Metroglyph Analysis of Morphological Variation in Indian Mustard (*Brassica juncea* L.)

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Abstract

Fifty accessions of Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] were evaluated to study the pattern of morphological variation for ten characters using Metroglyph analysis and the Index score method. The experiment was conducted to assess genetic variability and the contribution of specific morphological characters to grain yield at the Agricultural Research Farm, Janta Mahavidyalaya, Ajitmal, Auraiya (U.P.) during the Rabi season of 2021-22. Data on various economic characteristics of the plant were systematically recorded to facilitate comparative evaluations, employing metroglyph techniques. Highly significant differences with a substantial range of variability were observed among the genotypes for all traits. Anderson's Metroglyph analysis identified eight clusters, with Cluster III being the largest (13 genotypes), followed by Clusters I and IV, each comprising eight genotypes. Clusters VI, VII, and VIII consisted of five genotypes each, while Cluster II and V included 4 and 2 genotypes, respectively. The Index Score method was applied to assign a score to each character of the lines, indicating the worth of that genotype concerning the specific trait. Total index score values for the 50 germplasms ranged from 12 to 25. PR-21-35 exhibited the highest score (25), while PRE-21-02 showed the lowest score (14). Germplasms with an index score from 21 to 25 were categorized as the upper superior class, while those with scores between 15 to 20 constituted the lower class. These classifications provide valuable insights for a crossing program, enabling the harnessing of maximum variability for favourable combinations of traits. Additionally, the study helps identify the diversity in various characters among the 50 genotypes, facilitating informed decision-making in breeding programs.

Keywords: *Brassica juncea*, Metroglyph, Clusters, Index score, Intervarietal crosses

Introduction

Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] is a significant oilseed crop cultivated extensively in various agro-climatic regions worldwide. Its economic importance stems from its versatile applications in food, pharmaceutical, and industrial sectors, primarily attributed to its high oil content and nutritional value. Understanding the morphological diversity within *Brassica juncea* populations is crucial for breeding programs aimed at enhancing yield, oil quality, and stress tolerance.

Indian mustard [*Brassica juncea* (L.) Czern. & Coss.], is a notable oilseed crop cultivated extensively across diverse agro-climatic regions worldwide. Its economic significance is derived from its multifaceted applications in the food, pharmaceutical, and industrial sectors, predominantly due to its high oil content and nutritional value. Recognizing the morphological diversity within *Brassica juncea* populations holds pivotal importance for breeding programs focused on augmenting yield, oil quality, and stress tolerance.

Genetic divergence, the process wherein two or more populations of an ancestral species accumulate independent genetic changes (mutations) over time, often after becoming reproductively isolated, is crucial for populations to adapt to changing environments and is a principal mechanism underlying speciation. This phenomenon can be measured through Metroglyph Analysis (Anderson, 1957). Metroglyph analysis, a comprehensive morphometric approach, provides a detailed framework for characterizing and quantifying morphological variation within plant populations by integrating traditional morphometric measurements with geometric morphometrics. It offers a holistic perspective

on shape and size variations among plant structures, allowing researchers to discern subtle differences in plant morphology. This facilitates genotype discrimination and trait selection in breeding programs. The present investigation aims to elucidate the extent and patterns of morphological diversity among diverse Indian mustard germplasms. Through the application of metroglyph analysis, our objective is to identify key morphological traits contributing to population differentiation, thus providing valuable insights for *Brassica juncea* breeding programs. Furthermore, the insights derived from metroglyph analysis could inform future research endeavours focused on trait-specific breeding strategies and the conservation of genetic resources in *Brassica juncea.*

Material and Methods

The present investigation was conducted during the *Rabi* season of 2021-22 at the Agricultural Research Farm, Janta Mahavidyalaya, Ajitmal, Auraiya (U.P.), using fifty accessions of Indian mustard [*Brassica juncea* (L.) Czern. & Coss.]. The study aimed to assess genetic variability and the contribution of specific morphological characters to grain yield. Details of the experimental material are provided in Table 1. The genotypes were planted in a randomized block design with three replications. Each row was 3m in length, with a spacing of 40cm between rows and a plant-to-plant distance of 10cm. Thinning was conducted in the third week after sowing to maintain a distance of 10cm between plants. Fertilizer was applied at the rate of 80kg N, 40kg P_2O_5, and 40kg K_2O per hectare. Normal cultural practices and timely plant protection measures were followed to ensure the successful growth of the crop.

