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Changes in the fatty acids in seeds of interspecific hybrids between *Brassica napus* and *Brassica juncea*

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Abstract. Mustard (*Brassica juncea*) accessions from Sri Lanka have a fatty acid profile (FAP) dominated by the undesired erucic acid. Therefore, it is necessary to develop *B. juncea* lines with canola-quality FAP, carrying reduced erucic acid (<1%) and increased oleic acid (>50%). To improve the FAP, *B. juncea* accessions were hybridised with spring-type canola (*B. napus*) varieties grown in Australia. Interspecific crosses between three *B. napus* cultivars (♂) and *B. juncea* accessions (♀) gave crossability of 50–65%.

Embryo culturing on Lichter medium overcame post-germination barriers to obtain F_1 plants. Culturing of ovules 21 days after pollination was successful and embryos were independent of hormones in the culture medium and directly developed into plants. Seeds of interspecific hybrids had a FAP different from parental values, particularly for oleic and erucic acids. The low oleic acid (13%) in *B. juncea* increased to 23–26% in hybrids and high erucic acid in *B. juncea* (41%) declined to 21–23% in hybrids. Linoleic and linolenic acids showed little variation from parental values. FAP of F_1 hybrids shifted towards that of canola quality. The F_2 seeds had zero erucic acid and high oleic acid similar to or exceeding the canola parent. Successful interspecific hybridisation of *B. juncea* and *B. napus* was confirmed by altered FAP and molecular markers. Embryo rescue in interspecific hybrids of *B. juncea* and *B. napus* is a simple, powerful biotechnological tool to increase genetic diversity and transcend species barriers to transfer desired genes, between the species. By implementing a crossing strategy, there is a potential to improve the FAP of Sri Lankan mustard towards the canola type.

Additional keywords: embryo rescue, erucic acid, canola quality mustard.

Introduction

Brassica oilseed species are an important source of vegetable oil. Among the many *Brassica* species the most common oilseed crops cultivated commercially are rapeseed (*B. napus* L. and *B. campestris* L.) and mustard [*B. juncea* (L.) Czern and Coss]. In Sri Lanka mustard is used as a condiment and in the Indian subcontinent it is also an important oilseed crop. Sri Lanka has many local accessions of *B. juncea* which are cultivated as a subsidiary crop and have undergone selection by the subsistence farmer for desirable agronomic characters such as drought tolerance and resistance to pests and diseases (Andrahennadi *et al*. 1991).

Canola is a major oilseed crop in the USA, Canada, Europe, China and Australia. Canola-quality *B. napus* genotypes are adapted to temperate regions and do not flower in the tropics due to their thermo- and photosensitivity. It requires cooler conditions and irrigation and hence cannot be introduced to the tropics. *B. juncea* has valuable agronomic characteristics (enhanced seedling vigor, higher tolerance to drought, blackleg disease resistance, pre-harvest shattering) and is gaining importance as an alternative to canola *B. napus* in the drier regions of the USA, Canada and Australia. In the drier areas of western Canada, *B. juncea* has shown good adaptation and is grown as condiment mustard (Woods *et al*. 1991). In the dry regions of southern Australia *B. juncea* breeding lines have significantly out-yielded *B. napus* cultivars (Burton *et al*. 1999).

Oils are an important component in human nutrition, besides proteins and fats, particularly as a high-energy food and to absorb fat-soluble vitamins. The health conscious consumer is also concerned by the fatty acid composition of fats and oils in terms of saturated and unsaturated fats. Saturated fats elevate blood cholesterol levels and are considered undesirable. Erucic acid has been implicated in cardiac necrosis and inhibition of fatty acid oxidation in the heart in rats (Christopherson and Bremer 1972; McCutcheon *et al*. 1976). Oleic acid – a monounsaturated fatty acid is desirable nutritionally and increases shelf life. An ideal oil composition is low saturated fatty acids (<6%), high oleic acid (>50%), moderate linoleic (<40%) and low linolenic (<14%). Such a composition is available in canola oil from *B. napus*, but not in *B. juncea*.

