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Genetic Diversity and Yield Component Analysis in Indian Mustard [*Brassica juncea* (L.) Czern. and Coss.]

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ABSTRACT

Background: Researchers in Indian mustard have made minimal progress in breaking the yield barrier due to limited genetic resources. To increase productivity, a combination of molecular markers and morphological features is advised to boost productivity. The purpose of current work is to characterise polymorphic SSR markers, determine genotype variability, identify suitable parents for hybridization and understand key yield factors.

Methods: In 2020-21, twenty-eight Indian mustard varieties and advance breeding materials were evaluated at Ramakrishna Mission Vivekananda Educational and Research Institute'sexperimental field in Narendrapur, West Bengal, using statistical techniques to assess genetic estimates and molecular diversity using twentyidentified polymorphic SSR primers out of thirty tested primers.

Result: The study found that the number of siliqua per plant and primary and secondary branches are crucial yield components determined by correlation and path analysis. The high heritability of ten traits was observed. Genotypes were divided into four clusters with both Mahalanobis's D^2 and molecular diversity analysis, with 1000 seed weight and plant height showing the highest degree of divergence. But the composition of genotypes varied in two types of clusters. Ra1F09 and Ra2B02, SSR markers, showed greater PICvalues and heterozygosity among the twenty polymorphic SSR primers. Hybridization between PM25 and high oil content genotypes from cluster I, such as Sanjukta Asech and Kranti, is proposed to produce segregants with high yield, oil content and early maturity.

Key words: Genetic diversity, Indian mustard, Polymorphic information ccontent, SSR marker.

INTRODUCTION

Indian mustard, also known as *Brassica juncea* (L.) Czern. and Coss. is a major oilseed crop in India, with six cultivated species. The crop transported to India from China is an amphidiploid of *Brassica rapa* (2n=20) and *Brassica nigra* (2n=16). It is a member of the Brassicaceae family and self-pollinates 85-90% of the time. Rapeseed-mustard, a temperate zone crop, contains 38-42% oil, seeds are golden-yellow in colour. It is the third most significant oilseed crop in the world and is considered as a nutritious cooking medium. Major rapeseed-mustard producing countries are-Canada, China, Germany and France. The Yellow Revolution highlights India's significant role in the global mustard seed industry, ranking third in production. India with an area of 6.78 mha, 9.12 mt production and 1345 kg/ha productivity ranks second in area in rapeseed-mustard scenario of the world. It is used in various industries, including body massage, paints, varnishes, biofuel and chicken feed (Anonymous, 2020). The yield and productivity of Indian mustard are substantially reduced by numerous biotic and abiotic stress limitations. Mustard is absolutely insufficiently produced in India at a pitifully low rate of 1400 kg/ha. However, there is an increasing need for and use of mustard oil as the population increases and customer preferences change. According to Agricultural Statistics at a Glance (2019), roughly 57% of the edible oil consumed in India is imported, bringing in about Rs. 73,500 crores per year for the Government. Out of the predicted 34 million tonnes of edible oil we will use by 2025, 14 million tonnes must come from Indian mustard. There is a lot of demand on modern biotechnology and traditional plant breeding methods to create high yielding cultivars that can survive in a changing climate regime.

DNA-based molecular markers are increasingly used for cultivar identification, fingerprinting and diversification research due to rapid advancements in molecular biology techniques and increased availability of genetic resources (Pandey*et al*., 2015 and Singh *et al*., 2022). SSRs are currently preferred above other DNA-based markers by biotechnologists and plant breeders to evaluate genetic diversity and identify varietal attributes (Sharma *et al*., 2020).

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Short-duration mustard varieties usually suffer from low productivity. In West Bengal, winter being short, most of the cultivated varieties of Indian mustard are early maturing. But due to their low productivity, there is a need to identify and breed new genetic resources with high yields. Under this scenario, a study on character association is important as it serves the purpose of understanding the yield and the relationship of its contributing characters. This kind of study provides vital help to the breeder in the selection strategy of breeding materials. Furthermore, selection of parents based on genetic diversity, combining morphological traits and molecular markers, aids in greater confidence in selection process by limiting the influence of genotype-environment interaction (Pandey *et al*., 2015). This study uses path coefficient and correlation analyses to identify key yield-contributing factors in Indian mustard, assess genotype diversity through morphological attributes and SSR markers for parent identification in hybridization programmes and identify major production determinants for efficient breeding programmes.

