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RESEARCH ARTICLE

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Broadening the genetic base of Abbysinian mustard (*Brassica carinata* A. Braun) through introgression of genes from related allotetraploid species

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Abstract

Brassica carinata (BBCC, 2n = 34) has still to emerge as a major oilseed crop owing to poor agronomic attributes like long stature, long maturity duration and low seed yield. The restricted amount of genetic variability available in natural *B. carinata* necessitates utilization of new sources of variability for broadening its genetic base. Interspecific hybridization followed by selection in selfed and back cross progenies was employed to generate useful variability into *B. carinata* cv 'PC5' from elite lines of *Brassica napus* (AACC, 2n = 38) and *Brassica juncea* (AABB, 2n = 36). The morphological evaluation of 24 stable introgressed progenies revealed wide range of variability for key economic traits. The progenies with mean maturity duration of 161 ± 2.1 days, short stature of 139.5 ± 6.5 cm and seed yield per plant of 18.6 ± 2.0 g in comparison to the corresponding figures of 168 ± 4.6 days, 230.6 ± 12.7 cm and 12.0 ± 2.4 g in 'PC5' (recurrent parent) were recovered. Diversity analysis at morphological level revealed that 22 out of 24 stable introgressed progenies were grouped with *B. carinata* 'PC5' at average taxonomic distance of 1.19. The diversity at molecular level using 25 polymorphic and reproducible RAPD primers revealed that 19 out of 21 introgressed progenies grouped with *B. carinata* 'PC5' at a similarity coefficient of 0.68. The clusters in general represent a wide range of genetic diversity in the back cross lines of *B. carinata* as a result of introgression of genes from elite lines of *B. napus* and *B. juncea* parents.

Additional key words: interspecific hybridization; gene introgression; variability; genetic relatedness.

Introduction

Brassica carinata, which is also known as Abyssinian mustard, is native to northeastern Africa and thrives in climates and soil types similar to those found in its native region. It is a member of the Triangle of U species (U, 1935) in the agriculturally significant *Brassica* genus and is thought to have resulted from an ancestral hybridisation event between *Brassica nigra* (genome composition BB) and *Brassica oleracea* (genome composition CC) (Prakash & Hinata, 1980). *B. carinata* is cultivated as an oilseed crop in Ethiopia (Alemayehu & Becker, 2002), and its oil is more often used as lubricant or water repellant because of its generally high levels of undesirable glucosinolates and erucic acid (Getinet *et al*., 1997). This plant is also investigated to develop bio-fuel for jet engines. On October 29 of 2012, the first flight of a jet aircraft powered with 100% biofuel, made from *B. carinata*, was completed (http://www.asdnews.com/news-46032/First solely biofuel jet flight raises clean travel_hopes.htm). The crop is currently being evaluated as an option to the traditional canola/mustard cultivation, especially for low rainfall areas of the world. In its area of adoption, the crop has been shown to possess acceptable seed yield levels as well as resistance to various biotic and abiotic stresses (Getinet *et al*., 1996). In spite of these strong positive attributes, the crop

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Abbreviations used: PCR (polymerase chain reaction); PIC (polymorphic information content); PMC (pollen mother cells); RAPD (random amplified polymorphic DNA); UPGM (unweighted pair group method).

suffers from several agronomic limitations like long cycle, poor harvest index and long plant stature. Restricted level of natural variability for specified traits has greatly constrained the breeding programs aimed at overcoming these limitations (Song *et al.,* 1988; Prakash & Chopra, 1991). Several breeding options, like varietal hybridization, induced mutagenesis and to a limited extent artificial resynthesis from the progenitor diploid species, have been explored in the past with poor selection advances for yield and component traits. Induced mutagenesis has, however, helped significantly to improve seed quality profile (Barro *et al*., 2003). The artificial resynthesis of *B. carinata* from diploid progenitor species of *B. oleracea* and *B. nigra* has been attempted in *B. carinata* to enhance the spectrum of variability for key economic traits (Song *et al.,* 1993). However the resynthesis route has not been very productive as none of the two diploid progenitor species (*B. oleracea* and *B. nigra*) had any history of human selection pressure for evolution as an oilseed crop. Consequently the resynthesized *B. carinata* versions show poor breeding value. Present investigations were thus undertaken to generate variability for key economic traits in *B. carinata* through selective introgression from the related and agronomically superior amphiploid species like *B. napus* and *B. juncea*. Such an approach was also thought useful for mobilizing gross structural modifications that occurred in cohabiting genomes of *Brassica* allotetraploid during their evolution following natural amphiploidy (Song *et al.,* 1995). A departure from routine interspecific hybridization experiments was the deliberate use of donor species (*B. napus/B. juncea)* as female parent in both crosses to benefit from altered nucleo-cytoplasmic interactions.

