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Studies on Genetic Divergence among Greengram (*Vigna radiata* L.) Germplasm Accessions

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ABSTRACT

The present study was conducted at the Agricultural Research Station, Madhira, Telangana, India during *rabi* (October–December), 2017–18 to evaluate 39 diverse greengram germplasm accessions. The experiment was laid out in RBD replicated twice for 8 quantitative traits to study the nature and magnitude of genetic diversity among the accessions by multivariate analysis such as cluster analysis with D^2 Statistics and principal component analysis. 3 principal components viz., PC I, PC II and PC III contributed about 85.45% of total variance for the genotypes studied. The genotypes were grouped into 8 distinct clusters of which cluster I is the largest with a maximum number (16) of genotypes followed by cluster II and cluster VI with 6 genotypes each. Genotypes IC-436735, IC-261261, WGG-42 representing mono genotypic cluster signifies presence of diversity for creating variability through hybridization. Highest intra cluster distance of 47.27 was recorded for cluster VI and highest inter-cluster distance of 252.46 was observed between cluster IV and VIII. Data on cluster means for various traits showed that the highest mean value for number of clustersplant^{-1}, number of pods plant^{-1} and seed yield plant^{-1} was recorded by cluster VII. Percent contribution revealed that plant height contributed the most (32.11%) followed by seed yield plant^{-1} (21.86%) for total genetic divergence. The genotypes in the clusters with maximum inter cluster distance may be used as potential parents for developing high yielding greengram cultivars.

KEYWORDS: D^2 statistics, genetic divergence, germplasm, principal component analysis

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1. INTRODUCTION

Vigna is a widely cultivated legume all over the world. Greengram (*Vigna radiata* L. Wilczek) is the most widely distributed and cultivated species among *Vigna* species. It is the 3rd most important pulse crop in India next to chickpea and redgram. This self-pollinating legume belongs to the family Fabaceae. It is a diploid crop having chromosome number 2n=22 and originated from the Indo-Burma region of the Hindustan center (Nayak et al., 2022). In tropical countries of the world, it is a pre-dominant source of protein for vegetarian people, generally considered as the "poor man's meat" (Reddy, 2009) and it is grown as *kharif* (both as sole and intercrop), *rabi* and *summer* crop (Shweta, 2013, Kaur et al., 2015). Greengram is a short duration crop well suited for rotation and mixed farming, being a low water requirement crop suitable for drought tolerant crop, well adapted to a wide range of soil and also improve soil physical properties and fertility due to the presence of root nodules (Wanga et al., 2017). The yield of green gram has not increased substantially due to poor agronomic management practices applied, inherent low yield potential of cultivars and susceptibility to viral disease like MYMV (Mohan et al., 2021, Patel et al., 2021). Since it is a highly self-pollinated crop, variation existing within or between the species or varieties becomes important (Bisht et al., 2005, Angamuthu et al., 2018, Mehandi, 2015). Genetic diversity present in the germplasm accessions is an important tool for any plant breeding program (Das and Barua, 2018). One of the key factors in the greengram improvement programme is the lack of genetic diversity in the primary gene pool (Rahman and Al-Mansur, 2009, Kumar et al., 2011, Panigrahi and Baisakh, 2014). Genetic diversity provides a good opportunity for plant breeders to develop new and improved cultivars with desirable characteristics (Tabasum et al., 2010, Kaur et al., 2015). Assessment of phenotypic diversity or characterization of morphological and agronomical characters is the important role for the selection of proper parents in the genetic improvement or plant breeding program (Abbas et al., 2010, Panigrahi and Baisakh, 2014)

The utilization of diverse cultivars helps to tap a significant amount of genetic variability for the trait based yield improvement in greengram. The success of any breeding program depends on the immensity of the genetic variability present in these characters in the selected genotypes (Sneha et al., 2020). Mohan et al. (2021) reported the selection criteria for realizing higher seed yield through interpreting associated traits of yield. Study of genetic diversity in genetic resources is a critical factor for breeders to better understand the evolutionary and genetic relationships among populations, to select germplasm in a more systematic and efficient way and to perform and develop strategies to incorporate useful diversity in their breeding programs (Lavanyaet al., 2008, Mehandi et al., 2015, Angamuthu et al., 2018).

