Present status and future perspectives of breeding for seed quality in *Brassica* oilseed crops

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ABSTRACT

This review will discuss the present status and future perspectives of breeding for seed quality in *Brassica* oilseed crops. Rapeseed (canola) oil has an almost perfect fatty acid composition as a salad oil, high in oleic acid and essential polyunsaturated fatty acids. Further oil quality improvements include the development of very high oleic acid/low linolenic acid varieties for use in frying applications, and the creation of low and very low (zero) saturated fat oils for certain markets. Meal quality improvements will focus on fibre reductions through the creation of yellow seeded *B. napus* forms, especially reduction of lignin contents. Reduction of glucosinolate contents or their complete elimination is a further breeding goal. New *Brassica* oilseed crops, such as *B. juncea*, and *B. carinata*, are under development in various countries, leading to increased production and diversification of *Brassica* oilseeds worldwide. Each species has specific seed oil and meal quality challenges that need to be addressed, including modification of fatty acid compositions to improve oil quality. Complete elimination of allyl glucosinolate is a major objective for *B. juncea* and *B. carinata*. Many of these improvements will result from the interspecific transfer of the respective genes from *B. napus* and *B. rapa*. Progress made towards the various quality breeding objectives will be discussed and future research challenges highlighted.

Key words: *Brassica* oilseeds – seed quality – oil and meal

INTRODUCTION

Rapeseed (*Brassica napus*) became a major oilseed crop in Canada, Europe and Australia when quality varieties, low in erucic acid and glucosinolate content were developed and introduced into commercial production (Downey and Rakow, 1987). Quality improvements in both the oil and meal portions of the seed were key factors in the success of rapeseed as a new, high quality, edible oil and high quality animal protein meal. Future growth and possible market expansion of *Brassica* oilseeds will very much depend on our ability to further increase oil and meal quality of the dominant *B. napus* species to make it more competitive with other oilseeds; and to establish "canola-quality" types in *B. juncea* and *B. carinata* to provide better adapted high quality *Brassica* oilseed production platforms for areas with semiarid climates which are not well suited for the production of *B. napus* canola. The objective of this review is to provide an overview of the present status of seed, oil and meal quality breeding research in *Brassica* oilseeds, and to outline future quality breeding needs.

RESULTS AND DISCUSSION

A. <u>Fatty acid composition of zero erucic acid material</u>

The fatty acid composition of the zero erucic acid commercial Canadian *Brassica napus* crop in 2002 was typical for this species and similar to what was observed in the past over many years (Table 1). *Brassica napus* canola oil has high concentrations of oleic acid (about 60%), and contains moderate levels of linoleic (about 20%) and linolenic acid (about 10%). This fatty acid composition of a vegetable oil is considered ideal by many nutritionists for human nutrition, and superior to that of many other plant oils. Rapeseed oil also has the lowest saturated fatty acid content of any vegetable oil of about 7% of total fatty acids (total of C12:0 + C14:0 + C16:0 + C18:0 + C20:0 + C22:0 + C24:0), whereby palmitic acid (C16:0), with about 4% and stearic acid (C18:0) with about 2% of total fatty acids, are the major saturated fatty acids in rapeseed oil. A low saturated fat content of less than 7% is critical for labelling rapeseed oil as a low saturated fat product in the USA, the major customer of Canadian rapeseed oil.

Table 1: Fatty acid composition of *Brassica* oilseeds

Species	Variety and line	Fatty acid composition (% of total)							
		C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C22:1	Total Sat.
B. napus	Canada com.[1]	3.9	1.9	60.6	19.1	10.6	1.4	0.1	7.0
	AC Excel[2]	3.6	2.0	65.1	16.6	9.2	1.3	0.1	6.8
B. rapa	AC Parkland[2]	3.1	1.6	61.0	20.4	11.1	1.1	0.1	5.6
B. juncea	J90-4253[3]	4.3	2.1	43.6	34.6	11.9	1.1	0.1	7.6
B. carinata	BC_4F_2[4]	6.0	1.7	28.3	38.1	22.9	1.1	0.1	8.4
Modified lines									
B. napus	S86-69[4]	4.0	2.0	63.6	25.0	1.8	1.2	0.0	7.0
	M453[7]	3.8	1.2	72.6	7.7	10.1	1.6	0.1	6.1
	$F_5$5314-22[5]	3.9	2.0	77.8	8.7	2.8	1.4	0.1	7.4
	TO97-2268[5]	3.4	1.3	58.5	25.1	7.7	1.5	0.3	5.7
	$M_4$5906-6[5]	2.4	1.1	55.0	29.3	8.5	1.8	0.1	4.3
	$M_4$5908-31[5]	3.1	1.2	67.7	16.7	7.8	1.5	0.0	5.3
B. juncea	J00-6717[6]	3.4	2.8	63.8	16.0	10.6	1.2	0.0	7.2

