RAPESEED GENETIC RESEARCH TO IMPROVE ITS AGRONOMIC PERFORMANCE AND SEED QUALITY

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Abstract

Rapeseed (canola) is the major Canadian oilseed and average annual production was 6,871,600 metric tonnes for the 10 year period 1996-2005. *Brassica napus* is the only species grown, and only summer annual forms are cultivated in the short season areas of the western Canadian prairie. About 70% of the total production is exported, either as seed (50%) or oil (20%). We utilized inter-specific crosses between *B. napus* and related species to improve the disease resistance and seed quality of *B. napus*. We produced yellow-seed forms of *B. napus* from crosses with *B. rapa* and *B. juncea* which have higher seed oil content, and lower meal fibre content to improve the feed value of the meal. We also produced germplasm with high oleic/low linolenic acid content to improve the nutritional value of canola oil as well as its technological qualities for use in the production of solid fats without trans fatty acids. The content of saturated fats in canola oil was reduced to less than 5% of total fatty acids. Inter-specific methodology was also successful in the development of *B. juncea* mustard as an edible oilseed crop with high yield, disease resistance and seed quality for production in the semiarid regions of the Canadian prairie. This paper will describe the crossing approaches used to develop this germplasm and discuss future research activities for canola improvement.

Introduction

Oilseed rape (*Brassica napus*) is the major oilseed crop in Canada and was grown on 4,629,300 ha annually over the 10-year period 1996-2005 (Canada Grains Council, 2005) resulting in a average annual production of 6,871,600 metric tonnes for this 10-year period. The major areas of canola production are in western Canada where 99% of total production occurs. For the 10-year period 1994-2003, about one half of seed produced was exported as seed, 52.3% of all seed exports went to Japan. About one half of the total crop is crushed in Canada. For 2002-03 canola, about 85% of oil and 99.5% of meal was exported to the USA. Canola oil is favoured over other vegetable oil in the US because of its low saturated fat content of less than 7% of total fatty acids which allows it to be marketed as a low saturated fat oil. Canola meal is the preferred protein source for dairy cattle feed in California.

Breeding and genetic research at AAFC Saskatoon focuses on quality improvements of the *B. napus* canola crop (both the oil and meal), and on diversification of production through the development of canola quality types in *B. juncea* mustard (Rakow and Raney, 2003). The genomic relationships between species in the genus *Brassica* allow for interspecific transfer of quality traits between species, and the results of this work will be reviewed in this paper.

Materials and Methods

Plant Materials. Interspecific crosses between plants of different species were performed on greenhouse grown plants through emasculation of flowers at the bud stage and pollination of stigmas with fresh pollen from the male parent. We used standard "bud pollination techniques," developing interspecific embryos were allowed to mature on the female parent, no embryo rescue techniques were applied (Rashid et al. 1994). Interspecific hybrid plants were grown and backcrossed to the target species to produce and select plants with the desired introgressed quality traits. Several backcrosses had to be made to recover a true breeding line that exhibited the new trait in a genetically stable fashion. The agronomic performance and seed quality attributes of newly developed lines was evaluated in field nurseries and replicated yield tests at the AAFC Research Farm at Saskatoon, Canada.

Seed Chemical Analyses. The fatty acid composition of the seed oil was determined by the method of Thies (1971), except that gas chromatography of the methyl esters was performed with a supelcowax 10 fused silica capillary column at 210^{o}C using hydrogen as the carrier gas. The glucosinolate content of seed meal was determined by the gas chromatographic method of Thies (1980). Glucosinolates were extracted with 65% methanol, trimethylsilylation was carried out according to Landerouin et al. (1987) and gas chromatography of trimethylsilyl derivatives was on J & W DB-1 fused silica capillary column (Sosulski et al. 1984) at 280^{o}C using hydrogen as a carrier gas.

Results and Discussion

Development of yellow-seeded *Brassica napus*

The high fibre content of canola meal limits inclusion rates for canola meal into high protein animal feed, especially for pigs and poultry. The high fibre content is a result of the small seed size of canola 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 (Table 1).

