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RESEARCH ARTICLE

A comparative analysis of biparental and F_3 progenies of Indian mustard [*Brassica juncea* (L.) Czern & Coss]

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Abstract

The present investigation was carried out to assess the genetic variability generated as biparental progenies (BiPs) and F_3 populations from the crosses, RH 725 × Urvashi (cross I), DRMRIJ 31 × Urvashi (cross II) and PM 27 × Urvashi (cross III) in Indian mustard [*Brassica juncea* (L.) Czern & Coss] under field during *rabi* 2020-24. Both biparental and F_3 progenies exhibited significant variability for seed yield and most yield-related traits across all crosses, except for plant height in cross II. However, the mean performance of BiPs was generally higher than the corresponding F_3 progenies, indicating the generation of significant genetic variability, shedding light on the dynamics of genetic inheritance and expression within Indian mustard. While the BiPs showcased superior mean performance in pivotal traits, F_3 generation displayed competitive superiority in traits including days to 50% flowering, plant height, number of secondary branches per plant, number of seeds per siliqua, 1000-seed weight, biological yield per plant and oil content. It is evident that good recombination occurred from intermating, and also the dominance and epistasis components might have played some role in the increase in the mean of BiPs, compared to $F_{3.}$ For a few traits, the genetic variability carried over to the F3 generation and made selection effective. The study reflected that the use of genetically divergent parents such as RH 725, DRMRIJ 31 and PM 27 crossed with Urvashi enhanced recombination potential and the crosses seem to have facilitated the appearance of novel segregants in both F_3 and BiPs and offered enhanced opportunities for selecting transgressive segregants, giving enhanced yield and oil content gain.

Keywords: Biparental progenies, F_3 progenies, Indian mustard, mean, variability.

Introduction

Oilseed crops covered 23% of the global harvested area and experienced the fastest growth in both absolute (+114 million ha) and relative terms (+51%) (FAO 2023). India is the world's largest importer of vegetable oils, followed by China and the United States and is significantly dependent on imports to meet its needs for edible oils. The annual per capita consumption has climbed from 15.80 kg per person in 2012–13 to 19.70 kg per person in the year 2022-23. In terms of vegetable oil production, mustard contributes 31.49%, followed by Groundnut (19.81%) and Soybean (17.99%) (DAFW 2023). In recent times, domestic edible oil production has not been able to keep up with the rising consumption. Thus, the growth in imports of edible oils over the past few decades has increased to 174%, with imports accounting for 55.10% of total domestic demand in 2021–22 (DAFW 2023). This is further likely to go up in the coming years with the change in consumption patterns as well as rising living standards. Thus, there is a strong need to increase domestic oilseed production and productivity.

Indian mustard is a natural amphidiploid species (2n = 36) of *Brassica campestris* (2n = 20) and *Brassica nigra* (2n = 16). It is a self-compatible and largely self-pollinated crop. Mustard contains 36-42% oil, 17–25% protein, 8–10% fibre, 6–10% moisture and 10–12% extractable substances. The

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seeds are highly nutritive, containing 38–57% erucic acid, 5–13% linolic acid and 27% oleic acid. Mustard breeding programme largely aims at yield improvement, wider adaptability and higher oil content. However, progress is constrained by a narrow genetic base (Jenson 1970; Hosur et al. 2023).

Conventional breeding methods like pedigree, bulk and back crossing methods with some modifications impose restrictions on the chances of better recombinations because of the larger linkage blocks associated with the weakness of causing rapid homozygosity and low genetic variability. Nevertheless, outcrossing in Indian mustard is also very limited, as there is restricted free flow of genes among *Brassica* species (Rai 1980). Though the F_2 generation does carry a wide range of genetic variability, due to the presence of linkages, the recombinant potential may not be fully exploited (Clegg et al. 1972). Rather, inbreeding intensifies the linkage blocks. Additionally, unfavourable genetic linkages are likely to delay the selection advancement up to F_4 or even later generations. This may invite the risk of losing desirable recombinants. However, the high heterotic response observed in F_1 persists to a considerable extent in F_2 and subsequent advanced generations. Mating among randomly selected plants from the segregating generation of heterotic crosses needs to be exploited.

The biparental mating approach enlarges the spectrum of variation for diverse traits by releasing the latent variability due to forced recombinations and breakage of undesirable linkages, thus changing the character association pattern so that selection could be effective for a longer period (Pradeep and Sumalini 2003). Guddadamath et al. (2011) suggested biparental mating over other mating designs as it increases the desired variations for traits of interest. The present investigation was undertaken to systematically evaluate and compare the extent of genetic variability, mean performance, and range of traits expressed in biparental progenies (BiPs) and F_3 progenies of Indian mustard. The study was aimed at the assessment of the relative efficiency of the above-mentioned approaches in generating useful genetic variability for yield and oil-related traits, thereby providing a basis for their utilization in mustard breeding programmes.

Materials and methods

Planting materials, experimental design and location

The present experiment was carried out during the period 2020-2024 at the Agricultural Research Station, Ummedganj-Kota (Agriculture University, Kota). The four diverse genotypes of Indian mustard, RH 725, DRMRIJ 31, Pusa Mustard 27 (PM 27) as lines, and Urvashi as tester were crossed using the Line × Tester method (Kempthorne 1957) to produce F_1s. The details of the parents are tabulated in Supplementary Table S1.