Material and methods

Interspecific hybridization

Brassica carinata 'PC5' (BBCC) was crossed as male/recurrent parent with different elite cultivars of *B. juncea* (AABB) and *B. napus* (AACC). The varieties involved in the crossing programme were 'NHO 7-10' and 'MHO 18-1-184' (*B. napus*) and 'NUDH-YJ-4' and 'NJHO3-25' (*B. juncea*). At flowering the buds of the female *B. napus* and *B. juncea* plants that were about to open the following day were emasculated and immediately pollinated with fresh pollen from the male *B. carinata*. The pollinated buds were covered with glassine bags for one week to avoid contamination with foreign pollen. The F_1 seeds could be harvested from *B. napus* 'NHO 7-10' × *B. carinata* 'PC5' and *B. juncea* 'NUDH-YJ-4' × *B. carinata* 'PC5' crosses only. The F_1 plants of both interspecific crosses were backcrossed to *B. carinata* with the objective to eliminate unwanted A genome chromosomes and improve fertility and seed set. Backcross plants (BC_1) from both interspecific crosses which were morphologically similar to *B. carinata* and had high pollen grain stainability were backcrossed to recurrent parent as well as selfed to raise the BC_2 and BC_1 F_2 generations, respectively. The pollen fertility of hybrids and different advance generation derivatives was determined by staining pollen grains of mature undehisced anthers with 2% acetocarmine. Intensely stained and normal shaped pollen grains were scored as fertile while the unstained and collapsed ones were scored as sterile. Cytological investigations were carried out to confirm hybridity and assess genomic affinity between various genomes by studying meiotic configurations in F_1 hybrids and backcross generations. The flower buds were fixed around 6.00 to 8 a.m. in Carnoy's solution II (ethanol: chloroform: acetic acid; 6:3:1), containing a few drops of ferric acetate (a filtered solution of saturated ferric acetate made by adding ferric chloride to glacial acetic acid). After 48 hours of fixing, the young anthers were crushed in 2% acetocarmine on a slide and observed under inverted microscope to study the chromosome number and pairing behaviour of chromosomes in hybrids and backcross generations.

Morphological assessment of introgressed lines

The 24 BC_1 F_2/BC_2 progenies with high pollen fertility and morphologically similar to *B. carinata* along with 3 parental and 2 non-parental checks (Table 1) were raised in randomized block design with two replications at the Oilseed Research Farm of Punjab Agricultural University, Ludhiana, India. Each replication comprised of 24 entries grown in paired rows per entry with row length of 5 m. The row-to-row spacing was maintained at 30 cm. Data were measured with respect to pollen fertility (%), plant height (cm), primary branches per plant, secondary branches per plant, silique on main shoot, main shoot length (cm), siliqua length (cm), seeds per silique and seed yield per plant (g). Data were recorded on 10 random plants per entry in each

Table 1. Details of advanced introgression lines of *B. carinata* and their parents used in assessing variability and genetic diversity

Progenies	Cross combinations	Generation
BJC1, BJC8, BJC9, BJC19, BJC21, BJC23, BJC24, BJC28, BJC30, BJC31	(*B. juncea* × *B. carinata*) × *B. carinata*	BC_2
BNC1, BNC2, BNC3, BNC4, BNC5	(*B. napus* × *B. carinata*) × *B. carinata*	BC_2
SNC2, SNC3, NC55-10	(*B. napus* × *B. carinata*) × *B. carinata*	BC_1F_2
SJC9, SJC19, SJC22, SJC24, SJC25, SJC29, SJC30	(*B. juncea* × *B. carinata*) × *B.* carinata	BC_1F_2
PC5	*B. carinata*	Parent
NUDH-YJ-4, NJHO-325	*B. juncea*	Parent
NHO7-10, MHO-18-1-184	*B. napus*	Parent

replication and averaged to per plant basis. Besides these morphological traits, days to maturity and days to flowering was also recorded on per plot basis.

Data analysis

The mean data collected on various morphological traits varied with the unit of measurement; hence the means of morphological observations were standardized prior to cluster analysis by dividing these with standard deviation and subtracting the means for each trait. This allowed avoiding the bias in calculating Euclidean distances due to scale differences in the variables. The resulting Euclidean dissimilarity coefficients were calculated for 11 variables to find out the genotypic relationships using NTSYS (Numerical Taxonomic and Multivariate Analysis System), PC vers. 2.1 (Rohlf, 1998). Unweighted pair group method with arithmetic average (UPGMA) cluster analysis was performed producing a dendrogram, depicting relationships among the lines relative to morphological traits (Sokal & Michener, 1958).

RAPD analysis

Total genomic DNA from 21 stable BC_1F_2/ BC_2 progenies (progenies with high pollen fertility and morphologically similar to *B. carinata*) and three parental checks viz. *B. carinata* 'PC5', *B. juncea* 'NUDH-YJ-4' and *B. napus* 'NHO7-10' were isolated using the standard procedure of Bhaskar *et al*. (2002) at seedling stage. The DNA quantity and quality was assessed by electrophoresing DNA samples in agarose gel (0.8%) stained with ethidium bromide (1%), using 0.5X TBE electrophoresis buffer. Quantitative estimates of sample DNA were made by its visual comparison with DNA of known concentration. Quality of samples was judged based on whether the sample DNA formed a single high molecular weight band (good quality) or a smear (poor quality). About 100 random decamer primers were screened, out of which 25 primers were selected on the basis of their polymorphic nature (Table 2). PCR was performed in 25 μL of reaction mixture containing 1X *Taq* assay buffer, 0.5 units of *Taq* DNA polymerase, 200 μM of each dNTP (Banglore Genei Pvt. Ltd., India), 0.2 μM each primer and 50 ng of template DNA. The PCR reaction mix was placed in thermal cycler for amplification (MJ Research, PTC200). The PCR reaction was repeated thrice for each primer to ensure the reproducibility of RAPD results. The PCR amplification conditions for RAPD consisted of an initial step of denaturation at 94°C for 4 min followed by 44 cycles of denaturation at 94°C for 1 min and elongation at 72°C for 2 min followed by final step of extension at 72°C for 4 min. PCR products were fractionated on 1.2% agarose gel containing 0.5 μg μL^{-1} ethidium bromide. After separation, gels were illuminated using UV trans-illuminator and photographed using Gel Genius Photo Documentation System. Numbers of amplified bands were counted for each primer-genotype combination. RAPD bands were scored as present (1) or absent (0) and data were analysed using NTSYSpc version 2.1 (Rohlf, 1998). Similarity matrices generated according to the coefficient of Jaccard (Sneath & Sokal, 1973) were used to perform the cluster analysis using UPGMA (Sokal & Michener, 1958). Dendrograms indicating the estimated similarity of the newly developed lines with their parents were constructed with the tree programme of NTSYSpc.