MATERIALS AND METHODS

Field experiment

In 2020-21, Twenty-eight Indian mustard varieties and advance breeding materials (Table 1) were grown at the Experimental Farm of the IRDM Faculty Centre, RKMVERI,Narendrapur,West Bengal (latitude 22°43′N and longitude 88°40′E). The varieties and advance breeding materialswere planted using three replications of the randomized block design with the rows and the plants separated by 30 cm and 10 cm, respectively, on 15th November, 2020. Recommended agronomic practices were followed to grow the crop. Data on ten morphological traits such as days to maturity, plant height (cm), number of primary branches per plant, number of secondary branches per plant, number of siliqua per plant, siliqua length (cm), number of seeds per siliqua, 1000 seed weight (g), seed yield per plant (g) and oil content (%) were collected from ten randomly selected plants. The Analysis of Variance (ANOVA) was carried out for all ten morphological traits and genetic progress, heritability (broad sense) and genotypic and phenotypic coefficients of variation in percentage were estimated using formulas from Burton (1952) and Johnson *et al.* (1955), as detailed below:

Genotypic variance

It was computed using the formula.

$$Vg = \frac{MSG - MSE}{r}$$

Where:
Vg = Genotypic variance.
MSG = Mean square due to germplasm.
MSE = Error mean square.
r = Number of replications.

Phenotypic variance

It was calculated as:

$$Vp = Vg + Ve$$

Where:
Vp = Phenotypic variance.
Vg = Genotypic variance.
Ve = Error variance *i.e*., MSE.

Genotypic coefficient of variation (GCV) and Phenotypic coefficient of variation (PCV)

Both GCV and PCV were estimated by the formula suggested by the Burton (1952):

$$GCV (\%) = \frac{\sqrt{vg}}{\bar{x}} \times 100$$

Where:
Vg = Genotypic variance.
$\bar{X}$ = Varietal mean.

$$PCV (\%) = \frac{\sqrt{vp}}{\bar{x}} \times 100$$

Where:
Vp = Phenotypic variance.
$\bar{X}$ = Varietal mean.

Heritability

Heritability (h^2) in broad sense was calculated using the formula suggested by Allard (1960):

$$h^2 = \frac{vg}{vp} \times 100$$

and path analysis was performed using yield as dependent variable from the phenotypic correlation coefficients (Dewey and Lu,1957). All analyses were worked out following the software genes (Cruz, 2016) and OPSTAT (Sheoran, 2010).

DNA analysis and SSR genotyping

Genomic DNA was extracted from leavesof twenty-eight mustard genotypes' germination seeds at the 2-4 leaf stage using CTAB technique (Doyle and Doyle, 1987). After which RNase-A and phenol: chloroform: isoamylalcohol were used to purify the DNA. Nanodrop Lite (Thermo Scientific,USA) was used to quantify purified DNA (Pandey *et al*., 2018). 20 polymorphic markers were used for genotyping. Genotypes were grouped based on allelic size of PCR data. Polymorphic information content (PIC) and effective allele per locus (*Aep*) were calculated for microsatellite loci. NTSYS *Pc Ver.* 2.20 (Rohlf, 2000) software was used for molecular diversity analysis.

RESULTS AND DISCUSSION

The study (ANOVA) revealed significant differences among genotypes for ten morphological traits (Table 2). For every character that was observed, the phenotypic coefficient of variation (PCV) was greater than the corresponding genotypic coefficient of variation (GCV) (Table 2), though difference between two types of variability was not to a

considerable extent except primary branches and secondary branches per plant. Primary and secondary branches per plant, the number of siliqua per plant, the seed yield per plant and the length of the siliqua all displayed high PCV and high GCV (>20%) (Table 2). It's interesting to note that the heritability of these five characters ranged from high to moderate. High PCV, high GCV combined with high heritability indicate additive nature of gene action (Mandal *et al.*, 2022). Therefore, in order to improve the traits being better genetically controlled and exhibiting ideal variability, it would be advantageous to pick characters like seed yield, siliqua length, number of siliqua per plant, primary and secondary branches per plant. Similar finding was reported by Asheet *al.* (2023) in Indian mustard.

