Broadening the genetic base of *Brassica napus* canola by interspecific crosses with different variants of *B. oleracea*

Iftikhar R, Wang X, Rahman H[1]

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada

[1]Corresponding author (e-mail: habibur.rahman@ualberta.ca).

Abstract Broadening the genetic base of the C genome of *Brassica napus* canola by use of *B. oleracea* is important. In this study, the prospect of developing *B. napus* canola lines from *B. napus* × *B. oleracea* var. *alboglabra*, *botrytis*, *italica* and *capitata* crosses and the effect of backcrossing the F_1's to *B. napus* were investigated. The efficiency of the production of the F_1's varied depending on the *B. oleracea* variant used in the cross. Fertility of the F_1 plants was low – produced, on average, about 0.7 F_2 seeds per self-pollination and similar number of BC_1 seeds on backcrossing to *B. napus*. The F_3 population showed greater fertility than the BC_1F_2; however, this difference diminished with the advancement of generation. The advanced generation populations, whether derived from F_2 or BC_1, showed similar fertility and produced similar size silique with similar number of seeds per silique. Progeny of all F_1's and BC_1's stabilized into *B. napus*, although *B. oleracea* plant was expected, especially in the progeny of F_1 (ACC) owing to elimination of the A chromosomes during meiosis. Segregation distortion for erucic acid alleles occurred in both F_2 and BC_1 resulting significantly fewer zero-erucic plants than expected; however, plants with ≤15% erucic acid frequently yielded zero-erucic progeny. No consistent correlation between parent and progeny generation was found for seed glucosinolate content; however, selection for this trait was effective and *B. napus* canola lines were obtained from all crosses. Silique length showed positive correlation with seed set; the advanced generation populations, whether derived from F_2 or BC_1, were similar for these traits. SSR marker analysis showed that genetically diverse canola lines can be developed by using different variants of *B. oleracea* in *B. napus* × *B. oleracea* interspecific crosses.

Keywords *Brassica napus* • *Brassica oleracea* • *alboglabra* • *botrytis* • *italica* • *capitata* • interspecific cross • plant fertility • chromosome stability • canola quality

Introduction

The oilseed crop *Brassica napus* L. canola supplies about 15% of the total vegetable oil in the world. It is the major oilseed crop in the European Union, Canada, Australia, China and India, and makes significant contribution to their national economy. Therefore, improvement of this crop through breeding is important; for this, presence of adequate genetic diversity in the breeding population is essentially needed. The short history of domestication of *B. napus* (Prakash et al. 2012) and the breeding strategies, such as selection for low erucic acid and glucosinolate content (Friedt and Snowdon 2010) and the use of canola quality germplasm in repeated cycle of breeding, as well as growing of a specific form, such as spring, semi-winter or winter type, in a specific growing region and keeping these gene pools isolated from each other are some of the reasons for the lack of adequate genetic diversity in *B. napus* canola (Hasan et al. 2006; Cowling 2007; Fu and Gugel 2010; for review, see Rahman 2013). According to Gyawali et al. (2013), the Canadian and Australian populations carry the lowest richness of unique alleles as well as the least genetic diversity.

Genetic diversity analysis by use of molecular markers placed the European winter, Chinese semi-winter, rutabaga and spring type of *B. napus* into distinct gene pools (Diers and Osborn 1994; Hasan et al. 2006; Bus et al. 2011); and the genomes of the two diploid progenitor species *B. rapa* and *B. oleracea* are found to be genetically distinct from each other as well as from their corresponding genome of *B. napus* (Thormann et al. 1994; Abel et al. 2005). Efforts have been made to broaden the genetic base of the spring *B. napus* canola through the use of the European winter type (Butruille et al. 1999; Kebede et al. 2010) or Chinese semi-winter type *B. napus* (Udall et al. 2004, Qian et al. 2007, 2009) or rutabagas (Rahman et al. 2014). The diploid progenitor species *B. rapa* has been used in the breeding of Chinese (Liu 1985 cited by Chen et al. 2008; Qian et al. 2006; Mei et al. 2011; Li et al. 2013) as well as Canadian (Kubik et al. 1999; Attri and Rahman 2018) *B. napus*. Indeed, introgression of allelic diversity from *B. rapa* into *B. napus* has made the Chinese oilseed *B. napus* germplasm quite distinct from other *B. napus* germplasm (Qian et al. 2006), and this germplasm has shown the potential for increasing

heterosis in European winter and Canadian spring *B. napus* hybrid canola cultivars (Qian et al. 2005, 2007, 2009). In the case of the other parental species *B. oleracea*, very little effort have been made to utilize this gene pool in the breeding of *B. napus* canola. To our knowledge, only two accessions of kale (*B. oleracea* var. *alboglabra* and var. *acephala*) have been used for broadening the genetic base of spring (Bennett et al. 2012; Rahman et al. 2015) or Chinese semi-winter *B. napus* (Li et al. 2014).

Seeds of *B. oleracea* generally contain a high level of erucic acid in oil (> 40 %) and a high level of glucosinolate (GSL) (>100 µmol per g meal) in seed meal, and this species also show low crossability with *B. napus* (Bennett et al. 2008). High sterility in the progeny of *B. napus* × *B. oleracea* interspecific hybrids, due to meiotic abnormalities (Chiang et al. 1978; Bennett et al. 2012; Li et al. 2014), also imposes the difficulty of getting a euploid line from this interspecific cross. These are some of the factors that may have contributed to the reluctance of the use of *B. oleracea* gene pool in the breeding of *B. napus* canola despite wide diversity exists in *B. oleracea* (dos Santos et al. 1994; Simonsen, and Heneen 1995; Lázaro and Aguinagalde 1998; Izzah et al. 2013). Previously, we investigated the prospect of developing canola quality *B. napus* line from a *B. napus* × *B. oleracea* interspecific cross by use of a Chinese kale (var. *alboglabra*) accession (Rahman et al. 2015). Given the fact that wide morphological and genetic diversity exists in *B. oleracea*, it is important to study the different variants of this species for utilization of this wide diversity in breeding. It is also well-known that the allied species and exotic gene pool carry many alleles which are undersirable for *B. napus* canola; introduction of these alleles can disrupt the favourable allele combinations of the elite lines that have been established over cycles of breeding. In this context, limited backcrossing approach, as suggested by Falk (2010), can be advantageous for getting the benefit of the exotic alleles while maintaining the favourable allele combinations to some extent.