B. juncea genotypes with low erucic acid in their seed oil were first discovered in Australia in 1980. Early maturity, high-yielding Australian canola-quality *B. juncea* have been developed and are currently being crossed with higher oleic acid sources

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(Oram *et al*. 1999). In India, canola-quality *B. napus* was used to transfer the trait, high oleic acid content to *B. juncea* (Kaushik and Agnihotri 2000; Agnihotri *et al*. 2007). *B. juncea* plants with high oleic acid were selected and backcrossed to low erucic acid *B. juncea* to restore plant type. Similarly, Raney *et al*. (2003) in Canada crossed a *B. juncea* line with high erucic acid and low oleic acid with canola-quality *B. napus* followed by several backcrosses to *B. juncea*. They identified a canola-quality line from the BC5F5 generation.

Although *B. juncea* lines with canola-quality oil profiles have been developed in Canada (Raney *et al*. 2003) and Australia (Oram *et al*. 1999), such lines are suited to agro-climatic conditions in the country of origin. *B. juncea* accessions from Sri Lanka have a fatty acid profile (FAP) dominated by the undesired erucic acid (Iqbal *et al*. 2006). Therefore, it is necessary to develop *B. juncea* lines with canola-quality FAP, carrying reduced erucic acid (<1%) and increased oleic acid (>50%). In the absence of natural variation for these parameters, variation should either be introduced through mutagenesis or through hybridisation. Interspecific crosses between *B. juncea* and *B. napus* are usually incompatible due to pre- and post-fertilisation barriers such as pollination incompatibility, abortion of hybrid embryos and pre- and post-germination barriers (Nishiyama *et al*. 1991). Such barriers can now be overcome using biotechnological techniques of embryo rescue and *in vitro* culture of the embryo, enabling the transfer of genes across species barriers. Successful hybridisation will facilitate rapid improvement of the oil-quality parameters of Sri Lankan *B. juncea*.

Interspecific hybridisation has been achieved by ovary culture in *B. juncea* × *B. campestris* (Mohapatra and Bajaj 1988). Ovary, ovule and embryo culture were used by Bajaj *et al*. (1986) and Zhang *et al*. (2003) to recover hybrids of *B. juncea* × *B. napus*. Using embryo rescue, Ayotte *et al*. (1987) introduced genes for triazine resistance from *B. napus* to *B. oleracea* and Quazi (1988) transferred resistance to cabbage aphid from *B. oleracea* (cabbage) to *B. napus*. In India, canola-quality *B. napus* was used to transfer high oleic acid content to *B. juncea* by embryo rescue (Kaushik and Agnihotri 2000; Agnihotri *et al*. 2007). Besides transfer of genes across species, embryo rescue has also been used to resynthesise the allotetraploid *B. napus* from its diploid progenitor species *B. campestris* and *B. oleracea* (Olsson and Ellerström 1980).

Schelfhout *et al*. (2008) suggested that *B. juncea* would be a valuable source of genetic diversity in *B. napus* and vice versa if extensive backcrossing were not required to restore seed quality and agronomic traits. Selfing of interspecific hybrids should promote recombination among the shared A genomes of the two species and enhance genetic diversity from complex traits even if the selfed progeny revert back to one or the other parental genome.

To alter fatty acids in *B. juncea*, interspecific hybrid embryos should be recovered and a subsequent crossing strategy needs to be implemented to recover the parental plant type.

Embryo rescue is performed at various stages from the date after pollination. The hybrid embryos abort at various stages after pollination, depending on the genotype of the parental species. The objective of this study was to test the hypothesis that desirable genes for fatty acid composition can be transferred across the species from *B. napus* to *B. juncea* and stable embryos can be recovered or rescued *in vitro* and plants raised to maturity with an altered FAP.

Materials and methods

Three commercial spring-type canola cultivars of *B. napus* grown in Western Australia (Narendra, Outback and Monty) and *B. juncea* accessions (acc. 2180, acc. 0747, acc. 7700 and acc. 1099) obtained from the Plant Genetic Resources Centre, Gannoruwa, Sri Lanka, were grown in 13-cm-diameter pots using a standard potting mix in a greenhouse and watered daily at the Institute of Fundamental Studies, Kandy, Sri Lanka. Daytime temperatures were 26–30°C and relative humidity 70%.

