# Meal quality improvement in *Brassica napus* canola through the development of low fibre (yellow-seeded) germplasm

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**Abstract**

The importance of the yellow seed trait in improving the feed value of canola meal through fibre reductions has been reported in various *Brassica* species by several researchers over a number of years. A strong correlation has been reported between seed colour and meal fibre reduction, in particular reduction in lignin content. Research at AAFC Saskatoon has utilized various sources of "yellow seed colour genes" in related *Brassica* species, and these were incorporated into adapted spring annual lines and cultivars through interspecific crosses. Interspecific $F_1$ plants were repeatedly backcrossed to *B. napus* canola followed by reselection of true breeding yellow-seeded phenotypes after each backcross for several inbred generations before the next backcross was carried out. The resulting yellow-seeded lines were field tested under Canadian prairie growing conditions for several years. In all cases, fibre contents were strongly correlated to seed colour with lowest fibre contents observed in lines with strongest expression of the yellow seed trait. Intensive breeding work will be required to develop agronomically superior yellow-seeded (low fibre) canola cultivars and hybrids.

**Key words**: Brassica napus, yellow seed, low fibre meal.

## Introduction

The rapeseed (canola) oilseed crop in Canada is the summer annual form of *Brassica napus* with a low erucic acid content (<1% of total fatty acids) and a low glucosinolate content (<12 µmoles/g seed at 8% moisture). The seed oil must have a total saturated fat content of <7% of total fatty acids and the seed should have a low chlorophyll content of less than 25 mg/kg seed at maturity to qualify for the highest commercial grade, Canada No. 1, the basis for payment to the producer.

A high seed oil content is the most important seed quality objective in canola breeding because oil is the most important component of canola seed representing about 80% of its economic value. For this reason, minimum standards for oil content have been implemented for cultivar registration, together with minimum standards for meal protein contents. The average oil content of the Canadian canola crop as 42.8% at 8.5% seed moisture for the 10 year period 1995-2004 based on survey data by the Canadian Grain Commission (DeClerq, 2004). The average oil-free meal protein content, on an 8.5% moisture basis, for the same period was 40.4%. Oil and protein contents of individual farm fields (productions) can vary greatly due to environmental factors that affect plant growth and maturity.

Canola meal is viewed by the industry as a by-product of canola oilseed production and is used as an animal feed protein supplement. Canola meal has a high crude fibre content of about 12% compared to soybean meal containing about 4%, primarily a result of its small seed size. The meal is traded at a 35% price discount compared to soybean meal despite its good balance of amino acids (Bell, 1995). About 30% of the meal weight consists of seed coat tissue (hulls) which contains much less protein and more fibre than the seed embryo tissue. Hulls contain over 10% of total protein and 25% of gross energy of canola meal, and hulls in the meal depress levels of available energy and protein, as well as availability of amino acids and minerals (Bell, 1995). Therefore, a reduction in the hull content would be of interest to improve the nutritional value of the meal.

One possible and very efficient strategy to reduce the hull content in canola meal is the development of yellow-seeded cultivars which have been shown to have thinner seed coats than black-seeded cultivars, and meals produced from yellow seeds would therefore contain less hulls and a lower fibre content. This has clearly been shown in *B. rapa* (Stringam, et al. 1974), *B. juncea* (Woods, 1980) and *B. carinata* (Getinet et al. 1996) where yellow-seeded types do exist. Another advantage of the yellow seed trait is an increased oil content due to a larger embryo in yellow seed. *Brassica napus* cultivars and germplasm are black seeded and no yellow-seeded forms have ever been found in natural populations. Yellow-seeded *B. napus* has been developed through interspecific crosses with the objective to transfer the yellow seed trait from *B. rapa* into *B. napus* (Liu, 1983; Rahman, 2001), through resynthesis of *B. napus* based on crosses between yellow-seeded *B. rapa* and light seeded *B. alboglabra* (Shirzadegan, 1986; Chen et al. 1988) and through induced mutations (Sobrino-Vesperinas et al. 1991). More recently, yellow-seeded *B. napus* has also been successfully developed through interspecific crosses involving yellow-seeded forms of *B. juncea* and *B. carinata* in interspecific crosses with *B. napus* (Rashid et al. 1994).

This paper will review breeding work conducted at the AAFC Saskatoon Research Centre in regard to developing true breeding yellow-seeded forms of *B. napus* through interspecific crosses and improvements made in agronomic performance and seed quality.