The crosses between the parents with maximum genetic divergence are generally the most responsive for genetic improvement (Arunachalam, 1981). Genetically diverse parents with the purpose of combining desirable recombinant for specific trait improvement followed by appropriate selection in segregating generation in a self-pollinated crop like green gram would result in the development of better cultivars (Katiyar et al., 2009, Singh et al., 2014, Rekha et al., 2015, Patel et al., 2021). Multivariate statistics are very useful techniques to estimate genetic diversity and provide the most reliable evidence regarding the actual genetic distances between the tested genotypes. Among the techniques of multivariate, cluster analysis by means of the Mahalanobis generalized distance (D^2) statistics and principal component analysis are the most common techniques to estimate the variability of quantitative traits and to identify the superior genotypes (Sneha et al., 2020, Lavanya et al., 2008).

Knowledge about genetic diversity is an invaluable aid in crop improvement strategies. Cluster analysis in green gram would definitely help plant breeders to identify genetically diverse parents falling in different clusters (Kanavi et al., 2020) and the selection of genetically diverged parents is expected to throw superior and desirable segregants following crossing Jayamani and Sathya (2013). The present study of 39 greengram genotypes subjected to divergence studies by D^2 statistic.

2. MATERIALS AND METHODS

The experimental material for the present study consisted of 39 diverse germplasm accessions maintained at Agricultural Research Station, Madhira, was evaluated during *rabi* season (October–December), 2017–18. The farm is geographically located at 17°58' N Latitude, 78°44' East Longitude and an elevation of 189 m AMSL. The Soil is black clay vertisols. The germplasm lines were evaluated in a randomized block design with two replications. Each entry was planted in 2 rows of 4 m length with 30 and 10 cm spacing between and within rows. The recommended fertilizers doses of 16:50 kg ha^{-1} of N:P were applied. Observations on agro morphological quantitative traits viz., days to 50% flowering, days to maturity, plant height (cm), number of clusters $plant^{-1}$, number of pods $plant^{-1}$, 100 seed weight (g), seed yield $plant^{-1}$ (g) and seed yield (kg ha^{-1}) were recorded following standard procedures on randomly selected 5 plants $replication^{-1}$. Data on days to 50% flowering and days to maturity was noted on plot basis and the data was subjected to statistical analysis. Assessment of genetic

divergence was done using Mahalanobis D^2 statistic and the germplasm accessions were grouped into different clusters following Tocher's method as described using Generes statistical package.

Divergence was estimated by the multivariate analysis using Mahalanobis (1936) and D^2 statistic as described by Rao (1952). On the basis of D^2 values obtained, the variables were grouped into different clusters by employing Tocher's method (Rao, 1952). The percent contribution of each character to the total divergence was calculated by ranking each character on the basis of transformed uncorrelated values. Finally, the percent contribution for each character was calculated by taking the total number of ranks of all the characters to hundred. The data were analyzed statistically using the software WINDOSTAT, developed by INDOSTAT services Ltd. Hyderabad, India.

3. RESULTS AND DISCUSSION

The analysis of variance showed highly significant differences among the germplasm accessions for all the characters studied indicating the presence of considerable variability in the experimental material (Table 1).

Table 1: Analysis of variance for yield and yield components of greengram

Source of variation	d.f	Mean sum of squares							
		Days to 50% flowering	Days to maturity	Plant height (cm)	No. of clusters plant^{-1}	No. of pods plant^{-1}	100 seed weight (g)	Seed yield plant^{-1} (g)	Seed yield (kg ha^{-1})
Replications	1	0.05	0.82	0.30	0.01	2.73	0.16	1.82	116696.00
Genotypes	38	19.35**	14.14**	346.26**	5.85**	59.07**	0.58**	11.62**	726570.89**
Error	38	0.84	0.69	7.36	1.84	18.73	0.07	2.87	177958.07