The acid deterent digestion method was used to measure fibre contents in black versus yellow-seeded lines. The acid detergent fibre content of meal from black-seeded *B. napus* seed was 13.6% on a dry meal basis, as compared to 8.2% for meal from the yellow-seeded line, a reduction of 40%. The greatest reduction was observed in acid detergent lignin which was reduced from 5.4% to 1.3%, 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*.

Table 1: Agronomic and quality performance of yellow – and black-seeded *Brassica napus* canola

Lines	Yield	Oil	Protein	GSL	Fibre (%dry meal)		
	(kg/ha)	(%)	(%meal)	(µmol/g)	ADL	ADF	NDF
YN01-429	2030	49.1	46.9	12.9	1.3	8.2	14.9
46A65/Q2	1890	45.8	49.0	17.7	5.4	13.6	19.1
Diff							
(Y-B)	+140	+3.3	-2.1	-4.8	-4.1	-5.4	-4.2
Sig (p)	ns	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01
Station yrs	6	6	5	4	4	4	4

Oil by NMR, Protein by Leco combustion, GSL= total glucosinolates, by gas chromatography, Fibre by Ankom 200 Fibre Analyzer, ADL = acid detergent lignin, ADF = acid detergent fibre (cellulose + lignin), NDF = neutral detergent fibre (hemicellulose + cellulose + lignin + pectin).

Fatty acid composition of *Brassica* oilseeds

The fatty acid composition of the commercial Canadian *Brassica napus* canola crop in 2002 was typical for this species and similar to what was observed in the past over many years (Table 2). *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. Canola oil also has the lowest saturated fatty acid content of any vegetable oil of about 7% of total fatty acids A low saturated fat content of less than 7% is critical for labelling canola oil as a low saturated fat product in the USA, the major customer of Canadian canola oil.

Table 2: 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	Total Sat.
B. napus	Canada com.	3.9	1.9	60.6	19.1	10.6	7.0
	AC Excel	3.6	2.0	65.1	16.6	9.2	6.8
B. juncea	J90-4253	4.3	2.1	43.6	34.6	11.9	7.6
Modified lines							
B. napus	5314-22	3.9	2.0	77.8	8.7	2.8	7.4
	5906-6	2.4	1.1	55.0	29.3	8.5	4.3
	5908-31	3.1	1.2	67.7	16.7	7.8	5.3
B. juncea	J00-6717	3.4	2.8	63.8	16.0	10.6	7.2

Canada com.= Canada commerical *B. napus* canola crop 2002, Canadian Grain Commission harvest survey; AC Excel and experimental lines=Saskatoon Field Tests.

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*. 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* oil; it also has elevated saturated fat contents of 7.5%.

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 high in oleic and low in linolenic acid, for high temperature applications such as frying. We developed a novel *B. napus* line with >75% oleic acid and <3% linolenic acid from crosses of mutant lines. 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. Patent protection of plant lines hinders progress in this area in that it restricts the free flow of germplasm for further improvements of canola oil qualty. It is expected that about one half or more of the total canola acreage in Canada could be planted to high oleic/low linolenic acid cultivars in the future.

We successfully developed low saturated fat *B. napus* from interspecific crosses with *B. rapa* and through mutant induction. Line 5906-6 had a total saturated fat content of only 4.3% in combination with reduced oleic and elevated linoleic acid contents. Line 5908-31 had a total saturated fat content of 5.3%, but with high oleic (67.7%) and low linoleic acid (16.7%) contents.

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 *B. napus*, 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 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 2). This line had a total saturated fat content of 7.2%

Glucosinolate content of *Brassica* oilseeds

The average total glucosinolate content of the 2005 Canadian *B. napus* canola crop was 9 µmoles/g seed at 8.5% moisture level, making Canadian canola 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 3). The dominating alkenyl glucosinolate is 2-hydroxy-3-butenyl glucosinolate. This indicates strong hydroxylation capacity in *B. napus*.