The objective of this research was to evaluate the prospect of developing genetically diverse canola quality euploid *B. napus* lines by using different variants of *B. oleracea*, such as the Chinese kale (var. *alboglabra*), cauliflower (var. *botrytis*), broccoli (var. *italica*) and cabbage (var. *capitata*), in *B. napus* × *B. oleracea* interspecific crosses and following the approach of with or without backcrossing of the interspecific hybrids to the *B. napus* parent.

Materials and methods

Parental Materials

One canola quality (zero erucic acid and <15 µmol glucosinolates g^{-1} seed) spring type *B. napus* (AACC, 2*n* = 38) line A04-73NA and four *B. oleracea* lines/cultivars, viz. *B. oleracea* var. *alboglabra*-NRC, var. *botrytis* cv. BARI Cauliflower-1, var. *italica* cv. Premium Crop, and var. *capitata* cv. Balbro, were used. Seeds of *B. oleracea* var. *alboglabra* were obtained from the National Research Council (NRC), Saskatoon, Canada, BARI Cauliflower-1 from Bangladesh Agricultural Research Institute, Bangladesh, and cvs. Premium Crop and Balbro were obtained from Dr. Ron Howard, Alberta Agriculture and Rural Development, Brooks, AB, Canada from his germplasm colelction. All *B. oleracea* parents were of non-canola quality types (>40% erucic acid in seed oil and >80 µmol glucosinolates per g of seed meal). The following four interspecific crosses were made using the *B. napus* as female:

A04-73NA × *B. oleracea* var. *alboglabra*

A04-73NA × *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1

A04-73NA × *B. oleracea* var. *italica* cv. Premium Crop

A04-73NA × *B. oleracea* var. *capitata* cv. Balbro

The F_1 plants were produced through application of embryo culture technique (Bennett et al. 2008) and were grown in a growth chamber (20/15 ^{0}C day/night temperatures, 16 h photoperiod, and photosynthetic flux density of 450 µE m^{-2} s^{-1} at plant level). The plants were self-pollinated for F_2 seeds as well as backcrossed to the *B. napus* parent A04-73NA for backcross (BC_1) seeds:

(A04-73NA × *B. oleracea* var. *alboglabra*) × A04-73NA

(A04-73NA × *B. oleracea* var. *botrytis*) × A04-73NA

(A04-73NA × *B. oleracea* var. *italica*) × A04-73NA

(A04-73NA × *B. oleracea* var. *capitata*) × A04-73NA

Development of F_2- and BC_1-derived inbred lines

The F_2 and BC_1 populations were grown in a greenhouse (21°/18° ± 2°C day/night) and subjected to self-pollination with selection of individual plants for canola quality traits irrespective of their morphology (*B. napus* or *B. oleracea* type). Selection for zero erucic acid

started from F_2 generation, while analysis for glucosinolate content started one generation later as larger quantity of seeds was required for this analysis. The F_3 and BC_1F_2, F_4 and BC_1F_3, F_6 and BC_1F_5, and F_8 and BC_1F_7 populations of all four crosses were grown in a greenhouse in spring 2012, and in winter 2012-13, 2013-14 and 2014-15, respectively; the F_5 and BC_1F_4 populations of A04-73NA × *B. oleracea* var. *alboglabra* and A04-73NA × *B. oleracea* var. *botrytis* were grown in field plots in 2013 at the Edmonton Research Station of the University of Alberta and of A04-73NA × *B. oleracea* var. *italica* and A04-73NA × *B. oleracea* var. *capitata* were grown in a greenhouse in 2013; and the F_7 and BC_1F_6 population of all crosses were grown in field in 2014. Field plot size in 2013 and 2014 was 2.0 m long single row (unreplicated) with 50 cm space between the rows. In greenhouse, plants were grown in six-inch pots filled with SunGrow Sunshine Mix 4 (composed of sphagnum peat moss, fine perlite, dolomitic limestone, and gypsum), and the plants were watered daily and fertilized every week with 15-30-15 N-P-K fertilizer. Self-pollinated seeds were obtained by bagging of individual plants with transparent and micro-perforated plastic bags and the seeds were used to grow the next generation population. The detail of *B. napus* canola inbred line development from these interspecific crosses is presented in Fig. 1.

Plant fertility analysis

Plant fertility in the F_2 and BC_1 generation populations was estimated based on seed yield per plant (g), while plant fertility in the subsequent generations (F_3, BC_1F_2, F_4, BC_1F_3, F_5, BC_1F_4, F_6, BC_1F_5, and F_7, BC_1F_6) was estimated based on silique length (mm), number of seeds per silique, and seed yield per plant (g). For measurement of silique length, three to five siliques from the middle to upper half of the main raceme were measured and the mean values were used in data analysis. Seeds from the same siliques were counted and the average number of seeds per silique was calculated. Data of these traits were compared with the *B. napus* parent A04-73NA grown along with these populations to estimate plant fertility.

Fatty acid analysis

Erucic acid content was estimated in self-pollinated seeds of the F_2 and F_2-derived (F_3, F_4, F_5 and F_6) plants, and the BC_1 and BC_1-derived (BC_1F_3, BC_1F_4, and BC_1F_5) plants by gas chromatographic technique. For this, 0.2 g bulk seeds were used. The details of oil extraction and conversion to methyl esters, and gas chromatographic analysis of erucic caid content is reported elsewhere (Bennett et al. 2008).

Glucosinolate analysis

Total glucosinolate (GSL) content in seeds harvested from the F_2- and BC_1-derived populations (F_3, F_4, BC_1F_3, F_5, BC_1F_4, F_6, BC_1F_5, F_7, BC_1F_6) was determined by near-infrared spectroscopy (NIRS, FOSS NIRSystems model 6500) method. For this, 2.5 to 4.0 g self-pollinated seeds harvested from individual plants grown in greenhouse or 5.0 to 8.0 g open-pollinated bulk seeds harvested from several plants grown in field plots were used. Glucosinolate content was calculated on 8.5% moisture basis and reported as μmol/g seed.