Table 2. Primers, their sequence and level of polymorphism in BC_1F_2 and BC_2 progenies of *B. carinata*

Primer	Sequence (5' → 3')	No. of bands	Polymorphic bands	Polymorphic (%)	PIC
BG12	ACAGGTGCGT	6	6	100.0	0.81
BG13	ACAGGTGCTG	6	5	83.5	0.83
BG18	ACCCGGAAAC	6	6	100.0	0.79
BG23	ACCTTTGCGG	5	4	80.0	0.80
BG27	ACGCGCATGT	10	10	100.0	0.87
BG28	ACGGACGTCA	6	3	50.0	0.75
BG29	ACGGATCCTG	7	4	57.1	0.82
BG31	ACTGGCTCTC	7	5	71.4	0.83
BG35	AGCAGGTGGA	6	5	83.3	0.80
BG36	AGCCAGCGAA	5	3	60.0	0.70
BG39	AGCGTGTCTG	9	8	88.8	0.88
BG51	CAAAGCGCTC	10	10	100.0	0.87
BG52	CAATCGCCGT	10	10	100.0	0.88
BG57	CACCTTTCCC	5	5	100.0	0.79
BG60	CAGCACTGAC	8	7	87.5	0.86
BG62	CAGCGACAAG	4	4	100.0	0.70
BG64	CAGCTCACGA	7	6	85.7	0.82
BG88	CCTGCTCATC	6	4	66.6	0.80
BG93	CTCAGTCGCA	7	7	100.0	0.83
BG100	CTGATACGCC	3	3	100.0	0.65
BG104	CTGCTTAGGG	6	5	83.3	0.77
BG113	GACAGTCCCT	7	7	100.0	0.74
BG114	GACGGATCAG	6	5	83.3	0.82
BG121	GAGGTCCACA	11	9	81.0	0.89
BG123	GGAACCCACA	5	4	80.0	0.79
Total		168	146	86.9	

PIC: polymorphic information content.

Results

The interspecific hybrids exhibited intermediate plant morphology and were partially male fertile (15-20%). The cytological studies of *B. juncea* × *B. carinata* hybrids and its advanced backcross generations with *B. carinata* as recurrent parent (Fig. 1) showed F_1 plants with the expected somatic chromosome number (2n = 35). The predominant meiotic configuration was 11II +13I (Table 3). The bivalents are shown with small arrowheads in the Figs. 1 and 2. The BC_1 plants exhibited varying number of chromosomes from 28 to 35 with 12II + 11II + 3I as predominant meiotic configuration (Table 4). Meiotic analysis of representative

Table 3. Chromosome pairing in interspecific hybrid (F_1) of two crosses of *Brassica* species. Figures in parentheses indicate frequency

Cross combination	No. of PMCs observed	Mielotic configuration				
		11II + 1IV + 10I	12II + 1III + 9I	Mean bivalent frequency	Mean trivalent frequency	Mean quadrivalent frequency
B. napus × *B. carinata*	97	55 (0.57)	42 (0.43)	11.4	0.4	0.6
B. juncea × *B. carinata*	62	11II + 13I		12II + 1IV + 7I	Mean bivalent frequency	Mean quadrivalent frequency
		42 (0.68)		20 (0.32)	11.3	0.3

PMC: pollen mother calls.

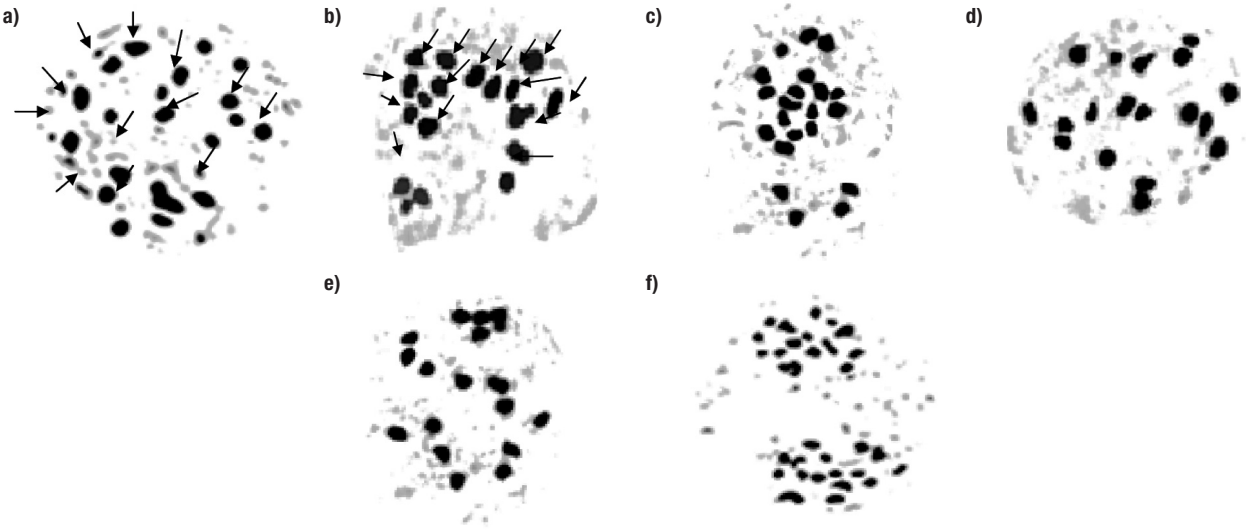

Figure 1. Meiotic configuration in pollen mother cells (PMCs) of *Brassica juncea* × *Brassica carinata* hybrid (a), BC_1 (b-c) and BC_2 (d-f). Distribution at anaphase: (a) 12II + 1IV + 7I, (b) 14II + 1III + 3I, (c) 17II, (d) 16II + 2I, (e) 17II, and (f) 17-17. Arrows indicate IIs.

