Development of *Brassica Napus* L. Ogu-INRA CMS Restorers Using Recurrent Full-Sib Selection.

Valeria Lobos-Sujo

University of Manitoba

Robert W. Duncan (✉ rob.duncan@umanitoba.ca)

University of Manitoba <https://orcid.org/0000-0002-0515-7520>

Research Article

Keywords: recurrent selection, full-sib intermating, *Brassica napus*, Ogu-INRA CMS, restorer

Posted Date: October 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1011114/v1>

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Abstract

The Ogu-INRA CMS system in canola and rapeseed (*Brassica napus* L.) uses a cytological variant of the radish- (*Raphanus sativus* L.) derived Ogu CMS pollination control system introduced through interspecific introgression. The restorers (R-lines) contain an introgression that is associated with poor agronomic performance due to a large undesired segment of the radish chromosome that was introgressed along with the *Rfo* gene. The introgression contains pentatricopeptide (PPR) motif repeats that confer fertility restoration abilities to the R-lines. The objective of this research was to test the hypothesis that multiple cycles of intermating will result in R-lines with improved agronomic performance. A base population was developed by designing five R-line by R-line crosses. Twelve plants from each initial cross were grown and chain-crossed at random, without selection, other than the presence of the *Rfo* gene. Twelve flowers from each plant were crossed and the remainder of the plant was selfed. Three intermating crossing cycles (C_0 , C_1 and C_2) were completed and each was selfed three times for evaluation. Total pod number, seeds per pod, a visual pod rating, thousand seed weight and yield were evaluated. The visual pod rating showed a positive correlation with seeds per pod. Improvements for all traits were found at C_0 and C_1 when compared to the best parent. Individual families from two of the crosses showed a yield increase of over 78 % from the best parent. This suggests that improvements in yield components can be obtained from intermating R-lines.

Introduction

Brassica species exhibit both cross-pollination and high levels of self-pollination (Rakow and Woods 1987). Due to this variation in pollination strategies, several breeding and selection methods can be used to improve *Brassica* populations (Kumar et al. 2015). Some of the most widely used breeding methods for *Brassica* species include pedigree breeding, backcross breeding, development of synthetic and composite varieties, development of hybrids, recurrent selection, mass selection, doubled haploid (DH) development, in vitro mutagenesis and genetic transformation (Prakash et al. 2009). Most of these methods were first evaluated in different crops such as maize and wheat. Around the 1940s, corn breeders developed the recurrent selection method to improve populations (Hallauer and Carena 2012). The first time the “recurrent selection” term was used, it described a strategy for specific combining ability aimed at high-yielding corn where one cycle of breeding was completed after selecting and intermating the best selfed hybrids that originated from a cross between a crossbred lot and a ‘tester line’ (Hull 1945). From this first recurrent selection method, variations were developed including reciprocal recurrent selection for half-sib (Comstock et al. 1949) and full-sib progenies (Hallauer and Eberhart 1970). Reciprocal full-sib selection was proposed as an efficient breeding method in which non-additive genetic effects, as well as additive effects were important in the expression of hybrid superiority (Peiris and Hallauer 2005). This method was used for producing hybrids (full-sib progenies) and selfed seed on the same plant, as well as early testing of single cross combinations (Hallauer and Eberhart 1970).

Systems that repeat the same process for several cycles are referred to as recurrent selection systems (Norman 1992). The process involves, production of a set of genotypes (individuals or families), evaluation of those genotypes, identification of the superior ones to be used as parents for the next generation and intermating the selected genotypes to produce the next generation, obtaining an improved generation (Norman 1992). This method has continued to evolve as breeders modify it for different crops and traits. In 2010, a recurrent selection strategy was evaluated in a greenhouse study of dry bean as a means to pyramid white mold resistance genes (Terán and Singh 2010). Phyto-recurrent selection was another variation on the method developed to choose superior-performing *Populus* and *Salix* genotypes for phytoremediation purposes (Zalesny et al. 2010). In this case, recurrent selection was conducted both in greenhouses and growth chambers before moving to the field to increase the speed of the evaluation and selection process. A marker-based recurrent selection strategy was successfully utilized in barley to increase heterozygosity and evaluate other yield components (Nandety and Geiger 2014).

Depending on the objective of each breeding program, there are other variables that can be modified within the recurrent selection method. Some of the most common recombination methods in cross-fertilizing species include diallel crossing, chain crossing, bulk entry and bulk plot (Darbeshwar 1992). The best methods for a small number of genotypes are diallel crossing and chain crossing (Darbeshwar 1992). In diallel crossing, the selected lines/families are intermated in all possible combinations by hand pollination (Darbeshwar 1992). Chain crossing involves the intermating between entries as described in Figure 1. This method is also referred to as a partial diallel where each entry is crossed to the same number of entries providing a balanced composite of the same number of crossed seed from each selected entry (family or genotype) to initiate the next cycle (Norman 1992). The combination of chain crossing and full sib recurrent selection produces a method to evaluate several combinations in a relatively small population and helps maintain a manageable number of entries in every cycle.