**p=0.01

3.1. *Principal component analysis and grouping of genotypes*

Partitioning of total variance through principal component analysis showed that three principal components viz PC I, PC II and PC III contributed about 85.45% of total variance for the germplasm lines studied (Figure 1). These three PCs i.e. PC I, PC II and PC III contributed 49.67, 26.87 and 8.90% of total variance (Table 2). These results were in agreement with the findings of Mohan et al. (2021) and Mehandi et al. (2015). The results obtained from PCA were further corroborated by cluster analysis using UPGMC (Unweighted Pair Group Method using Centroids). The 39 greengram germplasm accessions were grouped into eight distinct clusters. Cluster I is the largest with a maximum number (16) of genotypes followed by cluster II and cluster VI with 6 genotypes each, cluster IV with 5 genotypes, cluster V with 3 genotypes and cluster III, cluster VII and cluster VIII with single genotype each (Figure 2). The results of D2 analysis helped to identify diverse accessions from the available germplasm lines for use in crop improvement programmes. The varieties of these clusters may be used as parents in the crossing programme to generate breeding material with high

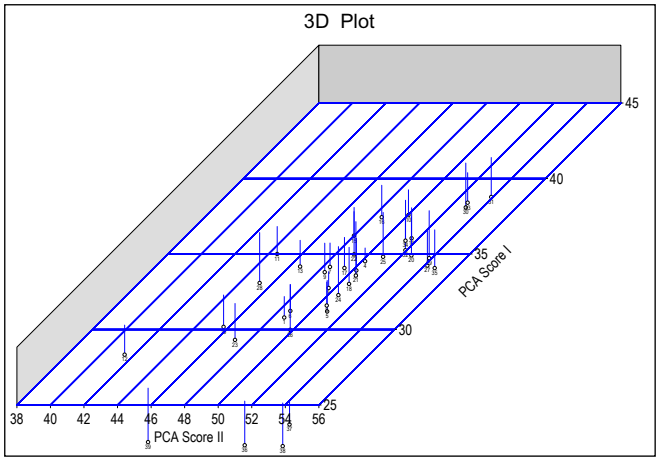

Figure 1: Principal component analysis diagram for greengram germplasm accessions

Table 2: Principal component analysis for yield component traits in greengram genotypes

		1 Vector	2 Vector	3 Vector
	Eigene value (Root)	662.082	358.267	118.677
	% Var. Exp.	49.673	26.879	8.904
	Cum. Var. Exp.	49.673	76.552	85.456
1	Days to 50% flowering	0.331	0.593	0.185
2	Days to maturity	0.276	0.557	0.218
3	Plant height (cm)	-0.829	0.358	0.109
4	Number of clusters plant^{-1}	-0.010	-0.029	-0.001
5	No. of pods plant^{-1}	-0.230	-0.115	0.694
6	100 seed weight (g)	0.255	-0.354	0.587
7	Seed yield plant^{-1} (g)	0.012	0.256	0.144
8	Seed yield (kg ha^{-1})	-0.094	-0.079	0.243