plants of each BC_2 progeny revealed a chromosome number of 2n = 34 and high (16.88) mean bivalent frequency (Table 5). In the cytological studies of F_1 and advanced backcross generations of *B. napus* × *B. carinata* with *B. carinata* as recurrent parent (Fig. 2) the pollen mother cells (PMCs) of *B. napus* × *B. carinata* F_1 plants revealed a somatic chromosome number of 2n = 36 with varied occurrence of univalents and bivalents (Ta-ble 2). The BC_1 of *B. napus* × *B. carinata* exhibited a varying number of chromosomes. The somatic chromosome number varied from 29 to 41 (Table 3). In BC_2 cytological studies revealed varied chromosome number (2n = 34-35) in different plants with 17 IIs being the predominant meiotic configuration (Table 4). Backcrossing selected BC_1 plants with *B. carinata* in both interspecific crosses, helped to improve the meiotic stability and consequently the pollen grain fertility. Selected BC_1 plants were also selfed to raise BC_1F_2 generation. Intensive selection for *B. carinata* type plants with higher pollen grain fertility was carried out. The results revealed that there were more BC_2 and BC_1F_2 plants with *B. juncea* parent than with *B. napus* parent due to the fact B genome has significant evolutionary divergence from A/C genomes. The assessment of morphological traits of 24 stable introgressed lines along with three parental and two non-parental checks revealed excellent variability in key economic traits among the introgression lines (Ta-

Table 4. Chromosome pairing in BC_1 progenies of the cross of two crosses of *Brassica* species. Figures in parentheses indicate frequency

Cross combination	No. of PMCs observed	Meiotic configuration							
(*B. napus* × *B. carinata*) × *B. carinata*	144	10II + 9I 8 (0.06)		11II + 13I 9 (0.06)		11II + 10I + 1IV 16 (0.11)		12II + 64 (0.45)	12II + 32 (0.22)	13II + 15 (0.10)
(*B. juncea* × *B. carinata*) × *B. carinata*	122	10II + 2III + 2I 5 (0.04)	11II + 1IV + 5I 21 (0.17)	12II + 1III + 3I 41 (0.34)	13II + 1IV + 4I 17 (0.14)	14II + 1III + 3I 15 (0.12)	16II + 1III 11 (0.09)	16II + 3I 6 (0.05)	17II + 1I 4 (0.03)	17II 2 (0.02)

PMC: pollen mother cells.

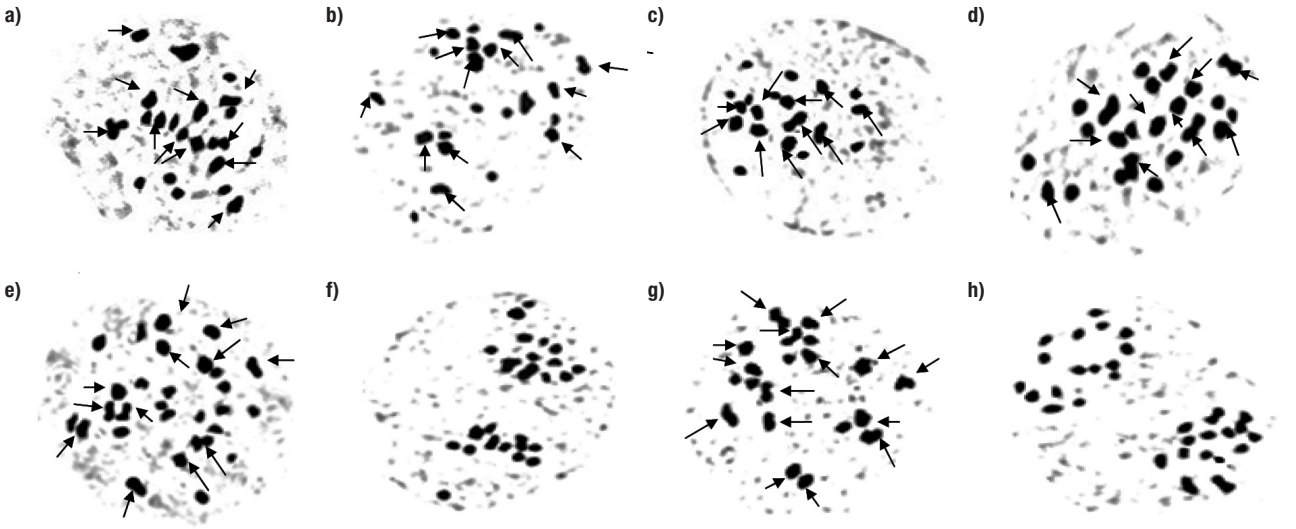

Figure 2. Meiotic configuration in the pollen mother cells (PMCs) of *B. napus* × *B. carinata* hybrid (a-b), BC_1 (c-f) and BC_2 (g-h) generations. Distribution at anaphase: (a) 11II + 1IV + 10I, (b) 12II + 1III+9I, (c) 10II + 9I, (d) 11II + 12I, (e) 12II + 16I, (f) 16-13, (g) 15II + 5I, and (h) 18-16. Arrows indicate IIs.

