Selection of near zero aliphatic glucosinolate *Brassica juncea* from an interspecific cross with *B. napus*

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ABSTRACT

Seed of 'zero' erucic acid, low glucosinolate *Brassica juncea* developed at the Saskatoon Research Centre had a low oleic acid content compared to canola. The glucosinolate content, although meeting the canola definition (<18 µmole total glucosinolate per g seed), was not as low as typical *B. napus* canola cultivars registered in Canada (<12 µmole total glucosinolate per g seed). 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) in order to improve the fatty acid composition, and in addition it was hoped that a further reduction in glucosinolate content could be achieved. The initial interspecific cross was followed by several backcrosses to *B. juncea* with half-seed selection for increased oleic acid phenotypes. Selection for low glucosinolate was achieved by vegetative tissue analysis of the selected seedlings, and by analysis of the seed produced. In the BC_2F_2 generation, plants were identified which had a near zero total aliphatic glucosinolate content. Several BC_4F_5 lines have been identified which not only perform well agronomically, but also have a total seed glucosinolate content of less than 7 µmole per gram seed (aliphatic glucosinolates <2 µmole per gram seed). Data from greenhouse grown plants is presented and the results of field trials of selected lines will be discussed. This germplasm will form the basis for new, improved varieties of *B. juncea* canola.

Key words: *Brassica juncea* – interspecific cross – glucosinolates

INTRODUCTION

Brassica juncea has been shown to have good adaptation to the hot dry 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 out perform 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 out yielded *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 a low oleic acid (18:1) content compared to canola (Raney *et al*., 1995a). Although they met the canola definition for glucosinolate (GSL) contents (<18 µmole/g seed), they were not as low as typical *B. napus* cultivars (<12 µmole/g: Rakow *et al.*, 1995). Efforts were made to select for lower contents of GSL, but the lowest achieved was about 12 µmole/g seed with J90-4253. 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 GSL *B. juncea* (Love *et al*., 1991), J90-4253, was crossed with a line of low linolenic *B. napus*. The F_1 was backcrossed to J90-4253. The BC_1F_1 progeny was advanced to BC_1F_2 via selfing and half-seed selection for high 18:1 and low linolenic acid (18:3) (Raney *et al*., 1995a). The BC_1F_3 and BC_2F_3 generations were backcrossed with J92-223, an improved 'canola quality' *B. juncea* (Rakow *et al.*, 1995). Followed by one more backcross to high yielding, high oil content, 'zero' erucic acid *B. juncea* at BC_3F_3. In between the backcrossing steps the progeny were advanced by selfing with half-seed selection for 'canola' fatty acid profile (increased 18:1, decreased 18:3)) and vegetative (leaf or bud) selection for low GSL content beginning with BC_2F_2. Refinement of fatty acid and GSL content selection was done on

5-20 seed bulk analyses of the selfed seed. When possible, progeny were evaluated in field nurseries. However, progeny failed to survive until 1999 (BC_4F_3 progeny evaluation). Fifty plants were harvested from code 88 of this nursery (accession TO99-5487). They and progeny from them were found to be among the lowest GSL lines in the 2000 and 2001 nurseries. In 2000 BC_4F_4 progeny were tested in a 2 rep 3m single row nursery. In 2001 BC_4F_4 to BC_4F_6 progeny were field-tested and a replicated yield test was grown of the 2000 nursery selections.

The fatty acid composition of seed samples was determined by gas chromatography of the methyl esters (Raney *et al.*, 1995b). The GSL 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

BC_2F_2 plants were identified with low GSL content (Table 1), 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 able to reselect low GSL progeny (Table 1). By the BC_4F_3 generation, the plants were healthy and normal in appearance and fertility. Table 2 compares low GSL BC_4F_3 and later progeny with parents and checks on seed harvested from field nurseries. Their aliphatic and total GSL is significantly lower than J92-4253 and other backcrossing parent lines. They have <8 µmoles/g seed total GSL compared to 12 for J90-4253

Table 1. Fatty acid and GSL content of pedigreed plants

Accession	Seed Gen.	Self Seed	Seed Anal.	Fatty acid (%) 18:1	18:2	18:3	GSL (µmole/g seed) Allyl	But[1]	TotA[2]	Total
TO93-0932-10	BC_1F_2	Few	0.5	49.8	34.0	6.4	Not Done.			
TO95-1318-1	BC_2F_2	Few	0.5	55.3	29.6	3.9				
TO96-1642	BC_2F_3	100	20	52.3	30.9	4.5	0.0	3.4	7.2	12.1
TO96-2087-2	BC_3F_2	150+	0.5	58.8	23.9	7.1	Not Done.			
TO96-3178	BC_3F_3	40	20	57.9	26.9	4.9	0.0	2.7	6.1	9.9
TO97-3419	BC_3F_4	50	10	52.7	29.2	6.0	0.0	12.7	13.6	17.3
TO99-5487	BC_4F_3	300+	20	51.3	30.8	7.4	0.0	1.8	2.0	4.6
TO99-5487-8*	BC_4F_4	300+	10	44.5	33.8	8.0	0.1	0.7	0.9	2.8
J99-88R2P13	BC_4F_4	Many	50	55.1	26.1	7.9	0.0	0.2	0.2	3.3
J99-88R2P15	BC_4F_4	Many	50	47.6	32.1	7.8	0.1	0.6	0.7	4.9

[1]3-butenyl. [2]Total aliphatic. [3]Code 6717 field 2000. [4]Plant 1 from code 6717 rep 1.

