DEVELOPMENTS IN THE BREEDING OF EDIBLE OIL IN OTHER *BRASSICA* SPECIES

G. RAKOW

Agriculture and Agri-Food Canada Research Centre, 107 Science Place, Saskatoon, Saskatchewan, S7N 0X2, Canada

ABSTRACT

This review summarizes research papers presented in the breeding/biotechnology session on oil quality in other *Brassica* species. The mustard species *B. juncea* and *B. carinata* have the potential to be grown as vegetable oilseed crops in many countries, especially under semiarid growing conditions. Canola quality *B. juncea* has been developed in Canada and was extensively field tested in Saskatchewan, Canada. The crop had superior yield and was highly resistant to blackleg. Seed oil contents must be further improved to make *B. juncea* competitive with *B. napus* canola in Canada. Work is underway to lower the levels of linoleic and linolenic acid in *B. juncea*. Zero erucic acid *B. carinata* has also been developed in Canada and work is ongoing in Spain. Work is in progress at Saskatoon, Canada, that will lead to low glucosinolate *B. carinata* opening the way for the development of canola quality forms in this species. Research towards a *B. napus* like fatty acid composition in *B. carinata* was successful. Canola quality forms have also been developed in *Sinapis alba* which will allow the production of canola quality *S. alba* seed. Research in India on *B. juncea* and Spain on *B. carinata* is aimed at developing canola quality forms in these two species. Research conducted in Germany explored the variation in fatty acid compositions in different *Brassica* species, and in China, a new source of low erucic acid has been identified in a wild *Brassica* species which is closely related to *Sinapis arvensis*.

BRASSICA JUNCEA

Brassica juncea mustard has been grown as a condiment crop on some 50,000 ha annually in the three prairie provinces of western Canda. *B. juncea* is better adapted to the semi-arid climate of these provinces, and is higher yielding than either of the two canola species *B. napus* and *B. rapa*. This observation was the basis for research aimed at the development of canola quality *B. juncea* through interspecific crosses. Zero erucic acid, low glucosinolate *B. juncea* has been developed at the Agriculture and Agri-Food Canada, Research Centre Saskatoon, Canada. This material was higher yielding than *B. napus* canola, but seed oil contents of *B. juncea* canola were approximately 5% lower than those of *B. napus* canola.

Rakow et al. (1995) initiated a breeding program with the objective to increase seed oil contents of *B. juncea* canola through cross breeding utilizing high oil content *B. juncea* germplam from Russia (Table 1). Twenty F_4 lines were yield tested at nine locations in western Canada in 1993. Table 1 summarizes the agronomic performance and seed quality of a few selected lines:

TABLE 1. Average agronomic performance and seed quality of selected *Brassica juncea* and*B. napus* at 9 locations in western Canada in 1993.

Entry	Yield % AC Elect	Mat. (days)	Height (cm)	Blackleg (0-5)	Oil (%)	GSL (µmoles/g meal)
B. juncea						
J90-2741	116	115	173	0.2	50.3	135.1
J90-4316	112	112	166	0.4	43.0	22.0
J92-223	123	112	163	0.2	44.5	19.2
B. napus						
AC Elect	100	107	130	4.0	47.2	6.6

B. juncea lines outyielded the *B. napus* canola cultivar AC Elect by 12 to 23%, an indication of its good adaptation to semi arid dryland agriculture. *B. juncea* was approximately one week later maturing than *B. napus*, and plants of *B. juncea* were 30 to 40 cm taller than *B. napus* plants. The later maturity of *B. juncea* would not hinder its production in the longer season growing areas of the southern prairies. *B. juncea,* is also highly resistant to blackleg. *B. juncea* seed is low in oil content (approximately 38%). It is essential that seed oil contents of *B. juncea* are increased to levels similar to those in *B. napus* cultivars to make *B. juncea* economically competitive with *B. napus* for the crushing industry. The research by Rakow et al. (1995) demonstrated that significant increases in oil contents of *B. juncea* seed can be achieved through the utilization of high oil content *B. juncea* germplasm of Russian origin. *B. juncea* line J90-2741, selected from crosses among Russian cultivars, had a 3% higher oil content than *B. napus* AC Elect, a high oil content Canadian *B. napus* cultivar (Table 1). *B. juncea* line J90-4316, one of the original canola quality lines had 43.0% oil. We were able to increase the oil content in *B. juncea* canola by 1.5% to 44.5% through cross breeding and selection (line J92-223, Table 1). This is a significant improvement in oil content, but the ultimate goal must be to develop *B. juncea* canola with seed oil contents similar to that of the *B. juncea* line J90-2741 which would make *B. juncea* a superior high oil content oilseed crop.