Ploidy analysis

Flow cytometric analysis for relative nuclear DNA content (or Partec value) was done on F_8 and BC_1F_7 generation plants grown in a greenhouse to estimate their approximate chromosome number — whether the plants were similar to the *B. napus* or *B. oleracea* parent. For this, two leaf samples, each of approximately 0.5 cm^2 in size, were collected from each plant (3-4 weeks old) and were chopped with a razor blade in extraction buffer (Partec GmbH, Münster, Germany). After that, the samples were filtered through 50 μm Partec CellTrics disposable filter and 1.6 ml nuclear fluorochrome DAPI (4,6-diaminido-2-phenylindole, Sigma, product no. D-9542) staining buffer was added. The samples were incubated for 1-2 min and analyzed by a Partec Ploidy Analyzer (Partec GmbH, Münster, Germany). Mean value of the two samples were used for data analysis.

Molecular marker analysis

Molecular marker analysis was done to study diversity of the interspecific cross-derived plants from the elite canola line A04-73NA. For this, a total of 76 F_4 and 111 BC_1F_3 generation plants of the four crosses and their parents were used. Genomic DNA of the plants was extracted from leaf samples of 3-4 weeks old plants grown in a greenhouse using a SIGMA DNA extraction kit (Sigma-Aldrich, St. Louis, MO) and following manufacturer's instructions, and the DNA was diluted to a concentration of 15 ± 5 ng μL^{-1} for polymerase chain reaction (PCR). A total of 366 SSR markers from nine C genome linkage groups (C1-C9) were tested on the five parents for polymorphism. These markers were obtained from Agriculture and Agri-Food Canada (AAFC) (299 markers) through a material transfer agreement, and the markers published by Cheng et al. (2009) (10 markers) and Li et al. (2011) (57 markers). Based on parental polymorphism, 102 markers from nine linakge groups were used to genotype the F_4 and BC_1F_3 plants. PCR was performed according to Kebede et al. (2010) and genotyping data was obtained by analysing the samples with a capillary ABI sequencer No. 3730 (Applied Biosystems, Foster City, CA).

Statistical analysis

Data recorded on different agronomic and seed quality traits were analyzed for basic descriptive statistics such as mean, range, standard deviation and standard error by using MS Excel, and comparison of different generation populations was done by using Statistical Analysis Software (SAS version 9.3).

For molecular marker data analysis, the marker amplicons were given a score of 1 when present in a sample and a score of 0 when absent. A binary data matrix based on these scores for different SSR markers was produced and used to calculate Dice genetic similarity coefficients (Nei and Li 1979) by using the software Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.2) (Rohlf 2000). These similarity coefficients were used for cluster analysis following unweighted pair-group method with arithmetic mean (UPGMA).

Results

Production of interspecific F_1 hybrids

A total of 214 fertilized ovules from 112 cross-pollinations of the four crosses were cultured *in vitro* from where 60 embryos were obtained which yielded 47 F_1 plants. Hybrid nature of the plants was confirmed through morphological comparision with the *B. napus* parent. Number hybrid plants obtained per pollination varied, depending on the crosses, from 0.19 to 0.96 with a mean of 0.42 (Table 1).

Production of F_2 and BC_1 generation population and their fertility

All F_1 plants showed high sterility and produced very little pollen; therefore, manual self-pollination of individual flower (bud) was done to obtain sufficient number of F_2 seeds. A total of 457 F_2 seeds of the four crosses were obtained from 594 manual self-pollinations and this translated to 0.77 (range 0.04 to 1.61) seeds per self pollination (Table 2a). On the other hand, backcrossing of the F_1 plants (female) to the *B. napus* parent (male) yielded 0.65 (range 0.26 to 2.41) seeds per pollination (Table 2b). Of the four crosses, the F_1 plants of *B. oleracea* var. *italica* produced the greatest number of F_2 and the cross involving *B. oleracea* var. *botrytis* produced the greatest number of BC_1 seeds per pollination. A total of 183 F_2 and 210 BC_1 plants were grown where 69% and 81% plants, respectively, of these two populations produced viable seeds (Table 2).

Growing F_3 and BC_1F_2, and subsequent generation populations

To capture maximum genetic diversity of the F_2 and BC_1 populations in their progeny, a total of 636 F_3 plants from all 127 families and 761 BC_1F_2 plants from all 170 families of the four crosses were grown (Table 2). About 60% of the F_3 plants and 40% of the BC_1F_2 plants of the four crosses produced viable seeds. Selection in F_3 and BC_1F_2 and in their progeny generations was done primarily for the two canola quality traits; however, plants with extremely low seed set in some cases were discarded. A total of 420, 740, 384 and 514 plants, respectively, of the F_4, F_5, F_6 and F_7 generations, and 549, 835, 422 and 639 plants, respectively, of the BC_1F_3, BC_1F_4, BC_1F_5 and BC_1F_6 generations of the four crosses were grown. An increase in the length of silique and number of seeds per silique was observed with the progression of generations. For example, the F_4 and BC_1F_3 populations, grown in greenhouse, on average, produced about 30

mm long silique carrying about 3 seeds per silique, while the F_6 and BC_1F_5 populations, grown in greenhouse, produced about 10 mm longer silique with about 3-times greater number of seeds per silique. Silique length and number of seeds per silique of the F_7 and BC_1F_6 populations, grown in field is presented in Fig. 2 (supplementary Table 1). No significant difference between the populations of the four crosses could be found for these two silique traits. These populations still produced about 10 mm shorter silique with fewer seeds than the *B. napus* parent. All F_8 and BC_1F_7 plants grown in greenhouse produced viable seed under self-pollination.

Erucic acid content in F_2 and BC_1, and in their progeny generations

Frequency distribution of the F_2 and BC_1 plants for erucic acid content in seed oil is presented in Fig. 3A and 3B, respectively, and scatter diagrams for the content of this fatty acid in different parent vs. offspring generations are presented in Fig. 3C, 3D and 3E. Erucic acid content in both F_2 and BC_1 population ranged from 0 to about 20%. Segregation for absence (<1%) vs. presence (>1%) of erucic acid in the F_2 population deviated significantly from the expected 1:3 ratio (χ^2 = 5.33, $p<0.05$); and in the BC_1 population, segregation deviated significantly from 1:1 ratio (χ^2 = 47.27, $p<0.001$). In both cases, significantly less number of plants fell into the zero erucic acid group (Fig. 3A, 3B). Considering additive effect of the erucic acid alleles, nine different genotypes resulting five phenotypes would be expected in the F_2 generation, as outlined by Rahman et al. (2015). Based on data of the 36 F_2 plants, only three phenotypic groups could be established while the two other groups of plants producing about 30% and 40% erucic acid could not be found (Fig. 3A); this deviation was apparently due to small population size. In case of the BC_1 population, four genotypes resulting three phenotypic classes with about 0%, 10% and 15% erucic acid would be expected in 2:1:1 ratio. The observed distribution of the BC_1 plants covered the expected range of variation for this fatty acid; however, the distribution deviated significantly from the expected distribution where significantly greater number of plants belonging to 10% erucic acid class was found (Fig. 3B).

