

# Broadening the variability of quality traits in rapeseed through interspecific hybridization with an application of immature embryo culture

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## Abstract

Interspecific hybridization is an important tool to transfer characters across species and develop synthetic amphidiploids, and therefore it has been widely applied for improving *Brassica* spp. The aim of our study was to determine whether the interspecific crosses can help increase the range of variability of traits connected with the higher value of rapeseed. An attempt was also made to investigate the environmental influence on the studied traits. For this reason, in our experiments, crosses between male sterile line of F8 generation and *B. campestris* ssp. *sarson*, Yellow sarson; *B. campestris* ssp. *pekinensis*; *B. carinata* and *B. juncea* were first attempted. Thereafter, hybrid seeds of 96 lines obtained by crossing *Brassica napus* male sterile line MS8 with three other *Brassica* species were tested for fiber and glucosinolates content using near-infrared reflectance spectroscopy (NIRS). Moreover, chromatography and spectrophotometric measurements of sinapine and tocopherols contents in those seeds were made. On the basis of the obtained results, it was found that all tested hybrid lines showed a great increase of variability for the studied quality traits when compared to the parental forms. As a result of our analyses, reduced glucosinolates hybrid lines were selected.

**Key words:** interspecific hybridization, quality traits, infrared reflectance spectroscopy (NIRS), glucosinolates, sinapine, fiber

## Introduction

The improvement of *Brassica* species has been mostly confined to the exploitation of the naturally occurring genetic variation in the cultivated species. The existing variation being limited, breeders have to resort to wide hybridization, which provides an important method of incorporation of desirable characteristics such as, resistance to disease, drought and frost. Moreover, artificial hybridization among the *Brassica* species may be useful for providing further approaches and novel traits in oilseed *Brassica* research and breeding programs. It is interesting especially because of the growing interest in rapeseed oil, which is connected with large possibilities of its use both for industrial and consumption purposes. In highly developed American countries as well as the European Union countries and China, there is an increasing emphasis on the oil quality (Olejniczak et al., 2007). The oil content in the seed varies from 30-45% depending on the species, the variety and

the climatic conditions under which it is grown. Following oil extraction, the remaining meal contains different nutritional and anti-nutritional compounds. The quality of rapeseed oil and meal depends, in particular, on the content of anti-nutritional compounds in the seeds (e.g. glucosinolates, sinapine and fiber). Among these, glucosinolates are the most important anti-nutritional compounds. The optimal content of glucosinolates in seeds, considered harmless at current cultivated varieties of oilseed rape, is in the range of 10-20  $\mu\text{M/g}$ . For many years an increased interest has been observed among *Brassica* researchers in the lowering of the sinapine content, which lowers the nutritional value of rapeseed meal (Wojciechowski et al., 1994; Velasco et al., 1998; zum Felde, 2005). Within the *Brassica* genera there is a large variation in sinapine content, which consequently creates the possibility of selecting lines with reduced sinapine synthesis (zum Felde, 2005). Furthermore, in rapeseed seeds, another important anti-nutri-

tional component is high fiber content. Compared with black or brown seeds, yellow-seeded rapeseed cultivars have a thinner seed coat, which is associated with the higher oil content in the seeds and a higher protein and lower fiber content in the meals (Chen and Heneen, 1992). For this reason, crosses between *Brassica napus*, and yellow seeded *Brassica campestris* ssp. *trilocularis* “Yellow sarson” were made. One of the most effective methods for increasing the variability of traits that impacts the quality of rapeseed products is interspecific hybridization. However, information on the genetic variation created through such crosses is meager. Therefore, the major objectives of the present study were to create a genetic variation through interspecific crosses of *B. napus* with chosen *Brassica* species and estimate variability parameters for different seed quality traits in the obtained hybrid lines.