Table 1: List of varieties and advance breeding materialsof India mustard with parentage.

Varieties and advance breeding materials	Year	Source
Pusa Tarak	2009	IARI, New Delhi
NRCHB-101	2008	DRMR, Bharatpur, Rajasthan
Bullet		West Bengal local landrace
PM 25	2009	IARI, New Delhi
PM 27	2011	IARI, New Delhi
PM 28	2011	IARI, New Delhi
Bankura black		West Bengal local landrace
Kranti	1982	GBPUA and T, Pantnagar, Uttarakhand
Shivani		West Bengal local landrace
Pusa Karishma	2004	IARI, New Delhi
Pusa Vijay	2008	IARI, New Delhi
PM 26	2010	IARI, New Delhi
PM 29	2013	IARI, New Delhi
PM 30	2013	IARI, New Delhi
C1-2		F6: 2nd selection: PM 21 × PM 22
C1-5		F6: 5th selection: PM 21 × PM 22
C2-5		F6: 4th selection: PM 24 × PM 30
C3		F6: 2nd selection: PM 21 × PM 25
C4-4		F6: Selection 4: PM 24 × PM 25
C4-1		F6: Selection 1: PM 24 × PM 25
C5		F6: Selection 2: PM 21 × PM 30
MS90-6		Shivani mutant
PM 24	2007	IARI, New Delhi
MS 1		Mutant Shivani (Selection 1) γ-ray 100 GY
Sarama	1984	PORS, West Bengal
Sanjukta Asech	1989	PORS, West Bengal
Bhagirathi	1984	PORS, West Bengal
Seeta	1982	PORS, West Bengal

Table 2: Analysis of variance (ANOVA) and genetic parametersfor ten quantitative traits of Indian mustard.

Source of variation	d.f.	DM	PH (cm)	NPBPP	NSBPP	NSPP	SL (cm)	NSPS	1000 SW (g)	SYPP (g)	OC (%)
Replication	2	18.726	3.251	0.063	5.157	118.585	0.264	0.080	0.004	0.090	0.257
Genotypes	27	397.588**	2297.399**	9.879**	113.430**	82176.247**	3.686**	8.105**	1.712**	292.153**	130.120**
Error	54	6.059	45.448	1.137	10.918	450.786	0.167	2.280	0.092	4.044	5.180
GCV (%)		8.870	15.999	44.339	55.199	58.390	22.946	10.493	17.169	62.063	17.001
PCV (%)		9.073	16.476	52.278	63.407	58.880	24.525	15.472	18.574	63.357	18.027
Heritability%		95.563	94.291	71.933	75.785	96.433	87.537	45.993	85.443	95.959	88.938

** significant at 1% level; DM: Days to maturity; PH (cm): Plant height; NPBPP: No. of primary branches/plant; NSBPP: No. of secondary branches/plant; NSPP: No. of siliqua/plant; SL (cm): Siliqua length; NSPS: No. of seeds/siliqua; 1000 SW: 1000 seed weight(g); SYPP(g): Seed yield/plant; OC (%): Oil content in percentage; d.f.: Degrees of freedom; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation.

Character association

The study (Table 3) found that the number of primary branches and secondary branches per plant, number of siliqua per plant, siliqua length significantly correlated with seed yield, suggesting that genotypes with higher mean values of these traits can yield higher crops (Mandal *et al*., 2022). On the contrary, oil content was negatively correlated with almost all variables. It was revealed further from inter-correlation study that the number of primary branches and secondarybranches per plant, siliqua length and number of siliqua per plant were interrelated significantly and positively with each other, suggesting that increasing these traits will strongly associate with co-related characters positively. Linkage and or pleiotropy may be responsible such associations.