Scatter diagram of the F_2 vs. F_3 and F_3 vs. F_4 population showed that the zero-erucic acid type stabilized for this phenotype. On the other hand, the parent generation plants with erucic acid content up to about 10% often gave zero-erucic progeny (Fig. 3C and 3D). In case of the population derived from the BC_1, parent-offspring relationship was studied in BC_1F_3 vs. BC_1F_4

populations. In this case, in addition to the zero and 10% erucic acid class, the 15% erucic acid class also yielded zero-erucic progeny (Fig. 3E). Despite the deviation from the expected segregation found in the progeny derived from this interspecific cross, selection for zero-erucic phenotype was quite effective; all F_7 and BC_1F_6 generation populations were indeed zero-erucic acid type (data not shown).

Glucosinolate (GSL) content in F_2- and BC_1-derived populations

The estimation of seed GSL content was started on seeds harvested from the F_3 and BC_1F_3 plants due to the lack of the quantity seeds needed for this analysis from the earlier generations. GSL content in the seeds harvested from 199 F_3 plants of the four crosses grown in greenhouse ranged from 5.2 to 77.4 µmol/g seed with a mean of 29.3 ± 15.1 SD (*B. napus* parent, 8.9 ± 1.7 SD µmol/g seed). In this generation, the mean GSL content of the four crosses was similar (26.3 ± 12.5 SD to 34.6 ± 12.7 SD). In case of the BC_1-derived population, GSL content in the seeds harvested from 343 BC_1F_3 plants of the four crosses varied from 8.1 to 81.7 µmol/g seed with a mean of 33.6 ± 16.6 SD (*B. napus* parent, 9.7 ± 0.9 SD µmol/g seed); mean GSL content of the four crosses varied from 29.0 ± 10.4 SD to 42.2 ± 22.1 SD µmol/g seed. Seed GSL content is known to be influenced by the environment (Mailer and Pratley 1990) as well as seed set of the plants (Holm et al. 1985), therefore, no stringent selection for this trait was performed in the early generation populations – seed with more than 50 µmol/g seed were primarily discarded; however, stronger selection for low GSL content was performed in F_5 and BC_1F_4 generation when higher fertility (seed set) of the plants was observed.

The F_2- and BC_1-derived populations were grown under different growth conditions (greenhouse or field) and this trait is known to be significantly influenced by environment (Mailer and Pratley 1990); therefore, GSL content data of the F_2 and BC_1-derived plants was adjusted based on GSL content of the *B. napus* parent A04-73NA. For this, the GSL content of A04-73NA was subtracted from the GSL content of the F_2- or BC_1-derived plants grown under same growth condition. This resulted either positive (GSL content higher than confidence limits of A04-73NA) or negative (GSL content lower than confidence limits of A04-73NA) or zero (falling within confidence limits of A04-73NA) values for the F_2- and BC_1-derived plants. Based on this data, scatter diagrams of the parent vs. offspring generation of the F_2- and BC_1-derived

populations are presented in Fig. 4.Correlation between the parent and offspring generation was positive and varied from 0.27 to 0.72 (Fig. 4); no consistent pattern of the strength of this relation between the parent and offspring generations grown in greenhouse or the parent generation grown in greenhouse and the offspring grown in field or *vice versa* was found. However, selection for low GSL content was effective in all populations. GSL content in the F_7 and BC_1F_6 generation populations, grown in field, varied from about 10 - 50 µmol/g seed with mean of 17.5 ± 6.6 SD to 28.2 ± 11.0 SD (*B. napus*: range 12.7 – 24.5; mean 19.6 ± 2.4 SD) (Fig. 5), respectively, and the population selected for growing the next generation population had mean GSL content less than 20 µmol/g seed (Supplementary Table 2). Thus, it was possible to develop canola quality *B. napus* lines from all four crosses, with or without backcrossing.

Ploidy analysis of the F_8 and BC_1F_7 populations

The F_8 and BC_1F_7 populations had similar relative nuclear DNA content (partec value) and all were statistically similar ($p > 0.05$) to the *B. napus* parent A04-73NA; none of the plants had nuclear DNA content similar to *B. oleracea* (Fig. 6; Supplementary Table 3). This suggests that all F_8 and BC_1F_7 plants stabilized into *B. napus* type ($2n = 38$).

Molecular marker analysis of the F_4 and BC_1F_3 population

A total of 102 SSR markers from the *Brassica* C genome amplified a total of 325 alleles specific to the four *B. oleracea* parents (103 of *B. oleracea* var. *alboglabra*, 93 of var. *botrytis*, 60 of var. *italica*, and 69 of var. *capitata*). Genetic similarity of the four variants of *B. oleracea*, var. *albograbra*, var *botrytis*, var. *italica*, and var. *capitata*, with the *B. napus* parent was 0.264, 0.255, 0.184, and 0.160, respectively.

Cluster analysis of the F_4 and BC_1F_3 populations showed that, wide diversity existed among the populations derived from the four interspecific crosses where several lines were genetically distinct from the *B. napus* parent (Fig. 7); however, few lines lack *B. oleracea* alleles and showed similarity with the *B. napus* parent. No distinct grouping of the F_4 and BC_1F_3 populations could be found in case of the populations derived from *B. napus* × var. *alboglabra* and *B. napus* × var. *botrytis* crosses. However, the F_4 and BC_1F_3 populations derived from the *B.*

napus × var. *italica* and *B. napus* × var. *capitata* crosses tended to form stronger genetically distinct groups where the most of the BC_1F_3 plants were genetically closer to the *B. napus* parent; the two *B. oleracea* parents involved in these crosses also showed greatest genetic distance from the *B. napus* parent.

Discussion

Breeding effort in the last few decades has substantially diversified the A genome of the Chinese *B. napus* through introgression of allelic diversity from its progenitor species *B. rapa* (Qian et al. 2006; Liu 1985 cited by Chen et al. 2008; Mei et al. 2011; Li et al. 2013). In the recent years researchers, such as Bennett et al. (2012) and Li et al. (2014) emphasized the need of diversifying the C genome of *B. napus* through the use of the progenitor species *B. oleracea*.

