# Phenotypic variations in plant progenies of interspecific crosses involving *Brassica juncea* / *B. carinata*

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**Abstract**

With the aim of transferring fungal disease resistance in Indian mustard, eight hybrids were generated, through *in vitro* ovule culture/ *in vitro* germination of seeds, using two brown seeded *Brassica juncea* genotypes; RESBJ-830 and RESBJ-837 as female parents, and the yellow seeded *B. carinata* variety Kiran as male donor. The hybrids showed intermediate morphological characteristics with pronounced male donor specific features; such as presence of red dots at the tip of the anthers (RA) and purple color (PC) at the junction of petioles, shoots, mid rib and green pods. Complete to partial pollen sterility was observed in the $F_2$ and $BC_1$ generations. A significant difference was observed in the seed coat color between the progenies of hybrids derived from the two *B. juncea* genotypes. While the $F_2$ seeds derived from RESBJ-830 were brown seeded, those from RESBJ-837 were yellow seeded, and both segregated in a ratio of 2 (Brown): 1 (Yellow) in $BC_1$ generation. Most of the plants in the $F_2$ generation resembled either the male parent *B. carinata* or were intermediate in morphology with about equal frequency, and only one plant resembled *B. juncea*. The $BC_1$ generation had a high percentage of plants resembling *B. juncea* (40%), followed by intermediate (37%), and *B. carinata* plant type (21%). A significant variation for branching pattern, leaf and floral morphology, inflorescence and siliquae arrangement was recorded in both $F_2$ and $BC_1$ generations. The *B. juncea* type plants in these generations resembled the female parent in most of the morphological traits except for length of the main shoot that was short in $F_2$ plants and long like the female parent in $BC_1$ plants. The variations observed for the presence of RA (85%) and PC (30%) in $F_2$ plants, were reduced to 40% and 17%, respectively, in $BC_1$ generation. An apetalous phenotype was also identified in both generations. The subsequent $BC_1F_2$ and $BC_2$ generations recovered *B. juncea* type brown/yellow seeded plants with the latter in higher percentage in $BC_2$ generation. The characteristic feature of the male donor, RA and PC, and short main shoot with distantly placed siliquae were observed only in plants derived from RESBJ-830. The genetic stability of the above-mentioned phenotypic traits and disease response to fungal diseases in advanced generations were evaluated.

**Key words**: interspecific hybridization, embryo rescue, backcross-progeny, morphological traits

## Introduction

Transfer of genes across the *Brassica* species has played an important role in improvement of several economically important traits such as oil and meal quality, and incorporation of resistance to fungal diseases (Gupta et al., 2006). This principle has played an important role in improvement of *B. juncea*, the most prominent oilseed *Brassica* in India and South East Asia (Agnihotri et al., 2003). The extent of variability generated through interspecific hybridization is phenomenally dependent on the breeding strategy adopted after successful establishment of the $F_1$ hybrids and plays an important role in recovering the desired plant type with trait of interest. The paper presents a comparison of the morphological/ phenotypic variation in the progenies derived from *B. juncea*/ *B. carinata* crosses attempted to transfer fungal disease resistance.

## Materials and Method

The *B. juncea* genotypes RESBJ-830 (EC446033) and RESBJ-837 (EC 446032) which have differential resistance response to *Pernospora parisitica* (Dr NI Nashaat, Personal communication) were used as female parents. The *B. carinata* cultivar Kiran (obtained from Prof SJ Kolte, GBPUAT, Pantnagar) was used as the pollen donor. The interspecific crossing and *in vitro* sequential embryo rescue were carried out as per Agnihotri (1993) and the regenerated plantlets were multiplied through axillary bud proliferation. The regenerated shoots were hardened and transplanted in the field. The morphological characteristics of the $F_1$ hybrids were recorded and these were bagged for self-pollination or backcrossed to the respective *B. juncea* parent. The inbred and backcrossed progenies were grown simultaneously in the successive *Brassica* growing season to study the phenotypic variability between these two populations.

