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-specific 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 confirm the hybrid nature of F1 plants. The F1 plants (2n=35) were intermediate for most of the morphological attributes. Although, the F1s showed poor pollen fertility, nevertheless, few seeds were obtained from open pollination. Meiotic analysis of F1 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 F1s 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 F2 progenies of the cross were estimated. In the F2 generation, more number of plants resembling *B. juncea* was observed indicating transgressive segregation. Present study also indicated that inter-specific 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, interspecific 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-specific 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 agronomic traits or cytoplasmic genes for inducing male sterility, Chromosome addition lines have also been generated to locate genes on specific 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, specifically *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.,
Thus, both *B. juncea* and *B. carinata* species have desirable characteristics and deficiencies. 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-specific hybridization and possible introgression of genes with useful agro-morphological traits of economic importance. While attempting inter-specific 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-specific 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 F1s. Additionally, patterns of variations in F2 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 F2 generation grown field conditions. Morphological comparisons were made for identifying the F1s 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 10oC. For meiotic analysis anthers were squashed in 1% aceticarmine and a total of 30 PMCs were analyzed at diakinesis/metaphase I stages of meiosis in F1 hybrid plants. For percentage pollen stainability, the pollen grains were stained in 1:1 (glycerine: aceticarmine) 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.

**F2 and generation advancement**

The seeds harvested from F1 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 F2 plants were grouped visually into four broad categories: *B. juncea* type, *B. carinata* type, intermediate type and any other type. From F1 segregating progenies four dwarf type *B. carinata* plants were selected and from F1 progenies one dwarf type *B. carinata* genotype DRMR-C-16-6 was selected (< 100 cm) maintained and advanced up to F6 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), siliqua length, seeds per siliqua, 1000 seed weight (g), Oil content (%) were made.

**Results and Discussion**

**Hybridization and morphological characteristics of F1 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-specific/inter-generic
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 F₁ hybrid plants (2n=20) obtained were found to be intermediate and vigorous in terms of many phenotypic features and inflorescence 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 F₁ plants inherited genomes of both parental species crossed. This is an advantage, since it would allow better selection for speci*c attributes in segregating progenies.

### Cytology of F₁ hybrids

Meiotic data of F₁ hybrids has been summarized in Figure 1, Table 2 and Table 3. Cytological analysis of F₁ hybrids (2n = 35) not only confirmed their hybridity but also indicated extent of genome homeology between the parents. Meiotic analysis of the F₁ hybrids of *B. juncea* and *B. carinata* showed a mixture of univalents, bivalent, trivalent and quadrivalent in a total of 30 PMCs analyzed. The F₁ hybrids showed predominance of univalents, which is a typical feature of wide hybrids (Kumar et al., 2013, 2015, 2018). The bivalents ranged between 2 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 F₁ 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 homeology with *B. juncea*.
indicating possibility of gene introgression 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, whereas only 25.9%

![Fig.1. Meiotic analysis of F₁ hybrids showing chromosome associations at diakinesis/metaphase I and chromosome distribution at anaphase I. 1. Metaphase I 11IV + 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.](image)

Table 2: Chromosome configuration at diakinesis/metaphase I in PMCs of F₁ hybrids *B. juncea* (cv. NRCDR-2) × *B. carinata* (NRCKR-304) hybrids (2n= 35)

<table>
<thead>
<tr>
<th>Chromosome configuration (number/PMC)</th>
<th>Number of PMCs observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>IV III II I</td>
<td></td>
</tr>
<tr>
<td>1 - 6</td>
<td>19</td>
</tr>
<tr>
<td>1 - 12</td>
<td>7</td>
</tr>
<tr>
<td>1 3</td>
<td>26</td>
</tr>
<tr>
<td>1 6</td>
<td>21</td>
</tr>
<tr>
<td>1 4</td>
<td>24</td>
</tr>
<tr>
<td>16 3</td>
<td>3</td>
</tr>
<tr>
<td>13 9</td>
<td>9</td>
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<td>12 11</td>
<td>11</td>
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<td>10 15</td>
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<td>7 21</td>
<td>21</td>
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<td>6 23</td>
<td>23</td>
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<td>5 25</td>
<td>25</td>
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<tr>
<td>4 27</td>
<td>27</td>
</tr>
<tr>
<td>2 31</td>
<td>31</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
</tr>
</tbody>
</table>

Range           0-1 0-1 2-16 3-27
Mean ± SE 0.7 0.04 0.1 0.05 7.7 3.9 18.6 7.6

indicating possibility of gene introgression 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, whereas 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)

<table>
<thead>
<tr>
<th>Chromosome distribution</th>
<th>PMCs observed</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equal distribution</td>
<td>7</td>
<td>25.93</td>
<td></td>
</tr>
<tr>
<td>Laggards at A I</td>
<td>15</td>
<td>55.56</td>
<td></td>
</tr>
<tr>
<td>Laggards at A II</td>
<td>5</td>
<td>18.51</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 interspecific hybrids offers an opportunity for the transfer of useful genes across the species.

**F₂ generation**

From the field grown F₂ 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 agromorphological traits. Wide phenotypic variations regarding plant types and metric traits were observed in F₁ 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 F₂ 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 F₂ progenies recorded higher mean pollen fertility (46.8%) compared F₁ progenies (22.4%).

The occurrence of *B. juncea* (the maternal parent) type plants in larger frequency compared with other three types in F₂ 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 F₂ generation of *B. juncea × B. rapa* crosses was also by Choudhary et al. (2002) and Choudhary and Joshi (2012b). Wide phenotypic variation and transgressive segregation for plant height, Secondary branches per plant, main raceme length etc. in the F₂ 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 F₂ and F₃ plants of the cross *B. rapa × B. nigra* for plant height, flowering 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 F₂ generation of *B. napus × B. carinata* and *B. juncea × B. rapa* interspecific crosses.

**Generation advancement**

From F₁ 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 F₂ 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), siliqua 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 agromorphological characteristics when compared with the variety of *B. carinata* (Kiran) (Table 5). Thus present

Table 4: Segregation for plant types in the F₂ generation of *B. juncea* (cv. NRCDR-2) and *B. carinata* (NRCKR-304) cross

<table>
<thead>
<tr>
<th>Cross</th>
<th>Plant type frequency</th>
<th>Number of Plants observed</th>
</tr>
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<tbody>
<tr>
<td><em>B. juncea</em> (cv:NRCR-2) × <em>B. carinata</em> (NRCKR-304)</td>
<td>44(43.1)*</td>
<td>30(29.43)</td>
</tr>
</tbody>
</table>

*Numbers in parenthesis indicate percentage*
study indicated that inter-specific 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 interspecific 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 F₁s. The study suggests that B. carinata has partial genome homology with B. juncea. In the F₂ generation, more number of plants resembling B. juncea was observed indicating transgressive segregation. Present study also indicated that inter-specific 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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