Observations were recorded on five randomly selected plants in each line and replication, and their mean values were utilized for statistical analysis. Data were collected on ten characters viz., day to 50% flowering, day to maturity, plant height (cm), number of primary branches per plant, number of secondary branches per plant, number of siliquae per plant, length of siliqua, number of seeds per siliqua, 1000-seed weight (g), and seed yield per plant (g). Analysis of variance for all characters of genotypes was conducted following a randomized block design. Mean values were then used to construct the metroglyph analysis according to the model proposed by Anderson (1957). In this analysis, glyphs were initially plotted based on two highly variable traits: seed yield per plant (X-axis) and number of siliquae per plant (Y-axis). All other characters were represented by rays on the glyph. Each ray denotes a specific character, divided into three equal classes (low, medium, and high) based on the range of variation. The length of the ray assigned to each character depends on the index scores of accessions for that particular character. Glyph positions and rays were employed to assess variability patterns and correlated traits for the evaluation of divergent groups. Each germplasm accession is assigned a unique number and represented as a glyph, which is the intersection point of mean values of X and Y coordinates.

The success of plant breeding for improving a trait depends on the availability of diverse germplasm, precise selection procedure and crossing programme. The experiment was conducted to evaluate the genetic potential of mustard genotypes for yield and yield component characters by Metroglyph analysis and developing a selection criterion. This method was used to assess genetic diversity by Dewan *et al.* (1992) in Indian mustard, Ghafoor and Ahmad (2005) in black gram, Khan *et al.* (2007) in cotton and Thakur *et al.* (2018) in Chickpea.

Table 1: The detail of genotypes under study

S. No.	Genotypes	Pedigree	S. No.	Genotypes	Pedigree
1	PRE-21	TM102×TC5	26	PRL-21	Pusa Vijay× Pusa Agrani
2	PRE-21	Seeta×Aravali	27	PRL-21	EC-552574×PRL-2007-3

S. No.	Genotypes	Pedigree	S. No.	Genotypes	Pedigree
3	PRE-21	NRCHB101-× NPJ173	28	PRL-21	EC552576×Vaibhav
4	PRE-21	PM-25	29	PRL-21	TM-2×IC264133
5	PRE-21	MCNR15-14×RH1402	30	PRL-21	NRCHB-101×DRMR2398
6	PRE-21	JM-2×JMM-927	31	PR-21	NP×EJ-17J-156
7	PRE-21	PM-28	32	PR-21	ACNMM-23
8	PRE-21	Seeta ×Varuna	33	PR-21	NPJ-153×RGN-73
9	PRE-21	JD-6	34	PR-21	Kranti
10	PRE-21	PUSAAgrani×NPJ112	35	PR-21	NRCHB101×DRMR2326
11	PRE-21	PRE2009-9×Kranti	36	PR-21	RGN73
12	PRE-21	BAUSM92-1-1×BR-40	37	PR-21	(PRB-04-3-04×Vruna)×PAB-9534
13	PRE-21	GM-1×RSK219	38	PR-21	RH1140×RH1372
14	PRE-21	RH1402×RH1401	39	PR-21	PBR210×PBR212
15	PRE-21	Pusa bold×NRCHB101	40	PR-21	JM-4×B-85
16	PRL-21	SEJ-2×HUJM -9964	41	PR-21	Ashirwad×GM-3
17	PRL-21	Vardan ×Maya	42	PR-21	Maya
18	PRL-21	NRCHB -101	43	PR-21	RH1140×RH1370
19	PRL-21	NPJ-113×EC399299	44	PR-21	Pusa bold×Urvashi
20	PRL-21	RH1402×RH1301	45	PR-21	NPJ-124×RB-50
21	PRL-21	NPJ-162×RGN-73	46	GIRIRAJ	DRMRIJ31
22	PRL-21	CS-56	47	ROHINI	KRB24
23	PRL-21	Vardan×Karnti	48	PM0031	-
24	PRL-21	RH1599×RH1402	49	CS-58	-
25	PRL-21	NPJ-153×Laxmi	50	CS-60	-

