Agronomic performance and blackleg disease reactions of yellow-seeded *Brassica napus* canola

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Abstract

The development of low fibre (yellow-seeded) *Brassica napus* canola cultivars and hybrids is a major focus of canola breeding research at the Saskatoon Research Centre of Agriculture Canada. Early generation interspecific derived true breeding yellow-seeded lines were low yielding and repeated backcrosses to black-seeded elite cultivars and lines had to be made to improve yield, blackleg resistance, standability and seed quality of yellow-seeded lines. We utilized the winter rape cultivar Express of NPZ Germany, to incorporate winter rape genetic material into spring annual lines through cross breeding. Selected spring annual lines derived from crosses with Express were crossed with the AAFC yellow-seeded line Rsyn 2-11 to develop agronomically improved, blackleg resistant, yellow-seeded lines through pedigree selection in progeny from these crosses. F_7 yellow-seeded lines derived from these crosses had improved seed yield, were highly resistant to blackleg and had acceptable maturity under Canadian short season growing conditions. Our research proved that winter rape can be successfully used in the breeding of agronomically improved, high quality, yellow-seeded spring *B. napus* lines for production in Canada.

Key words: *Brassica napus*, yellow seed, winter rape.

Introduction

The reduction in meal fibre content in *B. napus* canola to improve its nutritional value has been a breeding goal for the AAFC Saskatoon Research Centre for many years beginning with the observation by Stringam et al. (1974) that yellow-seeded lines of *B. rapa* had significantly lower meal fibre content than brown-seeded lines. We describe the development, agronomic performance and quality of lower fibre (yellow-seeded) *B. napus* germplasm in detail in another paper presented at this congress (Relf-Eckstein et al. 2007). Multiple interspecific crosses were made between yellow-seeded lines of *B. carinata*, *B. rapa* and *B. juncea* with black-seeded, canola-quality cultivars and lines of *B. napus*, followed by backcrosses to *B. napus* and re-selection of true yellow-seeded (low fibre) *B. napus* lines. The agronomic and seed quality improvement of early generation yellow-seeded *B. napus* was successfully accomplished through further backcrosses with elite, high yielding, blackleg resistant, and high oil and protein summer annual *B. napus* cultivars and lines.

We recognized that the genetic basis among summer annual, canola-quality germplasm available for this work was very narrow because of the common parentage of these cultivars for low erucic acid (Liho) and low glucosinolate (Bronowski) contents, and the requirement for early maturity for Canadian short season growing environments of about 100 days. This paper will report on our work to utilize winter rape germplasm to create a genetically different gene pool of yellow-seeded *B. napus* for the future breeding of low fibre (yellow-seeded) *B. napus* hybrid cultivars for production on the Canadian prairie.

Materials and Methods

The canola quality winter rape cultivar Express of NPZ, Germany, was crossed as female with the AAFC Saskatoon canola-quality breeding line N93-P1526 as male and the F_1 of this cross used as the male parent in a backcross to Express (female) to produce BC_1 plants. Segregating early flowering BC_1 plants were then used as male parents for a second backcross to Express to produce BC_2 populations and to incorporate as much as possible winter rape genetic material into summer rape. Pedigree selection was practised to isolate summer annual plants from these crosses that had acceptable maturity for Canadian growing conditions and early maturing BC_2F_3 plants were selected in the field.

Three BC_2F_3 plants selected from the Express winter rape crosses were crossed as females with the AAFC Saskatoon yellow-seeded *B. napus* line Rsyn 2-11 (Relf-Eckstein et al. 2007) as male parent in the greenhouse during the winter of 1998-99. A total of 192 F_1 plants were grown in the greenhouse to produce F_2 seed for progeny evaluation in a 2-replicate, single row nursery at Saskatoon in 2000. Thirty five F_2 rows were identified from nine of eleven crosses as having flowering and swathing maturity within two weeks of the AAFC *B. napus* cultivar AC Excel. The selected progeny had excellent straw strength, were seed shatter tolerant and had desirable plant phenotypes. A total of 429 blackleg-disease free, yellow to yellow-brown seeded F_2 plants were individually harvested from the second replicate of the nursery and seed colour measurements were made. Nineteen best yellow-seeded F_2 plants were identified and evaluated for agronomic performance and seed quality as F_3 progeny in a 2-replicate, double-row nursery at Saskatoon in 2001. Pedigree selection in combination with mass selection and early generation yield assessment of F_4 family groups was done in conjunction with blackleg disease evaluation in a disease nursery. The results of this work were reported previously (Relf-Eckstein & Rakow, 2003).

