# High stability oil *Brassica napus* from a cross low linoleic acid and low linolenic acid mutants: agronomic improvement through backcrossing to elite canola germplasm and reselection of extreme fatty acid profiles

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**Abstract**

At the 11th International Rapeseed Congress in Copenhagen, Denmark, the creation of high stability oil *Brassica napus* lines selected from a cross between low linoleic acid (8%) and low linolenic acid (2%) mutants was reported. Fatty acid profiles were achieved with oleic acid levels ranging up to 78% and linolenic acid levels as low as 1.6%. However, the agronomic performance of these lines was weak; the lines were very late maturing, up to 3 weeks later than adapted Canadian *B. napus* cultivars. Since then selected $F_4$ and $F_5$ lines were crossed to a black-seeded *B. napus* line, N99-508, an elite selection from the cross of Quantum and LG3260 and pedigreed to the $F_5$ generation. Half-seed selection for high stability oil profile and greenhouse selection for early flowering was performed on the $F_2$, $F_3$, $F_4$ and $F_5$ seeds and plants. At most generations the selected plants were selfed, but in order to improve the efficiency of recovery of the desired traits crosses were also made among certain $F_3$ plants representing different $F_2$ families. Selected $F_5$ and $F_6$ lines were tested in 2004 in a replicated single row, three meter field nursery. Greatly improved agronomic performance was observed including much earlier maturities approaching that of adapted canola cultivars. The fatty acid profile of many lines was observed to be high stability oil type and several lines exhibited a high stability fatty acid composition profile that even more extreme than that previously reported. In 2005 14 lines were retested in a three location, four replicate yield trial.

**Key words:** *Brassica napus*, high stability oil, fatty acid composition, oleic acid, linolenic acid

## Introduction

Frying stability of edible oil is inversely correlated to the degree of unsaturation of its fatty acids (Eskin et al. 1989, Przybylski et al. 1993, Warner and Mounts, 1993, Scarth and McVetty 1999, Xu et al. 1999, Carre et al. 2003). Linolenic acid (18:3) is the most important factor (Xu et al. 1999, Carre et al. 2003) and it has been demonstrated that even at levels as low as 1.6% it contributes to unacceptable odour under heating (Eskin et al. 1989) and there is a significant difference between 1.1% and 2.2% 18:3 (Carre et al. 2003). Oils with increased oleic (18:1) at the expense of linoleic (18:2) and 18:3 show even higher stability (Scarth and McVetty 1999). The optimum profile is 5 to 7% saturates, 67 to75% 18:1, 15 to 22% 18:2 and ⩽=3% 18:3 (Scarth and McVetty 1999). To overcome the shortcomings of canola oil for frying applications its is partially hydrogenated. However, hydrogenation leads to the formation of trans-fatty acids, particularily from 18:3 and trans-fats have been linked to ischemic heart disease (Stender and Dyerberg 2004). Because of these health concerns, in December 2002 Canada passed regulations requiring the labeling of food products as to the content of trans-fat (SOR/2003-11). As of 2004 Denmark banned the selling of oils containing >2% industrially produced trans-fat (Stender and Dyer berg, 2004). The American Heart Association Nutrition Committee has recommended reducing trans-fat comsumption to ⩽1% of energy (Lichenstein et al. 2006). Therefore, breeding efforts to develop high stability (HS) canola cultivars have intensified and high stability canola is in commercial production in Canada. This paper describes our efforts at developing at a agronomically competitive *B. napus* canola with increased levels of 18:1 and extremely low levels of 18:3 through pedigree selection.

## Material and Methods

*Greenhouse 2001/2004:* Eighteen selected HS $F_4$ and $F_5$ lines from a cross of low 18:1(2%) *B. napus* canola by low 18:2 winter *B. napus* (Raney et al. 2003) were crossed to N99-508 (a elite selection from a cross of Quantum and LG3260). Half-seed selection for HS oil profile and greenhouse selection for early flowering was performed on the $F_2$, $F_3$, $F_4$ and $F_5$ seeds and plants. Fatty acid profiles of half-seed selections were confirmed on 10-20 seed samples from harvested $F_3$, $F_4$, $F_5$ and $F_6$ seed. Further half-seed selection was then only done on plants with the most desirable fatty acid profiles. In this manner about a 10% selection pressure was achieved for each cycle of selection. At all generations the selected plants were selfed, but in order to avoid fixing of the desired traits at an early stage, crosses were also made among certain $F_3$ plants representing different $F_2$ families. These crossed $F_4$ lines were continued with another half-seed selection at the $F_5$ generation to the $F_6$ generation

*Field 2004/2005:* 286 selected $F_5$ and $F_6$ lines were tested in 2004 in a replicated single row, 3 meter field nursery. In 2005 14 $F_6$ and $F_7$ lines were retested in a 3 location, 4 replicate yield trial. Because of the lack of sufficient selfed seed for the replicated trials, open-pollinated seed from the 2004 nursery was used.

