Reduction in saturated fat content of *Brassica napus* canola through interspecifc crosses and mutagenesis

J. Philip Raney[*], Todd V. Olson, Gerhard Rakow,
Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, Saskatchewan, Canada, S7N 0X2, email: raneyP@agr.gc.ca

ABSTRACT

The U.S. Food and Drug Administration distinguishes edible oils on the basis of their saturated fat content. Low saturated fat oil is defined as oil containing less than one gram saturated fat per fourteen grams of total fat (7.1%). Until recently oil produced from Canadian canola seed could easily meet this criterion. However, with the shift in acreage towards *Brassica napus* the Canadian crop now averages 7% or more saturated fat endangering this market advantage. *B. napus* germplasm was identified having 6% or less saturated fat in breeding populations of the Saskatoon Research Centre derived from interspecific crosses with *B. rapa* and *B. alboglabra*. This material is now being used in the breeding program to develop low saturated fat *B. napus* varieties alone and in combination with other advanced seed quality traits such as ultra-low glucosinolate and low linolenic acid content, and yellow seed colour. Also the doubled haploid line TO97-2268 (6% total saturated fat), was treated with the chemical mutagen EMS in an endeavour to create mutant lines with even lower levels of saturated fat. M_3 seed of 30,000 individually harvested M_2 plants (field 1999) was analyzed for fatty acid composition and 21 plants with 4.5% to 5.1% total saturated fat were identified. Other plants with altered fatty acid composition were also identified. These mutant plants were advanced, by single seed descent, to the M_6 generation and 450 plants (290 low saturated fat descendants) were progeny evaluated in 2002. Single plant and field trial data will be discussed.

Key words: *Brassica napus* – saturated fat – interspecific cross – mutagenesis – canola

INTRODUCTION

The US FDA categorises edible oils according to their total saturated fat (TSF). Preferably, oils should be either “low in saturated fat” (< 7.1%) or “saturated fat free” (less than 3.6% saturated fat). Canadian canola oil is superior, ‘healthy’, edible oil in part because of its low TSF. This was achieved historically because the crop was a 50:50 mix of Argentine canola (7% TSF or more) and Polish canola (<6% TSF). Unfortunately, Polish canola has virtually disappeared as a crop species and thus TSF in the crop has been increasing until since 1997 the average TSF of the harvest survey is 7% or more (DeClerq and Daun, 2002). In response, the Canola Council of Canada (CCC) advocated that breeding for reduced TSF levels should become a high priority for Canadian breeders. This paper describes our progress towards that goal.

MATERIALS AND METHODS

RSYN1-43 (Raney and Rakow, 1995), a ‘zero’ aliphatic glucosinolate selection from interspecific crosses between yellow seeded *B. napus*, *B. rapa* and *B. alboglabra* was crossed with TO95-1299, a yellow seeded, low linolenic acid *B. napus* line. The F_1 of this cross was crossed with N93-1526, a high oil content, blackleg resistant line derived from a cross of adapted *B. napus* canola and an Australian blackleg resistant cultivar, Shiralee. The F_1 progeny of this three-way cross was used for doubled haploid (DH) production and was pedigree selected. A total of 631 DH, F4 and F_5 lines were field evaluated in 1997 and 1998 (Raney *et al.,* 1999). 19 selected lines were further evaluated in a 1999 replicated yield trial at two locations. The DH line, TO97-2268, was further evaluated in the 2000 Private Data Co-op trials. TO97-2268 was also treated with ethylmethanesulphonate (EMS) to create lines with even lower levels of TSF. Two mutagenic conditions were tried; buds were treated with 0.5% EMS in 5%DMSO, 0.05M NaPO4 pH 7 for 3h or seeds were treated with 1% EMS in 5%DMSO, 0.05M NaPO4 pH 7 for 4h. In 1999, a nursery of 30,000 M_2 plants was analysed for TSF. 21 plants with 4.5 - 5.1% TSF were identified. These plants were advanced, by single seed descent, to

the M_6 generation. M_3 and M_4 progeny were field evaluated in 2001, and in 2002 the M_6 progeny were evaluated. The fatty acid composition of seed was determined by gas chromatography (Raney *et al.,* 1995). The content of individual fatty acids is calculated as percent of total fatty acids (weight basis). TSF is the sum of the contents of lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), behenic (22:0) and lignoceric (24:0) acids. Glucosinolate content was measured by gas chromatography (Raney *et al.,* 1995). Oil content was measured by CW-NMR. Protein content was measured with a LECO FP-428 nitrogen analyzer.

RESULTS

The progeny of the above cross segregated for several seed quality parameters, including segregation for extremes of aliphatic glucosinolate, oil, linolenic acid content, colour and TSF. Analysis of the 1997 and 1998 field nursery indicated that there were significant differences between the lines. TSF varied from 5.4 to 8.1%. TSF content was not associated with any of the other traits. The 1999 yield trial indicated that there were significant differences between the selected lines for all measured seed quality parameters. Significant correlations were observed between the field nurseries and the yield trial for TSF. TO97-2268 was the highest yielding low TSF line averaging 84% of AC Excel. Table 1 illustrates that the 1% difference between the low TSF lines and normal lines was maintained over years and locations. In the 2000 private data Co-op trial (Table 2) TO97-2268 performed consistently over all zones. It averaged 1% lower than the checks for TSF content and was always < 7% TSF. It yielded 80-86% of the checks with low oil and protein. Glucosinolate content was within the range specified for canola.

