Figure 4-1. Schematic diagram of seeding the test-hybrids and their parents in the field experiments conducted in 2015 and 2016. An example shown with four inbred lines and their test-hybrids.

4.2.3 Field evaluation of the inbred lines for *per se* performance in RIL trial

Yield trials with the 93 lines used for production of test-hybrids were conducted at four locations in summer 2016: two trials conducted at Edmonton Research Station, namely ERS-1 and ERS-2, one at St. Albert Research Station and one trial at Killam Alberta, Canada. Open-pollinated seeds

harvested from the middle row of the plots of the inbred lines grown in test-hybrid trial in summer 2015 were used for these yield trials. Seeds were treated with Helix® to protect the young seedling from the damage caused by insect pests and diseases.

At each location, two replications were planted; plot size was 5.0 m × 1.8 m with six rows where 8.6 g seeds were seeded. Each replication included 93 inbred lines and 7 plots of the *B. napus* line A04-73NA as check. Due to large number of entries in the trial, each replication was divided into two blocks, each with 50 plots, and each block was further divided into two sub-blocks of 25 plots. Randomization of the accessions within each replication was done for all locations separately using PBTools 1.4 (International Rice Research Institute, Los Banos, Philippines) in such way that all sub-blocks, except one, have received two plots of A04-73NA and one had one plot of this check.

4.2.4 Agronomic and seed quality traits recorded in test-hybrid and inbred line trials

In addition to seed yield, different agronomic traits, such as days to flowering, days to maturity, and seed quality traits, such as seed oil, protein and glucosinolate content were recorded.

Days to flowering

Days to flowering data were recorded when approximately 50% of the plants in a plot had at least one open flower. Data recorded as Julian day and converted to number of days required to flower based on difference between the flowering and seeding date.

Plant height

Plant height was recorded at the end of flowering. For this, height (cm) of five randomly selected plants per plot was measured, and the mean values were used for statistical analysis.

Seed yield

The plots were sprayed with Reglone® desiccant when the siliques turned to yellow, and harvested about 10 days after desiccation with a plot combine. Seed yield data were recorded on whole plot basis, and converted to kg/ha at 8.5 % seed moisture content.

Seed quality traits

Seed oil, protein and glucosinolate contents were measured on open-pollinated seeds harvested from the plot following Near Infrared Reflectance Spectroscopy (NIRS) (Model 6500, Foss North America, Eden Prairie, MN) method. The detail of this method is presented in Section 2.2.7 of Chapter 2.

4.2.5 Statistical analysis

Data collected from the test-hybrid and inbred line trials were organized in MS Excel sheets. In case of the test-hybrid trial data, mid-parent heteroses (MPH) for all agronomic and seed quality traits were calculated using the trait values of the male and female parents and their test-hybrids (F_1) by use of the following formula:

$$\text{MPH (\%)} = \frac{F_1 - (P_1 + P_2)/2}{(P_1 + P_2)/2} \times 100$$

Heterosis over the *B. napus* parent A04-73NA (73NAH) was calculated using the following formula:

$$\text{73NAH (\%)} = \frac{F_1 - \text{A04 73NA}}{\text{A04 73NA}} \times 100$$

Data from four locations of the inbred line yield trial, and two years data of the test-hybrid trial were pooled and analyzed using the statistical computing and graphics software program 'R' (versions 3.3.2 and 3.4.2) (R Core Team, 2016). In analysis of both trials, year/location, replication and block were considered as random effects, while the cross (C) (A04-

73NA × Badger Shipper vs. A04-73NA × Bindsachsener), line type (L) (F_2- vs. BC_1-derived) and their interactions (C × L), and individual genotypes (inbred lines or test-hybrids) within the whole population were considered as fixed effects. The individual genotypes were considered as nested within C × L interactions. Having both fixed and random effect factors in the experiments, a linear Mixed-Effect model was fitted using 'lmer' function in 'lme4' package of R (Bates et al., 2015).

Analysis of variance was done separately for inbred lines, test-hybrids, MPH and 73NAH in the test-hybrid trial, and for inbred lines in the yield trial using 'anova' function in 'lmerTest' package of R (Kuznetsova et al., 2016) to partition the total variance into various components, and to test the level of their significance. LSmeans for the fixed effect factors were estimated for each trait using 'lsmeans' function in 'lsmeans' package of R (Lenth, 2016). Tukey's Honest Significant Difference test was done to compare the mean values of various fixed effect factors with each other and with the *B. napus* parent A04-73NA for different agronomic and seed quality traits.

The genetic distance (GD) between the inbred lines and the *B. napus* parent A04-73NA was calculated by subtracting the value of similarity coefficients from 1 (**Appendix: 3-3**). Pearson's correlation coefficients were calculated and tested against the null hypothesis of $\rho = 0$ for the level of significance using "corre.test" function in "psych" package of R (Revelle, 2017). The scatter diagram plots were created using MS Excel. The interaction graphs were created using 'plot' function in 'phia' package of R (De Rosario-Martinez, 2015), and the frequency distributions and boxplots were prepared using 'ggplots2' package of R (Wickham, 2009).

4.3 Results

4.3.1 Test-hybrid trial

4.3.1.1 Analysis of variance

Analysis of variance (ANOVA) for different agronomic and seed quality traits of the inbred lines and their test-hybrids evaluated in hybrid trials is presented in **Table 4-1**. The total variance was partitioned among the crosses (A04-73NA × Badger Shipper and A04-73NA × Bindsachsener) (C), the type of inbred line (F_2- or BC_1-derived) (L), the C × L interaction and the entries within the whole population. In case of the inbred line population, included in the test-hybrid trial, it was found that the crosses exerted significant effect on the variance for all agronomic and seed quality traits except for seed yield, plant height and seed protein content. Variation due to the type of inbred line was significant for all the traits except for seed yield and seed oil content ($p < 0.001$). In case of the test-hybrid population, variance due to cross and type of inbred line was significant for all the traits except for seed protein content. The C × L interaction played a significant role in the phenotypic variation for seed yield, days to flowering, seed oil and protein contents in the inbred line population, and for seed yield, oil and protein contents in the test-hybrid population. Significant variation for various traits also existed among the entries within the whole inbred line and test-hybrid populations ($p < 0.001$). Of the two major components of variance, the cross accounted for the majority of the variance for seed yield, oil and glucosinolate contents, while the type of inbred line accounted for the majority of the variance for days to flowering and plant height in both inbred and test-hybrid populations.

Analysis of variance (ANOVA) for mid-parent heterosis (MPH) and heterosis over the spring *B. napus* parent A04-73NA (73NAH) for different agronomic and seed quality traits is presented in **Table 4-2**. In case of MPH, the cross (C) exerted significant contribution to the total

variance for only seed yield, plant height and seed protein content. The type of line (L) contributed significantly to the observed phenotypic variation for seed yield and oil content, and C × L interaction was significant for seed yield, days to flowering and seed protein content ($p < 0.05$). Significant interaction between the cross and the type of inbred line indicated that the *B. oleracea* alleles of the two cvs. Bindsachsener and Badger Shipper can exert sizeable effect on MPH through the F_2- or BC_1-derived inbred lines (**Appendix: 4-3**). The variance for 73NAH for seed yield and oil content was significantly influenced by both the cross and the type of inbred line; while for seed glucosinolate content it was affected only by the cross, and for days to flowering and plant height it was affected only by the type of line. Highly significant variation within the whole population was found for both MPH and 73NAH for ($p < 0.001$) for most of the traits. Of the two major components of variance (cross and the type of line), greater variance for MPH for seed yield was found due to the cross.

Table 4-1. Mean square values derived from analysis of variance (ANOVA) of *per se* performance of the inbred lines of the two *B. napus* × *B. oleracea* crosses and their test-hybrids for different agronomic and seed quality traits based on pooled data of the test-hybrid trials conducted in 2015 and 2016

Source[1]	Df[3]	Error df[4]	MS[5]	F value	P value[6]	Error df	MS	F value	P value	Error df[4]	MS	F value	P value	Error df	MS	F value	P value
		Seed yield								Seed oil							
		Inbred lines				Test-hybrids				Inbred lines				Test-hybrids			
Cross [C]	1	241.8	509375.0	1.7	0.1961	253.1	6447872.0	19.6	0.0000***	262.2	99.0	59.5	0.0000***	257.1	17.4	12.5	0.0005***
Line type [L]	1	239.8	388934.0	1.3	0.2584	252.2	4517801.0	13.7	0.0003***	264	1.0	0.6	0.4282	258.1	12.7	9.2	0.0027**
C × L	1	242.3	3990686.0	13.2	0.0003***	252.7	5392363.0	16.4	0.0001***	256.6	90.2	54.3	0.0000***	251.4	40.4	29.1	0.0000***
Genotype (C × L)[2]	89	219.8	628243.0	2.1	0.0000***	231.7	494483.0	1.5	0.0084**	237.2	5.8	3.5	0.0000***	231.8	2.9	2.1	0.0000***
Residual			303100.0				329260.0				1.7				1.4		
		Days to flowering								Seed protein							
		Inbred lines				Test-hybrids				Inbred lines				Test-hybrids			
Cross [C]	1	239.4	36.9	7.9	0.0052**	256.3	16.3	4.3	0.0382*	264.7	0.9	0.6	0.4578	255.4	4.1	3.3	0.0686
Line type [L]	1	242.2	365.8	78.9	0.0000***	259.1	63.1	16.8	0.0001***	264.1	19.8	12.4	0.0005***	254.0	2.3	1.9	0.1681
C × L	1	214.3	33.7	7.3	0.0076**	243.5	0.0	0.0	0.9567	263.6	7.6	4.7	0.0307*	258.1	9.7	7.9	0.0053**
Genotype (C × L)[2]	89	246.6	20.6	4.4	0.0000***	250.5	8.4	2.2	0.0000***	235.3	2.7	1.7	0.0009***	230.7	1.9	1.6	0.0047**
Residual			4.6				3.8				1.6				1.2		
		Plant height								Seed glucosinolate							
		Inbred lines				Test-hybrids				Inbred lines				Test-hybrids			
Cross [C]	1	267.1	117.4	1.9	0.1638	251.0	467.3	10.1	0.0016**	225.7	940.6	302.9	0.0000***	258.4	159.6	77.9	0.0000***
Line type [L]	1	268.2	3829.3	63.6	0.0000***	252.3	2410.3	52.3	0.0000***	224.5	88.2	28.4	0.0000***	258.1	13.7	6.7	0.0104*
C × L	1	254.2	5.9	0.1	0.7551	232.3	86.2	1.9	0.1726	187.4	2.1	0.7	0.4158	258.1	0.0	0.0	0.8906
Genotype (C × L)[2]	89	253.7	265.6	4.4	0.0000***	251.9	128.2	2.8	0.0000***	245.5	22.7	7.3	0.0000***	258.1	8.1	4.0	0.0000***
Residual			60.2				46.1				3.1				2.0		

[1] Cross = *B. napus* A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper vs. *B. napus* A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener; Line type = F_2- vs. BC_1-derived; and genotype = 93 RILs of the two crosses. All these and their interactions were considered as fixed effect.

[2] Genotype (93 inbred lines or 93 test-hybrids) is nested within C × L; [3] df = degree of freedom

[4] Because of unbalanced data set, analysis of variance was synthesized with Satterthwaite approximation for denominator degrees of freedom and mean squares using "LmerTest" package of R (R Foundation for Statistical Computing)

[5] MS = Mean square; [6] Significance codes for p value of *** = < 0.001, ** = < 0.01 and * = < 0.05

Table 4-2. Mean square values derived from analysis of variance (ANOVA) of mid-parent heterosis (MPH) and heterosis over the spring *B. napus* parent A04-73NA (73NAH) for different agronomic and seed quality traits in the test-hybrids of the inbred lines of the two *B. napus* × *B. oleracea* crosses based on pooled data of the test-hybrid trials conducted in 2015 and 2016

Source[1]	Df[3]	Error df[4]	MS[5]	F value	P value[6]	Error df	MS	F value	P value	Error df[4]	MS	F value	P value	Error df	MS	F value	P value
		Seed yield								Seed oil							
		MPH[7]				73NAH[8]				MPH[7]				73NAH[8]			
Cross [C]	1	228.2	11046.9	31.6	0.0000 ***	180.0	8487.3	20.3	0.0000 ***	186.1	0.3	0.1	0.7681	240.5	137.1	25.4	0.0000 ***
Line type [L]	1	228.5	2501.8	7.1	0.0081 **	155.8	6137.2	14.7	0.0002 ***	187.3	59.0	15.0	0.0001 ***	244.1	90.2	16.7	0.0001 ***
C × L	1	228.7	2153.5	6.2	0.0139 *	132.5	7105.4	17.0	0.0001 ***	143.0	10.9	2.8	0.0980	216.9	153.5	28.4	0.0000 ***
Genotype (C × L)[2]	89	228.0	521.9	1.5	0.0096 **	230.9	520.2	1.2	0.0987	233.1	5.4	1.4	0.0272 *	242.8	13.1	2.4	0.0000 ***
Residual			350.1				417.5				3.9				5.4		
		Days to flowering								Seed protein							
		MPH[7]				73NAH[8]				MPH[7]				73NAH[8]			
Cross [C]	1	181.2	0.04	0.004	0.9519	200.9	59.4	3.3	0.0728	211.6	77.6	11.4	0.0009 ***	245.4	28.0	2.5	0.1143
Line type [L]	1	172.0	4.0	0.3	0.5676	192.1	210.8	11.6	0.0008 ***	215.3	9.5	1.4	0.2391	250.2	27.6	2.5	0.1171
C × L	1	129.8	64.3	5.3	0.0230 *	155.9	18.3	1.0	0.3187	176.7	46.0	6.8	0.0100 *	232.4	148.5	13.3	0.0003 ***
Genotype (C × L)[2]	89	234.5	19.9	1.6	0.0017 **	244.7	40.3	2.2	0.0000 ***	234.7	11.3	1.7	0.0012 **	241.7	22.3	2.0	0.0000 ***
Residual			12.1				18.3				2.6				11.2		
		Plant height								Seed glucosinolate							
		MPH[7]				73NAH[8]				MPH[7]				73NAH[8]			
Cross [C]	1	167.6	343.1	13.4	0.0003 ***	215.0	56.3	2.3	0.1309	168.2	163.6	2.9	0.0887	185.9	6452.4	76.4	0.0000 ***
Line type [L]	1	144.5	83.0	3.2	0.0737	204.7	991.3	40.5	0.0000 ***	167.4	66.7	1.2	0.2758	190.0	13.6	0.2	0.6892
C × L	1	116.7	33.9	1.3	0.2521	169.1	0.2	0.01	0.9269	123.3	7.6	0.1	0.7128	140.7	237.7	2.8	0.0957
Genotype (C × L)[2]	89	241.1	39.4	1.5	0.0053 **	247.4	69.5	2.8	0.0000 ***	230.7	100.9	1.8	0.0002 ***	237.5	263.1	3.1	0.0000 ***
Residual			25.6				24.5				55.8				84.5		

[1] Cross = *B. napus* A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper vs. *B. napus* A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener; Line type = F_2- vs. BC_1-derived; and genotype = 93 RILs of the two crosses. All these and their interactions were considered as fixed effect.
[2] Genotype (93 inbred lines) is nested within C × L; [3] df = degree of freedom
[4] Because of unbalanced data set, analysis of variance was synthesized with Satterthwaite approximation for denominator degrees of freedom and mean squares using "LmerTest" package of R (R Foundation for Statistical Computing)
[5] MS = Mean square; [6] Significance codes for p value of *** = < 0.001, ** = < 0.01 and * = < 0.05
[7] MPH = mid-parent heterosis; [8] 73NAH = heterosis over *B. napus* parent A04-73NA

4.3.1.2 Mean comparison

4.3.1.2.1 Seed yield (kg/ha^{-1})

Mean seed yield of the inbred population (3496.2 ± 1097.2 kg ha^{-1}) was significantly lower than that of the *B. napus* parent A04-73NA (3955.0 ± 1097.3 kg ha^{-1}), while mean seed yield of the test-hybrid population (4004.2 ± 1097.1 kg ha^{-1}) was statistically similar to that of A04-73NA (**Table 4-3**).

The F_2-derived inbred lines were at par with the BC_1-derived lines for seed yield; however, the test-hybrids of the F_2-derived lines out-yielded the test-hybrids of the BC_1-derived lines and showed significantly greater level of MPH (12.7 ± 1.9 vs. 6.8 ± 1.9 %) and 73NAH (7.4 ± 4.8 vs. -1.8 ± 4.9 %). While comparing the two crosses, performance of the two inbred line populations was statistically similar; however, the inbred lines of A04-73NA × Badger Shipper (BS) gave greater MPH than the inbred lines of A04-73NA × Bindsachsener (BD) (15.9 ± 2.0 vs. 3.6 ± 1.9 %) ($p < 0.001$) (**Table 4-3, Appendix: 4-2A**).

Individually, all four test-hybrid populations significantly surpassed the respective inbred populations for seed yield. Positive MPH for seed yield was found in all four populations of the two crosses (**Figure 4-3**). When comparing the four inbred populations for their performance in hybrids, the test-hybrid population of the F_2-derived lines of A04-73NA × Badger Shipper (BS-F) out-yielded the check by 519 kg ha^{-1} (**Table 4-3**). Of the 45 F_2- and 48 BC_1-derived inbred lines of the two crosses, only about 18 % lines of each of these two types out-yielded the check A04-73NA; however, about 71 % and 44 % test-hybrids of these two types of lines out-yielded A04-73NA (**Figure 4-2A**). The test-hybrids of the inbred lines 1362.166, 1362.173, 1362.174 and 1362.176 gave seed yield >4800 kg/ha (**Appendix: 4-14**). The level of

heterosis for seed yield also varied greatly within the test-hybrid population; MPH varied from -22.5 % to 43.3 % and 73NAH varied from -37.9 % to 38.5 %.

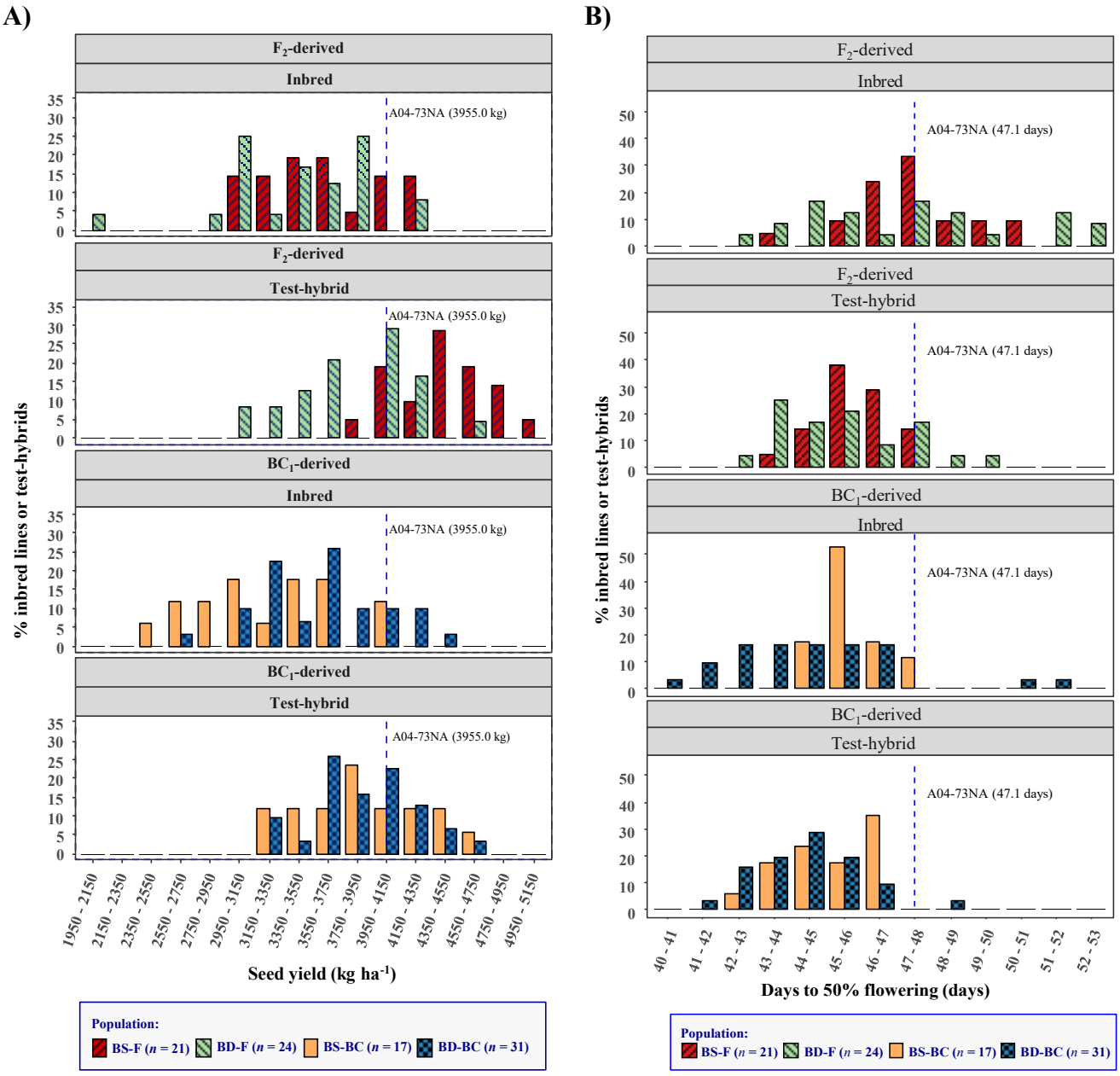

Figure 4-2. Frequency distribution for **A)** seed yield and **B)** days to flowering of the 45 F_2-derived (populations BS-F and BD-F) and 48 BC_1-derived (populations BS-BC and BD-BC) inbred lines of two *B. napus* × *B. oleracea* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA. Pooled data of 2015 and 2016 field trials are presented. The value for A04-73NA is indicated by dashed lines.

Table 4-3. Seed yield (kg/ha) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) for this trait. Mean data of the two field trials conducted in 2015 and 2016 presented.

Pedigree[1]	Line type (abbreviation)	No. entries	Inbred lines		Test-hybrids		MPH (%)		73NAH (%)	
			lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)	
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	3617.5 ± 1098.6 (3035.0 – 4310.1)	b[3]	4473.8 ± 1098.6 ***[2] (3903.3 – 5106.9)	a[3]	21.6 ± 2.4 (7.3 – 43.3)	a[3]	17.7 ± 5.1 (0.6 – 38.5)	a[3]
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	3234.3 ± 1099.5 (2517.6 – 4025.2)	c	3906.9 ± 1099.2 *** (3277.8 – 4661.4)	b	10.3 ± 2.7 (-22.3 – 32.5)	b	-1.4 ± 5.3 (-28.9 – 20.1)	b
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	3440.5 ± 1098.9 (1977.8 – 4194.0)	bc	3818.5 ± 1098.4 *** (3027.4 – 4620.7)	b	3.9 ± 2.6 (-21.3 – 26.4)	b	-2.9 ± 5.1 (-22.8 – 14.48)	b
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	3600.8 ± 1098.1 (2677.4 – 4391.3)	b	3883.1 ± 1098.2 ** (3244.5 – 4604.2)	b	3.4 ± 2.2 (-22.5 – 37.8)	b	-2.2 ± 5.0 (-37.9 – 38.4)	b
Pooled BS-cross	F & BC	38	3446.1 ± 1097.8 (2517.6 – 4310.1)	j	4220.2 ± 1097.8 *** (3277.8 – 5106.9)	i	15.9 ± 2.0 (-22.3 – 43.3)	i	8.1 ± 4.9 (-28.9 – 38.5)	i
Pooled BD-cross	F & BC	55	3530.9 ± 1097.5 (1977.8 – 4391.3)	j	3854.9 ± 1097.5 *** (3027.4 – 4620.7)	j	3.6 ± 1.9 (-22.5 – 37.8)	j	-2.5 ± 4.8 (-37.9 – 38.4)	j
Pooled F_2-derived	F	45	3523.1 ± 1097.7 (1977.8 – 4310.1)	y	4124.3 ± 1097.6 *** (3027.4 – 5106.9)	x	12.7 ± 1.9 (-21.3 – 43.3)	x	7.4 ± 4.8 (-22.8 – 38.5)	x
Pooled BC_1-derived	BC	48	3471.0 ± 1097.6 (2517.6 – 4391.3)	y	3891.6 ± 1097.6 *** (3244.5 – 4661.4)	y	6.8 ± 1.9 (-22.5 – 37.8)	y	-1.8 ± 4.9 (-37.9 – 38.4)	y
Pooled-all	F & BC	93	3496.2 ± 1097.2 (1977.8 – 4391.3)	n	4004.2 ± 1097.1 *** (3027.4 – 5106.9)	m	9.3 ± 1.5 (-22.5 – 43.3)		2.6 ± 4.3 (-37.9 – 38.5)	
Check (A04-73NA)			3955.0 ± 1097.3	aixm	3955.0 ± 1097.3	bjym				

[1] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

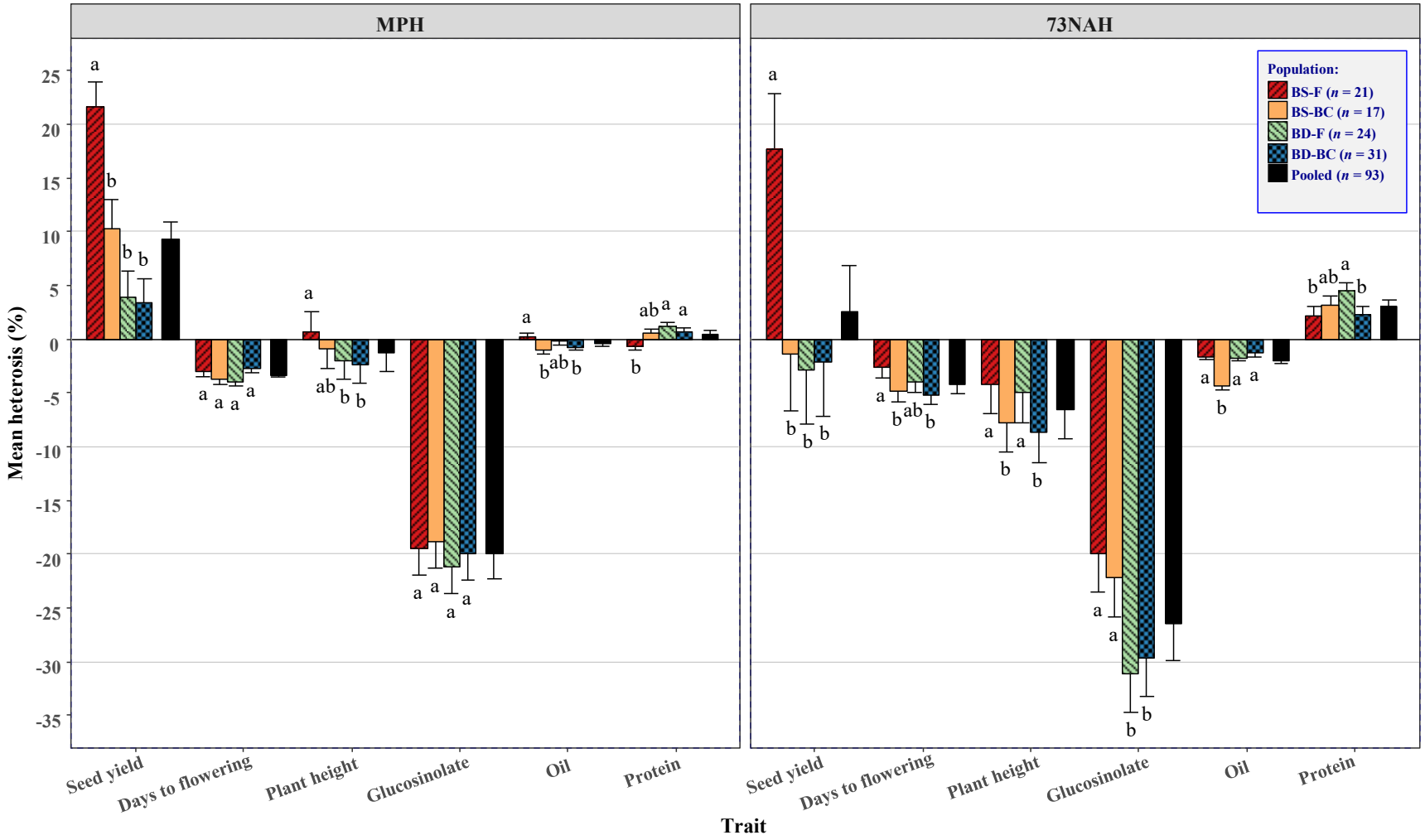

Figure 4-3. Comparison of the test-hybrids of the two F_2-derived (BS-F and BD-F) and two BC_1-derived inbred line populations (BS-BC and BD-BC) of the two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses for mid-patent heterosis (MPH) and heterosis over A04-73NA (73NAH) for different agronomic and seed quality traits. Mean data of the two field trials conducted in 2015 and 2016 presented.

4.3.1.2.2 Days to flowering (days)

The inbred lines of the whole population (BS-F, BS-BC, BD-F and BD-BC) took about 40.6 to 52.7 days to flower with a mean of 46.1 ± 1.8 days, which is about a day earlier than the spring *B. napus* parent A04-73NA (47.1 ± 1.8 days); while the test-hybrid population took 41.5 to 49.3 days to flower with a mean of 45.1 ± 1.8 days (**Table 4-4**). The level of MPH and 73NAH in the whole population varied from -9.5 to -1.3 % and -13.0 to 5.2 % with a mean of -3.3 ± 0.2 % and -4.3 ± 0.8 %, respectively. Of the 93 test-hybrids, more than 90 % of the test-hybrids gave negative values for MPH (87/93) and 73NAH (85/93) (**Appendix: 4-2B**).

Significantly delayed flowering was observed in the F_2-derived inbred lines (47.4 ± 1.8 days) and in their test-hybrids (45.7 ± 1.8 days) as compared to the BC_1-derived inbred lines (44.9 ± 1.8 days) and their test-hybrids (44.6 ± 1.8 days) (**Table 4-4**); however, the level of MPH in the hybrids of these two types of inbred lines was not significantly different (-3.5 ± 0.3 vs. -3.3 ± 0.3 %). While comparing the two crosses, the inbred population and their test-hybrids of A04-73NA × Bindsachsener flowered significantly later than those of A04-73NA × Badger Shipper; however, these two populations showed statistically similar level of MPH and 73NAH.

A greater number of test-hybrids (84/93) as compared to inbred lines (63/93) flowered earlier than A04-73NA (**Figure 4-2B**), and about 94 % (87/93) of the test-hybrids gave negative value for MPH. Compared to the F_2-derived inbred lines and their test-hybrids (19/45 and 37/45), a greater number of the BC_1-derived lines and their test-hybrids (44/48 and 47/48) flowered earlier than A04-73NA. The most early flowering inbred line (no. 1682.133) and the test-hybrid (no. 1682.140) was found in the BC_1-derived population of A04-73NA × Bindsachsener (BD-BC), which took 40.6 and 41.5 days, respectively, to flower; this is about a week earlier as compared to A04-73NA (**Appendix: 4-14**).

Table 4-4. Days to flowering (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) for this trait. Mean data of the two field trials conducted in 2015 and 2016 presented.

Pedigree[1]	Line type (abbreviation)	No. entries	Inbred lines		Test-hybrids		MPH (%)		73NAH (%)	
			lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)	
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	47.5 ± 1.8 (43.4 – 50.8)	a[3]	46.0 ± 1.8 **[2] (43.9 – 47.9)	b[3]	-3.0 ± 0.4 (-5.4 – 1.1)	a[3]	-2.7 ± 1.0 (-7.5 – 1.8)	a[3]
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	45.8 ± 1.8 (44.4 – 48.0)	b	45.0 ± 1.8 (42.4 – 46.8)	bc	-3.7 ± 0.5 (-9.5 – 1.1)	a	-4.8 ± 1.0 (-10.8 – -1.3)	b
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	47.3 ± 1.8 (42.1 – 52.7)	a	45.4 ± 1.8 *** (42.9 – 49.3)	b	-3.9 ± 0.4 (-7.9 – 0.7)	a	-4.0 ± 0.9 (-10.8 – 5.2)	ab
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	44.5 ± 1.8 (40.6 – 51.4)	c	44.4 ± 1.8 (41.5 – 48.7)	c	-2.8 ± 0.4 (-8.0 – 1.3)	a	-5.2 ± 0.9 (-13.0 – 1.9)	b
Pooled BS-cross	F & BC	38	46.7 ± 1.8 (43.4 – 50.8)	i	45.6 ± 1.8 *** (42.4 – 47.9)	j	-3.4 ± 0.3 (-9.5 – 1.1)	i	-3.7 ± 0.9 (-10.8 – 1.8)	i
Pooled BD-cross	F & BC	55	45.7 ± 1.8 (40.6 – 52.7)	j	44.8 ± 1.8 *** (41.5 – 49.3)	k	-3.4 ± 0.3 (-8.0 – 1.3)	i	-4.6 ± 0.9 (-13.0 – 5.2)	i
Pooled F_2-derived	F	45	47.4 ± 1.8 (42.1 – 52.7)	x	45.7 ± 1.8 *** (42.9 – 49.3)	y	-3.5 ± 0.3 (-7.9 – 1.1)	x	-3.3 ± 0.9 (-10.8 – 5.2)	x
Pooled BC_1-derived	BC	48	44.9 ± 1.8 (40.6 – 51.4)	y	44.6 ± 1.8 (41.5 – 48.7)	z	-3.3 ± 0.3 (-9.5 – 1.3)	x	-5.0 ± 0.9 (-13.0 – 1.9)	y
Pooled-all	F & BC	93	46.1 ± 1.8 (40.6 – 52.7)	n	45.1 ± 1.8 *** (41.5 – 49.3)	n	-3.3 ± 0.2 (-9.5 – 1.3)		-4.3 ± 0.8 (-13.0 – 5.2)	
Check (A04-73NA)			47.1 ± 1.8	aixm	47.1 ± 1.8	aixm				

[1] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

4.3.1.2.3 Plant height (cm)

Plant height of the inbred line population varied from 102.4 to 147.3 cm with a mean of 126.7 ± 16.3 cm, while plant height of the test-hybrid population varied from 117.9 to 145.9 cm with a mean of 132.3 ± 16.2 cm (**Table 4-5**). In other words, the relative height of the inbred lines and their test-hybrids varied from 72.5 to 104.2 % and 83.4 to 103.2 % as compared to the *B. napus* parent A04-73NA (141.3 ± 16.3 cm). Of the 93 inbred lines and their test-hybrids, 91 inbred lines (97.8 %) and 86 test-hybrids (92.5 %) were shorter than A04-73NA (**Figure 4-4A**). MPH in the whole test-hybrid population varied from -9.6 to 6.3 % with a mean of -1.3 ± 1.7 %, and 73NAH varied from -16.9 to 3.3 % with a mean of -6.5 ± 2.7 %.

The F_2-derived inbred lines (130.5 ± 16.3 cm) and their test-hybrids (135.3 ± 16.3 cm) were significantly taller than the BC_1-derived inbred lines and their test-hybrids (123.0 ± 16.3 and 129.4 ± 16.3 cm); however, no significant difference for the level of MPH could be found due to the type of inbred lines. In general, a greater proportion of the test-hybrids of the BC_1-derived lines (21/45) gave negative value for MPH as compared to the test-hybrids of the F_2-derived inbred lines (33/48) (**Appendix: 4-4A**).

Mean height of none of the four inbred or test-hybrid population was at par with A04-73NA; all were significantly shorter than this line – an effect of *B. oleracea* alleles on plant height is evident.

Table 4-5. Plant height (cm) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) for this trait. Mean data of the two field trials conducted in 2015 and 2016 presented.

