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Genetic diversity analysis using Mahalanobis' D^2 in Mungbean (*Vigna radiata* L.)

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Abstract

The present to assess Mahalanobis' D^2 statistic to quantify the genetic divergence among 50 distinct mungbean crosses based on 14 yield and yield-contributing traits. The field experiment was carried out at Research farm, Genetics and Plant Breeding, AKS University Satna (M.P). The analysis grouped the genotypes into six distinct clusters, confirming the presence of substantial genetic variability. The analysis of character contribution revealed that the genetic divergence was overwhelmingly driven by specific yield components. Number of Pods per Plant emerged as the paramount factor, single-handedly contributing a commanding to the total variation. This was followed by Number of Clusters per Plant and Biological Yield. These three traits collectively accounted for over of the observed divergence, underscoring their critical importance in genetic selection. Cluster mean analysis identified Cluster 2 as genetically superior, exhibiting the highest mean values for crucial traits like Seed Yield per Plant and Number of Pods per Plant. Crucially, the maximum inter-cluster distance (113.12) was recorded between Cluster 2 and Cluster 4 (characterized by late flowering and heavy seed weight). This high divergence suggests that hybridization utilizing genotypes from these two specific clusters offers the best strategy for maximizing heterosis, releasing genetic variability, and developing high-yielding, genetically distinct varieties in the mungbean breeding program.

Keywords: Divergence, clustering, mungbean, crosses

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek), popularly known as green gram, is a prominent self-pollinated legume believed to have originated in the Indian subcontinent or the Indo-Burmese region, where its cultivation dates back to at least 1500-1000 BC (Sastri and Mehra, 1973; Fuller, 2007). Green gram belongs to the family Leguminosae (Fabaceae), subfamily Papilionaceae, and has a diploid chromosome number of 2n = 22. This short-duration grain legume exhibits wide adaptability and is now an economically important crop extensively cultivated throughout South Asia (India, Pakistan. Bangladesh), Southeast Asia (Thailand, Vietnam, Myanmar, Indonesia), and East Asia (China). Its historical origin is supported by the works of pioneers in crop origin, including Decandole, Vavilov, and Zukoveskij. Green gram is considered one of the most important pulse crops in India, ranking third in production after Bengal gram (*Cicer arietinum*) and red gram (*Cajanus cajan*). It is versatile, being mainly utilized in making dhal, curries, soup, sweets, and snacks. The food value of mungbean lies in its high and easily digestible protein. The seeds typically contain approximately 22-28% protein, 1.0-1.5% oil (lipid), 3.5-4.5% crude fiber, 4.5-5.5% ash, and 62-65% carbohydrates on a dry weight basis. Recent research also highlights the presence of bioactive compounds, including polyphenols (like vitexin and isovitexin), which confer health benefits such as antioxidant, anti-inflammatory, and antihypertensive properties (Ganesan and Xu, 2018). The quantification of genetic diversity through biometrical procedures such as Mahalanobis D^2-statistics has made it possible to choose genetically diverged parents. The D^2 statistics also measure the degree of diversification and determine the relative proportion of each component character to the total divergence. The divergence analysis has a definite role to play in an efficient choice of divergent parents for hybridization to exploit maximum heterosis.

Material and Methods

The field experiment was carried out at Research farm, Genetics and Plant Breeding, AKS University Satna (M.P) In terms of geography and fertility, the experimental region was relatively uniform. The experimental material consisted of 67 genotypes received from the Research farm, Genetics and Plant Breeding, AKS University Satna (M.P). These genotypes were planted in a randomised complete block design with three replications. Table 3.1 includes information about these genotypes. The experimental material consisted of 67 genotypes received from the Research farm, Genetics and Plant Breeding, AKS University Satna (M.P). These genotypes were planted in a randomised complete block design with three replications. Table 3.1 includes information about these genotypes. The comprehensive two-season Mungbean research program begins in *Kharif* 2024-25. This stage concludes with a field experiment employing the Line × Tester mating design involving 12 Purelines selected as lines and 5 Testers along with 50 crosses (Table1). Data on following traits will be recorded on five randomly selected competitive plants from each plot. Observations of flowering and maturity will be recorded on plot basis as per the Mungbean descriptors developed by IBPGR and ICRISAT (1993). Standard package of practices will be adopted to raise the good crop. List of quantitative characters Days to 50% Flowering, Number of Primary Branches per Plant, Number of Secondary Branches per Plant, Days to Maturity, Plant Height (cm), Number of Clusters per Plant, Number of Pods per Cluster, Number of Pods per Plant, Number of Seeds per Pod, Pod Length (cm), 100 Seed Weight, Biological Yield per Plant (g), Harvest Index (%) and Seed Yield per plant.