In the present study, we investigated the prospect of developing canola quality euploid *B. napus* lines from *B. napus* × *B. oleracea* interspecific crosses involving different variants of this progenitor species, viz. Chinese kale, cauliflower, broccoli and cabbage. In addition to this, backcrossing of the F_1 to the *B. napus* parent was done with the hypothesis that fertile euploid *B. napus* ($2n = 38$) canola lines can be obtained relatively easily in the population derived from BC_1 as compared to the population derived from F_2 due to higher contribution of the *B. napus* genome. Indeed, plant fertility in BC_1 population was significantly higher than in F_2 – about 25% greater number of BC_1 plants produced viable seeds as compared to the F_2 popualtion. In contrast, when compared the BC_1F_2 and F_3 poulation, the F_3 population showed significantly higher fertility as compared to the BC_1F_2 population. This might be for the reason that the F_3 population passed through self-pollination for two times while the BC_1F_2 population passed through this only one time, and this resulted higher fertility in the F_3 population as compared to the BC_1F_2 population. However, the difference between the populations derived from the F_2 and BC_1 was not evident after an additional generation of self-pollination. Thus, the results from this study, by using four variants of *B. oleracea*, demonstrated that self-pollination of the *B. napus* × *B. oleracea* populations in their early generation directs the population towards the euploid *B. napus* type; indeed, some of the F_2- and BC_1-derived plants had seed set similar to *B. napus* in their early generation (data not presented). This indicates that the early generation interspecific hybrid plants with aneuploid chromosome composition largely produce unviable gametes;

however, only a couple of generation of self-pollination improve fertility of the plants to a great extent. Indeed, Rahman et al. (2011) and Li et al. (2014) obtained stable euploid *B. napus* plant ($2n = 38$) in F_2 and F_3 population of *B. napus* × *B. oleracea* interspecific crosses using a single variant of *B. oleracea* in their study. The low seed set in the early generation populations could also be due to the effect of self-incompatibility allele's introgressed from *B. oleracea*. However, the lack of consistent difference between the population derived from the cross involving var. *alboglabra* (self-compatible type) and the populations derived from the other three crosses (Table 2) suggests that aneuploidy played a greater role in this low seed set. Despite several challenges associated with *B. napus* × *B. oleracea* interspecific cross, canola quality *B. napus* lines were obtained form all four crosses involving different variants of *B. oleracea*.

The genome composition of the F_1 plants of the *B. napus* × *B. oleracea* crosses was ACC. Theoretically, it was expected that these F_1 plants will produce gametes with chromosome number ranging from 9C + 0A to 9C + 10A due to normal disomic segregation of the nine homologous chromosome pairs of the C genome and random assortment of the haploid set of the 10 A genome chromosomes. Based on this, it was expected to obtain progeny plants with variable chromosomes composition ranging from euploid *B. oleracea* (CC, $2n = 18$) to euploid *B. napus* (AACC, $2n = 38$) with majority being aneuploids with variable chromosome composition (CC + 1A to CC + 19A). The aneuploid plants were expected to show variable degree of sterility. Indeed, majority of the F_2 plants showed sterility and few *B. napus* type plant with high fertility was also obtained; however, no *B. oleracea* (CC, $2n = 18$) type plant could be obtained in F_2 or in subsequent generation populations of all four interspecific crosses. Thus, through working with different variants of *B. oleracea*, we demonstrate that the progeny of the ACC digenomic triploid stabilizes into amphidiploid *B. napus* type; this is apparently due to greater viability of the gametes carrying greater number of chromosomes of these two genomes. Brassica allopolyploids can tolerate aneuploidy including simultaneous loss and gain of the A- and C-genome chromosomes to some extent (Zhou et al. 2016); therefore, it is probable that some of the *B. napus* lines developed in this study are not 'true' amphidiploid. However, Qian et al. (2005) found that the digenomic AAC triploid, derived from *B. napus* × *B. rapa* interspecific crosses, mostly produces AC euploid gametes, and working with a single *B. oleracea* variant, Rahman et al. (2015) showed that the progeny of *B. napus* × *B. oleracea* interspecific cross stabilizes into *B. napus*. Similarly, Attri and Rahman (2018) also reported that the AAC progeny

of *B. napus* × *B. rapa* interspecific crosses stabilizes into *B. napus* type. Thus, it is highly likely that most of the *B. napus* lines developed in the present study are euploid. This knowledge has important implication in breeding that, no special effort for selection of *B. napus* type plants will be needed in the self-pollinated progeny of the digenomic triploids carrying the A and C genomes – population will mostly stabilize into *B. napus* type. Zaman (1988) and Rahman (2001) have also reported that the progeny of the amphidiploid *B. carinata* (BBCC, 2*n* = 34) × diploid *B. oleracea* mostly stabilizes into *B. carinata* type. In contrast, Pelé et al. (2016) developed AA (2*n* = 20) genome plants from (*B. napus* × *B. rapa*) × *B. napus* and (*B. napus* × *B. rapa*) × *B. rapa* crosses through selection of plants for chromosomeme number and composition. However, no selection was applied in the present study for chromosomeme number and composition and this might be the reason of stabilizing all plants into *B. napus* type. The A and C genomes of *B. napus* has close evolutionary relationship (Song et al. 1988); therefore, homoeologous pairing of chromosomes can occur (Mason et al. 2010; Cui et al. 2012) and this can result variable changes in the chromosomes including chromosomal recombination, translocation, and gain or loss of the entire or a part of the chromosome (Xiong and Pires 2011; Nicolas et al. 2012; Zhou et al. 2016). Occurrence of such chromosomal change in the populations developed in the present study cannot be ruled out; however, this investigation was beyond the scope of the present study.

