

RESEARCH ARTICLE

Genetic analyses of agronomic and seed quality traits of synthetic oilseed *Brassica napus* produced from interspecific hybridization of *B. campestris* and *B. oleracea*

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Abstract

The heritability, the number of segregating genes and the type of gene interaction of nine agronomic traits were analysed based on F₂ populations of synthetic oilseed *Brassica napus* produced from interspecific hybridization of *B. campestris* and *B. oleracea* through ovary culture. The nine traits—plant height, stem width, number of branches, length of main raceme, number of pods per plant, number of seeds per pod, length of pod, seed weight per plant and 1000-seed weight—had heritabilities of 0.927, 0.215, 0.172, 0.381, 0.360, 0.972, 0.952, 0.516 and 0.987 respectively, while the mean numbers of controlling genes for these characters were 7.4, 10.4, 9.9, 12.9, 11.5, 21.7, 20.5, 19.8 and 6.4 respectively. According to estimated coefficients of skewness and kurtosis of the traits tested, no significant gene interaction was found for plant height, stem width, number of branches, length of main raceme, number of seeds per pod and 1000-seed weight. Seed yield per plant is an important target for oilseed production. In partial correlation analysis, number of pods per plant, number of seeds per pod and 1000-seed weight were positively correlated with seed yield per plant. On the other hand, length of pod was negatively correlated ($r = -0.69$) with seed yield per plant. Other agronomic characters had no significant correlation to seed yield per plant. In this experiment, the linear regressions of seed yield per plant and other agronomic traits were also analysed. The linear regression equation was $y = 0.074x_8 + 1.819x_9 + 6.72x_{12} - 60.78$ ($R^2 = 0.993$), where x_8 , x_9 and x_{12} represent number of pods per plant, number of seeds per pod and 1000-seed weight respectively. The experiment also showed that erucic acid and oil contents of seeds from F₂ plants were lower than those of their maternal parents. However, glucosinolate content was higher than that of the maternal plants. As for protein content, similar results were found in the F₂ plants and their maternal parents. It was shown that the four quality traits, i.e. erucic acid, glucosinolate, oil content, and protein content, had heritability values of 0.614, 0.405, 0.153 and 0.680 respectively.

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Introduction

The oilseed *Brassica* species (*B. napus*, *B. rapa* or *B. campestris*, and *B. juncea*) are now the third most important source of edible vegetable oil in the world after palm and soybean oil. *Brassica* is a winter oil crop and the most important source of edible oil in China, expanding rapidly as a rotation crop following rice. The improvement of *Brassica* has mostly been confined to the exploitation of naturally occurring genetic variation in cultivated species.

The genus *Brassica* has 159 species, including a number of wild species that are potential sources of novel and economically important characteristics (Zhou 2001; Zhou *et al.* 2006). However, for breeding purposes, some of these species are not easy to characterize because of their wild origin, and the crosses are also often incompatible as a result of barriers to crossing such as the abortion of hybrid embryos (Liu 1984; Zhang *et al.* 2001; Momoh *et al.* 2002). Consequently, breeders have to resort to special techniques to achieve wide hybridization (Ayotte *et al.* 1987; Quazi 1988; Xu *et al.* 1995; Zhou *et al.* 2002a,b). Through these techniques, especially *in vitro* culture and embryo rescue, such

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interspecific crosses can be successfully carried out (McNaughten 1963; Agnihotri *et al.* 1991; Gu *et al.* 2003, 2004; Tang *et al.* 2003; Zhang *et al.* 2003b).

Brassica napus ($2n = 38$) is the natural allopolyploid from *B. campestris* ($2n = 20$) \times *B. oleracea* ($2n = 18$). The allo-tetraploid *B. napus* has been produced from its diploid progenitor species (*B. oleracea* and *B. rapa*) by sexual interspecific hybridization and somatic hybridization (Nishi 1980; Jourdan *et al.* 1989; Heath and Earle 1996; Zhang *et al.* 2001, 2004). Artificial hybridization of *B. campestris* and *B. oleracea* may be useful for providing further approaches and novel characteristics in oilseed *Brassica* research and breeding programmes, especially because the winter rapeseed *B. napus* is very important to China. Our previous experiments (Zhang *et al.* 2004) showed that, through embryo rescue (using ovary culture), seeds were formed and plantlets were successfully produced from the cross *B. campestris* \times *B. oleracea*. It was found that ovary age (days after pollination), cross combination and culture media were crucial to successful seed formation and number of seeds per ovary (Chen *et al.* 2000; Chen and Wojciechowski 2000; Zhang *et al.* 2004). Three novel synthetic oilseed *B. napus* populations were obtained from interspecific hybridization of *B. campestris* and *B. oleracea* through ovary culture (Zhang *et al.* 2004). The plants generated were subjected to colchicine treatment, and thus doubled haploid (DH) populations were obtained, and their agronomic traits and seed qualities were analysed.