### Materials and methods

The interspecific hybridization was performed in the glasshouse of the Genetics and Plant Breeding Department, Poznań University of Life Sciences (PULS) in Poznań, Poland. There were 4 different cross combinations and one control (Table 1). Depending on the availability of flower buds, 30-60 crosses were made for each cross combination. All hybridizations were performed with the application of an *in vitro* culture of isolated embryos according to the method described by Wojciechowski (1985, 1998). The immature embryos were isolated from young siliques at different developmental stages i.e. heart and early and late torpedo stages, 14-19 days after pollination. For the embryo culture, basal White (W, 1963), Murashige and Skoog (MS, 1962), Murashige and Skoog modified by Keller and Armstrong (MS<sub>k</sub>, 1977) and Nitsh and Nitsh (H<sub>3</sub>, 1969) media were applied (Fig. 1). During the season 2010/2011, ninety six F<sub>5</sub> hybrid lines were grown in two different locations i.e. the experimental fields in Poznań (Sołacz) and in the PULS experimental station Dłóń located 100 km south of Poznań. These two locations have different types of soil and weather conditions. In Poznań, the soil is light and sandy type (class IV in the soil valuation system), whereas in Dłóń, the soil is heavy (class III). During the growing season, the average temperature in Poznań was lower than in Dłóń, i.e. 11.9°C and 15°C, respectively. Similarly, the average rainfall was lower in Poznań (about 59.8 mm) than in Dłóń

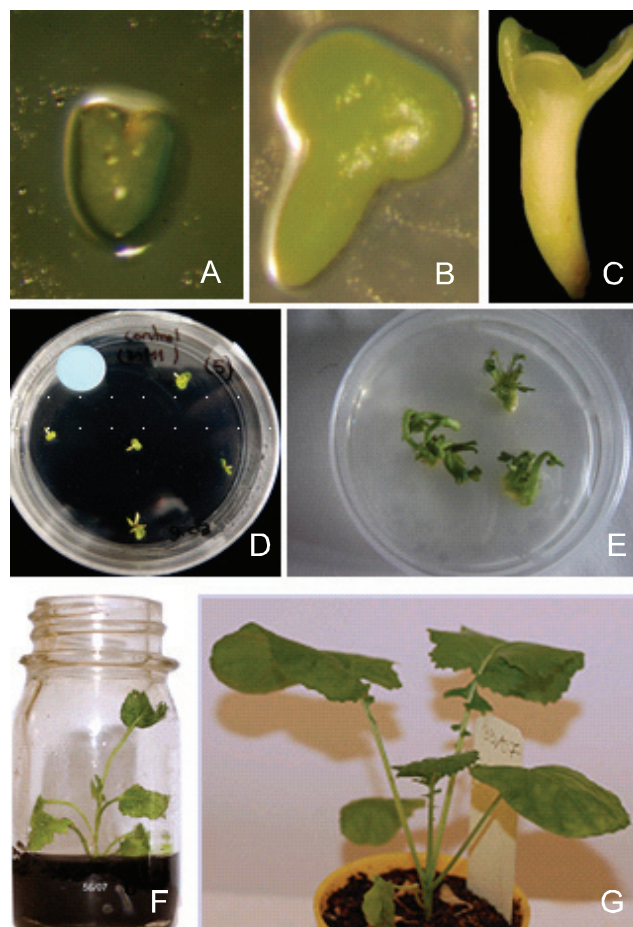


Fig. 1. Plant regeneration from Brassica hybrid embryos in vitro cultures: A-C) Hart, torpedo and late torpedo stage of *Brassica* hybrid embryos just after isolation from the silique; D) First day of incubation of hybrid embryos on MS medium; E) Regenerated seedlings on MS<sub>k</sub> medium; F) Regenerated plant on rooting H<sub>3</sub> medium; G) Regenerated plant in the soil

(70.5 mm). The seed samples for the analysis of glucosinolates, fiber and sinapine concentration were collected from ten self-pollinated plants from each tested line. Glucosinolates and fiber content was determined by near-infrared reflectance spectroscopy (NIRS) (Thies, 1982, Biston et al., 1988, Velasco et al., 1999). These analyses were performed at the Małyszyn Laboratory that belongs to Plant Breeding Strzelce. For the NIRS analyses, minimum 3 g of intact seeds were used. Such a seed probe was scanned on the NIRS Systems monochromator model 6500 (NIR Systems, Inc. Silver Springers, MD, USA). The sinapine content (total sinapic acid esters) was determined by spectrophotometric method. The sinapine content was calculated in accordance with the formula described by Wojciechowski et al. (1994). The compound was analyzed in the PULS laboratory in Poznań.