A recent study, evaluated the molecular mechanisms of recurrent selection improvement in *B. napus*, and reported that some of the desirable loci were maintained in the population while undesirable loci were lost (Zhao et al. 2016). The Ogu-INRA CMS system in *B. napus* uses a cytological variant of the radish- (*Raphanus sativus* L.) derived Ogu-INRA CMS pollination control system introduced through interspecific introgression (Sakai et al. 1996; Brown et al. 2003; Giancola et al. 2003). The restorers (R-lines) contain an introgression that is associated with poor agronomic performance due to a large undesired segment of the radish chromosome that was introgressed along with the *Rfo* gene (Delourme et al. 1998). The goal of the current research was to improve agronomic traits of Ogu-INRA CMS restorer genotypes using a full-sib recurrent selection strategy.

Materials And Methods

Population development

Nine *B. napus* fertility restorer genotypes were chosen with the best agronomic ratings evaluated in Winnipeg, Manitoba in 2013. The parental genotypes originated from crosses between R2000 (Primard-Brisset et al. 2005) and Castor (McVetty et al. 1997) (UM300, UM400, UM600, UM800, UM900, UM1000, and UM2000) or R2000 and Industry (UM500 and UM700) in 2010. A base population (C_0S_0) was developed by designing five R-line by R-line crosses (first intermating) (Table 1). Twelve plants from each initial cross (full-sibs) were grown and chain-crossed (Figure 1) at random, without any pre-selection other than the presence of an *Rfo* SCAR marker (Hu et al. 2008). Twelve flowers from each plant were crossed (C_1S_0 first full-sib intermating) and the remainder of

the plant was selfed (C_0S_1). All pods were harvested from each of the twelve plants keeping the selfed and the intermated pods separate. One seed from the intermated pods of each plant was chosen at random and planted. Twelve flowers from that plant were crossed again to create the next intermating cycle (C_2S_0 second full-sib intermating). The twelve plants at each cycle formed 'families' since they originated from the same cross and were only intermated among genotypes with the same background. The number of plants per family remained consistent throughout the experiments. Two full-sib intermating crossing cycles (C_1 and C_2) were completed and each was selfed twice in order to compare all populations after their second selfing (C_0S_2 , C_1S_2 and C_2S_2) for experiment one. This generation was selfed to compare the intermating cycles at their third selfing generation (C_0S_3 , C_1S_3 and C_2S_3) (Fig. 2) for experiment two.

Greenhouse conditions

Seeds for each genotype (during population development and experiments 1 and 2) were planted at a depth of 1 cm into plastic 4 x 3 well cell packs (13 cm x 13 cm x 5 cm) containing Sunshine Metro Mix potting soil (Sungro® Horticulture, 770 Silver Street Agawam, MA, USA). Plants were germinated in a growth room (temperature day 22 °C, night 18 °C, light cycle 16 hrs light, 8 hrs dark) and watered daily in the morning until soil reached field capacity. At the two-leaf stage [14 days after planting (DAP)] (growth stage 12 (Strauss 1997)) each plant was transferred to a plastic growers pot (14.5 cm x 15 cm) using Sunshine Metro Mix potting soil in an Argus controlled greenhouse (Argus Control Systems Ltd., Surrey, BC Canada) with the following specifications (temperature: high 25 °C, low 22 °C; relative humidity: 40-50 %; light cycle: 16 hrs light, 8 hrs dark) and watered each morning using an Argus-controlled flooding, until soil in the pots reached field capacity. Plants were fertilized twice during their growing cycle; once at the time of transplanting (growth stage 12) using Plant-Prod® 10-52-10 starter water soluble fertilizer (10 % N -52 % P2O5 – 10 % K2O) at a concentration of 15 g / 3.78 L and once as soon as flower buds raised above the youngest leaves (50 DAP) (growth stage 53) using Plant-Prod® 20-20-20 classic water soluble fertilizer (20 % N -20 % P2O5 – 20 % K2O) at a concentration of 15 g / 3.78 L. Insect populations were managed with Intercept™60 WP (imidacloprid, Bayer Environmental Science, Research Triangle Park, NC, USA) added several days after transplanting (20 DAP) (growth stage 18) using a concentration of 4.1 g/1000 seedlings. All mixing and application procedures were followed as per the manufacturer's label.

Experiment setup

The two experiments consisted of a selection of 60 (12 families for each of the 5 crosses) genotypes from each cycle (C_0 , C_1 and C_2) from which four plants were planted per replicate with three replicates for a total of 2,268 plants including the nine original parents as controls (four plants in each of the three replicates). Experiment one (evaluation after two selfings S_2) was conducted from June 2015 to September 2015. Experiment two (evaluation after three selfings S_3) was conducted from December 2015 to March 2016. The following traits were evaluated for each experiment: pod rating (based on the rating scale shown in figure 3. for the overall size, shape and distribution of the pods in the plant), number of pods (all seed producing pods in the plant were counted), thousand seed weight (TSW) (1000 seeds were counted and weighed), seeds per pod (SPP) (all seeds per pod were counted) and total yield per plant (g).

