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RESEARCH ARTICLE

Enhancing breeding potential in Indian mustard (*Brassica juncea* L. Czern & Coss): variability and association studies in three F_2 populations

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Abstract

This research was conducted to investigate the genetic variability, heritability and relationships among traits in F_2 populations of Indian mustard (*Brassica juncea* L. Czern & Coss) to identify practical breeding approaches for improving yield. Considerable variation was noted in traits associated with growth, yield and its components, highlighting the genetic diversity in the studied populations. Key characteristics such as the number of primary and secondary branches per plant, siliqua per raceme, racemes per plant and seed yield per plant exhibited high phenotypic and genotypic coefficients of variation (PCV and GCV), suggesting significant genetic variability. Traits with high heritability and genetic advance, including primary and secondary branches per plant, racemes per plant, siliqua per raceme and seed yield per plant, indicate a strong influence of additive genetic variance, making them ideal for direct selection. Through correlation and path analysis, key traits such as primary and secondary branches per plant, siliqua per raceme, number of racemes per plant and 1000-seed weight were identified as crucial for enhancing yield. The F_2 population from the cross TM-138-1 × KMR(E) 16-1 demonstrated superior breeding potential, as evidenced by higher mean values, broader absolute and standardized ranges, increased phenotypic coefficient of variation and a higher frequency of transgressive segregants compared to other crosses. These results offer valuable insights for breeders seeking to improve productivity and adaptability in oilseed crops.

Keywords: breeding potential; correlation; Indian mustard; path analysis; variability

Introduction

Brassicas are among the earliest domesticated crops developed by humans. Historical records and ancient scriptures indicate their cultivation began as early as 5000 BC. Evidence from the Neolithic age further supports this assertion, with mustard seeds discovered at Chanhudaro, a site of the Harappan civilization, dating to approximately 2300–1750 BC (1). Indian mustard (*Brassica juncea* L., Czern & Coss) is a member of the Brassicaceae family (also known as Cruciferae), which includes 3709 species and 338 genera (2). Six Brassica species are commonly grown in India, they include 3 diploid species and 3 amphidiploid species of which four species, *viz. Brassica juncea, Brassica napus, Brassica carinata* and *Brassica rapa* cvs. toria, yellow sarson and brown sarson are grown as oilseed crops. *B. juncea* covers more than 80 % of Indias' total rapeseed and mustard cultivation area, owing to its adaptability and resilience to biotic and abiotic stresses (3, 4).

Indian mustard oil is widely used for cooking (60-70 % of production), valued for its pungent flavor (from glucosinolate-derived allyl isothiocyanate) and high smoke point. Separately, 15-20 % is utilized in hair oil and Ayurvedic medicine for its anti-inflammatory properties. Industrially, 10-15 % is allocated to soap production, lubrication and tanning. Mustard leaves (per 100 g) provide ~250-500 % DV of vitamin K, ~100-150 % DV of vitamin A and flavonoid antioxidants. The oilcake byproduct (30-40 % protein) serves as animal feed and manure (5, 6).

Mustard cultivation has gained importance in South India, particularly in Karnataka (7). However, its productivity in the state remains significantly lower than the national average, largely due to the widespread use of traditional local cultivars, poor performance of landraces and introduced varieties, environmental fluctuations and the absence of pest - and disease-resistant genotypes against major biotic threats such as diamondback moth (*Plutella xylostella*), white rust (*Albugo candida*), Alternaria blight (*Alternaria brassicae*)

and aphid infestations (*Lipaphis erysimi*). Mustard in Karnataka is predominantly grown on marginal soils under rainfed conditions, primarily for household consumption. While mustard is the second most important oilseed crop in India, cultivated across 91.83 lakh hectares with a production of 132.59 lakh T and productivity of 1444 kg/ha, Karnatakas' contribution remains minimal, with only 4000 hectares under cultivation, producing 0.75 lakh T and a significantly lower productivity of 188 kg/ha (8). This significant disparity underscores the pressing need for targeted breeding programs and improved agronomic practices to enhance yields in Karnataka. Tackling these challenges is essential to keep pace with the increasing demand for edible oil, which is rising by 3-4 % each year, driven by population growth and higher living standards. To address climate challenges and elevate consumer engagement, it is essential to harness mustards' untapped industrial and medicinal value while innovating resilient, high-yielding seed and oil varieties tailored to thrive in Karnatakas' unique agro-climatic conditions, particularly in the Northern Transition Zone, ensuring sustainable growth and agricultural prosperity. In this context, the current study aims to evaluate the genetic variability within mustard populations to identify the F_2 population with better breeding potential to develop improved varieties tailored to this region.

Yield is a multifaceted characteristic shaped by numerous genetic and environmental factors, making direct selection for yield frequently inefficient. Consequently, analyzing the variability, heritability and correlations among traits that contribute to yield is essential. Key genetic metrics, including genotypic and phenotypic coefficients of variation, genetic advance and heritability, are vital for assessing population variability. Early segregating generations offer more significant genetic variability, enabling effective selection for desirable traits such as days to first flowering, days to maturity, plant height, primary branches per plant, secondary branches per plant, racemes per plant, siliquae per raceme, seeds per siliqua, siliqua length, 1000-seed weight and yield per plant. The use of F_2 populations in this study enables a comprehensive assessment of genetic variability and trait heritability assessment, which is critical for identifying superior genotypes.