Flowers of the female parent were opened and emasculated using fine forceps and fresh pollen from the male parent was transferred to the stigma. The pollinated flowers were covered with paper bags and tagged. Reciprocal crosses were undertaken for each of the crosses. Crossability was determined as a percentage of the pods formed for each cross combination.

Embryo rescue

Siliques were harvested 10 and 21 days after pollination. They were surface sterilised by wiping with 70% alcohol for 2 min and slit open along the suture and the seeds taken out. Seeds were sterilised by washing in 70% alcohol for 1 min followed with 0.5% sodium hypochlorite for 5 min and rinsed thrice in sterile water. The immature embryos were transferred to Lichter (1982) medium prepared with 3% sucrose, 3 g/L agar gel and pH adjusted to 5.7 before autoclaving at 121°C for 20 min at 15 psi. The embryos were cultured in Petri dishes (9.5 × 1.8 cm) and maintained under a photoperiod of 16 h provided by fluorescent bulbs (Thorn 40-W tropical daylight, Borehamwood, UK) at 26 ± 2°C. The developing embryos were acclimatised by transfer to liquid half-strength MS medium without sucrose followed by distilled water and placed in an environmental chamber for 1 week before potting in soil mix and transferred to a greenhouse. The mature plants were bagged at flowering to ensure self-pollination and seeds were collected for fatty acid analysis.

Data from the interspecific crosses and embryo rescue were analysed by one-way ANOVA using the statistics software MINITAB (Minitab 15 Statistical Software 2007, www.minitab.com).

Fatty acid analysis

Fatty acid composition of the seeds was determined by gas chromatography by the method developed at the Department of Crop Science, University of Göttingen (Thies 1971; Rücker and Röbbelen 1996). Seeds (200 mg) were ground in a mill and extracted with 1 mL Na-methylate-methanol (0.5 mol/L) for 20 min at 20°C and shaken. To this, 300 µL of iso-octane and 100 µL of 5% $NaHSO_4$ in water was added and shaken. The mixture was centrifuged for 3 min and ~200 µL of the upper phase was pipetted into a septum vial. From this 1.0–1.5 µL was injected into a gas chromatograph (Perkin Elmer 8600, Norwalk, CT, USA). The column was 0.25 mm × 25 m FFAP from Machery and Nagel, Duren, Germany, maintained at a temperature of 210°C isothermal, and 120 kPa H_2.

Mean values were obtained from three to four samples from each cross and duplicate gas chromatography measurements. Peaks were identified with a standard sample previously identified with standard fatty acids.

Molecular confirmation of F_1 hybrids

DNA extraction with leaf material of *B. juncea* and *B. napus* parents and F_1 hybrids was carried out by using a standard method (Chen and Roland 1999). Amplified fragment length polymorphism (AFLP) analyses were performed with minor modifications to the standard method described by Vos *et al*. (1995). Pre-amplification PCR were performed according to Vos *et al*. (1995). Selective amplifications were performed using 12 different selective amplification primer combinations, E11/M31, E11/M32, E11/M34, E10/M31, E10/M34, E12/M31, E12/M32, E13/M31, E13/M32 and E13/M34 (*Eco*RI primers were fluorescently labelled with HEX). Amplification products were purified by ethanol precipitation and separated by capillary electrophoresis using MegaBase 1000 (GE Healthcare Life Sciences, Piscataway, NJ, USA) automated DNA sequencer. AFLP bands (peaks) were scored as present (1) or absent (0) using Genetic Profiler software (GE Healthcare Life Sciences). Each peak was checked visually using electropherograms.

Simple matching indices (%) or Similarity index between the parents and F_1 hybrids were calculated using the following equation:

$$S = \frac{N_s}{(N_s + N_d)} \times 100 \qquad (1)$$

where, S = Similarity index, N_s = Number of similar bands, and N_d = Number of dissimilar bands. The analyses were carried out using SPSS/PC version 16.0 (www.spss.com).