## Results

The recovery and establishment of $F_1$ plantlets was greater *via* ovule culture (25%) than sequential embryo rescue (2.6%). Wrinkled and shriveled seeds produced from pods left *in vivo* invariably required an *in vitro* phase for germination. Eight hybrids including two from RESBJ-830 × Kiran (J0K) and six from RESBJ-837 × Kiran (J7K) were grown to maturity. The hybrids showed intermediate morphological traits with characteristic features of male donor such as purple color at the junction of petioles and stems, and presence of red dot at the tip of anthers (Figure 1). Anthers were partially male sterile with pollen fertility ranging from 19.5 to 39.2%. Self-pollination produced an average of 20 seeds per hybrid in J0K and approximately 40 seeds from J7K. The pod formation ranged from 38.8% in J7K and 41.8 % in J0K. Only nine backcross seeds were harvested following backcrossing to J0K. Self-pollinated seeds from J0K were dark brown resembling female, whereas seeds from J7K were yellowish brown in contrast to bright yellow in male donor. Seeds obtained from backcrossing were segregated in two brown: one yellow. The $BC_1$ and $F_2$ seeds were sown in single plant progeny rows to evaluate the respective plant populations.

$F_2$ *generation*: Only three plants (from 40 seeds) represent the two J0K hybrids and 39 plants (from 231 seeds) representing J7K were obtained. Three out of the six J7K hybrids did not germinate. A total of five $F_2$ single plant progenies were evaluated at the peak flowering stage (Table 1). The plants of J7K and J0K flowered at 76 and 88 days after sowing (DAS) compared to 50 and 68 DAS respectively for the female parent, while the pollen donor *B. carinata* had flowering at 114 DAS, indicating the days to 50% flowering as intermediate to both parents in each cross. The $F_2$ plants were categorized as resembling *B. carinata*, intermediate and *B. juncea* type. A high influence of male donor was observed in the $F_2$ plants from cross J7K that segregated in 1 *B. carinata* type:1 intermediate morphology type. All J0K plants were brown seeded while out of 35 plants from J7K, seven were yellowish brown and 28 were brown. Based on morphological traits, one plant from J0K and three from J7K were selected for progeny advancement.

$F_3$ *generation*: Two $F_3$ single plant progenies were evaluated further. Five plants from J0K and three from J7K were grown to maturity. The plants were late in flowering (102 DAS) and predominantly intermediate to their parents with medium height and profuse basal branching. Leaves were *B. carinata* type with sinuate margins and purple venation in J0K while in J7K leaves were partially dented having white venation with yellow chlorotic patches on the upper surface. Flowers resembled *B. carinata* with a characteristic red dot at the tip of the anthers. Plants with long pods possessed a shorter main shoot in J0K as compared to those from J7K.

$BC_1$ *generation*: The plants of J7K (25) and J0K (7) flowered at 70 and 84 DAS as compared to 50 and 68 DAS for the respective female parents. In J0K, more than 70% of the plants were intermediate type; but the leaves, flower and inflorescence of the remaining 30% of the plants resembled *B. juncea* type,. Variability in leaf, flower and inflorescence of plants raised from J7K was high and segregated in 13 (*B. juncea* type): 7 (intermediate): 5 (*B. carinata* type). The plant height and branching pattern was highly variable, 64% of the plants being medium in height and almost half of the plants exhibited basal branching. All J7K $BC_1$ plants, except one, had green color like the female parent. An apetalous flower phenotype plant was identified from progeny J7K-1 (Figure 1). More than 84% of the plants were *B. juncea* type with petiolate serrate margins, oval leaves and obtuse tip. The inflorescence had upright, partially appressed pods resembling the female parent J7. Only 40% of the plants possessed a red dot at the tip of the anthers. Plants from J7K-4 were completely sterile (Table 1). Four plants from J0K segregated in 1: 1 ratio for seed coat color, while 25 plants from J7K segregated in the ratio of 2: 1. In total, four plants selected from J0K and 11 from J7K were selfed and backcrossed to harvest $BC_1F_2$ and $BC_2$ seeds.

$BC_1F_2$ *generation*: $BC_1F_2$ seeds from four J0K-2 plants were forwarded, but seeds from only one plant germinated. Similarly out of 11 plants from J7K, seed from only five plants were germinated; one from each of J7K-2, J7K-3 and J7K-4, whereas the other two plants were from J7K-5. All germinated plants of J0K as well as $BC_1F_2$ seeds of J7K were derived from brown seeded plants except one (SPP-1) from J7K-5. A total of 71 $BC_1F_2$ plants; 12 from a single SPP of J0K and 35 derived from four SPP of J7K were grown and evaluated. The plants from J7K were intermediate type with medium height and above basal branching, while the plants from J0K had a pronounced influenced by the pollen donor *B. carinata* as reflected by presence of RA and purple color observed at the base of branches in all plants having intermediate leaf morphology. On the other hand the majority of the plants from J7K had *B. juncea* type leaf with a variation at tip and margins. The inflorescence of all plants was of a *B. juncea* type with dense pods except a few J0K plants with a short main shoot like *B. carinata*. Segregation for seed coat color was in the ratio of 1: 11 where 6 plants were with yellow seed coat color and 65 plants with brown seed coat color. Out of the six yellow seeded plants, 2 plants were from J0K and 4 from J7K (2 each from J7K-2 & -5). An unusual variant was identified from J7K-2 with a reddish tone in seed coat color (Figure 1).

