

Original Research Article

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Genetic Diversity Analysis Through D^2 Statistic for Quantitative Traits in Germplasm Lines of Green Gram [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

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Fifty-six germplasm lines of green gram [*Vigna radiata* (L.) Wilczek] were used to determine the extent of genetic diversity using Mahalanobis D^2 statistic. The analysis was carried out for 20 quantitative traits. The D^2 statistic performed on 20 quantitative traits grouped all 56 genotypes into eight divergent clusters. Cluster V (8559.766) had maximum intra-cluster distance while inter-cluster distance was highest between clusters III and VI(329997.938) Cluster means indicated that germplasm lines falling in cluster III and V possessed higher values for quantitative traits such as plant height, plant diameter, number of pods per cluster, number of pods per plant, number of seeds per pod, pod yield per plant, pod length, threshing %, leaf area, specific leaf weight, leaf area ratio, seed yield per plant, seed yield per plot and biological yield. Thus suggesting presence of high amount of genetic diversity between the genotypes of these clusters and expected to give higher frequency of transgressive segregants for higher yield. The present study revealed that Mahalanobis D^2 statistic may be successfully utilized for determining genetic diversity and relationships among germplasm lines of Green gram.

Introduction

Green gram [*Vigna radiata* (L.) Wilczek] is an important short duration food legume in the tropical and sub tropical countries of the world. Green gram is a diploid (2n) with the chromosome number 22, grown primarily in intercropping with wheat, maize, potato, etc., during the monsoon season and as a

monocrop in other seasons. India is the largest producer and consumer of pulses in the world. In India, mungbean is grown on an area of about 3 million hectares with the production of about 1 million tonnes and accounts for 55% of the total world acreage and 45% of total production (Rishi, 2009; Singh *et al.*, 2013). Pulses are the major source of dietary protein in vegetarian diets in most countries.

Among pulse crops, green gram is an important annual legume grown principally for its high protein seeds that are used as human food, by cooking, fermenting, milling or sprouting, making soups, curries, bread, sweets, noodles, salads, papad etc. (Singh *et al.*, 1988). Its cultivation in Asia is confined to India, Pakistan, Bangladesh, Myanmar, Indonesia, Philippines, Sri Lanka, Nepal, China, Korea, and Japan (Shanmuga Sundaram, 2001). Mungbean being a legume crop has the ability to fix atmospheric nitrogen (30–50 kg/ha).

Genetic diversity present in the germplasm lines is an important tool for any plant breeding program (Marilene *et al.*, 2012). Multivariate analysis by means of the Mahalanobis generalized distance (D^2) statistic is a powerful tool in quantifying the degree of divergence at the genotypic level and might be an efficient tool in the quantitative estimation of genetic diversity in mungbean genotypes (Mahalanobis, 1936). Success of the hybridization followed by selection depends largely on the selection of parents with high genetic diversity for traits of interest (Murthy and Arunachalam, 1966). The assessment of genetic variation would provide us a correct picture of the extent of genetic variation, further helping us to improve the genotypes responses to biotic and abiotic stresses (Panigrahi and Baisakh, 2014). The main objective of this study was to characterize mungbean germplasm lines for agronomic / quantitative traits for their genetic diversity and also for ancestral relationships.

Materials and Methods

The experiment was conducted in Replicated Augmented Design. Replicated treatments are tested in each block as in a RCBD. Fifty six (56) genotypes of green gram [*Vigna radiata* (L.) Wilczek] grown in 8 Blocks during

summer 2017 at experimental plot of College of Agriculture, Hassan, University of Agricultural Sciences, Bengaluru, Karnataka (India). The gross area of experiment was 302.5 m² and each block size was 3 x 3 m. The row spacing was 30 cm and inter plant distance was 10 cm. Observations were recorded on randomly chosen five competitive plants on 20 metric characters *viz.*, days to 50% flowering, days to 50% maturity, plant height, plant diameter, number of primary branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, pod length, number of seeds per pod, pod yield per plant, seed yield per plant, seed yield per plot, threshing percentage, biological yield, harvest index, 100 seed weight, leaf area, specific leaf weight and leaf area ratio.