Glucosinolate contents of *B. juncea* canola lines were 22.0 and 19.2 µmoles per 1 g oilfree meal for lines J90-4316 and J92-223, respectively (Table 1). The two lines were basically free of allyl glucosinolate the hot principal in mustard seed. However, these levels were three times the levels of glucosinolates observed in *B.napus* AC Elect seed meal, and further reductions in glucosinolate contents would therefore be highly desirable. We have evidence from selections carried out over the last two years in the field that further reductions in glucosinolate contents in *B. juncea* canola can be achieved.

Raney et al. (1995) crossed the canola quality *B. juncea* line J90-4253 with the low linolenic acid *B. napus* line C92-0226 and backcrossed the interspecific plant to *B. juncea* canola J92-223 with the objective to develop *B. juncea* with lower linoleic and linolenic acid contents. Fatty acid analyses of single seed of the BC_1F_2 seed generation identified plants with increased oleic and decreased linoleic and linolenic acid contents. The selections were continued in the BC_1F_3 and BC_1F_4 generations. It was noted that the material segregated for oleic and linoleic acid which would allow the development of 1) high oleic - low linolenic and 2) high linoleic - low linolenic *B. juncea*. Further backcrosses to *B. juncea* are required to develop better fertile *B. juncea* plants.

TABLE 2. Fatty acid composition of *Brassica napus*, *B. juncea* and interspecific plants derived from crosses between the two species.

	Fatty acid composition (% of total)					
Genotype	16:0	18:0	18:1	18:2	18:3	20:1
B. napus						
C92-0226	4.0	1.9	69.3	20.7	1.8	1.2
B. juncea						
J90-4253, J92-223	4.2	2.1	38.9	40.1	12.9	1.0
Interspecific[1]						
BC_1F_3, F_4mean[2]	5.3	4.3	57.4	24.3	5.5	1.1
BC_1F_3, F_4mean[3]	5.7	3.2	38.8	43.0	7.0	1.0

[1] = [*B. juncea* J90-4253 x *B. napus* C92-0226] x *B. juncea* J92-223

[2] = selection for high oleic acid

[3] = selection for high linoleic acid

B. juncea is an important oilseed crop in India, and the development of canola quality cultivars is attempted by several research groups in that country, and also in Bangladesh and Pakistan. All local cultivars are high in erucic acid and glucosinolate content. The introduction of foreign canola quality cultivars of *B. napus* and *B. rapa* failed repeatedly, because these cultivars were not adapted to the short photoperiod growing conditions in India. Agnihotri et al. (1995) produced zero erucic acid *B.juncea* from the cross (Eruca sativa x *B. rapa*) x *B. juncea*, and also zero erucic acid *B. napus* from the cross (*B. napus* x *Raphanobrassica*) x *B. napus*. Glucosinolate analyses revealed that all plants were high in glucosinolate content. The plants were therefore crossed with low glucosinolate *B. juncea* and *B. napus*, respectively, with the objective to develop adapted canola quality cultivars.

BRASSICA CARINATA

Brassica carinata mustard originated in Ethiopia and is grown as an oilseed crop in the highlands of that country. It has also potential as an oilseed crop in India, Spain and the United States. *B. carinata* is better heat and drought tolerant than any other *Brassica* species,and is therefore will adapted to oilseed production under semiarid conditions. *B. carinata* is potentially high yielding and has good disease resistance. As for *B. juncea*, canola quality forms of *B. carinata* are required before it can be utilized as an edible oilseed. Zero erucic acid *B. carinata* has been developed in Spain and in Canada, and work is in progress at Saskatoon, Canada, to develop low glucosinolate *B. carinata*.