Results

The number of successful crosses between *B. juncea* (♀) and *B. napus* (♂), although variable with the genotype of the *B. napus* parent, were not significantly different from each other. Crossability was moderate between the species ranging from 50 to 65%. Many of the pods were empty and the successful crosses produced 2.1–2.5 seeds per pod (Table 1). This is in contrast to 15 seeds per silique from selfing of the parents. The reciprocal crosses were less successful (5.2%, data not shown).

Embryo rescue was performed at various stages from the date after pollination. The hybrid embryos abort at various stages after pollination dependent on the genotype of the parental species. The culture of ovules 10 days after pollination was unsuccessful with all the crosses. After 21 days, it was possible to cut open the seed coat and squeeze out the embryo. The development stage of the embryo was 'walking-stick' stage. They were successfully cultured on Lichter (1982) medium. Phytohormones were not used in the cultures and callusing did not occur on any of the embryo cultures. The % of embryos germinated in different cross combinations was between 61 and 69% (Table 2). The germination of embryos *in vitro* were not statistically significant between the crosses ($P = 0.771$). The % of surviving hybrid plants ranged from 19 to 41% (Table 2).

To determine the success of the interspecific cross *B. napus* × *B. juncea* in improving FAP of Sri Lankan mustard accessions, the fatty acid contents of F_1 seeds were determined. Seeds from the interspecific hybrids had a FAP that differed from the parental values, particularly for oleic acid and erucic acid (Table 3 and 4). The low oleic acid in *B. juncea* (13%) increased to 23–26% in interspecific hybrids. The high erucic acid content in *B. juncea* (41%) declined to 21–23% in hybrids (Table 3). Linoleic and linolenic acid showed little variation from the parental values (Tables 3 and 4). The altered FAP of the F_1 seeds confirmed the success of the interspecific cross.

The F_1 seeds were planted and the plants selfed. Samples from the F_2 seeds from the cross *B. napus* (cv. Outback) × *B. juncea* (acc. 1099) and its reciprocal were analysed for their FAP (Table 4). This showed the F_2 plants tended strongly towards the canola parent. Seed set was much better than the F_1 plants, but required assisted pollination. The morphology of the plants resembled the canola parent. The prolonged vegetative period observed in canola before onset of flowering under local climate conditions was absent in the F_2.

The canola parent cv. Outback is a low linolenic and high oleic acid parent with a total fatty acid content of 97%. The unselected *B. juncea* parent is a high erucic acid and low oleic acid parent (Table 4). The F_2 plants from the selfed hybrids had zero erucic

Table 1. Interspecific crosses between *Brassica juncea* (♀) accessions (acc. 2180, acc. 0747, acc. 7700 and acc. 1099) and *B. napus* (♂) cultivars

Cross combination	No. of crosses	Pods set	% crossability	Seeds per pod
B. juncea × cv. Narendra	338	172	50.8	2.5
B. juncea × cv. Monty	293	184	62.7	2.1
B. juncea × cv. Outback	289	188	65.0	2.1

Table 2. Culture of F_1 embryos of *Brassica juncea*[A] × *B. napus* cultivars *in vitro*, 21 days after pollination, on Lichter medium (1982)

Cross combination	No. of embryos cultured	No. of embryos germinating (%)	Plantlets transferred to greenhouse (%)	Plants survival (%)
B. juncea × cv. Narendra	39	69.4 ± 8.2	67.8	19
B. juncea × cv. Monty	96	61.4 ± 22.3	57.1	12
B. juncea × cv. Outback	117	68.9 ± 15.3	65	41

[A]Accessions: 2180, 0747, 7700 and 1099.

Table 3. Selected mean fatty acid content (%) of seeds of F_1 hybrids of *Brassica juncea* × *B. napus* cvv. (±s.d.)

