Creation of HOLL spring *Brassica napus* from a cross between low linoleic and low linolenic acid mutants

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ABSTRACT

The fatty acid profile of canola oil is considered by many nutritionists to be ideal. However, the relatively high levels of linoleic acid and linolenic acid decrease the stability of the oil. They are also the progenitors of undesirable, *trans*-fatty acids found in partially hydrogenated canola oil. High oleic acid, low linolenic (HOLL) canola varieties alleviates much of these concerns. A low linolenic acid, spring *B. napus* line (S86-69, from Dr. Rachael Scarth, University of Manitoba, Canada) was crossed, reciprocally, with a low linoleic acid, winter *B. napus* line (M453, from the University of Göttingen, Germany) to combine the low linolenic acid trait of S86-69 with the low linoleic acid trait of M453 creating a HOLL phenotype. The F_1 generation displayed a very late spring phenotype; the F_2 generation segregated both winter and spring phenotypes. Half-seed selection for the HOLL profile was performed on F_2, and F_3 or F_4 seed; selected seedlings were greenhouse grown. The F_3, F_4, F_5 and F_6 generations were progeny tested in 1998, 2000 and 2001. HOLL phenotypes were recovered.

Key words: *Brassica napus* – fatty acid composition – oleic acid – linolenic acid – HOLL

INTRODUCTION

Canola is Canada's major edible oilseed crop and the world's third most important oilseed crop due to continuing demand for high quality edible oil. The fatty acid composition of canola oil, typically 60-65% oleic acid (18:1), 17-22% linoleic acid (18:2) and 8-11% linolenic acid (18:3) with <7% saturated fat, is considered by many nutritionists to be close to ideal. However, for the margarine and cooking oil uses, canola fatty acid composition is less than ideal and blending with other edible oils or hydrogenation is required. The moderately high levels of polyunsaturated fatty acids, 18:2 and particularly 18:3, increase the fluidity and oxidative instability of the oil, which affects its processing, storage and use. These fatty acids are the main progenitors of the undesirable, *trans*-fatty acids found in partially hydrogenated canola oil. Studies on low 18:3 cultivars (Scarth et al., 1988; Scarth et al., 1995) have demonstrated that this type of canola oil has improved odour, storage and frying stability compared with standard canola types (Eskin *et al.,* 1989; Przybylski *et al.*, 1993; Warner and Mounts, 1993). Deep frying applications, such as that used by fast fat restaurants, especially require a stable cooking oil that can be reused for long periods of time without effecting flavour. Oils with increased 18:1 at the expense of 18:2 and 18:3 also show even higher stability without extensive hydrogenation (Scarth and McVetty, 1999). The fatty acid profile for optimum frying performance is 67 to 75% 18:1, 15 to 22% 18:2 and <3% 18:3 (Scarth and McVetty, 1999). Oils with >75% 18:1 target the oleate market or can blended with conventional oils (Scarth and McVetty, 1999). This paper describes our progress towards developing a HOLL type canola.

MATERIALS AND METHODS

A low linolenic acid, spring *B. napus* line (S86-69, ~2% 18:3 acid, from Dr. R. Scarth, University of Manitoba, Canada) was crossed reciprocally with a low 18:2 acid, winter *B. napus* line (M453, ~8% 18:2, from the University of Göttingen, Germany) in order to combine the low 18:3 trait of S86-69 with the low 18:2 trait of M453. Half-seed selection for HOLL was performed on F_2 and F_3 seed; selected seedlings were selfed. In 1998 F_3 progenies of the 14 earliest F_2 plants were evaluated in a 2 replicate 3 metre single row nursery. One replicate received a hoop tent to preserve the integrity of the fatty acid composition. Due to the fatty acid compositon of the harvest seed, hoop tent seed (F_4) of three codes was chosen for another round of half seed selection. In 2000 and 2001 selected F_4, F_5 and F_6 progenies were tested in single row

nurseries. Hoop tents were used to preserve the integrity of the fatty acid composition. The fatty acid composition of seed was determined by gas chromatography (Raney *et al.*, 1995).

RESULTS

The F_1 generation from the crosses displayed a very late spring phenotype; succeeding generations segregated both winter and spring phenotypes. Plants displaying winter habit was discarded. Progeny rows grown in 1998, 2000 and 2001 were very late flowering and maturing and some progeny rows did not mature in time for harvest. Agronomic performance was generally rated as poor. The quality of seed grown in the hoop tents was generally better than the OP seed grown outside the tents. The low 18:2 parent, M453, could not be grown in the field because of its strong winter habit. Analysis of greenhouse harvested seed (Table 1) confirmed that it did indeed have altered fatty acid profile with an 18:2 content of about 9%. The concentration of 18:1 (~71%) was elevated and 18:3 (~10%) was normal or slightly elevated. The low 18:3 parent, S86-69, could be field grown. Analysis of harvested seed from each of the three nurseries (Table 1) demonstrated the expected fatty acid profile, 18:3 <2% with a slightly elevated concentration of 18:2 (25-26%). The check cultivar, AC Excel, exhibited the expected normal *B. napus* canola fatty acid profile (Table 1). Both S86-69 and AC Excel were earlier maturing than the progeny rows from the cross.

Table 1. Fatty acid composition of selected progeny grown in 1998, 2000 and 2001 nurseries.