*Chemical analysis*: Half-seed fatty acid composition was determined by a modification of the gas chromatography (GC) method of Thies (1971). Seeds were germinated overnight on moistened filter paper. The seeds were split. The inner cotyledon

and radical were preserved intact on 1% agar gel in microtiter plates at 4º until analysis was complete (2-4 days), selected and planted in pots in the greenhouse. The outer cotyledon was placed in a 250 µl autosampler vial insert and allowed to dry overnight. 25 µl hexane and 50 µl 0.8% metallic sodium in methanol were then added and the cotyledon ground with a glass rod. After 15 minutes 25 µl 0.2 M $NaPO_4$ pH 7 was added, methanol and hexane evaporated under a stream of air (1 minute), and 0.2 ml of heptane added. The samples were injected into a FID equipped gas chromatograph (model 6890 Agilent Technologies, Santa Clara, CA, USA; column: HP-Innowax, 7.5 m × 0.25 mm × 0.5 µm, hydrogen, constant flow, 1.3 ml/min; injector: 280°, 1 µl, split 1:40; oven: 190 - 240°, 20°/min., final time 0.6 min.; detector 300°). Individual fatty acids are expressed as percentages of all fatty acids detected. Bulk analysis of individual plants grown in the greenhouse was performed on 10-20 seed samples from harvested plants. Evaluation of seed from field rows and plots was performed on 3 gram samples taken from harvest envelopes. GC conditions were similar to the half-seed method. Reported oil and protein contents are NIR predictions. Total glucosinolate was measured by GC.

## Results

*2004 Nursery*: 2004 was a very, cool year with an early frost. Despite this many lines entered in the test reached maturity. Harvest was done in late September and early October. Relative to the 2000 and 2001 nurseries of the HS parent lines the overall agronomic performance of this nursery was much improved. Stands were better and maturity was generally within a week of the checks instead of the 3 weeks later seen with the HS parents earlier.

**Table 1: A comparison of fatty acid data of 14 lines selected from the 2004 Nursery**

| 04Code | Gen | Pedigree | N | 18:1 | s18:1 | 18:2 | s18:2 | 18:3 | s18:3 | TSF | sTSF | Oil |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10378 | $F_5$ | 7727 | 2 | 80.1 | 0.1 | 10.6 | 0.0 | 1.1 | 0.0 | 5.5 | 0.0 | 50.5 |
| 10379 | $F_5$ | 7727 | 2 | 78.8 | 0.6 | 10.4 | 0.7 | 2.3 | 0.1 | 5.7 | 0.0 | 50.7 |
| 10380 | $F_5$ | 7727 | 2 | 78.4 | 0.0 | 11.7 | 0.0 | 0.8 | 0.0 | 6.0 | 0.1 | 49.1 |
| 10384 | $F_5$ | 7727 | 2 | 79.0 | 0.2 | 10.9 | 0.0 | 0.9 | 0.0 | 6.0 | 0.1 | 46.5 |
| 10385 | $F_5$ | 7727 | 2 | 78.9 | 0.2 | 11.1 | 0.1 | 1.0 | 0.2 | 6.1 | 0.2 | 47.0 |
| 10389 | $F_5$ | 7727 | 2 | 76.4 | 1.9 | 13.1 | 1.6 | 1.2 | 0.1 | 6.4 | 0.1 | 47.7 |
| 10390 | $F_5$ | 7727 | 2 | 78.1 | 0.4 | 11.8 | 0.3 | 1.1 | 0.1 | 6.1 | 0.1 | 47.4 |
| 10395 | $F_5$ | 7727 | 2 | 77.9 | 0.1 | 11.8 | 0.0 | 1.2 | 0.0 | 6.2 | 0.0 | 48.7 |
| 10540 | $F_6$ | 7727x7719 | 2 | 79.1 | 0.4 | 10.6 | 0.2 | 1.0 | 0.0 | 6.1 | 0.0 | 48.0 |
| 10541 | $F_6$ | 7727x7719 | 2 | 78.4 | 0.2 | 11.5 | 0.1 | 1.0 | 0.0 | 6.1 | 0.1 | 47.9 |
| 10542 | $F_6$ | 7727x7719 | 2 | 77.3 | 0.9 | 12.4 | 0.5 | 1.1 | 0.2 | 6.1 | 0.2 | 47.4 |
| 10560 | $F_6$ | NTx7743 | 2 | 77.0 | 0.3 | 12.7 | 0.5 | 1.3 | 0.0 | 6.1 | 0.1 | 48.3 |
| 10580 | $F_6$ | NTxNT | 2 | 76.9 | 0.2 | 12.8 | 0.2 | 1.2 | 0.0 | 6.2 | 0.1 | 47.8 |
| 10605 | $F_6$ | 7718xNT | 2 | 70.5 | 0.2 | 18.6 | 1.0 | 0.9 | 0.1 | 7.1 | 0.8 | 45.1 |
| | | | | | | Checks | | | | | | |
| S86-69 | | | 6 | 58.8 | 0.7 | 28.4 | 0.4 | 2.3 | 0.1 | 7.3 | 0.2 | 44.1 |
| 46A65 | | | 26 | 61.4 | 0.5 | 19.5 | 0.4 | 9.9 | 0.3 | 6.2 | 0.2 | 47.5 |
| N99-508 | | | 32 | 59.7 | 1.4 | 18.5 | 0.5 | 12.4 | 0.3 | 6.6 | 0.2 | 49.5 |
| Nursery Mean | | | 237 | 74.8 | 4.3 | 12.7 | 4.5 | 3.1 | 1.7 | 6.3 | 0.4 | 46.3 |
| Nursery Median | | | 237 | 76.2 | | 11.3 | | 2.8 | | 6.3 | | 46.5 |
| | | | | | | Parents -2001 Nursery data | | | | | | |
| 7718 | | | | 73.4 | | 16.7 | | 0.9 | | 6.3 | | |
| 7719 | | | | 76.1 | | 13.7 | | 1.0 | | 6.5 | | |
| 7727 | | | | 76.3 | | 13.4 | | 0.9 | | 5.8 | | |
| 7743 | | | | 76.6 | | 9.5 | | 2.0 | | 7.4 | | |

