

Brassica juncea* with a canola fatty acid composition from an interspecific cross with *Brassica napus

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ABSTRACT

Seed of 'zero' erucic acid, low glucosinolate *Brassica juncea* developed at the Saskatoon Research Centre had low oleic acid content, and high contents of linoleic and linolenic acids compared to *B. napus* or *B. rapa* canola. To improve the oil quality, a line of this *B. juncea* germplasm was crossed with low linolenic acid *B. napus* canola (S86-69, 2% linolenic acid, a gift from Dr. Rachael Scarth, University of Manitoba, Canada) followed by several backcrosses to *B. juncea* with half-seed selection for increased oleic acid, decreased linolenic acid phenotypes after each backcross. The fatty acid composition of the selected half-seed seedlings was confirmed on greenhouse produced selfed seed of these seedlings. Often repeated half-seed selection cycles were performed before proceeding with the next backcross. Although the selected progeny seemed to improve with each succeeding backcross, either the plants did not produce enough seed for field evaluation or when field evaluated the progeny did not survive to maturity. Finally, BC₅F₃ progeny did survive in the field, and one BC₅F₅ line was identified which not only performed well agronomically, but also had a canola fatty acid composition (oleic acid >60%, linolenic acid ~11%). Data from greenhouse grown plants is presented and the results of field trials of selected lines are discussed. This germplasm will be used for the development of new, improved varieties of *B. juncea* canola.

Key words: high oleic acid – *Brassica juncea* – fatty acid composition – interspecific cross

INTRODUCTION

Brassica juncea has been shown to have good adaptation to the hotter drier areas of western Canada and is grown on >50,000 ha as condiment mustard crop (Woods *et al.*, 1991). In the semi-arid regions, *B. juncea* cultivars outperform both *B. napus* and *B. rapa* canola (Woods *et al.*, 1991). *B. juncea* lines are also resistant to blackleg and pod shattering. In the dry regions of southern Australia, early maturing *B. juncea* breeding lines have also significantly outyielded *B. napus* cultivars (Burton *et al.*, 1999). Lines of 'canola quality' *B. juncea* have also demonstrated yield advantage over *B. napus* canola (Rakow *et al.*, 1995). The first lines of 'canola quality' *B. juncea* (Love *et al.*, 1991) had low levels of oleic acid (18:1) content compared to canola (Raney *et al.*, 1995a). Although they met the canola definition for glucosinolate content (<18 µmole/g seed), they were not as low as typical *B. napus* cultivars (<12 µmole/g; Rakow *et al.*, 1995). In order to make improvements to these two important 'canola quality' characteristics, 'canola quality' *B. juncea* germplasm was crossed with low linolenic acid *B. napus* canola (Raney *et al.*, 1995a).

MATERIALS AND METHODS

'Zero' erucic acid, low glucosinolate *B. juncea* (Love *et al.*, 1991), J90-4253, was crossed with a line of low linolenic acid *B. napus*. The F₁ was backcrossed to J90-4253. The BC₁F₁ progeny was advanced to BC₁F₂ via selfing and half-seed selection for high 18:1 and low linolenic acid (18:3) (Raney *et al.*, 1995a). The BC₁F₃ and BC₂F₂ generations were backcrossed with J92-223, an improved 'canola quality' *B. juncea* (Rakow *et al.*, 1995). Followed by two more backcrosses to high yielding, high oil content, 'zero' erucic acid *B. juncea* at the BC₃F₃ and BC₄F₄ generations. In-between the backcrossing steps, the progeny was advanced by selfing with half-seed selection for 'canola' fatty acid profile (increased 18:1) and, where possible, vegetative (leaf or bud) selection for low glucosinolate contents. Confirmation of fatty acid and glucosinolate content was done on 5-20 seed bulk analysis of the selfed seed. When possible, progeny were evaluated in field nurseries, however, progeny failed to survive until 1999 (BC₅F₃

progeny evaluation). In 2000, BC₅F₅ progeny were tested in a 2 rep 3m single row nursery. In 2001 BC₅F₅ to BC₅F₇ progeny were field-tested and a replicated yield test was grown at Saskatoon of selections from the 2000 nursery.

