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Development of dwarf *B. carinata* genotype employing inter-specific hybridization between *B. juncea* and *B. carinata* and cyto-morphological studies for patterns of variation

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Abstract

Successful inter-speciûc hybrid plants were obtained through sexual hybridization between *Brassica juncea* cv. NRCDR-2 (2n=4x=36, AABB), and *B. carinata*, NRCKR-304 (2n=4x=34, BBCC) using the latter as a pollen parent. Morphological and cytological analyses were carried out to conûrm the hybrid nature of F_1 plants. The F_1 plants (2n=35) were intermediate for most of the morphological attributes. Although, the F_1s showed poor pollen fertility, nevertheless, few seeds were obtained from open pollination. Meiotic analysis of F_1 plants showed a predominance of univalents, a typical feature of wide hybrids. The occurrence of chromosome association ranging from bivalents (0–16), trivalent (0–1) and quadrivalent (0–1) in the F_1s indicated homeologous pairing between the AB and BC genomes. The study suggests that *B. carinata* has partial genome homeology with *B. juncea*. Patterns of variations in F_2 progenies of the cross were estimated. In the F_2 generation, more number of plants resembling *B. juncea* was observed indicating transgressive segregation. Present study also indicated that inter-speciûc hybridization in Brassica could be a potential source for generating variability besides broadening the genetic base and segregants with desirable agro-economical characteristics were selected for further advancement and evaluation.

Key words: *cytomorphology, homeologous pairing, interspeciûc hybrids, meiotic analysis, wide hybridization.*

Introduction

Brassica is economically one of the most important genus of the family Brassicaceae. It constitutes important sources of vegetables, cooking oil and condiments (Cardoza and Stewart, 2004). Narrow genetic variability in crop *Brassicas*, caused mainly due to intensive selection over past several decades, has jeopardized the crop improvement programmes (Cowling, 2007; Ananga *et al*., 2008). Fortunately, inter-speciûc hybridization offers great potential for the improvement of Brassica crop and is being widely utilized for expanding genetic variability, introgressing nuclear genes that conferred desirable agro-nomic traits or cytoplasmic genes for inducing male sterility, Chromosome addition lines have also been generated to locate genes on speciûc chromosomes and for construction of genetic maps, to elucidate homoeology between different genomes of Brassica (Prakash *et al*., 2009). Such crosses provides immense opportunities for generating genetic diversity and broadening the genetic base (Choudhary *et al*., 2002; Choudhary and Joshi, 2012a; 2012b) and are also useful to transfer valuable characters across the species (Sharma and Singh, 1992).

Brassica species are three basic diploid species, speciûcally *B. rapa* (AA, 2n=20), *B. nigra* (BB, 2n=16) and *B. oleracea* (CC, 2n=18), and three amphidiploids, each of which evolved as a natural allo-tetraploid following hybridization between pairs of the three diploids, namely *B. napus* (AACC, 2n=38), *B. juncea* (AABB, 2n=36) and *B. carinata* (BBCC, 2n=34) (Nagaharu, 1935). *B. juncea* (L.) Czern & Coss. (Indian mustard) is the predominantly cultivated Brassica species in the Indian subcontinent with yield potential of 15–30 q ha-1 (Meena *et al*., 2017). Although *B. juncea* is well adapted to drier conditions and mature earlier than other oilseed *Brassica* species (Kimber and McGregor, 1995). However, this species is susceptible to aphid, Alternaria blight, white rust and have a limited genetic variation for resistance to both biotic and abiotic stresses (Kumar *et al.,* 1997).

On the other hand, Ethiopian or Abyssinian mustard (*B. carinata* A. Braun, 2n=34) possesses has several agronomical important traits that are rare in other Brassica oil crops such as non-dehiscent siliquae and a much more developed and aggressive root system, heat, drought tolerance, tolerance to various biotic and abiotic stresses and availability of yellow-seeded germplasm (Jiang *et al*.,

2007) which makes potential oilseed crop under semi arid conditions as winter crop. Inspite of these positive attributes, it could not find favour with farmers because this crop suffers from many agronomic limitations like longer crop duration, poor harvest index, low oil content, short shoot length and small pod size *etc*.