Rajpoot *et al.* (2022) found a significantly positive correlation between siliqua number per plant and number of seeds per siliqua. Almost similar results were obtained in the present study, as highly positive correlation was found between siliqua number and seeds per siliquaconsistently at phenotypic and genotypic level (Table 3). This emphasizes that higher siliqua number would produce more number of seeds per siliqua through correlated response. Interestingly, both these traits are associated positively with seed yield. So, selection for higher siliqua number would augment seed yield and seed number as well.Similarly, like the report of Rajpoot *et al.* (2022) a significant negative correlation between 1000 seed-weight and seed numberper siliquawas also observed in our finding indicating that more number of seeds per siliqua would produce smaller seed size because these two characters are genetically linked negatively.

Path coefficient analysis

The path coefficient analysis on yield showed that number of siliqua per plant had the highest positive direct effect (Table 4). The number of secondary and primary branches also demonstrated positive direct effects on seed yield. However, siliqua length had a negative direct effect despite having a substantially positive correlation coefficient with seed yield (Table 4). Chaubey 2022 and Tiwari 2019 also reported similar findings. Although the association between seed yield and 1000 seed weight was only marginally favourable, it nevertheless had a good positive direct

Table 3: Phenotypic (upper diagonal) and genotypic (lower diagonal) correlation coefficients among ten traits of Indian mustard.

	DM	PH (cm)	NPBPP	NSBPP	NSPP	SL (cm)	NSPS	1000 SW (gm)	SYPP (gm)	OC (%)
DM	1.000	0.571**	0.036	0.027	0.072	0.120	0.006	0.039	0.237	-0.188
PH	0.575**	1.000	0.177	0.091	0.131	0.217	0.259	0.036	0.281	-0.151
NPBPP	0.037	0.179	1.000	0.848**	0.914**	0.773**	0.203	0.125	0.833**	-0.421*
NSBPP	0.028	0.090	0.856**	1.000	0.902**	0.743**	0.088	0.301	0.859**	-0.496**
NSPP	0.073	0.131	0.923**	0.909**	1.000	0.805**	0.323	0.137	0.925**	-0.395*
SL	0.122	0.217	0.784**	0.752**	0.815**	1.000	0.421*	0.011	0.744**	-0.419*
NSPS	0.007	0.261	0.202	0.084	0.329	0.420*	1.000	-0.565**	0.299	-0.031
1000 SW	0.041	0.036	0.125	0.303	0.137	0.011	-0.573**	1.000	0.289	-0.175
SYPP	0.238	0.281	0.839**	0.863**	0.926**	0.751**	0.302	0.289	1.000	-0.407*
OC (%)	-0.188	-0.151	-0.427*	-0.499**	-0.399*	-0.423*	-0.031	-0.176	-0.408*	1.000

**Significant at 1% level and *Significant at 5% level; DM: Days to maturity; PH (cm): Plant height; NPBPP: No. of primary branches/plant; NSBPP: No. of secondary branches/plant; NSPP: No. of siliqua/plant; SL(cm): Siliqua length; NSPS: No. of seeds/siliqua; 1000 SW(g): 1000 seed weight; SYPP(g): Seed yield/plant; OC (%): Oil content in percentage.

Table 4: Phenotypic path coefficient analysis of seed yield per plant in Indian mustard.

	DM	PH	NPBPP	NSBPP	NSPP	SL	NSPS	1000 SW	OC (%)	Correlation with seed yield/plant
DM	**0.184**	0.006	0.004	0.006	0.042	-0.011	0.002	0.009	-0.005	0.237
PH	0.105	**0.107**	0.019	0.019	0.077	-0.020	0.066	0.009	-0.004	0.281
NPBPP	0.007	0.002	**0.105**	0.176	0.539	-0.071	0.052	0.034	-0.011	0.833**
NSBPP	0.005	0.001	0.089	**0.207**	0.533	-0.068	0.023	0.083	-0.013	0.859**
NSPP	0.013	0.001	0.096	0.187	**0.590**	-0.074	0.083	0.039	-0.011	0.925**
SL	0.022	0.002	0.081	0.154	0.476	**-0.091**	0.109	0.002	-0.011	0.745**
NSPS	0.001	0.003	0.021	0.019	0.191	-0.039	**0.256**	-0.151	-0.002	0.300
1000 SW	0.006	0.001	0.013	0.064	0.084	-0.001	-0.143	**0.271**	-0.005	0.289
OC (%)	-0.035	-0.002	-0.044	-0.103	-0.233	0.038	-0.008	-0.047	**0.027**	-0.406