Developing heterotic F_1 hybrids or varieties relies on inbred parents possessing desirable trait combinations and strong general combining ability. To combine favourable traits from various parents, breeders often design crosses involving two, three, four, or multiple parents, producing recombinant inbred lines (RILs) that can be used as hybrid parents. Handling the early segregating generations from these extensive crosses to identify promising RILs in later stages requires significant resources. By eliminating underperforming crosses in the initial generations, breeders can prioritize potential crosses, optimize resource allocation and increase the chances of obtaining desirable RILs from large segregating populations of selected crosses (9-11).

The present study focuses on assessing genetic variability, trait relationships and path analysis within the F_2 generations of Indian mustard to gain deeper insights into the direct and indirect influences of various traits on yield, facilitating more informed selection choices. It also highlights the breeding potential of these populations by examining the emergence of transgressive segregants, which arise from the favourable combination of alleles from both parental lines. These outcomes offer valuable insights into genetic variability and aid in identifying promising crosses for developing high-yielding varieties, thereby enabling more precise and effective breeding approaches.

Materials and Methods

Research material

The study employed five high-yielding cultivars: KMR(E) 16-1, DRMR 4005, TM-210, TM-138-1 and TM-2776, along with three F_2 populations developed from the crosses TM-138-1 × KMR (E) 16-1, KMR(E) 16-1 × TM-210 and TM-2776 × DRMR 4005 (Table 1). These lines and crosses are selected based on their higher mean value and GCA values based on our previous work. In the crossing scheme, TM-138-1 and TM-2776 were female parents, while TM-210 and DRMR 4005 were male parents. KMR(E) 16-1 was utilized as a female and male parent. The F_1 generation was produced from the crossed seeds and F_2 seeds were subsequently generated through self -pollination of the F_1 plants.

Methodology

The three F_2 populations derived from the crosses TM-138-1 × KMR(E) 16-1, KMR(E) 16-1 × TM-210 and TM-2776 × DRMR 4005 consisted of 300 F_2 plants each and were evaluated at the Agricultural Research Station, Nippani, Karnataka, during the *Rabi* season of 2020-21.The experiment site is located in the Northern Transitional Zone of Karnataka at 16.20 °N, 74.20 °E and an altitude of 610 m above sea level. The F_2 populations and their parental lines were evaluated in an unreplicated experimental field trial with a row spacing of 0.45 m and an intra-row plant spacing of 0.10 m. The trial comprised 300 F_2 progeny plants and 50 plants from each parental line, all maintained at this spacing for analysis. Recommended agronomic practices and targeted pest and disease management measures were followed consistently during the crop growth period.

Data on eleven traits were collected from all the three hundred F_2 progeny plants in each of the three populations, along with ten plants from each parent, for eleven traits: days to first flowering, days to maturity, plant height, primary branches per plant, secondary branches per plant, racemes per plant, siliquae per raceme, seeds per siliqua, siliqua length, 1000-seed weight and yield per plant.

Table 1. Parental material and their sources

Sr. No.	Parents	Source
1	KMR(E) 16-1	Directorate of Rapeseed and Mustard Research, Bharatpur, Rajasthan
2	DRMR 4005	
3	TM-210	
4	TM-138-1	Bhabha Atomic Research Centre, Trombay, Mumbai
5	TM-2776	

The collected data was analyzed using a range of statistical methods, including the calculation of variability parameters such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad-sense heritability (h^2) and genetic advance as a percentage of the mean (GAM), following standard statistical procedures (12). Correlation and path coefficients were computed using MS-EXCEL and R Studio (13, 14). The mean values of the parents for all traits were assessed for significance by comparing them with the grand mean and critical difference values. Transgressive segregants, characterized by trait values surpassing the better parent by at least twice the standard deviation of the F_2 population, were also identified.

Results and discussion

Evaluation of genetic variability

Genetic variability metrics were computed for the F_2 populations derived from the three crosses and the results are summarized in Table 2. The phenotypic coefficient of variation (PCV) was higher than the genotypic (GCV) for all traits, indicating that genetic factors and environmental effects influenced the observed variation. High PCV and GCV values were observed for characteristics such as yield per plant, primary and secondary branches per plant and racemes per plant across all F_2 populations, indicating significant genetic variability. This variability can be effectively utilized for targeted selection. These results align with earlier research, including studies on secondary branches and racemes per plant and investigations into primary and secondary branches, siliquae per plant and seed yield per plant (15, 16). Conversely, traits like days to first flowering, days to maturity, plant height, seeds per siliqua and length showed moderate to low PCV and GCV, indicating its relatively lower genetic variability. This suggests limited potential for enhancing such characteristics through a selection process. The coefficient of variation quantifies the degree of observed variability, whereas heritability and genetic advance as a percentage of the mean (GAM) provide a more comprehensive understanding of the genetic factors influencing trait expression.

Heritability and genetic advance

High broad-sense heritability coupled with high genetic advance as a percentage of the mean was observed for traits such as primary and secondary branches per plant, racemes per plant, siliquae per raceme, siliqua length and seed yield per plant across all populations. This suggests that the expression of these traits is predominantly governed by additive gene action, rendering the selection process highly effective for these characteristics. Similar findings have been documented in previous studies for secondary branches per plant and racemes per plant (15, 17), as well as for seed yield per plant (17) and siliquae per plant (16).