Table 2: Analysis of variance (ANOVA) for ten characters of Indian mustard

Source of variation	d.f.	Days to 50 % flowering	Days to maturity	Plant height (cm)	No. of primary branches per plant	No of secondary Branches per plant	No. of siliqua per plant	Siliqua length(cm)	No. of seed per siliqua	1000-Seed weight(g)	Yield /plant(g)
Replication	2	1.43	37.53	810.43	4.01	17.49	15160.97	1.86	6.19	0.29	899.50
Treatment	49	20.94**	45.16**	905.89**	1.41**	3.26**	4094.05**	0.45*	1.83**	0.41*	77.82**
Error	98	2.78	14.46	307.64	0.88	2.68	1380.94	0.37	0.90	0.27	60.63

*, ** Significant at 5% and 1% levels, respectively

Results and Discussion

The germplasm lines were plotted with seed yield per plant on the X-axis and the number of siliquae per plant on the Y-axis, as these two characters exhibited a high genotypic coefficient of variation. The range of each character was divided into three equal classes (low, medium, and high), respectively, and each character was represented by a different length of the ray depending on its index score. Low, medium, and high scores were represented with 1, 2, and 3, respectively, according to their range of mean values (Table 3). Fifty advanced lines, including five check varieties, were graphically represented as a metroglyph (Figure 1). The distributions of germplasm lines of Indian mustard are given in (Table 4). Forty-five advance lines and five check varieties were grouped into eight complexes. Complexes I and IV contain eight lines each, complex II contains three advance lines and includes one check variety, complex V contains one advance line and includes one check, complex VI, VII, and VIII with five advance lines each were placed closely on the scattered plot (Figure 2). The advanced lines of group IV had medium values for both axes. The total index score values recorded for 50 germplasm lines ranged from 12 to 25 (Figure 1). The germplasm PR-21-35 showed the highest score (25), while PRE-21-2 showed the lowest score (12). The germplasms with an index score from 21 to 25 constituted the upper superior class, and the germplasms with an index score of 15 to 20 constituted the inferior class. Lende *et al*. (2013) also studied metroglyph and index score methods for morphological variations in 20 local collections and 9 promising varieties of mustard and grouped them into VI clusters. Several other researchers have suggested metroglyph analysis for the preliminary classification of genotypes in different crop plants (Khan *et al.*, 2007; Punitha *et al.*, 2010; Jha *et al.*, 2011).

Table 3: Index scores and signs used for ten characters in Metroglyph analysis of Indian mustard.

S. No.	Characters	Range	Scores 1 Value less than	Sign	Scores 2 Value between	Sign	Scores 3 Value more than	Sign
1	Days to 50% flowering	31.93 – 42.66	35.5	○	35.5-39.08	⊖	39.09	⊖
2	Days to %maturity	126.66 – 142.70	132	○	132.0-137.74	⊘	137.75	⊘
3	Plant height (cm)	126.10 – 199.36	150	○	150.0-174.94	⊶	174.95	⊶
4	No. of primary branches/plant	3.96 – 8.06	5.32	○	5.32-6.7	⚲	6.7	⚲
5	No. of secondary branches/plant	10.26 – 15.56	11.92	○	11.92-13.79	⚇	13.80	⚇
6	Siliqua/plant	182.70 – 343.40	236.27	○	236.27-289.84	⚭	289.85	⚭
7	Siliqua length (cm)	3.73 – 5.36	4.27	○	4.27-4.81	⊶	4.82	⊶
8	No. of seed/siliqua	10.60 – 14.56	11.92	○	11.92-13.24	⚲	13.25	⚲
9	1000-seed weight (g)	3.40 – 5.23	4.01	○	4.01-4.62	⚬	4.63	⚬
10	Seed yield/plant (g)	11.93 – 32.83	18.86	○	18.86-5.79	⚬	25.80	⚬