The four *B. oleracea* parents used in this study possess high content of erucic acid in seed oil and high content of glucosinolates in seed meal; therefore, segregation for these two traits in F_2 and BC_1 and in their progeny generations would primarily reflect the segregation of the C genome alleles. Segregation for erucic acid in an F_2 population of *B. napus* × *B. oleracea* and in a backcross population of (*B. napus* × *B. oleracea*) × *B. oleracea* was studied by Rahman et al. (2015) and Bennett et al. (2008); they found a significant deviation from the expected segregation for this trait in these populations. To our knowledge, no study has so far been conducted on segregation of erucic acid alleles in backcross population of (*B. napus* × *B. oleracea*) × *B. napus* interspecific cross. Backcrossing of the F_1 ($A^eC^eC^E$ where, e = zero erucic acid allele, E = high erucic acid allele) plants to the *B. napus* parent ($A^eA^eC^eC^e$) was expected to produce $A^eC^eC^e$, $A^eA^eC^eC^e$, $A^eA^eC^eC^E$ and $A^eC^eC^E$ genotypes for erucic acid alleles. The $A^eC^eC^e$ and $A^eA^eC^eC^e$ genotypes were expected to be virtually free (<1%) from erucic acid, while the plants of $A^eA^eC^eC^E$ and $A^eC^eC^E$ genotype were expected to produce about 10 % (8 – 15%) and

433 >15% erucic acid in seed oil, resectively (Chen and Heneen 1989; Bennett et al. 2008). However,
434 segregation distortion for this trait was observed in the present study where significantly less
435 number of plant was found in the zero erucic acid group and higher number of plants in 10%
436 erucic acid group ($A^eA^eC^eC^+$).
437 Seed glucosinolate (GSL) content is generally considered a quantitative trait controlled by
438 multiple gene loci (Kondra and Stefansson 1970; Rücker and Röbbelen 1994; Toroser et al.
439 1995; Howell et al. 2003; Hasan et al. 2008; Rahman et al. 2014) with both additive and non
440 additive effect of the genes (Hill et al. 2003). With this genetic control of the trait, Bahrani and
441 McVetty (2007) found significant correlation (r = 0.30 p =0.01) between the parent generation
442 (F_3) of *B. napus* grown in greenhouse vs. progeny generation (F_4) grown in field for this trait.
443 However, in the present study, correlation between the parent and progeny generation, based on
444 pooled data of the crosses, varied from 0.27 to 0.72. This wide variation is apparently due to the
445 difference between the type of populations used in these two studies as well as the growth
446 conditions. Despite these challenges, it was possible to develop low GSL lines from all four
447 interspecific crosses, irrespective of whether the F_1 plants were backcrossed to the low GSL *B.*
448 *napus* parent or not. This is apparently due to simpler segregation of the trait involving the
449 alleles only from the C genome.
450 In conclusion, this research demonstrated the feasibility of developing canola quality
451 genetically diverse euploid *B. napus* lines ($2n$ = 38) by using different variants of *B. oleracea* in
452 *B. napus* × *B. oleracea* interspecific cross. Furthermore, by the use of the different variants of *B.*
453 *oleracea*, this study also demonstrated that no special attention need to be paid for selection of *B.*
454 *napus* type plants in the self-pollinated progeny of this interspecific cross as all stabilizes into *B.*
455 *napus* type. Thus, re-constitution of the C genome of *B. napus* canola with the C genome of the
456 different variants of *B. oleracea* is expected to enrich the gene pool of this crop.
457
458 **Acknowledegments** H.R. gratefully acknowledges the Natural Sciences and Engineering
459 Research Council of Canada (NSERC) and Crop Production Services (CPS) for financial support
460 to this project. Authors also acknowledge technical staff of the Canola Program of the University
461 of Alberta for assistance in different routine operations, and Dr. Berisso Kebede for guidance in
462 molecular marker analysis.
463

Conflicts of interest The authors declare no conflicts of interest.

References

Abel S, Möllers C, Hecker HC (2005) Development of synthetic *Brassica napus* lines for the analysis of ‘‘fixed heterosis’’ in allopolyploid plants. Euphytica 146:157−163

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

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

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

Bennett RA, Séguin-Swartz G, Rahman H (2012) Broadening genetic diversity in canola (*Brassica napus* L.) using the C-genome species *B. oleracea*. Crop Sci 52:2030−2039

Bus A, Korber N, Snowdon RJ, Stich B (2011) Patterns of molecular variation in a species-wide germplasm set of *Brassica napus*. Theor Appl Genet 123:1413−1423

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

Chen BY, Heneen WK (1989) Fatty acid composition of resynthesized *Brassica napus* L., *B. campestris* L. and *B. alboglabra* Bailey with special reference to the inheritance of erucic acid content. Heredity 63:309−314

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

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

494 Chiang BY, Grant WF, Chiang MS (1978) Transfer of resistance to race 2 of *Plasmodiophora*
495 *brassicae* from *Brassica napus* to cabbage (*B. oleracea* var. *capitata*). II. Meiosis in the
496 interspecific hybrids between *B. napus* and 2x and 4x cabbage. Euphytica 27:81−93
497 Cowling WA (2007) Genetic diversity in Australian canola and implications for crop breeding
498 for changing future environments for crop breeding for changing future environments.
499 Field Crop Res 104:103−111
500 Chi C, Ge X, Gautam M, Kang L, Li Z (2012) Cytoplasmic and genomic effects on meiotic
501 pairing in *Brassica* hybrids and allotetraploids from pair crosses of three cultivated
502 diploids. Genetics 191:725–738
503 Diers BW, Osborn TC (1994) Genetic diversity of oilseed *Brassica napus* germplasm based on
504 restriction fragment length polymorphisms. Theor Appl Genet 88:662−668
505 dos Santos JB, Nienhuis J, Skroch P, Tivang J, Slocum MK (1994) Comparison of RAPD and
506 RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L.
507 genotypes. Theor Appl Genet 87:909−915
508 Falk DE (2010) Generating and maintaining diversity at the elite level in crop breeding. Genome
509 53:982−991
510 Friedt W, Snowdon R (2010) Oilseed rape. In Vollmann J, Rajcan I (eds) Oil Crops. Handbook
511 of Plant Breeding 4, Springer Dordrecht Heidelberg London New York, pp 91−126
512 Fu YB, Gugel RK (2010) Genetic diversity of Canadian elite summer rape (*Brassica napus* L.)
513 cultivars from the pre- to post-canola quality era. Can J Plant Sci 90:23−33
514 Gyawali S, Hegedus DD, Parkin IAP, Poon J, Higgins E, Horner K, Bekkaoui D, Coutu C,
515 Buchwaldt L (2013) Genetic diversity and population structure in a world collection of
516 *Brassica napus* accessions with emphasis on South Korea, Japan, and Pakistan. Crop Sci
517 53:1537−1545
518 Hasan M, Friedt W, Kühnemann JP, Freitag NM, Link K, Snowdon RJ (2008) Association of
519 gene-linked SSR markers to seed glucosinolate content in oilseed rape (*Brassica napus* ssp.
520 *napus*). Theor Appl Genet 116:1035−1049
521 Hasan M, Seyis F, Badani AG, Pons-Kuhnemann J, Friedt W, Lühs W, Snowdon RJ (2006)
522 Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet
523 Res Crop Evol 53:793−802