1= Canada commerical *B. napus* canola crop 2002, Canadian Grain Commission harvest survey; 2= Saskatoon 1997; 3=Saskatoon 1999, 4=Saskatoon 1998, 5=Saskatoon 2001, 6=Saskatoon 2000, 7=Saskatoon greenhouse

Brassica rapa, which at some time in the past was grown on about one half or more of the Canadian rapeseed acreage, has a somewhat different fatty acid composition in comparison to *B. napus* (Table 1). It has lower oleic and higher linoleic and linolenic acid contents. However, *B. rapa* has a signficantly lower saturated fat content of about 1.0 to 1.5% than *B. napus*. *Brassica juncea* is a potential oilseed crop for the semiarid Canadian prairies and many other countries of the world, such as China and India, and others, because of its better adaptation to dry and hot climates compared to *B. napus*. Zero erucic acid *B. juncea* germplasm was first developed in Australia (Kirk and Oram, 1981), but the oil of zero erucic acid cultivars and lines is low in oleic acid (about 45%) and high in linoleic acid (about 35%) in comparison to *B. napus* and *B. rapa* oil; it also has elevated saturated fat contents of 7.5% (Table 1). We are also interested in the development of *B. carinata* as an edible oilseed crop for dry areas, and produced zero erucic acid lines from interspecific crosses between normal erucic acid *B. carinata* and zero erucic acid *B. juncea*, followed by four backcrosses to *B. carinata* in combination with selection for zero erucic acid segregates after each backcross (Getinet *et al*. 1994). Zero erucic acid *B. carinata* has even lower oleic acid contents of about 30%, only half of that found in zero erucic acid *B. napus* cultivars. Its linoleic acid content is higher than that of *B. juncea* and its linolenic acid content is twice that of *B. napus* and *B. rapa* (Table 1).

B. Modification of fatty acid contents

Even though the fatty acid composition of *B. napus* is ideal from a nutritional standpoint, there are segments of the vegetable oilseed market that require a more stable oil for high temperature applications such as frying. The first objective was to develop low linolenic acid lines with less than 3% linolenic acid, which was achieved through mutation. The *B. napus* line S86-69 from the University of Manitoba is such an example which we used widely as a parent in *B. napus* breeding projects aimed at reducing linolenic acid. The second germplasm sources was a high oleic/low linoleic winter rape mutant M453 from the University of Göttingen in Germany. Crosses between these two lines resulted in high oleic/low linolenic acid lines such as the F_5 inbred 5314-22 with 77.8% oleic acid, 8.7% linoleic acid and 2.8% linolenic acid. The crossing strategy was to combine the low linolenic acid trait of S86-69 with the low linoleic acid trait of M453 and thus creating high oleic/low linolenic acid lines which was successful. Many breeding groups have developed similar quality lines utilizing mutagenesis, the leading firms in this area in Canada are Dow AgroSciences and Cargill. Several patents exist which protect various types of fatty acid profiles of oleic and linolenic acid (linoleic acid), and in certain cases in combinations with claims for low saturated fat content. Patent protection of plant lines is possible in the USA, but not in Canada. Patenting hinders progress in this area in that it restricts the free flow of germplasm for further improvements of rapeseed oil quality. It is expected that about one half or more of the total rapeseed acreage in Canada could be planted to high oleic/low linolenic acid cultivars in the future.