Pedigree[1]	Line type (abbreviation)	No. entries	Inbred lines		Test-hybrids		MPH (%)		73NAH (%)	
			lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)	
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	129.3 ± 16.3 (110.2 – 139.7)	b[3]	137.4 ± 16.3 ***[2] (128.5 – 144.5)	b[3]	0.7 ± 1.8 (-5.6 – 5.9)	a[3]	-4.2 ± 2.8 (-11.1 – 1.3)	a[3]
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	122.4 ± 16.3 (107.9 – 130.1)	c	130.1 ± 16.3 *** (117.9 – 138.9)	cd	-1.0 ± 1.8 (-7.7 – 6.3)	ab	-7.8 ± 2.8 (-16.1 – -1.7)	b
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	131.6 ± 16.3 (116.9 – 147.3)	b	133.5 ± 16.3 (118.5 – 144.6)	c	-2.0 ± 1.8 (-9.5 – 2.9)	b	-5.0 ± 2.8 (-14.2 – 3.3)	a
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	123.3 ± 16.3 (102.4 – 145.6)	c	129.1 ± 16.3 *** (119.3 – 145.9)	d	-2.4 ± 1.8 (-9.6 – 5.6)	b	-8.7 ± 2.8 (-16.9 – 2.2)	b
Pooled BS-cross	F & BC	38	126.2 ± 16.3 (107.9 – 139.7)	j	134.1 ± 16.3 *** (117.9 – 144.5)	j	-0.1 ± 1.8 (-7.7 – 6.3)	i	-6.0 ± 2.7 (-16.1 – 1.3)	i
Pooled BD-cross	F & BC	55	126.9 ± 16.3 (102.4 – 147.3)	j	131.0 ± 16.3 *** (118.5 – 145.9)	k	-2.2 ± 1.7 (-9.6 – 5.6)	j	-6.8 ± 2.7 (-16.9 – 3.3)	i
Pooled F_2-derived	F	45	130.5 ± 16.3 (110.2 – 147.3)	y	135.3 ± 16.3 *** (118.5 – 144.6)	y	-0.6 ± 1.8 (-9.5 – 5.9)	x	-4.6 ± 2.7 (-14.2 – 3.3)	x
Pooled BC_1-derived	BC	48	123.0 ± 16.3 (102.4 – 145.6)	z	129.4 ± 16.3 *** (117.9 – 145.9)	z	-1.7 ± 1.8 (-9.6 – 6.3)	x	-8.2 ± 2.7 (-16.9 – 2.2)	y
Pooled-all	F & BC	93	126.7 ± 16.3 (102.4 – 147.3)	n	132.3 ± 16.3 *** (117.9 – 145.9)	n	-1.3 ± 1.7 (-9.6 – 6.3)		-6.5 ± 2.7 (-16.9 – 3.3)	
Check (A04-73NA)			141.3 ± 16.3	aixm	141.3 ± 16.3	aixm				

[1] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

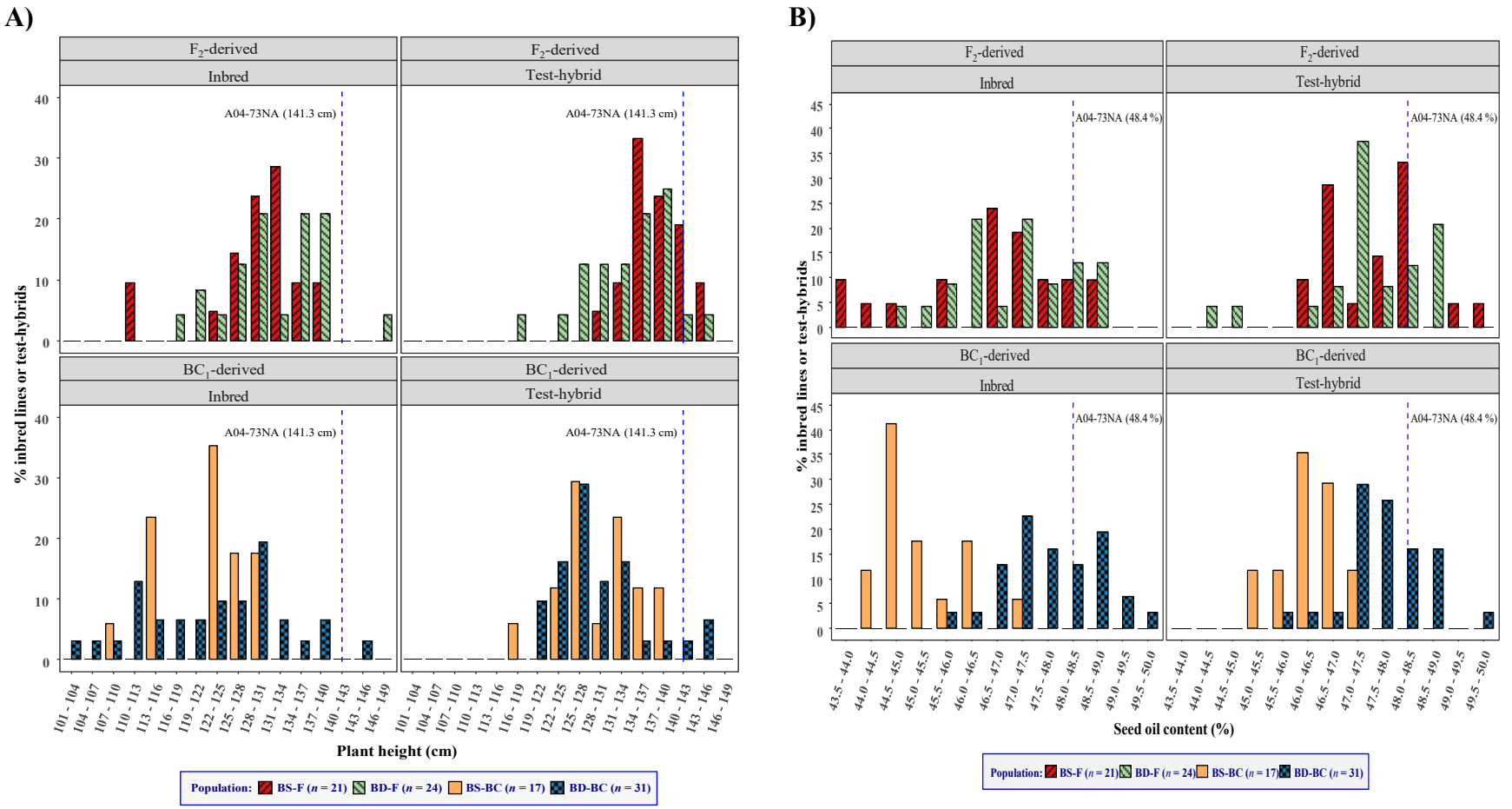

Figure 4-4. Frequency distribution for **A)** plant height and **B)** seed oil content of the 45 F_2-derived (populations BS-F and BD-F) and 48 BC_1-derived (populations BS-BC and BD-BC) inbred lines of two *B. napus* × *B. oleracea* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA. Pooled data of 2015 and 2016 field trials are presented. The value for A04-73NA is indicated by dashed lines.

4.3.1.2.4 Seed oil content

Seeds of the test-hybrid population had significantly higher oil than the inbred line population (47.4 ± 1.7 vs. 46.8 ± 1.7 %) ($p < 0.001$); however, both populations had significantly lower oil content as compared to the *B. napus* parent, A04-73NA (48.4 ± 1.7 %) (**Table 4-6**, **Figure 4-4B**). No significant difference was found between the F_2- and BC_1-derived inbred lines (46.7 ± 1.7 vs. 46.9 ± 1.7 %) and their test-hybrids (47.5 ± 1.7 vs. 47.3 ± 1.7 %) for seed oil content. None of the four inbred line populations had greater seed oil content than A04-73NA. The best inbred line (no. 1682.099) and test-hybrid (no. 1362.164) contained 49.7 % and 49.9 % seed oil, respectively, which is about 1.5 % higher than A04-73NA (**Appendix: 4-14**).

Average MPH and 73NAH of the whole population for seed oil content was -0.4 ± 0.2 % (range -3.5 to 3.2 %) and -2.1 ± 0.2 % (range -7.0 to 4.2 %), respectively. Of the four populations of the two crosses, only the F_2-derived population of A04-73NA × Badger Shipper exhibited positive MPH of 0.2 % (range -2.7 to 3.2 %). Of the 93 test-hybrids, a total of 34 test-hybrids (23 of the BC_1- and 11 of the F_2-derived inbred lines) exhibited a positive MPH. (**Appendix: 4-4B**).

Table 4-6. Seed oil content (%) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) for this trait. Mean data of the two field trials conducted in 2015 and 2016 presented.

Pedigree[1]	Line type (abbreviation)	No. entries	Inbred lines		Test-hybrids		MPH (%)		73NAH (%)	
			lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)	
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	46.7 ± 1.7 (43.7 – 48.9)	c[3]	47.6 ± 1.7 ***[2] (46.3 – 49.9)	b[3]	0.2 ± 0.3 (-2.7 – 3.2)	a[3]	-1.6 ± 0.3 (-4.2 – 4.2)	a[3]
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	45.3 ± 1.7 (44.3 – 47.1)	d	46.4 ± 1.7 *** (45.0 – 47.4)	c	-1.1 ± 0.3 (-2.9 – 0.5)	b	-4.3 ± 0.4 (-7.0 – -1.4)	b
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	46.8 ± 1.7 (41.0 – 48.7)	c	47.4 ± 1.7 * (44.3 – 48.9)	b	-0.2 ± 0.3 (-3.1 – 2.2)	ab	-1.7 ± 0.3 (-6.8 – 1.4)	a
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	47.8 ± 1.7 (45.6 – 49.7)	b	47.8 ± 1.7 (45.8 – 49.7)	b	-0.7 ± 0.3 (-3.5 – 2.2)	b	-1.3 ± 0.3 (-4.9 – 2.9)	a
Pooled BS-cross	F & BC	38	46.1 ± 1.7 (43.7 – 48.9)	k	47.1 ± 1.7 *** (45.0 – 49.9)	k	-0.4 ± 0.2 (-2.9 – 3.2)	i	-3.0 ± 0.3 (-7.0 – 4.2)	j
Pooled BD-cross	F & BC	55	47.4 ± 1.7 (41.0 – 49.7)	j	47.6 ± 1.7 (44.3 – 49.7)	j	-0.5 ± 0.2 (-3.5 – 2.2)	i	-1.5 ± 0.3 (-6.8 – 2.9)	i
Pooled F_2-derived	F	45	46.7 ± 1.7 (41.0 – 48.9)	y	47.5 ± 1.7 *** (44.3 – 49.9)	y	0.01 ± 0.2 (-3.1 – 3.2)	x	-1.7 ± 0.3 (-6.8 – 4.2)	x
Pooled BC_1-derived	BC	48	46.9 ± 1.7 (44.3 – 49.7)	y	47.3 ± 1.7 * (45.0 – 49.7)	y	-0.9 ± 0.2 (-3.5 – 2.2)	y	-2.8 ± 0.3 (-7.0 – 2.9)	y
Pooled-all	F & BC	93	46.8 ± 1.7 (41.0 – 49.7)	n	47.4 ± 1.7 *** (44.3 – 49.9)	n	-0.4 ± 0.2 (-3.5 – 3.2)		-2.1 ± 0.2 (-7.0 – 4.2)	
Check (A04-73NA)			48.4 ± 1.7	aixm	48.4 ± 1.7	aixm				

[1] *B. nap* = Spring *B. napus* canolaline A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

4.3.1.2.5 Seed protein content

Seed protein content in the 93 inbred line population varied from 24.5 to 31.3 % with a mean of 26.5 ± 1.1 SE %, and in their test-hybrids it varied from 24.5 to 28.9 % with a mean of 26.0 ± 1.1 SE %. Thus, the inbred line and the test-hybrid populations had about 1% higher seed protein than the *B. napus* parent, A04-73NA (25.3 ± 1.1). Of the 45 F_2- and 48 BC_1-derived inbred lines of the two crosses, more than 90 % (42 F_2- and 45 BC_1-derived) lines had higher protein content than A04-73NA; however, about 80 % (38/48) test-hybrids of the F_2-derived inbred lines and about 90 % (41/45) test-hybrids of the BC_1-derived inbred lines had higher protein content than A04-73NA (**Figure 4-5A**). All four inbred line populations of the two crosses and their test-hybrid populations had significantly higher protein content as compared to A04-73NA (**Table 4-7**). MPH (0.5 ± 0.3 %) and 73NAH (3.0 ± 0.7 %) for seed protein content was positive, however, very low (**Appendix: 4-5A**).

Table 4-7. Seed protein content (%) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) for this trait. Mean data of the two field trials conducted in 2015 and 2016 presented.

Pedigree[1]	Line type (abbreviation)	No. entries	Inbred lines		Test-hybrids		MPH (%)		73NAH (%)	
			lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)	
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	26.7 ± 1.1 (25.2 – 27.9)	a[3]	25.8 ± 1.1 ***[2] (24.5 – 27.2)	b[3]	-0.7 ± 0.4 (-4.3 – 3.0)	b[3]	2.2 ± 0.8 (-4.8 – 7.1)	b[3]
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	26.5 ± 1.1 (25.0 – 27.5)	ab	26.0 ± 1.1 (25.0 – 27.1)	ab	0.5 ± 0.4 (-2.1 – 2.6)	ab	3.2 ± 0.8 (0.1 – 7.5)	ab
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	26.9 ± 1.1 (24.5 – 31.3)	a	26.4 ± 1.1 (25.1 – 28.9)	a	1.2 ± 0.4 (-1.9 – 4.7)	a	4.5 ± 0.8 (0.2 – 11.5)	a
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	26.0 ± 1.1 (24.7 – 27.0)	b	25.8 ± 1.1 (24.7 – 27.3)	b	0.8 ± 0.4 (-2.8 – 3.0)	a	2.3 ± 0.8 (-2.1 – 6.9)	b
Pooled BS-cross	F & BC	38	26.6 ± 1.1 (25.0 – 27.9)	i	25.9 ± 1.1 *** (24.5 – 27.2)	i	-0.1 ± 0.3 (-4.3 – 3.0)	j	2.7 ± 0.8 (-4.8 – 7.5)	i
Pooled BD-cross	F & BC	55	26.4 ± 1.1 (24.5 – 31.3)	i	26.1 ± 1.1 * (24.7 – 28.9)	i	1.0 ± 0.3 (-2.8 – 4.7)	i	3.4 ± 0.7 (-2.1 – 11.5)	i
Pooled F_2-derived	F	45	26.8 ± 1.1 (24.5 – 31.3)	x	26.1 ± 1.1 *** (24.5 – 28.9)	x	0.3 ± 0.3 (-4.3 – 4.7)	x	3.3 ± 0.7 (-4.8 – 11.5)	x
Pooled BC_1-derived	BC	48	26.2 ± 1.1 (24.7 – 27.5)	y	25.9 ± 1.1 (24.7 – 27.3)	x	0.6 ± 0.3 (-2.8 – 3.0)	x	2.7 ± 0.7 (-2.1 – 7.5)	x
Pooled-all	F & BC	93	26.5 ± 1.1 (24.5 – 31.3)	m	26.0 ± 1.1 *** (24.5 – 28.9)	m	0.5 ± 0.3 (-4.3 – 4.7)		3.0 ± 0.7 (-4.8 – 11.5)	
Check (A04-73NA)			25.3 ± 1.1	cjzn	25.3 ± 1.1	cjyn				

[1] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

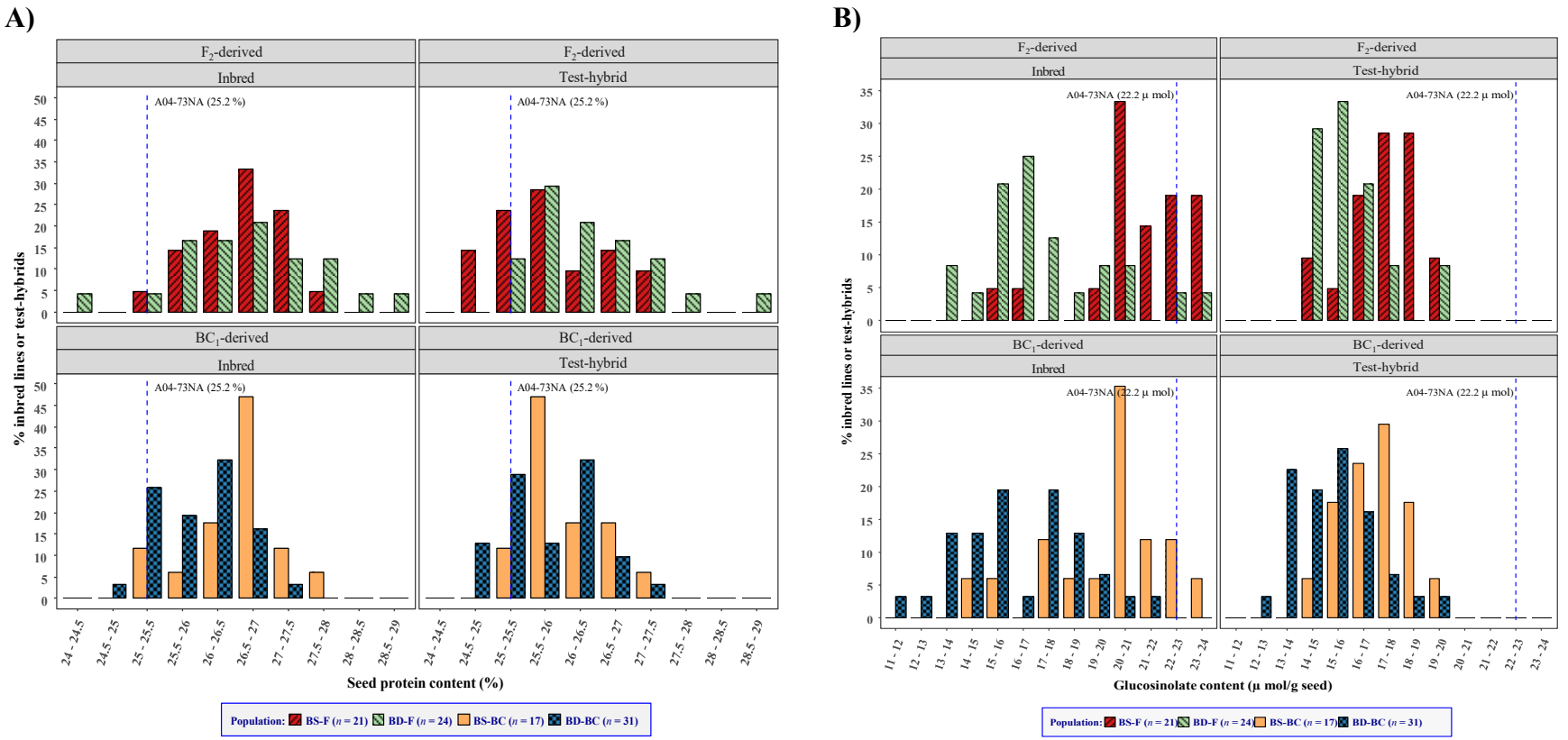

Figure 4-5. Frequency distribution for **A)** seed protein and **B)** seed glucosinolate contents of the 45 F_2-derived (populations BS-F and BD-F) and 48 BC_1-derived (populations BS-BC and BD-BC) inbred lines of two *B. napus* × *B. oleracea* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA. Pooled data of 2015 and 2016 field trials are presented. The value for A04-73NA is indicated by dashed lines. One outlier, inbred line no. 1363.202 (31.3 %) of population BD-F, was not included in inbred line frequency distribution for seed protein content.

4.3.1.2.6 Seed glucosinolate content (µmol/g seed)

The test-hybrid population (16.2 ± 0.4 µmol/g seed) had significantly lower GSL in seeds as compared to the inbred line population (18.3 ± 0.4 µmol/g seed) and A04-73NA (22.2 ± 0.4 µmol/g seed) (**Table 4-8**, **Figure 4-5B**). The F_2-derived inbred line population had significantly greater GSL content than the BC_1-derived inbred line population (19.0 ± 0.4 vs. 17.6 ± 0.4 µmol/g seed); however, no significant difference was found between the test-hybrid populations of the F_2- and BC_1-derived lines (16.5 ± 0.4 vs. 16.0 ± 0.4 µmol/g seed) as well as for the level of MPH and 73NAH for this trait. On an average, about -20 % MPH and -26 % 73NAH was observed for this trait. (**Appendix: 4-5B**).

To summarize, the magnitude of heterosis among the test-hybrids varied considerably for different agronomic and seed quality traits where seed yield exhibited the highest positive MPH (**Figure 4-3**). Overall, the four traits, seed glucosinolate content (-20.0 ± 2.4 %), days to flowering (-3.3 ± 0.2 %), plant height (-1.3 ± 1.7 %) and seed oil content (-0.4 ± 0.2 %), showed negative MPH, while seed yield (9.3 ± 1.5 %) and protein content (0.5 ± 0.3 %) showed positive MPH.

Table 4-8. Seed glucosinolate content (µmol/g seed) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) for this trait. Mean data of the two field trials conducted in 2015 and 2016 presented.

Pedigree[1]	Line type (abbreviation)	No. entries	Inbred lines		Test-hybrids		MPH (%)		73NAH (%)	
			lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)		lsmeans ± SE (Range)	
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	21.1 ± 0.4 (15.2 – 23.7)	b[3]	17.3 ± 0.5 ***[2] (14.4 – 19.2)	b[3]	-19.4 ± 2.5 (-26.0 – -12.5)	a[3]	-19.9 ± 3.6 (-38.1 – -7.0)	a[3]
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	19.7 ± 0.5 (14.4 – 23.9)	c	17.0 ± 0.5 *** (14.5 – 19.5)	b	-18.8 ± 2.5 (-24.9 – -12.3)	a	-22.2 ± 3.7 (-32.7 – -7.5)	a
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	17.3 ± 0.4 (13.2 – 23.8)	d	15.8 ± 0.4 *** (14.1 – 19.9)	c	-21.2 ± 2.5 (-31.1 – -9.8)	a	-31.1 ± 3.6 (-43.6 – -10.7)	b
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	16.4 ± 0.4 (11.7 – 21.8)	e	15.4 ± 0.4 ** (12.9 – 19.7)	c	-20.0 ± 2.4 (-32.1 – 0.1)	a	-29.7 ± 3.6 (-48.7 – -6.6)	b
Pooled BS-cross	F & BC	38	20.5 ± 0.4 (14.4 – 23.9)	j	17.2 ± 0.4 *** (14.4 – 19.5)	j	-19.1 ± 2.4 (-26.0 – -12.3)	i	-21.1 ± 3.5 (-38.1 – -7.0)	i
Pooled BD-cross	F & BC	55	16.8 ± 0.4 (11.7 – 23.8)	k	15.6 ± 0.4 *** (12.9 – 19.9)	k	-20.6 ± 2.4 (-32.1 – 0.1)	i	-30.4 ± 3.5 (-48.7 – -6.6)	j
Pooled F_2-derived	F	45	19.0 ± 0.4 (13.2 – 23.8)	y	16.5 ± 0.4 *** (14.1 – 19.9)	y	-20.3 ± 2.4 (-31.1 – -9.8)	x	-25.5 ± 3.5 (-43.6 – -7.0)	x
Pooled BC_1-derived	BC	48	17.6 ± 0.4 (11.7 – 23.9)	z	16.0 ± 0.4 *** (12.9 – 19.7)	y	-19.4 ± 2.4 (-32.1 – 0.1)	x	-26.0 ± 3.5 (-48.7 – -6.6)	x
Pooled-all	F & BC	93	18.3 ± 0.4 (11.7 – 23.9)	n	16.2 ± 0.4 *** (12.9 – 19.9)	n	-20.0 ± 2.4 (-32.1 – 0.1)		-26.5 ± 3.5 (-48.7 – -6.6)	
Check (A04-73NA)			22.2 ± 0.4	aixm	22.2 ± 0.4	aixm				

[1] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

4.3.2 RIL yield trial

4.3.2.1 Analysis of variance

Analysis of variance (ANOVA) for *per se* performance of the inbred lines for different agronomic and seed quality traits evaluated in the RIL trials is presented in **Table 4-9**. The results indicated that all the components of variance, viz. cross (C), type of line (L), their interaction (C × L) and the genotype of the individual inbred line exerted significant effect on the observed phenotypic variation for different agronomic and seed quality traits, except for days to flowering where the C × L interaction was found to be not significant. Of the two major components of variance, the cross accounted for majority of the variance for seed quality traits, such as seed oil, protein and glucosinolate contents, and the type of inbred line for agronomic traits, such as seed yield, days to flowering and plant height in this inbred line population.

Table 4-9. Mean square values derived from analysis of variance for different agronomic and seed quality traits of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses, viz. A04-73NA × Badger Shipper and A04-73NA × Bindsachsener

Source[1]	df[3]	Error df[4]	MS[5]	F value	P value[6]	Error df	MS	F value	P value
		Seed yield				Seed oil content			
Cross [C]	1	639.9	550477.0	4.7	0.0299 *	639.0	224.6	217.8	0.0000 ***
Line type [L]	1	639.9	3917909.0	33.7	0.0000 ***	639.1	5.9	5.7	0.0173 *
C × L	1	641.9	2510279.0	21.6	0.0000 ***	641.0	170.3	165.1	0.0000 ***
Genotype (C × L)[2]	89	627.1	319417.0	2.7	0.0000 ***	635.0	4.5	4.4	0.0000 ***
Residual			116262.0				1.0		
		Plant height				Seed protein content			
Cross [C]	1	643.2	432.1	7.8	0.0054 **	638.6	4.9	6.7	0.0099 **
Line type [L]	1	643.0	11617.5	209.4	0.0000 ***	638.7	4.9	6.6	0.0103 *
C × L	1	644.9	240.1	4.3	0.0379 *	640.7	7.7	10.4	0.0013 **
Genotype (C × L)	89	633.3	354.4	6.4	0.0000 ***	636.0	2.5	3.5	0.0000 ***
Residual			55.5				0.7		
		Days to flowering				Seed glucosinolate content			
Cross [C]	1	645.1	195.1	105.5	0.0000 ***	637.6	1222.3	514.8	0.0000 ***
Line type [L]	1	645.0	451.6	244.3	0.0000 ***	637.6	61.5	25.9	0.0000 ***
C × L	1	642.6	6.6	3.6	0.0595	639.0	594.7	250.5	0.0000 ***
Genotype (C × L)	89	595.7	24.8	13.4	0.0000 ***	636.6	32.1	13.5	0.0000 ***
Residual			1.8				2.4		

[1] Cross = *B. napus* A04-73NA × *B. oleracea* var. *capitata* cv. Badger Shipper vs. *B. napus* A04-73NA × *B. oleracea* var. *capitata* cv. Bindsachsener; Line type = F_2- vs. BC_1-derived and genotype = 93 RILs of the two crosses. All these and their interactions were considered as fixed effect

[2] Genotype (93 inbred lines) is nested within C × L; [3] df = degree of freedom

[4] Because of unbalanced data set, analysis of variance was synthesized with Satterthwaite approximation for denominator degrees of freedom and mean squares using "LmerTest" package of R (R Foundation for Statistical Computing)

[5] MS = Mean square; [6] Significance codes for p value of *** = < 0.001, ** = < 0.01 and * = < 0.05

4.3.2.2 Mean comparison

4.3.2.2.1 Seed yield

Seed yield of the inbred lines varied from 2782.4 to 3821.6 kg ha^{-1} with a mean of 3301.2 ± 371.1 kg ha^{-1} which was 335 kg ha^{-1} lower than mean seed yield of the check A04-73NA (3635.2 ± 373.7 kg ha^{-1}) (**Table 4-10, Appendix: 4-13**). The F_2-derived inbred lines (3364.1 ± 371.3 kg ha^{-1}) of the two crosses yielded significantly greater than the BC_1-derived inbred lines (3242.3 ± 371.3 kg ha^{-1}) ($p < 0.001$); however, average seed yield of these two populations was significantly lower than that of A04-73NA. A total of four inbred lines, 1362.166, 1362.180, 1682.100 and 1682.125, gave seed yield >3700 kg/ha; the maximum seed yield observed in the F_2- and BC_1-derived inbred lines was 3821.6 (no. 1362.180) and 3734.0 kg/ha (no. 1682.100), respectively (**Appendix: 4-15**). In general, greater proportion of the F_2-derived lines as compared to the BC_1-derived lines (13.3 vs. 6.3 %) out-yielded A04-73NA (**Figure 4-6**). Among the four populations of the two crosses, the F_2-derived lines of A04-73NA × Badger Shipper (BS-F) gave the highest seed yield (3398.5 ± 371.9 kg ha^{-1}), however, the BC_1-derived lines of the same cross gave the lowest yield (3128.1 ± 372.1 kg ha^{-1}) indicating the presence of strong C × L interaction for this trait.

Table 4-10. Performance of the inbred lines derived from the two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses, viz. A04-73NA × Badger Shipper and A04-73NA × Bindsachsener. Mean data of the four yield trials conducted in Alberta in 2016 presented

Pedigree[1]	Line type (abbreviation)	No. entries	Seed yield (kg ha^{-1})	Days to flowering (days)	Plant height (cm)	Seed oil content (whole) (%)	Seed protein content (whole) (%)	Seed glucosinolate content (µmol/g seed)
			Lsmeans ± SE (Range)	Lsmeans ± SE (Range)	Lsmeans ± SE (Range)	Lsmeans ± SE (Range)	Lsmeans ± SE (Range)	Lsmeans ± SE (Range)
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	3398.5 ± 371.9 b[2] (3016.0 – 3821.6)	54.3 ± 4.4 a[2] (51.5 – 56.1)	137.2 ± 6.9 b[2] (129.8 – 143.5)	46.2 ± 1.0 b[2] (44.7 – 47.7)	26.9 ± 1.2 a[2] (26.0 – 27.5)	19.9 ± 0.6 b[2] (16.6 – 22.7)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	3128.1 ± 372.1 d (2782.4 – 3378.0)	52.9 ± 4.4 c (51.6 – 55.4)	130.2 ± 7.0 c (123.2 – 138.0)	45.0 ± 1.0 c (43.8 – 46.3)	26.9 ± 1.2 a (25.5 – 27.8)	21.2 ± 0.6 a (17.2 – 23.8)
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	3333.9 ± 371.7 bc (2926.5 – 3671.9)	53.4 ± 4.4 b (50.1 – 58.3)	136.8 ± 6.9 b (121.3 – 148.9)	46.3 ± 1.0 b (45.1 – 48.2)	26.9 ± 1.2 a (25.7 – 28.4)	19.1 ± 0.6 c (15.3 – 23.9)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	3304.9 ± 371.5 c (2934.7 – 3734.0)	51.6 ± 4.4 d (48.0 – 56.6)	127.5 ± 6.9 d (109.9 – 143.3)	47.1 ± 1.0 a (45.5 – 48.5)	26.5 ± 1.2 b (25.3 – 27.6)	16.7 ± 0.6 d (12.9 – 23.5)
Pooled BS-cross	F & BC	38	3263.4 ± 369.6 k (2782.4 – 3821.6)	53.6 ± 4.4 i (51.5 – 56.1)	134.0 ± 6.9 j (123.2 – 143.5)	45.7 ± 1.0 j (43.8 – 47.7)	26.9 ± 1.2 i (25.5 – 27.8)	20.5 ± 0.6 j (16.6 – 23.8)
Pooled BD-cross	F & BC	55	3319.5 ± 369.5 j (2926.5 – 3734.0)	52.4 ± 4.4 j (48.0 – 58.3)	131.5 ± 6.9 k (109.9 – 148.9)	46.8 ± 1.0 i (45.1 – 48.5)	26.7 ± 1.2 j (25.3 – 28.4)	17.8 ± 0.6 k (12.9 – 23.9)
Pooled F_2-derived	F	45	3364.1 ± 371.3 y (2926.5 – 3821.6)	53.8 ± 4.4 x (50.1 – 58.3)	136.9 ± 6.9 y (121.3 – 148.9)	46.3 ± 1.0 y (44.7 – 48.2)	26.9 ± 1.2 x (25.7 – 28.4)	19.5 ± 0.6 y (15.3 – 23.9)
Pooled BC_1-derived	BC	48	3242.3 ± 371.3 z (2782.4 – 3734.0)	52.1 ± 4.4 y (48.0 – 56.6)	128.5 ± 6.9 z (109.9 – 143.3)	46.1 ± 1.0 z (43.8 – 48.5)	26.7 ± 1.2 y (25.3 – 27.8)	18.3 ± 0.6 z (12.9 – 23.8)
Pooled All	F & BC	93	3301.2 ± 371.1 n (2782.4 – 3821.6)	52.9 ± 4.4 n (48.0 – 58.3)	132.6 ± 6.9 n (109.9 – 148.9)	46.3 ± 1.0 n (43.8 – 48.5)	26.8 ± 1.2 m (25.3 – 28.4)	18.9 ± 0.6 n (12.9 – 23.9)
Check (A04-73NA)		7	3635.2 ± 373.7 aixm	53.6 ± 4.4 bixm	140.8 ± 7.0 aixm	47.0 ± 1.0 aixm	26.3 ± 1.2 bkzn	21.7 ± 0.6 aixm

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Mean comparison among the groups of populations. The alphabets a, b, c and d are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

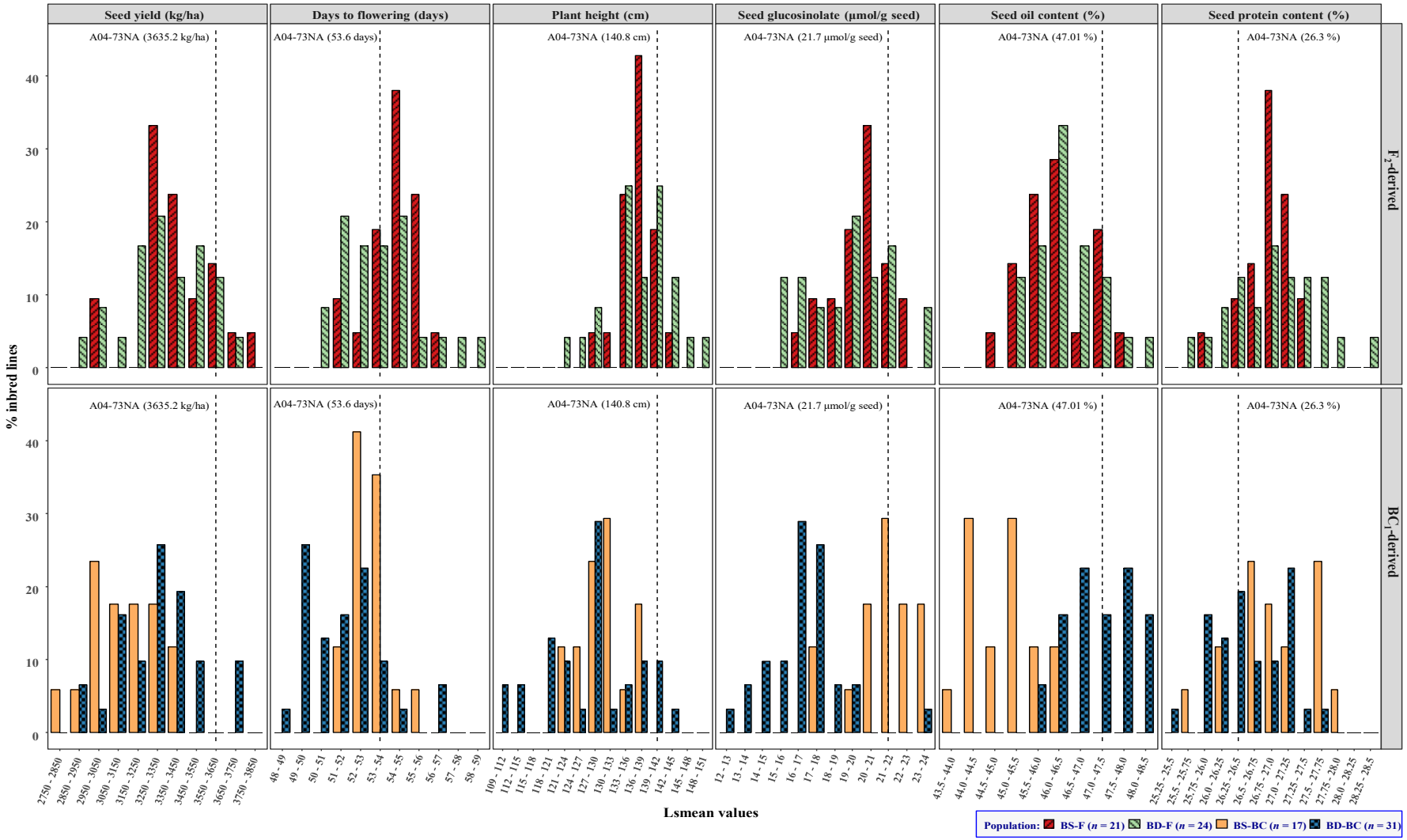

Figure 4-6. Frequency distribution of the least-squares means of seed yield, plant height, days to flowering, seed glucosinolate content, seed oil content and seed protein content of the 45 F_2-derived inbred lines (populations BS-F and BD-F) and 48 BC_1-derived inbred lines (populations BS-BC and BD-BC) of two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses evaluated in yield trial in summer 2016. The values for the *B. napus* parent A04-73NA is indicated as dashed lines.

4.3.2.2.2 Days to flowering

The inbred line population, on average, flowered significantly earlier (52.9 ± 4.4 days) than the *B. napus* parent, A04-73NA (53.6 ± 4.4 days) (**Table 4-10**). When compared the two types of inbred line populations with the spring *B. napus* parent, no significant difference was found between the F_2-derived inbred population (53.8 ± 4.4 days) and A04-73NA; however, the BC_1-derived population (52.1 ± 4.4 days) flowered about a day and half earlier than the F_2-derived population and A04-73NA.

A total of 58 (62.4 %) lines flowered earlier than A04-73NA, of which, 18 lines derived from F_2 (40 % of the total 45 lines) and 40 lines derived from BC_1 (83.3 % of the total 48 lines) (**Figure 4-6**). Most of the earliest flowering lines, 1682.131, 1682.133, 1682.137 and 1682.159, were found in the BC_1-derived population of A04-73NA × Bindsachsener (BD-BC), where the line no. 1682-131 took 48.0 days to flower, which is 5.6 days early as compared to A04-73NA (**Appendix: 4-15**).

4.3.2.2.3 Plant height

The height of the inbred lines varied from 109.9 to 148.9 cm with a mean of 132.6 ± 6.9 cm; this is significantly lower than that of the *B. napus* parent A04-73NA (140.8 ± 7.0 cm) (**Table 4-10**). The two types of inbred populations, the F_2- and the BC_1-derived, also differed significantly for plant height, where the F_2-derived population was taller than the BC_1-derived population by 8 cm (136.9 ± 6.9 vs. 128.5 ± 6.9 cm). Of the four populations of the two crosses, none was statistically similar to A04-73NA; the F_2-derived population of A04-73NA × Badger Shipper (BS-F) was the tallest (137.2 ± 6.9 cm) while the BC_1-derived population of A04-73NA × Bindsachsener (BD-BC) was the shortest (127.5 ± 6.9 cm). About 80 % of the F_2-derived inbred

lines (36/45) and 96 % of the BC_1-derived lines (46/48) were shorter than A04-73NA (**Figure 4-6**).

4.3.2.2.4 Seed oil content

Seed oil content in the inbred population varied between 43.8 % and 48.5 % with a mean of 46.3 ± 1.0 %; this is significantly lower than the oil content of A04-73NA (47.0 ± 1.0 %)(**Table 4-10**). When comparing the two types of inbred line populations, the F_2- and the BC_1-derived, both had similar seed oil content (46.3 ± 1.0 vs. 46.1 ± 1.0 %). Of the four inbred populations, oil content of only the BC_1-derived population of A04-73NA × Bindsachsener (BD-BC) was statistically similar to A04-73NA. A good number of the inbred lines had higher seed oil content than the spring canola parent A04-73NA (**Figure 4-6**). Of the two types of inbred lines, greater proportion of the BC_1-derived lines (17/48, 35.4 %) had higher seed oil content than A04-73NA as compared to the F_2-derived lines (10/45, 22.2 %). Seeds of the inbred lines 1363.180, 1682.100, 1682.108, 1682.128, 1682.130 and 1682.164 had >48 % oil (**Appendix: 4-15**).

4.3.2.2.5 Seed protein content

As opposed to seed oil content, the inbred population had significantly higher seed protein content (26.8 ± 1.2 %) as compared to A04-73NA (26.3 ± 1.2 %) ($p < 0.001$) (**Table 4-10**). When comparing the two crosses, inbred population of A04-73NA × Badger Shipper (BS) (26.9 ± 1.2 %) had only about 0.2 % higher seed protein as compared to the inbred population of A04-73NA × Bindsachsener (BD) cross (26.7 ± 1.2 %). This small difference for seed protein content was also found between the F_2- and BC_1-derived populations (26.9 ± 1.2 vs. 26.7 ± 1.2 %). More than 75 % (73/93) of the inbred lines had higher seed protein content than the check A04-73NA (**Figure 4-6**). The inbred line no. 1363.202 derived from F_2 of A04-73NA × Bindsachsener (BD-F) contained the highest seed protein (28.4 %) (**Appendix: 4-15**).