ble 6). High mean pollen grain stainability in many progenies emphasized their meiotic stability. There was a significant reduction in plant height in most of the lines as against the recurrent *B. carinata* parent ('PC5'). Days to maturity revealed a significant reduction in the progenies viz. BJC23, BJC30 and SNC2 over the check ('PC5'). The seed yield per plant revealed significant increase in the progeny SJC19 (18.6 ± 2.0 g) as against the recurrent *B. carinata* parent (12.0 ± 2.4 g). The mean morphological data of the cross progenies and their parents was subjected to diversity analysis. The results (Fig. 3) revealed that 22 out of 24 BC_2 and BC_1F_2 progenies were grouped with *B. carinata* 'PC5' at an average taxonomic distance of 1.19. Two progenies BJC 28 and SJC 24 were most diverse from all other progenies as well as the parents by a taxonomic distance of 2.88. The *B. napus* and *B. juncea* parental lines showed taxonomic distance of 1.61 with all introgression lines except two lines, BJC28 and SJC24.

We analyzed these 24 advanced BC_1F_2/BC_2 progenies, 21 progenies and 3 parental strains using 25 polymorphic and reproducible RAPD primers (Fig. 4).A total of 168 amplicons (Table 2) were obtained with an average of 6.72 bands per primer; 146 bands were polymorphic and the level of polymorphism was 86.9%. The PIC values were high for all the primers tested and ranged from 0.65 for BG100 to 0.89 for BG121, with an average of 0.80 for all the primers. The resultant dendrogram (Fig. 5) discriminated all the genotypes into three main groups with 21, 2, and 1 genotypes present in groups I, II, and III respectively. The 19 introgression lines in group I shared 68% similarity with the recurrent parent ('PC5') sharing 74% similarity

Table 5. Chromosome pairing in BC_2 progenies of two crosses of *Brassica* species. Figures in parentheses indicate frequency

Cross combination	No. of PMCs observed	Meiotic configuration			
(*B. napus* × *B. carinata*) × *B. carinata*	81	15II + 5I 17 (0.20)	16II + 3I 11 (0.14)	17II 53 (0.66)	Mean bivalent frequency 16.4
(*B. juncea* × *B. carinata*) × *B. carinata*	85	16II + 2I 10 (0.12)		17II 75 (0.88)	Mean bivalent frequency 16.88

PMC: pollen mother cells.

among themselves and 68% similarity with subgroup II of group I. The subgroup II, comprising 10 progenies including *B. juncea* parent (NUDH-YJ-4), shared 68% similarity among them. Two introgression lines (SJC22-2 and BJC23-2) formed a separate cluster at a similarity level of 59% with recurrent parent 'PC5' and 68% with *B. juncea* parent (NUDH-YJ-4). *B. napus* parent (NHO7-10) was grouped into a separate cluster with a similarity of 0.57 with all the genotypes, whereas NUDH-YJ-4 shared more than 69% similarity with 19 introgression lines. Both RAPD markers and morphological characteristics clustered the genotypes into three main groups. Group I comprised the majority of introgressed lines of *B. carinata* along with recipient *B. carinata* parent ('PC5'). *B. napus* and *B. juncea* were grouped together (group II) while group III contained progenies that were highly diverse from recipient *B. carinata* parent.

Discussion

Interspecific hybridization in the past among *Brassica* allotetraploids has helped to identify variation for earliness (Rao *et al*., 1993), yellow seed color (Szestowicka *et al*., 1995) and resistance to shattering (Prakash & Chopra, 1988, 1990). In the present work, *B. carinata* was crossed as a male with different elite cultivars of *B. juncea* and *B. napus* with the objective of enriching B and C genome(s) of *Brassica carinata* with B and C genomes of *B. juncea* (AABB) and *B. napus* (AACC) respectively. F_1 plants of both interspecific crosses were selfed as well as backcrossed to *B. carinata* to eliminate unwanted A genome chromosomes and improve fertility and seed set. The occurrence in *B. juncea* × *B. carinata* and *B. napus* × *B. carinata* of higher (8 and 9, respectively) number of bivalents than expected, and the presence of multi-

Table 6. Variability for morphological traits in introgressed progenies developed through interspecific hybridization