F_1 hybrids	Oleic acid C 18 : 1	Linoleic acid C 18 : 2	Linolenic acid C 18 : 3	Erucic acid C 22 : 1
B. juncea[A] × cv. Narendra	23.4 ± 2.6	20.8 ± 9.3	18.5 ± 2.4	23.0 ± 1.4
B. juncea[B] × cv. Monty	27.1 ± 0.8	20.1 ± 4.1	19.6 ± 2.3	23.21 ± 3.6
B. juncea[C] × cv. Outback	26.3 ± 4.7	14.0 ± 2.0	18.3 ± 2.0	21.8 ± 3.2

[A]Accessions: 2180, 7700 and 1099.
[B]Accessions: 1099, 7700 and 0747.
[C]Accessions: 0747, 2180 and 7700.

Table 4. Fatty acid profile (%) of parents and F_2 seeds from the cross *Brassica napus* (cv. Outback) × *B. juncea* (Acc. 1099) and its reciprocal (mean values ± s.d., from $n = 3$, 4 from duplicate measurements for each sample)

	Fatty acids	Acc. 16 : 0 Palmitic	Acc. 18 : 0 Stearic	Acc. 18 : 1 Oleic	Acc. 18 : 2 Linoleic	Acc. 18 : 3 Linolenic	Acc. 20 : 1 Eicosenoic	Acc. 22 : 1 Erucic
Parent	Outback	5.80 ± 0.01	2.25 ± 0.08	68.98 ± 0.56	16.06 ± 0.24	4.32 ± 0.26	1.04 ± 0.01	0
	acc. 1099	3.59 ± 0.09	0.92 ± 0.01	14.59 ± 0.12	17.09 ± 0.11	0.72 ± 0	10.08 ± 0.18	38.91 ± 0.03
F_2	Outback × acc. 1099	5.86 ± 0.05	2.21 ± 0.22	71.28 ± 1.04	14.98 ± 0.82	3.28 ± 0.43	0.94 ± 0	0
	Outback × acc. 1099	4.31 ± 0.15	2.58 ± 0.11	12.38 ± 0.07	22.75 ± 0.13	5.51 ± 0.01	6.8 ± 0.15	40.18 ± 0.08
	Outback × acc. 1099	5.40 ± 0.18	2.50 ± 0.16	69.72 ± 2.52	15.52 ± 1.40	3.29 ± 0.84	1.05 ± 0.06	0
F_2	acc. 1099 × Outback	5.51 ± 0.23	2.83 ± 0.26	69.42 ± 1.92	15.62 ± 1.32	3.02 ± 0.54	1.04 ± 0.06	0.02 ± 0.07
F_2	acc. 1099 × Outback	5.73 ± 0.08	2.29 ± 0.06	72.58 ± 0.63	14.03 ± 0.47	2.81 ± 0.18	0.98 ± 0.04	0

acid and high oleic acid similar to or exceeding the canola parent, except one cross which resembled the *B. juncea* parent in erucic acid and oleic acid content (Table 4). The small sample size did not show more segregation, with the segregants belonging to one of the parental genotypes.

To determine the degree of hybridity in the F_1 hybrids, three canola-quality *B. napus* cultivars and *B. juncea* accessions were crossed. The AFLP analysis of F_1 hybrids confirmed true hybridity of the hybrids obtained from crosses acc. 7700 × Narendra, acc. 2180 × Narendra, acc. 0747 × Narendra, acc. 7700 × Monty, acc. 2180 × Monty, acc. 0747 × Monty, acc. 7700 × Outback, acc. 2180 × Outback and acc. 0747 × Outback (Table 5).

According to Table 5, the F_1 hybrids of all cross combinations show partial similarity to their respective parents. For example the F_1 hybrid of the cross acc. 2180 × Monty shows 72% similarity to its *B. juncea* (acc. 2180) parent and 63% similarity to its *B. napus* (Monty) parent. A similar trend is observed for the rest of the F_1 hybrids. None of the F_1 hybrids have 100% similarity to their respective parents, which is a clear indication of their true

Table 5. Simple matching indices (%) between the parents and F_1 hybrids of *Brassica juncea* and *B. napus*. The similarity values for each cross combination are shown