4.3.2.2.6 Seed glucosinolate content

Seed glucosinolate content in the inbred lines varied between 12.9 and 23.9 µmol/g seed with a mean of 18.9 ± 0.6 µmol/g. The inbred population had 2.8 µmol/g lower seed glucosinolate than the *B. napus* parent A04-73NA (18.9 ± 0.6 vs. 21.7 ± 0.6 µmol/g seed) (**Table 4-10**). The population derived from A04-73NA × Badger Shipper (BS) (20.5 ± 0.6 µmol/g seed) had 2.7 µmol/g higher seed glucosinolate content as compared to the population derived from A04-73NA × Bindsachsener (BD) cross (17.8 ± 0.6 µmol/g seed). Similarly, the F_2-derived population (19.5 ± 0.6 µmol/g seed) had 1.2 µmol/g higher seed glucosinolate as compared to the BC_1-derived population (18.3 ± 0.6 µmol/g seed) ($p < 0.001$). Based on this, the F_2-derived population of A04-73NA × Badger Shipper (BS-F) supposed to have the highest glucosinolate content among the four populations derived from the two crosses; however, this population had the second highest level of glucosinolate which is apparently due to significant C × L interaction. Of the 93 inbred lines evaluated, 79 (84.9 %) lines, comprising 40 F_2-derived and 39 BC_1-derived, had lower glucosinolate content as compared to A04-73NA (**Figure 4-6**).

4.3.3 Relationship of genetic distance (GD) with MPH, performance of the inbred lines and test-hybrids

Coefficient of the correlation of the GD of the inbred lines from *B. napus* parent A0473NA (evaluated by SSR markers) with MPH, and *per se* performance of the inbred lines and the test-hybrids for different agronomic and seed quality traits is presented in **Table 4-11** and **Figure 4-7**. For seed yield, correlation of the GD with MPH, and *per se* performance of the inbred lines and the test-hybrids was positive and significant in case of A04-73NA × Badger Shipper (BD) ($r = 0.41$) cross; however, not significant in case of A04-73NA × Badger Shipper (BD) ($r = 0.08$)

cross. For other traits, no consistent correlation of the GD with MPH, *per se* performance of the inbred lines and the test-hybrids of the two crosses was found.

Table 4-11. Coefficient of correlation of the genetic distance (GD) of the inbred lines (derived from *B. napus* × *B. oleracea* var. *capitata* interspecific crosses) from the *B. napus* parent A04-73NA with MPH, *per se* performance of the inbred lines and test-hybrids for six agronomic and seed quality traits based on pooled data of the test-hybrid trials conducted in 2015 and 2016. The upper row contains *r*-values for A04-73NA × Badger Shipper cross (38 inbred lines), the middle row for A04-73NA × Bindsachsener cross (55 inbred lines) and the lower row for pooled data of the two crosses (93 inbred lines).

	MPH	Inbred line	Test-hybrid
	0.41	0.37	0.60
SY	0.08	-0.22	0.00
	0.31	**0.00**	**0.35**
	0.1	0.38	0.28
DTF	-0.08	0.66	0.58
	0.00	**0.57**	**0.50**
	0.22	0.42	0.53
PH	0.34	0.56	0.63
	0.35	**0.47**	**0.61**
	0.28	0.21	0.34
SO	0.01	-0.44	-0.36
	0.15	**-0.26**	**-0.12**
	-0.21	0.29	-0.05
SP	0.31	0.33	0.40
	-0.01	**0.32**	**0.19**
	0.07	0.61	0.41
GSL	0.00	0.46	0.35
	0.06	**0.56**	**0.44**

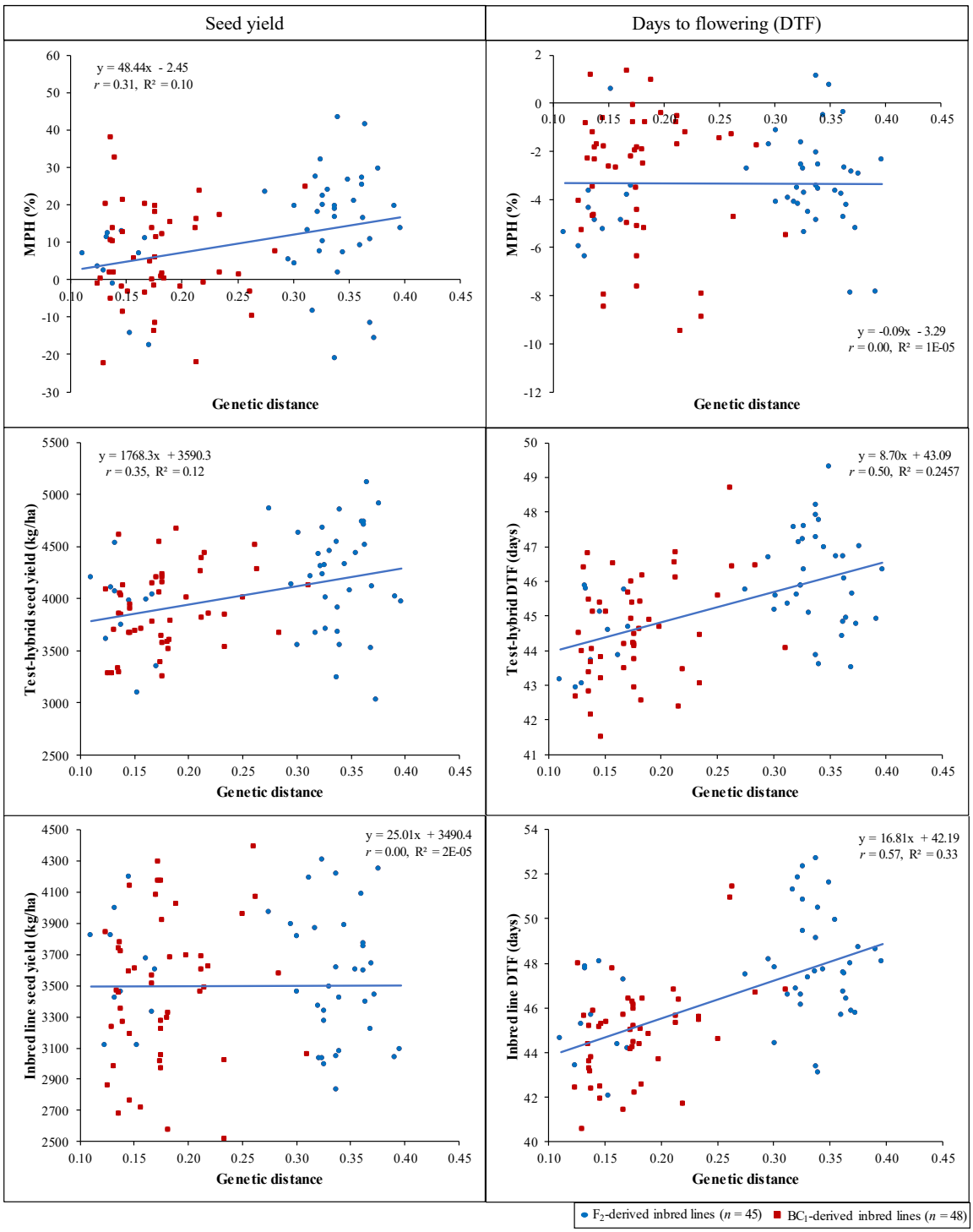

Figure 4-7. Correlation between genetic distance of the F_2 and BC_1-derived inbred lines, derived from two *B. napus* × *B. oleracea* interspecific crosses, from the spring *B. napus* parent A04-73NA and mid-parent heterosis (MPH), and *per se* performance of the inbred line and their test-hybrid for seed yield and days to flowering

4.3.4 Phenotypic correlation

Significant positive correlation was found between the performance of the inbred lines in yield-trial (large plot: 5 m × 1.8 m) vs. test-hybrid trial (small plot: 2 m × 1.3 m) for all agronomic and seed quality traits indicating the consistency of data from these two types of plots (**Table 4-12**). Of the different traits, the lowest correlation was observed for seed yield (r = 0.51***) and the highest correlation for days to flowering (r = 0.81***).

Table 4-12. Coefficient of correlation of the performance of the inbred lines tested in small (test-hybrid trial, THT) vs. large plot (yield trial, YT), and their correlations with mid-parent heterosis (MPH), heterosis over A04-73NA (73NAH) and hybrid yield.

Trait[1]	Inbred line performance in THT vs. YT	Inbred line performance in THT vs. test-hybrid performance	Inbred line performance in YT vs. test-hybrid performance	Inbred line performance in YT vs. MPH	Inbred line performance in THT vs. MPH	Inbred line performance in YT vs. 73NAH	Inbred line performance in THT vs. 73NAH	Test-hybrid performance vs. MPH	MPH vs. 73NAH
SY	0.51 ***[2]	0.42 ***	0.24 *	-0.03	-0.13	0.21 *	0.30 **	0.73 ***	0.83 ***
DTF	0.81 ***	0.75 ***	0.70 ***	0.04	-0.12	0.67 ***	0.68 ***	0.50 ***	0.60 ***
PH	0.66 ***	0.74 ***	0.53 ***	0.05	0.00	0.50 ***	0.71 ***	0.63 ***	0.67 ***
SO	0.73 ***	0.80 ***	0.59 ***	0.12	0.03	0.65 ***	0.74 ***	0.56 ***	0.65 ***
SP	0.53 ***	0.74 ***	0.33 **	0.00	0.00	0.40 ***	0.59 ***	0.59 ***	0.72 ***
GSL	0.76 ***	0.86 ***	0.70 ***	0.21 *	0.27 **	0.56 ***	0.76 ***	0.63 ***	0.79 ***

[1] Abbreviations: SY = seed yield; DTF = days to flowering; PH = plant height; GSL = seed glucosinolate; SO = seed oil; SP = seed protein
[2] Critical values of correlation at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) (df 91) are 0.20, 0.27 and 0.33, respectively

Performance of the inbred lines in either yield trial or test-hybrid trial did not show significant correlation with MPH except for seed glucosinolate (r = 0.21* and 0.27*, respectively); however, the inbred line performance in both type of trials was significantly positively correlated with 73NAH and test-hybrid performance for all traits suggesting that the inbred lines with higher trait value also tend to produce test-hybrids with greater trait value.

Table 4-13. Coefficient of correlation between six agronomic and seed quality traits in 93 inbred lines, derived from the two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses, based on pooled data of four yield trials conducted in 2016 (upper row) and pooled data of two test-hybrid trials conducted in 2015 and 2016 (middle row), and in 93 test-hybrids based on pooled data of two test-hybrid trials conducted in 2015 and 2016 (lower row)

Trait[1]	DTF	PH	SO	SP	GSL
SY	-0.01	0.14	0.41 ***	-0.37 ***	-0.19
	-0.08	0.28 **	0.29 **	-0.07	-0.30 **
	0.21 *[2]	0.37 ***	0.11	-0.07	0.16
DTF		0.80 ***	-0.21 *	-0.02	0.25 *
		0.72 ***	-0.13	0.07	0.19
		0.68 ***	-0.09	0.10	0.04
PH			-0.11	0.02	0.24 *
			0.12	0.02	0.04
			0.08	0.00	0.06
SO				-0.61 ***	-0.64 ***
				-0.71 ***	-0.50 ***
				-0.68 ***	-0.39 ***
SP					0.39 ***
					0.40 ***
					0.23 *

[1] Abbreviations: SY = seed yield; DTF = days to flowering; PH = plant height; GSL = seed glucosinolate; SO = seed oil; SP = seed protein

[2] Critical values of correlation at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) (df 91) are 0.20, 0.27 and 0.33, respectively

In yield trial, seed yield of the inbred lines did not show significant correlation with days to flowering, plant height and seed glucosinolate content; however, it did show a significant positive correlation with seed oil (r = 0.41***) and negative correlation with seed protein content (r = -0.37***) (**Table 4-13, Figure 4-8**). In case of test-hybrid trial, seed yield of the test-hybrids showed significant positive correlation with days to flowering (r = 0.21*) and plant height (r = 0.37***), but did not show significant correlation with any of the seed quality traits. Days to flowering exhibited significant positive correlation with plant height in both yield and test-hybrid trials (r = 0.80, 0.72 and 0.68). Seed oil showed a significant negative correlation with seed protein (r = -0.61, -0.71 and -0.68) and glucosinolate contents (r = -0.64, -0.50 and -0.39), and seed glucosinolate content showed a significant positive correlation with seed protein content (r = 0.39, 0.40 and 0.23) in both inbred line and test-hybrid populations in various trials. This

suggests that it would be difficult to develop a low glucosinolate line or hybrid with both high oil and protein contents through selection in this population.

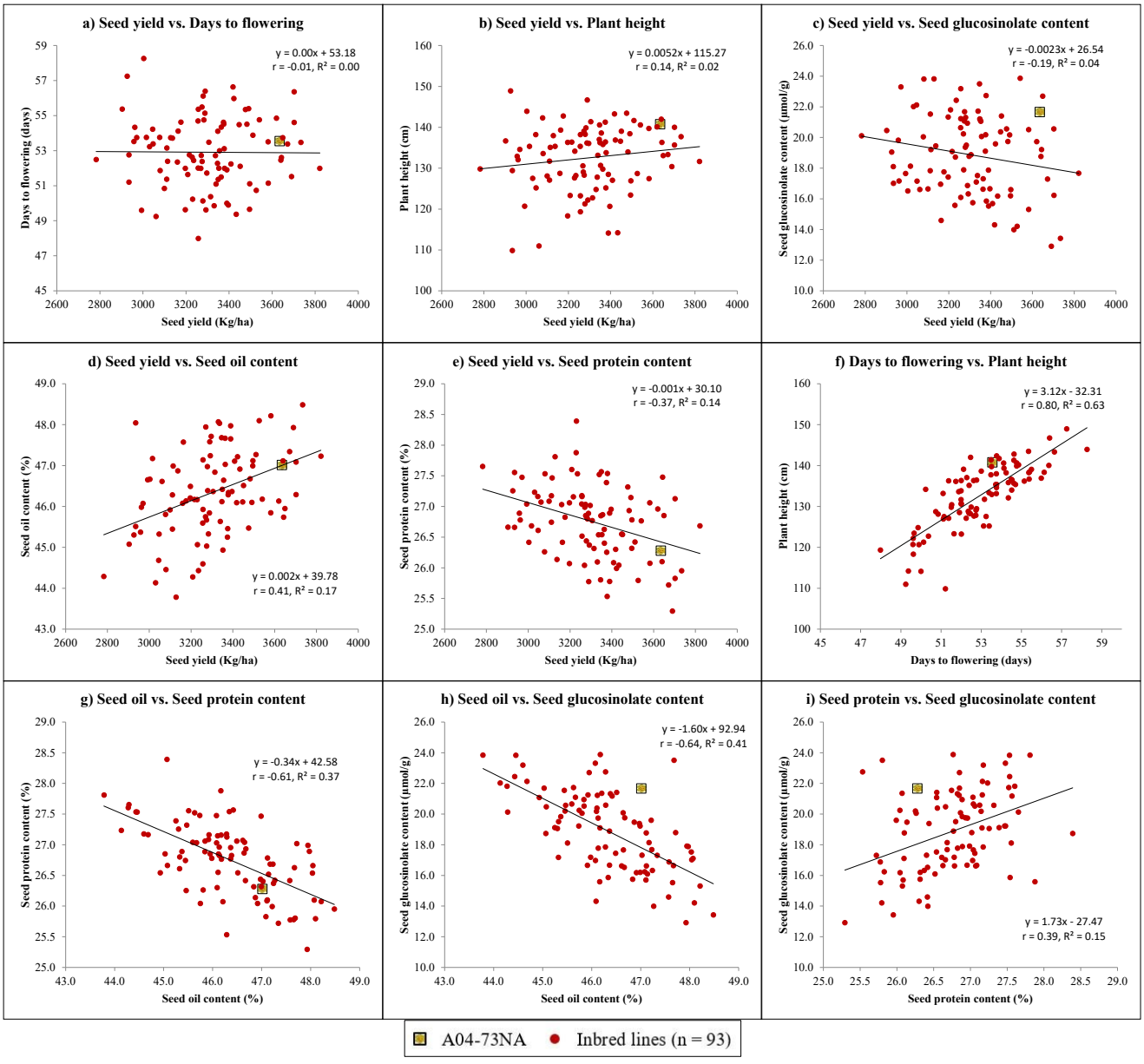

Figure 4-8. Phenotypic correlation between different traits in 93 inbred lines derived from the two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses, viz. A04-73NA × Badger Shipper and A04-73NA × Bindsachsener based on the pooled data of four yield trials conducted in 2016

4.4 Discussion

The development of hybrid canola cultivars has become the major focus for the seed companies in the last two decades (Bradshaw, 2016); currently, more than 95 % of the canola cultivars grown in Canada are hybrids. In the initial stage of hybrid breeding, heterosis for various agronomic and seed quality traits was studied by producing hybrids of different canola cultivars and lines available in different breeding programs (Grant and Beversdorf, 1985; Starmer et al., 1998). Later, researchers explored the exotic alleles of winter type *B. napus* to improve the level of heterosis for seed yield in spring canola hybrids (Butruille et al., 1999; Quijada et al., 2004) as well as to increase seed yield in open-pollinated cultivars (Kebede et al., 2010; Rahman, 2016). In the recent years, several researchers started to explore the two allied species, *B. rapa* and *B. oleracea*, for exotic alleles to increase the level of heterosis in *B. napus* (Qian et al., 2005; Zou et al., 2010; Jesske et al., 2013). Remarkably, Rahman et. al. (2016) observed higher MPH for seed yield in spring *B. napus* due to the alleles introgressed form *B. oleracea* as compared to the alleles from winter type, thus, showing potential use of the *B. oleracea* gene pool for heterosis breeding in *B. napus.*

In the present study, through working with a spring *B. napus* canola population carrying allelic diversity introgressed from *B. oleracea*, -3 to -4 % MPH (up to -9.5 %) and -3 to -5 % 73NAH was observed for days to flowering (**Table 4-4**). These negative values for heterosis resulted primarily from the effect of the *B. oleracea* alleles as the spring canola tester parent differed from the inbred lines predominantly for the C genome alleles of *B. oleracea*. Most test-hybrids (90 %) flowered earlier than the *B. napus* parent suggesting that the flowering time alleles of *B. oleracea*, on average, did not delay flowering in *B. napus*; instead, some of the alleles tended to improve the earliness in the test-hybrids. Rahman et al. (2011, 2017) also

reported that the alleles of *B. oleracea* can improve the earliness in spring *B. napus*. Grant and Beversdorf (1985a) also reported up to 8 % negative heterosis for this trait in spring *B. napus.*

In the present study, negative heterosis was also observed for plant height; this agrees with the results reported by Grant and Beversdorf (1985a) who reported -11.0 to 16.0 % heterosis for this trait where the majority of the hybrids gave negative heterosis. A negative heterosis for plant height is useful for breeding short statured plant which is considered a desirable trait.

Seed yield of the inbred line population (3496.2 ± 1097.2 kg/ha) used in this study was significantly lower (12 %) than the *B. napus* check A04-73NA (3955.0 ± 1097.3 kg/ha) while seed yield of the test-hybrid population (4004.2 ± 1097.1 kg/ha) was statistically similar to this check (**Table 4-3**). This suggests that the exotic alleles of *B. oleracea* mostly exerted a detrimental effect on *per se* performance of the inbred lines; however, some of the alleles seem to have exerted a positive effect on heterosis. Indeed, up to 43.3 % MPH and 38.5 % 73NAH was observed for seed yield in some of the test-hybrids where the best (5106.9 kg/ha) performing test-hybrid exhibited 29 % higher seed yield as compared to the check A04-73NA. On the other hand, seed yield in 25.8 % of the test-hybrids was less than the mid-parental value (up to -22.5 % MPH). In a similar study, Qian et al. (2007) also found comparable range of MPH for seed yield (-28 to 36 %, mean 15 %) in test-hybrids produced by crossing spring type *B. napus* to semi-winter *B. napus* lines carrying *B. rapa* alleles. Girke et al. (2012) reported, on average, 40 % reduction in seed yield in resynthesized *B. napus* lines, however, up to 17 % increased yield in their test-hybrids as compared to the natural spring *B. napus*; the level of MPH in their population varied from -3.5 to 47.2 %. They reported less extent of negative MPH than the present study. This is primarily due to poor performance of the resynthesized *B. napus* lines

which decreased the mid-parent value and hence gave higher MPH. However, the actual deleterious effect of some of the unfavorable alleles of *B. rapa* is evident from the low heterosis (up to 17 %) in the hybrids over the tester parent.

A high proportion of hybrids showing negative heterosis for seed yield is not normally seen in the hybrids produced from crossing of natural *B. napus* lines. For example, Sernyk and Stefansson (1983), by crossing spring canola cultivars of different geographical origin to a Canadian canola cultivar, and Shen et al. (2005), while working with spring × semi-winter type *B. napus*, reported a range of about 5 to 64 % positive MPH for seed yield. Grant and Beversdorf (1985) reported negative MPH (up to -16 %) in only 6.7 % (2/30) hybrid of spring *B. napus*. In the present study, the occurrence of such high proportion of the test-hybrids exhibiting negative heterosis for seed yield could be due to introgression of some of the unfavorable alleles of *B. oleracea* into the *B. napus* inbred lines. However, about 10.8 % test-hybrids gave ≥ 25 % MPH and 6.5 % test-hybrids gave ≥ 25 % 73NAH. This suggests that some of the *B. oleracea* alleles can considerably increase seed yield in spring *B. napus* canola hybrids. Identification of these alleles will be needed through further study.

As opposed to higher MPH for seed yield, oil content exhibited very low level of heterosis (range -3.5 to 3.2 %). Shen et al. (2005) also reported low level of MPH for seed oil content (range −1.55 to 7.44 %) as compared to yield (5.5 to 64.1 %). In fact, several studies have shown that a greater level of heterosis can be achieved for seed yield and almost no heterosis for seed oil content in *B. napus* (Riaz et al., 2001; Shen et al., 2005; Girke et al., 2012; Rahman et al., 2016). This is primarily for the reason that seed yield in *B. napus* is controlled by both additive and non-additive genes (Shen et al., 2005; Radoev et al., 2008; Basunanda et al., 2010), while oil content is mainly under additive gene control (Zhao et al., 2005; Delourme et

al., 2006). In the case of seed glucosinolate content, very high negative MPH (up to -32 %, mean -20 %) and 73NAH (up to -49 %, mean 27 %) observed in this population renders it is optional to reduce glucosinolate content in the inbred lines at a level lower than the canola quality standard as parents having slightly higher glucosinolate can produce hybrid with low glucosinolate content provided that one parent carry low glucosinolate property.

Significant effect of the cross (C), the type of line (L) and their interaction on the performance of the inbred lines and their test-hybrids for various traits observed in the present study suggests that the source of the *B. oleracea* alleles and the method used for the development of the inbred lines could have significant implication while using this allied species in the breeding of parental lines of hybrid cultivars. The wide diversity of the *B. oleracea* gene pool (Làzaro and Aguinagalde, 1998), therefore, needs to be investigated for the hunt of the alleles contributing to heterosis in spring *B. napus* canola, and these diverse variants needs to be subjected to similar methods of line development that has been used in the present study (lines derived from F_2 and BC_1).

In case of the A04-73NA × Badger Shipper cross, the genetic distance (GD) of the inbred lines from the *B. napus* parent A04-73NA was moderately correlated with the performance of the inbred lines and their test-hybrids, and MPH for seed yield; however, this correlation was not significant in case of the inbred lines derived from A04-73NA × Bindsachsener cross. This agrees with the results reported by Riaz et al. (2001), Qian et al. (2007), Girke et al. (2012) Jesske et al. (2013), Li et al. (2014b) Luo et al. (2016) and Rahman et al. (2016) who reported varied level of correlation, ranging from positive to negative, using different types of materials. This suggests that the performance of the test-hybrid or MPH is not uniquely a function of genetic distance between the parents. In other words, the greater genetic distance between the

parents does not always result better hybrid for seed yield. Hence, not only the non-additive effect genes but also the additive effect genes in the parents are important for realizing improvement in hybrids over the parents. This might be the reason of why the F_2-and BC_1-derived inbred lines did not differ for seed yield despite the former population carried a greater number of *B. oleracea* alleles; however, the difference became more evident and significant in the test-hybrid populations. This might be due to having greater number of favorable *B. oleracea* alleles in the F_2-derived lines that contributed to the non-additive effect of heterosis for seed yield in the test-hybrids.

In the present study, performance of the inbred lines showed significant association with the performance of the test-hybrids (**Table 4-12**) for different traits. This indicates the importance of the additive effect genes or general combining ability of the parents for greater seed yield in hybrids (Qian et al., 2007; Amiri-Oghan et al., 2012; Rahman et al., 2016). You et al. (2006) also found significant positive correlation between the performance of the RILs and test-hybrids for seed yield, plant height, heading date and number of panicles per plant in rice and suggested that selection of parents for their *per se* performance is important to achieve higher seed yield in hybrids. On the other hand, the lack of significant correlation between the inbred performance and MPH (**Table 4-12**) suggests the importance of non-additive effect of the genes in the control of the phenomenon heterosis.

Seed yield showed no correlation with days to flowering in the inbred lines; however, this correlation was slightly positive in the test-hybrids. Despite positive correlation in the test-hybrids, the mean number of days to flower reduced and mean seed yield increased demonstrating the feasibility of achieving favorable allelic combination that increase seed yield with the earliness of flowering. Seed yield, on average, showed a positive correlation with plant

height. Although, the test-hybrid population exhibited positive MPH for seed yield and slight negative MPH for plant height, the positive correlation between plant height and seed yield suggests that it will be difficult to select a high yielding hybrid with shorter plant stature from this population. Udall et al. (2004) and Girke et al. (2012) also found a positive correlation between seed yield and plant height (r = 0.51 to 0.74) while working with resynthesized *B. napus* lines. The correlation between plant height and days to flowering was found to be positive and significant as expected, and similar results reported by Ozer et al. (1999) and (Udall et al., (2006). Generally, taller plants result from prolonged vegetative growth phase which delays flowering; this might be due to the pleiotropic effect of the genes controlling flowering time on plant height (Quijada et al., 2006).

Seed yield displayed positive correlation with oil content and negative correlation with protein content agreeing results reported in the previous studies by Butruille et al. (1999), Ozer et al. (1999) and Girke et al. (2012). Therefore, it can be deduced that increased seed yield was not realized at the cost of oil content, in fact both of these traits can be improved simultaneously. A strong negative correlation was found between seed oil and protein contents both in inbred line and test-hybrid populations; this is in accordance with the results reported by several researchers, such as Ozer et al. (1999), Zhao et al. (2006), Girke et al. (2012), Huang et al. (2016) and Chao et al. (2017). The negative correlation between seed oil and seed protein content is apparently due to linkage between some of the high protein and low oil alleles, and competition for basic precursors, such as carbon, in the biosynthesis of these two seed constituents (reviewed by, Weselake et al., 2009).

Normally, it is desired to conduct field trials in a larger size plots if the required quantity of seed is available. In the present study, the inbred lines could be tested in large plots (5.0 × 1.8

m) trial. The test-hybrid seeds in the present study were produced manually and it was difficult to produce a large quantity of seed; therefore, the hybrid trial was seeded in small plots (2.0 × 1.3 m). By use of data of the inbred lines from large plots and from the test-hybrid trial, significant positive correlation was found between the performance of the inbred lines tested in these two types of plots for different agronomic and seed quality traits (**Table 4-12**). This suggests that reliable data can also be obtained from testing the materials in smaller size plots when large quantity seed is not available. Cuthbert et al. (2009, 2011) and Rahman et al. (2016) also tested test-hybrids in smaller size plots to study the phenomenon heterosis.

Greater value for standard error was observed, especially for seed yield, in the test-hybrid and the yield trials, i.e. in the test-hybrid trial, which was conducted over two years, the standard error of mean for seed yield of A04-73NA was 1097, and in the yield trial which was conducted over four locations it was 373. This was due to consideration of the year and the location in the test-hybrid and the yield trials, respectively, as random effects. In other words, the years and the locations in this experiment are random samples of possible "n" number of such experiments. Therefore, considering these factors as random effects facilitates extrapolation of the results over years and locations. However, when random factors are present in an experiment and a limited number of experiments are conducted, for example, only two years and four locations in the test-hybrid and the yield trials, respectively, were selected out of "n" number; this results into a small sample size and enlarged variance and standard error for the estimate of means (Borenstein et al., 2009). Therefore, more number of similar experiments over the years/locations needs to be conducted to precisely estimate standard error and thereby facilitating extrapolation of the results over many years and/or locations more accurately.

Chapter 5

General discussion and conclusions

5.1 General discussion

Brassica napus is the second most important oilseed crop worldwide (USDA ERS, 2015). Most present day canola cultivars carry the low erucic acid and low glucosinolate genes of two *B. napus* cultivars, Liho and Bronowski, respectively (Friedt and Snowdon, 2009). This bottleneck in the development of canola quality cultivars as well as the recent origin of this amphidiploid species (Chalhoub et al., 2014) from limited variants of the two diploid species, *B. rapa* and *B. oleracea*, and intensive breeding within a restricted gene pool resulted narrow genetic diversity in this crop (for review, see Rahman, 2013). Canada is the largest producer of *B. napus* canola where spring type cultivars are grown; genetic diversity in Canadian canola is narrow (Gyawali et al., 2013). Therefore, it is important to widen the genetic base of the Canadian spring canola breeding materials. Several researchers used the primary gene pool of *B. napus*, such as winter (Butruille et al., 1999; Quijada et al., 2004; Kebede et al., 2010) and semi-winter type (Qian et al., 2007) to broaden the genetic base of the spring type *B. napus* canola.

Bus et al. (2011) reported a lower genetic diversity in the C genome of *B. napus* as compared to the A genome. Alleles from *B. rapa* were introduced into *B. napus* by several researchers, especially in the breeding of Chinese semi-winter and spring type *B. napus* (Qian et al., 2006; Mei et al., 2011; Attri and Rahman, 2017). Though, it has been reported that the C genome can contribute alleles for the improvement of important traits in oilseed *B. napus* (Wang et al., 2014), only few cases can be found on the use of *B. oleracea* in the breeding of this crop; most focused on the transfer of disease resistance form *B. oleracea* into *B. napus* (Diederichsen

and Sacristan, 1996; Werner et al., 2008; Mei et al., 2015). Recently, transfer of the alleles from two variants of *B. oleracea*, viz. *alboglabra* (Rahman et al., 2015) and *acephala* (Li et al. 2014) have been undertaken by two research groups to broaden the genetic base of *B. napus*.

In this study, I used two cvs. of cabbage (*B. oleracea* var. *capitata*) to introgress unique alleles into spring *B. napus* canola. From these two *B. napus* × *B. oleracea* interspecific crosses, canola quality spring type *B. napus* type inbred lines carrying genome content of *B. oleracea* as much as 42% with a mean of 23.0 % were developed. Flow cytometric analysis **(Table 2-6)** showed that the inbred lines stabilized into euploid *B. napus* and they carried a greater number of *B. oleracea* alleles as compared to the *B. napus* lines developed by Rahman et al. (2015) (mean 19.0 %) from *B. napus* × *B. oleracea* var. *alboglabra* interspecific cross.

Low plant fertility was observed in the early generation population of this study – as commonly observed in interspecific cross due to meiotic anomalies (Fujii and Ohmido, 2011; Rahman, 2013); however, almost 100 % of the advanced generation lines were fully fertile and stabilized into *B. napus* type. The two F_2-derived populations carried greater number of *B. oleracea* alleles (33.9 and 25.0 %) as compared to the two BC_1-derived populations (20.2 and 15.8 %). When considering the theoretically expected number of *B. oleracea* alleles (50 % in F_2- and 25 % in BC_1-derived population), the BC_1-derived population carried a greater proportion (80.9 and 63.4 %) of the theoretically expected number of alleles as compared to the F_2-derived population (67.9 and 49.9 %). One of the reasons for this could be higher meiotic anomalies in early generation of the F_2-derived population; this is evident from lower fertility in the F_2 plants (32 %) as compared to the BC_1 plants (63 %) (**Table 2-2** and **Table 2-3**). The F_2 plants carrying greater proportion of *B. oleracea* genome might have shown greater sterility and hence eliminated many alleles from the population. In contrast, some non-parental alleles were

observed in the population which could have resulted from homoeologus chromosome pairing (Gaeta et al., 2007). Several other researchers have also reported non-parental alleles in the inbred lines derived from different interspecific crosses in *Brassica* (Qian et al., 2005; Xiao et al., 2010; Wu et al., 2014) as well as in wheat (Zhang et al., 2005). Thus, interspecific hybridization is an important tool for introgression of alleles from allied species as well as for creation of new alleles in this oilseed crop.

Some of the inbred lines and test-hybrids found to outperform the spring *B. napus* canola line A04-73NA for different traits including seed yield suggesting that the unique alleles introgressed from the C genome of *B. oleracea* have the potential of contributing to heterosis in this crop. The correlation of the genetic distance (GD) of the parents with MPH and performance of the test-hybrids for seed yield was found positive and significant for A04-73NA × Badger Shipper cross (r = 0.41 and 0.60), while no significant correlation was observed for A04-73NA × Bindsachsener cross. In similar studies, different researchers have reported different levels of correlation of GD, estimated by use of different types of genetic markers, with MPH and hybrid performance varying from positive to negative while working with both primary and secondary gene pools of this oilseed crop (Riaz et al., 2001; Qian et al., 2007; Girke et al., 2012; Jesske et al., 2013; Li et al., 2014b; Luo et al., 2016). This indicates that the genetic distance between the parents and MPH may not be a general feature in hybrid breeding, and it is difficult to predict hybrid performance or heterosis solely on the basis of genetic distance.

Some of the inbred lines and their test-hybrids flowered earlier than the *B. napus* parent A04-73NA; this agrees with the results reported by Rahman et al., (2011) that *B. oleracea* carry unique alleles which can improve the earliness of flowering in *B. napus*. The lack of correlation between the inbred line performance and MPH for most of the traits indicates that the alleles

contributing to the *per se* performance of the inbred lines are not necessarily associated with the genetic control of heterosis. However, positive correlation between *per se* performance of the lines and test-hybrid performance indicated that selection of hybrid parent lines with higher performance will be needed to achieve higher yield in the hybrids. Similar results have also been reported by Ertiro et al. (2013) and Miedaner et al. (2014) while working with maize and rye, respectively. This suggests that reduction in the number of parent lines based on *per se* performance in early stage in a hybrid breeding program, would reduce the number of hybrids to be produced and evaluated in field trials; thereby would increase the efficiency of a hybrid breeding program.

5.2 Conclusions

From the present study following conclusions can be inferred:

- Low fertility observed in the early generation population of *B. napus* × *B. oleracea* interspecific crosses apparently resulted from meiotic anomalies; however, fully fertile advanced generation inbred lines can be achieved through self-pollination of this interspecific hybrids.
- The inbred lines derived from this *B. napus* × *B. oleracea* interspecific crosses stabilized only into *B. napus* type.
- The advanced generation lines with zero erucic acid in oil and low glucosinolate in seed meal can be achieved from the progeny of canola quality *B. napus* × *B. oleracea* (non-canola quality) crosses; however, the achievement of low glucosinolate type would require greater number of generation as compared to the achievement of zero erucic acid type. One of the reasons of this is the involvement of a greater number of loci in the control of seed glucosinolate content.

- Several inbred lines derived from the *B. napus* × *B. oleracea* var. *capitata* crosses were genetically distinct from the *B. napus* parent demonstrating the usefulness of *B. oleracea* to widen the genetic base of the C genome of *B. napus*.
- The inbred lines developed based on the two cultivars of *B. oleracea* var. *capitata* (cvs. Badger Shipper and Bindsachsener) showed different level of heterosis; therefore, more cultivars of this variant as well as other variants of this species need to be studied as the potential source of alleles for heterosis in *B. napus* canola.
- The F_2- derived inbred lines retained greater number of *B. oleracea* alleles as compared to the BC_1-derived lines, and gave higher performing test-hybrids and showed a greater level of MPH for seed yield.
- Precise prediction of mid-parent heterosis (MPH) and performance of test-hybrids exclusively based on GD estimated by SSR markers is difficult.
- Higher correlation between the *per se* performance of the inbred lines and test-hybrids for different traits indicates that selection of parents for higher *per se* performance will result better performing hybrids.
- Inbred lines and test-hybrids with higher seed yield than the spring canola parent were identified.

5.3 Future research

This study provided information of the value of the *B. oleracea* var. *capitata* gene pool for the improvement of the *per se* performance of spring *B. napus* canola, as well as for the improvement of hybrid cultivars through increasing the level of heterosis for different agronomic traits including seed yield. Other variants of *B. oleracea*, which are known to be genetically distinct from var. *capitata* (Izzah et al., 2013; El-Esawi et al., 2016), need to be evaluated as

potential contributor of alleles to diversify the genetic base of *B. napus* and for increasing the level of heterosis in this crop. A comparative study by using the lines from the present study and the lines diversified in the A genome (lines from *B. napus* × *B. rapa*) can be conducted to understand the relative importance of the A and C genomes of the parental species of *B. napus* for heterosis for different traits in spring *B. napus*.

The inbred lines developed in this study can also be crossed with a large number of tester lines, as well as in a diallel mating design to estimate general and specific combining ability of these lines. These lines can also be used for mapping of QTL for different agronomic and seed quality traits including the phenomenon heterosis by genotyping the population with high density SNP markers. By converting these lines to male sterile lines, greater quantity of test-hybrid seeds can be produced for evaluation in replicated trials at multiple locations to investigate the G × E interactions on the phenomenon heterosis as well as to identify a stable hybrid for commercialization.

