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Research Article

Comparison of residual heterosis in F_2 and F_3 segregating **generations of Indian Mustard [***Brassica juncea* **(L.) Czern & Coss.] crosses**

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Abstract

The present investigation was undertaken to study the residual heterosis in F_2 and F_3 populations of Indian mustard [*Brassica juncea* (L.) Czern & Coss.]. The study material included eight parents and the 28 crosses of Indian mustard in two generations, namely F_2 and F_3 . The experiment was conducted over two years at Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal, during *Rabi* season 2020-21 and 2021-22. Out of the 28 crosses, three crosses namely, Npj-194 × DRMR-15-16, Npj-194 × RW-85-59 (Sarma) and Npj-194 × SKJM-05 were found to show significantly positive residual standard heterosis over the standard variety (SKJM-05) for both the characters siliquae per plant and seed yield per plant. This provides us evidence that by improving siliquae per plant, the character seed yield per plant can be improved. The promising cross populations which have shown significant standard heterosis for seed yield in F_2 and F_3 , can be utilized for the development of hybrid varieties, which would be very much beneficial to the marginal farmers of our country as they would be able to use the F_2 and F_3 seeds of the hybrid varieties without any compromise with seed yield.

Keywords: Residual relative heterosis, Residual heterobeltiosis, Residual standard heterosis, Indian Mustard, Segregating generations

INTRODUCTION

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is the most important oilseed crop, occupying a leading position in the Indian oilseed scenario and playing a crucial role in the country's oilseed economy. It accounts for 20.8% of the world's cultivated area and 10% of global production. India has an ever-increasing population. The standard of living is also rising, resulting in higher per capita consumption of edible oil. It has been estimated that by 2030, the annual growth in demand for edible oil

would be 3.54% and accordingly, per capita consumption of edible oil would increase to 23.1 kg/year. Thus, to achieve self-sufficiency in edible oil, 34.1 million tonnes of edible oil would be required, which is equivalent to about 102.3 million tonnes of oilseeds (DRMR 2011). By the end of fiscal year 2022, India produced more than 11 million tons of rapeseed and mustard (Statista, 2022). There is a growing demand for mustard oil in India which is mainly driven by the emerging food industry due to

increasing consumer awareness of the numerous health benefits associated with the use of mustard oil, like its anti-bacterial, anti-fungal, anti-viral, anti-inflammatory and most importantly, its anti-carcinogenic properties (Kaur *et al*., 2019).

Therefore, attainment of high production and productivity of Indian mustard is one of the major objectives in the country. The approach of heterosis breeding has been found to be one of the most successful options that has been employed for the improvement of *Brassica* varieties for quality and quantity of seed yield and other yield related parameters. With the help of heterosis studies, superior cross combinations of promising hybrids can be identified for developing high yielding varieties (Meena *et al*., 2015). Residual heterosis occurs when the yield of the segregating generation in some hybrids is likely to be higher than the check variety (Fu *et al*., 2014). It is the amount of heterosis shown by F_2 and its subsequent segregating generations. According to Burton and Brownie (2006), potential selection of hybrid crosses in the F_2 and F_3 generations might be to produce a greater degree of high yielding pure lines than other breeding methods that ignore the possibility of dominance. Hence, identification of better progeny in the early generation is essential for the potential selection process. According to Koseoglu *et al*. (2017), better progeny than that of the average of the parents at $F_{2/3}$ segregating populations are known as fruitful heterosis (HF). The residual heterosis in F_2 and F_3 populations over the parents is a desirable character and an indication that the superior performance of the $\mathsf{F}_{_{1}}$ generation is also reflected in later segregating generations, which indicates that the farmers who once buy seeds of a hybrid mustard variety can keep using the seeds for the next two-three generations without any significant reduction in yield, ultimately, reducing the cost of production of the crop (Duvick *et al*., 1999). This is applicable only with respect to the hybrid varieties prevalent in the market.

The study of residual heterosis has been exploited in several crops such as chickpea (Adak *et al*., 2017), wheat (Rajane *et al*., 2022), okra (Sabesan *et al*., 2016), Soybean (Bhartiya *et al*., 2014), rice (Balat *et al*., 2018), cucumber (Kumari *et al*., 2021), groundnut (Byadagi *et al*., 2018), brinjal (Varma *et al*., 2020) etc. But a very few studies were done on residual heterosis of Indian mustard. Therefore, the present study is an attempt to find the existence of residual heterosis that can be present in F_2 and $\mathsf{F}_\mathfrak{z}$ populations of Indian mustard so that the seeds of these populations can be used by our farmers without any significant reduction in yield.

