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Multivariate Analysis in Green Gram [*Vigna radiata* (L.) Wilczek] for Assessment of Genetic Diversity using D^2 Statistics

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ABSTRACT

Background: Greengram [*Vigna radiata* (L.) Wilczek] is one of the most significant edible pulse crop with a balanced nutritional profile that contains significant levels of bioactive compounds, dietary fiber, vitamins, minerals and protein. Genetic diversity is a fundamental requirement in any program aimed at enhancing yield through genetics. By effectively hybridizing genetically diverse parents, a significant heterotic response can be achieved in F1 hybrids, along with a wide range of variability in subsequent generations.

Methods: In the present study 36 genotypes of green gram were subjected to D^2 analysis for 13 varied characters. The experiment was conducted at experimental fields of Department of Genetics and Plant Breeding, Phagwara, Punjab during the month of March, 2024 in a randomized complete block design (RCBD) having three replications.

Result: Ten clusters were formed in which 16 genotypes were grouped into cluster I which was the largest cluster followed by cluster II (6), IV (3), cluster III, V, VI and VII had 2 genotypes while cluster VIII, IX and X accommodated only one genotype per cluster. Average intra-cluster and inter-cluster distance depicted that cluster VIII, IX and X have no intra-cluster distance as they possessed only one genotype each. The maximum intra-cluster distance was observed in cluster II (149.80) whereas, maximum inter-cluster distance was observed among the clusters I and V (1595.11). The least inter-cluster distance was exhibited among the clusters VI and X (219.75). Contribution percentage for genetic divergence ranged from 0.26 to 49.79%. Selection of desirable parents with these traits for improvement will give fruitful results as these traits have the maximum diversity.

Key words: Clusters, Genetic diversity, Inter-cluster distance, Intra-cluster distance, Selection.

INTRODUCTION

Greengram [*Vigna radiata* (L.) Wilczek] is one of the most significant edible pulse crops with chromosome number 22 (Karpechenko,1925). It is an annual short duration and is predominantly self-pollinated crop. This well-known and prehistoric pulse crop is native to Southeast Asia and is a member of the Fabaceae family (Rehman *et al.,*2009). This small, round legume, sometimes referred to as golden gram or mung bean, is widely grown and consumed throughout the world. It is a crop with a balanced nutritional profile that contains significant levels of bioactive compounds, dietary fiber, vitamins, minerals and protein (Gan *et al.,* 2017). To top it off, it is known to have more iron and folate than most other legumes (Keatinge *et al.,* 2011). There are also notable concentrations of essential amino acids including lysine, isoleucine, phenylalanine and leucine (Lambrides and Godwin, 2007). It is extensively treasured in India, especially by the country's large vegetarian population, because it offers an abundant supply of high-quality, readily digestible protein. It supplies high quality protein (22-24%) (Rahim *et al.,* 2010). Greengram has become a highly profitable short-duration grain legume crop (Kumari *et al.,* 2023) and due to its many desirable traits, such as increased flexibility, relatively drought tolerant, lower input requirements and the ability to improve soil fertility by fixing atmospheric nitrogen with the help of rhizobium.

Genetic diversity is a fundamental requirement in any program aimed at enhancing yield through genetics (Salman *et al.,* 2023). By effectively hybridizing genetically diverse parents, a significant heterotic response can be achieved in F1 hybrids, along with a wide range of variability in subsequent generations. Mahalanobis's D^2 statistic is

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a powerful tool for quantifying the level of variability at the genotype level. The significance of multivariate analysis has been strongly emphasized (Murty and Arunachalam, 1966). Several researchers have explored genetic diversity, clustering patterns, the relative contribution of different traits to divergence and the effectiveness of selection.

In this crop, the D^2 multivariate analysis technique has been effectively applied to choose divergent genotypes in order to take advantage of heterosis and to combine higher frequency of desired gene in a strain (Patil *et al.,* 2001). Breeders are able to choose appropriate genotypes with a broad genetic background and use them to start a breeding program by using knowledge of genetic diversity in conjunction with yield contributing characteristics. This will assist in selecting strains that may yield better segregants and are suitable for hybridization. Considering the aforementioned things, current research work was carried out to analyze 36 genotypes of green gram based on various quantitative traits utilizing multivariate techniques for screening elite genotypes which can be used as a parent in future hybridization programs.