It is known that the total yield of oilseed rape is affected by various agronomic traits such as branch number, length of main branch, number of pods per plant, etc. (Zhou *et al.* 1997; Leul and Zhou 1998, 1999). Correlations between agronomic traits and seed yield have been reported by several researchers (Liu 1984; Momoh and Zhou 2001; Momoh *et al.* 2004). However, genetic analyses of agronomic traits as well as seed quality characters have seldom been reported. Based on our previous experiments (Zhang *et al.* 2003a, 2004), this work has been extended and the present report deals with the genetic analyses of several quantitative traits of our artificially synthesized oilseed *B. napus* produced from interspecific hybridizations between *B. campestris* and *B. oleracea* through ovary culture. The major objectives were to consider the main characters useful to predict and increase yield as well as improve seed quality. The material used in this study is unique to our breeding programme, as we used the novel artificially synthesized oilseed *Brassica napus* produced from interspecific hybridization of *B. campestris* and *B. oleracea*. Their paternal parent (cv. Jingfeng-1) and two of the three maternal parents (cvs. Maoshan-3 and Youbai) showed good agronomic characters, and are grown widely in China. Another maternal parent (cv. Huarad) was from Sweden, and showed relatively good adaptation to local Chinese environments. All three crosses have much significance to oilseed breeding, especially because the F_1 and F_2 plants and seeds were obtained successfully through ovary culture

and chromosome doubling. It is very important to analyse the correlation between yield and agronomic and seed quality traits to determine the main target traits for increasing yield and improving seed quality, so that such crosses may be quickly and widely used in practice in rapeseed breeding and production. In addition, it is hoped that this method of analysis could be used in other related research.

Materials and methods

Plant material

Three cultivars of *B. campestris* syn. *B. rapa* (cv. Huarad from Sweden and cvs. Maoshan-3 and Youbai from China) were used as maternal parents and crossed with *B. oleracea* (cv. Jingfeng-1 from China) in the experimental field of Zhejiang University during April 2002. Flower buds of the maternal parents were emasculated 24–48 h before anthesis, and fresh pollen from the paternal parent was applied to the stigmas. The flowers were then protected with bags and tag-labelled. The ovaries were excised 9–12 days after pollination and cultured *in vitro* (Zhang *et al.* 2004). The resultant seeds were grown into F_1 plantlets and then chromosome doubling was induced by treatment with colchicine (Zhang *et al.* 2004), to produce DH plants which were transplanted into the experimental field in November 2002. From the cytological identification and progeny investigation, these regenerated plants were proved to be *B. napus* (Zhang *et al.* 2003a, 2004). Seeds from these F_1 DH plants were harvested in May 2003 and the F_2 seeds were sown in the field of the experimental farm at Zhejiang University during October 2003. In May 2004 the F_2 plants obtained were used for genetic analysis. The F_2 from the three crosses Huarad \times Jingfeng-1, Maoshan-3 \times Jingfeng-1 and Youbai \times Jingfeng-1 were called N_1 , N_2 and N_3 respectively (N stands for *napus*, *Brassica napus*).

Characterization of F_2 generations

Observations on various morphological characters such as plant height, stem width, branch position, length of main raceme, number of pods in main raceme, pod density in main raceme, length of pod, number of pods per plant, number of seeds per pod, number of primary branches, number of secondary branches, 1000-seed weight and seed yield per plant were recorded from the F_2 plants in May 2004. Ten plants with three replications were used in the experiment. At the same time, seed samples from these F_2 plants from each field plot were used to measure the erucic acid, glucosinolate, oil and protein contents using near-infrared reflectance spectroscopy (NIR System 5000; NIR Systems Inc., Silver Spring, USA) (Leonardo and Becker 1998; Wu *et al.* 2002).