The statistical method developed by P.C. Mahalanobis in 1928 is a numerical approach used for measuring genetic divergence based on collected data of different characters. This method is employed to analyze and quantify the genetic distance between individuals or populations. The intra-cluster distances was calculated formula used was ∑iDi2/n, where ∑iDi2 represented the sum of distances between all possible combinations (n) of the populations included within a cluster. This formula allowed for the computation of the average squared distances within a cluster, providing a measure of the intra-cluster variation.

Contribution of individual character towards divergence

In all the combinations each cluster was ranked on the basis of its combination towards divergence between two entries ($di = Yt^i - Yt^j$). Rank one is given to the highest mean difference and rank 'p' to the lowest difference where 'p' is the total number of characters. Percentage contribution of each character (X) towards genetic divergence was calculated using the formula:

$$X = \frac{N \times 100}{M}$$

where,

N = Number of genotype combinations where the character was ranked first.

M = All possible combinations of number of genotypes.

Table 1: List of 50 F1 hybrids Crosses

S. No	Crosses	S. No	Crosses
1	IC121301 X M1	26	PPU-911 X M5
2	IC121301 X M5	27	PPU-911 X M4
3	IC121301 X M4	28	PPU-911 X M3
4	IC121301 X M3	29	WGG-42 X M1
5	IC121301 X M2	30	WGG-42 X M3
6	IC-314694 X M3	31	WGG-42 X M4
7	IC-314694 X M2	32	WGG-42 X M5
8	IC-314694 X M1	33	IPM409-4 X M2
9	IC-314694 X M5	34	IPM409-4 X M1
10	IC-314523 X M3	35	IPM409-4 X M3
11	IC-314523 X M1	36	PS-16 X M5
12	IC-314523 X M2	37	PS-16 X M4
13	IC-314523 X M5	38	PS-16 X M1
14	IC-314523 X M4	39	PS-16 X M3
15	PM-6 X M2	40	IPM410-3 X M1
16	PM-6 X M5	41	IPM410-3 X M3
17	PM-6 X M3	42	IPM410-3 X M5
18	PM-6 X M1	43	PDM-139 X M5
19	BWCD-10 X M4	44	PDM-139 X M4
20	BWCD-10 X M2	45	PDM-139 X M3
21	BWCD-10 X M1	46	PDM-139 X M1 (IN LINE-19)
22	BWCD-10 X M3	47	IPM512-1 X M5 (IN LINE-20)
23	BWCD-10 X M5	48	IPM512-1 X M1 (IN LINE-21)
24	PPU-911 X M1	49	IPM512-1 X M3 (IN LINE-22)
25	PPU-911 X M2	50	IPM512-1 X M2 (IN LINE-24)

Table 2: List of parental lines

Female Line	Male Line
(F-1) PDM-139	(Male-1) NDU-16
(F-2) PS-16	(Male -2) PGRV-95016
(F-3) PPU-911	(Male -3) IC-76499
(F-4) IPMS-12-1	(Male -4) IPM205-7
(F-5) BMWCD-31	(Male -5) PANT-M-8
(F-6) BWCD-10	-
(F-7) WGG-42	-
(F-8) IC-121301	-
(F-9) PM-6	-
(F-10) IC-314694	-
(F-11) IC-314523	-
(F-12) IPM410-3	-