Residual effect: 0.150; DM: Days to maturity; PH (cm): Plant height; NPBPP: No. of primary branches/plant; NSBPP: No. of secondary branches/plant; NSPP: No. of siliqua/plant; SL (cm): Siliqua length; NSPS: No. of seeds/siliqua; 1000 SW (g): 1000 seed weight; SYPP (g): Seed yield/plant; OC: Oil content. Bold underlined figures indicate direct effects.

influence. Similar finding was reported by (Sultana, 2017). A significant positive association with seed yield followed by a significantly favourable direct effect on yield is required to identify the importance of yield components. The study suggests restructuring mustard plant types based on primary branches, secondary branches and the number of siliqua per plant to increase yield, identifying these traits as crucial yield traits.

Diversity in morphological characters of genotypes

The twenty-eightvarieties and advance breeding materials were grouped into four groups based on relative D^2 values for ten agro-morphological variables. Cluster-I had sixteen genotypes, while Cluster-II had eight. Cluster-III was made up of three genotypes, Sarama, PM30 and C3. Cluster-IV contained only PM25 (Table 5). The geographic origin of grouped genotypes was found from multiple locations in the cluster composition.In other words, genetic diversity was not correlated with geographic origin whichconfirmed previous observation by (Gupta *et al.*,1991). Clusters-I and IV had the greatest inter-cluster distance (D^2=45289.56), followed by clusters-III and IV (D^2=20542.58), suggesting that picking parents from these clusters would be rational for the hybridization program (Table 6). The relative contribution of characters showed that 1000 seed weight (73%) andplant height (10%) contributed most to divergence.

The study found significant differences in cluster means for various features, indicating a relationship between cluster and trait (Table 7). Cluster-IV had superior yield values along with some important yield components, while cluster-II had the highest values for siliquae length and number of siliqua per plant. To speed up breeding plans, creating variability or transferring targeted traits by breeding among diverse clusters is desirable.

Diversity based on molecular markers

DNA-based markers like RAPD, ISSR, RFLP, AFLP and SSR are used to assess genetic diversity in Indian mustard genotypes. SSR markers are more effective due to their higher polymorphism, repeatability and lower cost (Vieira *et al.*, 2016). The current study (Table 8) found a low average allele number per SSR marker (1.87), while Singh *et al.* (2022) found that the average number of alleles per SSR locus was 3.61. The high average number of alleles per locus may be the result of using a large number of genotypes and SSR markers. The study found that the PIC value of SSR markers, Ra1F09 and Ra2B02, was the most effective in distinguishing genotypes. The marker BRMS002 produced the largest genetic diversity among the twenty-eightvarieties and advance breeding materials, followed by BRMS011 and marker BRMS002 produced the greatest effective allele (Table 8). The dendrogram based on twenty SSR makers of

Table 5: Grouping of twenty-eight varieties and advance breeding materials of Indian mustard into different clusters based on Mahalanobis D^2 values of ten morphological traits.

Cluster	Total number	Varieties and advance breeding materials
I	16	Seeta, C4-1, C2-5, C1-5, Pusa Vijay, C4-4, Pusa Tarak, MS90-6, C5, C1-2, Pusa Karishma, Sanjukta Asech, Kranti, NRCHB-101, Bankura Black, PM24
II	8	Bullet, PM28, PM26, PM27, Bhagirathi, PM29, Shivani, MS-1
III	3	Sarama, PM30, C3
IV	1	PM25

Table 6: Intra and inter-cluster D^2 values among four clusters of Indian mustard.

	I	II	III	IV
I	1986.01	20540.05	9975.91	45829.56
II		3424.673	6278.80	8050.78
III			2461.27	20542.58
IV				0

Table 7: Mean values of ten morphological traits of Indian Mustard for different clusters.