MATERIALS AND METHODS

The present study was conducted over two years at Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, during *Rabi* seasons 2020-21 and 2021- 22. The experimental material for the study included eight parents and the 28 crosses of Indian mustard [*Brassica* *juncea* (L.) Czern & Coss.] in two generations, namely F₂ and F_3 . The crosses , based on inter-varietal hybridization, were obtained from a previous study made by Rout *et al*. (2021) during the *rabi* season of 2018-19. The F_{2} seeds were sown to develop F2 populations during *Rabi* 2020- 21. Subsequently, F_3 seeds were sown to develop F_3 populations during the following year 2021-22. The $\mathsf{F}_2^{\vphantom{\dagger}}$ and F_{3} populations were evaluated along with eight parents for the two consecutive years, in *Rabi* 2020-21 and 2021- 22. Data was recorded from five randomly selected plants from each parent, 20 plants of the 28 F_2 populations and 10 plants from the 28 F_3 populations per replicate. Observations were recorded for six morphological characters such as plant height (cm), primary branches per plant, secondary branches per plant, siliquae per plant, 1000 seed weight (g) and seed yield per plant (g). Morphological characters like plant height, primary number of branches, secondary number of branches etc. were found to exhibit significant and positive association with seed yield in a study conducted by Patel *et al.* (2006). The generation from F_2 to F_3 was advanced by bulking the seed in each of the 28 cross populations separately.

Residual relative heterosis, heterobeltiosis and standard heterosis (using SKJM-05 as the check variety) were estimated as the percentage of deviation of generation mean of F_2 (or F_3) from the mean parental value, better parental value and the standard variety, respectively. For the estimates of residual heterosis (F_2 and F_3) the data were calculated manually. The following formulae suggested by Rao *et al.* (1980) were followed for the calculation of different estimates of heterosis.

Estimation of residual heterosis:

Residual relative heterosis (%) = [{mean of F_2 or F_3 – mean of parent}/ mean of parent] ×100

Residual heterobeltiosis (%) = [{mean of F_2 or F_3 – mean of better parent}/ mean of better parent] ×100

Residual standard heterosis (%) = [{mean of F_2 or F_3 – mean of standard variety}/ mean of standard] ×100

Further, the statistical significance of all the estimates of heterosis was calculated through 't' test formulae suggested by Turner (1953).

RESULTS AND DISCUSSION

Results from ANOVA (**Table 1**) showed that the 36 entries consisting of eight parents and 28 F_2 populations in 2022-21 and F_3 populations in 2021-22 differed significantly for all the six characters in the present study, indicating the presence of large variability among the entries in both the F_2 and F_3 generations. The considerable variability between parents and the segregating population was also noted by Ara *et al*. (2013), in crosses between cultivars of *Brassica* species.

For the character plant height in F_2 , only six crosses

Table 1. Analysis of variance for seed yield and its attributing characters in Indian Mustard in F2 (2020-21) and F3 (2021-22) generations

 ** Significant (P ≤ 0.01); *Significant (P ≤ 0.05), PH = Plant height (cm), PBPP = Primary branches per plant, SBPP= Secondary branches per plant, SPP = Siliquae per plant, 1000 SW = Thousand seed weight (g), SYPP = Seed yield per plant (g)

showed significant residual relative heterosis ranging from -16.17% (Giriraj × RNWR- 09-3) to 18.64% (DRMR-15-16 × Kranti) (**Table 2**). Significantly positive residual relative heterosis was exhibited by only two crosses, namely, DRMR-15-16 \times Kranti and Npj-194 \times DRMR-15-16 . In F₃, 16 crosses showed significant residual relative heterosis that ranged from -33.04 (RNWR-09-3 × PHR-2) to 33.64 (DRMR-15-16 × RNWR-09-3). Out of them, only one cross DRMR-15-16 × RNWR-09-3 (33.64%) expressed significantly positive residual relative heterosis. In F_2 , 11 crosses showed significant residual heterobeltiosis that ranged from -20.41% (Giriraj × RNWR-09-3) to 17.65% (DRMR-15- 16 × Kranti). Significantly positive residual heterobeltiosis was shown by only two crosses, namely, Npj-194 × DRMR-15-16 and DRMR-15-16 × Kranti. In F_3 , 22 crosses expressed significant residual heterobeltiosis ranging from -39.87% (SKJM-05×PHR-2) to -22.07% (SKJM-05×Kranti). However, none of the crosses exhibited significantly positive residual heterobeltiosis. In F_2 , five crosses showed significant residual standard heterosis ranging from -15.75% (Giriraj × RNWR-09-3) to 15.98% (DRMR-15-16 × Kranti). On comparing all the residual heterosis for plant height, it was revealed that F_3 performed lower than F_2 for residual relative heterosis and heterobeltiosis, whereas for standard heterosis, F₃ performed better than the F_2 indicating the potential of the segregating population.