The reduction of saturated fat levels in *B. napus* was, until recently, a major research objective of the Canola Council of Canada, and was financially supported by research grants. We successfully developed low saturated fat *B. napus* from interspecific crosses with *B. rapa* (line TO97-2268, Table 1). We then used TO97-2268 for mutation induction and generated a number of mutants with further reduced saturated fat contents. The M_4 line 5906-6 had a total saturated fat content of only 4.3% in combination with reduced oleic and elevated linoleic acid contents. Another example is the M_4 line 5908-31 with a total saturated fat content of 5.3%, but with high oleic (67.7%) and low linoleic acid (16.7%) contents. It might be possible, through intercrossing of mutant lines, to further lower saturated fat contents in *B. napus* and potentially develop "zero" (<3.5%) saturated fat lines if there is a market need for such types.

Industry consultations during the development of *B. juncea* as a potentially new edible oilseed species for Canada concluded that the "wild-type" fatty acid composition of zero erucic acid *B. juncea* with only about 45% oleic acid and about 35% linoleic acid was unacceptable for a "canola-quality" oil. An increase in oleic acid to a minimum level of 55% (an increase by at least 10%) with a corresponding reduction in linoleic acid would be required to make the oil of *B. juncea* acceptable to Canadian oilseed crushers. We attempted this modification through an interspecific cross with the low linolenic acid line S86-69, followed by backcrosses to *B. juncea* in combination with reselection of plants with *B. napus*-like fatty acid composition before carrying out the next backcross. Five backcrosses to *B. juncea* were required to establish true breeding *B. juncea* plants with 36 chromosomes and normal meiotic behaviour (18 bivalents) which expressed the typical fatty acid profile of *B. napus*. The BC_5F_5 line J00-6717 is an example of this research containing 63.8% oleic, 16.0% linoleic and 10.6% linolenic acid in its seed oil (Table 1). This line had a total saturated fat content of 7.2%

C. Glucosinolate content of *Brassica napus* and *B. rapa*

The average total glucosinolate content of the 2002 Canadian *B. napus* rapeseed crop was 12 µmoles/g seed at 8.5% moisture level (ISO 9167-1: 1993, Canadian Grain Commission, Winnipeg, MB), making Canadian rapeseed a good source of high quality, high protein animal feed. Total glucosinolate content in *B. napus* seed consists of about 60% alkenyl glucosinolates and 40% indolyl glucosinolates (Table 2). The dominating alkenyl glucosinolate is 2-hydroxy-3-butenyl glucosinolate. This indicates strong hydroxylation capacity in *B. napus*. *Brassica rapa*, in contrast, has significant chain elongation activity from 3-butenyl to 4-pentenyl glucosinolate resulting in about equal amounts for these two glucosinolates in its seed. In addition, *B. rapa* exhibits also glucosinolate hydroxylation, and its indole glucosinolate content is similar to that of *B. napus* (Table 2).

Table 2: Glucosinolate content of *Brassica* oilseeds

Species	Variety and line	µmoles/g seed dry							
		All	But	Pent	HoBu	HoPe	Tot A	Tot I	Tot G
B. napus	AC Excel[1]	–	2.4	0.6	5.5	0.1	8.7	5.3	14.1
B. rapa	AC Parkland[1]	–	2.7	2.3	5.7	0.8	11.5	5.5	18.0
B. napus	TO97-3233[1]	–	0.2	0.0	0.3	0.0	0.5	3.6	4.1
	J01-1761[2]	–	0.0	0.0	0.1	0.0	0.1	1.1	1.2
B. juncea	AC Vulcan[3]	105.0	3.7	0.0	0.1	0.1	108.9	0.6	109.5
	J90-4316[3]	0.8	14.7	1.4	1.2	0.0	18.1	3.6	21.7
	J00-6866[3]	0.1	1.9	0.1	0.1	0.0	2.2	3.3	5.5

1=Saskatoon 1999, 4-replicate yield test; 2=Saskatoon 2001, 4-replicate yield test, 3=Saskatoon 2001, 2-replicate nursery

D. Elimilation of glucosinolate from *Brassica napus* seed

It has been demonstrated that further reduction of glucosinolates in rapeseed or their complete elimination will further increase the feed value of rapeseed meal. "Zero" (<1.0µmole/g seed) aliphatic glucosinolate lines of *B. napus* were identified in the F_3 generation of the double interspecific cross between yellow-seeded *B. napus* with *B. rapa* AC Parkland (yellow-seeded) and *B. alboglabra* (yellow-brown seeded). The zero alkenyl glucosinolate lines was then crossed with yellow-seeded, low linolenic acid *B. napus* and the cross pedigree selected. The F_6 line TO97-3233 was field evaluated in a yield test at Saskatoon in 1999 and had a total aliphatic glucosinolate content of 0.5 µmoles/g seed, 3.6 µmoles/g seed indole glucosinolates for a total glucosinolate content of 4.1 µmoles/g seed (Table 2). The seed was yellow-brown in colour and had a linolenic acid content of about 3% (data not shown).