In the F_2 population of each cross, four randomly selected females were crossed in the NCD II approach (Comstock and Robinson 1952), with four randomly selected males, resulting in 16 combinations per set. With two sets per cross and three crosses in total, 96 biparental progenies were generated. Simultaneously, selfing of the same F_2 plants was carried out to obtain 48 F_3 progenies (8 per set). During *rabi* 2023-24, evaluation of BiPs and F_3 progenies was carried out. The schematic flow chart of the crossing and evaluation timeline with progeny structure during the *rabi* seasons 2020-2024 is illustrated in Fig. 1.

Observations recorded

The observations namely, plant height (PH)(cm), number of primary branches per plant (NPB), number of secondary branches per plant (NSB), number of siliquae per plant (NSP), length of siliqua (LS in cm), number of seeds per siliqua (NSS), 1000-seed weight (SW) (g), biological yield per plant (BY in g), seed yield per plant (SY in g) and harvest index (HI in %) were recorded on ten randomly selected plants from each genotype. The phenological characters, such as days to 50% flowering (DF) and days to maturity (DM), were recorded on a plot basis. The oil content in per cent was estimated from the bulk of ten randomly selected plants from each plot through Near Infrared spectroscopy (NIR).

Statistical analysis

The mean values of each character were subjected to statistical analysis. The analysis of variance (ANOVA) in NCD II on plot basis for BiPs (suggested by Comstock and Robinson,

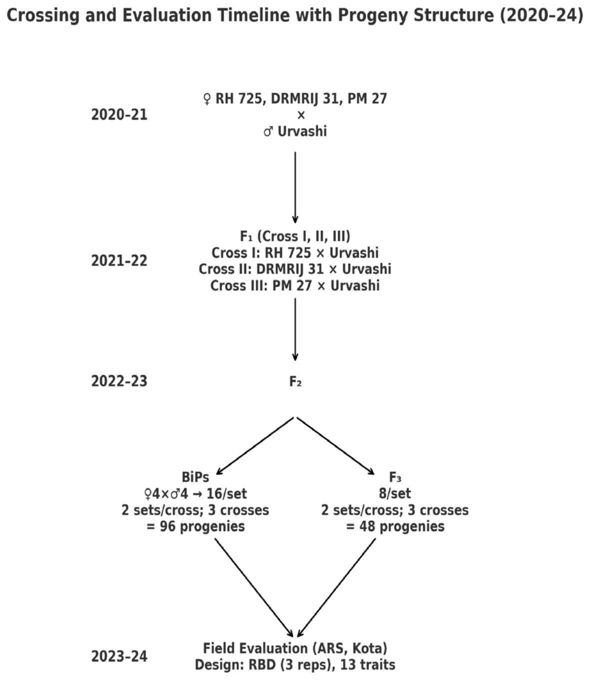


Fig. 1. Schematic diagram: Crossing and evaluation timeline with progeny structure during the period of *rabi* 2020-2024

1952) was carried out through RStudio, whereas ANOVA for F_3 generations was calculated in MS Excel as suggested by Snedecor and Cochran, 1968. The skeleton of ANOVA for NCD II and F_3 populations is presented in Supplementary Tables S2 and S3, respectively.

Results and discussion

Analysis of variance (ANOVA)

ANOVA provides insightful revelations regarding the complexity of genetic factors that influence yield and oil content traits; therefore, it was carried out on a plot basis in both the F_3 and BiPs populations of all three crosses. The estimates of ANOVA are given in Tables 1 to 2. The F_3 progenies in sets of all three crosses exhibited significant differences for all the characters under study except for plant height in sets of cross II. The significant mean squares attributed to males in the BiPs sets indicate considerable variation among half-sib progenies, reflecting genetic differences among the selected pollen parents. In this study, analysis of variance revealed that male effects were highly significant for all traits across the three crosses, except plant height in cross III. Similarly, female effects were significant for all traits in cross I, for all except siliqua length in cross II, and for all except plant height in cross III. Comparable results were reported earlier with significant differences due to both males and females for most traits, with the exception of the number of siliquae per plant. Sapkal et al. (2018) also noted the absence of significant variation for days to maturity in biparental progenies of mustard. Such exceptions, including siliqua length in cross II or plant height in cross III in the present study, suggest that either males or females contributed disproportionately to the observed variation, highlighting the existence of substantial genetic differences among randomly chosen parents.

Furthermore, the significance of mean squares for males × females implies that the behaviour of different males was not consistently mirrored across different females, and *vice versa*. This observation suggests that the variation observed arose from the interaction of genotypes from different male and female parents, emphasizing the importance of considering both parental contributions in understanding the genetic dynamics of the progeny. Significant male × female interactions were observed across the three crosses, indicating the creation of new recombination. In cross I, interactions were significant for most traits except plant height and primary branches. In cross II, they were significant for nearly all yield-contributing traits except harvest index. Similarly, in cross III, interactions were significant for all major traits except plant height. Overall, this highlights that biparental progenies exhibited significant variability for seed yield and most yield-related traits across all crosses. The present results confirm earlier findings that both selfed (Abdel-Moneam et al. 2015; Agdew et al. 2014) and biparental populations (Zeina 2002; Manickavelu et al. 2006; Mahalingam et al. 2011; Abdel-Moneam et al. 2015; Bisen et al. 2019) can generate useful variability.