In addition to the traits mentioned earlier, the F_2 population from the cross TM-138-1 × KMR(E) 16-1 showed high heritability along with high genetic advance as a percentage of the mean (GAM) for plant height (16, 18). Meanwhile, the other two populations demonstrated high heritability and GAM for the trait '1000-seed weight' (16, 19). Traits like days to first flowering and days to maturity displayed low genetic advance as a percentage of the mean (GAM) across

Table 2. Variability, heritability and genetic advance as per cent of mean for yield and its attributes in three F_2 populations

Trait	Crosses	Mean	Range		GCV (%)	PCV (%)	Heritability in broad sense (%)	GAM (%)
			Min	Max				
Days to first flowering	TM-138-1 × KMR(E) 16-1	31.27	26	36	5.28	6.33	69.63	9.07
	KMR(E) 16-1 × TM-210	26.63	24	29	4.09	5.15	63.28	6.86
	TM-2776 × DRMR 4005	33.06	26	37	4.59	5.66	65.66	7.65
Days to maturity	TM-138-1 × KMR(E) 16-1	82.05	76	86	2.12	2.41	77.56	3.85
	KMR(E) 16-1 × TM-210	83.43	80	86	1.25	1.74	75.22	2.7
	TM-2776 × DRMR 4005	83.06	80	90	1.76	2.06	73.2	3.1
Plant height (cm)	TM-138-1 × KMR(E) 16-1	139.87	90	225	11.82	13.1	81.39	21.97
	KMR(E) 16-1 × TM-210	153.05	110	200	8.11	9.01	81.09	15.05
	TM-2776 × DRMR 4005	145.73	105	200	7.69	9.24	69.25	13.18
Number of primary branches per plant	TM-138-1 × KMR(E) 16-1	6.69	2	12	33.33	36.47	83.52	62.74
	KMR(E) 16-1 × TM-210	5.54	3	13	31.45	33.78	87.12	60.34
	TM-2776 × DRMR 4005	5.33	2	10	31.42	34.74	81.8	58.53
Number of secondary branches per plant	TM-138-1 × KMR(E) 16-1	12.83	3	25	32.28	36.25	79.28	59.21
	KMR(E) 16-1 × TM-210	12.19	2	26	36.59	40.28	84.09	69.78
	TM-2776 × DRMR 4005	11.5	3	25	38.15	39.98	91.04	74.98
Number of racemes per plant	TM-138-1 × KMR(E) 16-1	25.61	10	40	26.21	27.82	88.79	50.88
	KMR(E) 16-1 × TM-210	21.52	8	36	27.27	32.03	71.27	47.43
	TM-2776 × DRMR 4005	23.89	10	40	23.98	27.31	77.1	43.37
Number of siliqua per raceme	TM-138-1 × KMR(E) 16-1	28.46	12	54	24.54	27.34	80.55	45.37
	KMR(E) 16-1 × TM-210	24.67	14	43	18.7	20.03	87.13	35.96
	TM-2776 × DRMR 4005	28.72	15	52	19.73	22.8	74.93	35.19
Seeds per siliqua	TM-138-1 × KMR(E) 16-1	15.01	8	24	12.78	14.88	73.74	22.61
	KMR(E) 16-1 × TM-210	14.43	6	24	20.57	22.98	80.89	37.95
	TM-2776 × DRMR 4005	11.99	6	20	22.48	25.88	75.42	40.22
Siliqua length (cm)	TM-138-1 × KMR(E) 16-1	5.49	3.5	8	16.43	16.65	97.59	33.38
	KMR(E) 16-1 × TM-210	6.61	3	8.5	13.24	15.17	77.29	23.82
	TM-2776 × DRMR 4005	5.35	3.5	8.5	15.17	17.39	76.04	27.25
1000-seed weight (g)	TM-138-1 × KMR(E) 16-1	4.61	3.6	5.6	10.17	11.06	85.12	19.27
	KMR(E) 16-1 × TM-210	4.76	3.8	5.8	11.7	13.62	73.81	20.72
	TM-2776 × DRMR 4005	4.23	3.2	5	13.59	15.03	78.37	24.81
Yield per plant (g)	TM-138-1 × KMR(E) 16-1	11.39	1.7	27	40.09	49.46	65.68	66.92
	KMR(E) 16-1 × TM-210	6.69	1.6	16.4	41.61	47.48	76.83	75.15
	TM-2776 × DRMR 4005	7.12	2.8	17	37.78	41.16	84.28	71.45

all populations, suggesting the influence of non-additive gene action and significant genotype-by-environment interactions. These factors limit the effectiveness of selection in early segregating generations (15, 16, 20).

High genotypic coefficient of variation (GCV), heritability and genetic advance as a percentage of the mean (GAM) were recorded for traits such as yield per plant, primary and secondary branches per plant and racemes per plant across all F_2 populations. These traits, showing significant genetic variation, could serve as excellent targets for improvement by selecting high-performing genotypes, making the selection process highly effective (21).

Correlation and path analysis

Correlation studies indicated a positive relationship between seed yield per plant and traits such as primary branches, secondary branches, racemes per plant, siliquae per raceme, 1000-seed weight and siliqua length in all F_2 populations (Fig. 1-3). These findings are consistent with the results of previous studies, which emphasized the critical role of these traits in improving seed yield (20, 22). Negative associations were observed with days to first flowering and days to maturity. Path coefficient analysis further identified traits such as primary and secondary branches per plant, siliqua per raceme, racemes per plant and 1000-seed weight as positively affecting seed yield (23, 24) (Fig. 4-6). These traits are critical determinants of seed yield. They should be prioritized in breeding programs, as suggested by previous studies (25).

Breeding potential

Furthermore, based on the values obtained from path analysis, we selected the traits with a higher positive direct effect on yield. These traits' means varied significantly across crosses, highlighting the influence of genetic background and environmental condition in which they have studied (26). F_2 plants derived from TM-138-1 × KMR(E) 16-1 showed higher seed yield per plant and wider variability, as evidenced by the high absolute and standardized ranges (Table 3). Similarly, high PCV and transgressive segregation frequencies in crosses like TM-138-1 × KMR(E) 16-1 and TM-2776 × DRMR 4005 underscore their breeding potential (Fig. 7 & 8). These results align with previous findings, which emphasized the importance of selecting crosses with high variability and superior trait means (10).