Statistical methods

The MINQUE (0/1) method (Zhu 1992; Zhu and Weir 1994) was used to estimate additive variance (V_A) components for each agronomic and seed quality trait. Phenotypic variance

(V_p) of each trait was also estimated. The estimates of variances were further used for calculating the heritability. Since this experiment used the DH populations, and we know that each DH line is genetically homozygous at all loci, its genetic variance was equal to the additive variance (Caranta and Palloix 1996). Consequently, with the DH population, its broad-sense heritability (h_b^2) was equal to the narrow-sense heritability (h_n^2). The number of genes (n) controlling each agronomic trait was estimated as: $n = (L - M)^2/V_A$, where L is extreme value and M the population mean of each agronomic trait (Choo and Reinbergs 1982a).

In this experiment, the coefficients of skewness and kurtosis of the traits were estimated using the SPSS 10.0 analysis system (SPSS 1999). If no gene interaction is found, the kurtosis will be smaller than zero, otherwise the kurtosis will be larger than zero. If duplicate interaction is observed, the skewness will be smaller than zero, but if the skewness is larger than zero, it will show that there is complementary interaction (Choo and Reinbergs 1982b; Zhang and Xue 1997; Yu *et al.* 1998). In this experiment, partial correlations of 13 agronomic traits were analysed. In addition, effects of the 12 agronomic traits on seed yield per plant were analysed using stepwise linear regression in the SPSS 10.0 analysis system.

Results

Heritability and gene interaction of nine quantitative traits

Heritability of nine quantitative traits was analysed based on populations of artificially synthesized *B. napus* produced through ovary culture from crosses between *B. campestris* and *B. oleracea*. It was shown that these nine traits—plant height, stem width, number of branches, length of main raceme, number of pods per plant, number of seeds per pod, length of pod, seed weight per plant and 1000-seed weight—had heritability values of 0.927, 0.215, 0.172, 0.381, 0.360, 0.972, 0.952, 0.516 and 0.987 respectively (table 1). Heritability values for plant height, number of seeds per pod, length of pod and 1000-seed weight were the highest >0.9, while the lowest heritability was observed for the number of

branches (0.172).

The numbers of genes controlling each trait were estimated. The results showed that number of genes controlling number of seeds per pod was the highest (21.7), followed by that for length of pod, seed yield per plant, length of main raceme, number of pods per plant and stem width (all over 10.0) (table 1). However, the numbers of genes controlling number of branches and plant height were 9.9 and 7.4 respectively, and the least number of genes was found for 1000-seed weight (only 6.4).

The skewness and kurtosis of the agronomic characters were tested. Gene interaction was found to be absent for plant height, stem width, number of branches, length of main raceme, number of seeds per pod and 1000-seed weight. However, gene interaction was detected for number of pods per plant, length of pod and seed weight per plant, and the kurtosis values were 0.740, 2.176 and 2.598 respectively (table 1). Complementary interaction of these three traits was also observed, and their skewness values were 0.557, 0.798 and 1.361 respectively.

Analysis of partial correlations

Partial correlations and one-way analysis of variance (ANOVA) of 13 agronomic traits—stem width, plant height, branch position, length of main raceme, number of pods, pod density in main raceme, length of pod, number of pods per plant, number of seeds per pod, number of primary branches, number of secondary branches, 1000-seed weight and seed yield per plant—were carried out. It was shown that the nine agronomic traits from the three crosses had significant differences at the 0.05 level of probability (table 2). The partial correlations of all 13 agronomic traits were analysed and the results are given in table 3. It was observed that there was a significantly negative correlation between seed yield per plant and length of pod, with a correlation coefficient of -0.69 . On the contrary, a significantly positive correlation was observed between seed yield per plant and number of pods per plant and 1000-seed weight, with correlation coefficients of 0.75 and 0.83 respectively.

Table 1. Estimated heritability (h_n^2) and the coefficients of skewness and kurtosis of the agronomic characters of F_1 generation from synthetic oilseed *B. napus*.