Table 4: Distribution of Indian mustard genotypes into different complex

Complex	Number of genotypes	Composition of clusters	Distribution
I	08	PR-21-37, PRL-21-22, PRL-21-21, PR-21-43, PRE-21-06, PR-21-38, PR-21-02, PRL-19	Low seed yield/plant and low siliqua/plant
II	04	PRL-21-17, PRE-21-07, CS-58-49, PRE-21-05	Moderate seed yield/plant and low siliqua/plant
III	13	PRE-21-03, PRE-21-11, PRE-21-15, PRE-21-12, PRE-21-01, PRE-21-9, PM0031-48, PRE-21-14, PRL-21-27, PR-21-36, PRL-21-23, PRE-21-41, Rohini-47	Low seed yield/plant and moderate siliqua/plant
IV	08	PRE-21-04, PRE-21-10, PRL-21-18, PR-21-44, PR-21-39, PR-21-45, PR-21-33, PRL-21-25	Moderate seed yield/plant and moderate siliqua/plant

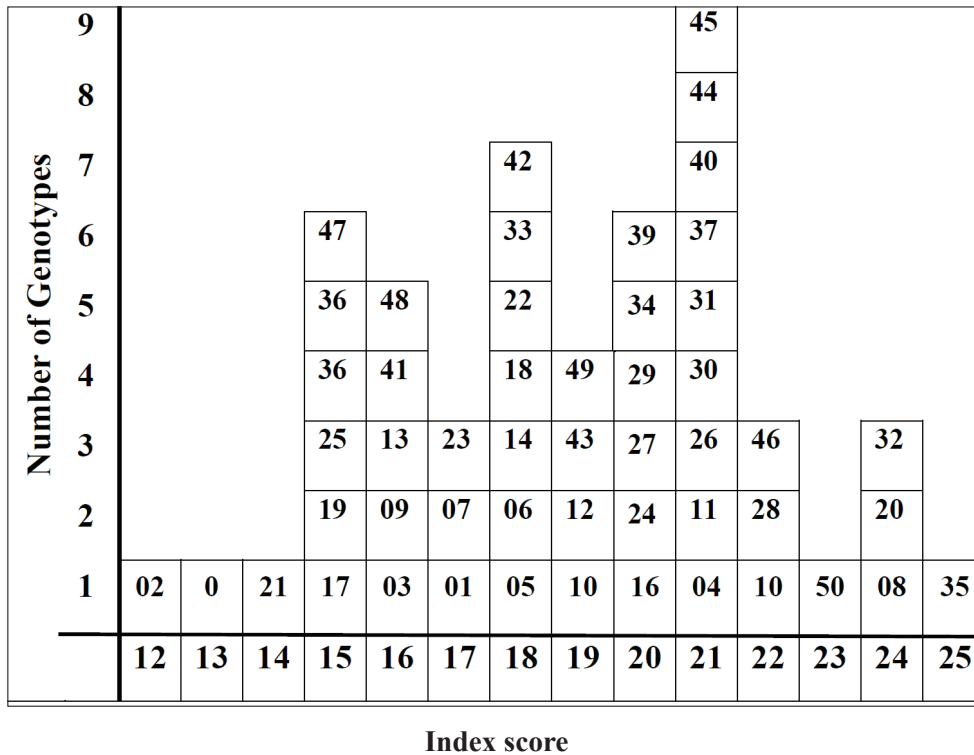| Complex	Number of genotypes	Composition of clusters	Distribution
V	02	PR-21-34, Giriraj-46	High seed yield/plant and moderate siliqua per plant
VI	05	PRL-21-16, PRL-21-20, PRL-21-29, PRL-21 -30, PR-21-31	Low seed yield/plant and high siliqua/plant
VII	05	PRE-21-08, PR-21-40, PRL-21-28, PRL-21-24, CS-60-50.	Moderate seed yield/plant and high siliqua/plant
VIII	05	PRE-21-13, PR-21-32, PRL-21-26, PR-21-35, PR-21-42	High seed yield/plant and high siliqua/plant
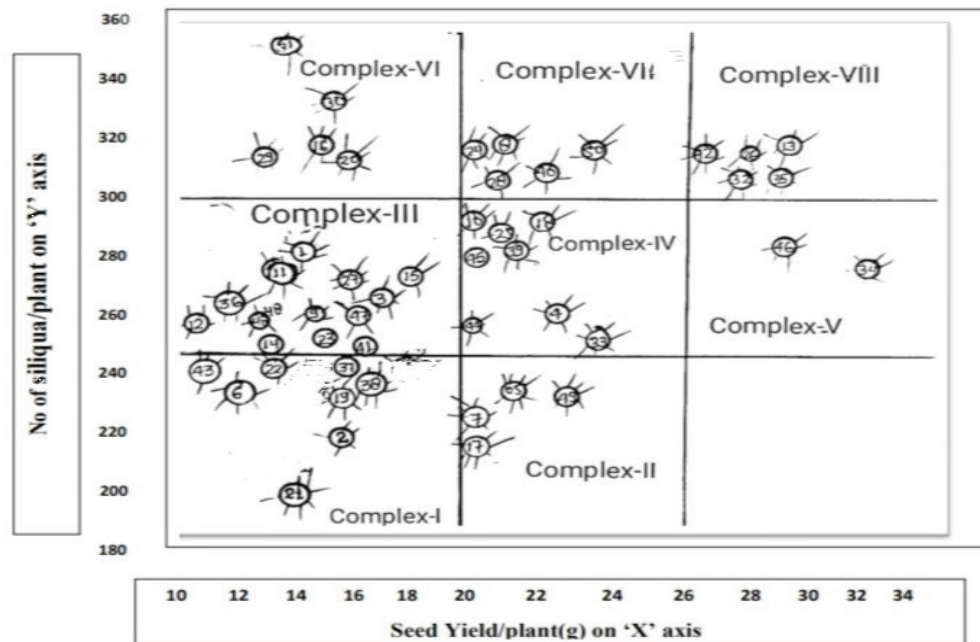
Fig 1: Scattered diagram of metroglyph analysis of Indian mustard germplasms