Plant materials

The material used in the study comprised of 56 germplasm lines of green gram [*Vigna radiata* (L.) Wilczek] obtained from different Research Institutions and Agricultural Research Stations of India (Table 1).

Statistical analysis

The data was subject to genetic divergence analysis using Mahalanobis D^2 statistic (Mahalanobis 1936) as suggested by Rao (1952). All the genotypes were grouped into respective clusters on the basis of D^2 values following Tocher's method. Twenty morphometric characters were evaluated for plant specimens from 56 germplasm lines.

Mahalanobis D^2 analysis

Mahalanobis (1936) D^2 analysis was used for assessing the genetic divergence among the test entries involving quantitative characters. The generalized distance between any two populations is given by the formula.

$$D^2 = \sum \sum \lambda_{ij} \sigma_{ai} \sigma_{aj}$$

Where,

D^2 = Square of generalized distance
 λ_{ij} = Reciprocal of the common dispersal matrix
 $\sigma_{ai} = (\mu_{i1} - \mu_i)^2$
 $\sigma_{aj} = (\mu_{j1} - \mu_j)^2$
 μ = General mean

Cluster of D^2 values

All $n(n-1)/2$ D^2 values were clustered using Tocher's method described by Rao (1952).

Intra cluster distance

Square of the intra cluster distance =

$$\frac{\sum D^2 i}{N}$$

Where,

$\sum D^2 i$ is the sum of distance between all possible combinations of the entries included in a cluster.
 n = Number of all possible combinations

Inter cluster distance

Square of the inter cluster distance =

$$\frac{\sum D^2 i}{n_i n_j}$$

Where,

$\sum D^2 i$ is the sum of distances between all possible combinations ($n_i n_j$) of the entries included in the clusters study.
 n_i = Number of entries in cluster i
 n_j = Number of entries in cluster j

Results and Discussion

Analysis of variance revealed that a wide range of variability existed for all the traits studied indicating the presence of significant variation among the genotypes. Based on the D^2 analysis, germplasm lines were grouped into eight different clusters as presented in the Table 2. Cluster II was the largest with fifteen genotypes followed by cluster I with fourteen, cluster IV with eleven, cluster III with six, cluster V with five, cluster VI with three and cluster VII and VIII were solitary clusters consisting only one genotype each. The mode of distribution of genotypes from different geographical regions into various clusters was at random indicating that the genotypes originating from different agro-climatic / geographical regions grouped together into different clusters showing no parallelism between genetic diversity and geographical distribution. Our results are on par with findings of Raje and Rao *et al.*, (2001), Venkateswarlu (2001), Dasgupta *et al.*, (2005), Makeen *et al.*, (2007), Tabasum *et al.*, (2010), Majumder *et al.*, (2011), Gunjeet Kaur *et al.*, (2015)

Intra and inter-cluster distances are presented in the Table 3. Average intra cluster distance ranged from 0.00 to 8559.77. The maximum intra-cluster (D^2) distance was recorded for Cluster V (8559.77) followed by Cluster II (2960.06), Cluster I (2395.56), Cluster III (1395.90), Cluster VI (1027), Cluster IV (909.99), cluster VII (0.00) and VIII (0.00). The highest intra-cluster distance recorded by cluster V indicates the presence of wide genetic diversity among the 5 genotypes viz., LGG-582, VBNGG-2, VGG04-007, LGG-577 and TARM-2013. Within the cluster, the maximum inter cluster D^2 distance value was found between cluster III and VI (329997.90) followed by cluster II and III (242988.80), cluster V and VI (187971.10), cluster III and IV (169053.60),

cluster VI and VIII (133744.00) and cluster VII and VI (120870.70). These results suggest that the genotypes grouped in different clusters may be used as potential parental lines for hybridization programmes to develop desirable genotypes as genetic diversity can be best exploited and chances of getting best transgressive segregants are more.