## Materials and Methods

*Creation of true yellow breeding* Brassica napus

Interspecific crosses were made at the University of Manitoba, Canada, (Dr. B.R. Stefansson) between yellow-seeded *B. carinata* as female and the black-seeded *B. napus* canola cultivar Regent and the $F_1$ backcrossed to Regent followed by selection of yellow-seeded plants in backcross generations. Selected yellow-brown seeded plants were then crossed with *B. rapa* yellow sarson and the resulting $F_1$ again backcrossed to Regent. Segregating generations of this second interspecific cross were selected for four generations for yellow seeded plants by Dr. D. Woods of AAFC Saskatoon and a yellow-brown seeded line, YSN80-1623 selected in the field.

YSN80-1623 was crossed as female with a $F_5$ generation inbred yellow-brown seeded AAFC *B. napus* canola quality line SZN73-1493, selected from crosses involving the rapeseed cultivars Target and Golden, zero erucic acid cultivars Oro and Zephyr and two low glucosinolate germplasm lines, Bronowski and S68-2895. The $F_5$ line YN86-37, selected from this cross, had yellow seed colour and was of canola quality.

YN86-37 was crossed in 1986, with a yellow-brown seeded *B. napus* introduction, obtained from the Svalöf seed company, R3608, resulting from the cross *B. napus* x *B. juncea* (yellow). The $F_5$ inbred line YN90-1016, selected from this cross was true breeding for the yellow seed trait. Selected yellow-seeded $F_3$ plants of this cross were crossed with the yellow-brown seeded *B. alboglabra* line 89-5402, derived from interspecific crosses with yellow-seeded *B. carinata* followed by backcrosses to *B. carinata* and reselection of yellow seeded plants. The $F_1$ of this cross was backcrossed to the yellow-seeded *B. napus* parent, and a yellow-seeded $BCF_2$ plant Rsyn 2-11 selected and this plant was further inbred to the $BCF_5$ generation to improve and stabilize the yellow seed trait.

Rashid et al. (1994) made two interspecific crosses: *B. napus* (Westar) x *B. juncea* (yellow) and *B. napus* x *B. carinata* (yellow) and backcrossed the two $F_1$ generations to *B. napus* (Westar). Twenty $BCF_2$ plants of the *B. juncea* interspecific cross were crossed with 20 $BCF_2$ plants of the *B. carinata* cross, and 18 doubled interspecific cross populations grown in field plots for selection of yellow-seeded plants. These were pedigree selected for several generations and a true yellow breeding, canola quality line, YN9592, identified in the $F_6$ generation.

*Improving agronomic performance and seed quality of yellow-seeded* B. napus

The yellow-seeded line YN90-1016 was crossed with the Australian blackleg resistant cultivar Shiralee and $F_1$ plants of this cross used as male parents and crossed with the black-seeded high oil content, AAFC Saskatoon canola line N89-53, a $F_4$ line derived from the cross Midas x Westar. The 3-way cross was selected for high oil content in plant-row progenies in the field and yellow seeds selected from highest oil content lines. The $F_6$ line YN97-262 was selected as a true breeding yellow seeded line in a field nursery in 1997.

Four black-seeded *B. napus* cultivars Magnum, Dunkeld, Range and Ebony were crossed as females with YN97-262 to improve yield, blackleg resistance, and oil and protein content of yellow-seeded *B. napus*. The $F_1$ Dunkeld x YN97-262 was crossed with the $F_1$ Magnum x YN97-262 to combine blackleg resistance with yield, and the $F_1$ Range x YN97-262 was crossed with the $F_1$ Ebony x YN97-262 to combine blackleg resistance with oil content. Double cross (DC) $F_1$ plants were interpollinated and DC $F_2$ plants grown in the field, followed by $F_3$ progeny evaluation of yellow-seeded DCF2 plants in a breeding nursery, and $F_4$ early generation yield assessments in replicated yield tests. The $F_5$ line YN01-429 was high yielding and had a high seed oil content and true breeding for the yellow seed trait which was confirmed in a $F_6$ plant-row progeny test in 2002.

The three yellow-seeded *B. napus* lines YN90-1016, Rsyn 2-11 and YN9592 were field tested in 4 replicate yield tests at Saskatoon, Scott and Melfort, Sask. from 2002-2004.

## Results

**Table 1: Yield and seed quality of yellow-seeded *Brassica napus* lines YN90-1016, Rsyn 2-11 and YN9592 of different interspecific origin and the agronomically improved true yellow-seeded line YN01-429 in comparison to the black-seeded cultivars 46 A65/Q2 in 4 replicate yield tests at Saskatoon, Scott and Melfort, Canada (total of 6 station years) from 2002 to 2004.**

| | Yield | | Oil[1] | Protein[2] | Colour[3] | ADL[4] | ADF[5] |
|---|---|---|---|---|---|---|---|
| Lines | kg/ha | % checks | %seed | % meal | (WIE) | % meal | %meal |
| YN90-1016 | 1270 | 67 | 43.3 | 48.0 | -22.4 | 1.2 | 7.8 |
| Rsyn 2-11 | 1190 | 63 | 42.6 | 48.3 | -22.9 | 1.0 | 7.7 |
| YN9592 | 1610 | 85 | 43.8 | 47.5 | -28.7 | 1.0 | 7.4 |
| YN01-429 | 2030 | 107 | 49.1 | 46.9 | -28.5 | 1.3 | 8.2 |
| 46 A65/Q2 | 1890 | 100 | 45.8 | 49.0 | -0.8 | 5.4 | 13.6 |
| SED | 380 | | 2.2 | 2.7 | | 0.7 | 1.0 |
| Station years | 6 | | 6 | 5 | 6 | 4 | 4 |