Thus, both *B. juncea* and *B. carinata* species have desirable characteristics and deûciencies. In view of these facts, the present investigation was conducted with a basic objective to combine the useful genetic attributes of *B. juncea* and *B. carinata*, to create genetic variation through inter-speciûc hybridization and possible introgression of genes with useful agro-morphological traits of economic importance. While attempting inter-speciûc and inter-generic crosses many have necessitated intervention in the forms of ovary culture, embryo rescue and protoplast fusion (Warwick and Black, 1993; Rieger *et al*., 1999) to overcome compatibility barriers. Here, we report successful synthesis of inter-speciûc hybrids between *B. juncea* (cv. NRCDR-2) and *B. carinata* (NRCKR-304) by sexual matings followed by their characterization in terms of morphological attributes, genome homology and differentiation pattern based upon crossability, meiotic behaviour of chromosome and fertility factors of the parents and their F_1s. Additionally, patterns of variations in F_2 derivatives and subsequent development of dwarf *B. carinata* genotype from segregants is also being reported.

Materials and Methods

Plant material

Seed samples of *B. juncea* (cv. NRCDR- 2) and *B. carinata* (NRCKR-304) were obtained from the germplasm section of the ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur.

Hybridization

Reciprocal inter-specific crosses between plants of the cultivated *B. juncea* (cv. NRCDR-2) and *B. carinata* (NRCKR-304) were attempted to produce inter-specific hybrids. Unopened flower buds of *B. juncea* (cv. NRCDR-2) were emasculated in the afternoon, covered with paper bags and pollinated with freshly collected pollens of *B. carinata* (NRCKR-304) in the following morning and covered again. The seeds collected from the crossed plants and parents were sown in earthen pots for evaluation and further advancement of progenies from F_2 generation grown field conditions. Morphological comparisons were made for identifying the F_1s and ascertained cytologically.

Cytological analysis

For meiotic observations flower buds of an appropriate size were collected from mature plant and fixed in freshly prepared carnoy's fluid (ethanol : chloroform : acetic acid - 6 : 3 : 1), supplemented with a drop of ferric chloride solution, for a minimum of 24 hours at room temperature and subsequently stored in 70% alcohol at 10^{o}C. For meiotic analysis anthers were squashed in 1% acetocarmine and a total of 30 PMCs were analyzed at diakinesis/metaphase I stages of meiosis in F_1 hybrid plants. For percentage pollen stainability, the pollen grains were stained in 1:1 (glycerine: acetocarmine) mixture and on an average five slides were scored for stainable pollen grains. Normal pollen grains were round, densely stained and were distinguishable from small, shrunken and lightly stained sterile pollen grains.

F_2 and generation advancement

The seeds harvested from F_1 plants were sown in the field. Based on morphological attributes such as plant appearance in comparison to parent species, leaf shape, size, colour and attachment to the stem, and inflorescence characteristics of the parent species, the F_2 plants were grouped visually into four broad categories: *B. juncea* type, *B. carinata* type, intermediate type and any other type. From F_2 segregating progenies four dwarf type *B. carinata* plants were selected and from F_3 progenies one dwarf type *B. carinata* genotype DRMR-C-16-6 was selected (< 100 cm) maintained and advanced up to F_6 generations. Comparison of performance of dwarf type *B. carinata* genotype (DRMR-C-16-6) for three years was made from 2016-17 to 2018-19. Observations on plant height (cm), days to maturity, primary branches per plant, main shoot length (cm), siliquae length, seeds per siliqua, 1000 seed weight (g), Oil content (%) were made.