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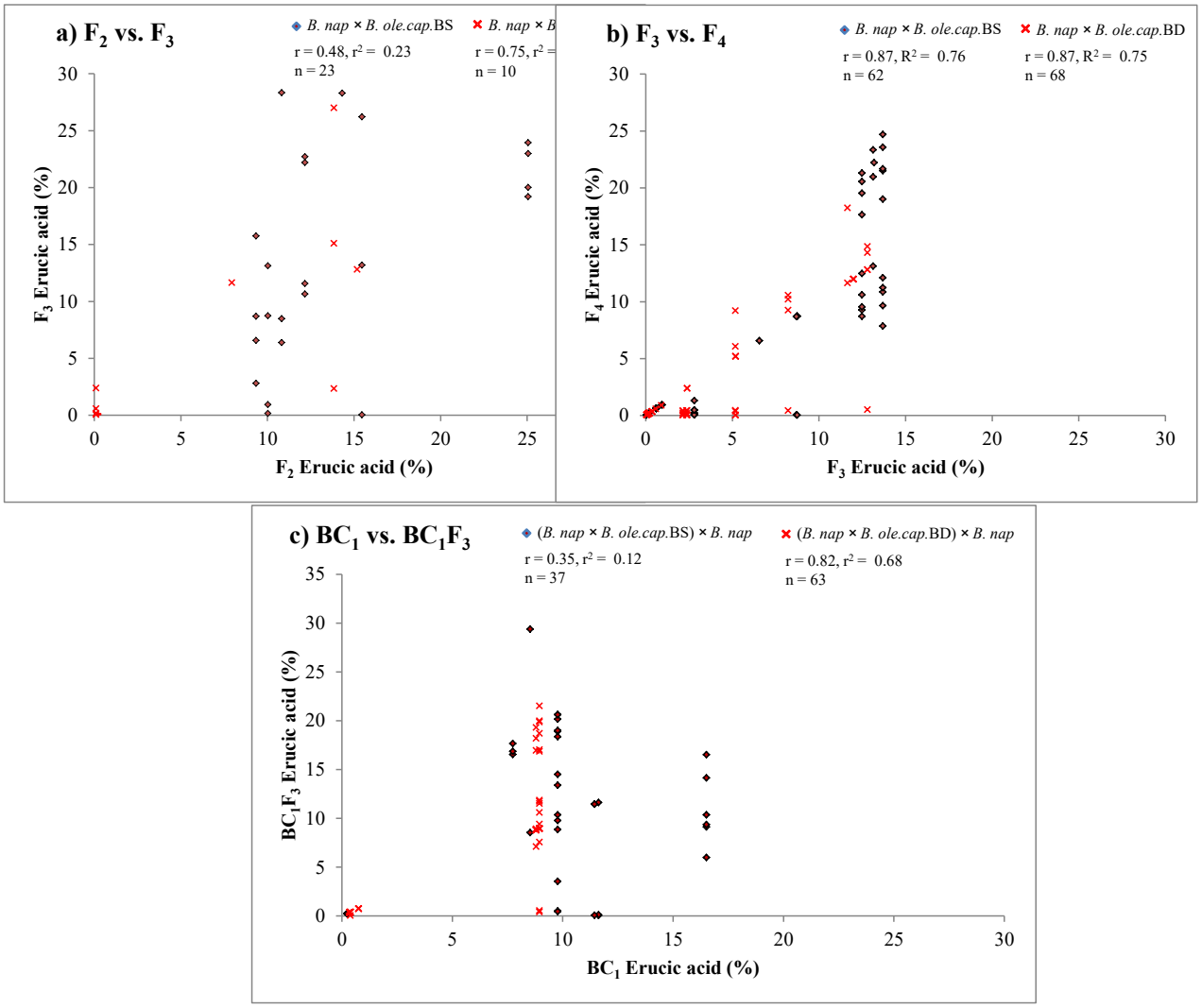
Appendices



Appendix: 2-1. Parents vs. offspring scatter plot for erucic acid content (%) for different generation populations of *B. napus* × *B. oleracea* interspecific crosses. The two F_2 and F_2-derived populations are *B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper and *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener crosses, and the two BC_1 and BC_1-derived populations are (*B. napus* × *B. oleracea* var. *capitata* cv. Badger Shipper) × *B. napus* and (*B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener) × *B. napus* crosses

Appendix: 3-1. Pedigree of the 93 inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses, viz. A04-73NA × Badger Shipper and A04-73NA × Bindsachsener investigated for SSR allele diversity, and evaluated in the test-hybrid and the yield trial

Pedigree[1]	Line type	Cross ID	Entry no.	F_2/ BC_1F_1 reg. no.[2]	F_3/ BC_1F_2 reg. no.	F_4/ BC_1F_3 reg. no.	F_5/ BC_1F_4 reg. no.	F_6/ BC_1F_5 reg. no.	F_7/ BC_1F_6 reg. no.[3]
B. nap × *B. ole.cap*.BS	F_2-derived	1362	1	1362.002-A1220 P1	1362.004-A1231	1362.034-A1242	1362.050-A1253	1362.103-A1264	1362.149-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	2	1362.002-A1220 P12	1362.007-A1231	1362.041-A1242	1362.055-A1253	1362.111-A1264	1362.152-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	3	1362.002-A1220 P12	1362.007-A1231	1362.041-A1242	1362.055-A1253	1362.112-A1264	1362.155-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	4	1362.002-A1220 P12	1362.007-A1231	1362.041-A1242	1362.055-A1253	1362.112-A1264	1362.156-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	5	1362.002-A1220 P12	1362.007-A1231	1362.041-A1242	1362.055-A1253	1362.113-A1264	1362.158-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	6	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.070-A1253	1362.137-A1264	1362.161-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	7	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.070-A1253	1362.138-A1264	1362.162-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	8	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.071-A1253	1362.139-A1264	1362.164-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	9	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.071-A1253	1362.139-A1264	1362.165-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	10	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.071-A1253	1362.140-A1264	1362.166-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	11	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.071-A1253	1362.141-A1264	1362.167-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	12	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.072-A1253	1362.143-A1264	1362.169-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	13	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.072-A1253	1362.143-A1264	1362.170-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	14	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.072-A1253	1362.144-A1264	1362.171-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	15	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.073-A1253	1362.145-A1264	1362.173-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	16	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.073-A1253	1362.145-A1264	1362.174-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	17	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.073-A1253	1362.146-A1264	1362.175-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	18	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.073-A1253	1362.147-A1264	1362.176-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	19	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.073-A1253	1362.147-A1264	1362.177-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	20	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.073-A1253	1362.148-A1264	1362.179-A1274
B. nap × *B. ole.cap*.BS	F_2-derived	1362	21	1362.002-A1220 P6	1362.018-A1231	1362.046-A1242	1362.073-A1253	1362.148-A1264	1362.180-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	22	1363.002-A1220 P11	1363.006-A1231	1363.034-A1242	1363.061-A1253	1363.104-A1264	1363.164-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	23	1363.002-A1220 P11	1363.006-A1231	1363.034-A1242	1363.061-A1253	1363.104-A1264	1363.165-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	24	1363.002-A1220 P11	1363.006-A1231	1363.034-A1242	1363.062-A1253	1363.105-A1264	1363.168-A1274

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Reg. no. = Registration number of a line

[3] No individual plant selection was made after F_7 and BC_1F_6; therefore, the same family number was maintained after F_7 and BC_1F_6

Appendix: 3-1. Continued…

Pedigree[1]	Line type	Cross ID	Entry no.	F_2/ BC_1F_1 reg. no.[2]	F_3/ BC_1F_2 reg. no.	F_4/ BC_1F_3 reg. no.	F_5/ BC_1F_4 reg. no.	F_6/ BC_1F_5 reg. no.	F_7/ BC_1F_6 reg. no.[3]
B. nap × *B. ole.cap*.BD	F_2-derived	1363	25	1363.002-A1220 P11	1363.006-A1231	1363.034-A1242	1363.063-A1253	1363.106-A1264	1363.170-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	26	1363.002-A1220 P11	1363.006-A1231	1363.034-A1242	1363.063-A1253	1363.107-A1264	1363.171-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	27	1363.002-A1220 P11	1363.006-A1231	1363.034-A1242	1363.063-A1253	1363.107-A1264	1363.173-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	28	1363.002-A1220 P18	1363.013-A1231	1363.046-A1242	1363.073-A1253	1363.119-A1264	1363.177-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	29	1363.002-A1220 P18	1363.013-A1231	1363.046-A1242	1363.073-A1253	1363.119-A1264	1363.178-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	30	1363.002-A1220 P18	1363.013-A1231	1363.046-A1242	1363.073-A1253	1363.120-A1264	1363.180-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	31	1363.002-A1220 P18	1363.013-A1231	1363.046-A1242	1363.073-A1253	1363.120-A1264	1363.181-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	32	1363.002-A1220 P18	1363.013-A1231	1363.046-A1242	1363.074-A1253	1363.121-A1264	1363.182-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	33	1363.002-A1220 P18	1363.013-A1231	1363.046-A1242	1363.075-A1253	1363.122-A1264	1363.183-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	34	1363.002-A1220 P18	1363.013-A1231	1363.046-A1242	1363.075-A1253	1363.123-A1264	1363.185-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	35	1363.002-A1220 P18	1363.013-A1231	1363.046-A1242	1363.075-A1253	1363.123-A1264	1363.186-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	36	1363.002-A1220 P18	1363.013-A1231	1363.047-A1242	1363.077-A1253	1363.124-A1264	1363.190-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	37	1363.002-A1220 P2	1363.015-A1231	1363.054-A1242	1363.087-A1253	1363.134-A1264	1363.194-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	38	1363.002-A1220 P2	1363.015-A1231	1363.054-A1242	1363.087-A1253	1363.134-A1264	1363.195-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	39	1363.002-A1220 P3	1363.017-A1231	1363.056-A1242	1363.091-A1253	1363.141-A1264	1363.197-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	40	1363.002-A1220 P3	1363.017-A1231	1363.057-A1242	1363.095-A1253	1363.149-A1264	1363.202-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	41	1363.002-A1220 P8	1363.022-A1231	1363.058-A1242	1363.096-A1253	1363.151-A1264	1363.205-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	42	1363.002-A1220 P8	1363.022-A1231	1363.058-A1242	1363.096-A1253	1363.151-A1264	1363.206-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	43	1363.002-A1220 P8	1363.022-A1231	1363.058-A1242	1363.096-A1253	1363.152-A1264	1363.207-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	44	1363.002-A1220 P8	1363.022-A1231	1363.058-A1242	1363.096-A1253	1363.152-A1264	1363.208-A1274
B. nap × *B. ole.cap*.BD	F_2-derived	1363	45	1363.002-A1220 P8	1363.022-A1231	1363.058-A1242	1363.096-A1253	1363.153-A1264	1363.211-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	46	1362.003-A6220 P7	1362.031-A1231	1681.014-A1242	1681.033-A1253	1681.066-A1264	1681.082-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	47	1362.003-A6220 P7	1362.031-A1231	1681.014-A1242	1681.033-A1253	1681.066-A1264	1681.083-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	48	1362.003-A6220 P7	1362.031-A1231	1681.014-A1242	1681.033-A1253	1681.067-A1264	1681.084-A1274

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Reg. no. = Registration number of a line

[3] No individual plant selection was made after F_7 and BC_1F_6; therefore, the same family number was maintained after F_7 and BC_1F_6

Appendix: 3-1. Continued…

Pedigree[1]	Line type	Cross ID	Entry no.	F_2/ BC_1F_1 reg. no.[2]	F_3/ BC_1F_2 reg. no.	F_4/ BC_1F_3 reg. no.	F_5/ BC_1F_4 reg. no.	F_6/ BC_1F_5 reg. no.	F_7/ BC_1F_6 reg. no.[3]
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	49	1362.003-A6220 P7	1362.031-A1231	1681.014-A1242	1681.033-A1253	1681.067-A1264	1681.085-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	50	1362.003-A6220 P7	1362.031-A1231	1681.014-A1242	1681.033-A1253	1681.067-A1264	1681.086-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	51	1362.003-A6220 P9	1362.033-A1231	1681.016-A1242	1681.034-A1253	1681.069-A1264	1681.090-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	52	1362.003-A6220 P9	1362.033-A1231	1681.016-A1242	1681.034-A1253	1681.070-A1264	1681.091-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	53	1362.003-A6220 P9	1362.033-A1231	1681.016-A1242	1681.034-A1253	1681.070-A1264	1681.092-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	54	1362.003-A6220 P9	1362.033-A1231	1681.016-A1242	1681.034-A1253	1681.072-A1264	1681.096-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	55	1362.003-A6220 P9	1362.033-A1231	1681.017-A1242	1681.035-A1253	1681.073-A1264	1681.097-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	56	1362.003-A6220 P9	1362.033-A1231	1681.017-A1242	1681.035-A1253	1681.073-A1264	1681.098-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	57	1362.003-A6220 P9	1362.033-A1231	1681.017-A1242	1681.035-A1253	1681.074-A1264	1681.100-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	58	1362.003-A6220 P9	1362.033-A1231	1681.017-A1242	1681.035-A1253	1681.075-A1264	1681.101-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	59	1362.003-A6220 P9	1362.033-A1231	1681.017-A1242	1681.035-A1253	1681.075-A1264	1681.102-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	60	1362.003-A6220 P9	1362.033-A1231	1681.017-A1242	1681.035-A1253	1681.075-A1264	1681.103-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	61	1362.003-A6220 P9	1362.033-A1231	1681.017-A1242	1681.035-A1253	1681.076-A1264	1681.104-A1274
(*B. nap* × *B. ole.cap*.BS) × *B. nap*	BC_1-derived	1681	62	1362.003-A6220 P9	1362.033-A1231	1681.017-A1242	1681.035-A1253	1681.076-A1264	1681.105-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	63	1363.003-A6220 P10	1363.025-A1231	1682.003-A1242	1682.023-A1253	1682.053-A1264	1682.099-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	64	1363.003-A6220 P10	1363.025-A1231	1682.003-A1242	1682.023-A1253	1682.054-A1264	1682.100-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	65	1363.003-A6220 P10	1363.025-A1231	1682.003-A1242	1682.023-A1253	1682.054-A1264	1682.101-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	66	1363.003-A6220 P10	1363.025-A1231	1682.003-A1242	1682.025-A1253	1682.059-A1264	1682.102-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	67	1363.003-A6220 P10	1363.025-A1231	1682.003-A1242	1682.025-A1253	1682.059-A1264	1682.103-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	68	1363.003-A6220 P10	1363.025-A1231	1682.003-A1242	1682.025-A1253	1682.060-A1264	1682.104-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	69	1363.003-A6220 P10	1363.025-A1231	1682.003-A1242	1682.025-A1253	1682.060-A1264	1682.105-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	70	1363.003-A6220 P10	1363.025-A1231	1682.003-A1242	1682.025-A1253	1682.061-A1264	1682.108-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	71	1363.003-A6220 P10	1363.025-A1231	1682.004-A1242	1682.027-A1253	1682.065-A1264	1682.113-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	72	1363.003-A6220 P2	1363.026-A1231	1682.005-A1242	1682.028-A1253	1682.070-A1264	1682.120-A1274

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Reg. no. = Registration number of a line

[3] No individual plant selection was made after F_7 and BC_1F_6; therefore, the same family number was maintained after F_7 and BC_1F_6

Appendix: 3-1. Continued…

Pedigree[1]	Line type	Cross ID	Entry no.	F_2/ BC_1F_1 reg. no.[2]	F_3/ BC_1F_2 reg. no.	F_4/ BC_1F_3 reg. no.	F_5/ BC_1F_4 reg. no.	F_6/ BC_1F_5 reg. no.	F_7/ BC_1F_6 reg. no.[3]
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	73	1363.003-A6220 P2	1363.026-A1231	1682.008-A1242	1682.029-A1253	1682.072-A1264	1682.124-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	74	1363.003-A6220 P2	1363.026-A1231	1682.008-A1242	1682.029-A1253	1682.072-A1264	1682.125-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	75	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.031-A1253	1682.074-A1264	1682.128-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	76	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.031-A1253	1682.075-A1264	1682.130-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	77	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.031-A1253	1682.075-A1264	1682.131-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	78	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.032-A1253	1682.076-A1264	1682.133-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	79	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.032-A1253	1682.077-A1264	1682.137-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	80	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.032-A1253	1682.078-A1264	1682.138-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	81	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.032-A1253	1682.079-A1264	1682.140-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	82	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.033-A1253	1682.080-A1264	1682.143-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	83	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.033-A1253	1682.081-A1264	1682.145-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	84	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.033-A1253	1682.082-A1264	1682.147-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	85	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.034-A1253	1682.083-A1264	1682.149-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	86	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.034-A1253	1682.083-A1264	1682.150-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	87	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.034-A1253	1682.084-A1264	1682.152-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	88	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.034-A1253	1682.084-A1264	1682.154-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	89	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.034-A1253	1682.085-A1264	1682.155-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	90	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.034-A1253	1682.085-A1264	1682.156-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	91	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.034-A1253	1682.086-A1264	1682.158-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	92	1363.003-A6220 P3	1363.027-A1231	1682.010-A1242	1682.034-A1253	1682.086-A1264	1682.159-A1274
(*B. nap* × *B. ole.cap*.BD) × *B. nap*	BC_1-derived	1682	93	1363.003-A6220 P4	1363.028-A1231	1682.013-A1242	1682.037-A1253	1682.093-A1264	1682.164-A1274

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Reg. no. = Registration number of a line

[3] No individual plant selection was made after F_7 and BC_1F_6; therefore, the same family number was maintained after F_7 and BC_1F_6

Appendix: 3-2. List of the 93 C genome SSR markers used for genotyping of the F_8 and BC_1F_7 inbred lines derived from *B. napus* × *B. oleracea* var. *capitata* interspecific crosses.

Sr. No.	Primer name	Primer #	Linkage group/s	Source	Total parental alleles	No. *B. oleracea* specific alleles	No. non-parental alleles[1]
1	sN2087†	2278	C1	AAFC	3	1	0
2	sN3734	2279	C1	AAFC	6	4	1
3	sN0691†	2286	C1	AAFC	2	1	1
4	sN11657†	2297	C1	AAFC	2	1	0
5	sR1078†	2299	C1	AAFC	5	2	0
6	sNRF94†	2300	C1	AAFC	2	1	0
7	sN3569F†	2301	C1	AAFC	3	1	0
8	sN12790†	2302	C1	AAFC	2	1	0
9	sN1834	2309	C1	AAFC	8	4	0
10	sN11675	2310	C1	AAFC	4	2	0
11	sN0842†	2311	C1	AAFC	5	2	2
12	sS2362†	2313	C1	AAFC	3	2	0
13	sR10417†	262	A2, C2	AAFC	3	1	1
14	sN3761†	315	A2, C2	AAFC	2	1	0
15	sNRE74 (a) †	2059	C2	AAFC	2	1	1
16	sN1825 (bNP) †	2062	C2	AAFC	3	1	0
17	sN1937 (aNP)	2063	C2	AAFC	2	0	1
18	sS2268 (bNP)	2065	C2	AAFC	6	3	0
19	sR2028 (aNP)	2069	C2	AAFC	5	3	1
20	sS2206 (aNP) †	2072	C2	AAFC	3	1	0
21	sORE66 (bNP) †	2075	C2	AAFC	3	2	0
22	sR1863 (aNP)	2082	C2	AAFC	5	4	0
23	sN2316†	110	C3	AAFC	3	2	0
24	CB10036A†	435	C3	Celera AgGen consortium	6	2	0

† sign indicates marker used to study inheritance of SSR alleles of either Badger Shipper or Bindsachsener or both the *B. oleracea* parents

[1] Non-parental alleles = alleles could not be detected in either *B. napus* or *B. oleracea* parents, but appeared in the advanced generation

Appendix: 3-2. Continued…

Sr. No.	Primer name	Primer #	Linkage group/s	Source	Total parental alleles	No. *B. oleracea* specific alleles	No. non-parental alleles[1]
25	CB10057	439	C3	Celera AgGen consortium	3	0	1
26	BoGMS0819†	1082	C3	Li et al. (2011) Mol. Breed. 28(4): 585–596	3	2	1
27	BoGMS0767†	1085	C3	Li et al. (2011) Mol. Breed. 28(4): 585–596	2	1	0
28	BoGMS0570†	1092	C3	Li et al. (2011) Mol. Breed. 28(4): 585–596	3	2	2
29	BoGMS0358†	1099	C3	Li et al. (2011) Mol. Breed. 28(4): 585–596	3	2	0
30	BoGMS0692†	1101	C3	Li et al. (2011) Mol. Breed. 28(4): 585–596	2	1	0
31	BoGMS1360	1104	C3	Li et al. (2011) Mol. Breed. 28(4): 585–596	4	2	2
32	BoGMS0953	1107	C3	Li et al. (2011) Mol. Breed. 28(4): 585–596	3	2	2
33	BoGMS0081†	1123	C3	Li et al. (2011) Mol. Breed. 28(4): 585–596	1	1	1
34	sN11670 (b)	2087	C3	AAFC	4	2	0
35	sS2277†	302	C4	AAFC	2	1	0
36	CB10109B†	731	C4	Celera AgGen consortium	3	1	0
37	Ra2-F11A†	764	C4	BBSRC microsatellite program	5	1	0
38	MR140†	982	C4	BBSRC microsatellite program	2	1	0
39	Ol11H02a†	989	C4	BBSRC microsatellite program	2	1	1
40	BRAS061†	990	C4	Celera AgGen consortium	3	1	1
41	CB10493†	994	C4	Celera AgGen consortium	5	2	1
42	sR0357†	2099	C4	AAFC	5	2	0
43	sNRG34†	2102	C4	AAFC	2	1	0
44	sN3685R (a) †	2113	C4	AAFC	6	2	0
45	sN3817 (a) †	2115	C4	AAFC	4	2	0
46	BnGMS347†	2200	C4	Cheng et al. (2009) TAG 118(6): 1121–1131	3	2	1
47	BnGMS681†	2225	C4	Cheng et al. (2009) TAG 118(6): 1121–1131	2	1	0
48	BoGMS0836	2233	C4	Li et al. (2011) Mol. Breed. 28(4): 585–596	6	3	2

† sign indicates marker used to study inheritance of SSR alleles of either Badger Shipper or Bindsachsener or both the *B. oleracea* parents

[1] Non-parental alleles = alleles could not be detected in either *B. napus* or *B. oleracea* parents, but appeared in the advanced generation

Appendix: 3-2. Continued…

Sr. No.	Primer name	Primer #	Linkage group/s	Source	Total parental alleles	No. *B. oleracea* specific alleles	No. non-parental alleles[1]
49	sN7410a†	607	A8, A10, C5, C8	AAFC	4	1	0
50	sORA84a†	616	A9, C5	AAFC	4	2	0
51	sN0758aNM	624	C1, C5, C7	AAFC	3	2	0
52	sN1945(bNP)†	709	A9, C5	AAFC	4	2	0
53	sN0761a†	721	A5, C5	AAFC	4	3	0
54	sORB17†	2445	C5	AAFC	4	1	1
55	sN12153I†	2448	C5	AAFC	4	2	2
56	sN11661†	2452	C5	AAFC	3	1	0
57	sN12503†	2453	C5	AAFC	2	1	0
58	sN12622	2476	C5	AAFC	4	2	0
59	sR0622	2477	C5	AAFC	3	0	0
60	Na10-C06	756	C6	BBSRC microsatellite program	3	2	0
61	FITO043†	897	C6	Iniguez-Luy et al. (2008) TAG 117(6): 977–985	4	2	2
62	BRMS-015†	991	C6	Suwabe et al. (2002) TAG 104(6–7): 1092–1098	2	1	1
63	sN3815†	2366	C6	AAFC	3	2	1
64	sS2352†	2373	C6	AAFC	5	2	1
65	sN11862	2374	C6	AAFC	4	2	0
66	sN12743J†	2379	C6	AAFC	7	2	0
67	sN9539†	2380	C6	AAFC	3	1	1
68	sNRD41 (a) †	2122	C7	AAFC	2	1	0
69	sORF37†	2393	C7	AAFC	3	2	1
70	sN1975	2410	C7	AAFC	4	2	1
71	sORA93†	2414	C7	AAFC	2	2	1
72	sS2225†	2416	C7	AAFC	3	2	2

† sign indicates marker used to study inheritance of SSR alleles of either Badger Shipper or Bindsachsener or both the *B. oleracea* parents

[1] Non-parental alleles = alleles could not be detected in either *B. napus* or *B. oleracea* parents, but appeared in the advanced generation

Appendix: 3-2. Continued…

Sr. No.	Primer name	Primer #	Linkage group/s	Source	Total parental alleles	No. *B. oleracea* specific alleles	No. non-parental alleles[1]
73	sNRD41†	2420	C7	AAFC	2	1	0
74	sN3825J†	2428	C7	AAFC	2	1	1
75	sN0706†	2431	C7	AAFC	3	2	2
76	sN2557†	240	C8	AAFC	2	1	0
77	CB10139†	489	C8	Celera AgGen Consortium	3	2	0
78	CB10028†	992	C8	Celera AgGen Consortium	3	2	1
79	BnGMS3†	2179	C8	Cheng et al. (2009) TAG 118(6): 1121–1131	2	1	1
80	BnGMS4†	2180	C8	Cheng et al. (2009) TAG 118(6): 1121–1131	4	2	0
81	BnGMS83†	2184	C8	Cheng et al. (2009) TAG 118(6): 1121–1131	3	2	0
82	BnGMS336†	2199	C8	Cheng et al. (2009) TAG 118(6): 1121–1131	2	1	1
83	BoGMS0468†	2244	C8	Li et al. (2011) Mol. Breed. 28(4): 585–596	3	2	0
84	BoGMS0868†	2248	C8	Li et al. (2011) Mol. Breed. 28(4): 585–596	3	2	2
85	FITO095†	751	C9	Iniguez-Luy et al. (2008) TAG 117(6): 977–985	3	1	1
86	BnGMS43†	2182	C9	Cheng et al. (2009) TAG 118(6): 1121–1131	3	1	0
87	BnGMS213†	2193	C9	Cheng et al. (2009) TAG 118(6): 1121–1131	3	1	0
88	BnGMS385†	2204	C9	Cheng et al. (2009) TAG 118(6): 1121–1131	2	1	0
89	BnGMS625†	2220	C9	Cheng et al. (2009) TAG 118(6): 1121–1131	3	1	1
90	BnGMS646†	2224	C9	Cheng et al. (2009) TAG 118(6): 1121–1131	2	1	0
91	BoGMS0845†	2257	C9	Li et al. (2011) Mol. Breed. 28(4): 585–596	3	2	1
92	BoGMS1283†	2258	C9	Li et al. (2011) Mol. Breed. 28(4): 585–596	3	2	1
93	sN4029†	3040	C9	Canola Program, the University of Alberta	3	2	1

† sign indicates marker used to study inheritance of SSR alleles of either Badger Shipper or Bindsachsener or both the *B. oleracea* parents

[1] Non-parental alleles = alleles could not be detected in either *B. napus* or *B. oleracea* parents, but appeared in the advanced generation

Appendix: 3-3. Dice coefficient of genetic similarity of the two *B. oleracea* var. *capitata* cultivars, *viz*. Badger Shipper and Bindsachsener, and 93 inbred lines derived from F_2 and BC_1 of two *B. napus* (A04-73NA) × *B. oleracea* var. *capitata* interspecific crosses with the spring *B. napus* canola line A04-73NA based on 93 SSR markers.

Entry No.	Reg. no.	Dice similarity with *B. napus*	GD (1-dice)	Entry No.	Reg. no.	Dice similarity with *B. napus*	GD (1-dice)
1	1362-149	0.6689	0.3311	25	1363-170	0.6554	0.3446
2	1362-152	0.6755	0.3245	26	1363-171	0.7043	0.2957
3	1362-155	0.6733	0.3267	27	1363-173	0.6986	0.3014
4	1362-156	0.6796	0.3204	28	1363-177	0.8544	0.1456
5	1362-158	0.6621	0.3379	29	1363-178	0.8896	0.1104
6	1362-161	0.6735	0.3265	30	1363-180	0.8294	0.1706
7	1362-162	0.6392	0.3608	31	1363-181	0.8467	0.1533
8	1362-164	0.8327	0.1673	32	1363-182	0.8618	0.1382
9	1362-165	0.8671	0.1329	33	1363-183	0.8673	0.1327
10	1362-166	0.7248	0.2752	34	1363-185	0.8383	0.1617
11	1362-167	0.6376	0.3624	35	1363-186	0.8707	0.1293
12	1362-169	0.6301	0.3699	36	1363-190	0.8759	0.1241
13	1362-170	0.6370	0.3630	37	1363-194	0.6309	0.3691
14	1362-171	0.6623	0.3377	38	1363-195	0.6087	0.3913
15	1362-173	0.6351	0.3649	39	1363-197	0.6599	0.3401
16	1362-174	0.6237	0.3763	40	1363-202	0.6267	0.3733
17	1362-175	0.6447	0.3553	41	1363-205	0.6733	0.3267
18	1362-176	0.6598	0.3402	42	1363-206	0.6821	0.3179
19	1362-177	0.6376	0.3624	43	1363-207	0.6779	0.3221
20	1362-179	0.6034	0.3966	44	1363-208	0.6503	0.3497
21	1362-180	0.6756	0.3244	45	1363-211	0.6621	0.3379
22	1363-164	0.6622	0.3378	46	1681-082	0.8179	0.1821
23	1363-165	0.6871	0.3129	47	1681-083	0.8428	0.1572
24	1363-168	0.6983	0.3017	48	1681-084	0.7657	0.2343

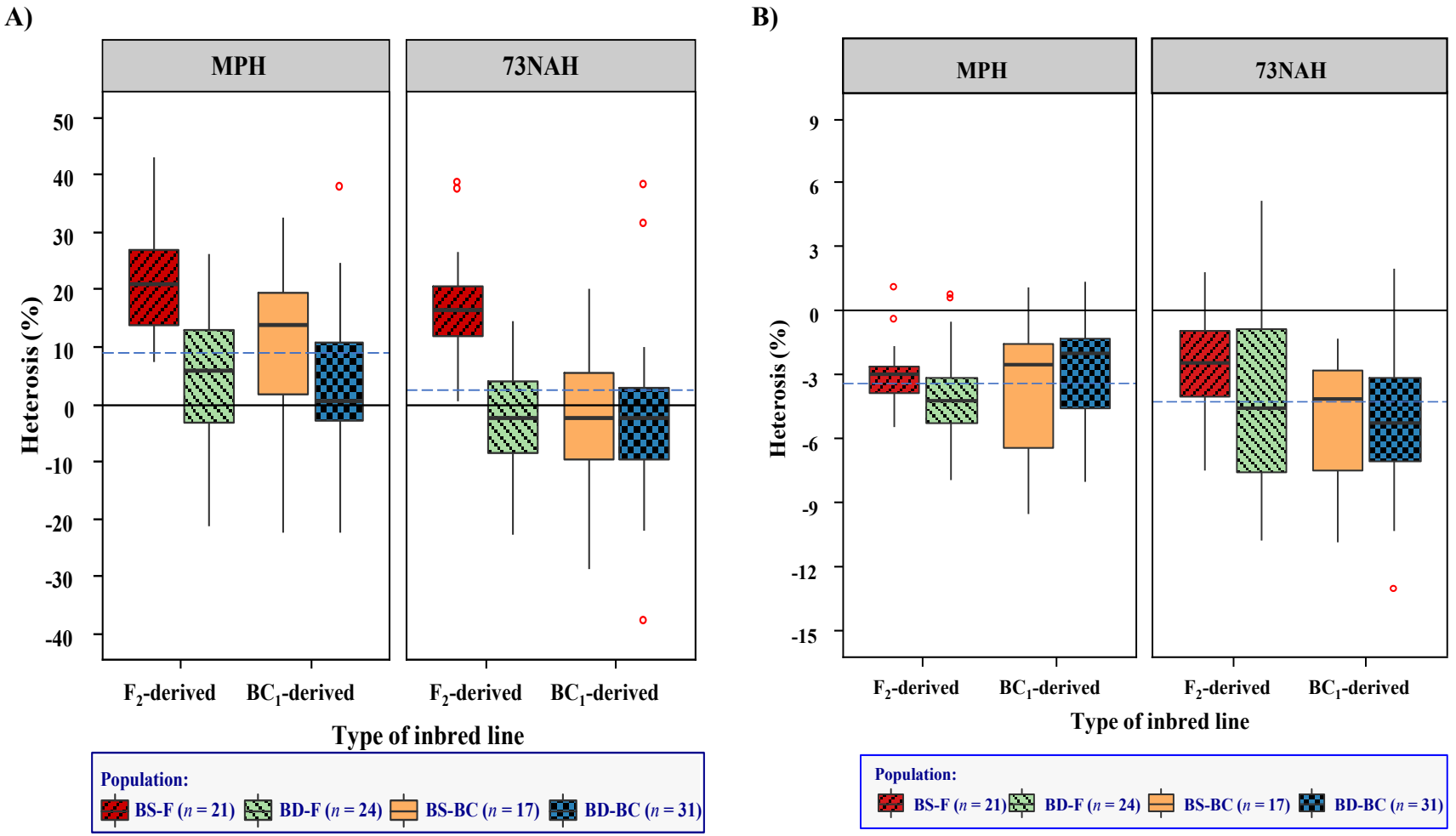
Appendix: 3-3. Continued…

Entry No.	Reg. no.	Dice similarity with *B. napus*	GD (1-dice)	Entry No.	Reg. no.	Dice similarity with *B. napus*	GD (1-dice)
49	1681-085	0.8649	0.1351	73	1682-124	0.7365	0.2635
50	1681-086	0.8733	0.1267	74	1682-125	0.7383	0.2617
51	1681-090	0.8239	0.1761	75	1682-128	0.7803	0.2197
52	1681-091	0.8533	0.1467	76	1682-130	0.8239	0.1761
53	1681-092	0.8684	0.1316	77	1682-131	0.8534	0.1466
54	1681-096	0.8600	0.1400	78	1682-133	0.8701	0.1299
55	1681-097	0.7869	0.2131	79	1682-137	0.8235	0.1765
56	1681-098	0.7657	0.2343	80	1682-138	0.8328	0.1672
57	1681-100	0.7869	0.2131	81	1682-140	0.8534	0.1466
58	1681-101	0.7841	0.2159	82	1682-143	0.8758	0.1242
59	1681-102	0.7881	0.2119	83	1682-145	0.8618	0.1382
60	1681-103	0.7491	0.2509	84	1682-147	0.8247	0.1753
61	1681-104	0.8328	0.1672	85	1682-149	0.8173	0.1827
62	1681-105	0.8105	0.1895	86	1682-150	0.8636	0.1364
63	1682-099	0.8161	0.1839	87	1682-152	0.8617	0.1383
64	1682-100	0.8267	0.1733	88	1682-154	0.8544	0.1456
65	1682-101	0.8267	0.1733	89	1682-155	0.8626	0.1374
66	1682-102	0.8251	0.1749	90	1682-156	0.8636	0.1364
67	1682-103	0.8239	0.1761	91	1682-158	0.8487	0.1513
68	1682-104	0.8013	0.1987	92	1682-159	0.8636	0.1364
69	1682-105	0.8188	0.1812	93	1682-164	0.7159	0.2841
70	1682-108	0.8239	0.1761		*B.ole.cap*.BS	0.1469	0.8531
71	1682-113	0.8289	0.1711		*B.ole.cap*.BD	0.1352	0.8648
72	1682-120	0.6890	0.3110				

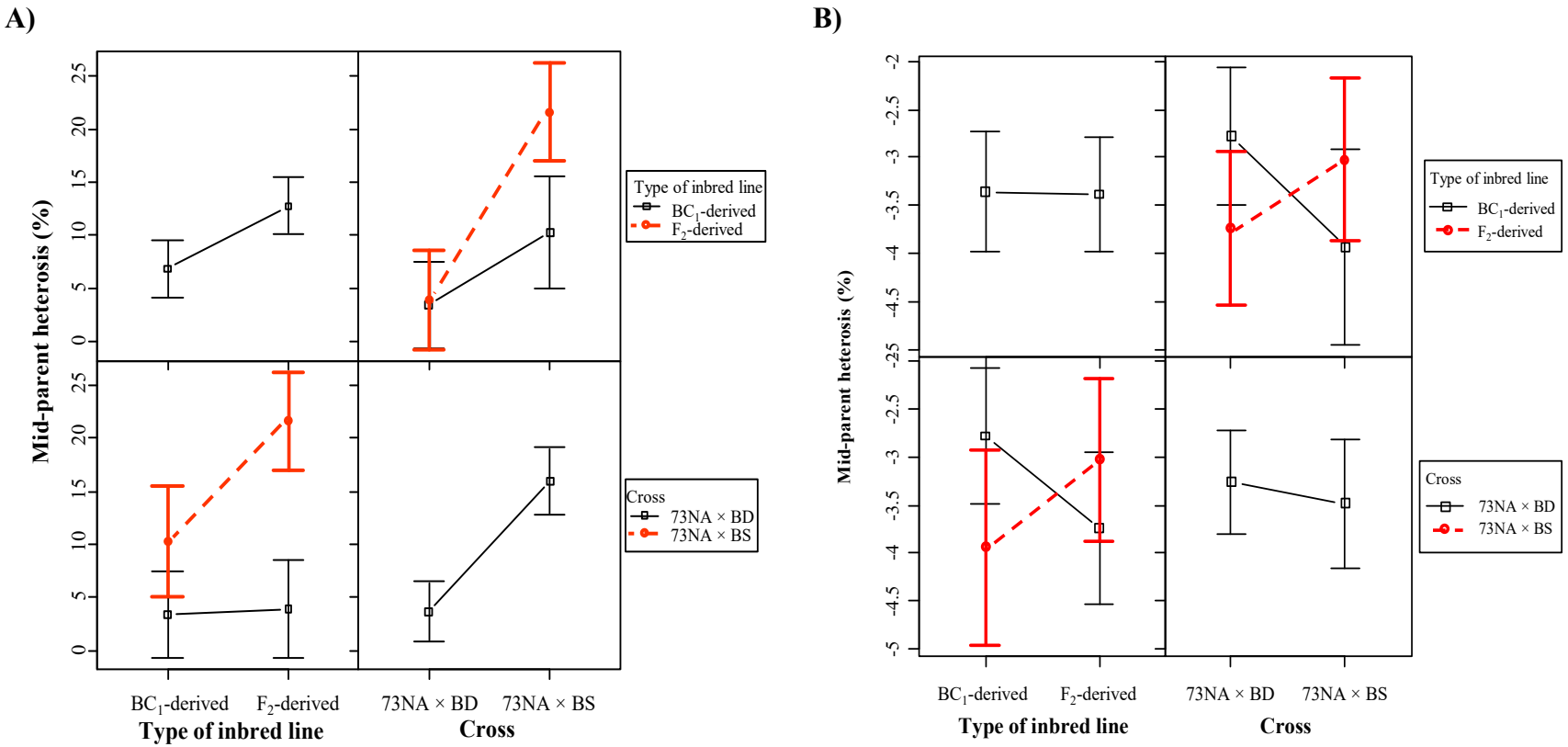
Appendix: 4-1. Mean square values for the random effects from the analysis of variance (ANOVA) of *per se* performance of the inbred lines of the two *B. napus* × *B. oleracea* crosses, their test-hybrids, and mid-parent heterosis (MPH) and heterosis over the spring *B. napus* parent A04-73NA (73NAH) for different agronomic and seed quality traits based on pooled data of the test-hybrid trials conducted in 2015 and 2016

