

## BREEDING FOR SPECIAL OIL QUALITY IN CANOLA/RAPSEED

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INTRODUCTION

The ability to manipulate the fatty acid composition of rapeseed (Brassica napus and B. campestris) through plant breeding was demonstrated with the dramatic reduction in erucic acid achieved in the 1970's. This change resulted in the creation of a new vegetable oil, canola, which now accounts for 60% of all vegetable oil products made in Canada. With increasing competition in the world oil market, the challenge is to maintain the position of canola in the top five of the world's vegetable oils, and to maintain Canada's position as a top exporter of quality canola seed and products. In order to do this, plant breeders, in consultation with nutritionists and processors, have to determine their breeding priorities to meet the market demands in the 1990's and beyond.

Since its inception in the 1950's, the breeding program at the University of Manitoba has focused on the improvement of quality in oilseed rape cultivars. The goal is the development of a product which is relatively inexpensive to process, free from contaminants and anti-nutritional factors, and with a favourable fatty acid composition. The definition of this last component depends on the end use of the oil. As new markets develop, cultivars can be developed which produce oils which meet specific nutritional, processing and industrial requirements. Our breeding program is developing canola cultivars with low levels of linolenic acid in the seed oil for the vegetable oil market and rapeseed cultivars with very high levels of erucic acid in the seed oil for the industrial oil market.

RESULTS AND DISCUSSIONDevelopment of high linoleic/low linolenic canola

The high linolenic acid content in canola is a source of oxidative rancidity and loss of flavour stability during storage. In some food applications such as industrial frying, the level of linolenic acid has to be less than 3%. This level can be achieved by partial hydrogenation, reducing one of the double bonds from C18:3 (linolenic acid) to C18:2 (linoleic acid). This is an expensive process and the formation of trans fatty acids raises some nutritional concerns. The alternative is to breed canola cultivars which have a reduced level of linolenic acid. It is well known that some plant families include species that do not synthesize linolenic acid but only linoleic acid in the oil. Researchers screened the available rapeseed germplasm for the genetic block between linoleic acid and linolenic acid. The necessary variation was not available. Therefore mutation experiments were initiated at the Institute for Plant Breeding, University of Göttingen in West Germany, to block the desaturation pathway between linoleic and linolenic acid. The chemical mutagen ethyl methanesulfonate (EMS), was used to treat seeds of the Canadian spring rapeseed cultivar, Oro, followed by selection of plants with an altered linoleic-linolenic fatty acid ratio in the seed oil (Rakow, 1973). Of particular interest was the mutation line M11 which had seed oil with a high level of linolenic acid but an altered ratio of linolenic to linoleic acid (Robbelen et al. 1975).

The M11 line was the source of variation used in the University of Manitoba low linolenic breeding program. There was evidence of deleterious effects from the mutation treatment as the plants of the M11 line were reduced in height and vigour and had a reduced seed set. After three years of selection within the M11 line for single plants accumulating seed oils with reduced linolenic and increased linoleic acid levels, lines with seed oil containing 3% linolenic and 28% linoleic acid were obtained. These low linolenic-high linoleic lines were crossed with the canola cultivar Regent, followed by further selection for oil quality and agronomic characteristics (Scarath et al. 1988). Line S81-2716 was a single plant selection in 1981, and was tested for quality and agronomic characteristics in the western Canadian Co-operative testing system for three years in 1983 to 1986. The excellent stability of the low linolenic character has been demonstrated under different environments, as shown over a five year period of yield testing and seed increase 1981 to 1986 (Table 1).

Table 1. Distribution of the C18 fatty acids: C18:1 (oleic), C18:2 (linoleic) and C18:3 (linolenic) over five years and three generations in the seed oil of the low linolenic strain S81-2716 (Stellar).

Year	Seed Source of S81-2716	Fatty Acid Composition (%)		
		C18:1	C18:2	C18:3
1981	Single Plant	65	24	2.4
1982	Nursery Row	65	24	2.1
1984	Increase	67	22	1.8
1985	Yield Trial	66	24	2.5
1986	Yield Trial	64	25	2.4

The oil has a very distinct fatty acid composition with the desired combination of low levels of linolenic acid combined with high linoleic acid content (Table 2). It was of great interest to test whether the reduction in the level of linolenic acid in the new canola cultivar Stellar improved the processing quality of the oil.

Table 2. Comparison of the fatty acid composition of the seed oil of the canola cultivar Westar and the low linolenic cultivar Stellar, analysed at the Grain Research Laboratory, Winnipeg, Canada.