Notably, BiPs of PM 27 × Urvashi did not show significant variations for plant height from any of the sources of variation, particularly due to males, females, or their interactions. Likewise, plant height displayed no significant variations in F_3 progenies of the same cross. Similar observations were reported by Manickavelu et al. (2006) and El-Shazly et al. (2023). This suggests that both selfing and biparental mating do not universally increase variability but act selectively depending on the genetic background.

Further, the differences in the significant characters in the biparental progenies among the three crosses indicate that the individuals involved in paired mating contributed differently, in every cross. The results are in accordance with Abdel-Moneam (2013) and El-Shazly et al. (2023) in cotton and Hasan and Deb (2023) in chickpea. However, unlike earlier reports in cereals and legumes, systematic comparisons between BiPs and F_3 progenies in Indian mustard are extremely limited.

Mean performance

In the context of the present investigation, the fundamental objective of employing biparental mating emerges as paramount: to optimize recombination, thereby disrupting the linkage between undesirable and desirable traits, and consequently amplifying the potential for identifying superior genotypes. Mean performance of a character is an important criterion for selecting superior segregates, because progenies with the highest mean values are effective for further selection. The results of the comparison of the mean performance and range of characters measured in BiPs and F_3 progenies for three crosses are presented in a concise summary in Table 3. The boxplot representation of mean and range for the progenies under study has been shown in Figs 2 to 4. The higher performing BiPs selected in all three crosses with their respective F_3 progenies are depicted in Table 4

The mean performance of BiPs was generally higher than the corresponding F_3 progenies in three crosses; thereby, noteworthy variations emerged, shedding light on the dynamics of genetic inheritance and expression within Indian mustard. Chand and Rao (2001) compared the effect of biparental mating with conventional breeding procedures in the half-sib progenies by using NCD II and proved that BiPs were more effective in improving the mean performance. Similar findings have been reported earlier (Kampli et al. 2002; Mahalingam et al. 2011; Koli et al. 2012; Abdel-Moneam et al. 2015) in different crops.

Within the biparental progenies resulting from the crosses I and II, a uniform elevation in mean performance across almost all traits within the biparental progenies compared to the F_3 generation, except days to maturity,

Table 1. Analysis of variance (NCD II) for thirteen characters under study in biparental progenies of all the three crosses in Indian mustard

Source of variation	DF	Mean sum squares					
		Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches per plant	No. of second. branches per plant	No. of siliquae per plant
RH 725 × Urvashi (cross I)							
Sets	1	32.67	0.04	75.26	0.57	7.71	2980.00
Replications in sets	4	0.67	0.17	272.85	0.18	0.48	92.90
Males in sets	6	48.47 **	4.24 **	261.97 *	1.76 **	12.39 **	5395.20 **
Females in sets	6	23.31 **	6.90 **	394.02 **	0.74 **	8.43 **	2083.80 **
Males × Females in sets	18	16.44 **	2.99 **	141.77	0.19	2.73 **	1485.70 **
Remainder among plots	60	1.67	0.58	103.97	0.11	0.25	109.80
DRMRIJ 31 × Urvashi (cross II)							
Sets	1	126.04	6.51	6329.40	0.01	30.96	661.61
Replications in sets	4	2.77	0.36	446.50	0.10	0.09	241.85
Males in sets	6	14.72 **	5.47 **	569.00 **	1.75 **	25.10 **	3015.91 **
Females in sets	6	15.10 **	4.77 **	101.90	0.29 **	5.82 **	1298.26 **
Males × Females in sets	18	5.16 **	2.27 **	65.00	0.46 **	8.02 **	2967.58 **
Remainder among plots	60	0.86	0.44	108.40	0.07	0.30	207.24
PM 27 × Urvashi (cross III)							
Sets	1	20.17	7.59	2978.17	0.38	2.44	1107.90
Replications in sets	4	0.51	0.68	155.56	0.06	0.05	475.60
Males in sets	6	70.22 **	7.61 **	602.91	1.05 **	5.97 **	3881.50 **
Females in sets	6	16.66 **	3.50 **	411.71	1.10 **	3.57 **	1895.50 **
Males × Females in sets	18	2.79 **	1.40 **	355.27	0.58 **	9.02 **	1667.90 **
Remainder among plots	60	0.49	0.39	275.93	0.13	0.21	158.10

*, ** Significant at 5 % and 1 % level, respectively; DF = degrees of freedom

biological yield per plant and 1000-seed weight in cross I and days to 50% flowering, days to maturity and number of seeds per siliqua in cross II. Conversely, in the context of the cross III, nuanced variations were observed. While the BiPs showcased superior mean performance in pivotal traits, F_3 generation displayed competitive superiority in traits including days to 50% flowering, plant height, number of secondary branches per plant, number of seeds per siliqua, 1000-seed weight, biological yield per plant and oil content.

Increased mean values in BiPs for the traits in three crosses might be due to pooling of favourable alleles because of recombination resulting from intermating (Vinayan and Govindarasu 2010). In addition, even dominance and epistasis components could play some role towards increase in increasing the mean of BiPs, compared to F_3 (Dwivedi and Singh 1978). Conversely, lower values of BiPs for days to 50% flowering (as in the case of cross II and III) and days to maturity (as in cross I and II) a favourable events, as early flowering and early maturity are significant in a breeding objective.

