Breeding *Brassica napus* canola for blackleg resistance and seed quality

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ABSTRACT

Genetic resistance to blackleg is an absolute requirement for the development of high yielding, *Brassica napus* canola for production in Canada. The high oil content, early maturing, blackleg susceptible *B. napus* line, N89-53, was crossed with two Australian blackleg resistant varieties, Dunkeld and Range, to incorporate blackleg resistance into N89-53. The F_2 generation of the double cross, derived from crosses between the two single crosses of N89-53 with Dunkeld and Range, was mass selected for maturity at Saskatoon in 1999. Selected F_2 plants were progeny tested (F_3, F_4 and F_5 generations) in 2000, 2001 and 2002, for the identification of adapted, blackleg resistant, high quality lines. Blackleg disease nurseries were grown in Wagga Wagga, Australia, 2000-02, and Melfort, Canada, 2002, to obtain information on blackleg reaction under two very different blackleg disease environments. In addition, two sequenced characterized amplified region (SCAR) markers, closely linked to the blackleg resistance gene in the French spring rape variety, Crésor, were used to characterize lines for blackleg resistance gene segregation. Results of this study suggested there may be additional genes for blackleg resistance present in the two Australian varieties which may be independent of the Crésor resistance gene. The blackleg resistance genes were successfully combined with the seed quality, early maturity, and adaptation to semiarid growing conditions on the western Canadian prairie, typical of the N89-53 parent.

Key words: *Brassica napus* – blackleg resistance – seed quality – molecular markers

INTRODUCTION

Blackleg disease, caused by *Leptosphaeria maculans*, is a major threat to *B. napus* canola production in Canada. Development of *B. napus* varieties that are resistant to the predominant isolates of *L. maculans* found in Canada (Kutcher *et al*., 1993), as well as the more virulent isolates found in Australia (Purwantara *et al*., 2000), will serve to protect the Canadian canola crop from possible future losses due to blackleg disease. Two Australian *B. napus* varieties, Dunkeld and Range, were presumed, based on their pedigree, to carry the blackleg resistance gene from the French spring rape variety Crésor, as well as other uncharacterized sources of blackleg resistance. The objective of this study was to incorporate blackleg resistance from Dunkeld and Range into high seed quality, well adapted, Canadian *B. napus*.

MATERIALS AND METHODS

The AAFC breeding line, N89-53, was chosen as the female parent because of its superior ability to synthesize oil, and adaptation to production on the prairies. The Australian varieties, Dunkeld and Range, were chosen as male parents based on performance in blackleg disease nurseries and yield trials in Canada (Table 1). Two SCAR markers developed by Somers *et al*., (1999), were found to each amplify a single fragment in Dunkeld and Range (++),with no amplification in blackleg susceptible, N89-53 (--). The two markers, separated by three centiMorgans (cM), flank the gene conferring adult stage resistance in Crésor. Ten F_1 plants from each of two single crosses were intercrossed to create a double cross. Forty double-cross F_1 plants were interpollinated and the F_2 seed was bulked. Plant progeny evaluation in field nurseries in Saskatoon were designed as 2-replicate, single or double row plots (3-m long, 60-cm spacing). Oil, protein and total glucosinolate content were measured on a dry seed basis using NMR, nitrogen combustion analysis (N x 6.25), and gas chromatography, respectively.

Blackleg disease survival (%) was based on first (seedling) count per row, relative to second(adult) plant count, at the disease nursery in Wagga Wagga, Australia. The survival of Westar determined a susceptible disease reaction category. Blackleg disease severity was based on a 0 – 5 rating scale (0=no symptoms, 5=plant dead) at disease nurseries in Saskatoon and Melfort.

Table 1. Blackleg disease severity rating, yield and seed quality of Dunkeld, Range, and checks, in replicated yield tests at Saskatoon, Scott and Melfort, Saskatchewan, and disease nurseries at Saskatoon and Melfort, Saskatchewan, Canada,1997-2000.

Entry	Blackleg (0-5)				Yield[1]	Oil[1]	Protein[1]	GSL[2]
	1997	1998	1999	2000	(kg/ha)	(%)	(%)	(μmol/g seed)
Dunkeld	0.64	0.34	1.06	0.30	1900	43.5	27.3	9.9
Range	0.28	0.16	0.30	0.11	2150	43.4	26.8	13.7
Quantum	0.90	0.39	0.92	0.25	2060	43.2	27.6	16.5
Westar	4.24	2.19	4.30	3.64	1870	44.1	26.8	14.9
LSD (5%)	-	-	-	-	470	2.7	1.7	1.0

[1] Average of 10 station-years (s.y.).
[2] GSL data from 1998 (3 s.y)

Table 2. Average yield, blackleg disease resistance and seed quality of six F_5 *Brassica napus* lines and the check variety 46A65, in a replicated yield test at Saskatoon, and disease nurseries in Melfort and Wagga Wagga, Australia, 2002.