Transgressive segregants, which combine favourable alleles from both parents, were identified in all populations. Notably, the cross TM-138-1 × KMR(E) 16-1 exhibited 31 transgressive segregants for seed yield per plant, while TM-2776 × DRMR 4005 and KMR(E) 16-1 × TM-210 had 25 and 12 segregants, respectively. These transgressive segregants present valuable genetic resources for developing superior recombinant inbred lines (RILs) in subsequent generations (Table 3). The importance of such segregants in expanding the genetic base has been highlighted in earlier studies.

Crosses with high trait mean, wider ranges and high PCV, such as TM-138-1 × KMR(E) 16-1, demonstrated more significant potential for selecting superior RILs. Selecting such crosses aligns with maximizing variability and genetic gain, as recommended in earlier studies (10, 27). Similar strategies have been successfully employed in mustard and groundnut to identify crosses with enhanced breeding potential (23, 28, 29).

Conclusion

This study underscores the critical role of genetic variability, heritability and trait correlations in evaluating the breeding potential of Indian mustard. Crosses such as TM-138-1 × KMR (E) 16-1 demonstrated superior agronomic traits, highlighting their promise for developing high-yielding genotypes. These findings provide actionable insights for breeders prioritizing seed yield and component traits. However, since the study was conducted at a single location, the results may reflect site-specific environmental influences. Future multi-location trials across diverse agroecological zones are recommended to validate the genotype stability and adaptability of advanced breeding lines derived from the selected superior crosses.

Table 3. Estimates of mean, absolute range and standardized range of F_2 plants derived from different crosses

Cross (P_1 × P_2)	Trait	Absolute range		Standardized range	Parental Means	
		Min	Max		P_1	P_2
TM-138-1 × KMR(E) 16-1	Number of primary branches per plant	2.00	12	1.49	4.33	6.30
	Number of secondary branches per plant	3	25	1.71	6.50	13.70
	Number of racemes per plant	10	40	1.17	20.67	25.80
	Number of siliqua per raceme	12	54	1.47	25.17	27.20
	Yield per plant (g)	1.7	27	2.22	8.97	10.52
KMR(E) 16-1 × TM-210	Number of primary branches per plant	3	13.00	1.80	6.30	5.29
	Number of secondary branches per plant	2	26.00	1.96	13.70	14.00
	Number of racemes per plant	8	36.00	1.30	25.80	17.43
	Number of siliqua per raceme	14	43.00	1.17	27.20	26.00
	Yield per plant (g)	1.6	16.40	2.21	10.52	6.04
TM-2776 × DRMR 4005	Number of primary branches per plant	2.00	10	1.50	5.17	4.29
	Number of secondary branches per plant	3.00	25	1.91	12.50	8.29
	Number of racemes per plant	10.00	40	1.25	20.50	23.57
	Number of siliqua per raceme	15.00	52	1.28	25.83	22.86
	Yield per plant (g)	2.80	17	1.99	8.32	7.49

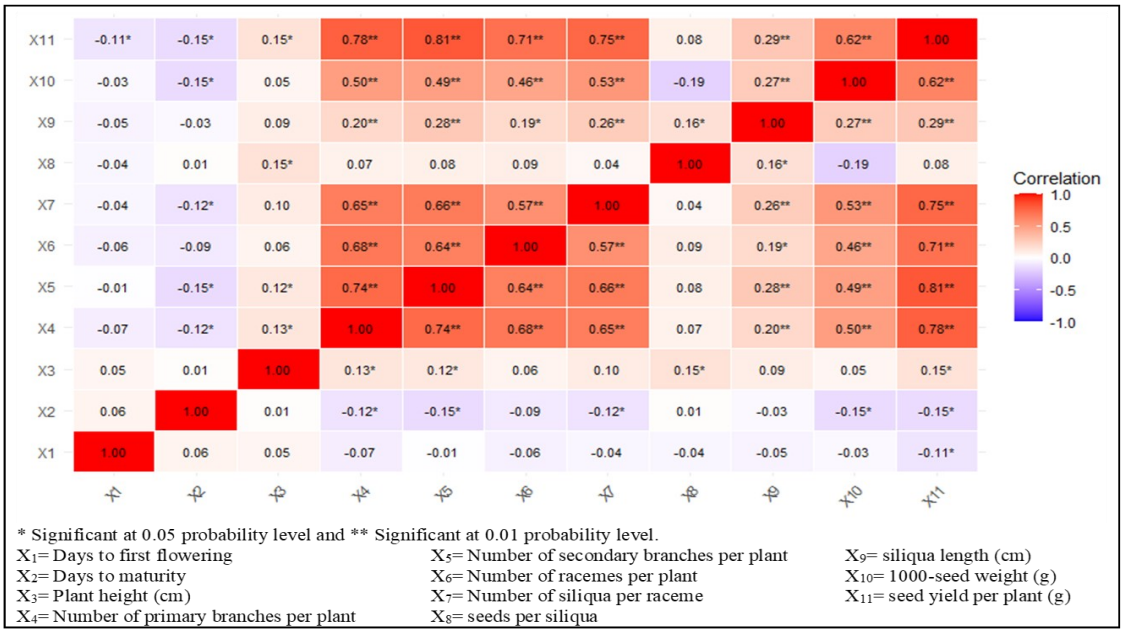


Fig. 1. Phenotypic correlation coefficient between yield and yield attributes in F_2 population of TM-138-1 × KMR(E) 16-1.