Additionally, 1000-seed weight (4.33 F_3 and 4.34 BiPs) in cross II and length of siliqua (4.85 F_3 and 4.87 BiPs) in cross III, which exhibited nearly equivalent mean performance between the BiPs and the F_3 generation, despite BiPs demonstrating slightly higher mean values. This phenomenon could potentially be attributed to the absence of genetic linkage (Mather et al. 1971).

Range

In terms of range distribution, the BiPs range exhibited a spread in both directions for several key traits across the

Length of siliqua (cm)	No. of seeds per siliqua	1000-seed weight (g)	Biological yield per plant (g)	Harvest index (%)	Oil content (%)	Seed yield per plant (g)
0.55	0.57	1.38	374.57	1.65	3.44	7.80
0.04	0.55	0.01	55.74	1.02	0.11	1.04
0.54 **	12.34 **	1.15 **	742.32 **	14.04 **	4.67 **	28.58 **
1.75 **	2.02 **	0.71 **	353.22 **	18.04 **	3.35 **	7.72 **
0.31 **	7.15 **	0.57 **	116.88 **	12.89 **	6.21 **	4.76 **
0.07	0.38	0.01	24.85	1.97	0.20	0.76
2.58	3.57	2.41	120.3	123.48	1.13	8.66
0.03	0.91	0.001	56.74	8.84	0.10	0.66
0.37 **	1.71 **	0.11 **	389.58 **	41.19 **	7.64 **	14.54 **
0.03	7.32 **	0.52 **	129.84 **	10.28 **	5.41 **	13.32 **
0.25 **	4.03 **	0.44 **	335.19 **	3.76	5.50 **	11.62 **
0.08	0.43	0.01	32.53	3.15	0.13	0.54
0.02	3.53	0.05	32.45	22.70	3.34	9.50
0.11	0.51	0.001	79.53	7.59	0.06	1.43
0.94 **	4.32 **	0.78 **	208.19 **	27.19 **	10.31 **	9.00 **
0.28 **	5.17 **	0.29 **	94.10 *	9.09 *	9.03 **	3.32 **
0.51 **	4.05 **	0.10 **	234.11 **	20.09 **	5.99 **	7.87 **
0.08	0.44	0.004	37.95	3.40	0.11	0.76

three crosses. In cross I, this wider range was observed for traits such as number of siliquae per plant, length of siliqua, number of seeds per siliqua, harvest index, oil content and seed yield per plant. Similarly, in cross II, traits like days to maturity, plant height, number of primary branches per plant and oil content displayed an expanded range in the biparental progenies. Cross III showed a wider range for traits including plant height, number of siliquae per plant, length of siliqua, 1000-seed weight, oil content and seed yield per plant in the biparental progenies. This broader range in biparental progenies suggests the creation of greater genetic variability, possibly due to the disruption of undesirable linkages that might obscure genetic variation within the small size of the F_2 generation (Prakash and Verma 2006). This phenomenon underscores the importance of parental selection in breeding programs, as well as the potential for creating novel genetic combinations that yield superior performance.

In the specific case of cross I, in days to maturity, the upper limit of the ranges remains static while the lower limit was shifted towards the lower side, which resulted in a desirable shift for early maturity. In cross II, the upper limit as well as the lower limit of the range decreased for days to 50% flowering, thereby increasing the selection chances for early flowering. However, in most of the traits, there was a shift of both upper limit and lower limit of the range towards the higher side. It indicated that despite the process of hybridization and genetic recombination, the progeny of cross II retained and even amplified certain desirable characteristics inherited from the parental lines.

Table 2. Analysis of variance for thirteen characters under study in F_3 progenies of all the three cross combinations in Indian mustard

Source of variation	DF	Mean sum squares				
		Days to 50% flowering	Days to maturity	Plant height (cm)	No. of primary branches per plant	No. of second. branches per plant
RH 725 × Urvashi (cross I)						
Sets	1	4.08	2.08	114.08	0.78	3.80
Replications in sets	4	1.29	0.77	176.33	0.04	0.10
F_3 progenies in sets	14	24.08 **	4.61 **	342.42 **	0.41 **	12.08 **
Remainder among plots	28	0.74	0.46	145.17	0.06	0.19
DRMRIJ 31 × Urvashi (cross II)						
Sets	1	31.69	1.69	793.49	0.12	6.45
Replications in sets	4	1.04	0.94	99.45	0.20	0.20
F_3 progenies in sets	14	8.70 **	2.35 **	274.88 *	0.52 **	6.52 **
Remainder among plots	28	1.78	0.60	99.77	0.08	0.18
PM 27 × Urvashi (cross III)						
Sets	1	12.00	0.19	1064.08	0.14	8.42
Replications in sets	4	1.04	0.63	296.57	0.08	0.09
F_3 progenies in sets	14	44.14 **	5.25 **	271.26	1.02 **	15.75 **
Remainder among plots	28	0.99	0.43	152.48	0.13	0.16