The cluster means of different characters for each of 20 characters are presented in Table 4. From the data we can conclude that considerable variation exists for all the traits studied. Results showed that genotypes in Cluster VII were early flowering (53.00 days) whereas genotypes in cluster VIII were late flowering (58.00 days). The genotypes in Cluster V and IV were of early maturity (68.00 days) whereas genotypes in cluster VIII were late in maturity (73.33 days). Cluster I exhibited highest mean for plant height (34.14 cm) whereas the cluster IV showed lowest (24.02 cm). Cluster I exhibited highest mean for plant diameter (40.12 cm) whereas the cluster VI showed lowest (28.77 cm). Number of Primary branches was highest in cluster VIII (4.10 branches) and lowest in cluster VI (1.27 branches). Number of clusters per plant was highest in cluster III (9.65) and was lowest in cluster VI (3.10). Number of pods per cluster was highest in VII (5.90) and lowest in cluster VI (2.90). Number of pods per plant was highest in cluster III (48.12) and was lowest in cluster VI (9.10). Pod length was highest in cluster VIII (7.65 cm) whereas it was lowest in cluster VI (6.00 cm). Number of seeds per pod was highest in cluster VIII (9.80) and was lowest in cluster VI (6.63). Pod yield per plant was highest in cluster III (25.29 g) and lowest in cluster VI (1.89 g). Threshing percentage was highest in cluster VIII (135.89 %) whereas lowest in cluster VI (48.12 %). Biological yield was highest in cluster III (38.76 g) and was lowest in cluster VI (3.49 g). Harvest index was highest in cluster VI (26.11 %) and lowest in cluster VIII (55.16

%). 100 Seed weight was highest in cluster IV (4.73 g) and lowest in cluster VIII (3.84 g). Leaf area was highest in cluster III (21.21 cm²) whereas lowest in cluster II (10.75 cm²). Specific leaf weight was highest in cluster VII (9.00 mg/cm²) whereas lowest in cluster III (5.67 mg/cm²). Leaf area ratio was highest in cluster I, II and III (0.19cm²/mg) whereas lowest in cluster VII (0.11 cm²/mg). Seed yield per plant was highest in cluster III (19.61 g) whereas lowest in cluster VI (0.88 g). Seed yield per plot was highest in cluster III (588.25 g) whereas lowest in cluster VI (26.32 g).

Therefore, genotype GG13-7 and GG13-6 can be considered for earliness and LGG-589 might fit in list of high yielding early duration green gram varieties. The promising genotypes having outstanding mean values for yield and yield component traits are; GG13-7 with lowest days to 50% flowering, LGG-596 with lowest days to 50% maturity, LGG-588 with highest value for plant diameter, pod yield per plant and biological yield. The genotypes VBNGG-2 had highest number of primary branches per plant, TM-962 had highest number of clusters per plant and number of pods per plant. Highest number of pods per cluster was exhibited by VGG04-007 while VBN-1 possessed highest pod length. Highest number of seeds per pod and leaf area was recorded by COGG-93 and highest plant height, seed yield per plant, seed yield per plot was witnessed by LGG-579. The highest values for threshing percentage were recorded by LGG-577, while highest harvest index was possessed by AKL-39. The genotype SML-668 had highest value for 100 seed weight. The highest specific leaf weight value was recorded for KKM13-44 and leaf area ratio for PM-110. Hence, crosses among these genotypes possessing larger genetic diversity are expected to exhibit high heterosis and might result in high yielding segregants with desired traits.