Velasco et al. (1995) reported results from mutation work aimed at developing zero erucic acid and low glucosinolate *B. carinata*. Seed of the *B. carinata* line C-101 was treated with EMS and the M_2 and M_3 generations analyzed for fatty acid composition and glucosinolate contents. The lowest level of erucic acid observed was 13.1% and it is likely that through further selections zero erucic acid plants could be identified in progenies of this plant. The information provided on variation of other fatty acids is interesting; however, it must be remembered that this variation is affected by the variation in erucic acid, and that true genetic differences in other fatty acids can only be established in a zero-erucic acid background. Glucosinolate contents observed ranged from a low of 75 μmoles for two plants to >270μmoles for 5 plants among 127 "mutants" analyzed. These "mutants" have to be progeny tested in order to demonstrate that the observed variation is in fact due to genetic factors and is not the result of environmental influences on glucosinolate contents.

FATTY ACID VARIATION IN BRASSICA

Rudloff (1995) studied fatty acid variation in different *Brassica* species. Fifty to sixty single seeds each were analyzed from 76 genotypes of nine *Brassica* species by gas chromatography. The collection had been grown in a field test in 1991, and the following species were tested: *Brassica napus, B. rapa, B. juncea, B. nigra, B. barrelieri, B. narinosa, B. nipposinica, Sinapis alba* and *Camelina sativa*. All genotypes were of the summer annual type. In *B. napus*, high oleic acid (maximum 77.6%) as well as low linolenic acid types (minimum 1.9%) were identified while erucic acid varied form 0 to 50.8%. This variation could be utilized in the breeding of high oleic/low linolenic acid cultivars of *B. napus*. Fatty acid variation in *B. rapa* genotypes was smaller than in *B. napus*. The dominating C_{18} fatty acid in *B. juncea* and *B. nigra* was linoleic acid, indicating strong desaturation activity in these species. This effect was particularly evident in zero erucic acid forms of *B. juncea*. It is interesting to note that *B.nipposinica* is low in erucic acid, and seeds with >70% oleic acid were identified in this species. *Sinapis alba* seed had the highest erucic acid contents (max. 59.2%). *Camelina sativa* seed had low erucic acid and high linolenic acid contents. Rudloff (1995) calculated correlations between individual fatty acids which are directly associated with each other in the fatty acid biosynthetic pathway. He found that the correlation between a particular pair of fatty acids varied greatly from negative to positive values. The only exception form this wide variation was the stable strong negative correlation between oleic acid and erucic acid.

NEW SOURCE FOR LOW ERUCIC ACID

Chen et al. (1995) studied the genomic constitution and seed oil fatty acid composition of a wild *Brassica* species, collected from fields in the Xinjiang autonomous region in northwestern China. The wild *Brassica* species had the same chromosome number as *Sinapis arvensis* (2n=18), and was morphologically similar to *S. arvensis*. It was crossed to *S. arvensis* to investigate pollen fertility and meiotic chromosome pairing in hybrid plants. Reciprocal crosses were easily accomplished with 7 seeds per pollination and pollen fertility of the hybrid plants was 96% identical to that of the parents. Hybrids exhibited normal meiosis with 9 bivalents at metaphase I. Erucic acid contents of seeds of the wild *Brassica* collection were approximately 22% with some seeds containing only 10 to 11% erucic acid. Progenies of the 10% erucic acid seeds yielded zero erucic acid individuals. The erucic acid trait showed a monogenic additive mode of inheritance. The authors concluded that the Xinjiang wild *Brassica* collection is phylogenetically closely related to *B. nigra* (2n=16).

SINAPIS ALBA

Sinapis alba has been grown as a condiment crop on the Canadian prairies for many years. It has good heat and drought tolerance and is well adapted to production in these areas. *S. alba* has good blackleg resistance and can outgrow flea beetle damage due to its vigorous early seedling growth. *S. alba* is highly shatter resistant and has a large bright yellow seed. Despite these many advantages, *S. alba* cannot be grown as an oilseed corp in Canada because the seed is high in erucic acid and glucosinolate content. It is now possible to develop canola quality *S. alba* because of the availability of zero erucic acid and low glucosinolate types.