Table 3: Glucosinolate content of *Brassica* oilseeds

Species	Variety and line	µmoles/g seed dry					
		All	But	Pent	HoBu	HoPe	Tot I
B. napus	AC Excel	–	2.4	0.6	5.5	0.1	5.3
B. napus	TO97-3233	–	0.2	0.0	0.3	0.0	3.6
	N01-1761	–	0.0	0.0	0.1	0.0	1.1
B. juncea	AC Vulcan	105.0	3.7	0.0	0.1	0.1	0.6
	J90-4316	0.8	14.7	1.4	1.2	0.0	3.6
	J00-6866	0.1	1.9	0.1	0.1	0.0	3.3

Variety and experimental lines Saskatoon field tests.

It has been demonstrated that further reduction of glucosinolates in canola or their complete elimination will further increase the feed value of canola meal. "Zero" (<1.0µmole/g seed) aliphatic glucosinolate lines of *B. napus* were identified in segregating generations 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 were then crossed with yellow-seeded, low linolenic acid *B. napus* and the cross pedigree selected. The 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. 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, 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 line N01-1761 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.

Standard condiment mustard cultivars such as AC Vulcan contain high concentrations of allyl glucosinolate in their seed (Table 3). The first step in meal quality improvement was the elimination of allyl glucosinolate from *B. juncea* seed (Love *et al*. 1990). The interspecific derived 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 2), 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.

Conclusions

Our goal is to develop true breeding, yellow-seeded cultivars of *B. napus* that are high yielding, resistant to diseases and have high oil and protein contents, and to convert the total Canadian canola acreage to yellow-seeded cultivars. Yellow-seeded cultivars will have an inherently higher seed oil content of 1 to 2% over black-seeded cultivars. The introduction of yellow-seeded cultivars will require identity preserved production of guarantee improved meal quality to the feed industry. Further reductions or the complete elimination of glucosinolates from canola seed will further improve the feed value of canola meal.

There is an increasing demand, in Canada and the USA, for temperature stable vegetable oils which will require the breeding of competitive high yielding cultivars with high oleic-low linolenic acid contents. Such oil is of great importance for the production of solid fats in that they require little or no hydrogenation thus significantly reducing or completely eliminating the formation of trans fatty acids. About one half of the total Canadian canola acreage could be planted to these cultivars in the future to meet market demand. For North America (USA) the breeding of cultivars with low contents of saturated fats is of importance. The total saturated fat content must be <7% to label products as "low in saturated fat." The classification as "zero saturated fat" is possible for products containing <3.5% saturated fatty acids.

The establishment of *B. juncea* canola will diversify canola production on the Canadian prairie, potentially increasing production and contribute to more sustainable oilseed production in Canada.

References

Canada Grains Council 2005. Field Crop Reporting Series, Catalogue 22-002, Statistics Canada.

Landerouion, A., Quinsac, A. and Ribaillier 1987. Optimization of silylation reactions to desulpho – glucosinolates before gas chromatography. World Crops: Production, Utilization, Description. Chapter 13: 26-37.

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

Rakow, G. and J.P. Raney, 2003. Present status and future perspectives of breeding for seed quality in *Brassica* oilseed crops. Proc. 11th Int. Rapeseed Cong., Copenhagen, Denmark. Vol. 1: pages 181-185.

Rashid, A., Rakow, G. and R.K. Downey 1994. Development of yellow-seeded *Brassica napus* through interspecific crosses. Plant Breeding 112: 127-134.

Sosulski, F.W. and K.J. Dabrowski 1984. Determination of glucosinolates in canola meal and protein products by desulfation and capillary gas liquid chromatography. J. Agr. and Food Chem. 32: 1172-1174.

Thies, W. 1971. Schnelle und einfache Analysen der Fettsäurezusammensetzung in einzelnen Raps-Kotyledonen. Z. Pflanzenzüchtg. 65: 181-202.

Thies, W. 1980. Analysis of glucosinolates via "on-column" desulfation. Proc. Symp. "Analytical Chemistry of Rapeseed and its Products." Winnipeg, Canada, pp. 66-71.