1=oil by NMR, 2=protein by LECO combustion, 3=seed colour by Method E313, white index, American Society for Testing and Materials. 4=ADL, acid detergent lignin, 5=ADF, acid detergent fibre (cellulose + lignin).

The true yellow-seeded lines derived from multiple interspecific crosses with yellow seeded donor species *B. carinata*, *B. rapa* yellow sarson and *B. juncea* for yellow seed colour, YN90-1016 and Rsyn 2-11 yielded 33% and 37%, respectively less seed than the average of the two black-seeded check cultivars 46 A65/Q2 (Table 1). Their oil and protein contents were also lower than those of the checks. YN9592 yielded 85% of checks and had also lower oil and protein contents. The seed colour scores of -22.4 to -28.7 represent a good yellow colour compared to -0.8 for the check cultivars. Acid detergent lignin contents of the three original yellow seeded lines ranged from 1.0% to 1.2% of meal dry weight which represents about an 80% reduction in ADL compared to the black seeded cultivars. Acid detergent fibre levels were also low in the yellow seeded lines and were reduced by more than 50%.

The agronomically improved yellow-seeded line YN01-429 yielded 7% more seed than check cultivars and had a 3.3% higher seed oil content associated with a lower meal protein content. ADL and ADF fibre levels were low and comparable to those of the original yellow-seeded lines in combination with a strong yellow seed phenotype. All lines were of canola quality.

## Discussion

We have shown that true breeding yellow-seeded lines of *B. napus* canola can be developed from interspecific crosses with related yellow seeded species of *B. carinata*, *B. rapa* yellow sarson and *B. juncea*. The recessive conditions of seed coat pigmentation genes, yellow seed, in the A-genomes of *B. rapa* (Stringam, 1980) and *B. juncea* (Vera et al. 1979) were successfully introgressed into the A-genome of *B. napus*, while yellow seed colour genes from the C-genome of *B. carinata* (Getinet & Rakow, 1997) were also successfully utilized and most likely incorporated into the C-genome of *B. napus*. A systematic genetic study on seed colour inheritance in *B. napus* is currently conducted to determine the number of genes involved in seed coat pigmentation in *B. napus* and to investigate their mode of inheritance.

The low seed yield of first generation yellow-seeded lines from interspecific crosses is most likely the result of aneuploidy and irregularities in meiotic cell division in these lines which we have observed (data not shown). These meiotic disturbances often lead to poor embryo development and premature seed ripening and seed abortion which causes low seed yield and reductions in seed oil content as observed in our material. However, systematic backcrossing of early generation yellow-seeded lines to agronomically superior, high oil and protein content elite cultivars or breeding lines will improve yield and quality of yellow-seeded lines as demonstrated by the development and performance of YN01-429. This line even outyields standard cultivars and has a significantly higher seed oil content. The higher oil content in YN01-429 results from a larger embryo in a bigger seed. The increase oil content in elite yellow-seeded lines will be of particular interest to the oilseed crushing industry, improving their crushing margins. YN01-429 has normal meiotic division, forming 19 bivalents and is fully fertile producing well developed seed of superior size.

We have shown that meal fibre contents, ADL and ADF in yellow seed meals are significantly reduced by about 75% and 50%, respectively. This reduction in meal fibre content will significantly increase the feed value of canola meal, and this has been documented in some animal feeding studies. Economic studies suggest that when adapted, high yielding yellow-seeded *B. napus* cultivars will be available for commercial production, the use of black-seeded cultivars will discontinue and the whole canola production will be of the yellow seed type (Evrard 2004). The commercial production of yellow-seeded cultivars of *B. napus* canola in Canada will improve the economic competitiveness of canola meal as a high quality protein feed supplement in animal feed relative to soybean meal.

## Conclusions

The development and commercial production of adapted, high yielding, disease resistant, high quality yellow-seeded *B. napus* cultivars and hybrids will establish a new standard for the rapeseed (canola) industry worldwide. We believe that low fibre, high oil (yellow-seeded) canola must be the next quality breeding goal for *B. napus* canola and all further future oil and meal quality improvements must be built on this technology platform for the benefit of the whole canola industry.

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