Statistical analyses

Analysis of variance of the individual intermating cycles, selfing generations and their interaction were conducted for each cross and the five traits using Agrobase® (Agronomix software, Winnipeg, Canada) as a complete block design. Experiments were analyzed separately due to the different generations (S_2 and S_3) evaluated in each experiment. Analyzing and comparing these experiments separately was facilitated by the control genotypes grown at each experiment. Least significant differences (LSD) were calculated to identify significant differences following the ANOVA results. The entire raw dataset was plotted using bivariate scatter plots for each pair of variables (10 panels pairing any two of the five yield components) using the pairplot function from Seaborn in Python (Seaborn - Michael Waskom, New York, USA <https://seaborn.pydata.org/>).

Results

Data from all traits were analyzed to detect the effects of intermating cycles and crosses for all of the yield components evaluated. The combined analysis of all crosses demonstrated a significant effect on most variables for all traits. A representative example of this analysis is shown in Table 2 for mean pod rating at S_2 and S_3 (ANOVA tables for all other traits can be found in Appendix 1).

A bivariate scatter plot shows the relationship between the five traits for all crosses combined (Fig. 4). As expected, yield has a linear positive correlation with and pod number ($r = 0.85$) (Fig. 4 d) and seeds per pod ($r = 0.80$) (Fig. 4 j) (Table 3). A negative correlation was found for seeds per pod and thousand seed weight ($r = -0.25$). The calculated correlation values between pod rating and the other traits was negative because the pod rating scale goes from 1 – 9 where one represents a plant with the best pods and 9 a represents a sterile plant (in contrast to all the other traits in which a higher number indicated a better performing plant). Pod rating exhibited a positive relationship with yield ($r = -0.43$) (Fig. 4 f) and seeds per pod ($r = -0.44$) (Fig. 4 i).

Regarding cycles of selfing, differences were found between the two greenhouse experiments (S_2 and S_3). In the S_3 generation, the means for pod number, yield and seeds per pod for all three cycles were higher compared to the S_2 generation (Table 4).

Highlights of the significant intermating effects for both experiments and each cross are presented for each trait individually (Table 5). Cross three had significant effects for the intermating cycles in all traits and both experiments; thus, cross three will be used to illustrate results for most traits. Data for all other crosses can be found in appendix 2.

Pod number

The analysis of the complete data set showed the largest mean pod number among all intermating cycles at C_0S_2 (Table 4). Mean pod number was the highest among all intermating cycles after the first cycle of full-sib intermating (C_1) in three of the five crosses evaluated at S_2 (Table 5). In the second experiment (S_3), cross 3 showed a larger mean pod number at C_2 . An evaluation of the individual number of families with improved mean pod number when compared to the best parent showed 7 improved families at C_1S_2 and no significant improvements between cycles at S_3 except for C_1 in cross 2 (Table 6). Data for the individual families at each intermating cycle in both experiments is shown in Table 7.

Pod Rating

During analyses of the entire data set, no significant differences were found between the intermating cycles in either experiment for pod rating (Table 4). Differences in the overall mean pod rating were found in three of the five crosses at S_2 and one cross in S_3 (Table 5). At S_2 , cycles 0 and 1 had the best mean pod rating scores. In contrast at S_3 , cross 3 showed an improvement in pod rating when compared to the best parent after two full-sib intermating cycles (C_2). Cross 5 included a parent with the worst field rating score (Table 1). However, a significant improvement was found at S_2 after one cycle of full-sib intermating (C_1) and at S_3 after one intermating cycle (C_0) (Table 5). Comparison of the mean pod rating for the individual families in cross 5 showed a similar trend with the improvement of one out of 12 families at S_2 after one cycle of full-sib intermating and 5 families out of 12 when compared to the best parent at C_0S_3 (Table 8).

Thousand seed weight

The analysis of the complete data set showed the largest mean TSW among all intermating cycles at C_0S_2 (Table 4). Cross 1 had a higher mean thousand seed weight at S_2 after one cycle of full-sib intermating (C_1) (Table 5). No significant mean TSW differences were found in the other four crosses for any intermating cycles in either experiment (Table 5). For cross 5, C_2 had the highest number of improved families (5/12) at S_2 . The same number of improved families (2/12) was observed in C_0 and C_1 at S_3 (Table 9).

Seeds per pod

No significant differences for SPP were found between the intermating cycles on either experiment when analyzing the total data set (Table 4). The cycle means for SPP showed one cycle of full-sib intermating (C_1) was better than the other intermating cycles for 2 of the 5 crosses at S_2 (Table 5). When comparing the means of individual families in every cycle at S_2 , only C_1 and C_2 had an improved number of seeds per pod when compared to the best parent (Table 10). No significant differences were found for the overall cycle means or the number of improved families when compared to the best parent at S_3 (Table 10).