For the character primary branches per plant in F_2 , five crosses expressed significant residual relative heterosis ranging from -32.17% (Npj-194 × PHR-2) to 22.98% [RW-85-59 (Sarma) × Giriraj] (**Table 2**). Significantly positive residual relative heterosis over the mid-parental value was exhibited by only one cross RW-85-59 (Sarma) × Giriraj (22.98). Similarly, in F_3 , 18 crosses showed significant residual relative heterosis ranging from 49.6% (SKJM-05 × Kranti) to 194.74% (Npj-194 × DRMR-15-16) and all of them expressed significantly positive residual relative heterosis. In F_2 , 11 crosses exhibited significant residual heterobeltiosis ranging from -75.4% (Npj-194 × Giriraj) to -16.82% [Npj-194 × RW-85-59 (Sarma)]. However, none

of the them were found to express significantly positive residual heterobeltiosis. Similarly, in F_3 , 15 crosses had expressed significant residual heterobeltiosis ranging from 48.33% [RW-85-59 (Sarma) × DRMR-15-16] to 172.37% (Npj-194 × DRMR-15-16) and all of them showed significantly positive residual heterobeltiosis. In F_{2} , the crosses exhibited neither significantly positive residual standard heterosis nor significantly negative residual standard heterosis over the standard variety. In F_3 , 20 crosses showed significant residual standard heterosis that ranged from 55.60% (RNWR-09-3 × PHR-2) to 180% [Npj-194 × RW-85-59 (Sarma)] and all of them exhibited significantly positive residual standard heterosis. Out of which Npj-194 × DRMR-15-16 had shown the highest positive residual standard heterosis of 180% over the standard variety SKJM-05. Overall, for all the residual heterosis, $\mathsf{F}_\mathfrak{\text{3}}$ performed better than the $\mathsf{F}_\mathfrak{\text{2}}$.

For the character secondary branches per plant, 17 crosses expressed significant residual relative heterosis in F_{2} that ranged from -64.95% (Npj-194 \times PHR-2) to 80.23% (Kranti × RNWR- 09-3) (**Table 2**). Out of which, seven crosses showed significantly positive residual relative heterosis in F_3 , 10 crosses expressed significant residual relative heterosis ranging from -51.72% (SKJM-05× Giriraj) to -29.52% [RW-85-59 (Sarma) × Giriraj]. Out of them, none of the crosses were found to express significantly positive residual relative heterosis. In F_2 , 16 crosses expressed significant residual heterobeltiosis ranging from -69.02% (Npj-194 × PHR-2) to 75.05% (Kranti × RNWR-09-3). Only two crosses, namely, Kranti × Giriraj and Kranti × RNWR- 09-3 exhibited significantly positive residual heterobeltiosis. In F_3 , 12 crosses expressed significant residual heterobeltiosis ranging from - 63.9% (SKJM-05 × Giriraj) to -34.51% [RW-85- 59 (Sarma) × Giriraj]. However, none of the crosses were found to exhibit significantly positive residual heterobeltiosis. In F_2 , nine crosses exhibited significant residual standard heterosis ranging from 78.9% (RNWR-09-3× PHR-2) to 203.42% (Kranti × PHR-2). All of these crosses expressed significantly positive residual standard

Table 2. List of crosses exhibiting positive and negative relative heterosis in F2 and F₃ for the six yield attributing characters in mustard **Table 2. List of crosses exhibiting positive and negative relative heterosis in F2 and F3 for the six yield attributing characters in mustard**

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heterosis. In F_{s} , three crosses showed significant residual standard heterosis ranging from 56.25% (Npj-194× SKJM-05) to 64.28% (Npj-194 × DRMR-15-16 and Kranti × Giriraj). All of them exhibited significantly positive residual standard heterosis. Overall, negative residual mid and better parent heterosis were revealed for both the generations and positive residual standard heterosis was observed for F_2 and F_3 generations. In all the cases, F_2 performed superior to the F_3 for the respective character.