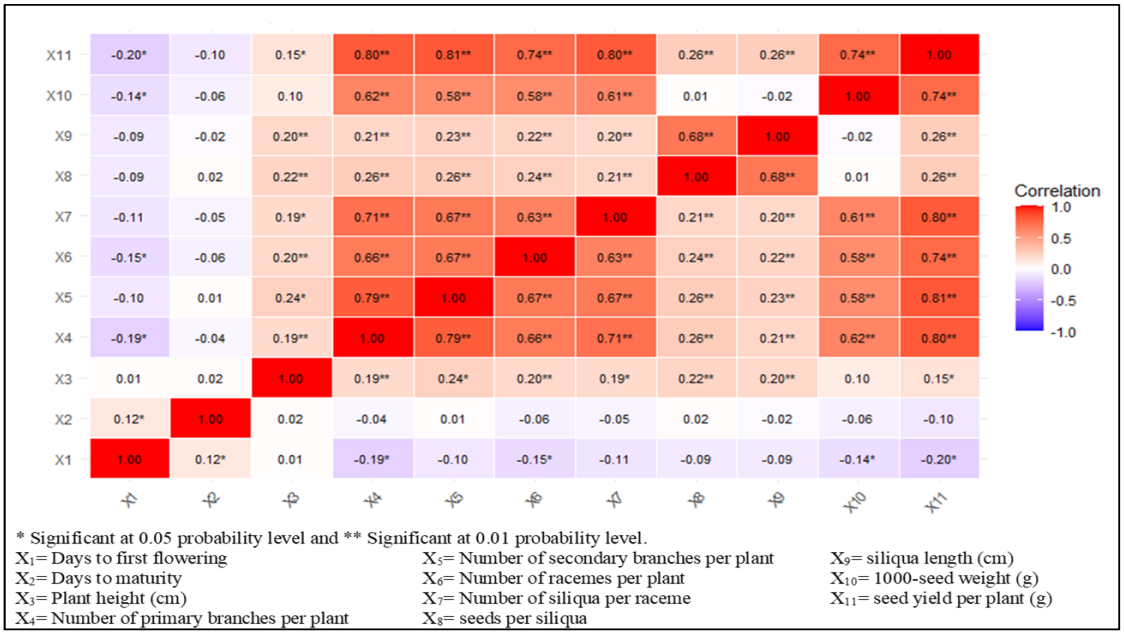


Fig. 2. Phenotypic correlation coefficient between yield and yield attributes in F_2 population of KMR(E) 16-1 × TM-210.

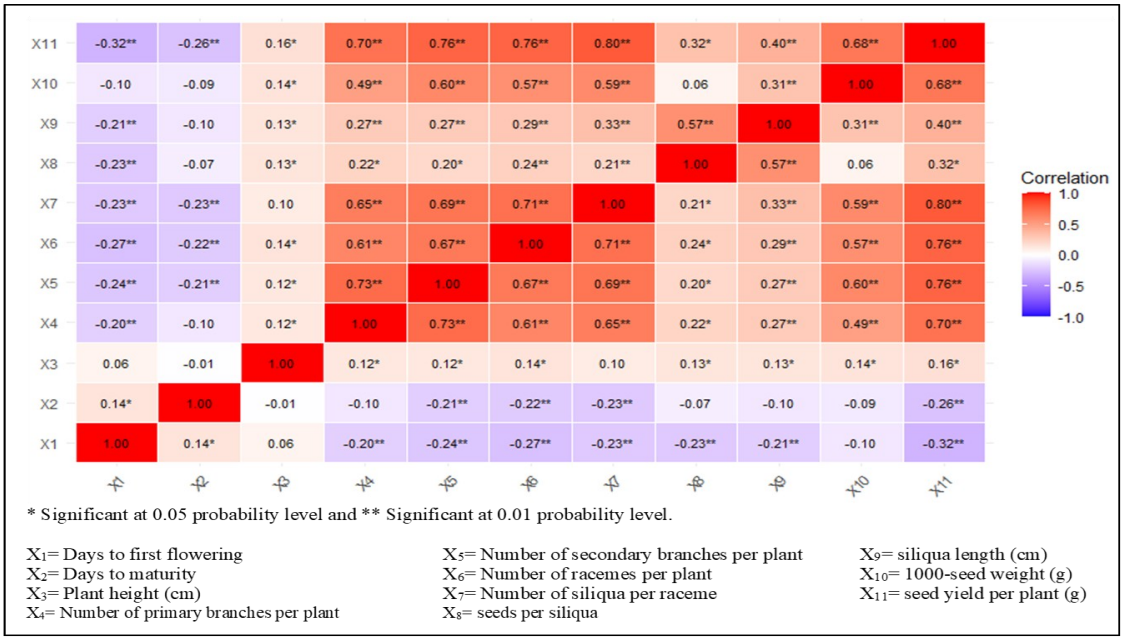
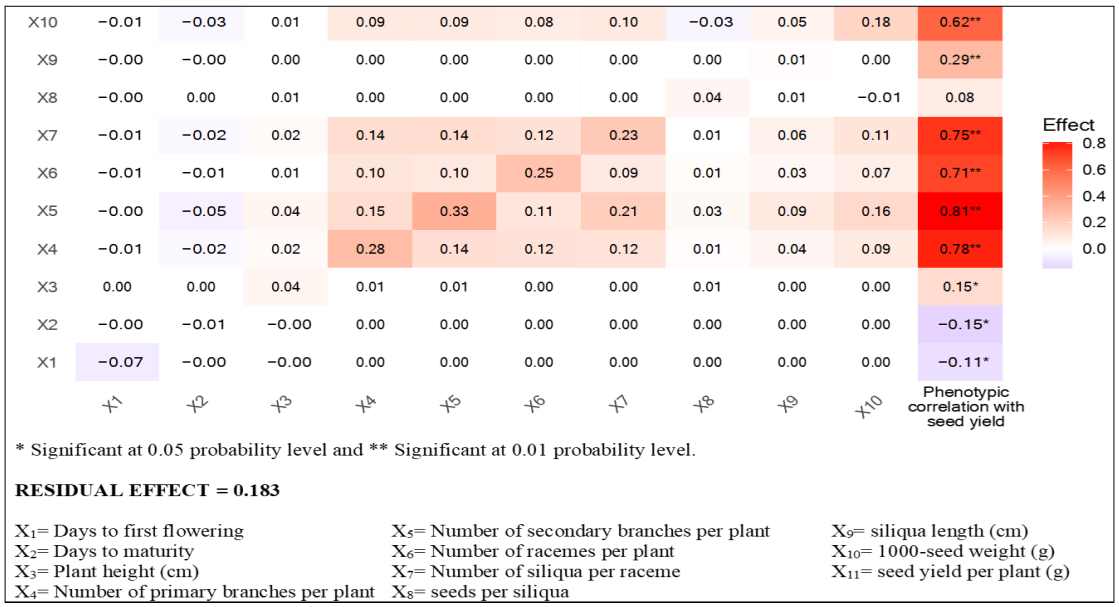


