Introgression of high oleic acid in Indian mustard through inter-specific hybridization

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Abstract

The rapeseed-mustard varieties presently being grown in India, contrary to the internationally accepted and nutritionally desired levels, have high erucic acid (40-50%) and low oleic acid (10-20%) in the seed oil. The high oleic acid is preferred for human nutrition, extended shelf life and better flavor. The present work was undertaken for introgression of high oleic acid content in *Brassica juncea* genotypes, TERI (OE) M07 and TERI (OE) M21, having 35-40% oleic acid. The early maturing *B. napus* genotypes TERI-Unnat [TERI (OE)R03; INGR 98002] and TERI-Shyamali [TERI (OE)R09; INGR 98005] having oleic acid 60-70%, were used as pollen donors for interspecific hybridization via sequential embryo rescue. The surface sterilized pollinated pistils were cultured *in vitro* on MS medium supplemented with Kinetin 1.0 ppm, napthalene acetic acid 0.1 ppm, gibberellic acid 1.0ppm and casein hydrolysate 10.0ppm. The excised ovules obtained from dissected ovaries were cultured on the same media till embryo emerged or rescued by dissecting ovules. The embryos were regenerated on MS medium containing one tenth concentration of growth hormones mentioned above for plantlet development. The plantlets were multiplied *in vitro* through axillary bud proliferation and 4-5 leaf growth stage plantlets were transplanted after hardening. These were selfed and back crossed to the *B. juncea* parent and the seeds were analyzed for their fatty acid profile through gas chromatography. The plants of F_1, BC_1 and BC_2 progenies were partially fertile having intermediate morphological traits, with oleic acid ranging from 36-68% and linolenic acid 5-11%. Plants having 45-50% oleic acid were forwarded in each generation. The plants were advanced till BC_4F_3 generation and stable incorporation of high oleic acid was confirmed. A strong association in plant type and oleic acid content was observed; the plants having upto 68-70%% oleic acid showed predominance of some of the *B. napus* morphological traits and the *B. juncea* type plants had oleic acid upto 50-53%. The advanced generation *B. juncea* lines were contributed to the All India Coordinated Research Project on Rapeseed-Mustard of Indian Council of Agricultural Research for multilocation evaluation.

Key words: *Brassica juncea*, interspecific hybridization, embryo rescue, fatty acid, half seed selection

Introduction

Among edible vegetable oils, rapeseed-mustard oil has the lowest amounts of saturated fatty acids (FA) and also contains nutritionally desired oleic acid along with two essential fatty acids, linoleic and linolenic. In India, *B. juncea* (Indian mustard) occupies 80% of the total acreage, followed by *B. rapa* and *B. napus* (rapeseed). However, the traditionally grown varieties of Indian mustard have high erucic acid and low oleic acid in the seed oil, thus so far the emphasis has been on successful reduction of erucic acid. Attempts towards introduction of variable fatty acid profile has recently reported generation of high oleic, low linolenic acid *B. juncea* in several countries (Stoutjesdijk et al. 1999, Oram et al. 1999, Potts et al. 1999) using either the RNAi or haploid mutagenesis approach. On the other hand in India recent efforts towards nutritional quality improvement have led to the development of several early maturing *B. napus* strains having low erucic acid/high oleic acid and the effort to popularize it with farmers is underway (Agnihotri et al 2004). However, *B. napus* cultivation is still restricted to a much smaller area as compared to *B. juncea*. Hence there is an imperative need to develop *B. juncea* varieties with desired fatty acid composition that are ideal for human nutrition and suitable to grow under wider agroclimatic conditions. Therefore, the present work was undertaken with the purpose to transfer high oleic acid trait from *B. napus* into *B. juncea* via application of *in vitro* embryo rescue, and to select the plants having comparatively high oleic acid and low linolenic acid content in the seed oil.

