Introgression of novel genetic variation in *Brassica carinata*

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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 duration, low oil content and hard to thresh siliqua. Interspecific hybridization followed by selection in selfed and backcross progenies was employed to introgress useful variability into *B. carinata* cv PC 5 from two related digenomics viz. *B. napus* (AACC, 2n=38) and *B. juncea* (AABB, 2n=36). Hybridity of *B. napus*×*B. carinata* and *B. juncea*×*B. carinata* crosses was confirmed through cytological and molecular analysis. Forty six F_3, BC_2 and BC_1 F_2 *B. carinata* type progenies were identified on the basis of morphology and high pollen grain stainability. Almost all these progenies had high bivalent frequency (16-17 II) indicating their relative genomic stability. Morphological and seed quality evaluation of these progenies indicated significant variation for key economic traits. Individual plants / progenies with low erucic acid (11.8% in BNC 2 vs. 45.5% in PC 5), high oil content (41.5% in BJC1 vs. 34.5% in PC 5), early maturity (157 days vs. 168 days in PC 5) and short stature (140 cm vs. 231 cm in PC 5) were selected. Diversity analysis (UPGMA) on the basis of morphological variation grouped 42 of the 46 progenies, with *B. carinata* cv. PC5 at a taxonomic distance of 1.09 inferring enlarged genetic base. At molecular (RAPD) level also 17 of the selected 21 progenies grouped with *B. carinata* cv. PC5 at a similarity coefficient of 0.63, confirming broadly the clustering based on morphological diversity. The use of identified variability in breeding programmes is likely to help in the development of early maturing, canola quality *B. carinata* cultivars having high oil content and desired morphotype. Increasing oil content to 41.5 per cent was a significant achievement of the study.

Key words: *B. carinata,* interspecific hybridization, gene introgression, variability, genetic relatedness

Introduction

Ethiopian mustard (*Brassica* carinata) can be a viable 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 yield levels as well as resistance to various biotic and abiotic stresses. In spite of these strong positive attributes, the crop suffers from several agronomic limitations. Restricted level of natural variability for several of agronomically desirable traits has greatly constrained the directed crop breeding programmes aimed at overcoming these limitations (Prakash and Chopra 1991). Resynthesis route for enlarging the basic germplasm base 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. Thus the resultant resynthesized *Brassica* carinata types show poor breeding value. Present investigations were, therefore, undertaken to explore the possibility of genetic enhancement in the crop through selective introgression from the related and agronomically superior digenomics species like B. napus and *B. juncea*. To benefit, from altered cytoplasmic interactions, donor species (B. napus / *B. juncea*) were used as female parent in both the crosses. Natural *B. carinata* is known to possess *B. nigra* cytoplasm.

Material and methods

'00' *B. juncea* cv. NUDH YJ-4 (AABB) and '0' erucic acid B. napus cv. MHO 7-10 (AACC) were selected as female parents for interspecific hybridization with *Brassica* carinata cv. PC5 (BBCC) as the pollen / recurrent parent. Standard procedure of selfing, backcrossing accompanied by selection for *B. carinata* morphotype in each generation, was followed to develop F3, BC1 F_2 and BC2 progenies in each combination. The pollen grain stainability of F_1 hybrids and advance generation derivatives was determined by squashing anthers in acetocarmine (2%). Cytological investigations were carried out to assess F_1 hybrids and various F3/BC1 F_2/BC2 segregants for assessing the chromosome number. Stable F3/BC1F_2/BC2 progenies were morphologically evaluated, and subjected to diversity analysis using variation in the DNA amplification generated through 25 decamer RAPD primers following the protocol described earlier (Bhaskar et al 2002). The polymorphism data from all the primers were used to estimate the similarity coefficients (NTSYS Pc version 2.02 e) on the basis of the number of shared amplified bands. Similarity was calculated with SIMQUAL function of NTSYS which computes a variety of similarity and dissimilarity coefficients (association coefficients) for qualitative/nominal data. The similarity matrix values based on DICE coefficients of similarity were calculated as

$$DIJ. = 2a/(2a+b+c)$$

where, a represented matched fragments and 'b' and 'c' were the unmatched fragments. The 2a+b+c were the total number of the fragments amplified in a particular set. The similarity matrix thus developed was used to generate a dendrogram.