Sources	df[1]	MS[2]	df	MS	df	MS	df	MS		MS	df	MS	df	MS		MS
	Seed yield								Seed oil							
	Inbred lines		Test-hybrids		MPH		73NAH		Inbred lines		Test-hybrids		MPH		73NAH	
Block	15	120750.0	15	122410.0	15	0.0	15	11.0	15	0.4	15	0.4	15	0.1	15	0.6
Replication	1	17389.0	1	40450.0	1	0.9	1	40.7	1	0.3	1	0.1	1	0.0	1	0.0
Year	1	2143357.0	1	2464021.0	1	1.6	1	0.0	1	6.5	1	5.7	1	0.0	1	0.0
Residual		303100.0		329260.0		350.1		417.5		1.7		1.4		3.9		5.4
Total	330		345		322		341		358		352		342		351	
	Days to flowering								Seed protein							
	Inbred lines		Test-hybrids		MPH		73NAH		Inbred lines		Test-hybrids		MPH		73NAH	
Block	15	0.5	15	0.6	15	0.3	15	0.9	15	0.5	15	0.6	15	0.4	15	1.7
Replication	1	1.2	1	0.7	1	0.0	1	1.1	1	0.0	1	0.0	1	0.0	1	0.5
Year	1	6.6	1	5.6	1	0.0	1	0.1	1	2.9	1	2.7	1	0.1	1	0.4
Residual		4.6		3.8		12.1		18.3		1.6		1.2		6.8		11.2
Total	360		363		352		362		358		352		342		351	
	Plant height								Seed glucosinolate							
	Inbred lines		Test-hybrids		MPH		73NAH		Inbred lines		Test-hybrids		MPH		73NAH	
Block	15	11.5	15	6.2	15	0.4	15	1.5	15	0.2	15	0.0	15	0.9	15	3.4
Replication	1	2.1	1	6.9	1	0.0	1	0.0	1	0.1	1	0.0	1	0.0	1	0.0
Year	1	516.3	1	594.0	1	5.8	1	14.5	1	0.1	1	0.0	1	10.8	1	23.5
Residual		60.2		46.1		25.6		24.5		3.1		2.0		55.8		84.5
Total	368		367		364		367		358		352		342		351	

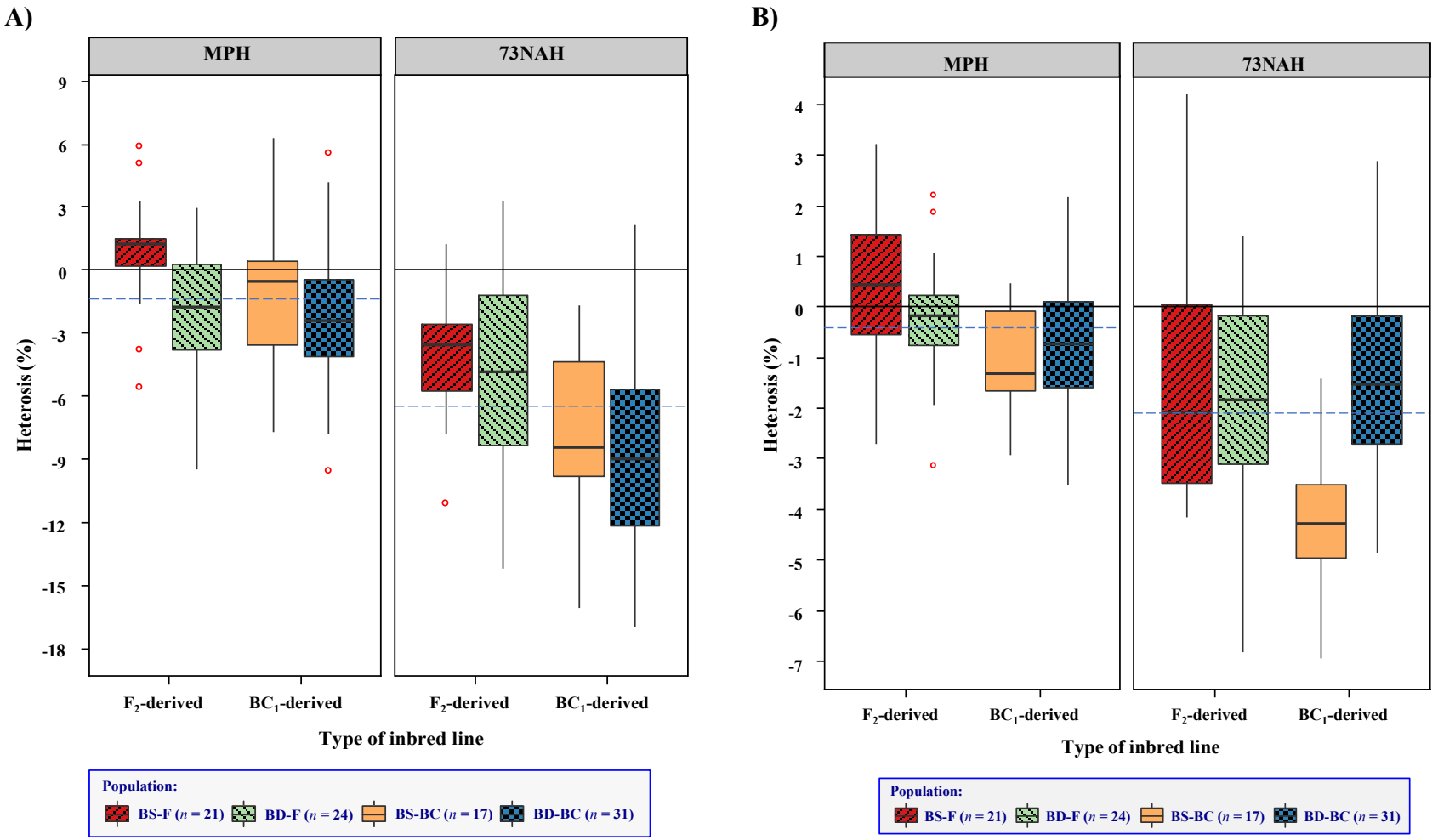
[1] df = degree of freedom
[2] MS = Mean square



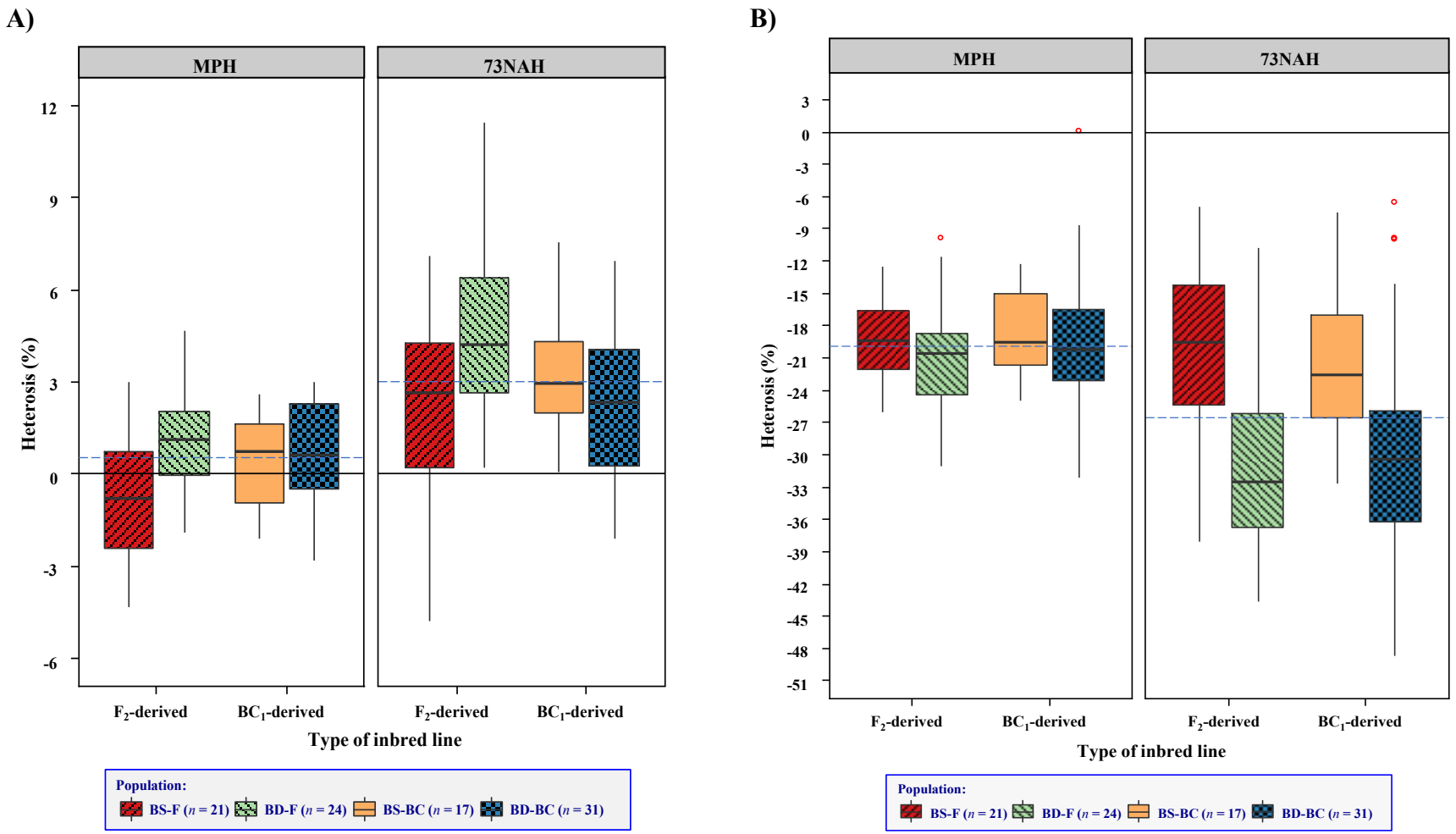
Appendix: 4-2. Boxplots showing the distributions of MPH (%) and 73NAH (%) for **A)** seed yield and **B)** days to flowering. The boxes cover interquartile range, and the whiskers cover the remaining variation; the solid line inside the box, the dashed line and the circles represent the median values, the mean values and the outliers, respectively.



Appendix: 4-3. The plots show interaction between the crosses (A04-73NA × Badger Shipper-BS and A04-73NA × Bindsachsener-BD) and line types (F_2- and BC_1-derived) for mean MPH for **A)** seed yield and **B)** days to flowering. The vertical bars indicate the confidence interval at 95 % probability level



Appendix: 4-4. Boxplots showing the distributions of MPH (%) and 73NAH (%) for **A)** plant height and **B)** seed oil content. The boxes cover interquartile range, and the whiskers cover the remaining variation; the solid line inside the box, the dashed line and the circles represent the median values, the mean values and the outliers, respectively.



Appendix: 4-5. Boxplots showing the distributions of MPH (%) and 73NAH (%) for **A)** seed protein and **B)** seed glucosinolate contents. The boxes cover interquartile range, and the whiskers cover the remaining variation; the solid line inside the box, the dashed line and the circles represent the median values, the mean values and the outliers, respectively.

Appendix: 4-6. Seed yield (kg/ha) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) in field trials conducted in 2015 and 2016.

Pedigree[1]	Line type (abbre.)	No. entries	Year-2015				Year-2016			
			Inbred lines	Test-hybrids	MPH (%)	73NAH (%)	Inbred lines	Test-hybrids	MPH (%)	73NAH (%)
			lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	2721.4 ± 315.1 ab[3] (1796.0 – 3558.6)	3535.1 ± 315.2 a ***[2] (2664.0 – 4179.3)	27.1 ± 5.0 a[3] (2.2 – 60.2)	20.9 ± 7.9 a[3] (2.0 – 46.7)	4606.8 ± 105.6 bc[3] (3745.6 – 5303.4)	5566.1 ± 105.6 a ***[2] (4788.3 – 6503.7)	17.3 ± 2.7 a[3] (2.3 – 41.7)	14.0 ± 5.0 a[3] (-4.0 – 45.4)
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	2477.0 ± 313.5 bc (1277.5 – 3216.5)	2757.8 ± 310.1 b (2063.9 – 3579.6)	4.1 ± 4.9 b (-36.2 – 38.2)	-2.8 ± 7.3 b (-32.5 – 27.4)	4429.6 ± 106.5 bc (2459.9 – 5378.2)	4935.5 ± 103.9 b *** (4013.3 – 5801.9)	4.3 ± 2.7 b (-16.4 – 30.8)	-1.4 ± 4.9 b (-23.4 – 20.6)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	1997.0 ± 326.0 c (607.7 – 2804.3)	2637.7 ± 320.8 b *** (1666.0 – 3607.2)	10.6 ± 5.7 ab (-57.1 – 51.9)	-9.3 ± 8.3 b (-67.7 – 34.2)	4276.1 ± 116.3 c (3410.2 – 5379.9)	5049.8 ± 114.7 b *** (4373.6 – 5952.9)	9.3 ± 2.9 ab (-7.0 – 29.0)	3.5 ± 5.1 b (-21.8 – 26.1)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	2459.6 ± 307.3 bc (1629.6 – 3565.2)	2766.9 ± 306.6 b * (1695.0 – 3716.5)	3.0 ± 4.1 b (-31.6 – 67.9)	-5.5 ± 6.6 b (-38.3 – 66.9)	4769.8 ± 96.7 ab (3403.0 – 5667.5)	5068.7 ± 101.6 b (4143.9 – 5961.4)	3.2 ± 2.5 b (-16.3 – 21.6)	0.6 ± 4.9 b (-20.8 – 22.4)
Pooled BS-cross	F & BC	38	2408.2 ± 306.8 j (607.7 – 3558.6)	3133.6 ± 305.7 i *** (1666.0 – 4179.3)	18.8 ± 3.8 i (-57.1 – 60.2)	5.8 ± 6.5 i (-67.7 – 46.7)	4458.8 ± 92.3 j (3410.2 – 5379.9)	5335.1 ± 92.0 i *** (4373.6 – 6503.7)	13.3 ± 2.3 i (-7.0 – 41.7)	8.7 ± 4.7 i (-21.8 – 45.4)
Pooled BD-cross	F & BC	55	2466.6 ± 301.6 j (1277.5 – 3565.2)	2762.9 ± 300.8 j ** (1695.0 – 3716.5)	3.5 ± 3.2 j (-36.2 – 67.9)	-4.2 ± 5.7 i (-38.3 – 66.9)	4621.4 ± 86.4 j (2459.9 – 5667.5)	5009.5 ± 87.5 j *** (4013.3 – 5961.4)	3.8 ± 2.2 j (-16.4 – 30.8)	-0.4 ± 4.7 j (-23.4 – 22.4)
Pooled F_2-derived	F	45	2599.2 ± 304.0 y (1277.5 – 3558.6)	3120.5 ± 303.0 x *** (2063.9 – 4179.3)	15.6 ± 3.5 x (-36.2 – 60.2)	9.0 ± 6.1 x (-32.5 – 46.7)	4512.3 ± 89.6 y (2459.9 – 5378.2)	5229.8 ± 88.6 x *** (4013.3 – 6503.7)	10.8 ± 2.2 x (-16.4 – 41.7)	6.3 ± 4.7 x (-23.4 – 45.4)
Pooled BC_1-derived	BC	48	2302.1 ± 303.3 y (607.7 – 3565.2)	2721.2 ± 302.4 y *** (1666.0 – 3716.5)	6.8 ± 3.5 x (-57.1 – 67.9)	-7.4 ± 6.1 y (-67.7 – 66.9)	4594.9 ± 87.8 y (3403.0 – 5667.5)	5061.9 ± 89.8 x *** (4143.9 – 5961.4)	6.3 ± 2.3 y (-16.3 – 29.0)	2.0 ± 4.7 x (-21.8 – 26.1)
Pooled-all	F & BC	93	2442.3 ± 298.5 n (607.7 – 3565.2)	2914.4 ± 298.0 m *** (1666.0 – 4179.3)	11.2 ± 2.5 (-57.1 – 67.9)	0.8 ± 5.2 (-67.7 – 66.9)	4554.9 ± 79.3 n (2459.9 – 5667.5)	5144.0 ± 79.6 m *** (4013.3 – 6503.7)	8.5 ± 2.0 (-16.4 – 41.7)	4.2 ± 4.6 (-23.4 – 45.4)
Check (A04-73NA)			2925.4 ± 298.7 aimx	2925.4 ± 298.7 bijmxy			5030.4 ± 86.9 aimx	5030.4 ± 86.9 bjmx		

[1] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

Appendix: 4-7. Days to flowering (days) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) in field trials conducted in 2015 and 2016.

Pedigree[1]	Line type (abbre.)	No. entries	Year-2015				Year-2016			
			Inbred lines	Test-hybrids	MPH (%)	73NAH (%)	Inbred lines	Test-hybrids	MPH (%)	73NAH (%)
			lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	48.3 ± 1.2 ab[3] (45.2 – 54.2)	46.6 ± 1.2 b **[2] (44.4 – 49.7)	-3.8 ± 0.8 a[3] (-7.7 – 0.9)	-4.1 ± 1.2 a[3] (-8.5 – -0.1)	46.3 ± 0.3 a[3] (41.0 – 48.9)	44.9 ± 0.3 a ***[2] (42.0 – 46.8)	-2.4 ± 0.5 a[3] (-5.4 – 3.3)	-1.6 ± 0.9 a[3] (-8.7 – 3.4)
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	48.8 ± 1.2 a (43.9 – 53.3)	47.0 ± 1.2 b *** (44.1 – 49.6)	-3.7 ± 0.7 a (-7.9 – 1.7)	-3.9 ± 1.1 a (-9.3 – 6.2)	45.6 ± 0.3 ab (38.9 – 52.6)	43.7 ± 0.3 b *** (39.8 – 48.9)	-4.1 ± 0.5 b (-9.2 – 1.7)	-4.0 ± 0.9 b (-13.0 – 6.8)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	48.2 ± 1.2 ab (45.7 – 50.1)	47.6 ± 1.2 ab (43.2 – 50.0)	-2.9 ± 1.0 a (-12.9 – 7.4)	-2.8 ± 1.3 a (-14.6 – 1.4)	43.8 ± 0.3 c (40.9 – 47.5)	42.6 ± 0.3 c ** (39.7 – 44.6)	-4.3 ± 0.5 b (-10.7 – 2.9)	-6.2 ± 0.9 c (-13.2 – -2.1)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	46.8 ± 1.1 b (43.0 – 51.4)	46.2 ± 1.1 b (43.9 – 49.1)	-2.9 ± 0.6 a (-11.3 – 2.4)	-4.5 ± 1.1 a (-12.5 – 4.5)	42.3 ± 0.3 d (37.7 – 52.1)	42.7 ± 0.3 c (39.1 – 48.2)	-2.8 ± 0.4 ab (-8.8 – 4.7)	-6.0 ± 0.8 c (-15.2 – 3.2)
Pooled BS-cross	F & BC	38	48.2 ± 1.1 ij (45.2 – 54.2)	47.1 ± 1.1 j ** (43.2 – 50.0)	-3.3 ± 0.6 i (-12.9 – 7.4)	-3.5 ± 1.0 i (-14.6 – 1.4)	45.2 ± 0.3 i (40.9 – 48.9)	43.9 ± 0.3 j *** (39.7 – 46.8)	-3.3 ± 0.4 i (-10.7 – 3.3)	-3.9 ± 0.8 i (-13.2 – 3.4)
Pooled BD-cross	F & BC	55	47.7 ± 1.1 j (43.0 – 53.3)	46.5 ± 1.1 j *** (43.9 – 49.6)	-3.3 ± 0.5 i (-11.3 – 2.4)	-4.2 ± 1.0 i (-12.5 – 6.2)	43.7 ± 0.3 j (37.7 – 52.6)	43.2 ± 0.3 k * (39.1 – 48.9)	-3.4 ± 0.3 i (-9.2 – 4.7)	-5.0 ± 0.8 j (-15.2 – 6.8)
Pooled F_2-derived	F	45	48.5 ± 1.1 x (43.9 – 54.2)	46.8 ± 1.1 y *** (44.1 – 49.7)	-3.8 ± 0.5 x (-7.9 – 1.7)	-4.0 ± 1.0 x (-9.3 – 6.2)	45.9 ± 0.3 x (38.9 – 52.6)	44.3 ± 0.3 y *** (39.8 – 48.9)	-3.2 ± 0.4 x (-9.2 – 3.3)	-2.8 ± 0.8 x (-13.0 – 6.8)
Pooled BC_1-derived	BC	48	47.3 ± 1.1 y (43.0 – 51.4)	46.7 ± 1.1 y (43.2 – 50.0)	-2.9 ± 0.6 x (-12.9 – 7.4)	-3.6 ± 1.0 x (-14.6 – 4.5)	42.8 ± 0.3 y (37.7 – 52.1)	42.7 ± 0.3 z (39.1 – 48.2)	-3.5 ± 0.4 x (-10.7 – 4.7)	-6.1 ± 0.8 y (-15.2 – 3.2)
Pooled-all	F & BC	93	47.9 ± 1.1 n (43.0 – 54.2)	46.8 ± 1.1 n *** (43.2 – 50.0)	-3.3 ± 0.4 (-12.9 – 7.4)	-3.8 ± 0.9 (-14.6 – 6.2)	44.3 ± 0.3 n (37.7 – 52.6)	43.5 ± 0.3 n *** (39.1 – 48.9)	-3.4 ± 0.3 (-10.7 – 4.7)	-4.5 ± 0.7 (-15.2 – 6.8)
Check (A04-73NA)			48.7 ± 1.1 aimx	48.7 ± 1.1 aimx			45.6 ± 0.3 bimx	45.6 ± 0.3 aimx		

[1] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

Appendix: 4-8. Plant height (cm) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) in field trials conducted in 2015 and 2016.

Pedigree[1]	Line type (abbre.)	No. entries	Year-2015				Year-2016			
			Inbred lines	Test-hybrids	MPH (%)	73NAH (%)	Inbred lines	Test-hybrids	MPH (%)	73NAH (%)
			lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	112.8 ± 2.2 b[3] (86.5 – 124.5)	118.6 ± 2.2 b **[2] (109.2 – 132.5)	-1.3 ± 1.1 a[3] (-12.6 – 8.0)	-7.2 ± 1.1 a[3] (-16.7 – 0.0)	144.1 ± 1.6 bc[3] (129.4 – 158.8)	154.3 ± 1.6 a ***[2] (142.2 – 163.7)	2.8 ± 0.5 a[3] (-2.5 – 7.1)	-1.1 ± 0.5 a[3] (-9.5 – 3.4)
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	116.0 ± 2.1 b (86.0 – 133.1)	115.9 ± 2.1 bc (99.1 – 128.1)	-4.4 ± 1.0 a (-16.3 – 4.1)	-8.0 ± 1.1 a (-20.2 – 2.1)	147.1 ± 1.6 b (128.3 – 165.5)	151.1 ± 1.6 b ** (135.8 – 164.4)	0.1 ± 0.4 b (-4.3 – 5.1)	-2.2 ± 0.5 a (-11.4 – 8.4)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	109.3 ± 2.3 bc (94.7 – 119.5)	116.6 ± 2.3 bc *** (98.5 – 132.0)	-1.3 ± 1.2 a (-13.6 – 8.1)	-8.2 ± 1.2 a (-21.7 – 3.2)	137.6 ± 1.6 d (121.8 – 145.8)	145.6 ± 1.6 c *** (136.7 – 152.2)	-0.6 ± 0.5 b (-3.8 – 5.4)	-6.7 ± 0.6 b (-12.9 – -2.2)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	104.8 ± 1.9 c (80.5 – 125.7)	110.5 ± 1.9 c *** (97.2 – 128.3)	-4.2 ± 0.9 a (-14.9 – 5.2)	-12.4 ± 1.0 b (-22.0 – 0.9)	141.3 ± 1.5 c (117.7 – 165.2)	147.4 ± 1.5 c *** (135.7 – 165.3)	-0.6 ± 0.4 b (-6.8 – 4.8)	-5.0 ± 0.4 b (-13.0 – 5.1)
Pooled BS-cross	F & BC	38	111.3 ± 1.9 j (86.5 – 124.5)	117.7 ± 1.9 j *** (98.5 – 132.5)	-1.3 ± 0.8 i (-13.6 – 8.1)	-7.7 ± 1.0 i (-21.7 – 3.2)	141.2 ± 1.5 k (121.8 – 158.8)	150.4 ± 1.5 j *** (136.7 – 163.7)	1.1 ± 0.4 i (-3.8 – 7.1)	-3.9 ± 0.4 i (-12.9 – 3.4)
Pooled BD-cross	F & BC	55	109.7 ± 1.8 j (80.5 – 133.1)	112.9 ± 1.8 k ** (97.2 – 128.3)	-4.3 ± 0.7 j (-16.3 – 5.2)	-10.2 ± 0.9 j (-22.0 – 2.1)	143.9 ± 1.5 j (117.7 – 165.5)	149.1 ± 1.5 j *** (135.7 – 165.3)	-0.2 ± 0.3 j (-6.8 – 5.1)	-3.6 ± 0.3 i (-13.0 – 8.4)
Pooled F_2-derived	F	45	114.5 ± 1.8 y (86.0 – 133.1)	117.2 ± 1.8 y (99.1 – 132.5)	-2.8 ± 0.8 x (-16.3 – 8.0)	-7.6 ± 0.9 x (-20.2 – 2.1)	145.7 ± 1.5 y (128.3 – 165.5)	152.6 ± 1.5 y *** (135.8 – 164.4)	1.5 ± 0.3 x (-4.3 – 7.1)	-1.7 ± 0.4 x (-11.4 – 8.4)
Pooled BC_1-derived	BC	48	106.4 ± 1.8 z (80.5 – 125.7)	112.7 ± 1.8 z *** (97.2 – 132.0)	-2.7 ± 0.8 x (-14.9 – 8.1)	-10.3 ± 0.9 y (-22.0 – 3.2)	140.0 ± 1.5 z (117.7 – 165.2)	146.8 ± 1.5 z *** (135.7 – 165.3)	-0.6 ± 0.3 y (-6.8 – 5.4)	-5.8 ± 0.4 y (-13.0 – 5.1)
Pooled-all	F & BC	93	110.3 ± 1.7 n (80.5 – 133.1)	114.8 ± 1.7 n *** (97.2 – 132.5)	-2.8 ± 0.6 (-16.3 – 8.1)	-9.0 ± 0.8 (-22.0 – 3.2)	142.8 ± 1.5 n (117.7 – 165.5)	149.6 ± 1.5 n *** (135.7 – 165.3)	0.4 ± 0.2 (-6.8 – 7.1)	-3.8 ± 0.3 (-13.0 – 8.4)
Check (A04-73NA)			126.4 ± 1.7 aimx	126.4 ± 1.7 aimx			155.5 ± 1.5 aimx	155.5 ± 1.5 aimx		

[1] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

Appendix: 4-9. Seed oil content (%) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) in field trials conducted in 2015 and 2016.

Pedigree[1]	Line type (abbre.)	No. entries	Year-2015				Year-2016			
			Inbred lines	Test-hybrids	MPH (%)	73NAH (%)	Inbred lines	Test-hybrids	MPH (%)	73NAH (%)
			lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	45.3 ± 0.6 b[3] (42.1 – 48.2)	46.3 ± 0.6 a ***[2] (44.7 – 48.8)	0.5 ± 0.5 a[3] (-4.1 – 5.1)	-1.1 ± 0.9 ab[3] (-5.4 – 5.8)	48.0 ± 0.2 b[3] (44.9 – 51.8)	49.0 ± 0.2 c ***[2] (47.0 – 51.7)	-0.1 ± 0.2 a[3] (-3.0 – 2.3)	-2.0 ± 0.3 b[3] (-5.3 – 2.6)
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	45.1 ± 0.6 b (39.5 – 47.2)	45.7 ± 0.6 b (42.4 – 47.0)	0.0 ± 0.5 a (-3.9 – 3.9)	-0.7 ± 0.8 a (-9.3 – 3.3)	48.5 ± 0.2 b (43.1 – 51.5)	49.2 ± 0.2 bc * (46.5 – 51.0)	-0.4 ± 0.2 a (-2.9 – 2.0)	-2.1 ± 0.3 b (-5.7 – 2.5)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	43.4 ± 0.7 c (42.0 – 44.2)	44.6 ± 0.7 c *** (43.0 – 45.9)	-0.7 ± 0.6 a (-4.6 – 3.5)	-4.2 ± 0.9 b (-8.8 – 0.2)	47.0 ± 0.3 c (45.4 – 50.1)	48.0 ± 0.3 d *** (47.0 – 49.7)	-1.3 ± 0.2 b (-2.8 – 0.7)	-4.3 ± 0.3 c (-6.3 – -1.9)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	45.5 ± 0.6 b (42.7 – 47.7)	45.6 ± 0.6 b (43.9 – 48.3)	-0.8 ± 0.4 a (-4.7 – 3.4)	-1.8 ± 0.7 ab (-6.8 – 5.1)	49.8 ± 0.2 a (48.5 – 51.9)	49.7 ± 0.2 ab (47.8 – 51.5)	-0.7 ± 0.2 ab (-3.4 – 1.0)	-1.1 ± 0.2 a (-4.3 – 1.6)
Pooled BS-cross	F & BC	38	44.4 ± 0.6 k (42.0 – 48.2)	45.5 ± 0.6 j *** (43.0 – 48.8)	-0.1 ± 0.4 i (-4.6 – 5.1)	-2.6 ± 0.6 i (-8.8 – 5.8)	47.6 ± 0.2 k (44.9 – 51.8)	48.6 ± 0.2 k *** (47.0 – 51.7)	-0.7 ± 0.1 i (-3.0 – 2.3)	-3.1 ± 0.2 j (-6.3 – 2.6)
Pooled BD-cross	F & BC	55	45.4 ± 0.6 j (39.5 – 47.7)	45.7 ± 0.6 j (42.4 – 48.3)	-0.4 ± 0.3 i (-4.7 – 3.9)	-1.3 ± 0.5 i (-9.3 – 5.1)	49.2 ± 0.2 j (43.1 – 51.9)	49.4 ± 0.2 j (46.5 – 51.5)	-0.5 ± 0.1 i (-3.4 – 2.0)	-1.6 ± 0.2 i (-5.7 – 2.5)
Pooled F_2-derived	F	45	45.2 ± 0.6 y (39.5 – 48.2)	46.0 ± 0.6 xy ** (42.4 – 48.8)	0.3 ± 0.4 x (-4.1 – 5.1)	-0.9 ± 0.6 x (-9.3 – 5.8)	48.3 ± 0.2 z (43.1 – 51.8)	49.1 ± 0.2 y *** (46.5 – 51.7)	-0.2 ± 0.1 x (-3.0 – 2.3)	-2.1 ± 0.2 x (-5.7 – 2.6)
Pooled BC_1-derived	BC	48	44.8 ± 0.6 y (42.0 – 47.7)	45.3 ± 0.6 y (43.0 – 48.3)	-0.8 ± 0.4 x (-4.7 – 3.5)	-3.0 ± 0.6 y (-8.8 – 5.1)	48.8 ± 0.2 y (45.4 – 51.9)	49.1 ± 0.2 y (47.0 – 51.5)	-1.0 ± 0.1 y (-3.4 – 1.0)	-2.7 ± 0.2 y (-6.3 – 1.6)
Pooled-all	F & BC	93	45.0 ± 0.6 n (39.5 – 48.2)	45.6 ± 0.6 n *** (42.4 – 48.8)	-0.3 ± 0.3 (-4.7 – 5.1)	-2.0 ± 0.4 (-9.3 – 5.8)	48.5 ± 0.2 n (43.1 – 51.9)	49.1 ± 0.2 n *** (46.5 – 51.7)	-0.6 ± 0.1 (-3.4 – 2.3)	-2.4 ± 0.1 (-6.3 – 2.6)
Check (A04-73NA)			46.5 ± 0.6 aimx	46.5 ± 0.6 aimx			50.2 ± 0.2 aimx	50.2 ± 0.2 aimx		

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

Appendix: 4-10. Seed protein content (%) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) in field trials conducted in 2015 and 2016.

Pedigree[1]	Line type (abbre.)	No. entries	Year-2015				Year-2016			
			Inbred lines	Test-hybrids	MPH (%)	73NAH (%)	Inbred lines	Test-hybrids	MPH (%)	73NAH (%)
			lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	27.9 ± 0.2 a[3] (26.5 – 29.1)	27.1 ± 0.2 ab ***[2] (25.9 – 29.2)	0.1 ± 0.8 a[3] (-3.8 – 6.3)	3.2 ± 1.1 a[3] (-3.0 – 11.1)	25.8 ± 0.3 a[3] (22.9 – 27.9)	24.9 ± 0.3 ab ***[2] (22.8 – 27.3)	-1.2 ± 0.4 b[3] (-5.4 – 5.5)	1.6 ± 0.5 b[3] (-5.8 – 7.6)
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	28.1 ± 0.2 a (26.0 – 31.4)	27.7 ± 0.2 a (26.0 – 29.5)	1.5 ± 0.8 a (-2.2 – 7.2)	4.0 ± 1.0 a (-1.1 – 12.7)	25.7 ± 0.3 a (23.0 – 30.8)	25.1 ± 0.3 a (23.9 – 27.7)	0.8 ± 0.4 a (-4.8 – 5.2)	3.9 ± 0.5 a (-2.3 – 10.2)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	27.6 ± 0.2 a (25.7 – 28.8)	27.2 ± 0.2 a (25.0 – 28.8)	0.7 ± 0.9 a (-4.3 – 5.9)	3.3 ± 1.2 a (-3.1 – 12.4)	25.6 ± 0.3 a (23.8 – 26.9)	25.0 ± 0.3 ab (24.0 – 26.0)	0.3 ± 0.4 a (-3.1 – 2.9)	2.9 ± 0.6 ab (-1.9 – 6.7)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	27.8 ± 0.2 a (26.1 – 29.0)	27.5 ± 0.2 a (25.6 – 28.9)	1.0 ± 0.7 a (-5.4 – 5.7)	3.3 ± 1.0 a (-5.4 – 10.0)	24.6 ± 0.3 b (23.1 – 27.0)	24.5 ± 0.3 bc (23.3 – 26.5)	0.6 ± 0.3 a (-3.0 – 4.8)	1.6 ± 0.4 b (-3.1 – 6.7)
Pooled BS-cross	F & BC	38	27.7 ± 0.2 i (25.7 – 29.1)	27.1 ± 0.2 i *** (25.0 – 29.2)	0.4 ± 0.7 i (-4.3 – 6.3)	3.3 ± 1.0 i (-3.1 – 12.4)	25.7 ± 0.3 i (22.9 – 27.9)	24.9 ± 0.3 i *** (22.8 – 27.3)	-0.5 ± 0.3 j (-5.4 – 5.5)	2.2 ± 0.4 i (-5.8 – 7.6)
Pooled BD-cross	F & BC	55	27.9 ± 0.1 i (26.0 – 31.4)	27.5 ± 0.1 I ** (25.6 – 29.5)	1.2 ± 0.6 i (-5.4 – 7.2)	3.6 ± 0.9 i (-5.4 – 12.7)	25.1 ± 0.2 j (23.0 – 30.8)	24.8 ± 0.2 i (23.3 – 27.7)	0.7 ± 0.2 i (-4.8 – 5.2)	2.7 ± 0.3 i (-3.1 – 10.2)
Pooled F_2-derived	F	45	28.0 ± 0.1 x (26.0 – 31.4)	27.4 ± 0.1 x *** (25.9 – 29.5)	0.8 ± 0.7 x (-3.8 – 7.2)	3.6 ± 0.9 x (-3.0 – 12.7)	25.8 ± 0.2 x (22.9 – 30.8)	25.0 ± 0.2 x *** (22.8 – 27.7)	-0.2 ± 0.3 x (-5.4 – 5.5)	2.7 ± 0.4 x (-5.8 – 10.2)
Pooled BC_1-derived	BC	48	27.7 ± 0.1 x (25.7 – 29.0)	27.4 ± 0.1 x * (25.0 – 28.9)	0.9 ± 0.7 x (-5.4 – 5.9)	3.3 ± 0.9 x (-5.4 – 12.4)	25.0 ± 0.2 y (23.1 – 27.0)	24.7 ± 0.2 x (23.3 – 26.5)	0.4 ± 0.3 x (-3.1 – 4.8)	2.2 ± 0.4 x (-3.1 – 6.7)
Pooled-all	F & BC	93	27.9 ± 0.1 m (25.7 – 31.4)	27.4 ± 0.1 m *** (25.0 – 29.5)	0.8 ± 0.6 (-5.4 – 7.2)	3.4 ± 0.8 (-5.4 – 12.7)	25.3 ± 0.2 m (22.9 – 30.8)	24.8 ± 0.2 m *** (22.8 – 27.7)	0.1 ± 0.2 (-5.4 – 5.5)	2.5 ± 0.3 (-5.8 – 10.2)
Check (A04-73NA)			26.5 ± 0.1 bjny	26.5 ± 0.1 bjny			24.2 ± 0.2 bknz	24.2 ± 0.2 cjny		

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

Appendix: 4-11. Seed glucosinolate content (µmol/g seed) (mean ± SE) of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses and their test-hybrids produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) in field trials conducted in 2015 and 2016.