Results

Mahalanobis' D^2 analysis, which provides a quantitative measurement of the link between geographic and genetic diversity based on a generalized distance, is a useful tool for evaluating the genetic differences across biological populations (Mahalanobis). By applying Tocher's approach to convert character-based correlated means into conventional uncorrelated means, the D^2 values are obtained. In this experiment, yield and its related variables were used to measure the genetic divergence across 50 distinguish crosses of mungbean. The analysis of character contribution, based on the frequency of being ranked first in the D^2 statistic, clearly identifies the key traits driving genetic divergence. The trait Number of Pods per Plant emerges as the paramount factor, single-handedly contributing a commanding 65.98% to the total genetic variation. This overwhelming dominance underscores its essential role in distinguishing the genotypes. Following this are Number of Clusters per Plant, contributing a significant 15.86%, and Biological Yield (g), which accounts for 12.41%.

These three yield-related components collectively command over 94% of the observed divergence, suggesting that the primary genetic differences reside in the yield structure of the evaluated germplasm. Conversely, a large group of seven traits, including Days to Flowering, Days to Maturity, Primary Branches, and Number of Seeds per Pod contributed 0%, indicating a high degree of genetic similarity or low variability for these characters across the genotypes. Minor contributors, such as 100 Seed Weight (g) and Harvest Index (%), accounted for less than 3% combined. For a successful breeding program, selection efforts must be heavily concentrated on improving the three major contributing traits to effectively exploit heterosis and create genetically distinct superior varieties (Table 3&4) These results were consonance by the findings of Fetemeh *et al.* (2012) [2] and Divyaramakrishnan *et al.* (2014) [1].

Table 3: Contribution table for various traits

Source	Times Ranked 1st	Contribution %
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	69	0.1586
7	0	0
8	287	0.6598
9	0	0
10	3	0.0069
11	12	0.0276
12	54	0.1241
13	8	0.0184
14	2	0.0046

Table 4: Contribution of individual characters towards genetic divergence

Cluster	Number of crosses	Crosses
I	7	27, 28, 12, 10, 26, 23, 1
II	7	15, 19, 4, 13, 14, 7
III	7	17, 20, 24, 21, 3, 5, 25
IV	6	11, 16, 18, 6, 29, 22
V	3	2, 9, 3
VI	1	8

Cluster Distances and Mean Values

The analysis of genetic divergence using Mahalanobis' statistic grouped the genotypes into 6 distinct clusters, and the resulting intra- and inter-cluster distances, as well as the cluster means, are interpreted below.

Intra- and Inter-Cluster Distances

The average value of intra- and inter-cluster distances are given in the table. Maximum differences among the genotypes within the same cluster (intra-cluster) were shown by Cluster 4 (31.62), followed by Cluster 3 (28.27), Cluster 1 (24.40), and Cluster 2 (22.54). Cluster 6 (0.00) showed the lowest (zero) intra-cluster distance, indicating all genotypes in this cluster are genetically identical for the traits studied. The inter-cluster distance was higher than the intra-cluster distance, indicating the presence of wide genetic diversity among the genotypes under study. Diversity among the clusters varied from 34.27 to 113.12 inter-cluster distances. Clusters 2 and 4 showed the maximum inter-cluster distance (113.12), followed by Clusters 2 and 6 (111.46). The lowest inter-cluster distance was noticed between Clusters 1 and 5 (34.27), followed by that between Clusters 1 and 3 (40.59) (Table 5). The maximum inter-cluster distance between Cluster 2 (highest mean for SYPP and NPP) and Cluster 4 (highest mean for DF and 100 SW) suggests that hybridization between genotypes belonging to these two clusters would likely result in the maximum heterosis and release of genetic variability. Findings of Goyal *et al.* (2021) [4] showed highest inter cluster distance in cluster II and cluster IV. Makram *et al.* (2018) [7] and Jadhav *et al.* (2021) [6] showed similar result supporting present study.