Fig. 3. Phenotypic correlation coefficient between yield and yield attributes in F_2 population of TM-2776 × DRMR 4005.

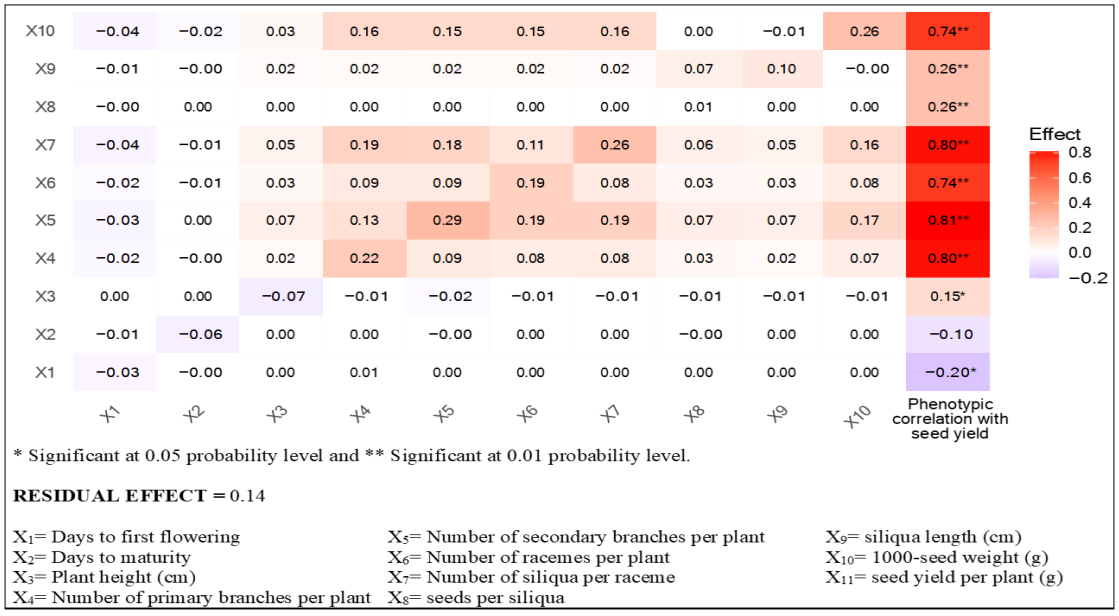


* Significant at 0.05 probability level and ** Significant at 0.01 probability level.

RESIDUAL EFFECT = 0.183

X_1= Days to first flowering
X_2= Days to maturity
X_3= Plant height (cm)
X_4= Number of primary branches per plant
X_5= Number of secondary branches per plant
X_6= Number of racemes per plant
X_7= Number of siliqua per raceme
X_8= seeds per siliqua
X_9= siliqua length (cm)
X_{10}= 1000-seed weight (g)
X_{11}= seed yield per plant (g)

Fig. 4. Direct (diagonal) and indirect effects of 10 characters on seed yield per plant at the phenotypic level in biparental F_2 population of TM-138-1 × KMR(E) 16-1.