Pedigree[1]	Line type (abbre.)	No. entries	Year-2015				Year-2016			
			Inbred lines	Test-hybrids	MPH (%)	73NAH (%)	Inbred lines	Test-hybrids	MPH (%)	73NAH (%)
			lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)	lsmeans ± SE[2] (Range)	lsmeans ± SE (Range)	lsmeans ± SE (Range)
B. nap × *B. ole.cap.*BS	F_2-derived (BS-F)	21	20.9 ± 0.5 b[3] (14.9 – 24.7)	17.3 ± 0.5 b ***[2] (14.4 – 19.4)	-20.6 ± 1.5 a[3] (-32.1 – -9.2)	-22.6 ± 2.0 a[3] (-43.5 – -2.6)	21.1 ± 0.2 a[3] (14.8 – 23.7)	17.2 ± 0.2 b ***[2] (14.3 – 19.1)	-17.9 ± 0.9 a[3] (-26.5 – -9.3)	-16.8 ± 1.1 a[3] (-37.0 – -0.9)
B. nap × *B. ole.cap.*BD	F_2-derived (BD-F)	24	17.5 ± 0.5 cd (12.6 – 23.7)	16.0 ± 0.4 b (13.0 – 20.2)	-24.3 ± 1.5 a (-41.4 – -0.3)	-37.1 ± 1.8 c (-54.0 – -12.5)	17.0 ± 0.2 c (12.4 – 25.3)	15.6 ± 0.2 c *** (13.1 – 19.4)	-18.1 ± 0.9 a (-26.9 – -11.7)	-26.0 ± 1.2 b (-41.7 – -6.1)
(*B. nap* × *B. ole.cap.*BS) × *B. nap*	BC_1-derived (BS-BC)	17	19.4 ± 0.5 bc (14.1 – 24.2)	16.7 ± 0.5 b ** (14.1 – 22.7)	-21.3 ± 1.8 a (-34.4 – -2.5)	-26.0 ± 2.3 ab (-42.9 – 1.4)	20.0 ± 0.2 b (14.6 – 23.9)	17.2 ± 0.2 b *** (14.9 – 18.7)	-16.1 ± 1.0 a (-24.9 – -8.9)	-18.6 ± 1.3 a (-30.0 – -8.0)
(*B. nap* × *B. ole.cap.*BD) × *B. nap*	BC_1-derived (BD-BC)	31	17.2 ± 0.4 d (12.3 – 24.1)	15.8 ± 0.4 b (12.0 – 21.0)	-22.5 ± 1.3 a (-52.5 – -2.3)	-32.3 ± 1.7 bc (-67.2 – -10.0)	15.8 ± 0.2 d (11.9 – 20.1)	15.0 ± 0.2 c * (12.8 – 17.6)	-18.3 ± 0.9 a (-27.7 – -8.8)	-28.0 ± 1.1 b (-42.2 – -3.7)
Pooled BS-cross	F & BC	38	20.2 ± 0.4 j (14.1 – 24.7)	17.0 ± 0.4 j *** (14.1 – 22.7)	-21.0 ± 1.2 i (-34.4 – -2.5)	-24.3 ± 1.6 i (-43.5 – 1.4)	20.6 ± 0.2 i (14.6 – 23.9)	17.2 ± 0.2 j *** (14.3 – 19.1)	-17.0 ± 0.8 i (-26.5 – -8.9)	-17.7 ± 1.0 i (-37.0 – -0.9)
Pooled BD-cross	F & BC	55	17.3 ± 0.3 k (12.3 – 24.1)	15.9 ± 0.3 j *** (12.0 – 21.0)	-23.4 ± 1.0 i (-52.5 – -0.3)	-34.7 ± 1.4 j (-67.2 – -10.0)	16.3 ± 0.1 j (11.9 – 25.3)	15.3 ± 0.1 k *** (12.8 – 19.4)	-18.2 ± 0.8 i (-27.7 – -8.8)	-27.0 ± 0.9 j (-42.2 – -3.7)
Pooled F_2-derived	F	45	19.1 ± 0.3 y (12.6 – 24.7)	16.6 ± 0.3 y *** (13.0 – 20.2)	-22.5 ± 1.1 x (-41.4 – -0.3)	-29.9 ± 1.5 x (-54.0 – -2.6)	18.9 ± 0.2 y (12.4 – 25.3)	16.4 ± 0.2 y *** (13.1 – 19.4)	-18.0 ± 0.8 x (-26.9 – -9.3)	-21.4 ± 0.9 x (-41.7 – -0.9)
Pooled BC_1-derived	BC	48	18.0 ± 0.3 y (12.3 – 24.2)	16.1 ± 0.3 y *** (12.0 – 22.7)	-21.9 ± 1.1 x (-52.5 – -2.3)	-29.1 ± 1.5 x (-67.2 – 1.4)	17.3 ± 0.1 z (11.9 – 23.9)	15.8 ± 0.2 z *** (12.8 – 18.7)	-17.2 ± 0.8 x (-27.7 – -8.8)	-23.3 ± 1.0 x (-42.2 – -3.7)
Pooled-all	F & BC	93	18.5 ± 0.3 n (12.3 – 24.7)	16.3 ± 0.3 n *** (12.0 – 22.7)	-22.2 ± 0.8 (-52.5 – -0.3)	-29.5 ± 1.2 (-67.3 – 1.5)	18.1 ± 0.1 n (11.9 – 25.3)	16.1 ± 0.1 n *** (12.8 – 19.4)	-17.6 ± 0.7 (-27.7 – -8.8)	-22.3 ± 0.8 (-42.2 – -0.9)
Check (A04-73NA)			23.7 ± 0.4 aimx	23.7 ± 0.4 aimx			21.1 ± 0.1 aimx	21.1 ± 0.1 aimx		

[1] *B. nap* = Spring *B. napus* line A04-73NA, *B.ole.cap.*BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap.*BD = *B. oleracea* var. *capitata* cv. Bindsachsener

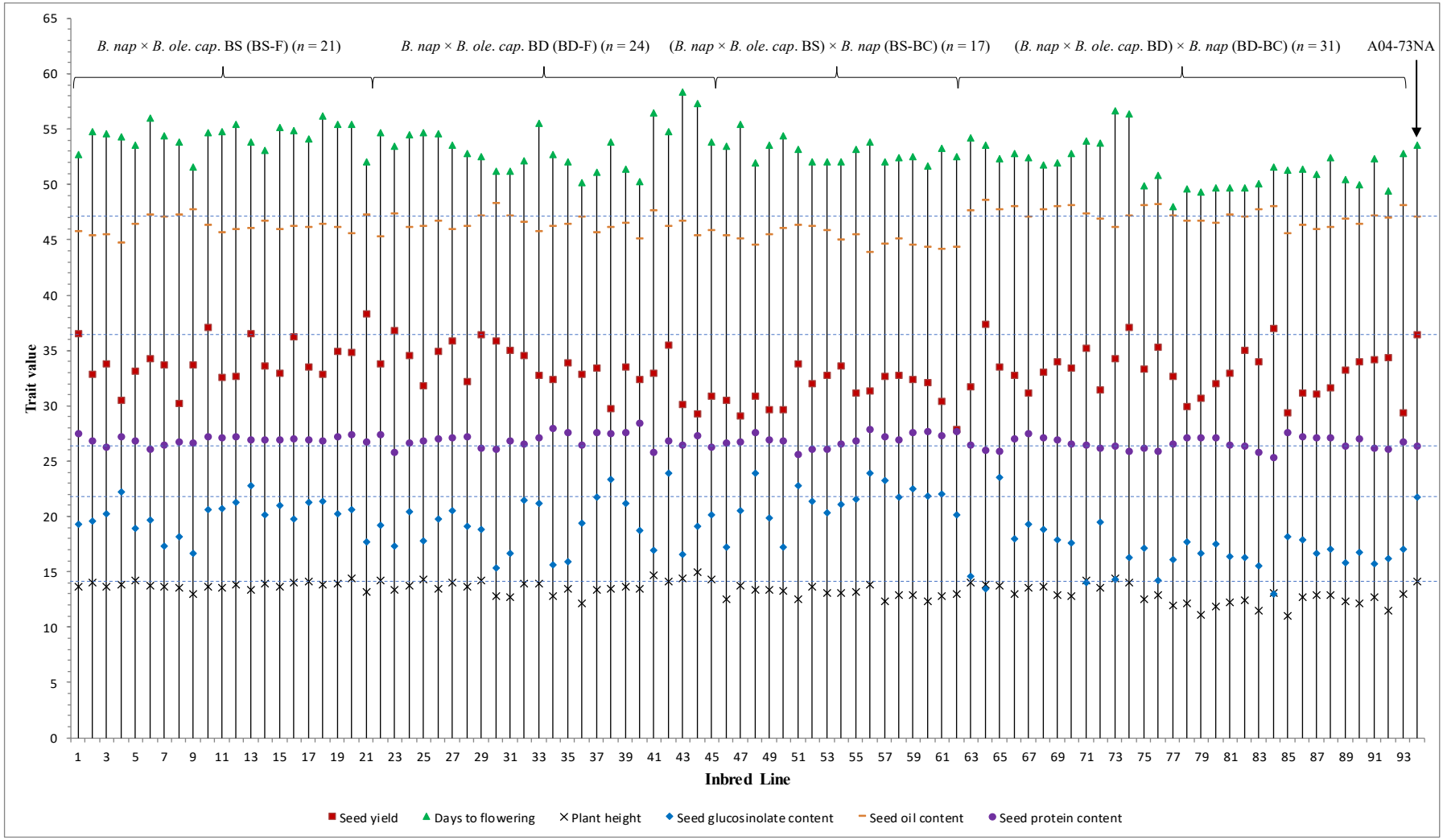
[2] Asterisks indicate the significance of difference between the inbred and their test-hybrid populations; p value of < 0.001 = ***, < 0.01 = ** and < 0.05 = *

[3] Mean comparison among the groups of populations. The alphabets a, b, c, d and e are used for comparison among the BS-F, BS-BC, BD-F, BD-BC and the check A04-73NA; the alphabets i, j and k are used for comparison among the pooled BS-cross, pooled BD-cross and the check A04-73NA; the alphabets x, y and z are used for comparison among the pooled F_2-derived, pooled BC_1-derived and the check A04-73NA; the alphabets m and n are used for comparison between the pooled data of the two crosses and the check A04-73NA

Appendix: 4-12. Mean square values for the random effects from the analysis of variance for different agronomic and seed quality traits of the inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses, viz. A04-73NA × Badger Shipper and A04-73NA × Bindsachsener

Sources	df[1]	MS[2]	df	MS
	Seed yield		Seed oil	
Block	3	1413	3	0.033452
Site	3	544233	3	3.692986
Replication	1	0	1	0.000502
Residual		116262		1.031393
Total	739		738	
	Plant height		Seed protein	
		Variance		Variance
Block	3	0.9845	3	0.0292
Site	3	192.8043	3	5.7618
Replication	1	0.1654	1	0
Residual		55.4747		0.7386
Total	741		738	
	Days to flowering		Seed glucosinolate	
		Variance		Variance
Block	3	0.005304	3	0.18641
Site	3	77.64114	3	1.17221
Replication	1	0.36813	1	0.03879
Residual		1.848638		2.37431
Total	741		738	

[1] df = degree of freedom
[2] MS = Mean square



Appendix: 4-13. Performance of the 45 F_2-derived inbred lines [populations BS-F (1 − 21) and BD-F (22 − 45)] and 48 BC_1-derived inbred lines (populations BS-BC (46 − 62) and BD-BC (63 − 93)] of two *B. napus* × *B. oleracea* interspecific crosses for different agronomic and seed quality traits evaluated in yield trial in summer 2016. Plant height is indicated as × 10 cm and seed yield as × 100 kg ha^{-1}. For all other traits, absolute values are given, where for days to flowering as days, for seed oil and protein content as % of the whole seed, and for seed glucosinolate as µmol/g seed. The *B. napus* parent A04-73NA (94) is included for comparison, the dashed lines indicate values for different traits in A04-73NA. *B. nap* = *B. napus* A04-73NA, *B. ole. cap*. BS = *B. oleracea* var. *capitata* cv. Badger Shipper and *B. ole. cap*. BD = *B. oleracea* var. *capitata* cv. Bindsachsener

Appendix: 4-14. Summary of agronomic and seed quality traits of the 93 inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses, and their test-hybrid produced by crossing with *B. napus* A04-73NA, and mid-parent heterosis (MPH, %) and heterosis over A04-73NA (73NAH, %) for these traits. Mean data of the two field trials conducted in 2015 and 2016 presented.

Reg. No.[1]	Ent.[2] No.	Seed yield (kg/ha)		Days to flowering (days)		Plant height (cm)		Seed oil content (%)		Seed protein content (%)		Seed glucosinolate content (μmol/g seed)	
		Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)
1362.149	1	4453.5 ± 1132.1 3494.6 ± 1132.1	23.7 ± 9.4 20.2 ± 11.3	45.1 ± 2.1 47.3 ± 2.1	-4.6 ± 1.8 -4.0 ± 2.3	134.5 ± 16.6 122.7 ± 16.6	1.3 ± 3.1 -5.7 ± 3.7	47.6 ± 1.8 45.5 ± 1.8	1.2 ± 1.0 -2.1 ± 1.2	26.7 ± 1.3 27.9 ± 1.3	0.7 ± 1.3 6.4 ± 1.9	17.1 ± 1.1 22.4 ± 1.1	-19.3 ± 4.4 -14.2 ± 5.8
1362.152	2	4671.7 ± 1143.7 3035.0 ± 1132.1	31.9 ± 10.9 15.8 ± 12.8	45.9 ± 2.1 46.1 ± 2.1	-1.6 ± 1.8 -2.5 ± 2.3	134.6 ± 16.6 112.8 ± 16.6	5.9 ± 3.1 -4.9 ± 3.7	46.7 ± 1.9 43.7 ± 1.8	1.4 ± 1.2 -4.2 ± 1.4	25.4 ± 1.3 26.9 ± 1.3	-1.7 ± 1.6 2.6 ± 2.1	16.7 ± 1.3 22.1 ± 1.1	-24.4 ± 4.9 -23.0 ± 6.4
1362.155	3	4003.2 ± 1132.1 3270.2 ± 1132.1	19.8 ± 9.4 10.3 ± 11.3	46.3 ± 2.1 50.8 ± 2.1	-5.4 ± 1.8 -1.7 ± 2.3	140.0 ± 16.6 139.7 ± 16.6	-1.5 ± 3.1 -3.1 ± 3.7	46.4 ± 1.8 44.6 ± 1.8	0.2 ± 1.0 -3.6 ± 1.2	25.4 ± 1.3 26.5 ± 1.3	-2.4 ± 1.3 -0.7 ± 1.8	16.4 ± 1.1 20.8 ± 1.1	-20.5 ± 4.4 -19.5 ± 5.8
1362.156	4	4418.`1 ± 1132.2 3372.0 ± 1132.2	27.3 ± 9.4 20.0 ± 11.3	45.6 ± 2.1 46.8 ± 2.1	-3.6 ± 1.8 -4.1 ± 2.3	136.6 ± 16.6 128.8 ± 16.6	0.7 ± 3.1 -4.1 ± 3.7	46.7 ± 1.8 44.3 ± 1.8	0.4 ± 1.0 -4.1 ± 1.2	25.7 ± 1.3 26.8 ± 1.3	-0.8 ± 1.3 2.7 ± 1.9	19.0 ± 1.1 23.5 ± 1.1	-13.9 ± 4.4 -7.0 ± 5.8
1362.158	5	3903.3 ± 1132.0 3046.8 ± 1132.0	18.6 ± 9.4 10.0 ± 11.3	43.9 ± 2.1 43.4 ± 2.1	-3.4 ± 1.8 -7.5 ± 2.3	128.5 ± 16.6 110.2 ± 16.6	1.4 ± 3.1 -11.1 ± 3.7	46.7 ± 1.8 43.8 ± 1.8	1.6 ± 1.0 -3.1 ± 1.2	25.6 ± 1.3 27.5 ± 1.3	-2.8 ± 1.3 1.6 ± 1.8	18.1 ± 1.1 23.4 ± 1.1	-23.0 ± 4.4 -23.7 ± 5.8
1362.161	6	4317.2 ± 1132.3 3334.7 ± 1132.3	22.1 ± 9.4 17.1 ± 11.3	47.2 ± 2.1 49.5 ± 2.1	-2.8 ± 1.8 -0.5 ± 2.3	138.4 ± 16.6 129.2 ± 16.6	1.9 ± 3.1 -3.2 ± 3.7	48.3 ± 1.8 47.0 ± 1.8	2.6 ± 1.0 2.4 ± 1.2	25.3 ± 1.3 26.6 ± 1.3	-4.3 ± 1.3 -3.7 ± 1.9	17.3 ± 1.1 20.2 ± 1.1	-22.0 ± 4.4 -27.6 ± 5.8
1362.162	7	4727.1 ± 1132.3 4086.2 ± 1132.3	8.9 ± 10.9 16.4 ± 12.7	44.4 ± 2.1 45.7 ± 2.1	-3.8 ± 1.8 -4.7 ± 2.3	135.9 ± 16.6 128.9 ± 16.6	1.5 ± 3.1 -2.0 ± 3.7	48.3 ± 1.8 47.4 ± 1.8	1.4 ± 1.0 1.0 ± 1.2	25.3 ± 1.3 26.9 ± 1.3	-3.2 ± 1.3 -0.1 ± 1.9	16.6 ± 1.1 21.0 ± 1.1	-26.0 ± 4.4 -30.0 ± 5.8
1362.164	8	4027.2 ± 1132.5 3331.4 ± 1132.5	10.9 ± 9.4 0.6 ± 11.3	45.7 ± 2.1 47.2 ± 2.1	-3.8 ± 1.8 -4.2 ± 2.3	140.8 ± 16.6 133.6 ± 16.6	1.3 ± 3.1 -2.7 ± 3.7	49.9 ± 1.8 48.9 ± 1.8	3.2 ± 1.0 4.2 ± 1.2	24.5 ± 1.3 25.2 ± 1.3	-3.9 ± 1.3 -4.8 ± 1.9	14.8 ± 1.1 17.0 ± 1.1	-24.8 ± 4.4 -33.2 ± 5.8
1362.165	9	4524.4 ± 1132.3 3996.4 ± 1132.3	12.3 ± 9.4 13.1 ± 11.3	45.8 ± 2.1 47.8 ± 2.1	-3.7 ± 1.8 -3.0 ± 2.3	139.4 ± 16.6 136.2 ± 16.6	0.2 ± 3.1 -1.9 ± 3.7	48.5 ± 1.8 48.9 ± 1.8	-0.5 ± 1.0 0.1 ± 1.2	25.8 ± 1.3 26.5 ± 1.3	0.2 ± 1.3 2.7 ± 1.9	14.4 ± 1.1 15.2 ± 1.1	-25.3 ± 4.4 -38.1 ± 5.8
1362.166	10	4858.6 ± 1132.4 3971.7 ± 1132.4	23.4 ± 9.4 20.4 ± 11.3	45.7 ± 2.1 47.5 ± 2.1	-2.7 ± 1.8 -1.6 ± 2.3	134.2 ± 16.6 125.2 ± 16.6	0.2 ± 3.1 -6.0 ± 3.7	48.2 ± 1.8 48.2 ± 1.8	0.4 ± 1.0 0.8 ± 1.2	25.5 ± 1.3 26.0 ± 1.3	-1.2 ± 1.3 -0.4 ± 1.9	17.7 ± 1.1 20.9 ± 1.1	-21.2 ± 4.4 -25.4 ± 5.8
1362.167	11	4705.2 ± 1133.1 3749.3 ± 1133.1	25.2 ± 9.4 25.1 ± 11.3	46.7 ± 2.1 46.7 ± 2.1	-0.4 ± 1.8 -0.8 ± 2.3	137.0 ± 16.6 125.3 ± 16.6	3.3 ± 3.1 -1.7 ± 3.7	47.9 ± 1.8 46.6 ± 1.8	0.5 ± 1.0 -1.6 ± 1.2	26.2 ± 1.3 27.1 ± 1.3	0.7 ± 1.4 4.8 ± 1.9	19.2 ± 1.1 23.1 ± 1.1	-13.8 ± 4.4 -10.3 ± 5.8
1362.169	12	4107.5 ± 1132.4 3642.3 ± 1132.4	10.5 ± 9.4 11.8 ± 11.3	45.6 ± 2.1 45.9 ± 2.1	-2.9 ± 1.8 -5.1 ± 2.3	143.1 ± 16.6 133.6 ± 16.6	1.3 ± 3.1 -3.6 ± 3.7	48.0 ± 1.8 47.4 ± 1.8	-0.6 ± 1.0 -2.1 ± 1.2	24.7 ± 1.3 26.0 ± 1.3	-2.4 ± 1.3 0.2 ± 1.9	17.8 ± 1.1 22.6 ± 1.1	-18.1 ± 4.4 -14.2 ± 5.8
1362.170	13	4505.2 ± 1132.2 3769.5 ± 1132.2	16.2 ± 9.4 10.4 ± 11.3	46.0 ± 2.1 47.5 ± 2.1	-2.7 ± 1.8 -2.1 ± 2.3	137.3 ± 16.6 132.3 ± 16.6	-1.6 ± 3.1 -6.1 ± 3.7	47.2 ± 1.8 47.8 ± 1.8	-2.7 ± 1.0 -4.1 ± 1.2	25.6 ± 1.3 25.5 ± 1.3	2.2 ± 1.3 4.2 ± 1.9	18.5 ± 1.1 22.6 ± 1.1	-13.2 ± 4.4 -7.4 ± 5.8
1362.171	14	4532.1 ± 1132.1 4213.9 ± 1143.9	19.6 ± 9.4 16.0 ± 11.3	47.9 ± 2.1 47.6 ± 2.1	1.1 ± 1.8 1.8 ± 2.3	144.5 ± 16.6 132.8 ± 16.6	5.1 ± 3.1 1.3 ± 3.7	47.6 ± 1.8 47.2 ± 1.8	-0.3 ± 1.0 -1.5 ± 1.2	26.0 ± 1.3 26.4 ± 1.3	0.2 ± 1.3 2.0 ± 1.8	18.0 ± 1.1 21.5 ± 1.1	-16.2 ± 4.4 -14.8 ± 5.8
1362.173	15	5106.9 ± 1132.3 3393.2 ± 1132.3	41.4 ± 9.4 37.4 ± 11.3	44.9 ± 2.1 46.4 ± 2.1	-4.3 ± 1.8 -5.3 ± 2.3	136.5 ± 16.6 125.7 ± 16.6	0.9 ± 3.1 -5.3 ± 3.7	46.6 ± 1.8 46.5 ± 1.8	-1.1 ± 1.0 -2.6 ± 1.2	26.8 ± 1.3 26.4 ± 1.3	3.0 ± 1.3 4.9 ± 1.9	17.5 ± 1.1 20.6 ± 1.1	-21.0 ± 4.4 -25.5 ± 5.8
1362.174	16	4900.9 ± 1132.1 4246.5 ± 1143.8	29.5 ± 9.4 26.6 ± 11.3	47.0 ± 2.1 48.7 ± 2.1	-2.9 ± 1.8 -2.0 ± 2.3	140.8 ± 16.6 133.3 ± 16.6	1.4 ± 3.1 -2.6 ± 3.7	46.9 ± 1.8 46.0 ± 1.8	-0.3 ± 1.0 -2.8 ± 1.2	26.7 ± 1.3 27.1 ± 1.3	0.6 ± 1.3 3.7 ± 1.8	16.9 ± 1.1 20.6 ± 1.1	-16.6 ± 4.4 -15.5 ± 5.8

[1] Reg. No. = Registration number
[2] Ent. No. = Entry number

Appendix: 4-14. Continued…

Reg. No.[1]	Ent.[2] No.	Seed yield (kg/ha)		Days to flowering (days)		Plant height (cm)		Seed oil content (%)		Seed protein content (%)		Seed glucosinolate content (µmol/g seed)	
		Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)
1362.175	17	4428.8 ± 1144.1 3598.8 ± 1144.1	20.8 ± 10.9 14.1 ± 12.8	46.7 ± 2.1 49.9 ± 2.1	-3.7 ± 1.8 -0.8 ± 2.3	140.5 ± 16.6 131.2 ± 16.6	1.5 ± 3.1 -3.0 ± 3.7	48.1 ± 1.9 47.0 ± 1.8	0.5 ± 1.2 -1.3 ± 1.4	25.4 ± 1.3 26.8 ± 1.3	-2.1 ± 1.6 2.4 ± 2.1	18.2 ± 1.3 19.7 ± 1.1	-17.7 ± 4.9 -24.5 ± 6.4
1362.176	18	4843.3 ± 1144.3 3419.8 ± 1132.4	43.3 ± 10.9 38.5 ± 12.8	47.7 ± 2.1 50.5 ± 2.1	-2.6 ± 1.8 0.8 ± 2.3	142.9 ± 16.6 138.2 ± 16.6	0.8 ± 3.1 -1.4 ± 3.7	46.3 ± 1.9 46.7 ± 1.8	-1.5 ± 1.2 -3.5 ± 1.4	27.1 ± 1.3 27.2 ± 1.3	0.8 ± 1.6 4.0 ± 2.1	18.0 ± 1.3 21.5 ± 1.1	-17.4 ± 4.9 -15.6 ± 6.4
1362.177	19	4733.3 ± 1132.7 3593.3 ± 1132.7	27.0 ± 9.4 23.4 ± 11.3	44.8 ± 2.1 47.6 ± 2.1	-4.8 ± 1.8 -3.7 ± 2.3	131.9 ± 16.6 130.6 ± 16.6	-3.8 ± 3.1 -7.8 ± 3.7	48.2 ± 1.8 48.1 ± 1.8	-1.0 ± 1.0 -1.9 ± 1.2	26.1 ± 1.3 26.6 ± 1.3	1.9 ± 1.4 5.5 ± 1.9	16.0 ± 1.1 20.2 ± 1.1	-21.3 ± 4.4 -21.3 ± 5.8
1362.179	20	3960.4 ± 1132.1 3090.9 ± 1132.1	13.6 ± 9.4 5.9 ± 11.3	46.3 ± 2.1 48.1 ± 2.1	-2.4 ± 1.8 -0.9 ± 2.3	131.4 ± 16.6 135.2 ± 16.6	-5.6 ± 3.1 -7.7 ± 3.7	49.1 ± 1.8 47.7 ± 1.8	1.5 ± 1.0 0.05 ± 1.2	24.6 ± 1.3 26.2 ± 1.3	-2.6 ± 1.3 1.5 ± 1.9	18.1 ± 1.1 23.7 ± 1.1	-19.4 ± 4.4 -13.7 ± 5.8
1362.180	21	4221.3 ± 1132.4 4310.1 ± 1132.4	7.3 ± 9.4 18.0 ± 11.3	45.8 ± 2.1 46.6 ± 2.1	-2.6 ± 1.8 -3.7 ± 2.3	135.6 ± 16.6 130.6 ± 16.6	-1.1 ± 3.1 -4.9 ± 3.7	46.7 ± 1.8 46.8 ± 1.8	-2.0 ± 1.0 -3.7 ± 1.2	27.2 ± 1.3 27.5 ± 1.3	2.8 ± 1.3 7.1 ± 1.9	18.3 ± 1.1 20.2 ± 1.1	-12.5 ± 4.4 -14.0 ± 5.8
1363.164	22	3232.0 ± 1144.3 3612.7 ± 1132.4	-21.3 ± 9.4 -15.1 ± 11.3	47.2 ± 2.1 49.1 ± 2.1	-2.1 ± 2 -1.1 ± 2.6	135.6 ± 16.6 134.9 ± 16.6	-3.0 ± 3.1 -6.6 ± 3.7	44.9 ± 1.8 45.1 ± 1.8	-3.1 ± 1.0 -5.9 ± 1.2	27.3 ± 1.3 27.6 ± 1.3	2.0 ± 1.3 5.3 ± 1.9	16.4 ± 1.1 17.1 ± 1.1	-20.6 ± 4.4 -32.2 ± 5.8
1363.165	23	4201.9 ± 1132.2 4191.8 ± 1144.0	12.9 ± 10.9 13.7 ± 11.3	45.3 ± 2.1 46.6 ± 2.1	-4.0 ± 1.8 -4.8 ± 2.3	139.4 ± 16.6 135.2 ± 16.6	0.0 ± 3.1 -2.7 ± 3.7	48.7 ± 1.8 48.6 ± 1.9	0.02 ± 1.2 -0.2 ± 1.2	25.1 ± 1.3 25.0 ± 1.3	1.7 ± 1.6 2.2 ± 1.9	14.5 ± 1.1 14.8 ± 1.3	-24.3 ± 4.9 -38.2 ± 5.8
1363.168	24	4620.7 ± 1132.3 3815.7 ± 1132.3	19.4 ± 9.4 14.5 ± 11.3	45.6 ± 2.1 47.8 ± 2.1	-4.2 ± 1.8 -3.8 ± 2.3	138.2 ± 16.6 137.9 ± 16.6	-1.9 ± 3.1 -4.4 ± 3.7	47.9 ± 1.8 48.3 ± 1.8	-0.7 ± 1.0 -0.5 ± 1.2	25.9 ± 1.3 25.6 ± 1.3	1.5 ± 1.3 2.4 ± 1.9	15.1 ± 1.1 16.6 ± 1.1	-28.2 ± 4.4 -40.9 ± 5.8
1363.170	25	4323.6 ± 1132.5 3887.6 ± 1132.5	7.0 ± 10.9 12.3 ± 12.8	47.0 ± 2.1 47.7 ± 2.1	-0.5 ± 1.8 0.4 ± 2.3	137.9 ± 16.6 139.6 ± 16.6	-1.6 ± 3.1 -1.3 ± 3.7	47.0 ± 1.8 46.4 ± 1.8	-0.4 ± 1.0 -1.9 ± 1.2	27.3 ± 1.3 27.2 ± 1.3	3.0 ± 1.3 5.4 ± 1.9	15.4 ± 1.1 16.8 ± 1.1	-20.5 ± 4.4 -29.8 ± 5.8
1363.171	26	4125.1 ± 1132.9 3894.4 ± 1132.9	5.2 ± 9.4 5.5 ± 11.3	46.7 ± 2.1 48.2 ± 2.1	-1.7 ± 1.8 -0.03 ± 2.3	137.7 ± 16.6 135.2 ± 16.6	1.2 ± 3.1 0.1 ± 3.7	47.1 ± 1.8 46.4 ± 1.8	-0.4 ± 1.0 -2.6 ± 1.2	26.5 ± 1.3 27.1 ± 1.3	0.3 ± 1.4 4.1 ± 1.9	15.0 ± 1.1 15.2 ± 1.1	-19.8 ± 4.4 -32.6 ± 5.8
1363.173	27	3546.8 ± 1132.7 3459.3 ± 1144.6	4.1 ± 10.9 -8.5 ± 11.3	45.2 ± 2.1 44.4 ± 2.1	-1.1 ± 1.8 -3.8 ± 2.3	134.3 ± 16.6 128.8 ± 16.6	2.9 ± 3.1 1.5 ± 3.7	47.2 ± 1.8 47.2 ± 1.8	-1.4 ± 1.0 -3.1 ± 1.2	25.7 ± 1.3 26.0 ± 1.3	0.4 ± 1.4 2.7 ± 1.9	14.2 ± 1.1 15.5 ± 1.1	-22.5 ± 4.4 -32.5 ± 5.8
1363.177	28	3969.8 ± 1132.6 4194.0 ± 1169.0	12.8 ± 18.8 -3.1 ± 12.8	45.1 ± 2.1 48.1 ± 2.1	-5.3 ± 1.8 -4.5 ± 2.3	127.6 ± 16.6 126.6 ± 16.8	-4.9 ± 3.4 -9.1 ± 3.7	47.0 ± 1.8 46.5 ± 1.9	-0.8 ± 1.2 -3.5 ± 1.2	26.8 ± 1.3 27.5 ± 1.3	0.0 ± 1.6 6.3 ± 1.9	14.1 ± 1.1 13.2 ± 1.3	-23.8 ± 4.9 -39.9 ± 5.8
1363.178	29	4197.9 ± 1132.1 3823.6 ± 1143.8	6.8 ± 10.9 6.1 ± 11.3	43.2 ± 2.1 44.7 ± 2.1	-5.4 ± 1.8 -7.3 ± 2.3	126.1 ± 16.6 130.4 ± 16.6	-9.4 ± 3.1 -14.2 ± 3.7	48.6 ± 1.8 47.4 ± 1.9	0.4 ± 1.2 1.0 ± 1.2	26.1 ± 1.3 26.6 ± 1.3	1.6 ± 1.6 3.8 ± 1.9	15.4 ± 1.1 16.4 ± 1.3	-19.1 ± 4.9 -33.5 ± 5.8
1363.180	30	3339.7 ± 1132.0 3603.1 ± 1132.0	-17.7 ± 9.4 -22.6 ± 11.3	44.7 ± 2.1 44.2 ± 2.1	-3.5 ± 1.8 -7.6 ± 2.3	124.6 ± 16.6 119.4 ± 16.6	-4.3 ± 3.1 -11.1 ± 3.7	47.9 ± 1.9 48.5 ± 1.9	-1.1 ± 1.2 -1.3 ± 1.4	26.8 ± 1.3 26.4 ± 1.3	2.7 ± 1.5 4.6 ± 2.1	16.2 ± 1.3 16.5 ± 1.3	-16.3 ± 4.9 -25.6 ± 6.4
1363.181	31	3091.8 ± 1132.0 3117.5 ± 1132.0	-14.6 ± 9.4 -22.8 ± 11.3	44.6 ± 2.1 42.1 ± 2.1	0.6 ± 1.8 -4.4 ± 2.3	128.7 ± 16.8 116.9 ± 16.6	0.1 ± 3.4 -8.1 ± 4.0	48.6 ± 1.8 48.7 ± 1.8	0.7 ± 1.0 1.2 ± 1.2	25.5 ± 1.3 25.6 ± 1.3	-0.1 ± 1.3 0.2 ± 1.8	15.5 ± 1.1 15.4 ± 1.1	-20.6 ± 4.4 -34.4 ± 5.8
1363.182	32	3739.7 ± 1132.1 3461.8 ± 1233.7	-1.4 ± 18.8 -8.9 ± 11.3	43.7 ± 2.1 45.7 ± 2.1	-4.9 ± 1.8 -5.6 ± 2.3	118.5 ± 16.6 121.1 ± 16.8	-7.9 ± 3.4 -12.8 ± 3.7	46.4 ± 1.8 45.9 ± 1.9	-0.1 ± 1.2 -2.0 ± 1.2	27.3 ± 1.3 28.3 ± 1.3	2.1 ± 1.6 6.7 ± 1.9	16.2 ± 1.1 16.4 ± 1.3	-11.5 ± 4.9 -21.5 ± 5.8

[1] Reg. No. = Registration number
[2] Ent. No. = Entry number

Appendix: 4-14. Continued…

Reg. No.[1]	Ent.[2] No.	Seed yield (kg/ha)		Days to flowering (days)		Plant height (cm)		Seed oil content (%)		Seed protein content (%)		Seed glucosinolate content (µmol/g seed)	
		Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)
1363.183	33	4060.4 ± 1132.2 3418.0 ± 1132.2	11.2 ± 9.4 3.3 ± 11.3	45.8 ± 2.1 47.8 ± 2.1	-4.4 ± 1.8 -4.7 ± 2.3	136.8 ± 16.6 132.1 ± 16.6	0.5 ± 3.1 -2.8 ± 3.7	47.3 ± 1.8 46.1 ± 1.8	0.3 ± 1.0 -2.3 ± 1.2	26.0 ± 1.3 26.7 ± 1.3	-0.1 ± 1.3 3.1 ± 1.9	14.1 ± 1.1 16.1 ± 1.1	-31.1 ± 4.4 -43.6 ± 5.8
1363.185	34	3980.6 ± 1132.5 3675.5 ± 1132.5	6.8 ± 9.4 2.3 ± 11.3	43.8 ± 2.1 44.3 ± 2.1	-4.9 ± 1.8 -8.4 ± 2.3	131.8 ± 16.6 122.8 ± 16.6	-2.1 ± 3.1 -9.8 ± 3.7	48.8 ± 1.8 47.8 ± 1.8	1.9 ± 1.0 1.4 ± 1.2	25.7 ± 1.3 26.9 ± 1.3	-1.9 ± 1.3 1.0 ± 1.9	14.4 ± 1.1 15.1 ± 1.1	-25.0 ± 4.4 -38.0 ± 5.8
1363.186	35	4100.9 ± 1132.3 3819.9 ± 1132.3	2.2 ± 9.4 0.8 ± 11.3	43.0 ± 2.1 45.3 ± 2.1	-6.4 ± 1.8 -7.9 ± 2.3	133.9 ± 16.6 136.4 ± 16.6	-3.7 ± 3.1 -5.3 ± 3.7	48.5 ± 1.8 48.3 ± 1.8	1.1 ± 1.0 1.4 ± 1.2	26.2 ± 1.3 26.2 ± 1.3	0.9 ± 1.3 2.1 ± 1.9	14.4 ± 1.1 13.2 ± 1.1	-23.6 ± 4.4 -40.8 ± 5.8
1363.190	36	3602.1 ± 1143.6 3113.7 ± 1166.3	3.3 ± 13.3 -12.5 ± 12.8	42.9 ± 2.1 43.4 ± 2.1	-6.0 ± 1.8 -10.8 ± 2.3	127.1 ± 16.6 127.9 ± 16.6	-4.1 ± 3.1 -6.6 ± 3.7	48.3 ± 1.8 47.2 ± 1.9	0.1 ± 1.2 -0.1 ± 1.2	25.3 ± 1.3 26.6 ± 1.3	-1.8 ± 1.6 2.1 ± 1.9	16.3 ± 1.1 19.4 ± 1.3	-18.9 ± 4.9 -33.3 ± 5.8
1363.194	37	3522.1 ± 1132.6 3220.0 ± 1144.4	-11.7 ± 10.9 -1.9 ± 11.3	43.5 ± 2.1 48.0 ± 2.1	-7.9 ± 1.8 -6.6 ± 2.3	135.3 ± 16.6 134.6 ± 16.6	-1.6 ± 3.1 -4.3 ± 3.7	47.3 ± 1.8 46.5 ± 1.8	-1.0 ± 1.0 -3.4 ± 1.2	25.7 ± 1.3 26.9 ± 1.3	-0.6 ± 1.4 3.1 ± 1.9	19.5 ± 1.1 23.8 ± 1.1	-15.0 ± 4.4 -10.7 ± 5.8
1363.195	38	4008.2 ± 1132.4 3041.8 ± 1132.4	19.6 ± 9.4 7.9 ± 11.3	44.9 ± 2.1 48.6 ± 2.1	-7.9 ± 1.8 -7.9 ± 2.3	131.6 ± 16.6 129.6 ± 16.6	-3.4 ± 3.1 -7.6 ± 3.7	47.4 ± 1.8 46.2 ± 1.8	-0.3 ± 1.0 -3.2 ± 1.2	26.4 ± 1.3 27.4 ± 1.3	1.7 ± 1.3 7.4 ± 1.9	19.9 ± 1.1 22.5 ± 1.1	-16.3 ± 4.4 -20.3 ± 5.8
1363.197	39	3543.5 ± 1132.4 3078.0 ± 1132.4	1.6 ± 10.9 -3.3 ± 12.8	43.6 ± 2.1 43.1 ± 2.1	-3.6 ± 1.8 -7.5 ± 2.3	129.9 ± 16.6 125.2 ± 16.6	-1.9 ± 3.1 -6.9 ± 3.7	46.8 ± 1.9 45.6 ± 1.8	0.2 ± 1.2 -1.8 ± 1.4	27.5 ± 1.3 28.8 ± 1.3	1.2 ± 1.6 6.7 ± 2.1	17.4 ± 1.3 20.7 ± 1.1	-20.8 ± 4.9 -24.7 ± 6.4
1363.202	40	3027.4 ± 1143.6 3441.6 ± 1166.7	-15.8 ± 13.3 -18.1 ± 12.7	44.7 ± 2.1 45.7 ± 2.1	-5.2 ± 1.8 -8.0 ± 2.3	134.4 ± 16.6 129.9 ± 16.6	-0.1 ± 3.1 -3.3 ± 3.7	44.3 ± 1.9 41.0 ± 1.9	0.2 ± 1.4 -6.8 ± 1.7	28.9 ± 1.3 31.3 ± 1.3	0.7 ± 1.9 11.4 ± 2.5	17.1 ± 1.3 18.8 ± 1.3	-19.4 ± 5.8 -27.9 ± 7.4
1363.205	41	3698.3 ± 1132.1 2994.6 ± 1132.1	10.0 ± 9.4 1.1 ± 11.3	47.6 ± 2.1 52.3 ± 2.1	-3.8 ± 1.8 2.5 ± 2.3	138.9 ± 16.6 138.2 ± 16.6	1.6 ± 3.1 1.8 ± 3.7	48.4 ± 1.8 48.6 ± 1.8	-0.3 ± 1.0 -0.6 ± 1.2	25.5 ± 1.3 24.5 ± 1.3	4.7 ± 1.3 5.6 ± 1.8	15.5 ± 1.1 15.4 ± 1.1	-9.8 ± 4.4 -18.5 ± 5.8
1363.206	42	3665.2 ± 1132.3 3867.7 ± 1132.3	-8.7 ± 9.4 -7.9 ± 11.3	47.6 ± 2.1 51.3 ± 2.1	-4.1 ± 1.8 -0.5 ± 2.3	144.6 ± 16.6 147.3 ± 16.6	0.6 ± 3.1 3.3 ± 3.7	46.9 ± 1.8 47.2 ± 1.8	-1.9 ± 1.0 -3.2 ± 1.2	26.7 ± 1.3 25.9 ± 1.3	4.7 ± 1.3 6.6 ± 1.9	15.4 ± 1.1 17.2 ± 1.1	-26.1 ± 4.4 -36.4 ± 5.8
1363.207	43	4305.9 ± 1132.4 3030.2 ± 1132.4	17.9 ± 9.4 3.0 ± 11.3	47.1 ± 2.1 51.8 ± 2.1	-4.2 ± 1.8 1.1 ± 2.3	138.9 ± 16.6 138.9 ± 16.6	0.2 ± 3.1 0.9 ± 3.7	48.1 ± 1.8 47.9 ± 1.8	0.04 ± 1.0 -0.2 ± 1.2	26.0 ± 1.3 26.2 ± 1.3	1.1 ± 1.3 2.8 ± 1.9	16.0 ± 1.1 17.1 ± 1.1	-18.2 ± 4.4 -26.3 ± 5.8
1363.208	44	4069.2 ± 1132.1 1977.8 ± 1143.8	26.4 ± 9.4 -7.0 ± 11.3	49.3 ± 2.1 51.6 ± 2.1	0.7 ± 2.0 5.2 ± 2.3	129.2 ± 16.6 130.7 ± 16.6	-5.7 ± 3.1 -9.3 ± 3.7	47.4 ± 1.8 44.7 ± 1.8	2.2 ± 1.0 -1.7 ± 1.2	26.5 ± 1.3 27.7 ± 1.3	-0.4 ± 1.3 4.3 ± 1.8	15.4 ± 1.1 20.7 ± 1.1	-30.6 ± 4.4 -32.9 ± 5.8
1363.211	45	3671.4 ± 1132.4 2831.7 ± 1166.5	16.5 ± 10.9 -7.1 ± 11.3	48.2 ± 2.1 52.7 ± 2.1	-4.9 ± 1.8 -0.9 ± 2.3	142.7 ± 16.6 138.5 ± 16.6	0.9 ± 3.1 -0.8 ± 3.7	47.3 ± 1.8 47.1 ± 1.8	-0.8 ± 1.0 -2.2 ± 1.2	26.8 ± 1.3 26.3 ± 1.3	3.9 ± 1.3 6.7 ± 1.9	16.0 ± 1.1 19.6 ± 1.1	-26.9 ± 4.4 -32.5 ± 5.8
1681.082	46	3504.9 ± 1144.5 2572.7 ± 1144.5	12.0 ± 10.9 -5.6 ± 12.8	45.4 ± 2.1 45.0 ± 2.1	-2.6 ± 2.0 -4.1 ± 2.3	138.3 ± 16.6 115.5 ± 16.6	6.3 ± 3.1 -4.6 ± 3.7	47.4 ± 1.8 46.4 ± 1.8	0.4 ± 1.0 -1.4 ± 1.2	25.2 ± 1.3 25.0 ± 1.3	0.7 ± 1.3 0.9 ± 1.9	14.5 ± 1.1 14.4 ± 1.1	-19.6 ± 4.4 -32.1 ± 5.8
1681.083	47	3703.1 ± 1144.1 2714.5 ± 1144.1	5.4 ± 10.9 -14.5 ± 12.8	46.5 ± 2.1 47.7 ± 2.1	-2.7 ± 1.8 -2.9 ± 2.3	133.2 ± 16.6 123.2 ± 16.6	1.3 ± 3.1 -5.0 ± 3.7	46.7 ± 1.9 46.3 ± 1.8	-1.4 ± 1.2 -3.5 ± 1.4	25.6 ± 1.3 25.4 ± 1.3	2.6 ± 1.6 3.0 ± 2.1	15.4 ± 1.3 15.3 ± 1.1	-21.7 ± 4.9 -32.7 ± 6.4
1681.084	48	3526.6 ± 1132.4 3020.1 ± 1132.4	1.6 ± 9.4 -12.1 ± 11.3	44.4 ± 2.1 45.5 ± 2.1	-8.0 ± 2.0 -7.5 ± 2.3	130.1 ± 16.6 126.8 ± 16.6	-3.5 ± 3.1 -8.9 ± 3.7	46.8 ± 1.8 45.0 ± 1.8	-1.4 ± 1.0 -6.0 ± 1.2	25.5 ± 1.3 26.8 ± 1.3	0.4 ± 1.3 6.1 ± 1.9	16.9 ± 1.1 22.2 ± 1.1	-24.9 ± 4.4 -25.1 ± 5.8