The fatty acid composition of seed samples was determined by gas chromatography of the methyl esters (Raney *et al.*, 1995b). The glucosinolate content of seed was determined by gas chromatography of the trimethylsilyl derivatives of the desulphoglucosinolates (Raney *et al.*, 1995b). Oil content was measured on dried, intact seed by CW-NMR.

RESULTS

Previously BC₁F₂ and BC₁F₃ plants were identified with elevated 18:1 content (Table 1, Raney *et al.*, 1995a), however, they were interspecific in nature, weak and largely infertile. Backcrosses to *B. juncea* were initiated. Analysis of greenhouse produced seed indicates that we were successful in preserving the high 18:1 trait to the BC₅F₇ generation (Table 1). By the BC₅F₃ generation, the plants were healthy and normal in appearance and fertility. Table 2 compares selected high oleic acid BC₅F₅ and later progeny (all closely related to accession TO0-5987) with parents and checks on seed harvested from field nurseries. Their 18:1 content is significantly higher than J92-4253, the original 'canola quality' *B. juncea* parent line. It is similar to *B. napus* canola (AC Excel). The 18:3 content is similar to *B. rapa* canola (10-12%). It is within the definition defined for C18 fatty acids for Canadian canola (>55% 18:1, <13% 18:3).

Table 1. Fatty acid and glucosinolate content in accession TO00-5987 pedigree

Accession	Seed Gen.	Self Seed	Seed Anal.	Fatty acid (%)			Glucosinolate (µmole/g sd)			
				18:1	18:2	18:3	Allyl	But ¹	TotA ²	Total
TO93-0933-7	BC ₁ F ₂	few	0.5	62.2	22.2	4.6				
TO94-1005-2	BC ₁ F ₃	few	0.5	63.1	23.7	3.8			Not done.	
TO95-1249-1	BC ₂ F ₂	15	0.5	55.5	26.9	10.3				
TO96-1751	BC ₃ F ₃	95	20	57.7	26.0	6.6	0.2	24.6	27.3	32.6
TO97-3360	BC ₄ F ₄	75	5	65.7	15.3	7.3	0.3	63.9	65.6	68.0
TO98-3788	BC ₄ F ₅	80	20	67.9	14.7	5.6	1.0	50.0	53.2	54.1
TO99-4923	BC ₅ F ₃	70	5	60.1	21.3	8.8	29.5	25.7	55.6	58.4
TO99-5399	BC ₅ F ₄	300+	10	58.1	21.8	9.6	35.2	30.9	66.7	71.5
TO00-5987 ³	BC ₅ F ₅	300+	10	62.3	16.7	8.9	35.2	30.9	66.7	71.5
6717R1-01 ⁴	BC ₅ F ₆	300+	50	65.2	15.3	9.6	27.3	19.9	47.6	53.9
TO01-6529-9	BC ₅ F ₇	300+	10	59.2	19.4	11.8	33.2	32.2	66.0	69.9
TO01-6529-15	BC ₅ F ₇	300+	10	59.2	17.9	12.9	26.0	24.9	51.6	56.3
TO01-6529-20	BC ₅ F ₇	300+	10	52.6	24.0	12.7	40.7	34.6	76.0	79.6

¹3-Butenyl. ²Total Aliphatic. ³Code 6717 field 2000. ⁴Plant 1 from code 6717 rep 1.