524 Hill J, Lethenborg P, Li PW, Rahman MH, Sørensen H, Sørensen JC (2003) Inheritance of
525 progoitrin and total aliphatic glucosinolates in oilseed rape (*Brassica napus* L). Euphytica
526 134:179–187
527 Holm SN, Rahman MH, Stølen O, Sørensen H (1985) Studies on pollination requirement in
528 rapeseed (*Brassica campestris*). In: Sørensen H (ed) Advances in the Production and
529 Utilization of Cruciferous Crops. Martinus Nijhoff Publ, Dordrecht, pp 245−253
530 Howell PM, Sharpe AG, Lydiate DJ (2003) Homoeologous loci control the accumulation of seed
531 glucosinolates in oilseed rape (*Brassica napus*). Genome 46:454−460
532 Izzah NK, Lee J, Perumal S, Park JY, Ahn K, Fu D, Kim G-B, Nam Y-W, Yang T-J (2013)
533 Microsatellite-based analysis of genetic diversity in 91 commercial *Brassica oleracea* L.
534 cultivars belonging to six varietal groups. Genet Resour Crop Evol 60:1967–1986
535 Kebede B, Thiagarajah MR, Zimmerli C, Rahman MH (2010) Improvement of open-pollinated
536 spring rapeseed (*Brassica napus* L.) through introgression of genetic diversity from winter
537 rapeseed. Crop Sci 50:1236−1243
538 Kondra ZP, Stefansson BR (1970) Inheritance of the major glucosinolates of rapeseed (*Brassica*
539 *napus*) meal. Can J Plant Sci 50:643−647
540 Kubik TJ, Hawkins GP, Stringam GR (1999) Cytological stability of doubled haploid lines
541 derived from interspecific crosses between *B. napus* L. and *B. rapa* L. Proc10th Intl
542 Rapeseed Congr, Australia
543 Lázaro A, Aguinagalde I (1998) Genetic diversity in *Brassica oleracea* L. (Cruciferae) and wild
544 relatives (2*n* = 18) using RAPD markers. Annals Bot 82:829−833
545 Li H, Chen X, Yang Y, Xu J, Gu J, Fu J, Qian X, Zhang S, Wu J, Liu K (2011) Development and
546 genetic mapping of microsatellite markers from whole genome shotgun sequences in
547 *Brassica oleracea*. Mol Breed 28:585−596
548 Li Q, Mei J, Zhang Y, Li J, Ge X, Li Z, Qian W (2013) A large-scale introgression of genomic
549 components of *Brassica rapa* into *B. napus* by the bridge of hexaploid derived from
550 hybridization between *B. napus* and *B. oleracea*. Theor Appl Genet 126:2073−2080
551 Li Q, Zhou Q, Mei J, Zhang Y, Li J, Li Z, Ge X, Xiong Z, Huang Y and Qian W (2014)
552 Improvement of *Brassica napus* via interspecific hybridization between *B. napus* and *B.*
553 *oleracea*. Mol Breed 34:1955–1963

554 Liu H (1985) Rapeseed genetics and breeding. Shanghai Science and Technology Press,
555 Shanghai pp 556–559
556 Mailer RJ, Pratley JE (1990) Field studies of moisture availability effects on glucosinolate and
557 oil concentration in the seed of rape, (*Brassica napus* L.) and turnip rape (*Brassica rapa* L.
558 var. *silvestris* (Lam.) Briggs). Can J Plant Sci 70:399–407
559 Mason AS, Huteau V, Eber F, Coriton O, Yan G, Nelson MN, Cowling WA, ChevreAM (2010)
560 Genome structure affects the rate of autosyndesis and allosyndesis in AABC, BBAC and
561 CCAB *Brassica* interspecific hybrids. Chromosome Res 18:655–666
562 Nicolas SD, Monod H, Eber F, Chèvre AM, Jenczewski E (2012) Non-random distribution of
563 extensive chromosome rearrangements in *Brassica napus* depends on genome organization.
564 Plant J 70:691–703
565 Mei J, Fu Y, Qian L, Xu X, Li J, Qian W (2011) Effectively widening the gene pool of oilseed
566 rape (*Brassica napus* L.) by using Chinese *B. rapa* in a 'virtual allopolyploid' approach.
567 Plant Breed 130:333–337
568 Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction
569 endonucleases. Proc Natl Acad Sci 76:5269–5273
570 Pelé A, Trotoux G, Eber F, Lodé M, Gilet M, Deniot G, Falentin C, Nègre S, Morice J,
571 Rousseau-Gueutin M, Chèvre A-M (2017) The poor lonesome A subgenome of *Brassica*
572 *napus* var. Darmor (AACC) may not survive without its mate. New Phytol 213: 1886–1897
573 Prakash S, Wu XM, Bhat SR (2012) History, Evolution, and Domestication of *Brassica* Crops.
574 Plant Breed Rev 35:19–84
575 Qian W, Chen X, Fu D, Zou J, Meng J (2005) Inter sub-genomic heterosis in seed yield potential
576 observed in a new type of *Brassica napus* introgressed with partial *Brassica rapa* genome.
577 Theor Appl Genet 110:1187–1194
578 Qian W, Li Q, Noack J, Sass O, Meng J, Frauen M, Jung C (2009) Heterotic patterns in rapeseed
579 (*Brassica napus* L.): II. Crosses between European winter and Chinese semi-winter lines.
580 Plant Breed 128:466–470
581 Qian W, Meng J, Li M, Frauen M, Sass O, Noack J, Jung C (2006) Introgression of genomic
582 components from Chinese *Brassica rapa* contributes to widening the genetic diversity in
583 rapeseed (*B. napus* L.), with emphasis on the evolution of Chinese rapeseed. Theor Appl
584 Genet 113:49–54