Fatty Acid		% of Westar Oil	% of Stellar Oil
Palmitic	16:0	3.6	4.1
Stearic	18:0	1.6	1.4
Oleic	18:1	57.1	59.1
Linoleic	18:2	20.8	28.9
Linolenic	18:3	11.5	3.3
Erucic	22:1	0.5	0.1
Others	-	4.9	3.1

A pilot plant performance evaluation of the oil was commissioned by the Canola Council of Canada at the POS plant in Saskatoon. The processing characteristics of the low linolenic oil showed a significant improvement over canola oil. Hydrogenation time was significantly reduced. For use as a salad oil, the low linolenic oil was evaluated by the Active Oxidation Method (AOM) and showed improved stability over Westar canola oil. As a liquid frying oil, the low linolenic oil had an increased stability of 87 AOM hours compared to 74 AOM hours for Westar and 24 AOM hours for soybean. The fry life of the two canola cultivars as the same, 240 hours compared to

168 hours for the soybean oil control (POS Technical Research Report No. 85-735).

The new oil thus showed the anticipated improvements which could potentially set a new standard for canola oil. The results justified further evaluation and test marketing of the low linolenic oil. In order to facilitate this process, the line S81-2716 was registered in 1987 as Stellar, the world's first canola-quality cultivar which produced seed oil with low levels of linolenic acid.

The improved oxidative stability of the low linolenic oil compared to conventional canola oil was shown in studies by Eskin et al. (1989). Measurements of five chemical indices of oxidation showed the low linolenic oil suffered less change when heated to 185° C than canola oil.

A recent study in France compared the room odour of the low linolenic canola oil from Stellar to that of oil from the canola cultivar Westar and a French rapeseed cultivar Bienvenu (Prevot et al. 1990). France specifically excludes oils with more than 2% linolenic from use for deep-fat frying and there is a perception that both rapeseed and soybean oil have an unpleasant odour when used in deep frying. The scores obtained by the low linolenic oil were significantly better than the other two oils, and the difference persisted through eight fryings. The scores of the low linolenic oil were comparable to those obtained by sunflower oil. This is a very significant finding, as it clearly links the amount of linolenic acid to the room odour. In combination with the results from the oxidative stability tests, the evidence is supportive of a potential market for the low linolenic oil as a frying oil.

The limitation to the production of Stellar is its agronomic performance. Three years of testing in the western Canadian Co-operative Trials indicated that Stellar was 20% lower yielding than the check Westar in all regions. The seed oil content of Stellar was lower than that of Westar by 11 g kg<sup>-1</sup> oil and the meal protein content was higher by 13 g kg<sup>-1</sup> oil free meal (Scarth et al. 1988). Is the source of the low linolenic variation limiting the productivity of the material?

To answer this question, we examined the data from five years of low linolenic breeding subsequent to the selection of Stellar in 1981. In 1984, F<sub>3</sub>'s from F<sub>2</sub> plants selected for maturity showed a range in linolenic acid from 1.7% to 4.7%, and a range in oil content from 40.5% to 47.0%. The correlation between the levels of linolenic acid and oil was not significant (Table 3).

Table 3. Correlation between the level of C18:3 (linolenic acid) and oil content in early generations of the low linolenic breeding program 1981-89.

YEAR	GENERATION	C18:3 <sup>a</sup>	SEED OIL <sup>b</sup>	CORRELATION
1981	F <sub>3</sub>	2.5	447	-0.04
1982	F <sub>3</sub>	2.4	468	-0.03
1984	F <sub>3</sub>	2.8	450	-0.01
1988	DH <sup>c</sup>	3.4	389	0.16
1989	DH	2.7	408	0.03

<sup>a</sup> mean % over lines <sup>b</sup> g kg<sup>-1</sup> <sup>c</sup> Doubled haploid lines

The yield trials in 1989 show the same picture - within the limited range of linolenic acid levels after selection, seed oil and yield are not correlated with the level of linolenic acid. There appears to be no genetic load carried with the low linolenic character which would result in lower oil content and yields. Low linolenic lines in test show the desired increases in oil and yield. These advances should improve the economics of producing low linolenic canola cultivars.

The stimulus for adopting the low linolenic character is coming from canola oil's traditional competitor in the vegetable oil market - soybean and from an unexpected source - a new edible oil flax. The Iowa State program under Dr. Fehr also had the priority of reducing the level of linolenic acid in soybean, from its level of 7% to 9%. Using mutation treatment and crossing to adapted cultivars, soybean selections have been developed with very low levels of linolenic acid, less than 3%. (Graef et al. 1988). No effect has been noted on agronomic performance in comparison with the adapted soybean cultivars.

The development of a low linolenic flax was stimulated by the decline of the traditional markets for flax and linseed oil. The high linolenic content which was so desirable for the drying qualities in paints and varnishes also limits the use of linseed oil as an edible product. Green and Marshall (1984) of the CSIRO in Australia conducted a mutation breeding program similar to that in soybean and canola. Two low linolenic lines were identified with intermediate levels of linolenic acid: 29% compared to the 45% to 55% in linseed cultivars. The lines were then crossed and the results was a reduction in linolenic acid to 1.6% with a corresponding increase in the linoleic acid content (Green et al. 1984). Commercial cultivars have been developed and some lines are under test in western Canada under exclusive contract. Dr. Rowland at the CDC, University of Saskatchewan also has an edible flax development program. Using mutation treatment and selection, lines have been developed with linoleic acid content of 65% and a linolenic acid levels of 2% (Rowland et al. 1990).