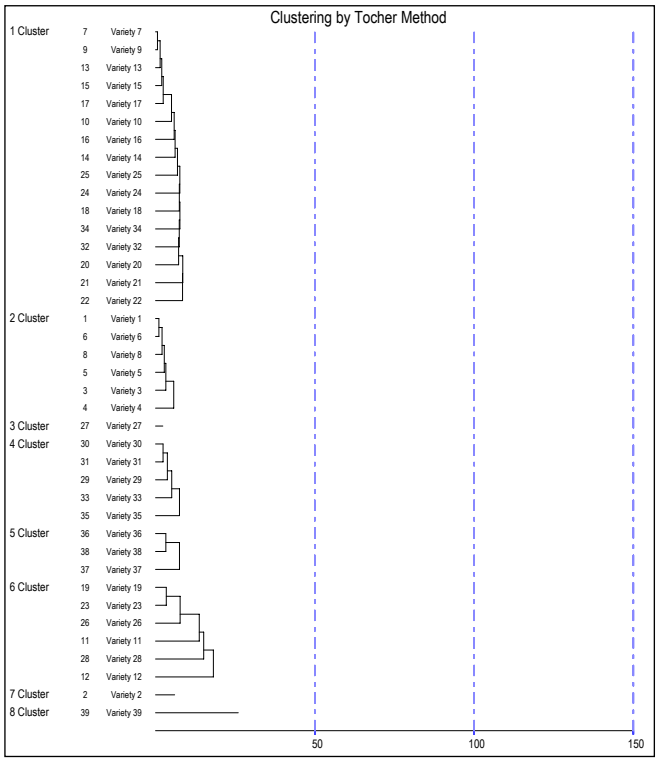

Figure 2: Dendrogram showing clustering of greengram germplasm accessions

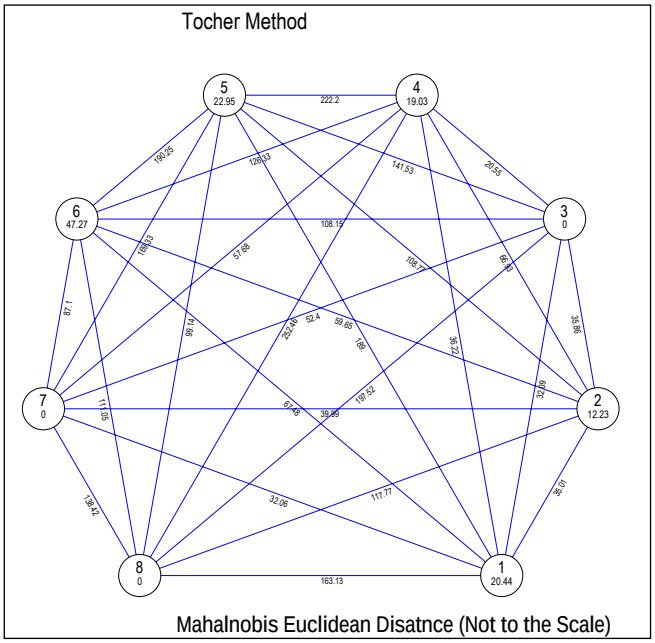

Figure 3: Mahalnobis euclidean distance

diversity.

3.2. *Cluster distances and cluster means*

The genetic divergence among the genotypes as indicated by intra and inter cluster distances for eight different clusters are presented in Table 3 (Figure 3). Highest intra cluster distance of 42.27 was recorded for cluster VI followed by cluster V (22.95), cluster I (20.44), cluster IV with 19.03 and cluster II with 12.23, thus suggesting that different genotypes included in these clusters might have different genetic architecture. The clusters with lowest intra cluster distance indicated that the genotypes resembled one another genetically and appeared to have evolved from a common gene pool (Patel et al., 2021). The inter cluster distance ranged from a minimum of 20.55 (between cluster III and IV) to a maximum of 252.46 (between cluster IV and VIII). The values of other inter cluster distances which are on the higher side are 222.20 (between cluster IV and cluster V), 197.52 (between cluster III and cluster VIII), 190.25 (between cluster V and cluster VI), 189.0 (between cluster I and cluster V), 169.33 (between cluster V and cluster VII) and 163.13 (between cluster I and VIII). Clusters III, VII and VIII are solitary clusters with intra cluster distance of 0.00. The perusal of mean in Table 3 revealed that inter-cluster distances were greater than intra-cluster distances revealing considerable amount of genetic diversity among the genotypes studied (Gadakh et al., 2013). Genotypes belonging to clusters with maximum intra-cluster distance are genetically more divergent and hybridization between divergent clusters is likely to produce wide variability with desirable segregants. The maximum amount of heterosis is expected from the

Table 3: Average Intra (in bold) and inter-cluster D^2 values of greengram genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII
I	20.44	35.01	32.09	36.22	189	67.48	32.06	163.13
II		12.23	35.86	66.93	108.77	59.65	39.99	117.77
III			0	20.55	141.53	108.15	52.4	197.52
IV				19.03	222.2	126.33	57.68	252.46
V					22.95	190.25	169.33	99.14
VI						47.27	87.1	111.05
VII							0	138.42
VIII								0

crosses with parents belonging to the most divergent clusters i.e., between cluster IV and VIII followed by parents in clusters of IV and clusterV and from parents in clusters III and cluster VIII. These results are in agreement with earlier reports of Sneha et al. (2020) and Mahalingam et al. (2018). The progenies derived from such crosses are expected to show wide variability, providing greater scope for isolating transgressive segregants in the advanced generations which can be used for selecting desirable genotypes for seed yield improvement in greengram (Panigrahi and Baisakh, 2014)