**Table 1.** The effectiveness of interspecific crosses between male sterile line of *Brassica napus* MS-8 (AACC = 38) and four *Brassica* species expressed by number of obtained embryos to mean number of well-developed ovules

Cross combination		Number			Ovules with embryos [%]	Regenerated plants (% plants/incubated embryos)	
♀	♂	analyzed pods	isolated ovules	embryos placed on media		on H3 medium	in soil
<i>B. napus</i> – MS8 line	<i>B. campestris</i> ssp. <i>trilocularis</i> “Yellow Sarson” – turnip rape	21	319	162	50.7	56.4	48.1
	<i>B. campestris</i> ssp. <i>pekinensis</i> – pekinense cabbage	34	158	97	61.3	62.1	43.1
	<i>B. carinata</i> – Ethiopian mustard	49	874	179	20.5	51.17	41.3
	<i>B. juncea</i> – Indian mustard	10	113	31	27.4	41.5	41.5
	<i>B. napus</i> – Lira Jet	6	83	82	98.8	100.0	100.0
Total		120	1547	551	35.6	50.0	43.7

**Table 2.** Glucosinolate content [ $\mu\text{mol/g}$ ] s.d.m in the seeds of interspecific hybrids obtained by crossing *Brassica napus* male sterile line MS-8 (AACC = 38) with yellow seeded *B. campestris* ssp. *trilocularis* “Yellow Sarson” – (A1A1 = 20), brown – seeded *B. campestris* ssp. *pekinensis* (A2A2 = 20), *B. carinata* (BBCC = 34) and with *B. juncea* – (AABB = 18) depending on the location – Dłóń (D), Poznań-Sołacz (S)

Hybrids and parental forms	Location	Alkene					Total glucosinolate content				
		mean	range	SLD <sub>0,05</sub>	SD	Var	mean	range	SLD <sub>0,05</sub>	SD	Var
♀ AC	S	18.60	–	–	–	–	20.78	–	–	–	–
AC x A1 (16 lines)	D	44.57	10.94-78.05	4.29	20.41	416.72	46.22	16.04-76.96	3.95	18.81	353.84
	S	36.03	6.43-69.40	5.82	20.56	422.85	37.73	10.94-69.10	5.18	18.31	335.27
♂ A1	S	57.18	–	–	–	–	56.87	–	–	–	–
AC x A2 (56 lines)	D	40.91	4.96-101.08	2.34	22.45	947.70	42.53	6.81-98.16	2.17	20.79	831.52
	S	40.40	10.48-64.99	3.86	13.21	174.57	41.34	14.08-62.45	3.48	11.91	141.83
♂ A2	S	46.14	–	–	–	–	7.28	–	–	–	–
AC x BC (10 lines)	D	43.03	10.82-79.56	5.00	17.12	293.03	44.18	16.03-77.52	4.55	15.56	242.15
	S	43.69	35.84-53.47	2.47	5.35	28.66	44.02	37.81-53.74	2.29	4.95	24.48
♂ BC	S	51.22	–	–	–	–	52.22	–	–	–	–
AC x AB (14 lines)	D	39.80	11.11-67.95	6.29	20.80	432.53	41.52	15.47-66.24	5.66	18.70	34.76
	S	40.16	9.70-63.54	5.74	19.46	417.35	39.82	12.34-61.29	5.19	18.23	331.12
♂ AB	S	53.13	–	–	–	–	56.28	–	–	–	–
Control – Lira Jet	S	9.83	–	–	–	–	12.84	–	–	–	–

The results of chemical analyses were verified by analysis of variance (ANOVA) in accordance with the established experimental method. ANOVA and the analysis of significant differences among the means was performed

with the use of STATISTICA computer package. The least significant difference (LSD) at  $P = 0.05$  level was calculated.