Yield

The analysis of the complete data set showed the highest mean yield among all intermating cycles at C_0S_2 (Table 4). Mean yield was highest following the initial intermating cycle (C_0) for crosses 1 and 2. Cross 2 showed an improved mean yield of 1.09 g, while the best parent had a mean yield of 0.77 g, in S_2 (Table 5). Crosses 3 and 5 exhibited higher mean yield at C_1 . The cycle with the highest number of improved families from all crosses was C_0 at S_2 (Table 11). The largest number of improved families for a single cross was found at C_1S_2 for cross 3. Data for the individual families of cross 3 at each intermating cycle in both experiments is shown in Table 12.

Improvement of individual families

Two of the crosses with the greatest improvements were crosses three and five. At least one of the twelve families from each of these crosses showed an increase of >36 % in pod number and yield when compared to the best parent after one cycle of full-sib intermating (C_1S_2) (Table 13).

Discussion

The goal of this study was to determine whether multiple cycles of intermating with recurrent selection would result in R-lines with improved agronomic performance. The cycle effects on all the crosses combined, would suggest that C_0 is the best for improving pod number, TSW and yield. However, when evaluating each cross individually and the number of improved families per cross and cycle, C_1 showed the most improvements when compared to the best parent.

Analyses from both experiments (including the parental genotypes) in a bivariate matrix facilitated the identification of relationship trends for all traits. The positive correlation between seeds per pod, total pod number and yield found in this study had been previously reported in other *Brassica* yield component interrelation studies (Olsson 1959; Ivanovska et al. 2007; Sabaghnia et al. 2010). A negative correlation was found for seeds per pod and thousand seed weight ($r = -0.25$). This is to be expected from 'poor pods' (6 - 8 in the pod rating scale (Fig. 3) which produce a maximum of 5 seeds that are larger and heavier than the average (Olsson 1959). A positive correlation was observed for yield (Fig. 4 j) and seeds per pod (Fig. 4 f) with the pod rating. This a good indicator for the accuracy of the pod rating scale (Fig. 3). Future studies can utilize this pod rating to predict high yielding restorer plants. The use of a pod rating scale facilitates selection of improved genotypes and potentially decreases the time needed to make selections on restorer genotypes.

The time of the year when the experiments were grown in the greenhouse had an effect on the performance of all the genotypes evaluated. The first experiment was planted in June and grew during the summer months where temperatures were higher (on average 10 °C higher for 2-3 hours a day at S_2 due

the cooling and shading limitations of the greenhouse), which had a negative effect on the plants. Parental genotypes (controls for both experiments) also exhibited lower scores for all the previously mentioned traits when compared to the S₃ experiment that was planted in December.

The initial step in any plant breeding strategy, is to identify plants with desirable traits among existing plant genotypes (or developing novel phenotypes if they are unavailable) (Ahmar et al. 2020). Due to the nature of the traits evaluated, selection could not take place before intermating the restorer genotypes. Random mating among the twelve genotypes per family was an important factor in this research. As observed in a soybean recurrent half-sib selection study, random mating can have a positive effect on the oil content of seeds but no significant results were found for yield or seed weight (Feng et al. 2004). Similar results were found in our study where mean seeds per pod and mean pod number were improved when compared to the best parent at cycles C₁ and C₂ (Table 5 and Table 10). Mean yield improved when compared to the best parent at C₀ in cross 2 and at C₁ in cross 3 at S₂ (Table 5). These results suggest that up to one cycle of random full-sib intermating can improve yield components in fertility restorer genotypes.

As described earlier, full-sib recurrent selection is a plant breeding method that has been well established and can break the linkage of disadvantageous alleles while maintaining the favorable alleles (Peiris and Hallauer 2005; Hallauer 2007). One of the major problems of the Ogu-INRA CMS restorer genotypes is the poor agronomic performance as a result of the radish-derived *Rfo* introgression on the C9 chromosome of *B. napus* (Primard-Brisset et al. 2005). In a recent study evaluating the genetic variation in a recurrent selection *B. napus* population, the C sub genome was found to be a repository for a wider range of selected regions with favorable loci contributing to agronomic traits (Zhao et al. 2016). At the same time, the authors report low genetic variation on chromosome C9, close to where it is speculated the *Rfo* gene was inserted (Zhao et al. 2016). In contrast with other recurrent selection studies where there was no improvement from the developed genotypes when compared to the parents (Urrea and Singh 1995), the present research shows that more than a third of the families (each with 12 genotypes) had improved seeds per pod, pod number and yield when compared to the best parent after one (C₁) and two cycles (C₂) of random full-sib intermating in select crosses. One family from cross 3 and one from cross 5 showed an improvement of over 70 % in yield when compared to the best parent after one cycle of full-sib intermating (C₁) and over 36 % in pod number (Table 13). This indicates that intermating restorer genotypes can produce improved restorer genotypes.

The ability to generate an improved restorer genotypes is of the utmost importance to produce improved canola and oilseed rape hybrids. Dozens of improved Ogu-INRA restorer genotypes were developed in this research, which can be further evaluated for their general combining ability in experimental hybrids. Future research should focus on developing new restorer by restorer crosses and evaluating them after intermating using marker-assisted selection strategy including the *Rfo* marker used in this experiment and new molecular markers targeting other QTL of interest.