MATERIALS AND METHODS

The experiment was conducted at experimental fields of Department of Genetics and Plant Breeding, Phagwara, Punjab during the month of March, 2024 in a randomized complete block design (RCBD) with three replications. Row spacing was kept at 45 cm and plant spacing was kept at 30 cm apart. All the recommended cultural practices were followed during crop raising. Thirty-six genotypes were analyzed for the variation and data were taken on 5 randomly selected competitive plants for each entry for thirteen traits *viz.,* days to 50% flowering, plant height, number of primary branches, days to maturity, number of clusters per plant, number of pods per plant, number of pods per cluster, number of seeds per pod, seed index, pod length, seed yield per plant, biological yield and harvest index.

A genetic divergence study was performed on the replicated data using Mahalanobis' D^2 statistics (Mahalanobis, 1928) as explained by Rao (1952). By employing Tocher's technique and the D^2 statistics, the lines were divided into several clusters. The recorded data were analyzed to assess the genetic divergence using computer software Windostat 8.6 version.

Genetic diversity using D^2 analysis

One of the best methods for calculating the genetic distance between genotypes based on allelic frequencies at a sample of loci is the Mahalanobis (1928) D^2 statistic. By using the pivotal condensation method of inversion matrix, original variable means were converted to uncorrelated variables. The sum of squares of differences between the values of the associated transformed variables served as the basis for calculating the D^2-values between genotypes.

The mean deviation for each pair of combination

$$di = Yi^1 - Yi^2$$

Where,

Yi = Transformed variables i = 1, 2, 3, 4, 5 …….p were calculated.

D^2 = Calculated as sum of the squares of the deviations, *i.e.:*

$$D^2 = \sum (Yi^1 - Yi^2)^2$$

Where,

p = Number of characters.

For formation of clusters, the general criteria of grouping as suggested by Tocher were followed in the present study (Rao, 1952). The criterion for clustering was that, any two populations in the same cluster should show a smaller D^2 value than those belonging to different clusters. After formation of clusters, average intra- and inter-cluster distance values were calculated and their relationships were diagrammed.

Average intra-cluster and inter-cluster distances were measured as:

Average intra-cluster distance ($D = \sqrt{D^2}$)

$$D^2 = \frac{\sum D_i^2}{n}$$

Where,

$\sum D_i^2$ = Sum of distance between all possible combinations of the two clusters populations in cluster.

n = Number of populations in cluster.

Average inter-cluster distance ($D = \sqrt{D^2}$)

$$D^2 = \frac{\sum D_{ij}^2}{n_i \times n_j}$$

Where,

D_{ij}^2 = Sum of distance between all possible combinations of the two clusters.

n_i = Number of populations in cluster i.

n_j = Number of populations in cluster j.

The inter-cluster distance was calculated by measuring the distance between clusters I and II, between I and IV and so on. Likewise, one by one cluster was taken and their distances from each other were calculated.

RESULTS AND DISCUSSION

In our current investigation, by using Tocher method (Rao, 1952) 36 genotypes have been grouped into 10 clusters based on D^2 values which are presented in Table 1 and a cluster diagram has been presented in Fig 2. Sixteen genotypes were grouped into Cluster I which was the largest cluster followed by cluster II (6), IV (3), cluster III, V, VI and VII each containing 2 genotypes while cluster VIII, IX and X accommodated only one genotype per cluster. Similar type of clustering pattern was observed in green gram by Bindu *et al.* (2023); Gadakh *et al.* (2013), Mehandi *et al.* (2015) and Chaudhary *et al.* (2015) also reported the similar results of having mono-genotypic cluster.

Table 1: Assignment of genotypes into various clusters.