[1] Reg. No. = Registration number
[2] Ent. No. = Entry number

Appendix: 4-14. Continued…

Reg. No.[1]	Ent.[2] No.	Seed yield (kg/ha)		Days to flowering (days)		Plant height (cm)		Seed oil content (%)		Seed protein content (%)		Seed glucosinolate content (µmol/g seed)	
		Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)
1681.085	49	3327.6 ± 1132.3 3469.0 ± 1143.8	1.6 ± 10.9 -15.1 ± 11.3	46.8 ± 2.1 44.3 ± 2.1	1.1 ± 2.0 -2.9 ± 2.3	127.6 ± 16.6 123.1 ± 16.6	-3.3 ± 3.1 -9.8 ± 3.7	46.9 ± 1.8 47.1 ± 1.9	-1.7 ± 1.2 -3.1 ± 1.2	25.9 ± 1.3 26.1 ± 1.3	1.4 ± 1.6 2.3 ± 1.9	16.6 ± 1.1 19.1 ± 1.3	-20.8 ± 4.9 -25.6 ± 5.8
1681.086	50	3277.8 ± 1132.5 2857.1 ± 1132.5	-0.03 ± 9.4 -8.2 ± 11.3	44.5 ± 2.1 48.0 ± 2.1	-5.3 ± 1.8 -3.4 ± 2.3	127.5 ± 16.6 122.7 ± 16.6	-3.0 ± 3.1 -9.5 ± 3.7	46.8 ± 1.8 46.3 ± 1.8	-1.5 ± 1.0 -3.8 ± 1.2	26.4 ± 1.3 26.6 ± 1.3	1.8 ± 1.3 4.3 ± 1.9	15.5 ± 1.1 17.1 ± 1.1	-18.4 ± 4.4 -25.7 ± 5.8
1681.090	51	4195.9 ± 1132.4 3051.0 ± 1144.1	19.5 ± 9.4 1.2 ± 11.3	44.1 ± 2.1 46.1 ± 2.1	-6.4 ± 1.8 -8.3 ± 2.3	123.9 ± 16.6 107.9 ± 16.6	-0.5 ± 3.1 -12.4 ± 3.7	46.2 ± 1.8 44.8 ± 1.8	-0.8 ± 1.0 -4.3 ± 1.2	25.5 ± 1.3 26.2 ± 1.3	-1.2 ± 1.3 0.1 ± 1.9	18.5 ± 1.1 21.0 ± 1.1	-12.8 ± 4.4 -13.5 ± 5.8
1681.091	52	3933.4 ± 1144.1 2759.2 ± 1144.1	21.2 ± 10.9 -3.0 ± 12.8	43.8 ± 2.1 45.3 ± 2.1	-8.5 ± 2.0 -8.7 ± 2.3	117.9 ± 16.6 114.9 ± 16.6	-7.7 ± 3.1 -16.1 ± 3.7	46.4 ± 1.9 44.9 ± 1.8	-1.0 ± 1.2 -5.0 ± 1.4	25.9 ± 1.3 26.8 ± 1.3	-0.1 ± 1.6 3.5 ± 2.1	18.1 ± 1.3 20.1 ± 1.1	-16.5 ± 4.9 -21.8 ± 6.4
1681.092	53	3689.3 ± 1143.8 2977.8 ± 1166.8	20.1 ± 10.9 5.6 ± 12.8	46.4 ± 2.1 45.6 ± 2.1	-2.4 ± 2.5 -4.3 ± 2.6	127.0 ± 16.6 115.5 ± 16.6	0.4 ± 3.1 -8.5 ± 3.7	46.3 ± 1.9 45.1 ± 1.8	-0.1 ± 1.2 -4.0 ± 1.4	25.7 ± 1.3 25.6 ± 1.3	-0.8 ± 1.6 1.3 ± 2.1	17.3 ± 1.3 18.2 ± 1.1	-14.3 ± 4.9 -18.9 ± 6.4
1681.096	54	4123.9 ± 1132.2 3266.8 ± 1144.1	32.5 ± 9.4 15.3 ± 11.3	45.1 ± 2.1 45.8 ± 2.1	-1.8 ± 1.8 -2.0 ± 2.3	124.2 ± 16.6 114.2 ± 16.6	-3.6 ± 3.1 -13.4 ± 3.7	45.0 ± 1.8 44.5 ± 1.8	-2.5 ± 1.0 -6.0 ± 1.2	26.7 ± 1.3 26.9 ± 1.3	2.0 ± 1.3 5.0 ± 1.9	18.4 ± 1.1 20.1 ± 1.1	-12.3 ± 4.4 -15.1 ± 5.8
1681.097	55	4377.8 ± 1143.7 3684.2 ± 1143.7	15.9 ± 10.9 13.8 ± 12.7	46.1 ± 2.1 45.3 ± 2.1	-1.7 ± 2.0 -1.5 ± 2.3	134.1 ± 16.6 129.9 ± 16.6	0.1 ± 3.1 -3.1 ± 3.7	45.6 ± 1.9 45.6 ± 1.8	-1.9 ± 1.2 -4.3 ± 1.4	26.7 ± 1.3 26.5 ± 1.3	1.6 ± 1.6 2.6 ± 2.1	16.9 ± 1.3 20.9 ± 1.1	-21.3 ± 4.9 -22.4 ± 6.4
1681.098	56	3841.1 ± 1144.3 2517.6 ± 1144.3	17.2 ± 10.9 -1.3 ± 12.8	43.0 ± 2.1 45.6 ± 2.1	-8.9 ± 2.0 -7.5 ± 2.3	132.0 ± 16.6 127.8 ± 16.6	0.4 ± 3.1 -2.6 ± 3.7	47.3 ± 1.9 45.4 ± 1.9	0.5 ± 1.2 -2.8 ± 1.4	25.0 ± 1.3 26.4 ± 1.3	-2.1 ± 1.6 1.1 ± 2.1	19.5 ± 1.3 23.9 ± 1.3	-13.6 ± 4.9 -7.5 ± 6.4
1681.100	57	3807.6 ± 1143.7 3604.4 ± 1143.7	-22.3 ± 9.4 -28.9 ± 11.3	46.8 ± 2.1 45.6 ± 2.1	-0.6 ± 2.0 -2.6 ± 2.3	133.6 ± 16.6 127.6 ± 16.6	0.1 ± 3.1 -4.3 ± 3.7	45.3 ± 1.8 44.8 ± 1.8	-2.9 ± 1.0 -6.9 ± 1.2	26.6 ± 1.3 26.8 ± 1.3	1.6 ± 1.3 4.7 ± 1.8	17.2 ± 1.1 20.8 ± 1.1	-17.8 ± 4.4 -17.0 ± 5.8
1681.101	58	4426.7 ± 1132.3 3487.3 ± 1132.3	23.6 ± 9.4 16.2 ± 11.3	42.4 ± 2.1 46.4 ± 2.1	-9.5 ± 1.8 -10.8 ± 2.3	126.7 ± 16.6 124.0 ± 16.6	-4.7 ± 3.1 -11.2 ± 3.7	45.7 ± 1.8 44.8 ± 1.8	-2.2 ± 1.0 -6.3 ± 1.2	27.1 ± 1.3 27.5 ± 1.3	2.6 ± 1.3 7.5 ± 1.9	18.0 ± 1.1 21.3 ± 1.1	-15.0 ± 4.4 -13.7 ± 5.8
1681.102	59	4251.8 ± 1132.4 3457.3 ± 1132.4	13.7 ± 9.4 4.6 ± 11.3	46.5 ± 2.1 46.8 ± 2.1	-0.8 ± 1.8 -1.3 ± 2.3	138.8 ± 16.6 130.1 ± 16.6	2.7 ± 3.1 -1.7 ± 3.7	46.3 ± 1.8 44.8 ± 1.8	0.0 ± 1.0 -3.2 ± 1.2	26.1 ± 1.3 27.1 ± 1.3	-1.0 ± 1.3 2.0 ± 1.9	17.5 ± 1.1 22.2 ± 1.1	-24.5 ± 4.4 -26.9 ± 5.8
1681.103	60	4001.0 ± 1132.3 3959.0 ± 1132.3	1.1 ± 9.4 -2.5 ± 11.3	45.6 ± 2.1 44.6 ± 2.1	-1.5 ± 1.8 -5.2 ± 2.3	132.2 ± 16.6 129.0 ± 16.6	-2.3 ± 3.1 -7.1 ± 3.7	46.5 ± 1.8 44.7 ± 1.8	-0.2 ± 1.0 -4.0 ± 1.2	25.9 ± 1.3 27.0 ± 1.3	-0.9 ± 1.3 2.5 ± 1.9	17.4 ± 1.1 21.3 ± 1.1	-20.6 ± 4.4 -22.6 ± 5.8
1681.104	61	3767.9 ± 1132.5 3560.6 ± 1132.5	-3.7 ± 9.4 -9.5 ± 11.3	43.5 ± 2.1 45.7 ± 2.1	-5.0 ± 2.0 -6.5 ± 2.6	128.0 ± 16.6 124.0 ± 16.6	-3.7 ± 3.1 -9.5 ± 3.7	46.6 ± 1.8 44.3 ± 1.8	0.2 ± 1.0 -4.4 ± 1.2	25.8 ± 1.3 27.0 ± 1.3	-1.2 ± 1.3 2.9 ± 1.9	16.8 ± 1.1 20.9 ± 1.1	-23.9 ± 4.4 -26.5 ± 5.8
1681.105	62	4661.4 ± 1132.5 4025.2 ± 1143.7	15.3 ± 10.9 20.1 ± 11.3	44.9 ± 2.1 44.8 ± 2.3	0.9 ± 2.5 -2.8 ± 2.6	136.3 ± 16.6 124.8 ± 16.8	4.9 ± 3.4 -4.4 ± 3.7	46.4 ± 1.8 44.8 ± 1.9	-1.3 ± 1.2 -4.8 ± 1.2	26.1 ± 1.3 26.7 ± 1.3	1.5 ± 1.6 3.8 ± 1.9	15.2 ± 1.1 17.1 ± 1.3	-22.1 ± 4.9 -30.4 ± 5.8
1682.099	63	3776.6 ± 1133.0 3680.6 ± 1133.0	0.2 ± 9.4 -1.7 ± 11.3	46.1 ± 2.1 46.4 ± 2.1	-0.8 ± 1.8 -1.5 ± 2.3	145.7 ± 16.6 137.0 ± 16.6	4.2 ± 3.1 2.1 ± 3.7	49.0 ± 1.8 49.7 ± 1.8	0.0 ± 1.0 1.7 ± 1.2	24.9 ± 1.3 24.7 ± 1.3	-0.4 ± 1.4 -1.7 ± 1.9	12.8 ± 1.1 13.8 ± 1.1	-28.1 ± 4.4 -41.4 ± 5.8
1682.100	64	4051.6 ± 1132.1 4171.3 ± 1132.1	-0.1 ± 9.4 1.8 ± 11.3	46.0 ± 2.1 45.0 ± 2.1	-0.1 ± 1.8 -2.5 ± 2.3	133.4 ± 16.6 127.1 ± 16.6	-0.4 ± 3.1 -5.4 ± 3.7	49.7 ± 1.8 49.2 ± 1.8	2.2 ± 1.0 2.9 ± 1.2	24.7 ± 1.3 25.5 ± 1.3	-2.8 ± 1.3 -2.1 ± 1.8	13.1 ± 1.1 13.9 ± 1.1	-31.3 ± 4.4 -45.3 ± 5.8

[1] Reg. No. = Registration number
[2] Ent. No. = Entry number

Appendix: 4-14. Continued…

Reg. No.[1]	Ent.[2] No.	Seed yield (kg/ha)		Days to flowering (days)		Plant height (cm)		Seed oil content (%)		Seed protein content (%)		Seed glucosinolate content (µmol/g seed)	
		Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)
1682.101	65	4536.9 ± 1132.2 4292.9 ± 1132.2	13.6 ± 9.4 31.6 ± 11.3	44.9 ± 2.1 44.1 ± 2.1	-0.8 ± 1.8 -3.1 ± 2.3	133.2 ± 16.6 130.4 ± 16.6	-1.8 ± 3.1 -5.1 ± 3.7	48.8 ± 1.8 48.7 ± 1.8	0.5 ± 1.0 0.3 ± 1.2	25.1 ± 1.3 25.3 ± 1.3	-0.2 ± 1.3 0.2 ± 1.8	18.8 ± 1.1 20.8 ± 1.1	-10.6 ± 4.4 -10.1 ± 5.8
1682.102	66	3386.4 ± 1132.4 3010.5 ± 1132.4	-1.9 ± 9.4 -9.9 ± 11.3	45.4 ± 2.1 46.3 ± 2.1	-2.0 ± 2.0 -3.7 ± 2.6	126.3 ± 16.6 129.8 ± 16.6	-6.9 ± 3.1 -10.5 ± 3.7	48.6 ± 1.8 49.5 ± 1.8	-0.4 ± 1.0 1.0 ± 1.2	25.4 ± 1.3 25.5 ± 1.3	0.5 ± 1.3 1.0 ± 1.9	15.7 ± 1.1 18.3 ± 1.1	-22.8 ± 4.4 -30.0 ± 5.8
1682.103	67	3562.8 ± 1132.1 2967.1 ± 1143.6	5.7 ± 10.9 -10.8 ± 11.3	42.9 ± 2.1 45.2 ± 2.1	-7.7 ± 1.8 -10.3 ± 2.3	131.4 ± 16.6 130.4 ± 16.6	-2.3 ± 3.1 -5.2 ± 3.7	47.5 ± 1.8 47.9 ± 1.9	-1.6 ± 1.2 -2.9 ± 1.2	26.3 ± 1.3 26.3 ± 1.3	2.0 ± 1.6 6.9 ± 1.8	16.4 ± 1.1 20.0 ± 1.3	-23.3 ± 4.9 -25.2 ± 5.8
1682.104	68	4001.8 ± 1132.4 3689.6 ± 1132.4	-2.2 ± 9.4 -8.3 ± 11.3	44.7 ± 2.1 43.7 ± 2.1	-0.5 ± 1.8 -3.3 ± 2.3	136.1 ± 16.6 132.1 ± 16.6	-0.6 ± 3.1 -3.4 ± 3.7	48.1 ± 1.8 49.0 ± 1.8	-1.1 ± 1.0 -0.4 ± 1.2	26.4 ± 1.3 26.1 ± 1.3	3.0 ± 1.3 4.7 ± 1.9	17.2 ± 1.1 19.6 ± 1.1	-16.8 ± 4.4 -21.2 ± 5.8
1682.105	69	3573.4 ± 1132.5 3289.5 ± 1132.5	0.6 ± 9.4 -3.8 ± 11.3	44.6 ± 2.1 44.3 ± 2.1	-2.0 ± 1.8 -4.2 ± 2.3	125.3 ± 16.6 124.8 ± 16.6	-6.4 ± 3.1 -12.3 ± 3.7	47.3 ± 1.8 48.5 ± 1.8	-1.9 ± 1.0 -1.3 ± 1.2	26.6 ± 1.3 26.5 ± 1.3	2.4 ± 1.3 4.0 ± 1.9	16.7 ± 1.1 19.0 ± 1.1	-19.7 ± 4.4 -25.9 ± 5.8
1682.108	70	3244.5 ± 1132.2 3219.1 ± 1132.2	-11.8 ± 9.4 -22.1 ± 11.3	43.7 ± 2.1 44.5 ± 2.1	-4.5 ± 1.8 -6.6 ± 2.3	127.9 ± 16.6 126.9 ± 16.6	-4.5 ± 3.1 -8.9 ± 3.7	47.6 ± 1.8 47.6 ± 1.8	0.3 ± 1.0 0.4 ± 1.2	26.2 ± 1.3 26.9 ± 1.3	-1.0 ± 1.3 0.3 ± 1.9	16.1 ± 1.1 17.8 ± 1.1	-22.3 ± 4.4 -31.1 ± 5.8
1682.113	71	4193.1 ± 1144.5 4082.9 ± 1143.6	4.7 ± 13.3 -0.5 ± 12.8	45.6 ± 2.1 46.4 ± 2.1	-2.3 ± 1.8 -2.5 ± 2.3	128.0 ± 16.6 138.0 ± 16.6	-9.6 ± 3.1 -10.8 ± 3.7	47.3 ± 1.9 47.1 ± 1.8	0.2 ± 1.2 -0.9 ± 1.4	26.4 ± 1.3 26.6 ± 1.3	0.5 ± 1.6 1.9 ± 2.1	13.2 ± 1.3 12.9 ± 1.1	-32.1 ± 4.9 -48.7 ± 6.4
1682.120	72	4120.7 ± 1133.5 3061.2 ± 1145.9	24.5 ± 9.4 0.6 ± 11.3	44.0 ± 2.1 46.8 ± 2.1	-5.5 ± 1.8 -5.1 ± 2.3	132.5 ± 16.6 130.7 ± 16.6	-3.1 ± 3.1 -6.0 ± 3.7	47.2 ± 1.8 45.5 ± 1.8	0.5 ± 1.0 -2.3 ± 1.2	26.5 ± 1.3 27.0 ± 1.3	1.4 ± 1.4 5.1 ± 1.9	17.9 ± 1.1 21.8 ± 1.1	-15.4 ± 4.4 -14.1 ± 5.8
1682.124	73	4270.8 ± 1144.4 4064.8 ± 1132.6	-9.9 ± 9.4 -9.6 ± 11.3	46.4 ± 2.1 51.4 ± 2.1	-4.8 ± 1.8 0.8 ± 2.3	138.3 ± 16.6 145.6 ± 16.6	-3.7 ± 3.1 -2.4 ± 3.7	47.5 ± 1.8 47.7 ± 1.8	-1.0 ± 1.0 -1.5 ± 1.2	26.2 ± 1.3 25.4 ± 1.3	2.9 ± 1.3 2.3 ± 1.9	13.9 ± 1.1 14.2 ± 1.1	-19.3 ± 4.4 -31.8 ± 5.8
1682.125	74	4512.0 ± 1144.2 4391.3 ± 1132.4	-3.4 ± 10.9 0.5 ± 12.8	48.7 ± 2.1 50.9 ± 2.1	-1.3 ± 1.8 1.9 ± 2.3	145.9 ± 16.6 139.9 ± 16.6	1.9 ± 3.1 0.1 ± 3.7	47.3 ± 1.9 47.6 ± 1.8	-2.0 ± 1.2 -3.3 ± 1.4	26.2 ± 1.3 26.3 ± 1.3	2.5 ± 1.6 4.6 ± 2.1	15.5 ± 1.3 17.1 ± 1.1	-23.5 ± 4.9 -31.9 ± 6.4
1682.128	75	3849.8 ± 1144.1 3622.7 ± 1144.2	-1.0 ± 10.9 -9.4 ± 12.8	43.4 ± 2.1 41.7 ± 2.1	-1.3 ± 1.8 -6.0 ± 2.3	129.6 ± 16.6 113.8 ± 16.6	2.6 ± 3.1 -6.9 ± 3.7	48.4 ± 1.9 48.9 ± 1.8	0.3 ± 1.2 1.9 ± 1.4	26.0 ± 1.3 25.7 ± 1.3	0.6 ± 1.6 -0.2 ± 2.1	16.0 ± 1.3 15.6 ± 1.1	-14.9 ± 4.9 -27.5 ± 6.4
1682.130	76	4152.8 ± 1132.4 3270.4 ± 1144.3	17.9 ± 9.4 5.0 ± 11.3	44.5 ± 2.1 46.0 ± 2.1	-5.1 ± 1.8 -7.2 ± 2.3	129.3 ± 16.6 118.5 ± 16.6	-1.5 ± 3.1 -10.4 ± 3.7	47.6 ± 1.8 48.8 ± 1.8	-2.2 ± 1.0 -1.7 ± 1.2	25.2 ± 1.3 25.4 ± 1.3	-0.7 ± 1.3 -0.6 ± 1.9	13.8 ± 1.1 13.1 ± 1.1	-21.6 ± 4.4 -38.0 ± 5.8
1682.131	77	3663.7 ± 1132.6 4141.1 ± 1144.6	-8.7 ± 10.9 -10.2 ± 11.3	43.2 ± 2.1 41.9 ± 2.1	-1.8 ± 1.8 -6.1 ± 2.3	121.9 ± 16.6 112.1 ± 16.6	-3.6 ± 3.1 -13.5 ± 3.7	48.2 ± 1.8 48.0 ± 1.8	0.0 ± 1.0 -0.2 ± 1.2	25.2 ± 1.3 25.5 ± 1.3	-0.6 ± 1.4 0.05 ± 1.9	13.7 ± 1.1 14.1 ± 1.1	-22.9 ± 4.4 -36.1 ± 5.8
1682.133	78	3270.0 ± 1166.8 3233.5 ± 1132.4	-22.5 ± 13.3 -37.9 ± 15.3	44.0 ± 2.3 40.6 ± 2.1	-0.9 ± 2.5 -6.8 ± 3.2	123.5 ± 17.0 112.1 ± 16.6	-3.0 ± 4.0 -16.9 ± 4.5	45.8 ± 1.9 47.2 ± 1.8	-3.5 ± 1.4 -4.9 ± 1.7	27.3 ± 1.4 26.8 ± 1.3	2.3 ± 1.9 5.8 ± 2.5	19.7 ± 1.5 18.1 ± 1.1	0.1 ± 5.8 -6.6 ± 7.5
1682.137	79	4230.8 ± 1144.2 3917.9 ± 1132.4	11.1 ± 10.9 8.0 ± 12.8	44.2 ± 2.1 42.2 ± 2.1	-1.9 ± 1.8 -7.6 ± 2.3	126.9 ± 16.6 107.9 ± 16.6	0.9 ± 3.1 -12.0 ± 3.7	48.0 ± 1.8 48.4 ± 1.8	-1.0 ± 1.0 -0.9 ± 1.2	25.2 ± 1.3 25.6 ± 1.3	-0.4 ± 1.3 0.4 ± 1.9	15.0 ± 1.1 15.4 ± 1.1	-20.2 ± 4.4 -32.6 ± 5.8
1682.138	80	4138.7 ± 1143.8 3513.7 ± 1132.3	20.1 ± 10.9 9.2 ± 12.8	44.2 ± 2.1 41.4 ± 2.1	1.3 ± 2.0 -3.5 ± 2.6	123.2 ± 16.8 102.4 ± 16.6	5.6 ± 3.4 -10.3 ± 4.0	48.3 ± 1.9 47.4 ± 1.8	0.9 ± 1.2 -0.1 ± 1.4	25.7 ± 1.3 26.4 ± 1.3	-0.6 ± 1.6 1.6 ± 2.1	14.3 ± 1.3 17.8 ± 1.1	-29.0 ± 4.9 -36.2 ± 6.4

[1] Reg. No. = Registration number
[2] Ent. No. = Entry number

Appendix: 4-14. Continued…

Reg. No.[1]	Ent.[2] No.	Seed yield (kg/ha)		Days to flowering (days)		Plant height (cm)		Seed oil content (%)		Seed protein content (%)		Seed glucosinolate content (µmol/g seed)	
		Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)	Test-hybrid Inbred line	MPH (%) 73NAH (%)
1682.140	81	3891.3 ± 1132.4 3186.0 ± 1132.4	12.4 ± 9.4 1.1 ± 11.3	41.5 ± 2.1 42.5 ± 2.1	-8.0 ± 1.8 -13.0 ± 2.3	119.3 ± 16.6 113.8 ± 16.6	-7.2 ± 3.1 -16.5 ± 3.7	48.0 ± 1.8 47.5 ± 1.8	-0.2 ± 1.0 -1.6 ± 1.2	25.0 ± 1.3 25.8 ± 1.3	-1.4 ± 1.3 0.4 ± 1.9	14.6 ± 1.1 17.2 ± 1.1	-27.2 ± 4.4 -36.4 ± 5.8
1682.143	82	4081.9 ± 1132.5 3844.3 ± 1132.5	-1.3 ± 9.4 -8.6 ± 11.3	42.7 ± 2.1 42.4 ± 2.1	-4.1 ± 1.8 -8.5 ± 2.3	122.2 ± 16.6 119.4 ± 16.6	-7.8 ± 3.1 -15.4 ± 3.7	47.7 ± 1.8 48.2 ± 1.8	-1.9 ± 1.0 -2.6 ± 1.2	25.7 ± 1.3 25.5 ± 1.3	2.5 ± 1.3 4.1 ± 1.9	14.2 ± 1.1 14.0 ± 1.1	-25.9 ± 4.4 -41.5 ± 5.8
1682.145	83	3840.3 ± 1143.8 3349.9 ± 1132.2	13.5 ± 13.3 8.3 ± 15.3	43.6 ± 2.1 42.4 ± 2.1	-1.8 ± 1.8 -5.7 ± 2.3	123.0 ± 16.6 112.5 ± 16.6	-2.9 ± 3.1 -12.7 ± 3.7	48.5 ± 1.8 48.7 ± 1.8	-0.1 ± 1.0 0.2 ± 1.2	25.0 ± 1.3 25.0 ± 1.3	0.01 ± 1.3 0.1 ± 1.9	14.6 ± 1.1 16.2 ± 1.1	-20.9 ± 4.4 -29.5 ± 5.8
1682.147	84	3632.5 ± 1145.8 4171.9 ± 1133.4	-13.9 ± 10.9 -14.1 ± 12.8	44.2 ± 2.1 44.2 ± 2.1	-3.5 ± 1.8 -6.8 ± 2.3	128.1 ± 16.6 130.1 ± 16.6	-5.6 ± 3.1 -9.7 ± 3.7	48.5 ± 1.8 48.5 ± 1.8	-0.3 ± 1.0 -0.2 ± 1.2	24.8 ± 1.3 25.3 ± 1.3	-1.8 ± 1.4 -1.8 ± 1.9	13.3 ± 1.1 11.7 ± 1.1	-18.4 ± 4.4 -36.5 ± 5.8
1682.149	85	3591.1 ± 1132.4 3320.6 ± 1132.4	1.5 ± 9.4 -9.1 ± 11.3	42.5 ± 2.1 42.5 ± 2.1	-5.2 ± 1.8 -10.0 ± 2.3	125.6 ± 16.6 111.1 ± 16.6	0.6 ± 3.1 -9.4 ± 3.7	47.2 ± 1.8 47.2 ± 1.8	-1.6 ± 1.0 -3.3 ± 1.2	26.1 ± 1.3 26.4 ± 1.3	1.7 ± 1.3 4.6 ± 1.9	15.8 ± 1.1 17.1 ± 1.1	-19.4 ± 4.4 -29.1 ± 5.8
1682.150	86	3284.5 ± 1132.4 2677.4 ± 1132.4	-5.3 ± 9.4 -19.4 ± 11.3	45.4 ± 2.1 45.2 ± 2.1	-1.2 ± 1.8 -3.2 ± 2.3	121.7 ± 16.6 106.2 ± 16.6	-2.3 ± 3.1 -14.2 ± 3.7	47.5 ± 1.8 46.8 ± 1.8	-0.7 ± 1.0 -2.8 ± 1.2	25.5 ± 1.3 26.2 ± 1.3	0.1 ± 1.3 3.5 ± 1.9	15.9 ± 1.1 18.3 ± 1.1	-18.8 ± 4.4 -23.2 ± 5.8
1682.152	87	4024.0 ± 1144.4 3716.3 ± 1144.4	1.7 ± 13.3 4.0 ± 12.8	44.0 ± 2.1 43.8 ± 2.1	-2.4 ± 1.8 -5.3 ± 2.3	122.5 ± 16.6 117.5 ± 16.6	-6.0 ± 3.1 -14.1 ± 3.7	46.4 ± 1.8 46.8 ± 1.9	-2.8 ± 1.2 -3.4 ± 1.2	26.6 ± 1.3 26.4 ± 1.3	2.6 ± 1.6 3.2 ± 1.9	15.7 ± 1.1 15.8 ± 1.3	-16.1 ± 4.9 -30.4 ± 5.8
1682.154	88	3661.0 ± 1144.1 3587.5 ± 1144.3	-2.1 ± 13.3 -2.7 ± 12.8	45.4 ± 2.1 45.1 ± 2.1	-0.7 ± 1.8 -1.5 ± 2.3	127.3 ± 16.6 126.0 ± 16.6	-2.9 ± 3.1 -6.8 ± 3.7	47.8 ± 1.9 47.3 ± 1.8	-1.5 ± 1.2 -3.3 ± 1.4	25.4 ± 1.3 25.7 ± 1.3	2.3 ± 1.6 4.0 ± 2.1	14.6 ± 1.3 15.0 ± 1.1	-23.0 ± 4.9 -36.7 ± 6.4
1682.155	89	4042.1 ± 1132.3 3776.9 ± 1132.3	9.9 ± 10.9 10.1 ± 12.8	42.1 ± 2.1 43.1 ± 2.1	-4.7 ± 2.0 -8.9 ± 2.6	126.2 ± 16.6 122.7 ± 16.6	-1.7 ± 3.1 -6.3 ± 3.7	47.2 ± 1.8 47.0 ± 1.8	-1.0 ± 1.0 -2.5 ± 1.2	26.3 ± 1.3 26.7 ± 1.3	1.3 ± 1.3 3.8 ± 1.9	15.1 ± 1.1 15.6 ± 1.1	-16.9 ± 4.4 -27.3 ± 5.8
1682.156	90	3844.8 ± 1132.6 3450.3 ± 1132.6	10.3 ± 9.4 -2.3 ± 11.3	43.3 ± 2.1 43.6 ± 2.1	-4.7 ± 1.8 -8.6 ± 2.3	128.1 ± 16.6 120.6 ± 16.6	-1.5 ± 3.1 -7.9 ± 3.7	48.3 ± 1.8 47.9 ± 1.8	-0.8 ± 1.0 -2.4 ± 1.2	25.3 ± 1.3 25.5 ± 1.3	1.6 ± 1.3 3.6 ± 1.9	15.6 ± 1.1 15.5 ± 1.1	-15.7 ± 4.4 -28.0 ± 5.8
1682.158	91	3678.5 ± 1132.5 3608.4 ± 1132.5	-3.5 ± 10.9 -5.7 ± 12.8	45.1 ± 2.1 45.4 ± 2.1	-2.7 ± 1.8 -4.4 ± 2.3	131.6 ± 16.6 128.1 ± 16.6	-3.2 ± 3.1 -8.8 ± 3.7	46.9 ± 1.8 47.0 ± 1.8	-2.3 ± 1.0 -4.0 ± 1.2	26.1 ± 1.3 26.3 ± 1.3	2.3 ± 1.3 5.3 ± 1.9	14.0 ± 1.1 14.9 ± 1.1	-22.5 ± 4.4 -33.9 ± 5.8
1682.159	92	4604.2 ± 1132.5 3737.3 ± 1132.5	37.8 ± 9.4 38.4 ± 11.3	42.8 ± 2.1 43.3 ± 2.1	-3.5 ± 1.8 -5.8 ± 2.3	127.2 ± 16.6 122.7 ± 16.6	-2.4 ± 3.1 -9.0 ± 3.7	47.9 ± 1.8 46.9 ± 1.8	0.9 ± 1.0 -0.5 ± 1.2	25.8 ± 1.3 26.7 ± 1.3	-0.8 ± 1.3 1.8 ± 1.9	16.7 ± 1.1 15.0 ± 1.1	-11.7 ± 4.4 -26.0 ± 5.8
1682.164	93	3663.9 ± 1144.0 3578.6 ± 1167.0	7.4 ± 10.9 0.3 ± 12.8	46.4 ± 2.1 46.7 ± 2.1	-1.8 ± 1.8 -2.8 ± 2.3	140.8 ± 16.6 133.1 ± 16.6	2.4 ± 3.1 -0.8 ± 3.7	47.1 ± 1.9 46.4 ± 1.9	-0.7 ± 1.2 -2.6 ± 1.4	26.3 ± 1.3 26.4 ± 1.3	1.4 ± 1.6 2.7 ± 2.1	16.9 ± 1.3 18.0 ± 1.3	-8.6 ± 4.9 -9.9 ± 6.4
A04-73NA	94	3955.0 ± 1097.3		47.1 ± 1.8		141.3 ± 16.3		48.4 ± 1.7		25.2 ± 1.1		22.3 ± 0.4	

[1] Reg. No. = Registration number
[2] Ent. No. = Entry number

Appendix: 4-15. Summary of agronomic and seed quality traits of the 93 inbred lines derived from two *B. napus* × *B. oleracea* var. *capitata* interspecific crosses. Mean data of the four yield trials conducted in Alberta, Canada in 2016 presented