Table 2. Seed quality characteristics of low GSL lines and checks in 1999, 2000 and 2001 nurseries at Saskatoon[1]

Accession	Plant Gen.	Year	Oil % dry	Fatty acid[1] 18:1	18:3	Glucosinolate (µmoles/g seed)[1] Allyl	But[2]	TotA[3]	TotI[4]	Total
J99-88	BC_4F_3	1999	41.0	49.8	9.7	0.0	1.4	1.6	3.8	6.0
J00-6765	BC_4F_4	2000	44.0	48.1	10.2	0.0	0.8	1.1	3.1	4.4
J00-6769	BC_4F_4	2000	45.6	52.5	10.1	0.0	1.1	1.2	3.1	4.4
J00-6866	BC_4F_4	2000	43.3	48.7	9.6	0.0	2.3	2.7	3.9	6.7
J00-6868	BC_4F_4	2000	44.2	50.1	8.9	0.3	1.1	1.9	3.4	5.7
J01-3808	BC_4F_5	2001	42.9	49.7	8.5	0.0	1.5	1.9	4.2	6.2
J01-3838	BC_4F_5	2001	42.9	51.2	8.9	0.0	1.5	2.0	4.4	6.5
J01-3822	BC_4F_5	2001	41.6	50.3	8.7	0.0	2.0	2.5	4.0	6.5
J01-3823	BC_4F_5	2001	41.2	49.6	8.9	0.0	2.4	3.0	4.1	7.3
J90-4253	CQ Bj	1999	41.1	42.9	11.9	0.2	6.7	7.7	3.6	12.2
J92-223	CQ Bj	2000	42.9	41.1	12.3	0.2	10.6	12.2	3.9	16.8
J92-223	CQ Bj	2001	42.7	43.7	10.99	0.2	10.4	13.0	4.4	18.0
TO98-4692-1	Hi Oil Bj	2001	43.5	47.2	10.75	0.6	33.6	37.3	3.4	41.4
S86-69	*B. nap.*	2000	44.8	62.2	3.6	0.0	2.0	5.0	4.4	11.1
AC EXCEL	*B. nap.*	2000	43.9	64.7	8.8	0.1	3.8	10.4	2.9	14.0

[1]Analysis on seed harvested from nurseries, avg. of 2 reps and 1 tent [2]3-butenyl. [3]Total aliphatic. [4] Total indolylic

Their fatty acid composition is also somewhat altered from the original 'canola quality' *B. juncea,* having around 50% oleic acid with a reduced linolenic acid content. When the low GSL lines were tested in a replicated yield trial (Table 3; 4m x 4-row plot, 4 replicates) they performed normally, some outyielded the canola cultivar, AC Excel. They were not significantly different from 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 of these was also within the normal range for canola and some were significantly improved over the original 'canola quality' *B. juncea* (compared to J90-4316, a sister line of J90-4253).

Table 3. Performance of low GSL lines and checks in 2001 yield test at Saskatoon

	Yield[1]		DTM[1]	Oil Content[1]		Glucosinolate (µmoles/g seed)[2]					
Accession	kg/ha	Rank	days	% dry	Rank	Allyl	But[3]	HOB[4]	TotA[5]	TotI[6]	Total
J00-6765	2493	2	86	44.2	6	0.0	2.6	0.1	2.9	3.9	6.9
J00-6769	2361	4	85	44.7	4	0.7	1.9	0.2	2.9	3.5	6.7
J00-6866	2326	5	85	43.7	10	0.1	1.9	0.1	2.1	3.3	5.6
J00-6868	2170	12	86	39.1	13	0.0	2.1	0.3	2.7	3.1	5.9
J90-4316	2451	3	85	41.5	12	0.8	14.7	1.2	18.0	3.6	22.7
J90-2741	2318	6	90	50	1	73.9	3.3	0.2	77.5	1.9	82.1
AC Vulcan	2296	8	80	39	14	105.0	3.7	0.1	108.8	0.6	111.9
AC Excel	1960	14	89	45.4	2	0.1	4.6	8.5	14.7	4.7	21.0

[1]Avg of 4 reps. [2]Analyzed 1 rep only. [3]3-butenyl. [4]2-hydroxy-3-butentyl. [5]Total Aliphatic. [6]Total indolylic.

DISCUSSION

By an intensive backcrossing and reselection regime, we were able to stabilise the low GSL trait (<7 µmoles/g seed) in BC_4F_4 and later progeny of accession J99-5487 from an interspecific cross of 'canola quality' *B. juncea* with *B. napus*. They have a normal *B. juncea* phenotype, are fully fertile and perform normally with improved oil content. This germplasm represents a step forward in meal quality for 'canola quality' *B. juncea*. Crosses are underway to combine this trait with the high oleic acid trait described in Raney *et al.,* 2003, poster #243.

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