Character	V_A	V_p	h_n^2	Mean	Genes (n)	Skewness	Kurtosis
Plant height (cm)	760.64**	819.88**	0.927**	153.10	7.4	0.950	-0.792
Stem width (cm)	1.266***	5.866***	0.215*	2.210	10.4	0.932	-0.966
No. of branches	0.164***	1.469***	0.172	15.466	9.9	0.525	-0.870
Length of main raceme (cm)	525.08***	1379.48***	0.381***	64.577	12.9	0.985	-0.665
No. of pods per plant	2047.46***	5680.96***	0.360**	425.38	11.5	0.557	0.740
No. of seeds per pod	65.04***	66.93*	0.972***	17.219	21.7	0.782	-0.668
Length of pod (cm)	7.666***	8.056***	0.952***	7.668	20.5	0.798	2.176
Seed weight per plant (g)	82.98***	160.68***	0.516***	35.310	19.8	1.361	2.598
1000-seed weight (g)	1.068***	1.081***	0.987***	4.186	6.4	0.490	-0.351

Significance at * $P = 0.1$, ** $P = 0.05$, *** $P = 0.01$.

Table 2. Analysis of variances of the 13 agronomic characters of F₂ generation from synthetic oilseed *B. napus*.

Source	SW	PH	BP	LMR	NPR	PD	LP	NPP	NSP	NPB	NSB	SWT	SWP
Between groups	10.73	4848.95	945.78	1117.86	835.56	0.04	2.38	63893.64	49.34	14.47	49.40	2.15	236.29
Within groups	0.08	87.62	49.49	21.77	112.60	0.01	0.49	17614.98	6.07	0.57	23.07	0.23	67.32
<i>F</i>	125.08	55.34	11.90	51.36	7.42	3.95	4.80	3.63	8.13	25.53	2.14	10.59	3.51
Significance	<0.001	<0.001	0.001	<0.001	0.011	0.054	0.034	0.065	0.012	<0.001	0.160	0.003	0.080

SW, stem width; PH, plant height; BP, branch position; LMR, length of main raceme; NPR, number of pods in the main raceme; PD, pod density in the main raceme; LP, length of pod; NPP, number of pods per plant; NSP, number of seeds per pod; NPB, number of primary branches; NSB, number of secondary branches; SWT, 1000-seed weight; SWP, seed weight per plant.

Table 3. Analysis of partial correlations of F₂ generation from synthetic oilseed *B. napus*.

	SW (X ₁)	PH (X ₂)	BP (X ₃)	LMR (X ₄)	NPR (X ₅)	PD (X ₆)	LP (X ₇)	NPP (X ₈)	NSP (X ₉)	NPB (X ₁₀)	NSB (X ₁₁)	SWT (X ₁₂)	SWP (Y)
X1	1.00												
X2	0.92**	1.00											
X3	0.72*	0.85**	1.00										
X4	0.97**	0.93**	0.66	1.00									
X5	0.67	0.90**	0.79*	0.73*	1.00								
X6	0.67*	0.77*	0.53	0.70*	0.83**	1.00							
X7	-0.63**	-0.46**	-0.18*	-0.62	-0.16	-0.12	1.00						
X8	0.67	0.44**	0.04**	0.67**	0.15	0.32	-0.74*	1.00					
X9	0.00	-0.22**	0.10*	-0.15*	-0.44*	-0.45	0.06**	-0.23	1.00				
X10	-0.83**	-0.81**	-0.74*	-0.76*	-0.69*	-0.73*	0.47	-0.33	-0.09	1.00			
X11	-0.62	-0.75*	-0.87**	-0.56	-0.78	-0.71*	0.13	0.89	-0.09	0.77*	1.00		
X12	-0.38	-0.35	-0.33	-0.25	-0.25	-0.34	0.29	-0.44	0.19	0.51	0.22	1.00	
Y	0.57	0.22	-0.02	0.53	-0.16	0.001	-0.69*	0.75**	0.39**	-0.26	0.92	0.83**	1.00

Abbreviations as in table 2.

Significance at **P* = 0.05, ***P* = 0.01.