*, ** Significant at 5 % and 1 % level, respectively; DF = degrees of freedom

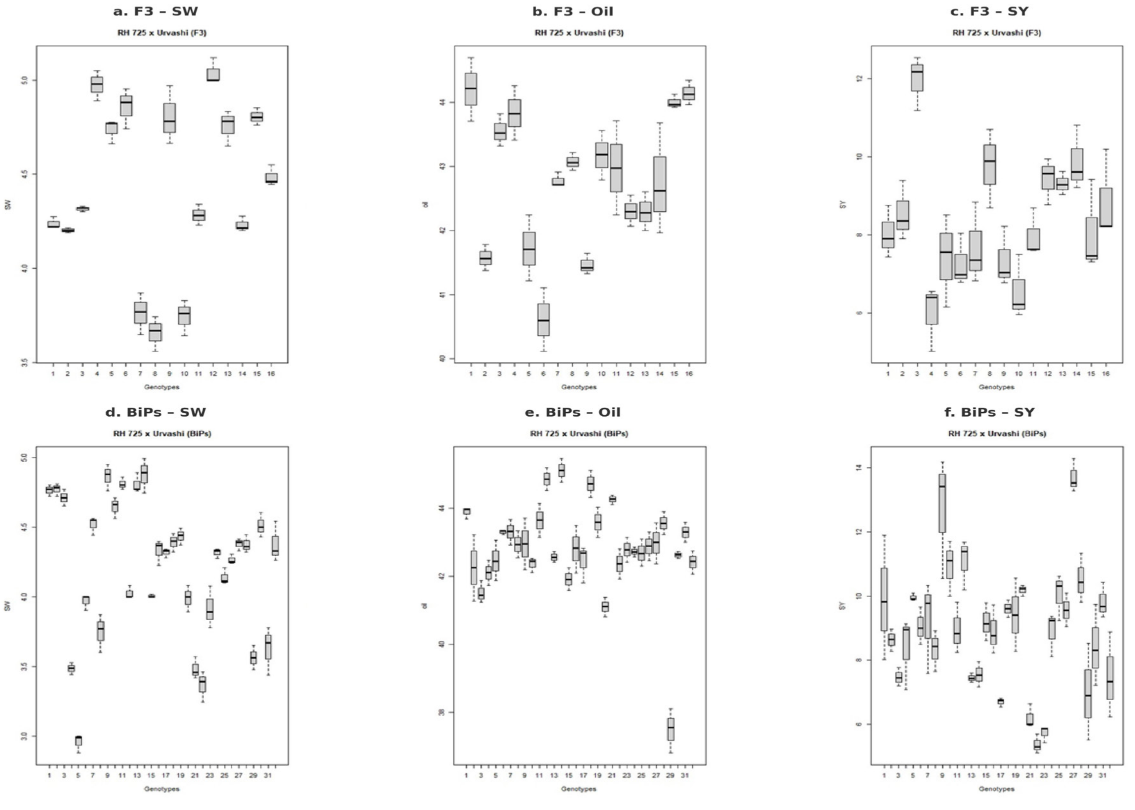


Fig. 2. RH 725 × Urvashi cross: Comparative boxplots of F_3 and BiPs populations derived from RH 725 × Urvashi cross. (a–c) show the F_3 population for seed weight (SW), oil content (Oil), and seed yield (SY). (d–f) show the BiPs population for the same traits, enabling comparison of performance between generations

No. of siliquae per plant		Length of siliqua (cm)		No. of seeds per siliqua		1000-seed weight (g)		Biological yield per plant (g)		Harvest index (%)		Oil content (%)		Seed yield per plant (g)	
845.04		0.24		29.93		0.37		15.66		2.24		0.59		0.11	
244.06		0.01		0.39		0.002		70.21		4.30		0.18		1.05	
1242.67	**	0.42	*	5.18	**	0.62	**	245.50	**	12.46	**	3.56	**	6.95	**
205.74		0.19		0.38		0.01		37.53		2.83		0.17		0.69	
9520.33		1.14		5.01		0.48		478.05		41.98		0.12		2.02	
176.35		0.11		0.99		0.01		14.87		3.19		0.31		0.48	
2913.59	**	0.30	*	3.45	**	0.72	**	347.34	**	10.52	**	4.42	**	11.61	**
133.84		0.13		1.40		0.01		24.9		3.02		0.16		0.75	
1668.40		0.67		0.88		0.03		5.84		1.29		6.96		0.002	
108.47		0.09		0.81		0.00		18.75		1.27		0.19		0.45	
1096.38	**	0.49	**	5.83	**	0.21	**	335.09	**	11.48	**	3.77	**	10.97	**
314.56		0.05		0.49		0.002		32.71		3.36		0.17		0.92	