After the identification of superior F_4 families, pedigree selection was applied in F_5 and F_6 generations utilizing plant-row progeny evaluations in 2-replicate, single-row breeding nurseries at Saskatoon in 2003. Twenty six yellow-seeded F_7 lines were evaluated in 4-replicate, 4-row plot yield tests for seed yield, plant height, maturity, seed weight, oil and protein content and fibre content at Saskatoon and Scott, Saskatchewan and Irricana, Alberta, test sites in 2004 and 2005. Check cultivars included in the tests were the official check cultivars 46 A65 and Q2 used in canola registration trials, the yellow seeded AAFC experimental line YN01-429 and the black-seeded line N89-53.

Resistance to blackleg disease of experimental lines was assessed in field disease nurseries naturally infested with *Leptosphaeria maculans* at the AAFC research farms Saskatoon and Melfort, Saskatchewan. The blackleg susceptible *B. napus* cultivar Westar was included in the tests to monitor disease pressure.

A mean blackleg severity rating was then calculated for each entry and severity ratings were converted to a percentage severity index with the following scale used to describe the level of resistance to blackleg disease. A rating of R (resistant) for a percentage severity index of <30% of Westar, MR (moderately resistant) for 30% to 49% of Westar, MS (moderately susceptible) for 50% to 69% of Westar and S (susceptible) for 70% to 100% of Westar.

Results

Table 1: Yield, height, maturity, seed weight, oil and protein content and acid detergent lignin content of nine yellow-seeded F_7 *Brassica napus* canola lines with winter rape genetic background, the AAFC *B. napus* lines YN01-429 (yellow-seeded) and N89-53 (black-seeded) and the official check cultivars 46 A65 and Q2 used for canola cultivar registration, in replicated yield tests at Saskatoon and Scott, Saskatchewan and Irricana, Alberta, in 2004 and 2005, average of 7 tests.

Entry	Yield kg/ha	Height (cm)	Maturity (days)	Seed Weight (g/1000s)	Oil (% seed)	Protein (% meal)	ADL[3] (% meal)
YN03-603	2470	119	101	2.82†	48.3	45.4‡	2.40‡
YN03-604	2460	119	102	2.72	47.6	46.4‡	2.49‡
YN03-607	2440	123	103	2.84‡	47.9	46.5‡	2.38‡
YN03-611	2480	118	102	2.93‡	48.5	46.3‡	2.56‡
YN03-612	2450	120	105‡	2.98‡	48.4	47.8‡	2.44‡
YN03-616	2440	119	103	2.72	48.2	46.0‡	2.39‡
YN03-652	1670‡	117	103	2.54	43.6‡	43.4‡	1.35‡
YN03-656	1920‡	121	104†	2.57	45.8‡	48.0‡	1.72‡
YN03-662	1890‡	123	106‡	2.74	45.9‡	48.2‡	1.69‡
YN01-429	2410	116	103	3.23‡	50.1‡	46.2‡	1.50‡
N89-53	2190†	111	98‡	2.81†	47.1	48.8†	7.31†
46A65	2460	115	102	2.52	47.8	50.4	7.72
Q2	2460	115	102	2.56	47.9	48.6‡	5.27‡
S.E.D.	120	1	1	0.11	0.6	0.6	0.23

1. Least-square means of entries evaluated in 4-replicate yield tests at Scott and Saskatoon, Saskatchewan and Irricana, Alberta, 2004-2005, 7 station-years data, n=210 observations.
2. Estimations based on NIR predictions based on calibration with NMR for oil content, Leco for protein and ANKOM digestion method for Acid Detergent Lignin content, five station-years data, n=150 observations.
3. ADL = Acid Detergent Lignin, a major component of fibre in canola meal.

† Pr >| t | 0.05 difference in least square means to 46A65.

‡ Pr >| t | 0.01 difference in least square means to 46A65.

The six F_7 lines YN03-603 to YN03-616 derived from the cross with one BC_2F_3 line and the yellow-seeded *B. napus* line Rsyn 2-11 had seed yields comparable to those of the check cultivars 46 A65 and Q2 and the yellow-seeded line YN01-429 (Table 1). The lines were similar in maturity to check cultivars, except for YN03-612 which was 3 days later maturing. They also had larger seed weights than the checks; their oil contents were similar to checks, but protein contents were significantly lower. The acid detergent lignin content (ADL) of the 6 lines was about 2.5% of dry meal weight which was only about one half of that of the check cultivar Q2 which has a lower ADL content than the check cultivar 46 A65.

The three F_7 lines YN03-652 to YN03-662 derived from the cross with a different BC_2F_3 line and the yellow-seeded *B. napus* line Rsyn 2-11 had lower seed yields than the check cultivars and had a tendency to mature later. They were lower in both, oil and protein contents than check cultivars, but had lower ADL contents, similar to those of the yellow-seeded line YN01-429. The low ADL contents of these three lines indicate a very good yellow seed colour since low ADL levels are very strongly correlated with the yellow seed phenotype.