Materials and Methods

B. juncea strains TERI(OE)M07 and TERI(OE)M21, having low erucic acid and early maturity were chosen as female parents and the *B. napus* strains TERI-Unnat [TERI (OE)R03] and TERI-Shyamli [TERI(OE)R09] having low erucic acid and high oleic acid (60 to 70%) were selected as pollen donors. The interspecific crosses and sequential embryo rescue were performed as per Agnihotri (1993) using ovary culture on MS medium (Murashige and Skoog 1962) supplemented with Kinetin (Kn) 1.0 ppm, napthalene acetic acid (NAA) 0.1 ppm, gibberellic Acid 1.0ppm and casein hydrolysate 10.0ppm followed by culture of ovules disected from the cultured ovaries on the same media till embryos were sufficiently enlarged for disection. The embryos were regenerated on MS medium containing one tenth concentration of growth hormones mentioned above for plantlet development. The plants obtained from each hybrid embryo were multiplied through axillary bud proliferation. The micropropagated plantlets were rooted on MS medium containing Kn 0.1 ppm, NAA 0.01ppm and casein hydrolysate 50 ppm for 2-3 weeks. The 8-10 cm tall plantlets at 3-5 leaf growth stage were hardened and grown in pots in a growth chamber (Conviron PGV-36) with 14 h, 25°C/ 10 h, 15°C day/night cycles and 60% relative humidity. The hardened plants were transplanted in the field during 1998-99. The plants having closer resemblance to *B. juncea* plant type, with fully/partial pollen fertility were backcrossed (BC) to low erucic acid *B. juncea* female parent for restoration of plant type. The BC_1 self and open pollinated seeds were collected and used for fatty acid analysis by GC as per Kaushik and Agnihotri (1997). Recurrent backcrossing was performed till the fourth backcross and at each progeny level high oleic acid content was used as the selection criteria.

Results

Eight F_1 hybrids were realized and these showed intermediate morphological characteristics with *B. napus* type leaves. Some of these plants had either poor or profuse branching giving a bushy appearance. The flowers from these plants had rudimentary anthers with partial pollen fertility. Three F_1 hybrids had *B. juncea* type agronomic features while the rest resembled more to the *B. napus* parent. Only a few BC_1 seeds were obtained, with a majority of them being shriveled, and thus could not be subjected to fatty acids analysis. For this reason the open pollinated seeds set on the selected plants were analyzed for their fatty acids content. Only a few BC_1 seeds were obtained from four F_1plants resembling *B. juncea*, having partially or well developed anthers and good seed set. These were chosen for further progeny advancement and the seeds were grown in single plant progenies with recurrent backcrossing during three consecutive *Brassica* growing seasons. The BC_1 progenies segregated for three distinct plant types; resembling *B. juncea* (11-48%), resembling *B.napus* (10-18%) and intermediate type (41-90%). Thereafter, only the individual plants that had *B. juncea* characteristics were selected for recurrent BCs. Despite predominantly *B. juncea* type agro-morphological features the BC progenies showed segregation for leaf morphology wherein after each BC the proportion of plants with *B. juncea* type leaves increased by about 50%. While all the BC progenies had low erucic acid and moderate to low linoleic acid, the oleic acid content was higher in plants with *B. napus* type leaf morphology (Table 1). After $BC_{4,}$ the inbred lines were developed from plants selected for >50% oleic acid content. The FA profile of these lines is depicted in Figure 1. The high oleic acid trait was stable in these lines, however the *B. napus* type leaf morphology was a predominant feature in the lines having >60% oleic acid.

Table 1: The FA profile in recurrent backcross lines derived from *B. juncea/B. napus* interspecific crossing.

Generation/ parents	*FA profile (%)							
	Plants with *B. juncea* type leaves				Plants with *B. napus* type leaves			
	Oleic	Linoleic	Linolenic	Erucic	Oleic	Linoleic	Linolenic	Erucic
B. juncea ♀ parents	34.4-40.6	36.3-36.5	15.2-16.4	0-2	-	-	-	-
B. napus ♂ parents	-	-	-	-	59.5-70.2	13.3-20.9	12.5-7.3	0-2
BC_1	38.5-41.9	36.5-42.3	7.8-10.8	0-2	36.9-58.2	27.4-40.2	5.1-11.3	0-2
BC_2	40.2-50.0	28.9-40.1	12.2-22.5	0-2	45.6-62.0	23.5-27.4	6.1-11.1	0-2
BC_3	41.2-49.6	20.9-38.6	11.3-19.7	0-2	59.3-63.5	19.5-20.1	6.8-11.0	0-2
BC_4	44.1-50.5	16.2-32.1	9.1-15.4	0-2	64.1-68.4	14.6-19.5	6.7-10.8	0-2