585 Qian W, Sass O, Meng J, Li M, Frauen M, Jung C (2007) Heterotic patterns in rapeseed
586 (*Brassica napus* L.): I. Crosses between spring and Chinese semi-winter lines. Theor Appl
587 Genet 115:27–34
588 Rahman MH (2001) Production of yellow-seeded *Brassica napus* through interspecific crosses.
589 Plant Breed 120:463–472
590 Rahman H (2013) Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic
591 germplasm. Can J Plant Sci 10:24–29
592 Rahman H, Peng G, Yu F, Falk KC, Kulkarni M, Selvaraj G (2014) Genetics and breeding for
593 clubroot resistance in Canadian spring canola (*Brassica napus* L.). Can J Plant Path 36
594 (S1):122–134
595 Rahman MH, Bennett RA, Yang RC, Kebede B, Thiagarajah MR (2011) Exploitation of the late
596 flowering species *Brassica oleracea* L. for the improvement of earliness in *B. napus* L.: an
597 untraditional approach. Euphytica 177:365–374
598 Rahman H, Kebede B, Zimmerli C, Yang RC (2014) Genetic study and QTL mapping of seed
599 glucosinolate content *Brassica rapa* L. Crop Sci 54:537–543
600 Rahman H, Bennett RA, Séguin-Swartz G (2015) Broadening genetic diversity in *Brassica*
601 *napus* canola: Development of canola-quality spring *B. napus* from *B. napus* × *B. oleracea*
602 var. a*lboglabra* interspecific crosses. Can J Plant Sci 95:29–41
603 Rohlf FJ (2000) NTSYS-pc numerical taxonomy and multivariate analysis system. Exeter
604 Software, New York, NY
605 Rücker B, Röbbelen G (1994) Inheritance of total and individual glucosinolate contents in seeds
606 of winter oilseed rape (*Brassica napus* L.). Plant Breed113:206–216
607 SAS Institute (2011) SAS Statistical Analysis Software Version 9.3. SAS Inst., Cary, NC
608 Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction
609 fragment length polymorphisms (RFLPs). 1. Genome evolution of diploid and
610 amphidiploid species. Theor Appl Genet 75: 784–794
611 Simonsen V, Heneen WK (1995) Genetic variation within and among different cultivars and
612 landraces of *Brassica campestris* L. and *B. oleracea* L. based on isozymes. Theor Appl
613 Genet 91:346–352

614 Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994) Comparison of RFLP
615 and RAPD markers to estimating genetic relationships within and among cruciferous
616 species. Theor Appl Genet 88:973–980
617 Toroser D, Thormann CE, Osborn TC, Mithen R (1995) RFLP mapping of quantitative trait loci
618 controlling seed aliphatic glucosinolate content in oilseed rape (*Brassica napus* L.). Theor
619 Appl Genet 91:802–808
620 Udall JA, Quijada PA, Polewicz H, Vogelzang R, Osborn TC (2004) Phenotypic effects of
621 introgressing Chinese winter and resynthesized *Brassica napus* L. germplasm into hybrid
622 spring canola. Crop Sci 44:1990–1996
623 Xiong Z, Pires CJ (2011) Karyotype and identification of all homoeologous chromosomes of
624 allopolyploid *Brassica napus* and its diploid progenitors. Genetics 187:37–49
625 Zaman MW (1988) Limitations for introgression of yellow seed coat colour in *Brassica napus*. J
626 Swedish Seed Assoc 98:157–161

Table 1. Production of *Brassica napus* × *B. oleracea* interspecific hybrids through application of *in vitro* ovule culture technique

Cross[1]	No. pollination	No. ovules cultured	No. ovules/ pollination	No. embryo to solid media	No. embryo/ pollination	No. F_1 plantlet to soil	No. F_1/ pollination
B.nap × *B.o.albo*	29	55	1.90	9	0.31	8	0.28
B.nap × *B.o.bot*	42	26	0.62	14	0.33	8	0.19
B.nap × *B.o.ital*	26	51	1.96	27	1.04	25	0.96
B.nap × *B.o.capi*	15	82	5.47	10	0.67	6	0.40
Total	112	214	0.52	60	0.54	47	0.42

[1]*B.nap* = *B. napus* line A04-73NA, *B.o.albo* = *B. oleracea* var. *alboglabra*, *B.o.bot* = *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1, *B.o.ital* = *B. oleracea* var. *italica* cv. Premium Crop, *B.o.capi* = *B. oleracea* var. *capitata* cv. Blabro.

Table 2. Production of F_2 and BC_1 seeds and development of subsequent generation populations of four *Brassica napus* × *B. oleracea* interspecific crosses.

(a) F_2 and subsequent generation populations

Cross[1]	No. bud pollination of F_1 plants	No. F_2 seeds harvested	No. F_2 seeds/self-pollination	No. F_2 plants grown	No. F_2 plants produced seeds	% fertile F_2 plants	No. F_3 plants grown	No. F_3 plants produced seeds	% fertile F_3 plants
B.nap × *B.o.albo*	219	67	0.31	60	37	61.7	170	137	80.6
B nap × *B.o.bot*	97	66	0.68	60	35	58.3	126	74	58.7
B.nap × *B.o.ital*	200	321	1.61	60	53	88.3	290	127	48.1
B.nap × *B.o.capi*	78	3	0.04	3	2	66.7	50	42	84.0
Total	594	457	0.77	183	127	69.4	636	380	59.7

(b) BC_1 and subsequent generation populations

Cross	No. crosses made	No. BC_1 seeds harvested	No. BC_1 seeds/ pollination	No. BC_1 plants grown	No. BC_1 plants produced seeds	% Fertile BC_1 plants	No. BC_1F_2 plants grown	No. BC_1F_2 plants produced seeds	% Fertile BC_1F_2 plants
(*B.nap* × *B.o.albo*) × *B.nap*	130	81	0.62	60	52	86.7	243	108	44.4
(*B.nap* × *B.o.bot*) × *B.nap*	73	176	2.41	60	52	86.7	253	111	43.9
(*B.nap* × *B.o.ital*) × *B.nap*	220	62	0.28	60	51	85.0	163	37	22.7
(*B.nap* ×*B.o.cap*) × *B.nap*	117	30	0.26	30	15	50.0	102	47	46.1
Total	540	349	0.65	210	170	81.0	761	303	39.8

[1]*B.nap* = *B. napus* line A04-73NA, *B.o.albo* = *B. oleracea* var. *alboglabra*, *B.o.bot* = *B. oleracea* var. *botrytis* cv. BARI Cauliflower-1, *B.o.ital* = *B. oleracea* var. *italica* cv. Premium Crop, *B.o.capi* = *B. oleracea* var. *capitata* cv. Blabro.