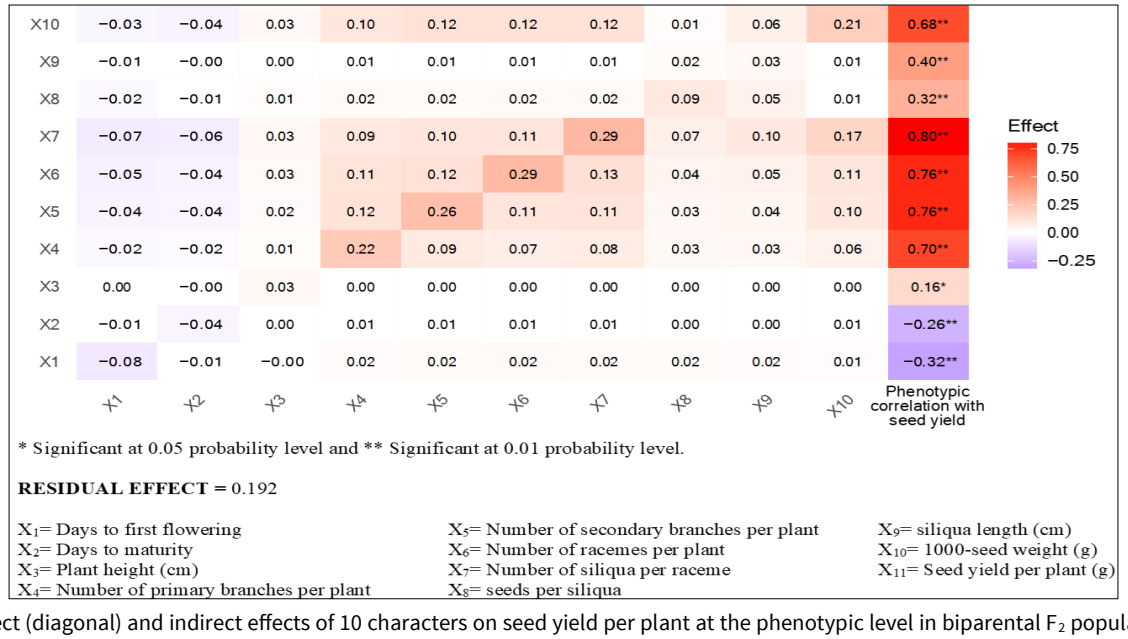


* Significant at 0.05 probability level and ** Significant at 0.01 probability level.

RESIDUAL EFFECT = 0.14

X_1= Days to first flowering
X_2= Days to maturity
X_3= Plant height (cm)
X_4= Number of primary branches per plant
X_5= Number of secondary branches per plant
X_6= Number of racemes per plant
X_7= Number of siliqua per raceme
X_8= seeds per siliqua
X_9= siliqua length (cm)
X_{10}= 1000-seed weight (g)
X_{11}= seed yield per plant (g)

Fig. 5. Direct (diagonal) and indirect effects of 10 characters on seed yield per plant at the phenotypic level in biparental F_2 population of KMR (E) 16-1 × TM-210.



* Significant at 0.05 probability level and ** Significant at 0.01 probability level.

RESIDUAL EFFECT = 0.192

X_1= Days to first flowering
X_2= Days to maturity
X_3= Plant height (cm)
X_4= Number of primary branches per plant
X_5= Number of secondary branches per plant
X_6= Number of racemes per plant
X_7= Number of siliqua per raceme
X_8= seeds per siliqua
X_9= siliqua length (cm)
X_{10}= 1000-seed weight (g)
X_{11}= Seed yield per plant (g)

Fig. 6. Direct (diagonal) and indirect effects of 10 characters on seed yield per plant at the phenotypic level in biparental F_2 population of TM-2776 × DRMR 4005.