Table 2. Seed quality characteristics of TO00-5987 related lines and checks in 1999, 2000, 2001 nurseries

Accession	Plant Gen.	Year	Oil % dry	Fatty acid (%) ¹					GSL ²	
				18:1	18:2	18:3	22:1	Satd	TotA ³	Total
TO00-5987	BC ₅ F ₅	2000	39.9	58.6	19.3	11.7	0.0	7.8	53.9	55.8
TO00-5987	BC ₅ F ₅	2001	44.5	60.9	17.6	11.2	0.4	7.4	59.4	63.6
TO01-6529-9	BC ₅ F ₇	2001	44.2	62.7	16.4	11.3	0.1	7.2	52.9	57.7
TO01-6529-15	BC ₅ F ₇	2001	44.3	63.4	15.5	11.3	0.2	7.3	53.7	58.7
TO01-6529-20	BC ₅ F ₇	2001	44.8	63.2	15.8	11.1	0.3	7.1	52.2	57.4
J90-4253	Check	1999	41.1	43.6	34.6	11.9	0.1	7.6	8.4	13.7
J92-223	Check	2000	42.9	41.1	37.4	12.3	0.0	6.5	12.2	16.8
T099-5406-5	Check	2000	43.1	45.4	33.1	12.5	0.0	6.8	32.9	36.1
S86-69	Check	2000	45.0	61.4	27.3	1.9	0.0	7.0	3.2	10.1
S86-69	Check	2001	44.5	62.6	24.3	2.9	0.2	7.4	7.0	15.2
AC Excel	Check	2000	45.8	64.7	17.6	8.8	0.0	6.4	10.4	14.0
AC Excel	Check	2001	44.8	63.7	18.4	8.8	0.0	6.9	6.0	12.1

¹Analysis on seed harvested from nurseries, avg. of 2 reps and 1 tent ²Glucosinolates (µmole/g seed). ³Total Aliphatic.

When accession TO00-5987 was tested in a replicated yield trial (Table 3; 4m x 4-row plot, 4 replicates) it was ranked no. 1 for yield, significantly outyielding the canola cultivar, AC Excel. It was equal to or better than other *B. juncea* lines such as J90-2741 (high oil line) and AC Vulcan (condiment mustard cultivar). Maturity was in the normal range for *B. juncea*. Oil content was also within the normal range for canola and significantly improved over the original 'canola quality' *B. juncea* (compared to J90-4316 a sister line of J90-4253). Saturated fat content is at near normal levels for canola (Tables 2 and 3). During the course of backcrossing the low glucosinolate profile of J90-4253 was lost (all tables).

Table 3. Performance of TO00-5987 and checks in 2001 yield at Saskatoon

Accession	Yield ¹		DTM days	Oil Content ¹		Fatty acid (%) ²					GSL ⁴
	kg/ha	Rank		% dry	Rank	18:1	18:2	18:3	22:1	Satd ³	
TO00-5987⁵	2513	1	84	44.2	7	56.2	21.5	10.7	1.2	7.0	53.0
J90-4316	2451	3	85	41.5	12	43.7	32.9	12.7	0.8	6.9	22.7
J90-2741	2318	6	90	50.0	1	18.3	17.3	9.2	37.9	5.2	82.1
AC Vulcan	2296	8	80	39.0	14	20.2	21.6	12.1	24.1	5.5	111.9
AC Excel	1960	14	89	45.4	2	64.8	17.6	8.4	0.0	6.9	21.0

¹Avg of 4 reps. ²Analysed 1 rep only. ³Saturated fat content of total fatty acids. ⁴Total glucosinolate content (μ mole/g seed), 1 rep only. ⁵Seed source was from 2000 nursery code 6717.

DISCUSSION

By an intensive backcrossing and reselection regime, we were able to stabilise the high oleic acid trait previously observed in early interspecific lines of *B. juncea* by Raney *et al.* (1995a) in progeny of accession TO00-5987 (BC₅F₅ generation). These progeny are shown to have fatty acid composition similar to *B. napus* and *B. rapa* canola. They also have a normal *B. juncea* phenotype, are fully fertile and perform normally. This germplasm represents a second source of endogenous high oleic acid oil content *B. juncea*, the first having been previously patented (Potts *et al.*, 2001).

(Arial 10 point, bold) ACKNOWLEDGEMENTS

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