Field	F_2	F_4/F_3	Planting		Fatty Acid (% of total)[2]						
Code	Plant[1]	Plant	Gen.	Year	16:0	18:0	18:1	18:2	18:3	20:1	22:1
Progeny											
1	3246-1		F_3	1998	3.7	1.7	73.5	14.5	2.8	1.2	0.1
7723[3]	3246-1	5314-8	F_5	2000	3.6	1.8	76.6	11.2	2.5	1.6	0.1
3873[4]	3246-1	5314-8	F_6	2001	3.8	2.0	73.8	11.9	2.6	1.7	0.5
3884[3]	3246-1	5314-22	F_5	2001	3.9	2.0	77.8	8.7	2.8	1.4	0.1
2	3248-1		F_3	1998	3.9	1.4	76.0	11.2	3.1	1.4	0.1
7729[3]	3248-1	5316-6	F_5	2000	3.4	2.1	76.0	11.5	2.6	1.3	0.1
3891[3]	3248-1	5316-13	F_5	2001	3.9	2.2	74.8	12.4	2.3	1.2	0.1
9	3255-1		F_3	1998	3.8	1.7	75.7	12.2	3.3	1.3	0.0
7741[3]	3255-1	5318-18	F_5	2000	3.5	1.9	77.8	9.5	3.0	1.4	0.1
3880[4]	3255-1	5318-18	F_6	2001	3.9	1.9	75.2	11.1	3.3	1.4	0.1
3896[3]	3255-1	5318-23	F_5	2001	4.4	2.4	73.0	12.7	1.6	1.3	0.1
7743[3]	3255-1	5318-25	F_5	2000	3.6	2.3	76.6	9.5	2.0	1.4	0.2
3881[4]	3255-1	5318-25	F_6	2001	4.3	2.5	71.3	13.0	2.4	1.4	0.1
7708	3260-2	5311-1	F_4	2000	3.8	1.5	73.8	14.6	2.0	1.5	0.0
3861[4]	3260-2	5311-1	F_5	2001	4.0	1.4	73.0	15.5	1.8	1.4	0.1
7712	3260-2	5311-17	F_4	2000	3.5	1.6	75.1	13.2	1.8	1.6	0.1
3864[4]	3260-2	5311-17	F_5	2001	4.4	1.9	70.8	15.0	1.8	1.4	0.2
7713	3260-2	5311-19	F_4	2000	3.3	2.2	72.2	15.0	2.3	1.5	0.1
3865[4]	3260-2	5311-19	F_5	2001	4.1	2.2	70.1	14.1	1.9	1.4	0.2
Parents											
S86-69	Parent			1998	4.0	2.0	63.6	25.0	1.8	1.2	0.0
S86-69	Parent			2000	3.9	1.8	62.9	25.6	1.8	1.3	0.0
S86-69	Parent			2001	4.1	2.2	61.2	26.4	1.9	1.2	0.1
M453[5]	Parent			GH	4.0	1.1	71.3	8.8	10.2	1.7	0.1
Check											
AC Excel	Check			1998	4.2	2.0	66.8	17.1	6.4	1.3	0.1
AC Excel	Check			2000	3.8	1.7	63.4	18.4	8.7	1.4	0.0
AC Excel	Check			2001	3.8	2.0	66.1	16.3	8.1	1.3	0.0

[1]3246-1 & 3248-1 F_2 progeny from M453 by S86-69; 3255-1 & 3260-2 F_2 progeny from S86-69 by M453. [2]Analysis of seed harvested from hoop tents. [3]Seed used for the final round of selection was F_4 seed from the 1998 hoop tent of the F_3 progeny row. [4]Seed used for planting nursery was from hoop tent of previous year nursery. [5]Analysis reported is the average of selfed seed harvested from greenhouse grown plants.

Table 1 also illustrates that a number of progeny demonstrating a HOLL phenotype could be selected from this cross. HOLL-type progeny were found in both of the reciprocal crosses, eliminating the possibility of a cytoplasmic influence on the results. Levels of 18:1 observed ranged from ~70% to nearly 78%. Levels of 18:3 ranged from 1.6% to 3.3%. For entries grown in 2001 directly from hoop tent seed of the 2000 nursery, there was good agreement between the two years of fatty acid data, especially for 18:3 (see Note 4 of Table 1).

DISCUSSION

By a half-seed pedigreed selection regime, we were able to select high oleic acid, low linolenic acid (HOLL) phenotypes from a cross of low linolenic acid and low linoleic acid parents (S86-69 x M453 and visa versa). The recovered HOLL phenotypes would ideal for use as frying oil without hydrogenation. As such this germplasm could prove to be useful source for this trait in future HOLL canola cultivars. Lines with a HOLL phenotype have been reported before. Among others (Scarth and McVetty, 1999), Wong and Swanson (1991) reported the creation of a HOLL phenotype with through microspore mutagenesis and Debonte and Hitz (1996) reported the generation of HOLL genotypes through genetic modification technologies. Identity preserved production of HOLL canola is already occurring on the Canadian prairies. This germplasm is another source of endogenous high oleic acid, low linolenic acid spring *B. napus* canola, but it requires considerable breeding to improve agronomic performance and maturity.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical support of the Don Rode, Cliff Powlowski, Joanne Relf-Eckstein, Doug Hennigan, Gerald Serblowski, Dawnne Campbell, Lynnelle Harris and Tracey Schell without whose able assistance this project could not have succeeded.

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