Raney et al. (1995) began the development of canola quality *S. alba* by combining the zero erucic acid and low glucosinolate traits through cross breeding. Canola quality plants were identified in the F_2 generation of the cross (Tables 3).

TABLE 3. Glucosinolate content and fatty acid compositoin of canola quality *Sinapis alba*

	Glucosinolates (µmoles		Fatty acid composition (%)				
Genotype	Hydroxy-benzyl	Hydroxy-butenyl	18:1	18:2	18:3	20:1	22:1
92-6669[1]	0.0	27.7	32.8	9.5	11.8	12.0	28.8
BHL-926[2]	207.3	2.6	64.3	10.7	16.8	1.9	0.4
F_1	133.9	4.7	nd[3]	nd	nd	nd	nd
F_2	0.1	6.8	61.5	11.9	17.5	2.7	0.5
F_3	0.0	4.3	58.0	15.4	17.2	2.2	0.2

[1] = low glucosinolate [2] = low erucic acid [3] = not determined

S. alba seed contained hydroxybenzyl glucosinolate at levels of approximately 200 µmoles/g meal. The low glucosinolate strain was basically free of hydroxybenzyl glucosinolate but had an elevated hydroxybutenyl glucosinolate content (Table 3). It is possible to reduce the levels of hydroxybutenyl glucosinolate content in zero hydroxybenzyl glucosinolate segregants through selection. The F_1 generation was intermediate in hydroxybenzyl glucosinolate content. The fatty acid composition of zero erucic acid *S. alba* was characterized by low levels of linoleic acid and high levels of linolenic acid. The oil content of *S. alba* was approximately 30% under Saskatchewan growing conditions which is too low for a vegetable oilseed crop. Research is underway at Saskatoon to increase oil content of *S. alba* seed.

MODIFICATION OF BRASSICA OIL FATTY ACID COMPOSITION

Zero erucic acid *B. carinata* has recently been developed and these lines had significantly higher linoleic and linolenic acid contents than standard zero erucic acid *B. napus* and *B. rapa* (Table 4). The total amount of linoleic and linolenic acid in zero erucic acid *B. carinata* was >70% of total fatty acids. This indicated very strong desaturation activity in *B. carinata*. Raney et al. (1995) crossed zero erucic acid *B. carinata* with low linolenic acid *B. napus*. They backcrossed the interspecific F_1 generation to both, *B. carinata* and *B. napus*. The objective of the *B. carinata* backcross was to develop zero erucic, low linolenic acid *B. carinata* while the backcross to *B.napus* was aimed at developing high linoleic-low linolenic *B. napus* with a sunflower oil-like fatty acid composition.

The backcross to *B. carinata* yielded plants in the BC_1F_5 generation (selected for high oleic acid) that had a *B. napus* like fatty acid composition. If these selections prove to be genetically stable, it might be possible to develop *B. carinata* with a canola quality type oil composition, an important step towards the development of *B. carinata* as an oilseed crop. The backcross to *B. napus* yielded lines with significantly increased linoleic acid contents while maintaining relatively low linolenic acid contents.

TABLE 4. Fatty acid composition of *Brassica carinata* and B. *napus* lines derived from interspecific backcrosses

	Fatty acid composition (% of total)					
	Genotype	16:0	18:0	18:1	18:2	18:320:1
Parents						
B. carinata	5.7	1.2	18.6	37.6	34.7	1.0
B. napus	3.7	2.0	65.6	23.2	2.2	1.4
Backcrosses to *B. carinata*						
BC_1F_5 high 18:1	4.5	3.4	60.2	24.1	4.4	1.7
BC_1F_5 high 18:2	5.0	1.5	26.5	51.5	12.9	1.2
B. napus						
BC_1F_5	7.1	1.3	43.8	38.6	6.8	1.0

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