Cluster number	DM	PH (cm)	NPBPP	NSBPP	NSPP	SL (cm)	NSPS	1000 SW (g)	SYPP (g)	OC (%)
I	128.0	167.82	2.60	6.13	152.66	4.00	12.91	4.13	8.04	40.74
II	133.13	184.41	5.74	16.73	479.73	5.95	13.90	4.46	26.63	34.10
III	114.78	140.80	4.80	15.44	395.02	5.01	13.46	4.54	20.88	35.04
IV	149.00	212.33	6.00	18.33	451.67	5.43	13.90	4.60	37.87	32.97

DM: Days to maturity; PH (cm): Plant height; NPBPP: No. of primary branches per plant; NSBPP: No. of secondary branches per plant; NSPP: No. of siliqua per plant; SL(cm): Siliqua length; NSPS: No. of seeds per siliqua; 1000 SW (g): 1000 seed weight; SYPP (g): Seed yield per plant; OC (%): Oil content in percentage.

Table 8: Polymorphic information content (PIC) value, Heterozygosity or genetic diversity and effective alleleof twenty-eight varieties and advance breeding materials of Indian mustard on twenty SSR markers.

Primer name	PIC value	Heterozygosity or genetic diversity	Effective allele
Ra1F09	0.72	0.22	1.29
Ra2B02	0.67	0.24	1.31
Ra1F03	0.64	0.32	1.49
Ra2C07	0.53	0.34	1.52
BRMS003	0.64	0.35	1.57
BRMS030	0.55	0.40	1.70
BRMS011	0.43	0.57	1.78
BRMS033	0.45	0.54	2.17
BRMS040	0.44	0.52	2.08
BRMS005	0.33	0.47	1.89
BRMS002	0.51	0.69	3.22
MR52a	0.36	0.50	2.00
BRMS011	0.34	0.39	1.64
BrgMS13	0.38	0.48	1.92
Ra1-F06	0.39	0.49	1.96
BrgMS90	0.39	0.51	2.04
BrgMS746	0.41	0.48	1.92
Ni2A01	0.38	0.48	1.92
SB3751	0.36	0.49	1.96
ENA21	0.35	0.51	2.04
Mean	0.46	0.45	1.87

Table 9: Clustering of twenty-eight varieties and advance breeding materials of Indian mustard based on twenty SSR markers.

Cluster	Sub-cluster	Total number	Varieties and advance breeding materials
I		5	Sarama, PM 26, C3, PM 29, MS-1
II	IIA	12	PM 27, C4, PM 28, Pusa Vijay, PM 24, C2-5, MS90-6, PM 25, NRCHB-101, C1-5, Seeta, Bhagirathi
	IIB	4	Shivani, Pusa Karishma, C5, C1-2
III		3	PM 30, Sanjukta Asech, Kranti
IV		4	Bullet, Pusa Tarak, C4-1, Bankura Black

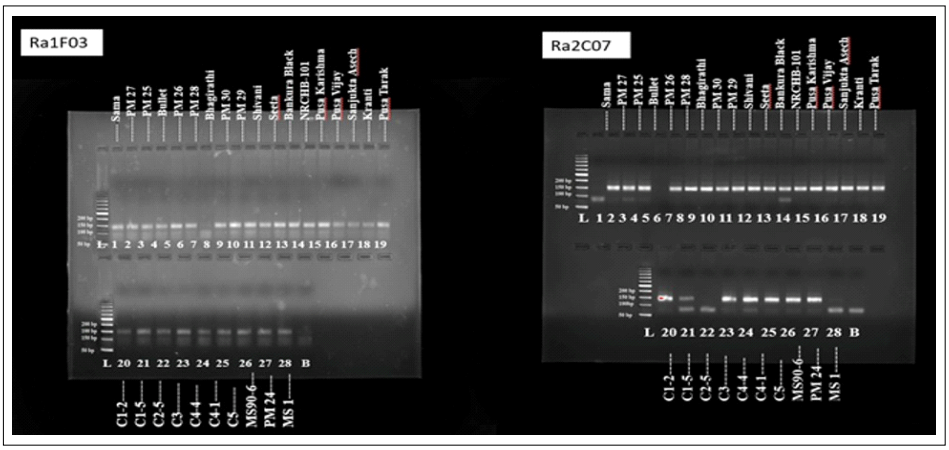

Fig 1: Electrophoregram of different mustard varieties and advance breeding materials (1-28) by using Ra1F03 and Ra2C07 SSR markers (where L=50bp ladder; B=Blank).