For siliquae per plant, in F_2 , 13 crosses showed significant residual relative heterosis ranging from -22.19% [RW-85-59 (Sarma) × SKJM-05] to 59.31% (Kranti × Giriraj) (Table 2). Out of which, seven crosses had expressed significantly positive residual relative heterosis. In F_{3} , five crosses expressed significant residual relative heterosis ranging from 41.05% (RNWR-09-3× PHR-2) to 84.24% (Npj-194× SKJM-05). All of them expressed significantly positive residual relative heterosis with Npj-194 × SKJM-05 as the best performer. In F_{2} , 14 crosses expressed significant residual heterobeltiosis ranging from -22.78% [RW-85-59 (Sarma) × SKJM-05] to 200% (SKJM-05 × PHR-2). Eight of them had expressed significantly positive residual heterobeltiosis with SKJM-05 × PHR-2 as the best performer. In F_3 , five crosses showed significant residual heterobeltiosis ranging from -33.79% [RW-85- 59 (Sarma) × PHR-2] to 76.78% (Npj-194 × SKJM-05). Out of them, four crosses expressed significantly positive residual heterobeltiosis and only one cross, RW-85-59 (Sarma)× PHR-2 showed significantly negative residual heterobeltiosis. In $\mathsf{F}_2^{}$, seven crosses exhibited significant residual standard heterosis ranging from 25.20% (DRMR-15-16 × SKJM-05) to 60.60% (Kranti × Giriraj) and all of them expressed significantly positive residual standard heterosis. In F_3 , six of the crosses showed significant residual standard heterosis ranging from 53.95% (RNWR-09-3× PHR-2) to 126.00% [(Npj-194 × RW-85- 59 (Sarma)]. All of them showed significantly positive residual standard heterosis. Here, F_3 performed well for all the forms of residual heterosis especially the crosses involving Npj-194 as a parent.

For the character 1000 seed weight, in F_{2} , 13 crosses expressed significant residual relative heterosis ranging from -36.38% (Npj-194 × SKJM-05) to 84.90% [RW-85- 59 (Sarma) × PHR-2] (**Table 2**) with only three crosses exhibiting significantly positive residual relative heterosis. In F_3 , six crosses exhibited significant residual relative heterosis ranging from -27.82% (2×5) to 50.15% (Npj-194 × SKJM-05). Five of them showed significantly positive residual relative heterosis. The other five crosses showed significantly negative residual relative heterosis in F_3 . In F_2 , 12 crosses expressed significant residual heterobeltiosis that ranged from -48.00% [SKJM-05, RW-85-59 (Sarma)× RNWR-09-3] to -24.96% (SKJM-05 × Giriraj) and none of them showed significantly positive residual heterobeltiosis. In F_{3} , three crosses showed significant residual heterobeltiosis ranging from -29.91%

[RW-85-59 (Sarma) × Kranti] to 48.35% (Npj-194 × SKJM-05). In F_2 , majority of the crosses, i.e., 25 out of 28 crosses exhibited significant residual standard heterosis that ranged from -54.62% [RW-85-59 (Sarma) × RNWR-09-3] to 48.86% [RW-85-59 (Sarma) × PHR-2]. Out of them, only two crosses, namely RW-85-59 (Sarma) × PHR-2 and Giriraj × RNWR-09-3 exhibited significantly positive residual standard heterosis. In F_3 , only four crosses exhibited significant residual standard heterosis ranging from -24.02% [RW-85-59(Sarma) × Kranti] to 49.25% (SKJM-05 × Giriraj). Out of them, three crosses, exhibited significantly positive residual standard heterosis over the standard variety SKJM-05. Overall, F_3 performed better than the F_2 for all the types of residual heterosis.