Cross combination	F_1 (2180 × Monty)	F_1(7700 × Monty)	F_1 (0747 × Monty)	F_1 (7700 × Narendra)	F_1 (0747 × Narendra)	F_1 (2180 × Narendra)	F_1 (1099 × Narendra)	F_1 (7700 × Outback)	F_1 (2180 × Outback)	F_1 (0747 × Outback)	F_1 (1099 × Outback)
acc. 2180	72%	–	–	–	–	–	–	–	–	–	–
Monty	63%	–	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 7700	–	75%	–	–	–	–	–	–	–	–	–
Monty	–	70%	–	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 0747	–	–	79%	–	–	–	–	–	–	–	–
Monty	–	–	64%	–	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 7700	–	–	–	69%	–	–	–	–	–	–	–
Narendra	–	–	–	68%	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 0747	–	–	–	–	77%	–	–	–	–	–	–
Narendra	–	–	–	–	69%	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 2180	–	–	–	–	–	72%	–	–	–	–	–
Narendra	–	–	–	–	–	81%	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 1099	–	–	–	–	–	–	73%	–	–	–	–
Narendra	–	–	–	–	–	–	76%	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 7700	–	–	–	–	–	–	–	79%	–	–	–
Outback	–	–	–	–	–	–	–	60%	–	–	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 2180	–	–	–	–	–	–	–	–	76%	–	–
Outback	–	–	–	–	–	–	–	–	73%	–	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 0747	–	–	–	–	–	–	–	–	–	79%	–
Outback	–	–	–	–	–	–	–	–	–	63%	–
	–	–	–	–	–	–	–	–	–	–	–
acc. 1099	–	–	–	–	–	–	–	–	–	–	66%
Outback	–	–	–	–	–	–	–	–	–	–	77%

hybridity. The common genomes in the amphidiploid parents is indicated by >50% similarity values.

Discussion

Interspecific hybridisation with canola-quality *B. napus* offers a new strategy to improve the quality of fatty acids in *B. juncea* towards that of canola. We adopted the strategy of crossing the *B. juncea* accessions acclimatised to local agro-climatic conditions with canola-quality *B. napus* and embryo rescue *in vitro* to recover the interspecific embryos. The experiments supported our hypothesis that stable embryos can be recovered from the interspecific cross *B. napus* × *B. juncea* by embryo rescue and desirable genes for fatty acids can be transferred to *B. juncea*.

The interspecific crosses in this study between *B. napus* cultivars (male parent) and *B. juncea* (female parent) were successful (50–65%), however, the reciprocal crosses were much less successful (<5%). The parental influence in interspecific crosses between *B. napus* and *B. juncea* is not consistent. Schelfhout *et al*. (2006, 2008) observed *B. napus* as the most successful maternal parent in interspecific crosses with *B. juncea*. Zhang *et al*. (2003) obtained a better response with *B. juncea* (male) × *B. napus*. In contrast Bajaj *et al*. (1986) found the reciprocal cross between *B. napus* and *B. juncea* to be more successful. Similar genotypic dependence on interspecific hybridisation between *B. napus* × *B. juncea* were also reported by Honma and Summers (1976), as well as in intergeneric crosses between *Brassica* and *Sinapis* species (Momotaz *et al*. 1998). Thus the genotypic influence on the interspecific crossability is strong and a pool of genotypes from both species should be crossed reciprocally to identify the best combination of genotypes.

Embryo rescue and tissue culture can assist in the recovery of hybrid embryos from interspecific crosses. In this study embryos cultured after 21 days were independent of hormones in the culture medium. Bajaj *et al*. (1986) and Zhang *et al*. (2003) applied the hormones NAA and BAP to recover embryos from the interspecific cross *B. napus* × *B. juncea*. Embryo rescue from the intergeneric crosses of *Brassica* and *Sinapis* (Honma and Summers 1976) and interspecific crosses between *B. rapa* and *B. oleracea* also required hormones (BA and NAA) in the basal medium (Zhang *et al*. 2003).