$BC_2$ *generation*: $BC_2$ seeds from four J0K-2 plants were forwarded, but seeds from only one plant were germinated. Similarly out of 11 plants from J7K, seeds from one plant each of J7K-2 and J7K-3, and two plants of J7K-5 were germinated. All plants of J0K and J7K except one SPP J7K-5 (SPP-1) were derived from brown seeded plants. A total of 17 $BC_2$ plants, 4 from J0K and 13 from J7K were grown to maturity and evaluated. All plants in $BC_2$ were *B. juncea* type but with characteristic feature of the male donor. Plants were medium in height with above basal branching. Very minor variation was observed in leaf morphology in the $BC_2$ progeny. Leaves were petiolate, oval with serrate margins and coarse textured at the adaxial surface except J0K leaves that were round with obtuse tip. Floral morphology was similar to their female parents and the red dot on the anther was absent in all the plants. Inflorescences also resembled the female parents with long main branches and upright appressed dense pods, except J0K that possessed open scattered pods like the male donor. Good pod formation and seed set was achieved with variation in seed coat color. Stability for seed coat color was recorded in the second backcross and plants of J0K were all yellow seeded. However, J7K plants segregated almost equally into yellow and brown seeded plants. The yellow seeded $BC_2$ plants from J7K resembled the male donor, but the bright yellow seeded plants from J0K were distinctly different from male donor which had brownish yellow seed coat color.

**Table 1: Agro-morphological variations in $F_2$ and $BC_1$ populations of *B. juncea*/ *B. carinata* crosses**

| Genotypes [no of plants screened] | Height | Plant type @ | Leaf morphology | Inflorescence/ pods | Seed coat color |
|---|---|---|---|---|---|
| RESBJ 837 ♀<br>RESBJ 830 ♀ | 194 – 194 cm (M) | J | Dark green, acute pointed tip Serrate margins, petiolate, narrow from tip course texture | Raceme type, long main branch, upward, appressed dense pods | Brown |
| Kiran ♂ | 246 cm (T) | K | Sap green, obtuse tip, round from top, slight serrate, white veins, broad from top and bottom, smooth texture | Raceme type, short main branch, upright, open scattered pods | Bright yellow |
| $F_2$ | | | | | |
| J0K [3] | M | 66% - J<br>34% - I | I to J type, oval to elliptical tip, clasping upper leaves dentate margins | Raceme type, short main branch, upright, partially appressed pods | Brown |
| J7K [39] | M | 50% - K<br>50% - I | Petiolate, smooth texture, partially serrate, obtuse tip, light green | 53.8% - I<br>46.1% - K | 17.9% plants yellow rest brown |
| **$BC_1$** | | | | | |
| J0K [7] | M | 100%- I | K to I type, oval round or elliptical tip, course texture | Raceme type short main branch, appressed pods | 28.5% plant yellow seeded |
| J7K [25] | T= 20%<br>M = 64%<br>D = 16% | 52% - J<br>28% - K<br>20% - I | 16%- K<br>84% - J | 16%- K<br>84% - J | 32% plants yellow seeded |

Plant height T= tall > 200 cm; M= moderate (170- 200 cm); D= dwarf (⩽ 170cm), @ K = *B. carinata* type; J = *B. juncea* type; I = Intermediate