Reg. No.[1]	Entry no.	Cross[2]	Line type	Seed yield (kg/ha)	Days to flowering (days)	Plant height (cm)	Seed oil content (%)	Seed protein content (%)	Seed glucosinolate (µmol/g seed)
				lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)
1362.149	1	*B. nap* × *B.ole.cap*.BS	F_2-derived	3643.4 ± 390.1 (2851.4 – 4155.2)	52.6 ± 4.4 (40.0 – 61.2)	136.3 ± 7.4 (120.0 – 150.2)	45.7 ± 1.0 (44.3 – 48.1)	27.5 ± 1.2 (24.4 – 29.1)	19.2 ± 0.8 (16.5 – 21.1)
1362.152	2	*B. nap* × *B.ole.cap*.BS	F_2-derived	3283.5 ± 390.1 (2275.1 – 3737.1)	54.8 ± 4.4 (42.6 – 64.2)	139.9 ± 7.4 (126.5 – 153.8)	45.3 ± 1.0 (44.1 – 47.1)	26.8 ± 1.2 (24.0 – 28.1)	19.5 ± 0.8 (16.9 – 21.4)
1362.155	3	*B. nap* × *B.ole.cap*.BS	F_2-derived	3375.8 ± 390.2 (2348.3 – 4091.7)	54.5 ± 4.4 (43.5 – 61.9)	136.5 ± 7.4 (122.2 – 147.2)	45.5 ± 1.0 (43.8 – 47.0)	26.3 ± 1.2 (23.9 – 28.1)	20.2 ± 0.8 (18.7 – 21.9)
1362.156	4	*B. nap* × *B.ole.cap*.BS	F_2-derived	3045.8 ± 390.2 (2083.4 – 3726.4)	54.2 ± 4.4 (42.0 – 63.7)	138.2 ± 7.4 (121.0 – 147.6)	44.7 ± 1.0 (43.5 – 47.4)	27.2 ± 1.2 (23.7 – 28.7)	22.1 ± 0.8 (19.9 – 23.7)
1362.158	5	*B. nap* × *B.ole.cap*.BS	F_2-derived	3306.2 ± 390.1 (2267.4 – 4105.4)	53.5 ± 4.4 (43.0 – 60.8)	141.4 ± 7.4 (126.0 – 150.0)	46.4 ± 1.0 (45.2 – 49.5)	26.8 ± 1.2 (23.0 – 28.6)	18.9 ± 0.8 (17.1 – 19.8)
1362.161	6	*B. nap* × *B.ole.cap*.BS	F_2-derived	3422.9 ± 390.1 (2513.5 – 3986.4)	56.0 ± 4.4 (43.0 – 65.1)	136.9 ± 7.4 (121.5 – 152.7)	47.2 ± 1.0 (44.6 – 50.2)	26.0 ± 1.2 (22.4 – 28.6)	19.6 ± 0.8 (16.7 – 21.3)
1362.162	7	*B. nap* × *B.ole.cap*.BS	F_2-derived	3364.3 ± 390.3 (2448.7 – 4109.9)	54.3 ± 4.4 (41.9 – 63.6)	135.8 ± 7.4 (115.5 – 152.4)	47.0 ± 1.0 (44.4 – 51.5)	26.4 ± 1.2 (22.0 – 28.8)	17.3 ± 0.8 (16.5 – 17.9)
1362.164	8	*B. nap* × *B.ole.cap*.BS	F_2-derived	3016.0 ± 390.1 (2530.3 – 3677.7)	53.8 ± 4.4 (41.5 – 62.9)	135.4 ± 7.4 (121.9 – 149.5)	47.2 ± 1.0 (45.2 – 50.8)	26.7 ± 1.2 (22.6 – 28.5)	18.1 ± 0.8 (16.5 – 20.4)
1362.165	9	*B. nap* × *B.ole.cap*.BS	F_2-derived	3363.6 ± 390.2 (2315.5 – 4113.9)	51.5 ± 4.4 (40.9 – 57.7)	129.8 ± 7.4 (105.9 – 145.9)	47.7 ± 1.0 (45.8 – 51.0)	26.6 ± 1.2 (22.5 – 28.7)	16.6 ± 0.8 (15.7 – 17.6)
1362.166	10	*B. nap* × *B.ole.cap*.BS	F_2-derived	3702.6 ± 390.1 (2780.3 – 4208.4)	54.6 ± 4.4 (42.5 – 62.8)	135.7 ± 7.4 (121.1 – 150.5)	46.3 ± 1.0 (44.5 – 50.4)	27.1 ± 1.2 (22.5 – 28.9)	20.6 ± 0.8 (19.3 – 21.5)
1362.167	11	*B. nap* × *B.ole.cap*.BS	F_2-derived	3256.3 ± 390.3 (2126.8 – 3863.2)	54.7 ± 4.4 (42.0 – 63.7)	135.4 ± 7.4 (121.0 – 148.0)	45.6 ± 1.0 (43.9 – 48.3)	27.0 ± 1.2 (23.3 – 28.8)	20.6 ± 0.8 (18.3 – 22.0)
1362.169	12	*B. nap* × *B.ole.cap*.BS	F_2-derived	3257.3 ± 392.9 (2120.8 – 4063.9)	55.4 ± 4.4 (43.0 – 63.7)	138.3 ± 7.5 (125.3 – 149.3)	45.9 ± 1.0 (44.2 – 47.9)	27.2 ± 1.2 (24.6 – 28.9)	21.2 ± 0.8 (19.1 – 23.1)
1362.170	13	*B. nap* × *B.ole.cap*.BS	F_2-derived	3649.4 ± 390.1 (2550.2 – 4385.5)	53.8 ± 4.4 (42.0 – 62.7)	133.1 ± 7.4 (116.3 – 141.6)	45.9 ± 1.0 (44.4 – 48.9)	26.9 ± 1.2 (23.4 – 28.7)	22.7 ± 0.8 (20.9 – 23.7)
1362.171	14	*B. nap* × *B.ole.cap*.BS	F_2-derived	3356.2 ± 390.1 (2398.4 – 4237.1)	53.0 ± 4.4 (41.1 – 62.9)	138.6 ± 7.4 (120.0 – 152.8)	46.6 ± 1.0 (44.5 – 50.6)	26.9 ± 1.2 (22.4 – 29.1)	20.1 ± 0.8 (18.1 – 22.6)
1362.173	15	*B. nap* × *B.ole.cap*.BS	F_2-derived	3288.3 ± 390.2 (2423.1 – 3913.2)	55.1 ± 4.4 (42.0 – 63.4)	136.2 ± 7.4 (119.1 – 149.7)	45.8 ± 1.0 (44.4 – 48.4)	26.9 ± 1.2 (23.2 – 28.6)	20.9 ± 0.8 (18.5 – 22.5)
1362.174	16	*B. nap* × *B.ole.cap*.BS	F_2-derived	3620.1 ± 392.9 (2417.5 – 4464.8)	54.9 ± 4.4 (42.6 – 64.2)	140.2 ± 7.4 (121.5 – 154.5)	46.1 ± 1.0 (43.9 – 50.0)	27.0 ± 1.2 (22.0 – 29.3)	19.7 ± 0.8 (17.8 – 21.3)
1362.175	17	*B. nap* × *B.ole.cap*.BS	F_2-derived	3348.0 ± 390.3 (2553.9 – 4111.4)	54.1 ± 4.4 (43.5 – 62.2)	140.6 ± 7.4 (129.4 – 151.4)	46.1 ± 1.0 (44.2 – 50.0)	26.8 ± 1.2 (22.4 – 29.0)	21.2 ± 0.8 (19.0 – 23.5)

[1] Reg. No. = Registration number

[2] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

Appendix: 4-15. Continued…

Reg. No.[1]	Entry no.	Cross[2]	Line type	Seed yield (kg/ha)	Days to flowering (days)	Plant height (cm)	Seed oil content (%)	Seed protein content (%)	Seed glucosinolate (µmol/g seed)
				lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)
1362.176	18	*B. nap* × *B.ole.cap*.BS	F_2-derived	3280.2 ± 390.2 (2607.1 – 3853.6)	56.1 ± 4.4 (44.0 – 63.9)	138.3 ± 7.4 (130.9 – 150.9)	46.4 ± 1.0 (44.1 – 50.2)	26.8 ± 1.2 (22.2 – 29.2)	21.3 ± 0.8 (18.6 – 22.5)
1362.177	19	*B. nap* × *B.ole.cap*.BS	F_2-derived	3491.2 ± 390.2 (2881.1 – 4022.1)	55.4 ± 4.4 (42.1 – 65.7)	138.5 ± 7.4 (124.9 – 147.9)	46.1 ± 1.0 (43.7 – 49.0)	27.1 ± 1.2 (24.1 – 29.7)	20.2 ± 0.8 (18.9 – 22.4)
1362.179	20	*B. nap* × *B.ole.cap*.BS	F_2-derived	3476.3 ± 390.1 (2395.1 – 4284.5)	55.4 ± 4.4 (41.4 – 65.6)	143.5 ± 7.4 (130.9 – 151.6)	45.5 ± 1.0 (43.7 – 48.8)	27.3 ± 1.2 (23.3 – 28.9)	20.6 ± 0.8 (17.7 – 22.7)
1362.180	21	*B. nap* × *B.ole.cap*.BS	F_2-derived	3821.6 ± 390.1 (2668.4 – 4898.5)	52.0 ± 4.4 (40.5 – 59.4)	131.7 ± 7.4 (119.1 – 143.0)	47.2 ± 1.0 (45.0 – 50.4)	26.7 ± 1.2 (22.9 – 29.3)	17.7 ± 0.8 (16.7 – 18.8)
1363.164	22	*B. nap* × *B.ole.cap*.BD	F_2-derived	3374.7 ± 390.2 (2551.5 – 3915.3)	54.6 ± 4.4 (42.4 – 64.7)	141.3 ± 7.4 (125.2 – 151.5)	45.2 ± 1.0 (43.5 – 49.2)	27.4 ± 1.2 (23.3 – 29.3)	19.1 ± 0.8 (16.9 – 21.3)
1363.165	23	*B. nap* × *B.ole.cap*.BD	F_2-derived	3671.9 ± 390.2 (2983.1 – 3636.2)	53.4 ± 4.4 (42.0 – 60.7)	133.4 ± 7.4 (111.0 – 148.4)	47.3 ± 1.0 (45.1 – 51.5)	25.7 ± 1.2 (21.3 – 27.8)	17.3 ± 0.8 (13.7 – 20.1)
1363.168	24	*B. nap* × *B.ole.cap*.BD	F_2-derived	3447.4 ± 390.2 (2456.6 – 4144.3)	54.5 ± 4.4 (43.0 – 61.7)	136.6 ± 7.4 (119.1 – 157.7)	46.1 ± 1.0 (43.9 – 48.3)	26.5 ± 1.2 (23.1 – 28.6)	20.4 ± 0.8 (18.7 – 22.9)
1363.170	25	*B. nap* × *B.ole.cap*.BD	F_2-derived	3176.4 ± 392.9 (2106.6 – 3785.6)	54.6 ± 4.4 (42.0 – 62.9)	142.8 ± 7.4 (132.3 – 153.9)	46.1 ± 1.0 (44.7 – 49.8)	26.8 ± 1.2 (22.7 – 28.6)	17.8 ± 0.8 (14.7 – 20.8)
1363.171	26	*B. nap* × *B.ole.cap*.BD	F_2-derived	3482.8 ± 390.2 (2764.9 – 4361.8)	54.5 ± 4.4 (42.6 – 61.9)	134.0 ± 7.4 (125.3 – 144.8)	46.7 ± 1.0 (44.8 – 49.8)	26.9 ± 1.2 (23.2 – 29.0)	19.8 ± 0.8 (17.1 – 20.6)
1363.173	27	*B. nap* × *B.ole.cap*.BD	F_2-derived	3581.8 ± 390.1 (2459.7 – 3964.2)	53.5 ± 4.4 (41.0 – 63.7)	139.8 ± 7.4 (125.1 – 149.4)	45.8 ± 1.0 (44.4 – 49.6)	27.1 ± 1.2 (22.7 – 29.0)	20.5 ± 0.8 (17.3 – 22.9)
1363.177	28	*B. nap* × *B.ole.cap*.BD	F_2-derived	3216.1 ± 390.2 (2235.3 – 4145.7)	52.8 ± 4.4 (41.0 – 60.0)	136.4 ± 7.4 (122.2 – 151.8)	46.2 ± 1.0 (44.3 – 48.9)	27.2 ± 1.2 (23.6 – 29.1)	19.1 ± 0.8 (17.3 – 20.3)
1363.178	29	*B. nap* × *B.ole.cap*.BD	F_2-derived	3640.5 ± 390.2 (2667.1 – 4153.5)	52.5 ± 4.4 (40.1 – 61.7)	142.0 ± 7.4 (124.8 – 153.5)	47.1 ± 1.0 (45.1 – 49.9)	26.1 ± 1.2 (23.0 – 28.2)	18.8 ± 0.8 (18.0 – 20.1)
1363.180	30	*B. nap* × *B.ole.cap*.BD	F_2-derived	3582.2 ± 390.2 (2539.5 – 4345.4)	51.1 ± 4.4 (38.1 – 58.7)	127.5 ± 7.4 (110.0 – 146.3)	48.2 ± 1.0 (46.4 – 52.3)	26.1 ± 1.2 (21.2 – 28.1)	15.3 ± 0.8 (13.7 – 16.9)
1363.181	31	*B. nap* × *B.ole.cap*.BD	F_2-derived	3496.2 ± 390.1 (2673.9 – 3999.1)	51.1 ± 4.4 (38.4 – 59.2)	126.9 ± 7.4 (111.5 – 140.9)	47.1 ± 1.0 (45.7 – 50.0)	26.8 ± 1.2 (23.4 – 28.4)	16.6 ± 0.8 (15.2 – 19.0)
1363.182	32	*B. nap* × *B.ole.cap*.BD	F_2-derived	3451.7 ± 390.1 (2684.1 – 3989.1)	52.1 ± 4.4 (39.9 – 60.3)	139.1 ± 7.4 (125.5 – 146.4)	46.5 ± 1.0 (45.9 – 47.1)	26.5 ± 1.2 (24.5 – 27.7)	21.4 ± 0.8 (20.3 – 22.4)
1363.183	33	*B. nap* × *B.ole.cap*.BD	F_2-derived	3274.2 ± 390.1 (2397.3 – 3734.5)	55.5 ± 4.4 (42.0 – 64.8)	139.1 ± 7.4 (117.2 – 150.3)	45.7 ± 1.0 (44.6 – 46.6)	27.0 ± 1.2 (24.8 – 28.4)	21.1 ± 0.8 (20.0 – 22.1)
1363.185	34	*B. nap* × *B.ole.cap*.BD	F_2-derived	3229.3 ± 390.1 (2206.9 – 4201.3)	52.6 ± 4.4 (40.5 – 63.4)	127.7 ± 7.4 (107.5 – 137.3)	46.2 ± 1.0 (44.4 – 49.6)	27.9 ± 1.2 (23.9 – 29.6)	15.6 ± 0.8 (13.4 – 17.0)

[1] Reg. No. = Registration number
[2] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

Appendix: 4-15. Continued…

Reg. No.[1]	Entry no.	Cross[2]	Line type	Seed yield (kg/ha)	Days to flowering (days)	Plant height (cm)	Seed oil content (%)	Seed protein content (%)	Seed glucosinolate (µmol/g seed)
				lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)
1363.186	35	*B. nap* × *B.ole.cap*.BD	F_2-derived	3379.5 ± 390.2 (2405.4 – 4170.1)	52.0 ± 4.4 (40.5 – 59.7)	133.8 ± 7.4 (116.0 – 146.7)	46.3 ± 1.0 (44.5 – 48.6)	27.5 ± 1.2 (24.5 – 29.3)	15.9 ± 0.8 (15.5 – 16.7)
1363.190	36	*B. nap* × *B.ole.cap*.BD	F_2-derived	3278.8 ± 390.1 (2260.1 – 2570.3)	50.1 ± 4.4 (38.5 – 58.2)	121.3 ± 7.4 (100.1 – 140.2)	47.0 ± 1.0 (45.5 – 49.8)	26.4 ± 1.2 (22.6 – 28.0)	19.4 ± 0.8 (17.3 – 23.6)
1363.194	37	*B. nap* × *B.ole.cap*.BD	F_2-derived	3339.9 ± 390.2 (2627.8 – 4278.7)	51.1 ± 4.4 (38.0 – 59.2)	133.2 ± 7.4 (115.4 – 148.4)	45.6 ± 1.0 (44.9 – 47.5)	27.5 ± 1.2 (24.9 – 28.6)	21.7 ± 0.8 (20.8 – 22.2)
1363.195	38	*B. nap* × *B.ole.cap*.BD	F_2-derived	2970.5 ± 390.2 (2518.5 – 3429.4)	53.8 ± 4.4 (42.0 – 60.9)	134.6 ± 7.4 (122.6 – 147.0)	46.1 ± 1.0 (44.2 – 49.4)	27.5 ± 1.2 (23.9 – 29.2)	23.3 ± 0.8 (21.8 – 25.1)
1363.197	39	*B. nap* × *B.ole.cap*.BD	F_2-derived	3349.8 ± 390.1 (2241.2 – 4102.3)	51.4 ± 4.4 (40.0 – 58.9)	136.3 ± 7.4 (118.9 – 148.3)	46.4 ± 1.0 (44.2 – 49.5)	27.6 ± 1.2 (23.9 – 29.5)	21.2 ± 0.8 (20.4 – 22.7)
1363.202	40	*B. nap* × *B.ole.cap*.BD	F_2-derived	3231.1 ± 390.1 (2276.0 – 3859.7)	50.2 ± 4.4 (38.5 – 58.5)	134.2 ± 7.4 (117.7 – 161.3)	45.1 ± 1.0 (43.6 – 48.1)	28.4 ± 1.2 (24.3 – 30.3)	18.7 ± 0.8 (18.0 – 19.4)
1363.205	41	*B. nap* × *B.ole.cap*.BD	F_2-derived	3289.5 ± 390.1 (2443.5 – 3961.8)	56.4 ± 4.4 (43.4 – 65.2)	146.7 ± 7.4 (135.3 – 159.9)	47.6 ± 1.0 (45.7 – 50.4)	25.8 ± 1.2 (22.2 – 27.6)	16.9 ± 0.8 (14.0 – 18.1)
1363.206	42	*B. nap* × *B.ole.cap*.BD	F_2-derived	3541.1 ± 390.2 (2586.1 – 4143.8)	54.8 ± 4.4 (44.1 – 63.9)	140.6 ± 7.4 (123.1 – 158.2)	46.2 ± 1.0 (44.3 – 49.3)	26.8 ± 1.2 (22.7 – 28.6)	23.9 ± 0.8 (21.5 – 27.6)
1363.207	43	*B. nap* × *B.ole.cap*.BD	F_2-derived	3003.8 ± 390.2 (2075.2 – 3414.5)	58.3 ± 4.4 (44.6 – 68.2)	143.9 ± 7.4 (130.7 – 156.8)	46.7 ± 1.0 (45.7 – 48.1)	26.4 ± 1.2 (23.6 – 27.9)	16.5 ± 0.8 (14.0 – 18.0)
1363.208	44	*B. nap* × *B.ole.cap*.BD	F_2-derived	2926.5 ± 390.2 (2053.4 – 3629.5)	57.3 ± 4.4 (44.4 – 67.8)	148.9 ± 7.4 (124.1 – 164.7)	45.3 ± 1.0 (44.6 – 47.3)	27.3 ± 1.2 (24.1 – 28.5)	19.1 ± 0.8 (16.7 – 21.1)
1363.211	45	*B. nap* × *B.ole.cap*.BD	F_2-derived	3078.2 ± 390.1 (1963.9 – 3949.6)	53.8 ± 4.4 (41.9 – 63.9)	142.3 ± 7.4 (129.0 – 159.5)	45.8 ± 1.0 (44.6 – 48.6)	26.3 ± 1.2 (22.2 – 28.2)	20.1 ± 0.8 (17.4 – 21.4)
1681.082	46	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3047.5 ± 390.1 (1817.5 – 3592.2)	53.4 ± 4.4 (40.5 – 61.8)	125.2 ± 7.4 (113.9 – 135.0)	45.3 ± 1.0 (44.3 – 47.2)	26.6 ± 1.2 (23.4 – 28.2)	17.2 ± 0.8 (14.3 – 18.4)
1681.083	47	*B. nap* × *B.ole.cap*.BS	BC_1-derived	2903.2 ± 390.1 (2105.1 – 3404.8)	55.4 ± 4.4 (42.6 – 65.8)	136.7 ± 7.4 (114.0 – 160.9)	45.1 ± 1.0 (43.8 – 46.0)	26.7 ± 1.2 (24.5 – 28.3)	20.5 ± 0.8 (16.3 – 22.5)
1681.084	48	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3080.4 ± 390.2 (2180.7 – 3742.3)	51.9 ± 4.4 (41.0 – 59.3)	133.6 ± 7.4 (109.4 – 152.2)	44.5 ± 1.0 (42.9 – 48.0)	27.5 ± 1.2 (23.2 – 29.3)	23.8 ± 0.8 (22.7 – 25.3)
1681.085	49	*B. nap* × *B.ole.cap*.BS	BC_1-derived	2957.9 ± 390.2 (2031.5 – 3437.6)	53.5 ± 4.4 (41.0 – 62.9)	132.9 ± 7.4 (115.7 – 147.4)	45.4 ± 1.0 (43.8 – 46.9)	26.9 ± 1.2 (24.3 – 28.6)	19.8 ± 0.8 (19.2 – 21.1)
1681.086	50	*B. nap* × *B.ole.cap*.BS	BC_1-derived	2962.0 ± 390.2 (1804.0 – 4000.2)	54.3 ± 4.4 (41.5 – 63.2)	132.1 ± 7.4 (112.1 – 148.0)	46.0 ± 1.0 (44.8 – 47.9)	26.8 ± 1.2 (23.7 – 28.4)	17.2 ± 0.8 (16.2 – 19.0)
1681.090	51	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3378.0 ± 390.2 (1949.0 – 4292.3)	53.1 ± 4.4 (41.5 – 60.7)	125.2 ± 7.4 (112.7 – 135.9)	46.3 ± 1.0 (45.1 – 47.6)	25.5 ± 1.2 (22.9 – 27.1)	22.7 ± 0.8 (21.0 – 24.5)

[1] Reg. No. = Registration number
[2] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

Appendix: 4-15. Continued…

Reg. No.[1]	Entry no.	Cross[2]	Line type	Seed yield (kg/ha)	Days to flowering (days)	Plant height (cm)	Seed oil content (%)	Seed protein content (%)	Seed glucosinolate (µmol/g seed)
				lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)
1681.091	52	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3197.0 ± 392.8 (1970.5 – 4003.3)	52.0 ± 4.4 (40.0 – 57.9)	136.3 ± 7.5 (115.2 – 152.3)	46.2 ± 1.0 (45.1 – 47.9)	26.1 ± 1.2 (23.1 – 27.6)	21.3 ± 0.8 (18.8 – 22.7)
1681.092	53	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3269.4 ± 390.1 (2115.3 – 4030.6)	52.0 ± 4.4 (40.0 – 60.3)	130.6 ± 7.4 (104.6 – 149.2)	45.7 ± 1.0 (44.8 – 48.0)	26.0 ± 1.2 (23.0 – 27.2)	20.2 ± 0.8 (18.2 – 21.4)
1681.096	54	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3352.8 ± 390.2 (2094.4 – 4487.7)	52.0 ± 4.4 (40.4 – 59.1)	130.7 ± 7.4 (105.5 – 155.5)	44.9 ± 1.0 (44.2 – 46.4)	26.5 ± 1.2 (24.0 – 27.8)	21.1 ± 0.8 (20.7 – 21.9)
1681.097	55	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3111.2 ± 390.2 (1961.4 – 3583.8)	53.2 ± 4.4 (42.5 – 60.7)	131.8 ± 7.4 (114.2 – 148.3)	45.4 ± 1.0 (44.1 – 47.2)	26.7 ± 1.2 (23.9 – 28.2)	21.5 ± 0.8 (19.9 – 22.5)
1681.098	56	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3128.9 ± 390.1 (2298.8 – 3605.2)	53.7 ± 4.4 (42.1 – 61.5)	138.0 ± 7.4 (123.8 – 152.9)	43.8 ± 1.0 (43.2 – 44.7)	27.8 ± 1.2 (26.2 – 28.5)	23.8 ± 0.8 (21.9 – 26.1)
1681.100	57	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3257.1 ± 390.1 (2203.3 – 3929.6)	52.0 ± 4.4 (40.9 – 59.9)	123.2 ± 7.4 (104.7 – 144.5)	44.6 ± 1.0 (43.7 – 47.1)	27.2 ± 1.2 (23.8 – 28.2)	23.2 ± 0.8 (21.0 – 25.2)
1681.101	58	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3275.2 ± 390.1 (2442.4 – 4045.1)	52.4 ± 4.4 (41.5 – 59.7)	128.4 ± 7.4 (103.6 – 147.5)	45.0 ± 1.0 (43.0 – 47.4)	26.9 ± 1.2 (23.8 – 28.7)	21.7 ± 0.8 (20.3 – 24.0)
1681.102	59	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3235.7 ± 390.4 (2687.3 – 4037.3)	52.5 ± 4.4 (41.0 – 60.3)	128.2 ± 7.4 (108.5 – 145.3)	44.4 ± 1.0 (43.2 – 45.6)	27.5 ± 1.2 (25.5 – 28.7)	22.4 ± 0.8 (21.1 – 25.7)
1681.103	60	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3208.4 ± 390.3 (2311.4 – 4039.9)	51.6 ± 4.4 (40.9 – 60.3)	123.3 ± 7.4 (105.1 – 137.5)	44.3 ± 1.0 (42.9 – 47.6)	27.6 ± 1.2 (23.6 – 28.9)	21.8 ± 0.8 (19.8 – 24.1)
1681.104	61	*B. nap* × *B.ole.cap*.BS	BC_1-derived	3031.0 ± 390.1 (2063.5 – 3763.5)	53.2 ± 4.4 (41.5 – 61.7)	127.5 ± 7.4 (106.2 – 138.6)	44.1 ± 1.0 (42.3 – 47.3)	27.2 ± 1.2 (23.7 – 29.1)	22.0 ± 0.8 (21.1 – 23.2)
1681.105	62	*B. nap* × *B.ole.cap*.BS	BC_1-derived	2782.4 ± 390.2 (2223.1 – 2914.1)	52.5 ± 4.4 (40.5 – 61.7)	129.8 ± 7.4 (109.4 – 140.9)	44.3 ± 1.0 (42.9 – 47.8)	27.7 ± 1.2 (23.5 – 28.9)	20.1 ± 0.8 (19.0 – 20.7)
1682.099	63	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3163.6 ± 390.2 (1687.7 – 4209.3)	54.1 ± 4.4 (42.0 – 62.4)	139.4 ± 7.4 (121.1 – 156.9)	47.6 ± 1.0 (46.1 – 49.8)	26.4 ± 1.2 (23.2 – 27.9)	14.6 ± 0.8 (13.6 – 15.1)
1682.100	64	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3734.0 ± 390.2 (2766.4 – 4857.4)	53.5 ± 4.4 (41.5 – 61.6)	137.7 ± 7.4 (119.6 – 150.6)	48.5 ± 1.0 (45.9 – 51.5)	26.0 ± 1.2 (22.7 – 28.3)	13.4 ± 0.8 (12.0 – 14.7)
1682.101	65	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3346.6 ± 390.2 (2366.5 – 4357.6)	52.3 ± 4.4 (40.0 – 60.9)	137.1 ± 7.4 (119.3 – 144.2)	47.7 ± 1.0 (45.7 – 51.9)	25.8 ± 1.2 (21.1 – 28.1)	23.5 ± 0.8 (21.2 – 26.4)
1682.102	66	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3270.8 ± 390.1 (2263.7 – 4259.1)	52.7 ± 4.4 (41.5 – 59.5)	129.1 ± 7.4 (112.6 – 144.3)	47.9 ± 1.0 (45.7 – 52.1)	27.0 ± 1.2 (22.6 – 28.7)	17.9 ± 0.8 (16.5 – 19.1)
1682.103	67	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3114.5 ± 390.2 (2050.0 – 3784.2)	52.4 ± 4.4 (40.0 – 60.9)	135.1 ± 7.4 (117.9 – 147.2)	47.0 ± 1.0 (45.4 – 49.6)	27.5 ± 1.2 (24.8 – 29.0)	19.2 ± 0.8 (17.3 – 22.6)
1682.104	68	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3296.7 ± 390.2 (2331.0 – 4665.1)	51.7 ± 4.4 (40.5 – 59.7)	136.1 ± 7.4 (113.9 – 154.9)	47.7 ± 1.0 (46.5 – 51.9)	27.0 ± 1.2 (22.4 – 28.3)	18.8 ± 0.8 (16.0 – 20.1)

[1] Reg. No. = Registration number
[2] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

Appendix: 4-15. Continued…

Reg. No.[1]	Entry no.	Cross[2]	Line type	Seed yield (kg/ha)	Days to flowering (days)	Plant height (cm)	Seed oil content (%)	Seed protein content (%)	Seed glucosinolate (µmol/g seed)
				lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)
1682.105	69	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3389.7 ± 390.2 (2169.5 – 4138.6)	51.9 ± 4.4 (40.0 – 60.4)	128.5 ± 7.4 (109.9 – 142.2)	48.0 ± 1.0 (46.5 – 51.2)	26.9 ± 1.2 (23.2 – 28.7)	17.9 ± 0.8 (15.7 – 19.9)
1682.108	70	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3336.8 ± 390.1 (2330.6 – 4036.8)	52.7 ± 4.4 (40.9 – 62.2)	127.8 ± 7.4 (113.6 – 137.7)	48.0 ± 1.0 (45.9 – 51.0)	26.5 ± 1.2 (23.1 – 28.6)	17.5 ± 0.8 (15.6 – 19.3)
1682.113	71	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3511.0 ± 390.1 (2549.7 – 4264.8)	53.9 ± 4.4 (41.5 – 63.8)	141.7 ± 7.4 (130.1 – 149.9)	47.3 ± 1.0 (46.0 – 49.9)	26.4 ± 1.2 (22.9 – 28.1)	14.0 ± 0.8 (12.1 – 14.7)
1682.120	72	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3137.5 ± 390.2 (2506.7 – 3532.2)	53.7 ± 4.4 (41.0 – 63.7)	135.4 ± 7.4 (123.7 – 145.2)	46.9 ± 1.0 (45.4 – 49.3)	26.1 ± 1.2 (22.8 – 27.7)	19.5 ± 0.8 (18.3 – 21.4)
1682.124	73	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3418.8 ± 390.2 (2576.3 – 3750.6)	56.6 ± 4.4 (45.0 – 64.2)	143.3 ± 7.4 (130.9 – 152.3)	46.1 ± 1.0 (43.8 – 49.6)	26.3 ± 1.2 (21.7 – 28.7)	14.3 ± 0.8 (11.8 – 15.2)
1682.125	74	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3702.9 ± 390.2 (2642.3 – 4186.9)	56.4 ± 4.4 (44.9 – 66.3)	140.0 ± 7.4 (131.0 – 148.7)	47.1 ± 1.0 (44.2 – 50.8)	25.8 ± 1.2 (21.1 – 28.8)	16.2 ± 0.8 (14.0 – 17.9)
1682.128	75	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3331.1 ± 390.2 (2075.3 – 4019.6)	49.9 ± 4.4 (36.5 – 59.4)	124.8 ± 7.4 (96.1 – 141.7)	48.1 ± 1.0 (47.0 – 49.6)	26.1 ± 1.2 (23.5 – 27.5)	17.1 ± 0.8 (15.9 – 17.8)
1682.130	76	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3526.6 ± 390.2 (2374.1 – 4484.6)	50.7 ± 4.4 (39.0 – 60.3)	128.7 ± 7.4 (109.3 – 140.7)	48.1 ± 1.0 (46.6 – 50.9)	25.8 ± 1.2 (22.6 – 27.3)	14.2 ± 0.8 (13.0 – 15.4)
1682.131	77	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3257.4 ± 390.2 (2130.7 – 4091.8)	48.0 ± 4.4 (36.0 – 54.2)	119.3 ± 7.4 (101.4 – 140.7)	47.1 ± 1.0 (44.9 – 50.0)	26.5 ± 1.2 (22.8 – 28.6)	16.1 ± 0.8 (14.8 – 17.6)
1682.133	78	*B. nap* × *B.ole.cap*.BD	BC_1-derived	2993.1 ± 390.2 (1903.4 – 3597.9)	49.6 ± 4.4 (36.5 – 57.8)	120.7 ± 7.4 (102.0 – 145.2)	46.6 ± 1.0 (43.7 – 50.1)	27.0 ± 1.2 (23.3 – 29.4)	17.7 ± 0.8 (16.2 – 20.2)
1682.137	79	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3061.4 ± 390.1 (1741.2 – 4064.6)	49.2 ± 4.4 (36.5 – 56.4)	111.0 ± 7.4 (95.0 – 124.3)	46.6 ± 1.0 (44.9 – 48.8)	27.1 ± 1.2 (24.0 – 28.7)	16.6 ± 0.8 (15.1 – 17.4)
1682.138	80	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3197.2 ± 390.3 (2080.8 – 4046.3)	49.6 ± 4.4 (36.5 – 59.3)	118.3 ± 7.4 (94.3 – 139.2)	46.5 ± 1.0 (44.6 – 50.4)	27.1 ± 1.2 (22.7 – 28.8)	17.4 ± 0.8 (15.2 – 19.0)
1682.140	81	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3292.6 ± 390.1 (1935.2 – 3369.7)	49.6 ± 4.4 (37.0 – 57.3)	122.2 ± 7.4 (94.9 – 148.2)	47.2 ± 1.0 (45.6 – 49.8)	26.4 ± 1.2 (23.0 – 28.1)	16.3 ± 0.8 (14.9 – 17.8)
1682.143	82	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3495.3 ± 390.2 (2075.5 – 4403.7)	49.7 ± 4.4 (37.5 – 57.2)	123.4 ± 7.4 (99.9 – 141.4)	47.0 ± 1.0 (45.8 – 49.1)	26.3 ± 1.2 (23.2 – 27.8)	16.2 ± 0.8 (14.3 – 19.8)
1682.145	83	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3389.9 ± 390.1 (1554.3 – 4254.2)	50.0 ± 4.4 (37.0 – 58.2)	114.1 ± 7.4 (97.6 – 132.9)	47.7 ± 1.0 (46.0 – 49.0)	25.8 ± 1.2 (23.4 – 27.6)	15.5 ± 0.8 (14.6 – 16.2)
1682.147	84	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3690.1 ± 390.2 (2677.6 – 4365.8)	51.5 ± 4.4 (38.5 – 60.7)	130.4 ± 7.4 (108.1 – 148.4)	47.9 ± 1.0 (46.4 – 50.6)	25.3 ± 1.2 (21.5 – 27.2)	12.9 ± 0.8 (11.2 – 14.2)
1682.149	85	*B. nap* × *B.ole.cap*.BD	BC_1-derived	2934.7 ± 390.2 (1596.9 – 4073.2)	51.2 ± 4.4 (37.0 – 62.2)	109.9 ± 7.4 (91.9 – 119.8)	45.5 ± 1.0 (44.0 – 48.4)	27.6 ± 1.2 (24.1 – 29.2)	18.1 ± 0.8 (17.3 – 19.3)

[1] Reg. No. = Registration number
[2] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener

Appendix: 4-15. Continued…

Reg. No.[1]	Entry no.	Cross[2]	Line type	Seed yield (kg/ha)	Days to flowering (days)	Plant height (cm)	Seed oil content (%)	Seed protein content (%)	Seed glucosinolate (µmol/g seed)
				lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)	lsmeans (Range)
1682.150	86	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3111.0 ± 390.2 (2171.3 – 3542.7)	51.4 ± 4.4 (38.0 – 61.7)	127.1 ± 7.4 (105.2 – 141.7)	46.3 ± 1.0 (44.7 – 49.6)	27.2 ± 1.2 (23.3 – 29.0)	17.8 ± 0.8 (16.8 – 19.6)
1682.152	87	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3100.1 ± 390.2 (1610.2 – 4159.3)	50.9 ± 4.4 (38.5 – 59.7)	128.1 ± 7.4 (100.6 – 138.6)	45.9 ± 1.0 (44.7 – 48.1)	27.1 ± 1.2 (23.9 – 28.8)	16.7 ± 0.8 (15.7 – 17.1)
1682.154	88	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3160.2 ± 390.2 (2206.9 – 4160.3)	52.4 ± 4.4 (39.5 – 62.0)	128.8 ± 7.4 (106.6 – 143.5)	46.1 ± 1.0 (43.8 – 49.1)	27.0 ± 1.2 (23.2 – 29.2)	17.0 ± 0.8 (15.1 – 18.7)
1682.155	89	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3314.6 ± 390.2 (2229.9 – 4151.2)	50.4 ± 4.4 (38.0 – 58.8)	122.7 ± 7.4 (103.4 – 138.9)	46.8 ± 1.0 (44.5 – 50.0)	26.3 ± 1.2 (22.6 – 28.6)	15.7 ± 0.8 (15.6 – 16.2)
1682.156	90	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3396.5 ± 390.2 (2305.3 – 3937.4)	49.9 ± 4.4 (36.5 – 59.7)	120.7 ± 7.4 (98.4 – 137.0)	46.4 ± 1.0 (45.0 – 50.2)	27.0 ± 1.2 (22.5 – 28.6)	16.7 ± 0.8 (15.0 – 17.9)
1682.158	91	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3408.9 ± 390.1 (2279.3 – 3418.7)	52.3 ± 4.4 (41.5 – 61.0)	127.1 ± 7.4 (102.0 – 149.2)	47.1 ± 1.0 (45.2 – 49.7)	26.1 ± 1.2 (22.8 – 28.4)	15.7 ± 0.8 (13.8 – 17.0)
1682.159	92	*B. nap* × *B.ole.cap*.BD	BC_1-derived	3433.8 ± 390.2 (2185.5 – 4463.4)	49.4 ± 4.4 (36.5 – 57.2)	114.2 ± 7.4 (98.6 – 130.5)	46.9 ± 1.0 (44.2 – 49.7)	26.0 ± 1.2 (22.5 – 28.1)	16.2 ± 0.8 (15.0 – 17.4)
1682.164	93	*B. nap* × *B.ole.cap*.BD	BC_1-derived	2934.8 ± 390.2 (2427.1 – 3439.8)	52.8 ± 4.4 (42.5 – 60.9)	129.5 ± 7.4 (110.6 – 142.7)	48.0 ± 1.0 (45.8 – 50.7)	26.7 ± 1.2 (23.3 – 28.6)	17.0 ± 0.8 (15.6 – 18.1)
A04-73NA	Check	*B. napus*		3635.2 ± 373.7 (2619.6 – 4358.8)	53.6 ± 4.4 (42.2 – 62.3)	140.8 ± 7.0 (125.6 – 151.8)	47.0 ± 1.0 (45.6 – 49.4)	26.3 ± 1.2 (23.0 – 28.1)	21.7 ± 0.6 (20.7 – 23.2)

[1] Reg. No. = Registration number
[2] *B. nap* = Spring *B. napus* canola line A04-73NA, *B.ole.cap*.BS = *B. oleracea* var. *capitata* cv. Badger Shipper, *B.ole.cap*.BD = *B. oleracea* var. *capitata* cv. Bindsachsener