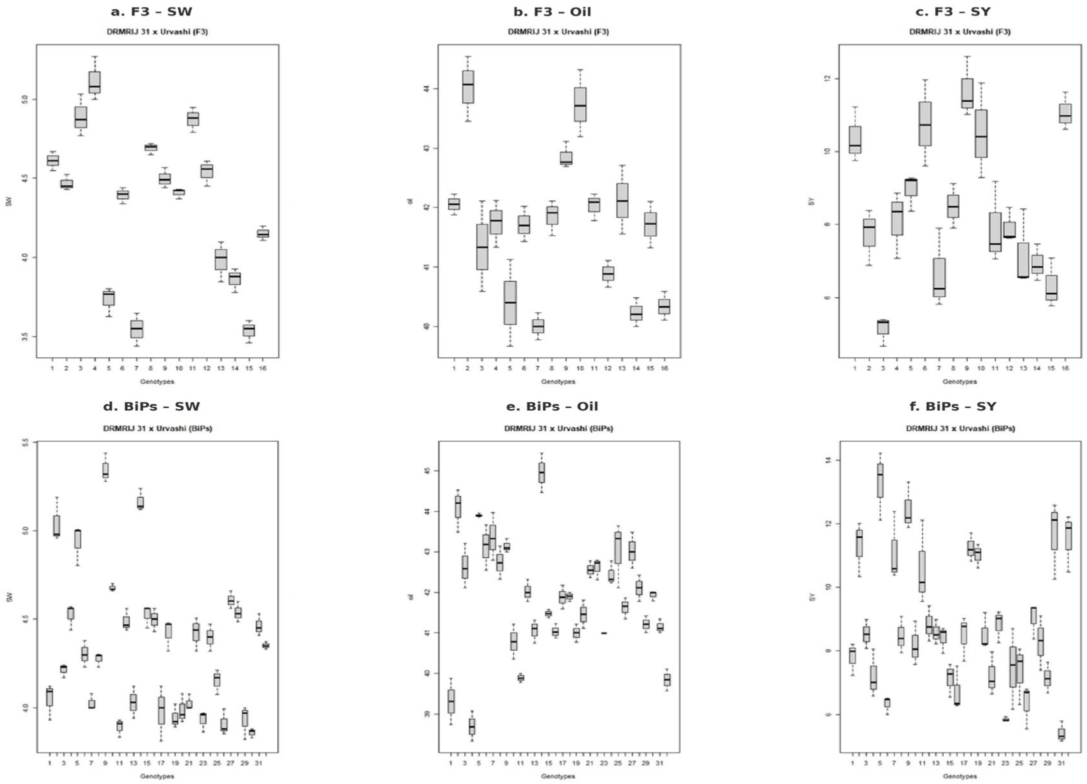


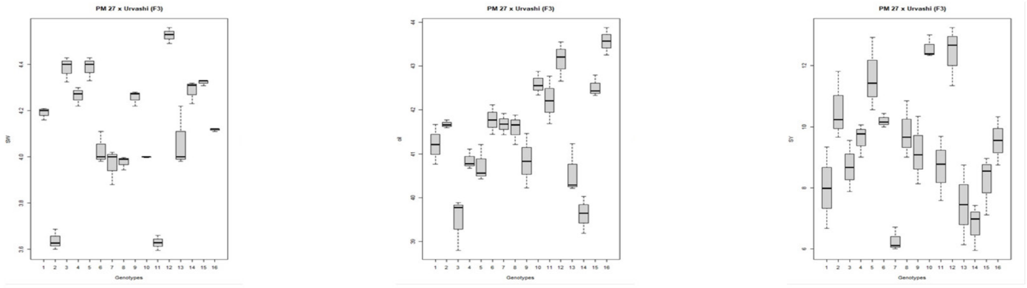
Fig. 3. DRMRUJ 31 × Urvashi cross: Comparative boxplots of F_3 and BiPs populations derived from DRMRUJ 31 × Urvashi cross. (a–c) show the F_3 population for seed weight (SW), oil content (Oil), and seed yield (SY). (d–f) show the BiPs population for the same traits, enabling comparison of performance between generations

Table 3. **Mean and range of thirteen characters under study, in F3 and BiPs for all three crosses in Indian mustard**

Characters	RH 725 × Urvashi (Cross I)						DRMRIJ 31 × Urvashi (Cross II)						PM 27 × Urvashi (Cross III)					
	F_3			BiPs			F_3			BiPs			F_3			BiPs		
	Range		Mean	Range		Mean	Range		Mean	Range		Mean	Range		Mean	Range		Mean
	Min.	Max.		Min.	Max.		Min.	Max.		Min.	Max.		Min.	Max.		Min.	Max.	
DF	47.33	58.00	52.38	48.67	59.67	52.54	48.00	54.00	50.40	45.33	52.67	49.88	45.00	60.00	52.54	48.00	58.67	51.77
DM	128.67	132.67	130.33	126.33	132.67	130.02	127.67	130.67	129.19	126.33	131.33	128.97	128.00	132.33	130.69	128.33	133.33	130.76
PH	166.67	216.33	194.79	181.67	209.33	195.93	170.27	204.50	187.60	167.17	207.77	190.57	175.40	215.03	199.51	157.10	222.33	198.31
NPB	3.43	4.93	4.17	3.60	5.40	4.51	3.20	4.47	3.82	3.07	5.07	4.06	3.67	5.60	4.58	3.93	6.07	4.85
NSB	1.87	8.00	4.50	3.00	9.47	5.29	1.60	6.73	3.91	2.40	9.40	4.83	2.60	11.00	6.08	3.07	9.24	6.01
NSP	104.48	177.34	135.08	101.67	220.80	159.72	95.67	201.03	130.58	101.87	215.90	151.34	149.82	212.48	180.97	134.99	235.63	181.97
LS	4.46	5.65	4.99	4.25	5.83	5.01	4.17	5.83	5.02	4.63	6.01	5.04	4.30	5.76	4.85	4.03	5.99	4.87
NSS	9.60	14.47	11.66	9.40	14.87	12.79	10.17	14.87	12.80	10.53	15.13	12.48	10.37	15.47	13.10	10.80	15.80	12.81
SW	3.66	5.04	4.43	2.96	4.87	4.21	3.54	5.12	4.33	3.86	5.35	4.34	3.63	4.53	4.13	3.53	4.61	4.04
BY	36.20	71.94	47.55	29.10	65.26	47.18	23.80	63.29	43.82	27.80	66.05	44.85	34.90	67.58	52.06	27.85	64.94	51.09
HI	15.00	22.03	17.83	14.32	23.02	19.17	14.84	23.97	19.65	16.12	24.62	19.77	14.07	21.49	18.14	15.62	25.25	19.36
OC	40.61	44.20	42.78	37.49	45.11	42.81	40.00	44.02	41.69	38.70	44.96	41.91	39.48	43.57	41.50	38.35	45.77	40.99
SY	5.99	11.97	8.38	5.37	13.70	8.95	5.13	11.67	8.48	5.42	13.30	8.76	6.27	12.58	9.35	6.25	13.42	9.74