Entry	Yield	Blackleg			(% seed)	
	(% 46A65)	(0-5)[2]	% Survival[3]	Crésor markers	Oil	Protein
N01-780	131	0.36	64%	(--)	46.1	25.9
N01-738	117	0.80	59%	(++)	46.1	26.3
N01-727	160	1.32	48%	(±)	48.4	22.9
N01-956	139	1.08	45%	(++)	48.1	23.4
N01-839	93	0.40	47%	(±)	44.9	27.4
N01-1061	91	0.08	63%	(++)	46.2	25.7
46A65	100	n/a	n/a	n/a	46.4	25.6
LSD (5%)	19	n/a	n/a	n/a	0.9	0.9

[1]Average yield of 46A65 = 1091 kg/ha =100%, LSD=214 kg/ha.
[2]Blackleg severity rating, Melfort, 2002. Susceptible check, Westar = 3.68, Dunkeld = 0.12, Range = 0.04.
[3]Blackleg survival, Wagga Wagga, 2002. Susceptible check, Westar = 33.2%, Dunkeld = 50%, Range = 57%

The F_2 seed was sown in a field plot in 1999, and 4,378 F_2 plants were individually harvested. DNA was extracted from a 10-seed bulk from each of 1,737 F_2 plants mass selected for maturity and best quality F_3 seed, and 1,272 F_2 plants were (++) and 465 F_2 plants (--) for the Crésor markers. Blackleg disease survival was evaluated on F_3 plant-rows, from 50 (++) and 50 (--) F_2 plants, Australia, 2000; 34 (++) were resistant and16 (++) susceptible, 33 (--) were resistant and 17(--) susceptible. A two-replicate, F_3 plant-row nursery was sown at Saskatoon, 2000, and 112 agronomically superior F_3 rows from the total 1,737 F_3 rows selected. Within-row selection for absence of stem lesion and canker symptoms resulted in 560 F_3 plants being individually harvested from one replicate (five plants from each selected F_3 row). Twelve seeds from each of the 560 F_3 plants were sown in the glasshouse, winter 2000-01, and DNA was extracted from the tissue of 4,808 F_4 plants; 4,008 F_4 plants were (++), 785 (--), and 15 F_4 plants were recombinant (±). Blackleg survival was evaluated on F_4 plant rows, from 50 homozygous (++) and 50 homozygous (--) F_3 plants, Australia, 2001; 31 (++) were resistant and16 (++) susceptible, 41 (- -) were resistant and 7 (--) susceptible. Early generation seed yield and quality was evaluated in two F_4 nurseries at Saskatoon in 2001. A total of 112 F_4 lines, each made up from reserve F_4 seed of five-F_3 plants from the 2000 nursery, were tested in a double row nursery, with 46 superior F_4 composite lines identified. Seed quality of the 560 F_3 plants was evaluated in a single row nursery, and 59 F_4 rows from the 46 F_4 composite lines were selected. Using blackleg survival and early generation yield and seed quality data, 20 superior F_4 lines, representing 13 of the 112 F_3 rows in 2000, were selected.

RESULTS

Twenty selected F_5 lines were sown in a 4-replicate test at Saskatoon, and disease nurseries at Melfort and Wagga Wagga, Australia in 2002. Performance from six of these F_5 lines was compared to 46A65 (Table 2). The lines N01-780 (--), and N01-738 (++)showed excellent levels of blackleg resistance in Canada and Australia, with good oil content and high seed yield; N01-727(±) and N01-956 (++) had significantly higher oil content and seed yield, with good resistance in Australia and Canada. The lines, N01-839 (±) and N01-1061(++) had lower seed yields. However, N01-839 had significantly higher protein content along with good blackleg resistance. N01-1061 had one of the highest survival scores in Australia, the highest resistance in Canada, and oil content only slightly below that of 46A65. The SCAR markers segregated in both the F_2 and F_4 plant populations (F_2: χ^2= 7.5, P=0.01-0.001, F_4: χ^2=201.1, P<0.001) using a 3:1 single gene model for resistance (++). Lines recombinant (±) for either of the flanking markers were identified in 0.3% of the F_4 plants.

DISCUSSION

The breeding strategy employed in this study was based on a double cross to create a heterogeneous/heterozygous F_2 breeding population. This enabled the recovery of recombinant lines expressing the genes for high oil content, early maturity and adaptation from the female parent, N89-53, with genes for blackleg resistance and protein content from the male parents, Dunkeld and Range. A 6% selection pressure had to be applied for the selection of 112 F_3 plant rows with desirable agronomy and early maturity. This was followed by a 45% selection intensity in the early generation F_4 yield test, for seed yield and quality, a 12% selection intensity among F_4 progeny for high oil and/or protein content , and identification of blackleg resistant lines using Australian and western Canadian disease nurseries and SCAR markers. The SCAR markers segregated in a 3:1 ratio indicating that the single-gene adult stage resistance of Crésor was carried in the F_2 and F_4 populations. No correlation was found between the SCAR markers and blackleg survival in Australia. The higher than expected (0.09%) number of blackleg resistant lines that were (--) for the Crésor marker may be explained by the presence of additional loci in Dunkeld and Range, conferring blackleg resistance in both Australia and Canada. These loci may be independent from the linkage group (LG6) associated with the Crésor gene suggested by Rimmer *et al*., (1999).

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