Entry	Pollen fertility (%)	Days to flowering	Days to maturity	Plant height (cm)	Primary branches plant^{-1}	Secondary branches plant^{-1}	Siliques on main shoot	Main shoot length (cm)	Silique length (cm)	Seeds per silique	Seed yield per plant (g)
BNC1	60.0±2.1	109.5±2.4	169±2.5	196.4±13.0	22.6±2.5	89.4±±10.0	15.2±2.1	35.6±2.5	4.5±0.2	10.8±1.5	12.3±1.2
BNC2	86.6±4.2	104.5±11.2	171±2.7	214.5±20.5	23.3±1.8	86.2±11.2	24.5±2.0	41.5±2.5	4.0±0.5	11.4±1.1	13.2±1.1
BNC3	62.4±8.3	107.2±726.5	168±2.3	179.8±6.5	16.9±1.5	68.1±7.9	17.7±3.0	32.8±2.9	4.1±0.2	8.3±0.9	13.7±1.4
BNC4	60.8±7.2	104.4±0.9	172±2.5	199.8±4.1	28.4±2.4	122.2±16.8	24.2±4.3	46.6±3.1	4.1±0.5	10.6±1.0	11.5±1.9
BNC5	80.5±8.0	110.2±2.1	170±2.6	188.1±±8.3	32.2±3.8	68.0±8.2	17.0±3.6	33.2±2.0	4.1±0.6	12.0±0.7	10.7±1.3
SNC2	82.3±5.4	107.5±7.2	164±3.2	185.2±15.9	21.2±3.1	60.8±9.5	21.5±5.8	45.8±4.1	4.1±0.4	10.8±1.5	10.8±1.2
BJC1	70.2±6.2	103.5±0.8	173±2.5	196.0±19.3	21.5±4.5	100.8±8.0	15.5±2.9	34.2±3.5	4.3±0.1	13.2±0.5	10.6±2.2
BJC8	65.1±3.2	114.2±1.3	171±3.4	224.5±14.9	23.8±3.6	73.8±15.0	29.2±1.2	45.0±1.8	3.6±0.3	5.8±0.7	12.5±1.8
BJC9	70.3±5.2	116.8±2.9	170±3.2	214.2±12.7	16.7±4.2	59.5±10.4	22.8±3.9	34.3±1.7	3.2±0.4	9.4±0.4	10.3±0.4
BJC19	60.5±1.9	123.5±0.3	175±3.6	141.2±7.8	19.6±9.7	25.6±12.7	10.5±1.5	17.3±4.0	3.2±0.7	4.1±0.2	12.6±2.5
BJC21	60.0±2.5	107.4±2.5	176±4.1	185.5±20.5	23.5±6.4	56.5±11.0	22.2±0.5	37.5±2.2	2.6±0.3	10.3±0.5	8.6±0.3
BJC23	84.0±6.7	87.8±±.7	161±2.1	139.5±6.5	20.5±4.6	29.7±5.4	18.5±2.1	32.7±5.4	4.0±0.5	10.2±0.3	15.0±2.0
BJC24	68.0±5.2	108.6±2.3	172±3.7	211.6±8.5	23.6±1.6	78.2±9.5	26.1±3.6	47.2±4.3	4.3±0.2	12.5±0.7	13.9±1.9
BJC28	91.4±5.5	110.0±2.7	171±4.9	208.0±12.4	24.8±3.2	96.8±21.7	20.8±1.9	35.6±2.1	4.7±0.3	9.3±1.0	15.8±2.0
BJC29	70.5±4.3	106.4±3.1	165±4.2	206.0±10.6	26.9±5.2	51.6±7.5	20.4±4.1	29.5±3.6	4.7±0.4	11.7±0.2	12.3±1.4
BJC30	100.0	117.5±4.2	162±3.2	217.5±6.5	23.3±2.1	61.5±8.3	29.2±6.4	34.3±8.2	4.2±0.3	12.9±0.5	14.0±2.0
BJC31	100.0	108.6±1.5	169±5.1	215.3±3.4	23.2±±.1	76.4±6.1	32.3±2.6	61.3±6.5	4.9±0.2	11.8±1.0	15.2±2.1
SJC9	60.2±5.2	119.0±2.3	175±4.6	201.4±9.2	16.8±2.3	41.0±2.7	26.2±1.9	34.4±2.9	3.1±0.1	9.2±1.0	11.5±1.7
SJC19	75.1±5.4	113.4±3.9	172±4.9	285.4±5.3	18.2±1.5	41.8±7.4	29.6±3.4	36.5±2.3	3.6±0.4	16.5±0.7	18.6±2.0
SJC22	90.0±4.2	112.0±2.0	174±5.1	210.3±10.6	32.6±5.0	49.6±8.4	36.3±9.3	42.6±5.7	3.5±0.2	11.7±1.2	10.0±0.7
SJC24	77.5±12	106.5±4.2	169±3.8	200.4±8.7	20.4±2.9	70.2±7.3	22.1±3.4	40.2±2.8	3.5±0.1	9.5±0.5	12.0±2.5
SJC25	65.±±6.7	115.2±0.2	173±5.3	155.6±8.9	15.6±4.9	38.6±8.0	30±6.6	35±7.2	3.2±0.2	12.4±1.2	10.5±1.2
SJC29	100.0	112.3±1.5	168±2.7	188.4±4.6	25.8±6.5	48.4±10.6	23.3±3.2	33.2±4.4	4.2±0.3	11.9±0.6	10.5±1.8
SJC30	100.0	117.5±3.8	170±4.6	217.5±14.3	23.3±2.6	61.5±12.3	29.2±1.6	34.3±2.2	4.2±0.1	12.4±0.4	12.4±3.6
NUDH-YJ-4	100.0	84.0±3.5	152±3.9	219.0±9.6	12.8±4.3	22.8±5.9	60.8±8.4	73.0±3.7	3.6±0.4	13.4±0.5	10.2±2.9
PC5	100.0	105.±2.8	173±4.6	230.6±12.7	19.0±3.2	62.4±6.5	28.8±7.2	45.2±3.2	4.1±0.6	14.4±0.6	12.0±2.4
NHO7-10	100.0	80.0±2.3	153±3.7	136.7±10.8	7.4±3.1	13.4± 7.6	79.5±6.8	76.2±4.1	6.0±0.2	23.1±0.2	10.7±2.9
MHO-18-1-184	100.0	82.5±2.6	150±4.8	135±13.6	8.2±2.9	15.4±9.3	81.2±7.4	74.4±2.9	5.4±0.2	24.1±0.5	11.4±1.9
NJHO-325	100.0	83.7±3.2	149±4.3	210±9.8	10.3±3.1	21.4±8.9	62.2±6.0	76.2±2.8	4.2±0.3	14.5±0.4	12.3±2.0