Table 5: Inter and Intra Cluster distances

Cluster		Group. 1	Group. 2	Group. 3	Group. 4	Group. 5	Group. 6
Group.	1	24.4	60.59	40.59	71.09	34.27	109.87
Group.	2	60.59	22.54	77.42	113.12	44.26	111.46
Group.	3	40.59	77.42	28.27	46.93	41.53	103.03
Group.	4	71.09	113.12	46.93	31.62	75.83	108.53
Group.	5	34.27	44.26	41.53	75.83	21.43	100.11
Group.	6	109.87	111.46	103.03	108.53	100.11	0

Cluster Mean Analysis

The cluster mean analysis of the 14 traits revealed distinct differences among the clusters, facilitating the identification of those with favourable values for specific characteristics. Cluster 2 exhibited the most promising combination of yield and yield-contributing traits, showing the highest mean values for Number of Pods per Cluster (6.85), Number of Pods per Plant (179.62), Harvest Index (%) (99.76%), and Seed Yield per Plant (g) (35.29 g), though it recorded the lowest 100 Seed Weight (g) (2.90 g). Conversely, Cluster 6 possessed the highest mean for Number of Clusters per Plant (81.43), Number of Seeds per Pod (11.37), and Pod Length (cm) (7.17 cm), but demonstrated the lowest means for Number of Primary Branches per Plant (5.47), Days to Maturity (65.67), Number of Pods per Cluster (4.10), Biological Yield per Plant (g) (0.06 g), and Seed Yield per Plant (g) (21.33 g). Cluster 4 was characterized by late flowering and maturity, showing the highest means for Days to 50% Flowering (42.13 days), Days to Maturity (70.07), and 100 Seed Weight (g) (3.65 g), along with the lowest means for Number of Secondary Branches per Plant (6.17), Number of Clusters per Plant (18.01), Number of Pods per Plant (95.00), and Number of Seeds per Pod (10.20) (Table 6). Cluster 3 had the highest means for Number of Primary Branches per Plant (7.26) and Plant Height (cm) (65.76 cm). Cluster 5 displayed the lowest mean for Days to 50% flowering (33.33 days) and Plant Height (cm) (45.41 cm), but the highest mean for Number of Secondary Branches per Plant (9.88). Finally, Cluster 1 was highest for Biological Yield per Plant (g) (0.32 g). Cluster 2 showed the highest mean for the primary yield traits SYPP (35.29 g), NPP (179.62), and NPPC (6.85), identifying its members as superior for direct yield improvement. Cluster 4 (high DF and 100 SW) or Cluster 3 (high NPBPP and PH) are the most divergent groups and can be used as donor parents in crosses with Cluster 2 for maximizing genetic recombination. Rahangdale *et al.* (2021 & 2023) [8, 10], Gupta *et al.* (2021) [5] reported similar result and these results were also supported by the findings of Gokulakrishnan *et al.* recorded highest mean value of yield related traits in cluster IV and V.

Table 6: Cluster means for various yield and yield attributing traits

		DF	NPBPP	NSBPP	DM	PH	No. of c/p	No. of p/c
Group.	1	37.95	6.23	6.91	69.86	48.82	27.61	6.07
Group.	2	38.67	6.2	7.11	69.5	50.77	42.19	6.85
Group.	3	37	7.26	7.08	69.54	65.76	22.96	5.91
Group.	4	42.13	5.63	6.17	70.07	46.53	18.01	5.73
Group.	5	33.33	6.56	9.88	67.78	45.41	32.18	6.09
Group.	6	35.33	5.47	7.27	65.67	52.4	81.43	4.1
		No. of p/p	**No. of s/p**	**PL**	**100 SW**	**BYPP**	**HI**	**SYPP**
Group.	1	149.13	11.3	6.22	3.08	0.32	98.92	29.38
Group.	2	179.62	10.8	6.41	2.9	0.08	99.76	35.29
Group.	3	126.25	10.51	5.95	3.2	0.11	99.54	24.9
Group.	4	95	10.2	6.16	3.65	0.09	99.59	22.59
Group.	5	151.78	10.78	6.71	2.98	0.08	99.69	26.32
Group.	6	109.23	11.37	7.17	3.29	0.06	99.72	21.33