Results

The interspecific hybrids of B. napus×*B. carinata* and *B. juncea*×*B. carinata* were successfully developed using field pollinations. Hybridity of the two cross combinations was confirmed from morphological, cytological and molecular analysis. Phenotypically, F_1 plants exhibited intermediate plant morphology and were partially male fertile (15-20%). Cytological analysis of 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 and 11II + 1IV + 10I was the prominent (.57) meiotic configuration. The mean bivalent, trivalent and quadrivalent frequencies were 11.4, 0.4 and 0.5 respectively. *B. juncea*×*B. carinata* hybrid also showed expected somatic chromosome number (2 n= 35) whereas 11II + 13I was the predominant meiotic configuration. BC1 generation harboured maximum meiotic diversity. Backcrossing selected BC1 plants with the *B. carinata* in both the instances, helped to improve the meiotic stability and consequently the pollen grain fertility. In BC2, a significant proportion of plants had 2n= 34 somatic chromosome number and 17II as the predominant meiotic configuration. Selected BC1 plants were also selfed to raise $BC1F_2$ generation. Significant differences for plant morphology and pollen fertility were apparent in each progeny. Cytological analysis of individual F3 plants led to the identification of plants with euploid chromosome number (2n = 34). Studies indicated 17II or 16II + 2I as meiotic configurations for *B. carinata* type plants in both the crosses. Interestingly, proportion of PMC's with 17II was substantially higher in *B. juncea*×*B. carinata* (0.74) combination as compared to B. napus×*B. carinata* (0.25).

Data for important morphological traits of the progenies selected for high pollen grain stainability (⩾70%) and morphological resemblance with B.carinata is presented in Table 1. Days to maturity were generally skewed towards late maturing *B. carinata* parent. For plant height also the trend was towards taller *B. carinata* parent. However, substantially dwarfer progenies were isolated from B. napus×*B. carinata* combination. The primary branch number was substantially higher in introgression lines than any of the parents used in both the hybrid combinations. Progeny means for other yield contributing traits like pod intensity, seed/ siliqua and plant yield were lower for interspecific derivatives than the superior parent(s) for the trait (Table 1). In spite of the lower progeny mean values for desired traits, individual progenies with superior trait expression were recorded. For example, significantly higher seed yield than the recurrent parent PC5 was recorded for BJC 23, BJC 28, BJC 30 and BJC 31. For quality traits, there was a general shift towards positive trait expression. This was evident from higher oil (41.5%) content in many of the progenies such as BJC1, BNC 5 when compared to *B. carinata* parent (34.3%). Intermediate estimates (20-30%) were observed for erucic acid content in all the selected progenies whereas individual plants having only 11.8 per cent erucic acid were also identified. No change was observed for mean glucosinolate content. Individual plants with ⩽ 70 μ moles glucosinolates per gram of defatted meal were identified in three BC2 progenies (BNC 3, BJC 30 and BJC 24).

The mean morphological data of all the stable F3/$BC1F_2$/BC2 progenies after standardization were subjected to diversity analysis using the software programme NTSYS pc version 2.02e (Rholf 1998). Out of the evaluated 46 progenies, 31 grouped with recipient *B. carinata* parent PC 5 at a taxonomic distance of 0.60; 11 lines related to PC 5 at a taxonomic distance of 1.09 whereas four progenies viz. BNC 5, BNC 5-10, BJC 28 and SJC 24 appeared most divergent from the recipient parent. Of the 46 progenies, twenty one selected progenies (2n=34) and three parental strains were also subjected to diversity analysis using variation in DNA amplification generated through 25 RAPD primers. Clustering was done using the symmetric matrix of similarity coefficient and a dendrogram based on UPGMA using SHAN module of NTSYS PC version 2.02 (Rholf 1998) was obtained. The resultant dendrogram, representing diversity level from 0.57 to 0.92, categorized 24 genotypes into four groups (12, 9, 2, 1). Group I lines comprising recurrent parent PC 5 shared 70 percent similarity among themselves and 68 percent with lines of group II. Nine introgression lines of group I were more than 75 percent similar to PC 5. In all 19 introgression lines shared 68 per cent similarity with recurrent parent. Two introgression lines (SJC 22-2 and BJC-23-2) formed a separate cluster at a similarity level of 59 percent with recurrent parent PC 5. B. napus parent (cv. NHO 7-10) was grouped into a separate cluster with a similarity of 0.57 with all the genotypes whereas *B. juncea* parent NUDHYJ-4 shared more than 68 per cent similarity with 19 introgression lines.