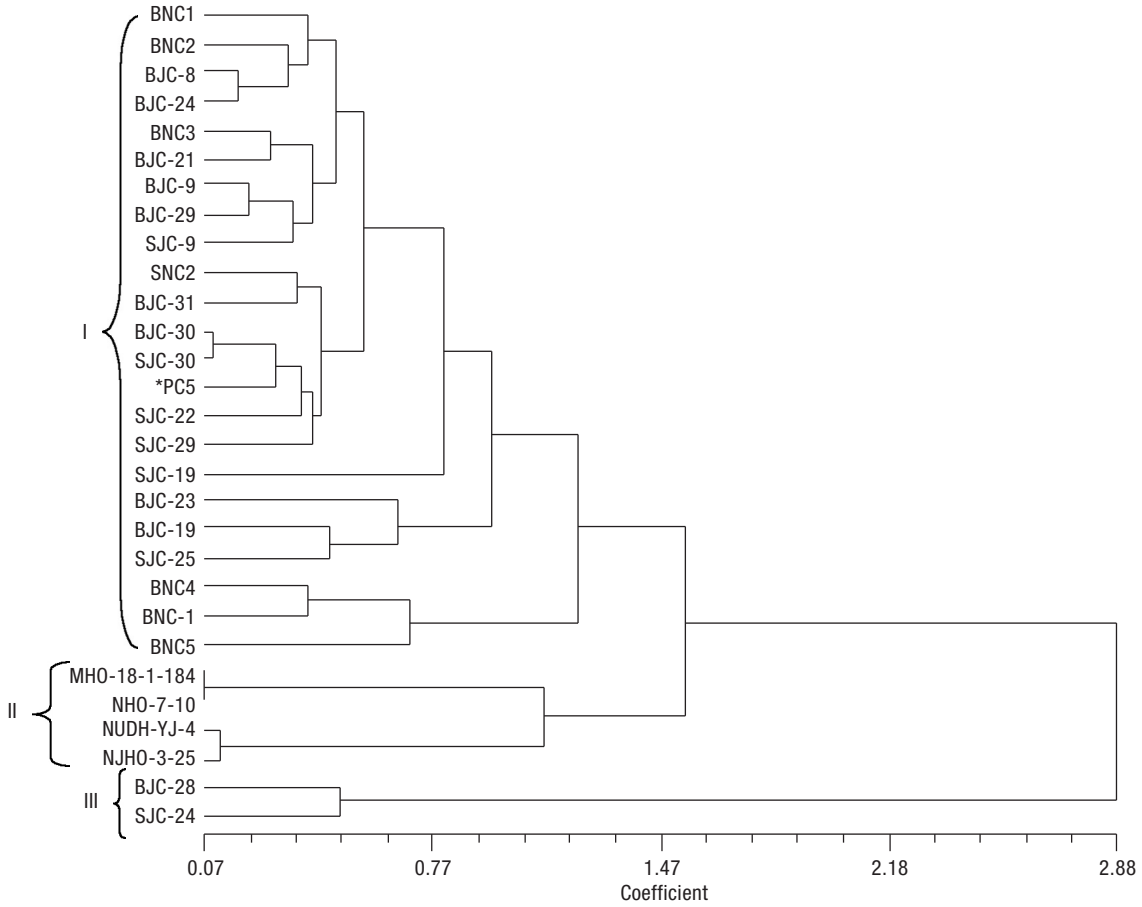

Figure 3. Dendrogram showing morphological diversity in 24 (BC_2, BC_1F_2) introgression lines of *Brassica carinata* and three parental and two non-parental lines of allodiploids.

valents may only be attributed to the intragenomic autosyndetic pairing due to inherent homologies in the three genomes (Roebbelen, 1960) and to some extent, allosyndetic pairing (between A/B and A/C) in addition to homologous paring between the chromosome belonging to the BB (8) or CC (9) genomes. The occurrence of trivalents and quadrivalents in many cells of both interspecific crosses, possibly resulted from the allosyndetic pairing (Attia & Roebbelen, 1986) which could increase the variability in *B. cari-*

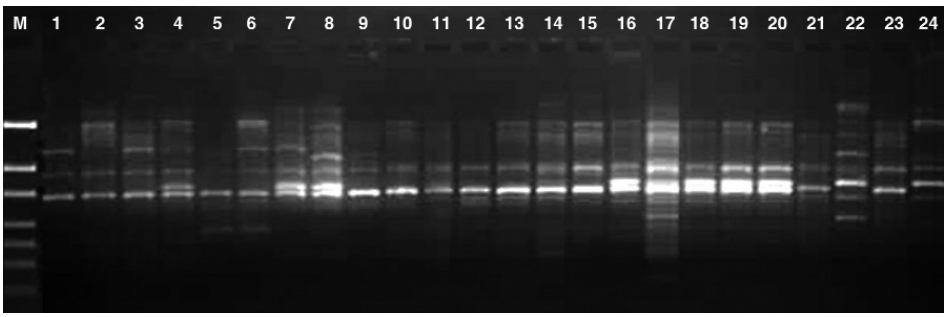

Figure 4. RAPD profile of 21 advanced introgression lines of *B. carinata* and three parental lines (*B. napus, B. juncea* and *B. carinata*) by using the primer BG93. Lanes: (1-21) advanced introgression lines of *B. carinata,* (22) *B. napus* parent, (23) *B. juncea* parent and (24) *B. carinata* parent. Marker (M) is 1-kb DNA ladder (New England Biolabs).