From table 3, it can also be seen that number of branches, including primary and secondary branches, had a significantly negative correlation with plant height, branch position and pod density, and their coefficients were -0.81, -0.74, -0.73 and -0.75, -0.87, -0.71, respectively, but there was no significant correlation between 1000-seed weight and all the other 12 agronomic traits. A significantly negative correlation was observed between length of pod and three agronomic traits (stem width, plant height and branch position), and their correlation coefficients were -0.63, -0.46 and -0.18, respectively.

Analysis of linear regression of seed yield per plant and other agronomic traits

In the present experiment, the linear regression of seed yield per plant and other agronomic traits was analysed. The results showed that the stepwise method of linear regression could be used, since there was significant difference between seed yield per plant and other agronomic traits (*F* = 283.11, *P* < 0.001) (table 4A). It was observed that three characters (number of pods per plant, number of seeds per plant and 1000-seed weight) had a significant effect on seed yield per plant, and all the *P* values were <0.001 (table 4B). The coefficients of the linear regression equation were estimated, and were as follows: $y = 0.074x_8 + 1.819x_9 + 6.72x_{12} - 60.78$ ($R^2 = 0.993$).

Analysis and heritability of seed quality traits

The seed samples from each cross were used to measure erucic acid, glucosinolate, oil and protein contents using near-infrared reflectance spectroscopy. It was observed that glucosinolate content of the seeds from the three F₂ progenies were higher than those from their maternal parents and the contents reached 98.1, 127.9 and 99.3 μmol/g, respectively (table 5), but the erucic acid and oil contents of the F₂ plants were lower than in their maternal parents at 20.5, 23.9 and 27.3%, and 33.4, 31.4 and 35.3% respectively. Protein content was similar in the F₂ plants and their maternal parents. The heritability of the four seed quality traits was also analysed. It was shown that erucic acid, glucosinolate, oil and protein content had heritability values of 0.614, 0.405, 0.153 and 0.680 respectively.

Discussion

Through analysis of the heritability of nine quantitative traits based on three populations of oilseed *B. napus* synthesized artificially through ovary culture from crosses between *B. campestris* and *B. oleracea*, it has been shown that number of seeds per pod, length of pod and 1000-seed weight are less affected by the environment, and these characters could be used to select directly for breeding purposes.

Table 4. Analysis of linear regression of seed yield per plant and other agronomic traits of F₂ generation from synthetic oilseed *B. napus*.

(A) ANOVA (d)

Model	SS	df	MS	F	P
1. Regression	627.217	1	627.217	13.667	0.006 (a)
Residual	367.148	8	45.893		
Total	994.364	9			
2. Regression	894.712	2	447.356	31.424	0.000 (b)
Residual	99.652	7	14.236		
Total	994.364	9			
3. Regression	987.389	3	329.130	283.113	0.000 (c)
Residual	6.975	6	1.163		
Total	994.364	9			

(a) Predictors: (constant), number of pods per plant. (b) Predictors: (constant), number of pods per plant, number of seeds per pod. (c) Predictors: (constant), number of pods per plant, number of seeds per pod and 1000-seed weight. (d) Dependent variable: seed yield per plant.

(B) Coefficients (a)

Model	Unstandardized coefficients		Standardized coefficients		
	B	Std. Error	B	t	P
1. (Constant)	3.860	8.876		0.435	0.675
Number of pods per plant (x_8)	0.065	0.018	0.794	3.697	0.006
2. (Constant)	-16.324	6.791		-2.404	0.047
Number of pods per plant (x_8)	0.056	0.010	0.686	5.615	0.001
Number of seeds per pod (x_9)	1.405	0.324	0.530	4.335	0.003
3. (Constant)	-60.778	5.344		-11.374	<0.001
Number of pods per plant (x_8)	0.074	0.003	0.891	21.328	<0.001
Number of seeds per pod (x_9)	1.819	0.104	0.686	17.561	<0.001
1000-seed weight (x_{12})	6.720	0.753	0.415	8.929	<0.001

(a) Dependent variable: seed yield per plant (y), $y = 0.074x_8 + 1.819x_9 + 6.72x_{12} - 60.78$ ($R^2 = 0.993$).

Table 5. Mean of seed quality traits of maternal and F₂ generation from synthetic oilseed *B. napus*.