Declarations

Acknowledgements: The authors acknowledge Judith Nugent-Rigby for her research contribution, as well as the National Science and Engineering Research Council of Canada, Bunge, DL Seeds and the University of Manitoba for their funding support and the Consejo Nacional de Ciencia y Tecnología, Mexico for the Ph.D. scholarship to V Lobos-Sujo (349578).

Statements and Declarations: The authors declare that they have no conflict of interest nor any financial or non-financial interests.

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Tables

Table 1. *Brassica napus* L. parental genotypes used to create the initial five crosses (C₀S₀) evaluated using a recurrent selection strategy.

Cross	Female parent		Male parent	
	ID	Field score ^a	ID	Field score
1	UM300	6	UM800	5
2	UM400	5	UM900	5
3	UM500	5	UM700	5
4	UM600	5	UM1000	5
5	UM700	5	UM2000	7

^aField score is given on a scale from 1 – 9 where 1 represents the best row. Score is based on the overall agronomic performance including pod number, length of pods, shape of pods, lodging resistance and vigor.

□

Table 2. Combined analysis of variance for all *Brassica napus* L. restorer crosses and cycles (C₀, C₁, C₂) of intermating for pod rating at S₂ and S₃^a.

	Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	Pr>F
S ₂	Total	629	529.38			
	Rep	2	0.07	0.03	0.07	0.9244
	Cycle	2	0.40	0.20	0.42	0.6585
	Family	13	16.90	1.30	2.71	0.0010
	Cross	4	210.64	52.66	109.65	0.0000
	Cycle x cross	8	13.20	1.65	3.44	0.0007
	Residual	600	246.95	0.44		
S ₃	Total	629	492.68			
	Rep	2	0.473	0.23	0.36	0.6987
	Cycle	2	3.48	1.74	2.65	0.0717
	Family	13	11.22	0.86	1.31	0.2017
	Cross	4	51.60	12.90	19.58	0.0000
	Cycle x cross	8	30.57	3.82	5.80	0.0000
	Residual	600	395.32	0.65		

^a S₂ and S₃ correspond to the evaluation after two and three selfings for experiment 1 and 2 respectively.

Table 3. Correlation matrix for all yield components and pod rating for the complete data set of *Brassica napus* L. fertility restorer crosses at all intermating cycles (C₀, C₁, C₂) in the two greenhouse experiments (S₂ and S₃).

	Pod number	TSW ^a	Yield	Seeds per pod
TSW (g)	0.06			
Yield (g)	0.85	0.00		
Seed per pod (No.)	0.49	-0.25	0.80	
Pod rating ^b	-0.37	0.08	-0.43	-0.44

^a Thousand seed weight.

^b Pod rating scale from 1 – 9 where 1 represents a full straight pod with more than 25 seeds; 2 represents a straight pod with 15 - 25 seeds; 3 represents a curved pod with 10-15 seeds; 4 represents a straight pod with 7 - 9 seeds; 5 represents a curved pod with 6-8 seeds; 6 represents an uneven pod with 4-5 seeds; 7 represents a curved pod with 2-3 seeds; 8 represents a pod with only 1 seed; 9 represents an aborted pod with no seed.

Table 4. Means for all yield components and pod rating measured for the complete data set of *Brassica napus* L. fertility restorer crosses at all intermating cycles (C₀, C₁, C₂) in the two greenhouse experiments (S₂ and S₃).

	S ₂				S ₃			
	C ₀	C ₁	C ₂		C ₀	C ₁	C ₂	
Mean Pod (No.)	41.01*	40.98	37.15	LSD ^b 2.74	73.69	75.72	75.58	LSD 3.18
Mean Pod Rating ^a	4.55	4.49	5.54	LSD 0.12	3.94	4.10	4.10	LSD 0.14
Mean SPP ^c (No.)	5.00	5.09	4.86	LSD 0.40	9.61	9.53	9.55	LSD 0.45
Mean TSW ^d (g)	3.70*	3.65	3.58	LSD 0.10	3.74	3.71	3.70	LSD 0.07
Mean Yield (g)	0.82*	0.81	0.66	LSD 0.08	2.54	2.57	2.54	LSD 0.13

*Significantly best among all intermating cycles at p = 0.05.

^a Pod rating scale from 1 – 9 where 1 represents a full straight pod with more than 25 seeds; 2 represents a straight pod with 15 - 25 seeds; 3 represents a curved pod with 10-15 seeds; 4 represents a straight pod with 7 - 9 seeds; 5 represents a curved pod with 6-8 seeds; 6 represents an uneven pod with 4-5 seeds; 7 represents a curved pod with 2-3 seeds; 8 represents a pod with only 1 seed; 9 represents an aborted pod with no seed.

^b LSD least significant difference.

^c SPP represents seeds per pod.

^d TSW represents thousand seed weight.