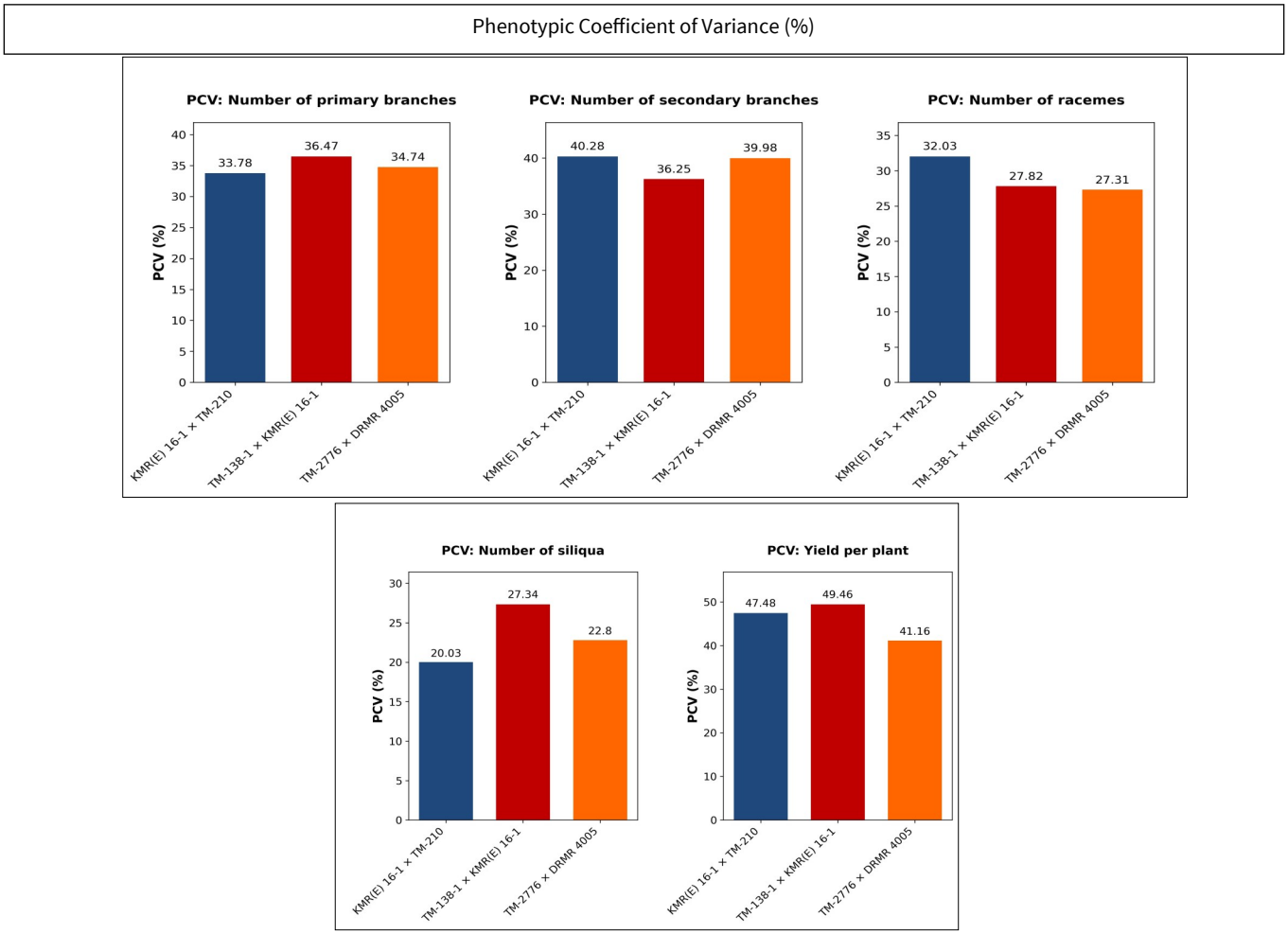


Fig. 7. Graph depicting differences in estimates of phenotypic co-efficient of variance of F_2 individuals.

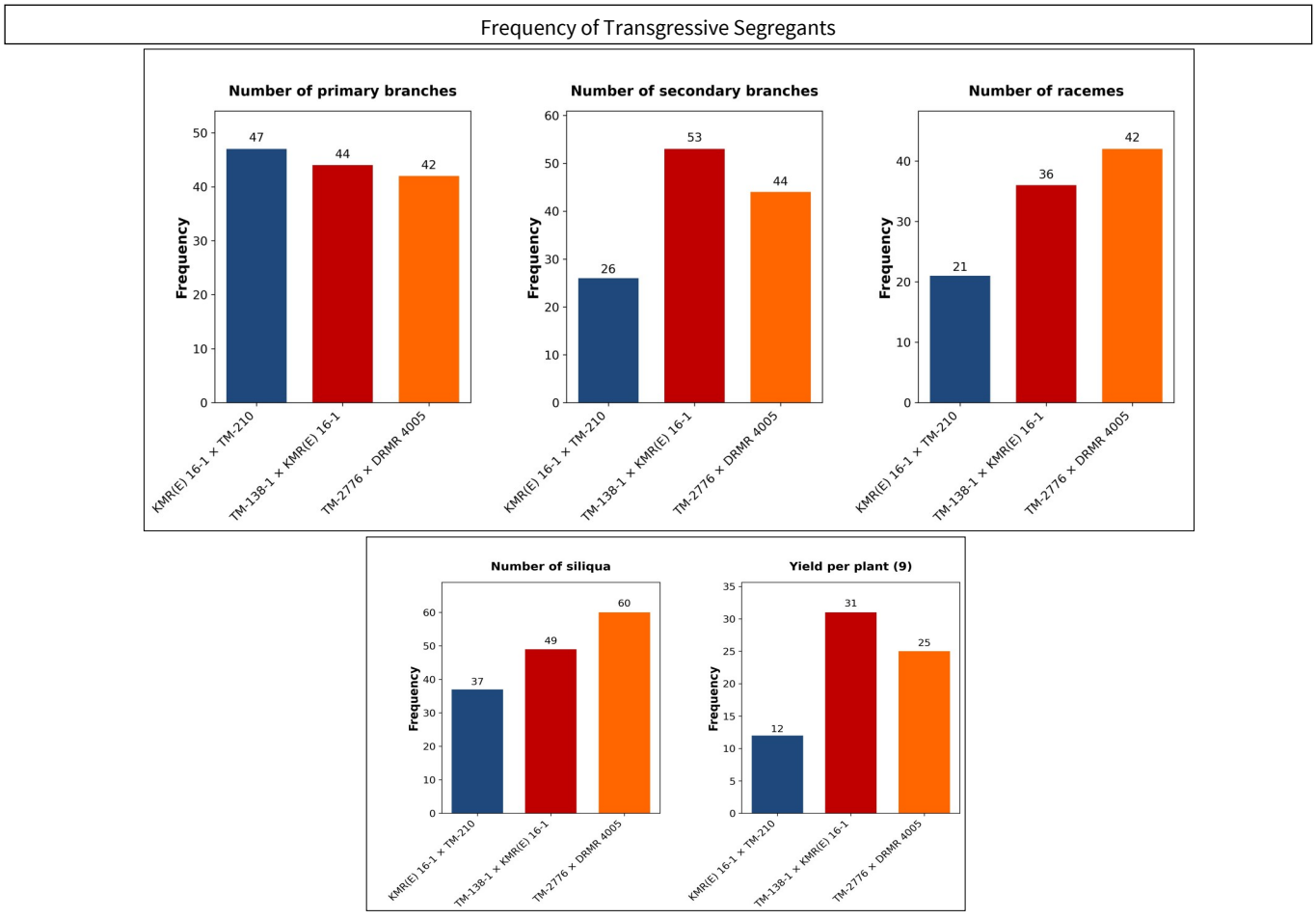


Fig. 8. Graphs depicting differences in frequency of transgressive segregants.

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Authors' contributions

BPMP, BRP and SBR conceptualized the experiment. BPMP, BRP, and SBR designed and planned the experimental layout. BPMP and VA collected the data. BPMP analyzed the data. BPMP, BVS, KS and MP contributed to draft preparation. All authors reviewed the manuscript.

Compliance with ethical standards

Conflict of interest: The authors assert they have no conflict of interest in this research.

Ethical issues: None

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