* N – number of rows; TSF – total saturated fat; s18:1, s18:2, s18:3, sTSF – standard deviation of the rows for the respective fatty acid or fatty acid group; Oil – average oil content (% dry seed) NT – not tested in 2000.

Because of the cool conditions most of the HS parent lines included in the nursery did not produce harvestable seed and therefore no fatty acid is reported for them from this nursery. After fatty acid analysis was completed on this nursery it was evident that many lines possessed or nearly possessed the desired HS profile (Table 1) and there was very good agreement between the replicate rows (Table 1 –standard deviation data). The fatty acid profile seen in the HS parents was fully recovered in several lines (Table 1, 10378, 10380, 10384, 10385, 10390, 10540). In fact it was observed that some lines may contain ⩽1% 18:3 (10380, 10384, 10605). Line 10605 demonstrated that it is possible to obtain this extremely low level of 18:3 without extreme elevation of 18:1. Oil contents were also a considerable improvement over the HS parents with many approaching the oil content of the checks and N99-508. Because of the cool conditions most of the HS parent lines included in the nursery did not produce harvestable seed and therefore no fatty acid is reported for them from this nursery. Reported is 2000 nursery hoop tent data of those parent lines which are ancestors to the 14 selected lines from the 2004 nursery. The 2000 data explains the likely source of the 1% or lower 18:3 progeny.

*2005 Yield Trial*: One of the three sites seeded was destroyed by hail, so only the two Saskatoon sites is reported (Table 2). On average the yield of the 14 selections was about 80% of the check cultivar, 46A65. The best yield was 88% of the check

(10379). This, although not acceptable yet, does represent a very substancial improvement over the original HS selections, which could not be tested because of their extreme lateness and poor performance. Oil content of the selections was quite improved as well, the best line (10378) was nearly equal to the check and the worse case (10560) was 2.2% lower. The protein content of several lines was within acceptable levels. The fatty acid data of the selections in general confirmed the observations of the 2004 nursery, but 18:1 was somewhat lower (1-4%) and 18:3 was somewhat higher (~1%) in all cases. They all still meet or exceeded HS criteria. All lines contained 0.1% or less erucic acid and the total saturated fat content (12:0 to 24:0) of most lines met or exceeded the canola standard of 7% or less. As expected, the glucosinolate content of the selections did not meet canola standards. The decline in the fatty acid profile of the selections is explained by the outcrossing to inferior individuals which could have occurred in the 2004 nursery (see nursery mean and median, Table 1), since open-pollinated seed was used to plant the 2005 replicated yield trials.