Table 5. Means for all yield components measured for the two parental genotypes and all intermating cycles (C₀, C₁, C₂) with 12 families cycle and all 5 *Brassica napus* L. fertility restorer crosses in two greenhouse experiments (S₂ and S₃).

	S ₂							S ₃				
	Parents		C ₀	C ₁	C ₂	Parents		C ₀	C ₁	C ₂		
Cross1												
Mean Pod (No.)	53.53	54.81	49.35	51.91*	44.55	LSD 4.97	^b 59.92	94.42	75.01	80.17	76.01	74.4
Mean Pod Rating ^a	3.08	2.75	3.63	3.63	3.49	LSD 0.21	3.83	4.17	3.99	4.13	4.59	1.0
Mean SPP ^c (No.)	8.24	6.79	7.22	7.27	6.94	LSD 0.88	13.81	9.16	10.31	10.56	9.69	1.0
Mean TSW ^d (g)	3.21	4.09	4.01*	3.72	3.40	LSD 0.20	4.00	3.96	3.84	3.74	3.89	1.0
Mean Yield (g)	1.54	1.27	1.37*	1.35	1.01	LSD 0.18	3.31	3.40	2.89	3.08	2.79	1.0
Cross 2												
Mean Pod (No.)	45.83	23.97	54.09*	32.69	37.54	LSD 5.45	55.00	66.22	66.46	68.46	70.15	1.5
Mean Pod Rating	4.25	4.89	4.61*	5.05	4.76	LSD 0.25	4.08	2.67	4.46	4.33	4.24	1.0
Mean SPP (No.)	4.60	3.10	5.21	4.52	5.95*	LSD 0.79	12.23	11.51	9.24	8.56	9.43	1.0
Mean TSW (g)	3.63	3.52	3.72	3.60	3.47	LSD 0.25	3.71	3.43	3.84	3.89	3.78	1.0
Mean Yield (g)	0.77	0.29	1.09*	0.57	0.80	LSD 0.16	1.96	2.47	2.25	2.20	2.42	1.0

Table 5.
Continued

	S ₂						S ₃					
	Parents		C ₀	C ₁	C ₂		Parents		C ₀	C ₁	C ₂	
Cross 3												
Mean Pod (No.)	27.25	25.92	33.25	43.35*	32.49	LSD ^b 4.07	95.08	83.58	79.79	81.22	88.37*	16
Mean Pod Rating ^a	4.64	4.83	5.30	4.87*	5.08	LSD 0.33	4.00	5.00	4.39	4.35	3.92*	10
Mean SPP ^c (No.)	3.31	3.47	3.79	3.76	3.35	LSD 0.86	13.44	5.92	7.82	8.04	7.71	10
Mean TSW ^d (g)	3.69	3.50	3.63	3.76	3.81	LSD 0.19	3.13	4.34	3.91	3.98	3.85	10
Mean Yield (g)	0.32	0.32	0.47	0.67*	0.34	LSD 0.10	3.91	2.09	2.37	2.44	2.48	10
Cross 4												
Mean Pod (No.)	33.42	35.47	26.56	28.73	29.61	LSD 3.73	62.97	98.83	80.06	78.61	74.69	17
Mean Pod Rating	4.67	4.72	5.16	4.76*	5.13	LSD 0.26	3.67	3.67	3.81	3.77	3.94	10
Mean SPP (No.)	4.46	4.41	4.41	4.47*	2.94	LSD 0.62	11.50	10.66	8.64	10.04*	9.69	10
Mean TSW (g)	3.67	3.60	3.52	3.61	3.62	LSD 0.22	3.18	3.54	3.58	3.46	3.55	10
Mean	0.53	0.56	0.42	0.48	0.36	LSD	2.24	3.68	2.42	2.68	2.45	10

Table 5. Continued

	S ₂					S ₃						
Cross 5	Parents		C ₀	C ₁	C ₂		Parents		C ₀	C ₁	C ₂	
Mean Pod (No.)	55.33	25.92	41.81	48.21*	41.55	LSD 5.22 ^b	87.42	95.08	67.11	70.14	68.67	I 6
Mean Pod Rating ^a	3.86	4.83	4.03	4.14	4.23	LSD 0.16	3.00	4.00	3.05*	3.90	3.80	I C
Mean SPP ^c (No.)	7.61	3.47	4.39	5.43*	5.13	LSD 0.79	10.69	13.44	12.07*	10.47	11.26	I C
Mean TSW ^d (g)	2.84	3.50	3.62	3.52	3.66	LSD 0.14	3.29	3.13	3.54	3.45	3.43	I C
Mean Yield (g)	1.34	0.32	0.75	1.00*	0.79	LSD 0.17	3.16	3.91	2.78*	2.44	2.55	I C

Means were calculated from 3 replicates (each with 4 individuals).

* Significantly best among intermating cycles at 0.05.

^a Pod rating scale goes from 1-9 where 1 represents a full straight pod with more than 25 seeds; 2 represents a straight pod with 15 - 25 seeds; 3 represents a curved pod with 10-15 seeds; 4 represents a straight pod with 7 - 9 seeds; 5 represents a curved pod with 6-8 seeds; 6 represents an uneven pod with 4-5 seeds; 7 represents a curved pod with 2-3 seeds; 8 represents a pod with only 1 seed; 9 represents an aborted pod with no seed.