DF = Days to 50 % flowering; DM = Days to maturity; PH = Plant height (cm); NPB = No. of primary branches per plant; NSB = No. of secondary branches per plant; NSP = No. of siliquae per plant; LS = Length of siliqua (cm); NSS = No of seeds per siliqua; SW = 1000-seed weight (g); BY = Biological yield per plant (g); HI = Harvest index (%); OC = Oil content (%); SY = Seed yield per plant (g).

a. F3 – SW b. F3 – Oil c. F3 – SY



d. BiPs – SW e. BiPs – Oil f. BiPs – SY

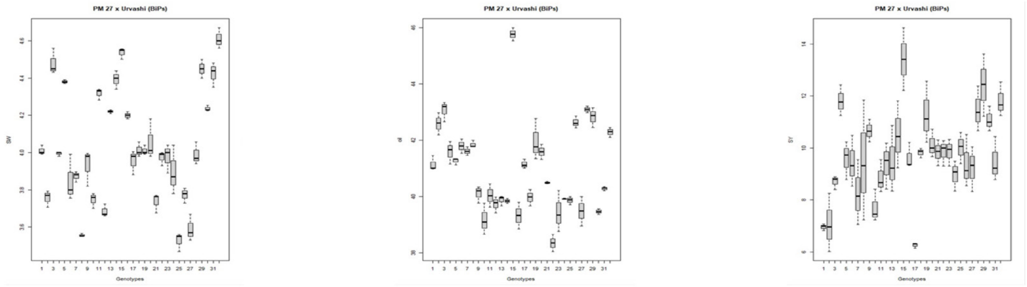


Fig. 4. PM 27 × Urvashi cross: Comparative boxplots of F_3 and BiPs populations derived from PM 27 × Urvashi cross. (a–c) show the F_3 population for seed weight (SW), oil content (Oil), and seed yield (SY). (d–f) show the BiPs population for the same traits, enabling comparison of performance between generations

In cross III, the range was shortened in BiPs for the days to 50% flowering and the number of secondary branches per plant, which reduced the extent of variability in BiPs. The observation that the range of some characters in the BiPs was lower than in the F_3 generation can be attributed to the effects of biparental mating. This mating strategy may induce a certain degree of inbreeding, consequently leading to a reduction in genetic variability.

In the present study of Indian mustard, the adoption of biparental mating was expected to produce effects similar to phenotypic assortative mating (Agdew et al. 2014). This outcome was anticipated because there was only a single sowing date, ensuring that individuals mating at a given time would have synchronous flowering and thus be phenotypically similar in terms of days to 50% flowering and date of maturity in F_3 and BiPs of all three crosses.

For siliquae per plant, BiPs consistently exhibited a 20–30% wider range compared to F_3 across all three crosses. This broader variability implies that biparental mating captured greater recombination potential, which is crucial for identifying transgressive segregants. In seed yield, BiPs outperformed F_3 by 15–20% on average, while also maintaining a wider distribution. Such combined gains in both mean and variability emphasize the superiority of BiPs as a breeding resource. Similar trends were reported by Mahalingam et al. (2011) in rice, Agdew et al. (2014) in barley, where biparental progenies enhanced yield-related variability over selfed generations, supporting the general applicability of this approach.

In terms of oil content, cross II was particularly noteworthy, where BiPs recorded an expanded range that was ~12% higher than F_3 lines. This demonstrates that biparental mating not only augments yield attributes but also enhances quality traits, an important consideration for edible oil crops. Comparable results were also obtained in mustard, where biparental progenies contributed to improved oil quality variability (Sapkal et al. 2018). Thus, the present findings confirm that the biparental approach is effective in Indian mustard, a crop otherwise constrained by a narrow genetic base.

Notably, when comparing the performance of the crosses, cross III continued to demonstrate the highest major yield and yield-contributing characteristics. This observation suggests the preservation of parental traits while simultaneously fostering broader variability within the progeny population. Furthermore, the wider variability observed within cross I suggest the possibility of tapping into a broader genetic pool, which could be valuable for future breeding efforts aimed at enhancing yield and productivity in Indian mustard.