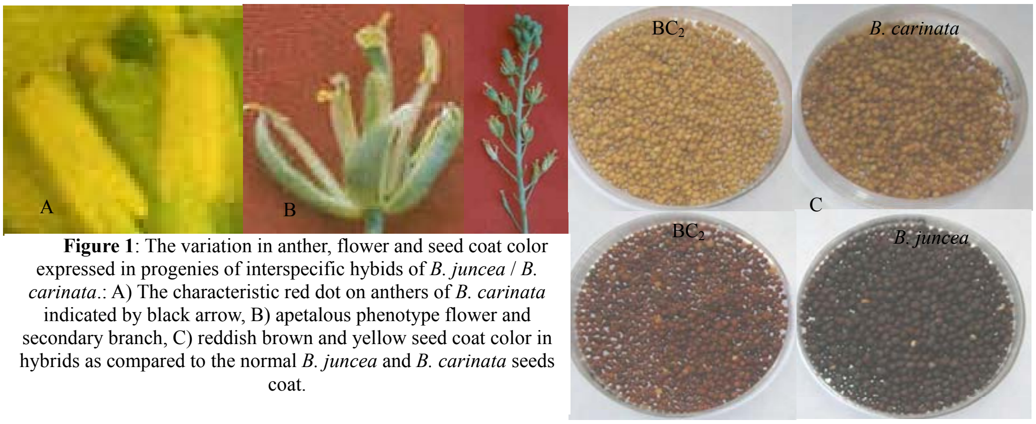

**Figure 1**: The variation in anther, flower and seed coat color expressed in progenies of interspecific hybids of *B. juncea* / *B. carinata*.: A) The characteristic red dot on anthers of *B. carinata* indicated by black arrow, B) apetalous phenotype flower and secondary branch, C) reddish brown and yellow seed coat color in hybrids as compared to the normal *B. juncea* and *B. carinata* seeds coat.

## Discussion and Conclusion

Limited seed set in hybrids and poor seed germination in subsequent progenies supports the presence of post fertilization barriers in the interspecific cross *B juncea / B. carinata*. Significant variation in leaf shape, flower color, branching pattern, pod size and seed coat color was observed. High frequencies of intermediate type plants were observed in $F_2$ progeny from *B. juncea*/*B. carinata* (72%) followed by those resembling the male donor (20%). The segregation pattern of $F_2$ plants indicated a high recombination rate with more influence from the male donor. The high frequency of plants resembling the maternal parent and the intermediate type of plants in the $F_2$ generation in an interspecific cross have been reported earlier by Subudhi and Raut (1994) in *B. juncea*×*B. napus* and by Choudhary et al. (2002) in *B. juncea*×*B. rapa*. The wide phenotypic variation and transgressive segregation for several morphological traits in $F_2$ and $BC_1$ might have resulted from recombination and/or eventual segregation of aneuploids (Olsson, 1960). Taxonomic and cytogenetic studies indicate that hybrids of tetraploid/digenomic species possess high amount of pairing and thus reflect a greater variation in morphological traits as well as in subsequent generations in *Brassica* species (Nishiyama et al., 1991). One of the significant outcomes was identification of an apetalous phenotype. The apetalous trait is desirable in *Brassicas* that has been related to higher seed yielding capacity and tolerance to sclerotinia rot in *B. napus* (Rakow and Séguin-Swartz, 1999; Zhao and Wang, 2004). Although apetalous phenotypes have been reported in *B. napus* and *B. rapa*, to our knowledge, this is the first report for identification of apetalous morphology in early segregating populations in *B. juncea*. A slow transition to *B. juncea* type plants was observed with the two recurrent backcrosses. Probably the chromosomes of C genome were not excluded during recombination at meiosis during subsequent backcrossing with the *B. juncea* (AABB) which needs to be correlated with appropriate cytological studies. A characteristic red dot present at the tip of the anthers on $F_1$ and $F_2$ plants derived from *B. juncea*/*B. carinata* progenies diminished with successive advanced generations ($BC_1$, $BC_2$ and $BC_1F_2$). Presence of purple color, though segregating, was maintained throughout the progenies. The partial homeology that exists between A, B and C genomes indicates interspecific transfer of gene/gene-complexes between the three genomes (Choudhary and Joshi, 1999). The identification of yellow seeded plants in the subsequent generations is most significant. The presence of a dominant repressor gene in yellow seeds that inhibit expression of seed coat pigment synthesis, and absent in brown seeded plants has been reported (Getinet and Rakow, 1997). The $BC_1$ plants of all cross combinations and $F_2$ plants from cross J7 segregated into yellowish-brown and brown color seeds, suggesting partial dominance of brown over yellow seed coat color.

The progenies forwarded after vigorous selection for morphological traits (as well as fungal disease resistance) at each generation, resembled their female parent in almost all-important morphological traits at much advanced stage except for seed coat color. Although *B. carinata* has been used earlier to incorporate economically important traits in *B. juncea*, detailed study of the possible variation obtained in agro- morphological traits in hybrids and advanced progenies derived from *B. juncea*/*B. carinata* is not available The advance generation genotypes resembling *B. juncea* developed through this hybridization program are being evaluated further for their response to fungal diseases.

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