Cluster	Number of genotypes within a cluster	Name of genotype within a cluster
I	16	MH 934, ML 2056, IPM99-125 (Meha), Tilak moong, MH1451, MGG 347, MGG 296, MGG 351, WGG 37, KM11-586, TARM-2, COGG 1276, IPM 2-3, KM11- 582, Samrat
II	6	RMG 1004, MASCO-44, KM11-585, RMG 1091, ML 818, Pusa Baisakhi
III	2	RMG 1030, SML668
IV	3	MGG 348, ADT-3, SML 832
V	2	MGG 295, SMI 1115
VI	2	Virat Gold, IPM 2-3
VII	2	Gujarat Moong-4, JLM- 1754
VIII	1	KM11-587
IX	1	JLM-1748
X	1	JLM-1702

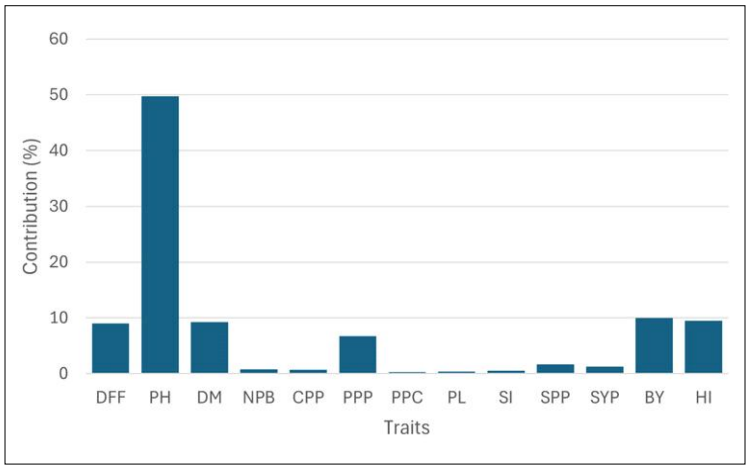

Fig 1: Contribution of traits towards genetic divergence.

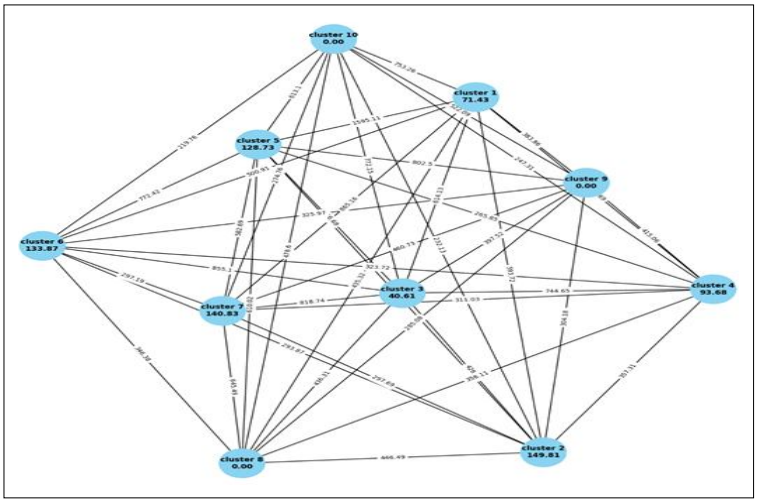

Fig 2: Cluster diagram depicting intra-cluster and inter-cluster distance.

Table 2: Cluster mean of 10 clusters for yield and yield attributing traits in green gram.

Clusters and traits	I	II	III	IV	V	VI	VII	VIII	IX	X
DFF	40.10	52.00	48.77	50.33	42.54	35.33	32.66	59.50	41.77	42.66
PH	68.13	33.50	43.77	36.33	40.95	42.66	52.2	60.63	33.74	55.66
DM	65.12	85.00	71.05	59.33	65.33	66.66	69.33	77.33	69.66	75.66
NPB	3.16	3.16	4.61	2.66	3.00	3.66	5.33	5.16	2.22	2.33
CPP	6.35	6.50	6.77	8.66	4.00	5.00	4.33	6.66	5.55	6.33
PPP	16.81	27.16	20.83	21.00	12.66	14.83	30.33	33.16	28.88	26.33
PPC	3.43	6.50	5.11	3.00	3.00	5.66	4.33	7.83	5.77	6.33
PL	8.42	6.38	6.87	7.33	8.33	6.50	6.33	7.28	7.81	8.20
SI	4.14	4.16	5.33	4.00	4.66	4.83	2.66	7.50	3.22	3.33
SPP	10.85	7.51	7.55	7.66	8.50	8.50	8.00	8.00	9.88	8.33
SYP	6.52	10.00	8.33	5.00	8.00	5.16	9.33	13.00	7.44	6.66
BY	42.52	24.00	44.11	31.33	37.83	30.83	42.66	41.16	25.00	22.66
HI	14.14	29.33	16.88	11.66	29.00	12.00	25.66	29.66	23.88	19.66

Cluster means for different traits

The cluster means for various yield attributing traits are presented in Table 2 and the comparison indicates Cluster I, VII and cluster VIII had better cluster means for more than a single trait. Similar findings were reported by Garg *et al.* (2017) and Jadhav *et al.* (2023).