The next step was a further reduction of indole glucosinolate contents to lowest possible concentrations. A "zero" (~1.0 µmoles/g seed) indole glucosinolate *B. rapa* selection, based on yellow sarson crosses (Ph.D. thesis of Dr. Paul Dribnenki, Saskatoon), was crossed with the "zero" aliphatic glucosinolate *B. napus* line to introgress the low indole glucosinolate trait from *B. rapa* into *B. napus*. Two backcrosses were made to *B. napus* to reconstitute a true breeding *B. napus* plant. The BC_2F_9 line J01-1761 was field evaluated at Saskatoon in a 2-replicate row nursery in 2001 and had a total aliphatic glucosinolate content of 0.1 µmoles/g seed, 1.1µmoles of indole glucosinoaltes for a total glucosinolate content of 1.2 µmoles/g seed.

E. Reducing glucosinolate content in *Brassica juncea*

Standard condiment mustard cultivars such as AC Vulcan contain high concentrations of allyl glucosinolate in their seed (Table 2). The first step in meal quality improvement was the elimination of allyl glucosinolate from *B. juncea* seed (Love *et al*. 1990). The interspecific F_4 line J90-4316 contained less than 1.0 µmoles of allyl glucosinolate, the 3-butenyl glucosinolate content was about 15 µmoles/g seed. The line contained low concentrations of indole glucosinolate, and had a total glucosinolate content of about 22 µmoles/g seed.

Progeny from crosses and backcrosses with *B. napus* described above, which led to the isolation of *B. juncea* lines with *B. napus* fatty acid composition (line J00-6717, Table 1), were also selected for further reduced glucosinolate content. These selections resulted in the isolation of very low alkenyl and total glucosinolate content lines. Line J00-6866 contained only 2.2 µmoles/g seed of alkenyl glucosinolates, 3.3 µmoles/g seed of indole glucosinolates for a total glucosinolate content of 5.5 µmoles/g seed.

Table 3: Fibre contents (% dry meal basis) and % reductions in meal from yellow seeds in comparison to meal from black/brown seeds in *Brassica napus*, *B. rapa* and *B. juncea*

		ADF[1]		ADL[2]		NDF[3]		CEL[4]		HCE[5]	
Species & Cultivar	Colour	% act.	% red.	% act.	% red.	% act.	% red.	% act.	% red.	% act.	% red.
B. napus											
AC Excel	black	20.2		7.9		26.2		12.3		5.7	
YN93-1016	yellow	11.2	45	1.9	76	19.2	26	9.3	24	8.0	–
B. rapa											
Echo	brown	21.3		8.9		27.3		12.4		6.1	
yell. sarson	yellow	12.8	40	1.1	88	21.1	23	11.7	6	7.6	–
B. juncea											
Com. brown	brown	16.0		4.7		25.5		11.2		9.2	
Domo	yellow	11.2	30	0.9	81	21.5	16	10.2	9	9.8	–

1= acid detergent fibre; 2= acid detergent lignin; 3=natural detergent fibre; 4=cellulose, ADF-ADL, 5=hemicellulose, NDF-ADL

F. Improving meal quality through fibre reduction

The high fibre content in rapeseed meal limits inclusion rates for rapeseed meal into high protein animal feed, especially for pigs and poultry. The high fibre content is a result of the small seed size of rapeseed compared to soybean. It has been known for some time that meal from yellow-seeded lines has much lower meal fibre contents than meal from black-seeded lines due to their thinner seed coat. The yellow seed trait is well established in *B. rapa*, *B. juncea* and *B. carinata*, while no yellow-seeded forms exist in natural populations of *B. napus*. However, true breeding yellow-seeded forms of *B. napus* have now been developed through interspecific crosses with related yellow-seeded *Brassica* species. The productivity (yield), disease resistance and seed quality (oil and protein content) of new, yellow-seeded, "canola-quality" lines of the AAFC Saskatoon Research Centre is at least comparable, and in certain aspects, superior, to that of standard black-seeded *B. napus* cultivars.