Discussion

Interspecific hybridization among *Brassica* digenomics in the past has helped to identify variation for earliness, yellow seed colour and resistance to shattering in the past. In the present instance, *Brassica* 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 *B. carinata* (BBCC) following introgression from B and C genome of *B. juncea* (AABIBI) and B. napus (AACICI) respectively. The occurrence of higher than expected (8 and 9, respectively) number of bivalents, in two F_1 combinations (ACBB, ABCC) and presence of multivalents may be attributed to homologous pairing between A, B and C genome chromosomes as well as to allosyndetic pairing between A/B and A/C. The occurrence of trivalents and quadrivalents in many cells in BC 1 of both interspecific crosses, possibly resulted from the allosyndetic pairing (Attia and Roebbelen 1986) which could increase the genetic variability in *B. carinata* through introgression of desirable genes from A genomes of B. napus and *B. juncea* (Prakash, 1973). Significantly enlarged genetic base was also emphasized by morphological and molecular diversity analysis. Successful introgression was apparent from widely enlarged genetic base in terms of transfer of desirable characteristics like earliness, dwarf plant type, high oil content and low erucic acid. Fernandez-Martinez et al. (2001) have in the past reported a genetic stock 25-1 of Ethiopian mustard characterized by seed oil with no erucic acid from interspecific crosses with *B. juncea* and *B. napus*.

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. The large genetic distance between the evaluated interspecific derivatives was expected due to genomic dissimilarities with B. napus and *B. juncea*. The extremely higher number of polymorphic RAPD bands recorded in the present study reflects the diversity of the introgressed progenies. Grouping of *B. carinata* cv PC5 with 11 introgressed lines at a similarity level of more than 70 per cent is a strong indicator of reconstitution of *B. carinata* genome after interspecific hybridization with related species. A similar approach can be successfully used in other alloploid species for enhancing germplasm base.

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Table 1. Variation for various morphological and seed quality traits in selected F3 /BC1 F_2/ BC2 progenies of the *Brassica* interspecific crosses

Combination	Generation	Progenies	Pollen fertility (%)	Maturity (days)	Plant ht.(cm)	Primary branches	Siliqua intensity/cm	Seeds/ silqua	Yield (%)	Oil (%)	Erucic acid	Glucosinolates
B.juncea×*B. carinata*	F_3	8	69.9 ± 2.9	165.0± 1.1	220.3± 10.6	24.8± 2.1	1.6± 1.5	7.9 ± 0.6	12.5 ± 1.7	32.9 ± 0.6	25.7 ± 3.0	97.2 ± 7.0
	BC_2	11	76.4± 4.1	169.5 ± 1.2	196.2 ± 11.2	22.5 ± 4.4	1.6± 1.1	10.1± 0.5	12.8 ± 1.7	35.2 ± 0.5	30.5 ± 1.2	108.9 ± 7.9
	BC_1 F_2	7	81.1 ± 4.8	171.6± 2.1	208.3 8.8	21.8 ± 3.7	1.3 ± 0.8	11.9± 0.8	12.2 ± 1.9	35.3± 0.4	22.1 ± 2.2	107.7 ± 6.0
B.napus×*B. carinata*	F_3	14	77.2± 1.7	162.2± 0.9	236.9± 3.1	18.6± 1.3	1.6± 0.6	9.4± 0.3	11.9± 0.9	31.8± 0.9	30.7± 2.2	102.0± 26.2
	BC_2	5	70.4± 6.0	170.0± 0.9	195.7± 10.5	24.7± 2.4	1.9± 0.5	12.8± 1.3	12.3± 1.4	35.2± 0.6	22.2± 2.2	108.9± 8.0
	BC_1 F_2	1	82.3± 5.4	164.5± 0.3	185.2± 15.9	21.2± 3.1	2.1± 0.3	10.8± 1.5	10.8± 1.2	35.3± 0.4	19.3± 1.7	107.7± 6.0
B.carinata cv PC5	-	-	100.0	169.0	230.6	19.0	1.6	14.4	13.5	34.3	45.4	107.6
B.juncea cv.NUDH YJ-4	-	-	100.0	152.0	219.0	12.9	1.2	13.6	10.2	43.4	1.2	28.3
B.napus cv. NHO 7-14	-	-	100.0	155.0	141.2	7.0	1.0	22.4	9.5	45.1	45.2	76.3