Contribution of traits towards genetic divergence

The contribution of 13 characters towards genetic divergence is presented in Table 3 and Fig 1. Contribution percentage for genetic divergence ranged from 0.26 to 49.79%. It is revealed that plant height was the major contributor towards genetic divergence (49.79%). Similar result was found by Sridhar *et al.* (2022) and Kaur *et al.* (2023), followed by biological yield (9.96%), harvest index (9.53%), days to maturity (9.27%), days to 50% flowering (8.98%), number of pods per plant (6.76%), number of seeds per pod (1.69%), seed yield per plant (1.31%), number of primary branches (0.78), number of clusters per plant (0.73%), seed index (0.50%), pod length (0.36%) and the least contributor was number of pods per cluster (0.26%).

Intra-cluster and inter-cluster distance

Among the ten clusters formed, the intra-cluster distance ranged from 0 to 149.80 as depicted in Fig 2 and Table 4. The maximum intra-cluster distance was observed in the cluster II (149.80), followed by cluster VII (140.82), cluster VI (133.87), cluster V (128.72), cluster IV (93.67), cluster I (71.43), cluster III (40.00) and the intra-cluster distance for clusters VIII, IX and X was 0 because they had only one genotype in each.

Inter-cluster distance for these clusters ranged from 219.75 to 1595.11. Maximum inter-cluster distance was

Table 3. Contribution of yield and attributing traits towards genetic divergence.

Traits	Contribution (%)
DFF	8.98
PH	49.79
DM	9.27
NPB	0.78
CPP	0.73
PPP	6.76
PPC	0.26
PL	0.36
SI	0.50
SPP	1.69
SYP	1.31
BY	9.96
HI	9.53

DFF- Days to 50% flowering; PH- Plant height; DM- Days to maturity; NPB- Number of primary branches; CPP- Number of clusters per plant; PPP- Number of pods per plant; PPC- Number of pods per cluster; PL- Pod length; SI- Seed index; SPP- Seeds per pod; SYP- Seed yield per plant; BY- Biological yield; HI- Harvest index.

Table 4: Average intra-cluster and inter-cluster distance (D^2) among 10 clusters.

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
Cluster I	71.43	593.72	614.13	1026.69	1595.11	500.90	865.16	435.31	383.86	753.25
Cluster II		149.80	426.94	357.30	570.71	293.87	297.68	446.48	304.17	232.12
Cluster III			40.60	744.65	786.68	855.10	818.74	436.30	397.51	772.14
Cluster IV				93.67	265.85	323.71	311.03	356.10	415.06	247.31
Cluster V					128.72	772.41	582.69	610.02	802.50	613.10
Cluster VI						133.87	297.18	346.38	325.96	219.75
Cluster VII							140.82	645.49	460.72	274.76
Cluster VIII								0	285.07	478.6
Cluster IX									0	522.08
Cluster X										0

Bold figures indicate intra-cluster distance.

observed among the clusters I and V (1595.11), followed by cluster III and VI (855.10), cluster V and cluster IX (802.50), cluster III and V (786.68) cluster VII and cluster VIII (645.49) cluster II and cluster V (570.71). The least inter-cluster distance was exhibited among the clusters VI and X (219.75). The result was in accordance with the reports of Singh *et al.,* (2014), Rekha *et al.* (2015) and Sridhar *et al.,* (2022).

CONCLUSION

To deduce, genetic diversity is most likely influenced by plant height and on the basis of cluster means, cluster I, VII and cluster VIII had better cluster means for more than a single trait. The genotypes in these cluster depicted better mean values for more than single yield attributing traits such as days to 50% flowering, number of primary branches, pod length, number of seeds per pod, pods per plant, pods per cluster, seed index, seed yield per plant and harvest index. The maximum intra-cluster distance was observed in cluster II and the maximum inter-cluster distance was observed among clusters I and V. Hence, choosing the genotypes from these clusters for crossing programs will be rewarding and fruitful as they have many desirable traits.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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