Results and Discussion

Hybridization and morphological characteristics of F_1 hybrids

Inter-specific hybrid derived from cross NRCDR-2 (*B. juncea*) × *B. carinata* (NRCKR-304) through sexual hybridization was confirmed through cyto-morphological studies. Inter-specific hybrid plants were obtained when *B. carinata* was used as a male parent, and all the hybrids grew up to maturity. NRCKR-304 is a registered germplasm for early maturity, long main shoot and bold seed. One hundred and Thirty eight buds were pollinated from which 20 siliquae with a total of 29 seeds were obtained. Out of the 29 seeds only three the plants, were found to be hybrid and thus the percentage success of crossability was 10.34%. While attempting inter-speciûc/inter-generic

Table 1: Plant morphological characteristics of parents and the F_1 hybrids of *B. juncea* (cv. NRCDR-2) × *B. carinata* (NRCKR-304)

Characteristics	*B. juncea* (cv. NRCDR-2)	*B. carinata* (NRCKR-304)	F_1 hybrids
Plant height(cm)	195	155	85
Days to 50% flowering	46	38	41
Days to maturity	147.5	132	127
Primary branches per plant	6.8	6.5	7.2
Secondary branches per plant	20.5	17.3	17.8
Main raceme length (cm)	82.6	85.4	60
Siliqua length (cm)	5.6	3.7	2.9
Seeds per siliqua	15.7	16.0	10.2
1000 seed weight (g)	5.3	5.6	2.8
Corolla length (cm)	0.7	1.2	1.4
Corolla width (cm)	0.3	0.7	0.7
Siliqua texture	Smooth	Smooth	Undulated
Seed colour	Dark Brown	Dark Yellow	Light brown
Leaf colour	Medium green	Medium green	Light green
Leaf hairiness	Sparse	sparse	Sparsely hairy
Leaf margin	Serrate	Entire	Dentate
Leaf lobes	Present	Absent	Present
Petal colour	Yellow	Yellow	Light yellow
Oil content (%)	41.5 41.8	40.03	41.3

crosses most of authors necessitated the intervention in the forms of ovary culture, embryo rescue and protoplast fusion (Warwick and Black, 1993; Rieger *et al*., 1999) to overcome compatibility barriers.

The F_1 hybrid plants (2n=20) obtained were found to be intermediate and vigorous in terms of many phenotypic features and inûorescence attributes of both the parents. These observations are in congruence with earlier reports (Choudhary and Joshi, 2012a, b; Choudhary *et al*., 2000). The hybrid plants were medium in height, profusely branched and intermediate to their parents for most of the morphological and inflorescence attributes. The leaves were petiolate, lobed and lyrately pinnatified. The dentation of leaf margin was noted to be crenate type with acute tip and petal colour (light yellow) was intermediate to the parents. The seeds of hybrids were small in size and light brown in colour. The hybrid plants had smaller siliquae as compared to the parents (Table 1). The present investigation reports the successful hybridization between *B. juncea* and *B. carinata* even when it is involved as a male parent. It is quite useful in maintaining the cytological background of the crop species in distant hybridization programmes. The occurrence of characteristics from both progenitor species in the hybrids indicates that the F_1 plants inherited genomes of both parental species crossed. This is an advantage, since it would allow better selection for speciûc attributes in segregating progenies.