The pictorial representation of germplasms (Figure 2) derived from the scattered plot of metroglyph analysis serves as a measure of relative genetic distance among the germplasms. The metroglyph analysis proves to be valuable for the preliminary classification of germplasm and its divergence study. When coupled with an index score, metroglyph analysis becomes a powerful tool for preliminary screening and classification of genotypes, especially when dealing with a large number of germplasm lines. Germplasms with highly diverse characteristics, based on their total index score, include PR-21-35, PR-21-42, CS-60-50, Giriraj-46, and PR-21-45, while germplasms with less diversity include PRE-21-2, PR-21-21, PRE-21-3, PRE-21-, and PR-21-19. The scoring procedure employed in metroglyph analysis can be utilized in the preliminary screening of a large number of genotypes to select germplasm with a desirable combination of various characters influencing seed yield per plant and the number of siliquae per plant in index scores.

Number of Genotypes	12	13	14	15	16	17	18	19	20	21	22	23	24	25
9										45				
8										44				
7							42			40				
6			47				33		39	37				
5			36	48			22		34	31				
4			36	41			18	49	29	30				
3			25	13	23	14	43	27	26	46			32	
2			19	09	07	06	12	24	11	28			20	
1	02	0	21	17	03	01	05	10	16	04	10	50	08	35

Index score

Fig. 2: Pattern of distribution of 50 genotypes of Indian mustard

The findings of the present study suggest that genotypes from distinct groups can be utilized in crossing programs to maximize variability and identify favourable combinations of traits. Additionally, the study contributes to assessing the diversity of various characters among the 50 genotypes. This information is crucial for identifying and planning crosses that may yield desired results. Such studies play a key role in arranging cross-combinations to optimize outcomes, providing valuable insights for efficient breeding strategies.

The studies revealed that these genotypes with high index scores and fell into different clusters can be crossed to have maximum variability of good combinations of characters. The information could help identify and engineer the crosses that could be attempted to obtain desired results. This kind of study will help with the arrangement of cross combination for the best possible results (Bahadra and Akhtar, 1991; Sachan *et al.*, 2003; Khan *et al.*, 2005).

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