Table 1. Average TSF of selected lines, parents and checks in field nurseries and trials

	Total saturated fat content (%)						
Line	Progeny	1997[1]	1998[2]	1999[3]	1999[3]	1999[4]	2001[1]
TO97-2268	DH	5.7	6.5	6.5	6.3	6.0	5.7
TO97-2175	DH	5.8	5.8	6.6	6.3		
TO97-2308	DH	5.7	6.0	6.4	6.2		
TO97-2388-4	Pedigreed		5.6	6.4	6.2		
TO97-3230-14	Pedigreed		5.4	6.0	6.7		5.7
TO97-3234-9	Pedigreed		5.8	6.4	6.2		5.8
RSYN1-43	Parent	6.3	6.9	7.0	6.9		
TO95-1299	Parent	5.7	6.4	7.3	6.6		
N93-1526	Parent	6.8	6.9	7.0	7.0		
AC Excel	*B. napus* Chk		7.3	7.5	7.3	7.1	6.7
N89-53	*B. napus* Chk		7.3	7.3	7.3		
AC Parkland	*B. rapa* Chk		5.8	5.9	5.6		5.4

[1]Row nursery at Saskatoon. [2]Yield trail at Saskatoon. [3]Yield trial at Scott. [4] M_2 single plant nursery at Saskatoon.

Table 2. Comparison of TO97-2268 to checks in the 2000 Private Data Co-op Trials

Zone	Entry	Sites	Yield[1]	% Oil	% Prot.	TGSL[2]	TSF[3]	HiTSF[4]
Long	TO97-2268	4	86	43.8	43.8	13.4	6.3	6.7
	AC Excel	4	88	43.8	45.0	10.7	7.4	7.8
	Defender	4	107	44.3	46.1	10.0	7.3	7.4
	Legacy	4	105	44.6	46.1	9.4	7.1	7.5
Mid	TO97-2268	7	80	44.5	44.3	14.3	6.0	6.6
	AC Excel	7	100	47.7	44.2	10.6	6.8	7.0
	Defender	7	101	47.0	44.9	10.1	6.8	7.1
	Legacy	7	99	47.4	44.4	9.0	6.6	7.0
Short	TO97-2268	3	86	44.8	46.7	18.8	5.7	5.8
	AC Excel	3	94	48.2	47.2	14.2	6.5	6.9
	Defender	3	112	46.2	48.1	13.3	6.6	7.0
	Legacy	3	94	46.2	49.4	11.7	6.6	7.1

[1] Percent yield of the average of the checks. [2]Total glucosinolate. [3]Average TSF. [4]Highest TSF observed in each zone.

Lines resulting from mutagenic treatment of both buds and seeds that were a further 1% lower than T097-2268 were found (Table 3). In some progeny families the initial observation seen on the individual M_2 plants was confirmed on the M_3 and M_4 progeny rows and there was strong agreement between the TSF content observed on M_3 row and all the M_4 progeny rows. The lowest level of TSF observed in a single M_4 progeny was 4.3%. These levels of TSF are 2% lower than standard *B. napus* (AC Excel) and even lower than *B. rapa* (AC Parkland).

Table 3. Fatty acid composition of selected M_2 families in the 1999 and 2001 nurseries.

Family	Fatty acid (%)										
	EMS	Seed Source	n	16:0	18:0	18:1	18:2	18:3	20:0	22:0	24:0	TSF
684-3	Buds	M_2 1999 Plant	1	2.9	1.1	50.8	30.2	10.6	0.4	0.3	0.1	4.9
		M_3 2001 row	1	2.6	1.2	55.6	28.3	8.3	0.4	0.2	0.2	4.7
		M_4 2001 rows	14	2.6	1.2	55.1	28.5	8.7	0.4	0.3	0.2	4.7
1499-20	Seed	M_2 1999 Plant	1	2.5	1.4	56.6	26.4	9.1	0.5	0.3	0.2	4.9
		M_3 2001 row	1	2.4	1.4	60.8	24.4	7.3	0.5	0.3	0.2	4.8
		M_4 2001 rows	15	2.4	1.4	60.8	24.2	7.3	0.5	0.3	0.2	4.8
891-12	Seed	M_2 1999 Plant	1	2.8	0.9	53.8	28.0	10.6	0.4	0.3	0.1	4.5
		M_3 2001 row	1	3.0	1.2	56.5	27.0	8.7	0.4	0.3	0.2	5.2
		M_4 2001 rows	14	2.8	1.2	56.9	26.8	8.6	0.4	0.3	0.2	4.9
1195-12	Buds	M_2 1999 Plant	1	3.2	0.8	55.3	24.1	12.4	0.4	0.3	0.2	4.8
		M_3 2001 row	1	3.1	1.0	58.7	24.4	9.0	0.4	0.3	0.2	5.1
		M_4 2001 rows	11	3.1	1.0	58.7	24.0	9.3	0.4	0.3	0.2	5.1
2268	No	S_3 2001 rows	5	3.3	1.3	59.3	24.7	7.6	0.5	0.3	0.2	5.7
AC Excel	No	2001 rows	3	3.6	1.9	65.8	16.8	8.4	0.6	0.3	0.2	6.7
Parkland	No	2001 rows	6	3.2	1.4	57.6	21.6	13.3	0.4	0.3	0.2	5.4

DISCUSSION

The work described here illustrates that reduced levels of TSF can be introgressed into *B. napus* through interspecific crosses. Levels approaching *B. rapa* (< 6%) can be achieved in this way. Line TO97-2268 has shown a consistent low level of TSF over years and locations. If Argentine canola cultivars were developed with similar TSF levels there would be no danger that the Canadian canola crop would ever exceed 7% again. Further reductions up to 1% can be achieved through mutagenesis and lines having < 5% TSF are on the horizon.

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