Cytology of F_1 hybrids

Meiotic data of F_1 hybrids has been summarized in Figure1, Table 2 and Table 3. Cytological analysis of F_1 hybrids (2n = 35) not only confirmed their hybridity but also indicated extent of genome homoeology between the parents. Meiotic analysis of the F_1 hybrids of *B. juncea* and *B. carinata* showed a mixture of univalents, bivalent, trivalent and quadrivalent in a total of 30 PMCs analyzed. The F_1 hybrids showed predominance of univalents, which is a typical feature of wide hybrids (Kumar *et al*., 2013, 2015, 2018). The bivalents ranged between 02 to 16 in various PMCs. The average chromosome association in the hybrid was of 0.13IV + 0.19III + 7.69II + 18.63I. The number of univalents and bivalents ranged from 3 - 27 and 2 - 16, respectively, whereas number of trivalent and quadrivalent never exceeded one per PMC. Chromosome pairing in the F_1 hybrids could be interpreted as a result of auto as well as allosyndesis within and between, respectively, the AB and BC genomes (Choudhary and Joshi, 2012a, Prakash, 1974). From the observations the occurrence of chiasmatic and heteromorphic pairing, multivalent associations in the form of trivalents and quadrivalents, though only in a few PMCs, and more importantly, the formation of up to 16 bivalents could be accounted for by autosyndesis within the AB and BC genomes, clearly indicating homoeologous pairing between chromosomes of AB and BC genomes. Thus, it appears *B. carinata* has some homoeology with *B. juncea*

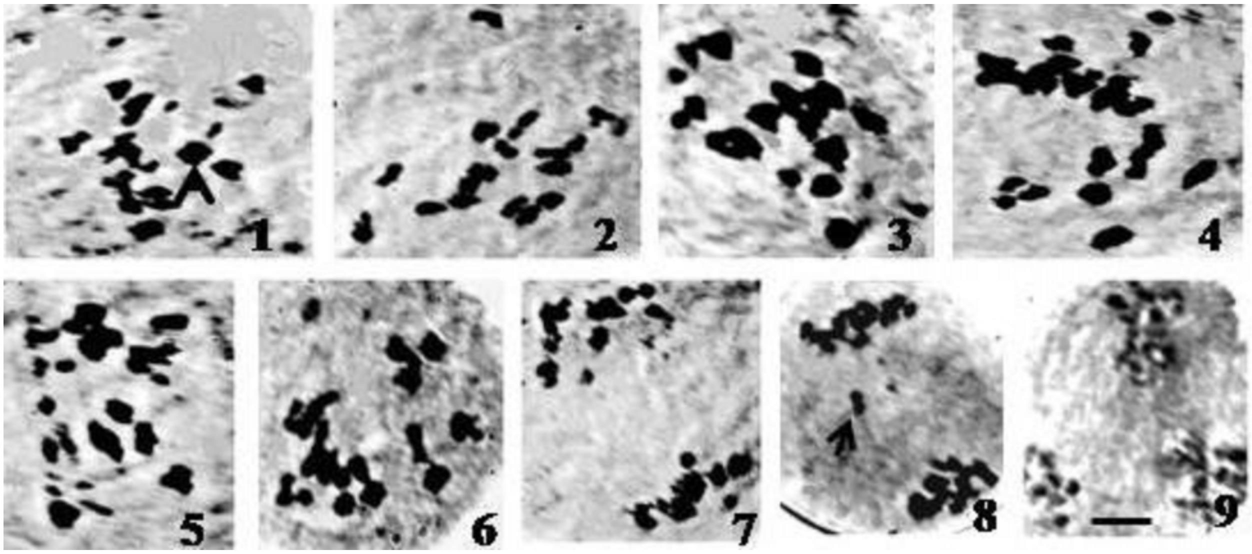

Fig.1. Meiotic analysis of F_1 hybrids showing chromosome associations at diakinesis/metaphase I and chromosome distribution at anaphase I. 1. Metaphase I 1IV + 12II + 7I (Quadrivalent marked by arrow head), 2. Metaphase I 6II + 23I, 3. Diakinesis 10II + 15I, 4. Diakinesis 7II + 21I, 5. Metaphase I 8II + 19I, 6. Diakinesis 6II + 23I, 7. Anaphase I, 8 Anaphase I with laggards (marked by arrow), 9 Anaphase II (showing intermixing of chromatids at one pole), Bar: 10 µm.