For the character seed yield per plant in F_2 , 17 crosses showed significant residual relative heterosis ranging from -61.26% (Npj-194 × SKJM-05) to 51.50% (Kranti × RNWR-09-3) (**Table 2**). Out of them, only two crosses, namely, SKJM-05 × Giriraj and Kranti × RNWR-09-3 showed significantly positive residual relative heterosis In F_3 , only four crosses exhibited significant residual relative heterosis ranging from -31.62% (Giriraj × PHR-2) to 76.86% (Npj-194 × DRMR-15-16). Out of them, three crosses exhibited significantly positive residual relative heterosis. In F_{2} , majority of the crosses, i.e., 21 out of the 28 crosses expressed significant residual heterobeltiosis ranging from -68.67% (Giriraj × RNWR-09-3) to 43.50% (Kranti × RNWR-09-3). However, only one cross Kranti × RNWR-09-3 (43.50%) exhibited significantly positive residual heterobeltiosis over the better parent RNWR-09-3. In F_3 , six crosses showed significant residual heterobeltiosis ranging from - 50.46% (DRMR-15- 16× PHR-2) to -9.47% (SKJM-05× RNWR-09-3) and none of them showed significantly positive residual heterobeltiosis. In F_2 , 12 crosses exhibited significant residual standard heterosis ranging from -54.52% (Giriraj × RNWR-09-3) to 46.11% (DRMR-15-16 × RNWR-09-3) In F_3 , only one cross DRMR-15-16 × Giriraj with significantly positive residual standard heterosis of 45.77% was found. Overall, F_3 performed better than the F_2 for residual mid and better parent heterosis whereas, for standard heterosis both the generations performed equally, irrespective of the crosses.

In the present study, the residual relative heterosis, heterobeltiosis and standard heterosis in desired direction were found for all the characters for both the generations. This retention of heterosis may be due to the presence of transgressive segregants and tight linkage between some of the favorable genes that are controlling the characters (Sabesan *et al*., 2016). There are many causes for residual heterosis. According Kumar *et al*. (2002), low inbreeding depression might be the reason for residual heterosis. It is also found that heterosis was expressed in both the positive and negative directions for most of the characters. The positive and negative expressions indicated the role of dominant and recessive genes in the

inheritance of the characters (Rajane *et al*., 2022). The **Table 2** shows the crosses that exhibited a positive and negative residual heterosis for the characters for both the generations. Sabesan *et al*. (2016) suggested that negative heterosis in F_2 population indicates that those characters suffer from severe inbreeding depression.

From **Table 2**, it is also evident that some notable crosses in both the generations performed well for given one or more characters such as DRMR-15-16 × Kranti, Npj-194 × DRMR-15-16, Npj-194 × RW-85-59 and Npj-194 × SKJM-05, DRMR-15-16 × Giriraj and Kranti × RNWR-09-3. The number of mustard crosses with positive residual heterosis (relative, heterobeltiosis and standard heterosis) in F_2 and F_3 generations are depicted in Fig **1**. Hence, out of all the 28 crosses, some of the crosses were chosen based on their superior performances for the characters under study in F_2 and F_3 generations as shown in **table 3**.

Out of the 28 crosses, three crosses namely, Npj-194 \times DRMR-15-16, Npj-194 \times RW-85-59 (Sarma) and Npj-194 × SKJM-05 were found to show significantly positive residual standard heterosis over the standard variety for two characters namely, siliquae per plant and seed yield per plant. This provides us evidence that by improving siliquae per plant, the character seed yield per plant can be improved. Therefore, it may be inferred that the crosses identified are useful to study the residual heterosis and also to improve the particular character in the breeding programme under present study. Since the development of hybrid seed of Indian mustard is not adequately developed in India, search for the crosses manifesting heterosis largely due to additive gene effects should be attempted to make proper use of residual heterosis.

The present study was done to find out whether residual heterosis is available in Indian mustard and if it can be utilized in the segregating generations. For economic application, similar kind of research can be conducted by including the commercially available hybrid varieties of Indian mustard. The promising cross populations in the present study, which have shown significant standard heterosis for seed yield in F_2 and F_3 , can be utilized for the development of hybrid varieties, which would be very much beneficial to the marginal farmers of our country as they would be able to directly use the F_1 followed by indirectly using the F_2 and F_3 seeds of the promising hybrid varieties, without any significant compromise with seed yield.

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Table 3. Promising crosses and their residual heterosis performance for the different characters

Fig. 1. Number of crosses with positive residual heterosis (relative heterosis, heterobeltiosis and standard heterosis) in F₂ and F₃ generations of mustard

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