From the nutritional standpoint, the debate is still going on as to the status of linolenic acid as an omega-3 fatty acid. The two most common omega-3 fatty acids found in fish oils and some vegetable oils are eicosapentaenoic acid (20:5) or EPA and docosahexaenoic acid DHA (22:6). There is evidence that the  $\alpha$ -linolenic acid in soybean and canola oils can be converted to EPA. Interest in EPA was stimulated by the low incidence of heart disease among Greenland Eskimos, despite their high-fat high-cholesterol diet. The heavy reliance on fish in their diet focused attention on the omega-3 fatty acids and their effect in reducing platelet aggregation, one component of a heart attack (Kinsella 1986). However, while Eskimos may have the lowest incidence of coronary heart disease, they do have a high incidence of stroke, which can be attributed to inefficient platelet clotting in the brain. The debate over the relative merits of the different types of fats in human diets continues and will determine to some extent the acceptance of the low linolenic oil as a new standard for canola oil.

#### Development of high erucic/ low glucosinolate rapeseed

In keeping with the development of special quality oils for niche markets, the University of Manitoba breeding program has been developing rapeseed cultivars with high levels of erucic acid. Erucamide, a derivative of erucic acid, is the primary established market. It is used as an anti-block or slip-promoting agent in the production and functioning of plastic films. Erucic acid can also be used to form brassylic acid, a 13 carbon acid and pelargonic acid. Brassylic acid may be polymerized to form a flexible and moisture resistant nylon. The co-product pelargonic acid has a market as a component of plasticizers and synthetic lubricants.

A very topical new use is the development of an alcohol ester of

rapeseed oil (AERO) that holds promise as a clean-burning fuel. Researchers at the University of Idaho were quoted as saying that the ignition characteristics are more than adequate and engine modifications for AERO usage would result in even better performance. The estimates are that one acre of rapeseed is capable of producing 100 gallons of oil, at a cost of \$1.18 to \$1.31 a gallon (Peterson 1990).

The common thread through all these diverse markets is that the uses are only feasible if a competitively priced and stable source of erucic acid is available. Most commercially available erucic acids are in the 85-90% purity range, obtained by commercial distillation of the fatty acids in rapeseed oil. The levels available in rapeseed range from 40% to 55%.

The University of Manitoba breeding program targeted the development of a high erucic cultivar with canola quality meal, to achieve the maximum economic benefit for producers and crushers. The source of the high erucic character was a B. napus summer rape strain introduced from Sweden. This strain was grown in isolation for 4 years at the University of Manitoba and single plants with high erucic acid content in the seed oil were selected. These lines were then crossed to the high erucic acid cultivar, Reston, followed by selection of high erucic-low glucosinolate plants in the F<sub>2</sub> and F<sub>3</sub> generation. F<sub>4</sub> lines were selected for high seed yield and high oil and meal protein contents. Line S82-4362 was derived from a single F<sub>3</sub> plant selected in 1982 (Scarath et al. 1991). After four years of testing in the western Canadian Rapeseed Co-operative tests, support for registration was sought in 1989 and the strain was registered as Hero.

Hero produces seed oil with erucic acid levels above 50% and canola quality meal with 15  $\mu\text{mol}$  glucosinolates g<sup>-1</sup> oil-free meal. This represents a commercially economic combination of erucic acid for industrial purposes and a canola quality meal for the feed industry. Hero has good agronomic performance, with seed yields 14% lower than Westar. Hero is equal in maturity to Westar, with seed oil content equal to Westar and seed protein content 18 g kg<sup>-1</sup> higher. Seed chlorophyll levels are relatively low (Scarath et al. 1991).

The University of Manitoba breeding program aims for further improvements in oil quality by introducing the low linolenic character into the high erucic acid background. High levels of linolenic acid decrease the oxidative stability of the oil. Partial hydrogenation is the method of choice for removing linolenic acid. It is an expensive process and causes the formation of unwanted by-products. Therefore, the reduction of linolenic acid would be of benefit to the industrial oil. Lower levels of linoleic acid are also desirable in applications that require very high stability. The reduction of factors such as the level of free fatty acids, chlorophyll, and glucosinolates would all improve oil quality, and are breeding objectives in the program.

#### SUMMARY

The potential for modifying the fatty acid composition of canola/rapeseed oil appears to be limited not by available or acquired variation, but instead by the imagination and ability of plant breeders to anticipate the nutritional and industrial requirements of the future.

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