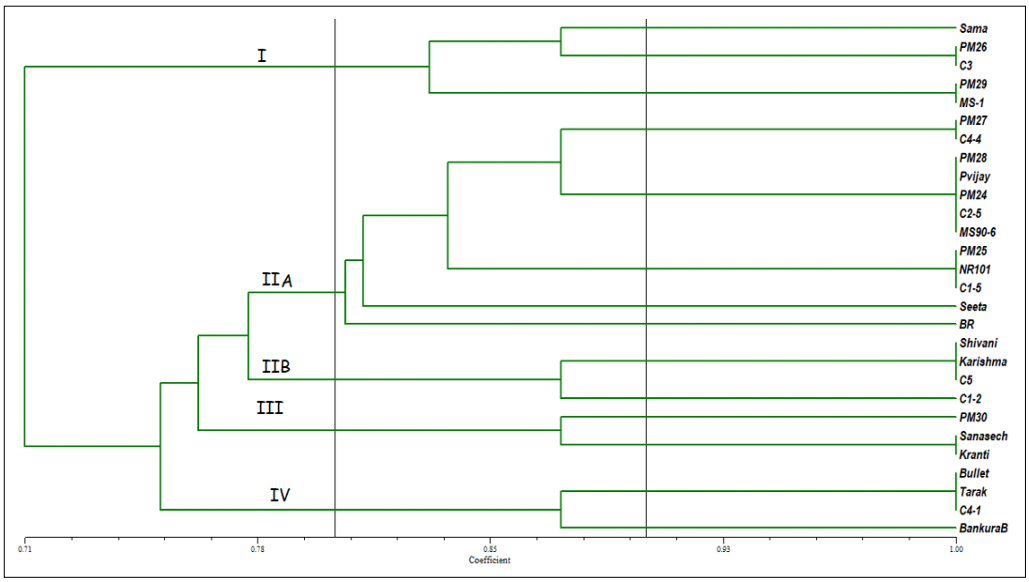

Fig 2: Dendrogram of twenty-eight Indian mustard varietiesand advance breeding materials based on scoring of SSR markers.

twenty-eight mustard varieties and advance breeding materials revealed four major clusters, with Cluster-II having the highest number of genotypes. Sub-cluster IIA contained PM27, C4, PM28, Pusa Vijay, PM24, C2-5, MS 90-6, PM25, NRCHB-101, C1-5, Seeta and Bhagirathi. Cluster-III contained PM30, Sanjukta Asech and Kranti (Table 9 and Fig 1). Though four clusters were formed using both molecular markers and morphological traits (Table 9, Table 5 and Fig 2), the composition of clusters varied between the two methods, with the genotypes PM28 and PM30 having a consistent uniform position in both types of clusters.

The study found good genetic diversity between PM25 and Sanjukta Asech or Kranti, as they are placed in different clusters irrespective of SSR based or morphometric analysis (Table 5 and Table 9). PM25 in cluster-IV had high yield and yield components, while the remaining two genotypes *i.e*., Sanjukta Asech and Kranti belonging to cluster-I had high oilcontent (Table 5 and Table 7). Hybridization between PM25 and Sanjukta Asech or Kranti could develop desired segregants high yield, high oil content and early maturity.

CONCLUSION

The study highlights the importance of siliqua number per plant, secondary and primary branches in yield factors, emphasizing the need for more attention in plant selection and desirable segregants.The genetic diversity of varieties and advanced breeding materials is evaluated at the morphological and molecular levels. Effective allele size and heterozygosity were highest in marker BRMS002. All twenty SSR markers that produced clear bands had moderately high PIC values. These SSR markers can be used to differentiate between mustard varieties and advanced breeding materials. In terms of both morphological and molecular diversity, the varieties and breeding materials formed an equal number of clusters. However, the accessions' composition differed between the two types of clusters in nearly every cluster.Premium breeding materials with high yield and oil content can be produced by carefully selecting parents for a hybridization programme based on the genetic diversity, particularly the molecular distance between accessions, combined with the performance of morphological traits.

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Conflict of interest

Authors of this research paper have no conflicts of interest.

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