The cluster means for 8 traits included in the present study are shown in Table 4. The lowest mean value for days to first flowering and days to maturity was recorded by cluster VIII. The highest mean value for number of clusters plant^{-1}, number of pods plant^{-1} and seed yield plant^{-1} was recorded by cluster VII. Cluster VIII recorded the highest mean for 100 seed weight followed by cluster VII and cluster I. Hence crossing between these genotypes can be better exploited for genetic introgression studies (Sneha et al., 2020, Sen and De, 2017).

Table 4: Cluster mean among green gram genotypes

Cluster	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of clusters plant^{-1}	No. of pods plant^{-1}	100 seed weight (g)	Seed yield plant^{-1} (g)	Seed yield (kg ha^{-1})
I	41.25	65.88	22.13	6.38	19.87	3.79	7.97	1993
II	39.58	64.42	32.33	6.46	19.38	3.17	6.74	1682
III	43.5	69	29.5	4.9	15.95	3.14	5	1253
IV	45	69.2	20.84	7.45	24.21	3.55	8.62	2155
V	39.83	65.83	65.5	9.02	28.52	3.16	9.4	2350
VI	36.33	61.75	24.29	7.31	23.26	3.54	8.53	2132
VII	39.5	66.5	30.65	10.75	32.25	4.39	15.65	3905
VIII	36	60	52.5	7.1	22	4.95	11	2761

3.3. % contribution towards genetic divergence

The relative contribution of different traits included in the present study towards genetic divergence is shown in Table 5. Plant height contributed the most (32.11%), followed by seed yield plant^{-1} (21.86%), seed yield (kg ha^{-1}) (15.92%), number of pods plant^{-1} (14.17%), 100 seed weight (13.22%), days to 50% flowering (0.53%), number of clusters plant^{-1}(0.95%) and days to maturity (1.21%). The grouping of germplasm lines based upon their genetic divergence into different clusters is shown in Table 6. This information can also be used to assess the genetic divergence among the genotypes for framing an effective breeding programme for selection of parents for yield gain in greengram genotypes under study. These results are in agreement with earlier

Table 5: Relative contributions of yield and yield components to genetic diversity in greengram

Character	Times ranked 1st	Contribution %
Days to 50% flowering	4	0.53
Days to maturity	9	1.21
Plant height (cm)	238	32.11
Number of clusters plant^{-1}	7	0.95
Number of pods plant^{-1}	105	14.17
100 seed weight (g)	98	13.22
Seed yield plant^{-1} (g)	162	21.86
Seed yield (kg ha^{-1})	118	15.92

Table 6: Clustering pattern and grouping of greengram accessions

Cluster	No. of accessions	Genotypes
I	16	IC-436515, IC-436534, IC-436568, IC-436575, IC-436631, IC-436541, IC-436624, IC-436571, IC-436714, IC-436707, IC-436636, IC-436865, IC-436823, IC-436653, IC-436660, IC-436672
II	6	IC-249568, IC-282136, IC-436528, IC-282091, IC-261274, IC-282074
III	1	IC-436735
IV	5	IC-436781, IC-436797, IC-436763, IC-436827, IC-436921
V	3	IPM-02-14, MGG-347, MGG-295
VI	6	IC-436646, IC-436681, IC-436723, IC-436543, IC-436746, IC-436557
VII	1	IC-261261
VIII	1	WGG-42

reports of Sneha et al. (2020), Singh and Singh (2012).

4. CONCLUSION

While selecting parents for hybridization inter-cluster distance must be taken into consideration that may provide a wide spectrum of variation in the segregating generations. Among the 39 genotypes IC-436781, IC-436797, IC-436763, IC-436827, IC-436921 belonging to cluster IV and WGG-42 belonging to cluster VIII has the highest inter cluster distance and can be used for hybridization programme to get superior transgressive segregants. The characters like seed yield plant^{-1}, number of pods plant^{-1}, 100 seed weight should be given importance for further improvement of yield and yield components.

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