^b LSD least significant difference.

^c SPP represents seeds per pod.

^d TSW represents thousand seed weight.

Table 6. Number of *Brassica napus* L. restorer families^a with mean pod number significantly higher than the best parent per cycle of intermating (C₀, C₁, C₂) at S₂ and S₃.

	S ₂			S ₃		
	C ₀	C ₁	C ₂	C ₀	C ₁	C ₂
Cross1 BP: 59.08	0	0	0	BP: 94.42	0	0
Cross2 BP: 45.83	2	0	0	BP: 66.22	0	1
Cross3 BP: 27.25	4	7	3	BP: 95.08	0	0
Cross4 BP: 35.47	0	0	1	BP: 98.83	0	0
Cross5 BP: 55.33	1	1	0	BP: 95.08	0	0

^a Twelve total number of families per cycle.

^b BP represents best parent.

Table 7. Individual family mean pod number values for each cycle (C₀, C₁, C₂) of cross 3 at the second (S₂) and third selfing (S₃).

Family	S ₂ ^a			S ₃ ^b		
	C ₀	C ₁	C ₂	C ₀	C ₁	C ₂
1	45.33*	63.75*	28.00	84.00	93.92	85.33
2	41.27	53.39*	25.33	71.42	93.42	75.83
3	41.50	40.75	22.83	75.92	85.92	85.58
4	10.69	65.58*	33.19	75.67	69.08	82.50
5	19.66	51.77*	44.80*	81.00	81.17	94.00
6	14.47	47.61*	46.25*	58.67	86.25	92.67
7	16.76	41.58	34.85	79.75	73.00	89.75
8	18.16	51.58*	31.39	61.25	61.08	103.00
9	21.41	29.00	32.83	69.08	76.67	78.33
10	57.66*	46.17*	42.53*	98.58	80.75	80.08
11	61.92*	22.14	26.33	93.58	81.08	91.33
12	63.50*	36.41	33.33	89.42	76.08	100.08
UM500 ^c	27.25	24.67	24.67	95.08	95.08	95.08
UM700 ^c	25.91	20.50	20.50	83.58	83.58	83.58

*Significantly better than the best parent at 0.05.

a LSD, least significant difference (P = 0.05) 15.25 for all S₂ data.

b LSD (P = 0.05) 25.66 for all S₃ data.

c Parents were grown within the same experiment as control samples without any intermating.

Table 8. Number of *Brassica napus* L. restorer families^a with mean pod rating significantly better than the best parent per cycle of intermating (C₀, C₁, C₂) at S₂ and S₃.

	S ₂			S ₃			
	C ₀	C ₁	C ₂	C ₀	C ₁	C ₂	
Cross 1 BP ^b : 2.75	0	0	0	BP: 3.83	0	0	0
Cross 2 BP: 4.25	0	0	0	BP: 2.66	0	0	0
Cross 3 BP: 4.64	0	0	0	BP: 4.00	0	1	4
Cross 4 BP: 4.66	0	0	0	BP: 3.66	0	2	0
Cross 5 BP: 3.86	0	1	0	BP: 3.00	5	0	0

^a Twelve total number of families per cycle.

^b BP represents best parent.

Table 9. Number of *Brassica napus* L. families^a with significantly improved mean thousand seed weight when compared to the best parent in all three cycles of intermating (C₀, C₁, C₂) for all crosses.

		S ₂			S ₃			
		C ₀	C ₁	C ₂	C ₀	C ₁	C ₂	
Cross 1	BP ^b : 4.08	0	0	0	BP: 4.00	0	0	1
Cross 2 BP: 3.71		0	0	0	BP: 3.63	0	0	0
Cross 3 BP: 3.69		2	3	3	BP: 4.33	1	0	0
Cross 4 BP: 3.67		0	0	1	BP: 3.54	0	0	2
Cross 5 BP: 3.50		0	2	5	BP: 3.29	2	2	0

^a Twelve total number of families per cycle.

^b BP represents best parent.

Table 10. Number of *Brassica napus* L. restorer families^a with mean seeds per pod significantly higher than the best parent per cycle of intermating (C₀, C₁, C₂) at S₂.

	S ₂				S ₃					
	C ₀	C ₁	C ₂		C ₀	C ₁	C ₂			
Cross 1 BP ^b : 8.23	0	0	0	BP: 13.08	0	0	0			
Cross 2 BP: 4.60	0	1	5	BP: 12.23	0	0	0			
Cross 3 BP: 3.46	0	1	2	BP: 13.43	0	0	0			
Cross 4			BP: 11.49	0	1	0	BP: 11.49	0	0	0
Cross 5 BP: 7.61	0	0	0	BP: 13.43	0	0	1			

^a Twelve total number of families per cycle.

^b BP represents best parent.