Table 2: Chromosome configuration at diakinesis/metaphase I in PMCs of F_1 hybrids *B. juncea* (cv. NRCDR-2) × *B. carinata* (NRCKR-304) hybrids (2n= 35)

	Chromosome configuration (number/PMC)				Number of PMCs observed	
	IV	III	II	I	Number	Frequency (%)
	1	-	6	19	1	3.3
	1	-	12	7	1	3.3
		1	3	26	1	3.3
		1	6	21	1	3.3
		1	4	24	1	3.3
			16	3	1	3.3
			13	9	1	3.3
			12	11	1	3.3
			10	15	1	3.3
			9	17	3	10.0
			8	19	1	3.3
			7	21	2	6.7
			6	23	8	26.7
			5	25	2	6.7
			4	27	3	10.0
			2	31	2	6.7
Total					30	
Range	0-1	0-1	2-16	3-27		
Mean ± SEm	0.7 0.04	0.1 0.05	7.7 3.9	18.6 7.6		

indicating possibility of gene inrogression through conventional means. Numerous disjunctional abnormalities including late disjunction of bivalents, and laggards were observed at anaphase I and II (Table 2) was recorded in a few cells, which is similar to the study reported by Choudhary and Joshi (2012b). Majority of PMCs (74%) analyzed showed laggards univalents or bivalents at anaphase I and II, where as only 25.9%

Table 3: Chromosome distribution at anaphase I and II in hybrids of *B. juncea* (cv. NRCDR-2) × *B. carinata* (NRCKR-304) hybrids (2n= 35)

Chromosome distribution	PMCs observed	
	Number	Percentage
Equal distribution	7	25.93
Laggards at A I	15	55.56
Laggards at A II	5	18.51
Total	27	

showed equal distribution of chromosomes at anaphase I. However, a few cells were recorded with normal distribution of chromosomes resulting in some fertile pollen grains in the hybrids. The drastic decrease in pollen fertility (22.4%) and reduced seed set recorded in the present hybrids might be due to meiotic irregularities and segregational anomalies (Singh, 1993). The occurrence of homoeologous pairing between *B. juncea* and *B. carinata* chromosomes and few seeds set by inter-speciûc hybrids offers an opportunity for the transfer of useful genes across the species.

F_2 generation

From the ûeld grown F_2 segregating progenies, a total of 102 plants in which 44 (*B. juncea* type), 30 (*B. carinata*), 17(intermediate type) and 11(other type) obtained were used to record observations on different agro-morphological traits. Wide phenotypic variations regarding plant types and metric traits were observed in F_2 populations. The pattern of segregation for plant types showed high recovery of *B. juncea* type plants (43.31%) as compared to *B. carinata* type (29.4%), intermediate category (16.6%) and other types (10.9%) (Table 4). High frequency of the maternal and intermediate types of plants in F_2 generation of *B. juncea* × *B. rapa* crosses was also by Choudhary *et al*. (2002). In all, five plants were found to be completely male sterile. With regards to pollen fertility of F_2 progenies recorded higher mean pollen fertility (46.8%) compared F_1 progenies (22.4%).

The occurrence of *B. juncea* (the maternal parent) type plants in larger frequency compared with other three types in F_2 generation might resulted from a greater viability of female gametes with higher chromosome number (Subudhi and Raut, 1994). High frequency of the maternal and intermediate types of plants in F_2 generation of *B. juncea* × *B. rapa* crosses was also by Choudhary *et al*. (2002) and Chuodhary and Joshi (2012b). Wide phenotypic variation and transgressive segregation for plant height, Secondary branches per plant, main raceme length etc. in the F_2 generation might have resulted from recombination and or eventual segregation of aneuploid forms arising in the populations. This suggested the possibility of improving the derivatives through selection for these traits. This could be explained why Olsson (1960a, 1960b) found wide variation in F_2 and F_3 plants of the cross *B. rapa* × *B. nigra* for plant height, ûowering and maturity, siliqua size and pollen fertility. High-yielding physiological variants from segregants of the *B. campestris* × *B. nigra* cross are reported by Prakash (1973). On the similar hypothesis, Rao *et al*. (1993) and Choudhary *et al*. (2002) reported transgressive segregants for yield components from the F_2 generation of *B. napus* × *B. carinata* and *B. juncea* × *B. rapa* inte-rspecific crosses.