The timing of embryo rescue is important to recover embryos that can directly develop into plantlets. Early-stage embryos in our experiments, 10 days after pollination, did not develop further while embryos cultured after 21 days developed into plants without an intervening callus stage. Early-stage embryos had a higher tendency to form calli on culture medium with auxins and cytokinins (Bajaj *et al*. 1986; Zhang *et al*. 2003) from which plant regeneration is time consuming and labour intensive. Mature embryos developed directly into plants as in the present study. Bajaj *et al*. (1986) also found that younger interspecific embryos from *B. napus* × *B. juncea* regenerated fewer plants than embryos older than 15 days after pollination. Older embryos of *B. napus* × *B. juncea* avoided callusing and regenerated more plants.

Schelfhout *et al*. (2006) observed substantial F_1 sterility in the cross *B. napus* × *B. juncea* in both cross directions and *B. napus* was the most successful maternal parent. The authors suggest that this indicates unbalanced chromosome behaviour among the Brassica genomes A, B, and C, especially when *B. juncea* was used as the female parent. We obtained similar results with our interspecific crosses between *B. napus* × *B. juncea*. Schelfhout *et al*. (2006) also observed that backcrossing to *B. juncea* resulted in low fertility as also observed by other studies (Roy 1980). Thus, generating sufficient variation from the segregating F_1 generation and selecting the desirable genotypes to be carried forward would be a better alternative.

Schelfhout *et al*. (2008) made interspecific crosses between canola-quality *B. napus* and near-canola-quality *B. juncea*. The dominant morphological type of the F_2 was that of the *B. napus* parent, with segregations in the later generations of a few *B. juncea* morphological types. These results are similar to our study, where F_2 seeds from *B. napus* (cv. Outback) × *B. juncea* (acc. 1099) and the reciprocal crosses had zero erucic acid and high oleic acid similar to canola, except one sample which was similar to *B. juncea*.

Similar results were obtained by Schelfhout *et al*. (2008), where lines from *B. napus* × *B. juncea* interspecific crosses showed the seed quality of some *B. juncea*-type progeny was improved beyond the parental type with higher oleic acid and lower glucosinolates. They further observed transgressive segregation for agronomic and quality traits from the selfed and backcrossed interspecific progeny.

Mustard, *B. juncea*, is a popular oil seed crop in South Asia. However, it has an unfavourable FAP dominated by the long-chain erucic acid (C 22 : 1). This study determined the possibility of *in vitro* embryo rescue of interspecific hybrids of *B. napus* and *B. juncea*. A successful interspecific hybridisation was confirmed by analysis of the F_1 seeds for fatty acids which showed a change in the erucic and oleic acids towards the canola type. Linoleic and linolenic acids, which were almost similar in *B. napus* and *B. juncea* remained more or less unchanged. The crossing strategy to alter the FAP in *B. juncea* is to initially recover F_1 hybrids and backcross to the desired parent. AFLP molecular markers confirmed the true hybridity of interspecific hybrids.

The lack of canola-quality progeny from wide crosses to introgress desirable traits from *B. juncea* into canola is a problem for canola breeders (Schelfhout *et al*. 2008). However, a study conducted by Weerakoon *et al*. (2010) using AFLP molecular markers showed that the *B. juncea* genotypes in Sri Lanka has a high level of genetic variation due to the low level of exploitation of *B. juncea* germplasm. This study has shown that canola-quality oil can be obtained from interspecific cross progeny through selfing and embryo rescue of the F_1 progeny.

The recovery of plants from interspecific crosses is dependent on the genotypic combinations, maturity of the embryo at time of rescue and culture medium. It is suggested to initially cross the available genotypes from the two species in all possible combinations. Embryo rescue should be attempted at a later stage, 15–20 days after pollination. A simple culture medium, without hormones, should be initially used for embryo rescue and hormones used subsequently, only if necessary.

Embryo rescue in interspecific hybrids of *B. juncea* and *B. napus* is a simple and powerful biotechnological tool to increase genetic diversity and transcend species barriers to transfer desired genes, between the species. It offers new

possibilities to improve a crop cultivated by subsistence farmers in Sri Lanka. By implementing a crossing strategy, the fatty acid composition of mustard can be improved towards canola quality, and introduce a nutritionally healthy oilseed crop.

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