Despite the expected variability reduction in F_3, it outperformed BiPs in some cases, evident that one or more cycles of biparental mating would have a beneficial impact on a particular population of Indian mustard. The enhanced magnitude of variability in some F_3 progenies is attributed to segregation and fixation of additive alleles during selfing, which exposed latent variation not fully visible in the F_2 generation. This phenomenon is particularly prominent

Table 4. **Per se performance of top 5 biparental progenies among three crosses based on seed yield per plant, compared to their respective highest performing F_3 progenies**

S.No.	BiPs	SY (g)	Other important ancillary characters of the progenies				
			NPB	NSP	NSS	SW (g)	Oil (%)
RH 725 × Urvashi (cross I)							
1.	S27	13.70	5.27	211.97	14.87	4.38	42.98
2.	S9	12.72	5.40	189.52	14.73	4.86	42.96
F_3 progeny		11.97	4.20	149.87	12.93	4.32	43.55
DRMRIJ 31 × Urvashi (cross II)							
3.	S5	13.30	4.67	215.90	12.53	4.94	43.91
4.	S9	12.46	4.60	186.27	14.27	5.35	43.14
F_3 progeny		11.67	3.87	201.03	10.17	4.50	42.85
PM 27 × Urvashi (cross III)							
5.	S15	13.42	6.07	235.63	12.8	4.54	45.77
F_3 progeny		12.58	5.60	212.48	14.00	4.00	42.59

in traits governed by fewer major genes, such as flowering and maturity. Additionally, the use of genetically divergent parents (RH 725, DRMRIJ 31, PM 27 crossed with Urvashi) enhanced recombination potential. The differences in adaptation and yield backgrounds of these parents likely facilitated the appearance of novel segregants in both F_3 and BiPs.

Given India's heavy reliance on edible oil imports (~60% of domestic demand), strategies that broaden the genetic base and enhance variability are urgently needed. Nevertheless, the scant amount of work is available on biparental mating in Indian mustard. The present study exposed BiPs' performance over the F_3 progenies, which gives breeders a significant opportunity to gain insights into the complex genetic makeup of this important oilseed crop.

In conclusion, the results of the present investigation revealed that yield and oil content gain were realized by way of expanding genetic variability among all three crosses. The F_3 progenies in sets of all three crosses, RH 725 x Urvashi, DRMRIJ 31 x Urvashi and PM 27 x Urvashi exhibited significant differences for the characters under study, except, plant height. Analysis of variance revealed significant differences among males, females, and their interactions for nearly all traits. Cross II had a higher mean performance compared to other crosses, whereas Cross I showed a wider range of variability.

The evidence from this study suggests that incorporating biparental mating can accelerate the development of mustard genotypes with higher seed yield and improved oil content. Such an approach has the potential to directly contribute to India's edible oil self-reliance goals under initiatives like the National Mission on Edible Oils–Oilseeds (NMEO-OS).

Supplementary material

Supplementary Tables S1 to S3 are provided, which can be accessed at www.isgpb.org

Authors' contribution

Conceptualization of research (RB, PKPM); Designing of the experiments (RB, PKPM); Contribution of experimental materials (PKPM, NRK, HM, YT, RKM); Execution of field/lab experiments and data collection (RB, PKPM, NRK, HM, YT, RKM); Analysis of data and interpretation (RB, PKPM); Preparation of the manuscript (RB, PB).

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Supplementary Table S1. Details of the parents used for biparental mating design

S. No.	Parents	Origin / Source	Year of release	Salient features
1.	RH 725	CCSHAU, Hisar	2018	Timely sown; rainfed condition; bold seeded; long and semi-apprised siliquae
2.	DRMRIJ 31	DRMR, Bharatpur	2013	Timely sown; irrigated; high yielder; bold seeded; high oil content
3.	PM 27	IARI, New Delhi	2011	Early sown; irrigated; moderately tolerant to high temperature at seedling as well as at maturity stages
4.	Uravashi	CSAUA&T, Kanpur	2001	High temperature tolerant; drought tolerant

Supplementary Table S2. Analysis of variance (ANOVA) in NCD II on plot basis

Source of variation	Degree of freedom	Mean squares (M.S.)	Expected M.S.
Sets	(s-1)		
Replications in sets	s(r-1)		
Males in sets	s(m-1)	M1	$\sigma_e^2 + r\sigma_{mf}^2 + rf\sigma_m^2$
Females in sets	s(f-1)	M2	$\sigma_e^2 + r\sigma_{mf}^2 + rm\sigma_f^2$
Males x Females in sets	s(m-1) (f-1)	M3	$\sigma_e^2 + r\sigma_{mf}^2$
Remainder among plots	s(mf-1) (r-1)	M4	σ_e^2

Where,
s = the number of sets,
r = the number of replications,
m = the number of males,
f = the number of females,
M1 = the mean sum of squares due to males in sets,
M2 = the mean sum of squares due to female in sets,
M3 = the mean sum of squares due to males x females in sets,
M4 = the error meansum of squares among plots,
σ_e^2 = the error variance,
σ_m^2 = the progeny variance arising from genetic difference among male parent,
σ_f^2 = the progeny variance arising from genetic difference among female parents,
σ_{mf}^2 = the progeny variance arising from interaction of male and female parents.

Supplementary Table S3. Analysis of variance (ANOVA) in F_3 on plot basis

Source of variation	*Degree of freedom*	*Mean squares (M.S.)*	*F ratio*
Sets	(s-1)	MS_s	MS_s / MS_e
Replications in sets	s(r-1)	MS_r	MS_r / MS_e
F_3 progenies in sets	s(p-1)	MS_p	MS_p / MS_e
Remainder among plots	s(mf-1) (r-1)	MS_e	

Where,
s = the number of sets,
r = the number of replication,
p = the number of parents (males + females),
MS_s = the mean sum of squares due to sets,
MS_r = the mean sum of squares due to replications in sets,
MS_p = the mean sum of squares due to F_3 progenies in sets,
MS_e = the error meansum of squares among plots.