Note: Days to 50% Flowering (DF), Number of Primary Branches per Plant (NPBPP), Number of Secondary Branches per Plant (NSBPP), Days to Maturity (DM), Plant Height (cm), Number of Clusters per Plant (NCPP), Number of Pods per Cluster (NPPC), Number of Pods per Plant (NPP), Number of Seeds per Pod (NSP), Pod Length (cm), 100 Seed Weight, Biological Yield per Plant (g) (BYPP), Harvest Index (%) (HI) and Seed Yield per plant (SY)

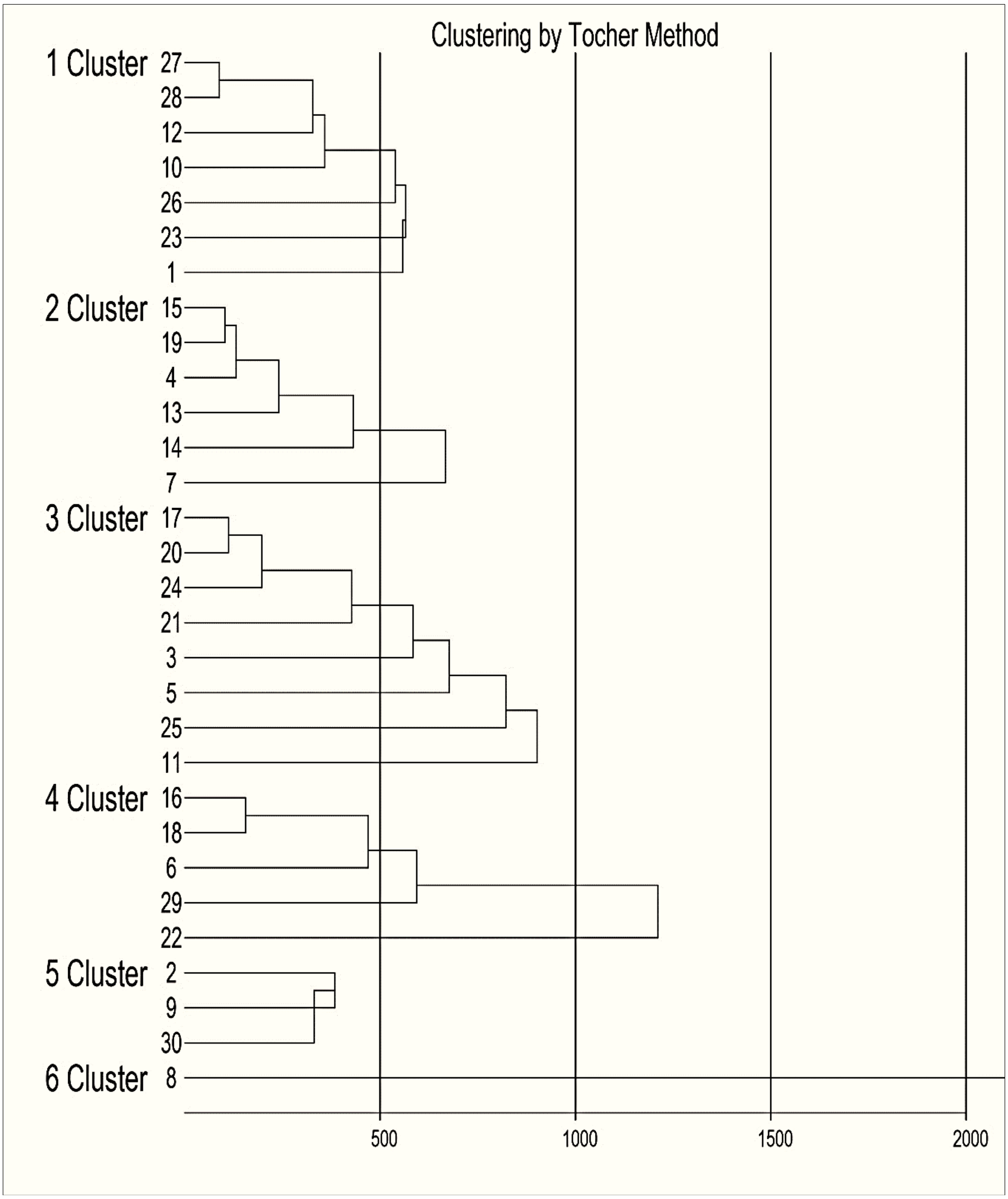

Fig 1: Clustering among various crosses

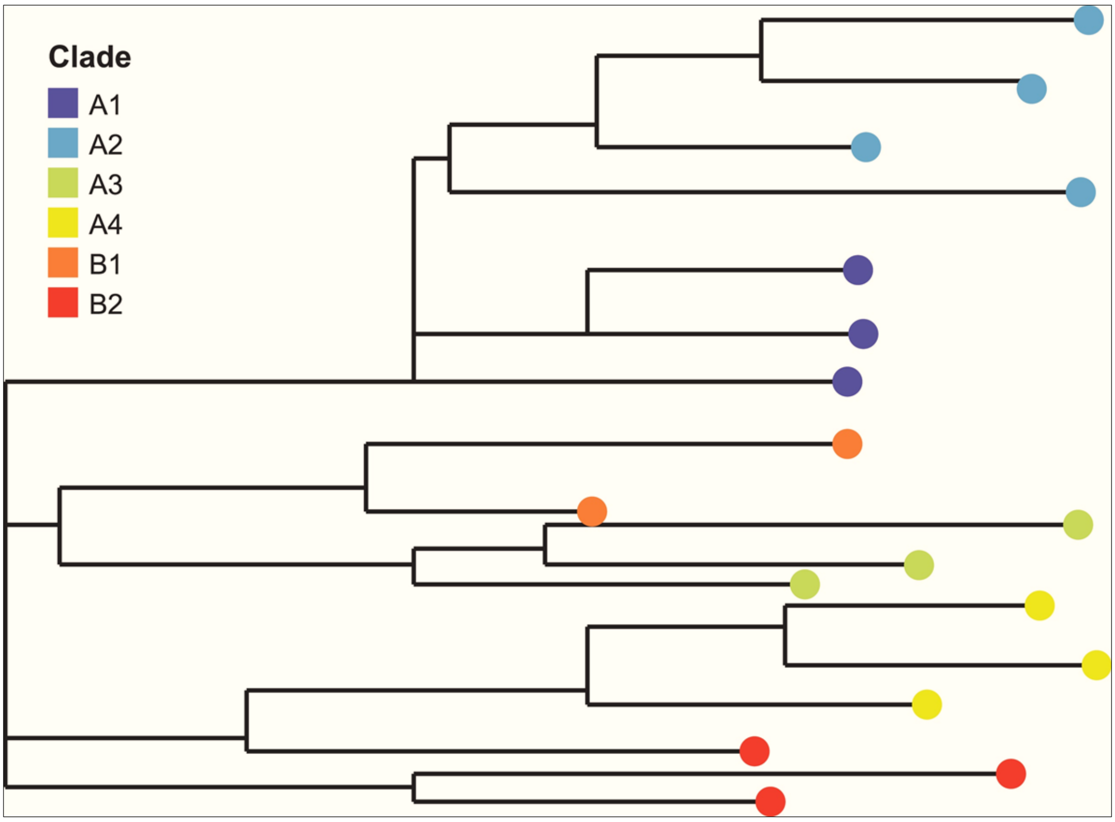

Fig 2: Visual representation of various cluster

Conclusion

The present investigation entitled "Genetic diversity analysis using Mahalanobis' D^2 in mungbean (*Vigna radiata* L.)" was carried out to estimate the significant difference among 50 crosses derived from 13 lines and 5 tester. Mahalanobis' D^2 analysis of 50 mungbean crosses grouped the genotypes into 6 distinct clusters, indicating substantial genetic divergence. Number of Pods per Plant was the overwhelmingly dominant trait, contributing 65.98% to the total genetic variation, followed by Number of Clusters per Plant (15.86%). These yield-related traits collectively account for over 94% of the divergence. Cluster 2 showed the highest mean for key yield components (Seed Yield per Plant and Number of Pods per Plant), identifying it as genetically superior. The maximum inter-cluster distance (113.12) was found between Cluster 2 and Cluster 4, making them the ideal parental combination for hybridization to exploit maximum heterosis and create new, superior varieties.

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