There was no direct comparison of the agronomic performance of the new yellow-seeded lines tested with either of the parents of the crosses from which these lines were derived. Rsyn 2-11 was yield tested in replicated yield tests in comparison to the yellow-seeded line YN01-429 from 2002 to 2004 where its yield was only 63% of that of check cultivars, it had 3.2% lower oil content and 0.7% lower protein content than the checks and had an ADL content of 1.0% of dry meal weight (Relf-Eckstein et al. 2007).

Table 2: Blackleg severity ratings (0-5) of nine yellow-seeded F_7 *Brassica napus* canola lines with winter rape genetic background, the yellow-seeded AAFC Saskatoon line YN01-429, and the check cultivars Q2 (blackleg resistant) and Westar (blackleg susceptible) in blackleg disease nurseries at Saskatoon and Melfort, Saskatchewan, in 2004 and 2005.

Entry	2004		2005		Average	
	Saskatoon	Melfort	Saskatoon	Melfort	(0-5)	(% Westar)
YN03-603	0.59	1.62	0.80	1.10	1.17	34.6
YN03-604	0.45	1.27	0.37	0.85	0.83	24.5
YN03-607	0.56	1.50	0.91	0.92	1.11	32.7
YN03-611	0.38	1.44	0.71	0.83	0.99	29.3
YN03-612	0.47	1.54	1.01	0.92	1.16	34.1
YN03-616	0.59	2.22	0.95	1.14	1.44	42.3
YN03-652	0.24	0.35	0.18	0.24	0.26	7.6
YN03-656	0.34	0.78	0.30	0.23	0.44	12.9
YN03-662	0.36	0.77	0.39	0.64	0.60	17.7
YN01-429	0.96	1.95	1.05	1.10	1.37	40.3
Q2	0.45	0.59	0.37	0.60	0.52	15.3
Westar	2.52	3.94	3.39	2.85	3.39	100.0

The blackleg disease severity ratings varied somewhat among the nine experimental lines but were generally in the resistant to moderately resistant category (Table 2). All four tests had good levels of blackleg infestations from 2.52 for Westar at Saskatoon in 2004 to 3.94 for Westar at Melfort in 2004 which allowed a reliable estimation of blackleg resistance of candidate lines.

Discussion

BC_2F_3 plants and progeny with winter rape Express genetic background were two weeks later maturing than the AAFC *B. napus* check cultivar AC Excel which is unacceptable for cultivation of such lines in the Canadian prairies. There is great danger that such late maturing lines produce high chlorophyll contents in seed at maturity and the risk of frozen seed at harvest cannot be tolerated. The cross of these lines with the true spring annual yellow-seeded line Rsyn 2-11 resulted in F_7 progeny with acceptable maturity (Table 1). The seed yield of lines derived from these crosses was similar to that of check cultivars clearly indicating the positive contribution of winter rape genetic material in increasing seed yields of spring type material, in this case by about 40% over that of Rsyn 2-11 through a single cross in combination with pedigree selection.

The improvement in oil and protein content over the yellow-seeded spring rape parent Rsyn 2-11 is also significant, but similar or even greater improvements in oil content are possible through crosses with adapted, high quality summer annual cultivars and breeding lines (Relf-Eckstein et al. 2007). The line YN01-429 is an excellent example for possible oil content increases from crosses with summer forms.

The reselection of the yellow seed trait from the crosses described above is challenging and requires several generations of intensive selections after the cross. The results of this research indicated a negative relationship between yellow seed colour (as indicated by ADL levels) and seed yield. The best yellow seeded lines (low ADL levels) had the lowest yields which complicates the breeding of high yielding, good yellow-seeded (low fibre) lines in crosses with Rsyn 2-11. This is not necessarily a strong genetic relationship that can be overcome through intensive cross breeding and backcrossing with elite quality lines as we have demonstrated in other crosses also aimed at the development of high yielding, high oil content and good yellow-seeded *B. napus* lines (Relf-Eckstein et al. 2007).

The winter rape genetic background for blackleg disease resistance in one parent was contributing significantly to high levels of blackleg resistance in newly selected yellow-seeded F_7 lines (Table 2). The resistance to blackleg observed in these lines must have its origin in the winter rape cultivar since the yellow-seeded Rsyn 2-11 parent was only partially resistant to blackleg.

Winter rape represents a genetically distinct gene pool from summer rape and can make a significant contribution to the genetic diversification of *B. napus* canola for the breeding of improved cultivars and hybrids. Our results support conclusions drawn by other researchers attempting to utilize winter rape for the genetic improvement of summer rape (Diers & Osborn 1994, Butruille et al. 1999, Quijada et al. 2004).

Conclusions

The systematic utilization of *B. napus* winter rape germplasm in the development of a second gene pool of genetically diverse from summer rape has great potential for canola hybrid breeding. Our goal is to produce true yellow-seeded (low fibre) lines that have genetic contributions from winter rape which could produce high quality, yellow-seeded hybrid cultivars in crosses with lines of our spring (summer) annual yellow-seeded lines.

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