Table 11. Number of *Brassica napus* L. restorer families^a with mean yield significantly higher than the best parent per cycle of intermating (C₀, C₁, C₂) at S₂ and S₃.

		S ₂				S ₃		
		C ₀	C ₁	C ₂		C ₀	C ₁	C ₂
Cross 1	BP ^b : 1.54	0	0	0	BP: 3.39	0	0	0
Cross 2	BP: 0.76	3	0	1	BP: 2.47	0	0	1
Cross 3	BP: 0.32	3	4	1	BP: 3.90	0	0	0
Cross 4	BP: 0.56	0	0	1	BP: 3.67	0	0	0
Cross 5	BP: 1.37	0	1	0	BP: 3.91	0	0	0

^a Twelve total number of families per cycle.

^b BP represents best parent.

Table 12. *Brassica napus* L. individual family mean yield (g) values for each cycle (C₀, C₁, C₂) of cross 3 at the second (S₂) and third selfing (S₃).

Family	C ₀		C ₁		C ₂	
	S ₂ ^a	S ₃ ^b	S ₂	S ₃	S ₂	S ₃
1	0.73*	2.49	1.01*	2.83	0.21	1.95
2	0.50	1.88	0.83*	2.77	0.14	2.19
3	0.48	2.07	0.59	2.48	0.17	2.27
4	0.05	1.69	1.38*	2.06	0.84*	3.06
5	0.22	1.78	0.55	2.20	0.58	2.66
6	0.18	1.91	0.66	2.52	0.49	2.54
7	0.17	2.51	0.47	1.99	0.44	2.51
8	0.22	1.85	1.37*	2.44	0.21	2.68
9	0.23	2.36	0.57	2.48	0.22	1.95
10	0.93	3.05	0.59	2.06	0.42	2.23
11	1.09*	2.86	0.28	2.03	0.19	2.53
12	1.07*	2.71	0.43	2.28	0.26	2.18
UM500 ^c	0.32	3.91	0.32	3.91	0.32	3.91
UM700 ^c	0.32	2.09	0.32	2.09	0.32	2.09

*Significantly better than the best parent at 0.05.

^a LSD, least significant difference (P = 0.05) 0.39 for all S₂ data.

^b LSD (P = 0.05) 0.70 for all S₃ data.

^c Parents were grown within the same experiment as controls without intermating.

Table 13. Comparison of the best *Brassica napus* L.family after one cycle of full-sib intermating (C_1) and the best parent of crosses 3 and 5 S_2 .

	Cross 3 family 4 ^a	Best parent ^b	Increase %	Cross 5 family 9 ^a	Best parent	Increase %
Pod number	65.58	24.67	165.82	75.67	55.33	36.76
Yield (g)	1.37	0.32	328.12	2.37	1.33	78.19

^a Data for each family is based on a mean of 3 replicates each with four individuals.
^b Data for the best parent is based on a mean of 3 replicates each with four individuals.

Figures

Figure 1
Chain crossing. Entry number 1 is crossed with entry number 2, which is in turn crossed with entry number 3 and so forth, until the nth entry, which is then crossed with entry number 1 to complete the chain.

Figure 2

Development of three *Brassica napus* L. subpopulations using recurrent selection. Each population began with a cross at C0S0. A total of 12 plants were grown per cross. From each plant, 12 flowers were crossed to develop the next cycle (C1S0) while the remainder of the plant was selfed (C0S1). One seed from the 12 intermated pods of each plant was chosen at random and planted. Twelve flowers from the new plant were crossed again to create the next intermating cycle (C2S0). Replicated experiment 1 analysed the 3 cycles after two selfings and replicated experiment 2 followed the same populations after three selfings.

Figure 3

Pod rating scale for *Brassica napus* L. pods. The score assigned represents a mean visual rating of the pod development on the entire plant. 1 represents a full straight pod with more than 25 seeds; 2 represents a straight pod with 15 - 25 seeds; 3 represents a curved pod with 10-15 seeds; 4 represents a straight pod

with 7 - 9 seeds; 5 represents a curved pod with 6-8 seeds; 6 represents an uneven pod with 4-5 seeds; 7 represents a curved pod with 2-3 seeds; 8 represents a pod with only 1 seed; 9 represents an aborted pod with no seed.

Figure 4

Evaluation of data trends in *Brassica napus* L. restorer genotypes. Scatter plots generated from greenhouse means for 5 yield components measured with four plants per replicate for all cycles with the parents as controls. Boxes are read as pairs of variables for example, bivariate data trends for pod number and trends for TSW (thousand seed weight) (a) and bivariate data trends for pod number and rating (b) 1 Pod rating scale from 1 – 9 where 1 represents a full straight pod with more than 25 seeds; 2 represents a straight pod with 15 - 25 seeds; 3 represents a curved pod with 10-15 seeds; 4 represents a straight pod with 7 - 9 seeds; 5 represents a curved pod with 6-8 seeds; 6 represents an uneven pod with 4-5 seeds; 7 represents a curved pod with 2-3 seeds; 8 represents a pod with only 1 seed; 9 represents an aborted pod with no seed

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