## Results

### *The effectiveness of the crosses*

The hybridization efficiency was expressed by the number of obtained embryos to the mean number of well-developed ovules (Table 1). The highest efficiency was observed for *B. napus* MS8 line x *B. campestris* ssp. *trilocularis* "Yellow sarson". In this cross combination, plants were obtained from 48.1% of isolated embryos. In other combinations, plants were regenerated from 41.3 to 43.7% of isolated embryos.

### *The seed analysis*

Hybrid seeds from 96 lines were harvested and examined for anti-nutrient content.

### *Glucosinolates (GLS) content*

The alkene GLS content in the analyzed parental forms ranged from 18.60  $\mu\text{mol/g}$  seeds (MS8 line) to 53.13  $\mu\text{mol/g}$  (*B. juncea*), and the total GLS content spanned from 20.78 to 56.28, respectively as in the case of alkene GLS content (Table 2). The hybrid seeds of four cross combinations exhibited intermediate values for GLS content between the parental forms, and in the case of alkene, the GLS content ranged from 36.03 (MS8 x *B. campestris* ssp. *trilocularis*, harvested in Poznań) to 44.57  $\mu\text{mol/g}$  seeds harvested in Dłóń. Similar to alkene GLS content, the highest values of total GLS content were observed in the hybrid lines harvested in Dłóń (46.22) and Poznań (37.73). It is noteworthy that among the analyzed hybrid lines there were some lines with significantly lower and higher GLS content. The lowest GLS content was noted in Dłóń for MS8 x *B. campestris* ssp. *pekinensis* hybrids (6.81) and in Poznań for MS8 x *B. campestris* ssp. *trilocularis* hybrid lines (10.94).

The average value of alkene GLS content ( $\mu\text{mol/g}$  seeds) in the control seeds of *B. napus* – Lira Jet variety was lower than in all analyzed hybrid lines in both location. Also the total GLS value showed a similar dependence.

### *Fiber content*

The fiber content in the hybrid and parental seeds was expressed as the average value of the neutral detergent fiber (NDF) and the acid detergent fiber (ADF) as a subtraction of the NDF. It was shown that the NDF average content was the lowest for yellow seeded *B.*

*campestris* ssp. *trilocularis* "Yellow sarson" (22.92%) compared to other tested parental and hybrid forms (Table 3).

Although there was no significant difference in the NDF average content among the hybrids, the fiber levels were slightly lower in Dłóń than in Sołacz. In Dłóń, the NDF values ranged in MS8 x *B. campestris* ssp. *trilocularis* "Yellow sarson" hybrid lines from 21.93% to 28.38%. For the other hybrids, some lines with lower fiber content than the parental forms were selected.

### *Sinapine content*

The sinapine content in seeds of parental forms ranged from 0.76% in *B. carinata* to 2.04% in MS8 line, while in the hybrid lines, it ranged from 0.96% (MS8 x *B. carinata*, harvested in Poznań) to 2.72% (MS8 x *B. campestris* ssp. *pekinensis*), harvested in Dłóń (Table 4).

Generally, in all analyzed hybrid lines, lower values of the average sinapine content compared with the controls was observed.

The value of the variability parameters for the analyzed quality traits in hybrid seeds differed between the crosses. A significant variation was observed for all the parameters except the sinapine content. The widest range was recorded for alkene glucosinolates (4.96-101.08  $\mu\text{mol/g}$  of seeds for MS8 x *B. campestris* ssp. *pekinensis* in Dłóń) followed by the ADF for the same hybrid line and location (15.75-24.63%). The analyses of the variance for glucosinolate content exhibited high values for almost all the tested hybrid lines except one MS8 x *B. carinata*, which had a very low variance value (28.66 for alkene GLS) and (24.48 for GLS sum) in Poznań. The variance for fiber content in hybrid seeds was lower than for the GLS and ranged from 0.23 (MS8 x *B. campestris* ssp. *pekinensis* in Poznań) to 1.54 (MS8 x *B. campestris* ssp. *pekinensis* in Dłóń) for the ADF, and from 1.34 (MS8 x *B. carinata* in Poznań) to 11.35 (MS8 x *B. campestris* ssp. *pekinensis* in Dłóń) for the NDF, respectively (Table 3). Even lower were the variance values for the sinapine content that ranged from 0.01 in Poznań to 0.08 in Dłóń.