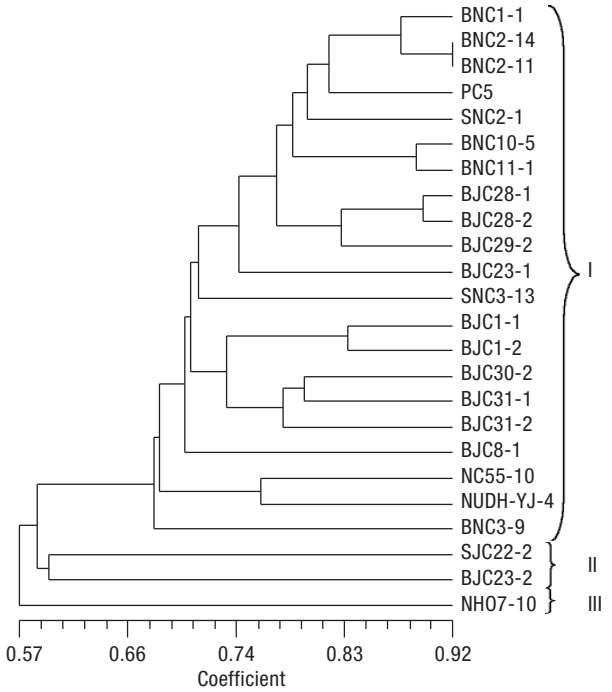

Figure 5. Dendrogram showing molecular diversity in 21 (BC_2 and BC_1F_2) introgression lines of *Brassica carinata* and three parents (*B. napus, B. juncea* and *B. carinata*).

nata through introgression of desirable genes from A genomes of *B. napus* and *B. juncea* (Prakash, 1973). Previously the interspecific hybridization between *B. carinata* and *Brassica rapa* produced hybrids only when *B. carinata* was used as a female parent. One of the four hybrid plants was completely male sterile while the remaining had 4.8, 8.6 and 10.9% stainable pollen respectively. The occurrence of maximum 11 bivalents as well as up to 44.8% cells with multivalent associations in the form of trivalents (0-2) and quadrivalents (0-1) in the trigenomic triploid hybrid (ABC, 2n = 7) revealed intergenomic homoelogy among A, B, and C genomes (Choudhary *et al*., 2000). The A and C genomes are closer to each other than either is to B genome (Pradhan *et al*., 1992; Axelsson *et al*., 2000). Morphological assessment of introgressed lines indicated a positive trait shift, possibly following introgression of genes governing short plant stature from the *B. napus* parent. *B. carinata* is a tall growing crop and there is a need for development of varieties with a stature at par with that of *B. napus* or *B. juncea*. Rao *et al.* (1993) was successful in developing short stature plants in backcrosses of *B. napus* × *B. carinata*. Mean primary and secondary branches per plant showed a significant increase in majority of the backcross progenies as compared to both parents. *B. carinata* is a long duration crop and development of varieties having maturity at par with *B. napus* and *B. juncea* is an agronomic necessity to fit in the paddy- oilseed cropping sequence prevalent in most of the parts of the country. Nuclear genes for desirable agronomic traits have been incorporated from related sources exploiting non-homologous recombination following sexual/somatic hybridization like earliness to oil rape leading to release of cultivars like 'Norin 16' and 'Asahi-Natane' in Japan (Namai *et al*., 1980). Development of 10 lines of new type of rapeseed through introgression of genes from Chinese *B. rapa* to Chinese normal rapeseed (*B. napus*) (Qian *et al*., 2006) thereby revealing that Chinese *B. rapa* could significantly diversify the genetic basis of the rapeseed and play an important role in the evolution of Chinese rapeseed.

The extremely higher number of polymorphic RAPD bands recorded in the present study underlines the ability of the RAPD technique to detect polymorphism, and reflects the diversity of the introgressed progenies. The clusters in general represented a wide range of genetic diversity generated in the back cross progenies of *B. carinata* as a result of introgression of genes from *B. napus* and *B. juncea* parents. Previously, Kumar *et al.* (2003) achieved more or less similar clustering in cotton genotypes with the use of RAPD markers and morphological characteristics.The large genetic distance between the evaluated interspecific derivatives was expected to be due to genomic dissimilarities with *B. napus* and *B. juncea*. The germplasm of *B. napus* was successfully widen by introgression of the A(r) subgenome of *B. rapa* (A(r)A(r)) and C(c) of *B. carinata* [(B(c)B(c)C(c)C(c)] into natural *B. napus* [A(n)A(n)C(n)C(n)] and the selected new-typed *B. napus* had a balanced genetic base (Li *et al*., 2007). The success of genetic enrichment of *B. carinata* was apparent in the present studies from introgression of agronomically desirable variability as well as demonstrated enlargement of genetic base.

In summary, a comparatively high degree of interspecific crossability within the genus *Brassica* provides a very large reservoir of agronomic and biochemical characteristics which may be transferred and selected from species to species. Although no cultivars have been released at present as a direct result of reconstitution of species through interspecific hybridization, this study reveals that there is a great scope of genes introgression into *B. carinata* for des-

ired agronomic traits, especially early maturity and short stature plants, through interspecific hybridization involving elite lines of *B. juncea* and *B. napus*; allowing so to broaden the restricted level of natural variability for these specific traits. The study will serve as a milestone for future research work on this aspect.

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