The acid deterent digestion method was used to measure fibre contents in black/brown versus yellow-seeded lines (Table 3). The acid detergent fibre content of meal from black-seeded *B. napus* seed was 20.2% on a dry meal basis, as compared to 11.2% for meal from the yellow-seeded line, a reduction of 45%. The greatest reduction was observed in acid detergent lignin which was reduced from 7.9% to 1.9%, a reduction of 76%. There was also a reduction in neutral detergent fibre and cellulose contents while hemicellulose contents were actually higher in meal from yellow seeds. Similar observations were made in seed meals from brown versus yellow-seeded *B. rapa* and *B. juncea*.

FUTURE PROSPECTS

The focus in *B. napus* quality breeding at AAFC Saskatoon will be on the development of agronomically improved, blackleg disease resistant, yellow-seeded *B. napus* cultivars, adapted to production on the western Canadian dry prairie. We will develop yellow-seeded cultivars of *B. napus* with standard fatty acid composition, as well as fatty acid modified low linolenic acid cultivars and high oleic/low linolenic acid cultivars. The low saturated fat content of 4.5 to 5.0% will also be incorporated into all *B. napus* cultivar types. The major objective of this reseach is to increase rapeseed meal quality as a high protein animal feed. The commercial production of these different quality types will require segregated production systems to meet customer needs. The possible incorporation of herbicide tolerance into yellow-seeded *B. napus* cultivars is under discussion, as is the development of hybrid cultivars. We believe that we have all the germplasm that is needed to initiate an aggressive commercial breeding program to achieve these goals. In a few years, the Canadian *B. napus* rapeseed crop will be yellow-seeded.

The *B. juncea* oilseed crop in Canada will be yellow-seeded because of the availabilty of yellow-seeded forms in this species contributing to the improvement of the feeding value of the meal. Present quality research focusses on the combination of the *B. napus*-type fatty acid composition with very low (or "zero) total glucosinolate content to further improve both the quality of the oil and the meal. The need for herbicide tolerance in *B. juncea* oilseed is being discussed, and hybrid cultivar development is in progress. As in *B. napus*, we have all the needed germplasm to start a commercial breeding project. *Brassica juncea* oilseed could potentially occupy a significant acreage of the Canadian rapeseed crop. The first two oilseed *B. juncea* cultivars, Arid and Amulet, developed in the collaboration with the Saskatchewan Wheat Pool, have been registered for contract production last year.

Quality research in *B. carinata* has progressed to the zero erucic acid stage, and some lines with reduced glucosinolate content have been developed. However, there is a lot of work to do to modify fatty acid composition and to completely eliminate allyl glucosinolate from the seed, which is a prerequisite for its acceptance as an animal feed by the industry. *Sinapis alba* quality research has produced zero erucic acid, zero hydroxy-benzyl and zero benzyl glucosinolate populations, and the prospects for its development as a highly drought and heat tolerant oilseed species with excellent disease and insect resistances are promising.

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REFERENCES

Downey, R.K. and G. Rakow, 1987: Rapeseed and mustard. In: W.R. Fehr (editor), Principles of cultivar development, Vol. 2, Crop species, Macmillan Publishing Company, New York, pages 437-486.

Kirk, J.T.O and R.N. Oram, 1981: Isolation of erucic acid-free lines of *Brassica juncea*: Indian mustard now a potential crop in Australia. J. Aust. Inst. Agric. Sci. 47: 51-52.

Getinet, A., G. Rakow, J.P. Raney and R.K. Downey, 1994: Development of zero erucic acid Ethiopian mustard through an interspecific cross with zero erucic acid Oriental mustard. Can. J. Plant Sci. 74: 793-795.

Love, H.K., G. Rakow, J.P. Raney and R.K. Downy, 1990: Development of low glucosinolate mustard. Can. J. Plant Sci. 70: 419-424.