*Values represent range

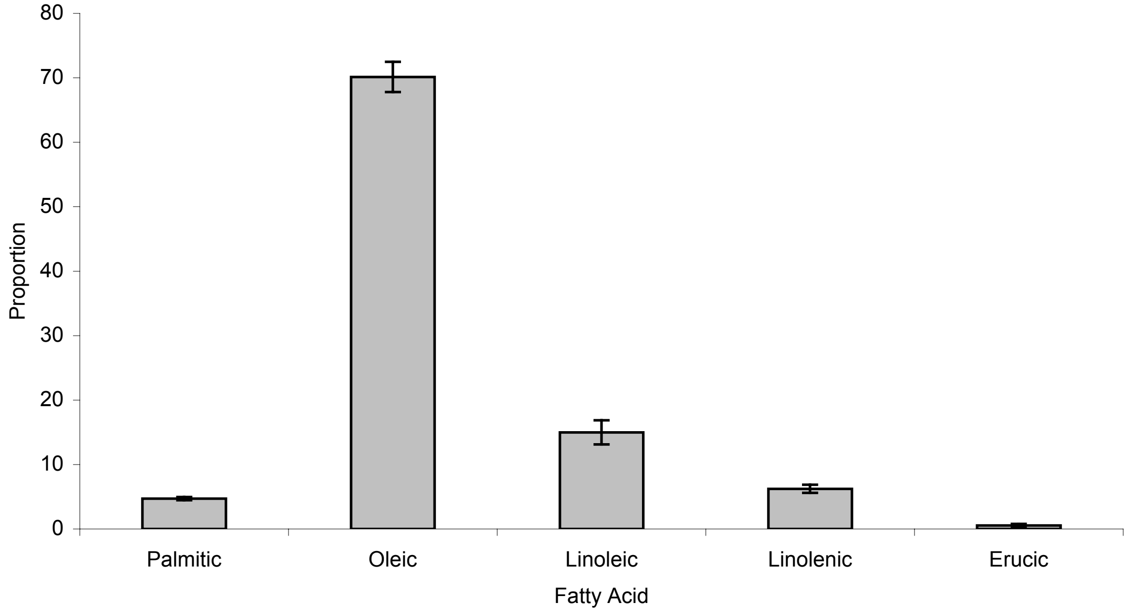

Figure 1: The FA profile of BC_3F_4 plants with high oleic acid content and *B. napus* type leaf morphology. Each histogram represents average FA proportion estimated from three replicate GLC analyses of pooled self pollinated seeds of six lines. The bars indicate standard error of mean.

Discussion

B. juncea and *B. napus* share a complementary A-genome and the complementarity between the B and C genome has recently been established (Hasterok et al., 2005). Thus the two species share elasticity for horizontal gene transfer and this premise was tested in the present work for transferring the high oleic acid trait across the species boundary. Such interspecific crosses have been carried out for development of yellow seeded *B. napus*, transferring resistance to blackleg from *B. juncea* to *B. napus* (Roy, 1978), vice versa for white rust (Gugel et al., 1999) and for transferring low glucosinolate genes to *B. carinata* using *B. juncea* as a bridge species (Getinet et al., 1997). However, the linkage of morphological trait such as *B. napus* leaf characteristics to a biochemical trait, high oleic content in the present study, has not been reported yet. Such a phenomenon is possible if recurrent backcrossing created an alien *B. napus* chromosome substitution in *B. juncea* background and the genes for high oleic and *B. napus* type leaf characteristics were contributed from this genomic region. However, detailed cytological/ molecular confirmation is required to prove this hypothesis.

Conclusions

The wide hybridization, aided through in vitro ovary/ ovule/ embryo rescue has remained one of the important tools for introgression of economically important traits across species barrier (Shivanna, 1996). In *B. juncea* the increase in oleic acid content with a corresponding reduction in erucic acid content is less pronounced as compared to *B. napus,* where a quantum jump in the amount of oleate results from reduction of erucic content to zero (Banga et al. 1998). Similar results are obtained in the present study, where an increase in oleic acid content upto 50% has been obtained in plants resembling *B. juncea* against the oleic acid content of upto 70% in plants still having leaf type resembling *B. napus.*

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