**Table 2: Yield, oil, protein and fatty acid comparison of lines entered into the 2005 replicated yield trial**

| | Yield -%46A65 | | | Oil | Protein | Saskatoon site 1 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 04CODE | Loc1 | Loc2 | Avg | %dry | %dry | 18:1 | 18:2 | 18:3 | TSF | GSL |
| 10378 | 81 | 73 | 77 | 47.9 | 23.4 | 76.4 | 12.0 | 2.3 | 6.3 | 60.1 |
| 10379 | 90 | 85 | 88 | 47.3 | 24.1 | 76.0 | 11.7 | 2.9 | 6.5 | 59.0 |
| 10380 | 81 | 84 | 83 | 47.0 | 23.8 | 75.3 | 13.0 | 2.0 | 6.7 | 52.0 |
| 10384 | 79 | 63 | 71 | 47.4 | 23.9 | 75.6 | 12.7 | 2.0 | 6.5 | 39.7 |
| 10385 | 78 | 89 | 83 | 46.4 | 24.3 | 75.4 | 12.8 | 2.3 | 6.5 | 57.8 |
| 10389 | 78 | 83 | 81 | 46.2 | 23.9 | 72.8 | 15.2 | 2.0 | 6.9 | 25.8 |
| 10390 | 72 | 71 | 71 | 47.4 | 23.4 | 75.7 | 12.0 | 2.5 | 6.7 | 16.7 |
| 10395 | 72 | 73 | 72 | 46.4 | 23.6 | 73.8 | 12.9 | 2.5 | 7.0 | 18.4 |
| 10540 | 77 | 73 | 75 | 46.6 | 24.7 | 75.3 | 12.7 | 2.2 | 6.6 | 22.4 |
| 10541 | 77 | 75 | 76 | 47.5 | 24.3 | 75.5 | 12.7 | 1.9 | 6.7 | 68.7 |
| 10542 | 80 | 75 | 78 | 46.9 | 24.4 | 75.1 | 13.4 | 2.1 | 6.3 | 22.3 |
| 10560 | 86 | 81 | 83 | 45.9 | 24.6 | 73.4 | 14.0 | 2.9 | 6.8 | 21.0 |
| 10580 | 78 | 80 | 79 | 46.0 | 25.3 | 74.2 | 13.6 | 2.1 | 7.0 | 48.8 |
| 10605 | 90 | 79 | 84 | 46.8 | 24.0 | 69.4 | 18.8 | 1.7 | 7.4 | 22.8 |
| N99-508 | 107 | 103 | 105 | 49.1 | 23.2 | 60.0 | 19.5 | 11.0 | 6.9 | 11.5 |
| YN01-429 | 108 | 119 | 113 | 51.3 | 21.3 | 58.4 | 22.2 | 9.9 | 6.8 | 12.6 |
| InVigor2663 | 114 | 105 | 110 | 47.5 | 23.7 | 59.8 | 19.3 | 11.6 | 6.7 | 8.9 |
| 46A65 | 100 | 100 | 100 | 48.1 | 24.6 | 61.8 | 19.5 | 9.7 | 6.2 | 15.6 |

* Loc1 – Saskatoon site 1 seeded May 14, 2005; Loc2 – Saskatoon site 2 seeded May 26, 2005; Avg – average of 2 sites; Oil – average oil content over 2 sites; Protein – average protein content over 2 sites; TSF – total saturated fat (12:0 to 24:0); GSL – total glucosinolate, µmoles/gram seed.

## Discussion

From the 2004 nursery data it is evident that extremely low levels of l8:3 can be effectively selected in HS *Brassica napus*. The benefit of this extreme low level of 18:3 will be frying oils with improved stability, odour and imparted flavour. The 2005 yield trial confirms the 2004 nursery, but demonstrates the effect of outcrossing on 18:1 and 18:3 contents. Pleines and Friedt (1989) found that 18:3 is under partial maternal control, which explains why the outcrossing to inferior individuals which would have occurred in the 2004 nursery did not appreciably affect the 18:3 values of that nursery, but would affect the levels seen in 2005 nursery when open-pollinated seed was used to plant the test. These 14 selected line represent a very significant agronomic improvement over the original HS lines (Raney et al. 2003), but improvements still have to be made in yield, maturity, oil and glucosinolates. Therefore, another cross to elite germplasm is necessary.

## Conclusions

We claim here the development of *Brassica* napus lines having high stability edible oil with up to 70-80% oleic and as little 1% or less linolenic acid. This trait has been recovered from the original cross (Raney et al. 2003) and now with improved agronomics from a cross with a standard canola line (N98-508). If canola cultivars are developed which have this high stability oil profile, in particular the 1% linolenic acid, improved cooking oils can be developed which will extend the frying stability and other characteristics of high stability oil. We next plan to incorporate the high stability oil trait into elite yellow-seeded germplasm making yellow-seeded high stability oil canola, which will also have enhanced uses for the low fibre meal.

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