## Discussion

The results of this study show that the analyzed hybrid lines differ in their glucosinolate, fiber and sinapine content. In the experiments, it was observed that four

**Table 3.** Fiber content [%] in the seeds of interspecific hybrids obtained by crossing male sterile line of *Brassica napus* MS-8 (AACC = 38) with yellow – seeded *B. campestris* ssp. *trilocularis* “Yellow Sarson” – (A1A1 = 20), brown – seeded *B. campestris* ssp. *pekinensis* (A2A2 = 20), *B. carinata* (BBCC = 34) and with *B. juncea* – (AABB = 18) depending on the location – Dłóń (D), Poznań-Sołacz (S)

Hybrids and parental forms	Location	ADF					NDF				
		mean	range	NIR <sub>0.05</sub>	SD	Var	mean	range	NIR <sub>0.05</sub>	SD	Var
♀ AC	S	18.53	–	–	–	–	24.58	–	–	–	–
AC x A1 (16 lines)	S	19.95	18.35-21.89	0.25	0.88	0.78	25.57	22.16-29.98	0.58	2.04	4.16
	D	19.31	17.24-21.34	0.22	1.04	1.08	24.39	21.93-28.38	0.33	1.57	2.46
♂ A1	S	19.34	–	–	–	–	22.92	–	–	–	–
AC x A2 (56 lines)	S	19.97	19.20-21.21	0.14	0.47	0.23	25.01	23.01-29.12	0.37	1.27	1.60
	D	19.48	15.75-24.63	0.12	1.17	1.54	24.85	18.94-29.34	0.22	2.11	11.35
♂ A2	S	19.48	–	–	–	–	24.85	–	–	–	–
AC x BC (10 lines)	S	20.10	18.35-21.48	0.40	0.86	0.73	25.05	22.47-26.35	0.53	1.16	1.34
	D	19.34	17.60-21.13	0.28	0.96	0.92	24.60	21.34-28.22	0.52	1.79	3.20
♂ BC	S	21.02	–	–	–	–	24.68	–	–	–	–
AC x AB (14 lines)	S	20.33	19.13-21.20	0.54	0.82	0.68	25.09	23.29-27.68	1.15	1.76	3.10
	D	20.30	19.13-21.18	0.17	0.56	0.31	25.92	22.82-28.61	0.49	1.62	2.62
♂ AB	S	20.87	–	–	–	–	24.72	–	–	–	–
Control – Lira Jet	S	19.12	–	–	–	–	25.60	–	–	–	–

**Table 4.** Sinapine content [%] in the seeds of interspecific hybrids obtained by crossing male sterile line of *Brassica napus* MS-8 (AACC = 38) with yellow – seeded *B. campestris* ssp. *trilocularis* “Yellow Sarson” – (A1A1 = 20) brown – seeded *B. campestris* ssp. *pekinensis* (A2A2 = 20), *B. carinata* (BBCC = 34) and with *B. juncea* – (AABB = 18) depending on the location – Dłóń (D), Poznań-Sołacz (S)