Generation advancement

From F_3 progenies (dwarf *B. carinata* type) one dwarf type *B. carinata* genotype DRMR-C-16-6 was selected (< 100 cm) maintained through selfing and advanced up to F_7 generations. Comparison of performance of dwarf type *B. carinata* genotype (DRMR-C-16-6) for three years was made from 2016-17 to 2018-19. Observations on plant height (cm), days to maturity, primary branches per plant, main shoot length (cm), siliquae length, seeds per siliqua, 1000 seed weight (g), Oil content (%) were made (Table 5) along with *B. carinata* (cv. Kiran), and both the parents NRCKR-304 (*B. carinata*) and *B. juncea* (cv. NRCDR-2) respectively. Mean values of plant height (83.7 cm), days to maturity (128.7), Secondary branches (22.1) and oil content 41.4% were found to be higher when compared with parents *B. carinata* (NRCKR-304)and *B. juncea* (cv. NRCDR-2). Dwarf genotype *B. carinata* (DRMR-C-16-6) was observed to be superior almost all agro-morphological characteristics when compared with the variety of *B. carinata* (Kiran) (Table 5). Thus present

Table 4: Segregation for plant types in the F_2 generation of *B. juncea* (cv. NRCDR-2) and *B. carinata* (NRCKR-304) cross

Cross	Plant type frequency				
	B. juncea (cv. NRCDR-2)	*B. carinata* (NRCKR-304)	Intermediate	Other	Number of Plants observed
B. juncea (cv.NRCDR-2) ×*B. carinata* (NRCKR-304)	44(43.1)*	30(29.4.3)	17(16.6)	11(10.9)	102

*Numbers in parenthesis indicate percentage

Table 5: Comparison of performance of dwarf type *B. carinata* genotype (DRMR-C-16-6) for three years from 2016-17 to 2018-19 (average data) along with parents

Characteristics	DRMR-C-16-6 (*B. carinata*)	NRCKR-304* (*B. carinata*)	Kiran (*B. carinata*)	NRCDR-2 ** (*B. juncea*)
Days to maturity	128.67	136	162.67	143.53
Plant height (cm)	83.66	158.33	234	206
Primary branches	10.3	5.27	9.17	6.47
Secondary branches	22.13	13.7	21.3	15.53
Main shoot length (cm)	58.33	89.5	38.33	89.1
Siliqua length (cm)	3.83	3.7	3.4	5.27
Seeds per siliqua	15.43	16.3	12.7	15.97
1000 seed weight (g)	4.77	5.17	3.23	5.25
Oil content (%)	41.43	40.44	39.0	41.4

parents = *NRCKR-304, **NRCDR-2

study indicated that inter-speciûc hybridization in Brassica could be a valuable source for generating genetic diversity for broadening base and segregants with desirable attributes can be valuable source for further crop improvement of *B. carinata* and as well as *B. juncea*.

Conclusion

Successful synthesis of interspeciûc hybrids between *B. juncea* (cv. NRCDR-2) and *B. carinata* (NRCKR-304) by sexual mating followed by their characterization in terms of morphological attributes, genome homology and differentiation pattern based upon crossability, meiotic behaviour of chromosome and fertility factors of the parents and their F_1s. The study suggests that *B. carinata* has partial genome homeology with *B. juncea*. In the F_2 generation, more number of plants resembling *B. juncea* was observed indicating transgressive segregation. Present study also indicated that inter-speciûc hybridization in Brassica could be a potential source for generating variability besides broadening the genetic base and segregants with desirable agro-economical characteristics were selected for further advancement and evaluation and subsequent selection of dwarf type *B. carinata* genotype.

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