Seed quality	Maternal			F ₂			h_n^2
	Hauarad	Maoshan-3	Youbai	N ₁	N ₂	N ₃	
Erucic acid (%)	23.559	34.107	37.697	20.459	23.934	27.327	0.614*
Glucosinolate (μ mol/g)	59.265	111.697	67.536	98.067	127.851	99.315	0.405*
Oil content (%)	42.276	36.797	38.432	33.372	31.369	35.261	0.153*
Protein content (%)	38.634	42.616	42.476	43.003	42.609	43.594	0.680**

N₁, N₂ and N₃ are F₂ generation from the crosses Hauarad×Jingfeng-1, Maoshan-3×Jingfeng-1 and Youbai×Jingfeng-1 respectively.

Significance at * $P = 0.05$, ** $P = 0.01$.

However, heritability of number of branches was very low (only 0.172), indicating that variation in number of branches was more affected by the environment (table 1). Thus, number of branches was not a good target character for direct selection in breeding programmes. However, in other researches, it has been reported that heritability of 1000-seed

weight was 0.34 and was more affected by environment (Yu *et al.* 1998). This is probably due to the different genotypes (cultivars) used in those experiments from those used in our study. In addition, heritability of plant height and number of seeds per pod were 0.68 and 0.74 respectively (Yu *et al.* 1998), which is consistent with the results reported here.

Therefore, selecting characters with higher heritability as targets is very important for breeding purposes and can save labour and time.

According to estimated coefficients of skewness and kurtosis of the traits tested, gene interaction was not found for plant height and 1000-seed weight. The numbers of genes controlling these traits were 7.4 and 6.4 respectively. Similar results have been reported by other researchers (Yu *et al.* 1998). Therefore, the selection of plant height and 1000-seed weight must be strict, since their heritability is higher. Similarly, selection of number of pods per plant, length of pod and seed yield per plant should also be strict, because gene interaction was observed and showed complementary interaction, but for stem width and number of branches early selection should not be strict because the heritabilities were lower and were only 0.215 and 0.172 respectively.

Partial correlation analysis showed that number of pods per plant, number of seeds per pod and 1000-seed weight were positively correlated with seed yield per plant. On the contrary, length of pod was negatively correlated with seed yield per plant, with correlation coefficient of -0.69 . Other agronomic characters had no significant correlation with seed yield per plant. This was consistent with the results of other researches (Han and Liu 1993; Li *et al.* 2001). In addition, it was shown that branch position, number of pods in the main raceme and number of primary branches were negatively but insignificantly correlated with seed yield, whereas stem width, plant height and length of main raceme showed positive but insignificant correlation with seed yield. Therefore, for improving oilseed yield, plants with more pods per plant and seeds per pod and higher 1000-seed weight should be selected as the target for the next procedure. At the same time, improving number of branches was also beneficial for higher yield.

Seed yield per plant is an important target for oilseed production. In this experiment, the linear regression of seed yield per plant and other agronomic traits was analysed. It was observed that three characters (number of pods per plant, number of seeds per plant and 1000-seed weight) had significant effects on seed yield per plant. Similar results could also be obtained by analysing partial correlation of all agronomic traits. Therefore, number of pods per plant, number of seeds per pod and 1000-seed weight should be given more attention as the main targets of selection during early breeding procedure. Similar results have been reported by other researchers (Li *et al.* 2001).

In the present experiment, the seed samples from each cross were used to measure erucic acid, glucosinolate, oil and protein contents. It was observed that glucosinolate content of three F_2 plants was higher than that of the respective maternal parent. This is probably due to the fact that the maternal plants have high glucosinolate content and glucosinolate has high heritability value of 0.405. Additionally, glucosinolate content may also be affected by the *B. oleracea* cv. Jingfeng-1 used as the paternal plants. This experiment

also showed that oil content of the F_2 plants was lower than that of their maternal parents, but the heritability was only 0.153. It was shown that the cross with *B. oleracea* could lead to decrease of oil content which was greatly affected by the paternal plants. As for protein content, similar results were found in the F_2 plants and their maternal parents, and the heritability reached 0.680. In addition, it was observed that erucic acid content of the F_2 plants was lower than that of the maternal parents. This indicated that erucic acid could be reduced if the proper cultivar of *B. campestris* is intercrossed with that of *B. oleracea*.

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