Hybrids and parental forms	Dłóń					Poznań – Solacz				
	Mean	Range	NIR <sub>0.05</sub>	SD	VAR	Mean	Range	NIR <sub>0.05</sub>	SD	VAR
♀ AC	–	–	–	–	–	2.04	–	–	–	–
AC x A1 (16 lines)	1.40	1.13-2.01	0.05	0.19	0.04	1.21	1.02-1.51	0.12	0.16	0.03
♂ A1	–	–	–	–	–	0.88	–	–	–	–
AC x A2 (56 lines)	1.52	1.3-2.72	0.09	0.28	0.08	1.22	1.04-1.68	0.05	0.14	0.01
♂ A2	–	–	–	–	–	0.91	–	–	–	–
AC x BC (10 lines)	1.15	1.09-1.19	0.05	0.14	0.02	1.14	1.09-1.53	0.05	0.12	0.01
♂ BC	–	–	–	–	–	0.76	–	–	–	–
AC x AB (14 lines)	1.09	0.90-1.19	0.16	0.14	0.02	0.96	0.60-1.36	0.04	0.12	0.01
♂ AB	–	–	–	–	–	1.09	–	–	–	–
Control – Lira Jet	–	–	–	–	–	1.47	–	–	–	–

parental forms (*B. campestris* ssp. *sarson*, *B. campestris* ssp. *pekinensis*; *B. carinata* and *B. juncea*) demonstrate a much higher average of the alkene glucosinolate content than the hybrid lines. Rapeseed lines and cultivars may be recommended for inclusion in the human diet due to their low specific glucosinolate content. Consequently, we see a possible application of the hybrid lines we identified?/obtained? in the future breeding programs. In oilseed rape, however, the level of genetic diversity in the adopted winter oilseed rape breeding material with double low seed quality (zero erucic, low glucosinolate content) is relatively low due to a lengthy selection procedure of better lines with respect to the seed oil and meal quality. The variation in the amount and pattern of glucosinolates in *Brassica* plants is attributed to genetic and environmental factors, including plant age, temperature, water stress and soil type (Rosa, 1997). Comparison of the two locations, i.e. the fields in Poznań and Dłóń, revealed a greater variability in the glucosinolate and sinapine content in the tested hybrid lines in Dłóń. This variability may be influenced by better environmental conditions in this region. Since the thinner seed coat meal from the yellow-seeded lines has much lower fiber content than the meal from black-seeded lines, yellow-seeded species of *Brassica* were also used for hybridization for the experiment. However, as per our results, the fiber content in hybrid lines showed variability in hybrid lines when compared to the parental forms and the control variety, especially in MS8 x *B. campestris* ssp. *trilocularis* and MS8 x *B. campestris* ssp. *pekinensis* hybrid lines.

The elimination of sinapine content will improve the flavor, palatability and nutritional properties of canola seeds and canola meal. Although several methods for removing sinapine have been reported (Fenwick et al., 1979; Dabrowski et al., 1989, Tayanian and Henkel, 1991, Wojciechowski et al., 1994), none has been proven economical thus far (Wang et al., 1998). Conventional plant breeding or genetic engineering would be a more efficient long term means of lowering or eliminating sinapine levels. Success of a conventional plant breeding program depends on finding genetic variability for sinapine content and developing a suitable analytical methodology to select plants with low sinapine content in the seed. Average sinapine concentrations among the four hybrid lines ranged from 1.09 to 1.52% in Dłóń, and from 0.96 to 1.22% in Poznań. Our results were si-

milar to those obtained by Taylor et al. (1993) for six cultivars of cabbage, broccoli and cauliflower (0.74-1.20%). The results obtained in our experiment show that there is a high variability in sinapine content, especially among the haploid lines originating from crosses of *B. napus* with *B. juncea*. This suggests that it might be possible, by selection, to develop rapeseed varieties that are low in sinapine content.

## Conclusions

Generally, all the tested hybrid lines displayed a great increase in the range of variability for the studied quality traits, especially in the glucosinolate and fiber content when compared to the parental forms and the control varieties.

On the basis of the conducted analyses, reduced glucosinolates hybrid lines were selected (7.54-15.76 µmol/g dry weight of seeds).

The environmental impact on the tested quality traits was observed for both, the glucosinolates and the sinapine content. A higher range of variability for the above-mentioned traits was observed in Dłóń.

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