Table.1 List of germplasm used in the study and their source

Sl. No.	Genotype	Source	Sl. No.	Genotype	Source
1	Selection- 4- Check	UAS, Raichur	29	LGG-572	RARS, Guntur
2	DGG-1- Check	ARS, Bidar	30	PM-110	RARS, Guntur
3	Barimung- Check	UAS, Raichur	31	LGG-577	RARS, Guntur
4	KKM-3	UAS, Bangalore	32	IC-436624	IIPR, Kanpur
5	Harsha	UAS, Raichur	33	IC-436723	IIPR, Kanpur
6	VBN-1	Coimbatore	34	IC-413316	IIPR, Kanpur
7	BGS-9	ARS, Bidar	35	IC-436746	IIPR, Kanpur
8	KM13-16	ARS, Bidar	36	VGG10-010	Coimbatore
9	KM13-19	ARS, Bidar	37	VGG04-011	Coimbatore
10	KM13-39	ARS, Bidar	38	VGG04-007	Coimbatore
11	GG13-7	ARS, Bidar	39	COGG-93	Coimbatore
12	GG13-6	ARS, Bidar	40	VBNGG-2	Coimbatore
13	KM13-44	ARS, Bidar	41	TARM-2013	Coimbatore
14	GG13-10	ARS, Bidar	42	VGG04-005	Coimbatore
15	SML-668	ARS, Bidar	43	COGG-920	Coimbatore
16	KM13-9	ARS, Bidar	44	VGG07-003	Coimbatore
17	IPM99-125	ARS, Bidar	45	VGG10-002	Coimbatore
18	LGG-596	RARS, Guntur	46	VGG-112	Coimbatore
19	LGG-572	RARS, Guntur	47	IC-92048	NBPGR, Akola
20	LGG-450	RARS, Guntur	48	AKL-103	NBPGR, Akola
21	LGG-583	RARS, Guntur	49	AKL- 39	NBPGR, Akola
22	LGG-590	RARS, Guntur	50	AKL-106	NBPGR, Akola
23	LGG-588	RARS, Guntur	51	AKL-225	NBPGR, Akola
24	LGG-589	RARS, Guntur	52	AKL-95	NBPGR, Akola
25	LGG-579	RARS, Guntur	53	AKL-194	NBPGR, Akola
26	LGG-562	RARS, Guntur	54	AKL-212	NBPGR, Akola
27	LGG-582	RARS, Guntur	55	AKL-195	NBPGR, Akola
28	LGG-585	RARS, Guntur	56	AKL-211	NBPGR, Akola

Table.2 List of different cluster formed from 56 mungbean genotypes

Cluster number	Number of genotypes	Name of the genotypes
I	14	LGG-450, VGG04-011, LGG-583, PM-110, LGG-572, VGG10-002, VGG04-005, LGG-573, IC-413316, KKM-3, VGG07-003, LGG-596, LGG-588, Selection-4
II	15	KM13-16, KM13-19, AKL-195, BGS-9, GG13-7, IPM99-125, AKL-212, KM13-39, VGG-112, AKL-194, KM13-9, AKL-225, AKL-95, AKL-103, IC-436624
III	6	TM-962, COGG-93, LGG-589, VGG10-010, LGG-585, LGG-579
IV	11	KM13-44, IC-92048, COGG-920, GG13-6, VBN-1, Barimung, Harsha, DGG-1, LGG-590, SML-668, AKL-106
V	5	LGG-582, VBNGG-2, VGG04-007, LGG-577, TARM-2013
VI	3	GG13-10, AKL-211, AKL-39
VII	1	IC-436746
VIII	1	IC-436723

Table.3 Intra and Inter cluster distance of 56 genotypes using Mahalonobis D^2 analysis

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	2395.557	40370.133	90405.266	14325.948	27689.197	78636.539	7312.957	11729.597
Cluster II		2960.056	242988.844	8463.021	124285.242	9458.265	70811.992	79202.133
Cluster III			1395.901	169053.625	25584.635	329997.938	54153.438	53738.402
Cluster IV				909.989	73759.844	28312.922	34126.000	41053.754
Cluster V					8559.766	187971.141	11732.245	12340.687
Cluster VI						1027.017	120870.719	133743.953
Cluster VII							0.000	5134.390
Cluster VIII								0.000

Table.4 Cluster wise mean performance of 56 mungbean genotype for yield and its related traits

Character	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Days to 50% Flowering	56.70	54.13	55.50	53.27	54.60	54.67	53.00	58.00
Days to 50% Maturity	70.86	70.67	73.33	68.00	68.00	71.00	69.00	73.00
Plant Height	34.14	25.46	33.95	28.11	30.76	24.02	27.47	30.10
Plant Diameter	40.12	29.81	39.42	34.91	38.03	28.77	33.72	31.60
Primary Branches	3.34	1.83	3.18	2.15	3.64	1.27	2.90	4.10
Clusters/ Plant	7.46	4.19	9.65	5.70	7.10	3.10	5.80	4.60
Pods/ Cluster	4.78	2.97	5.13	4.10	5.58	2.90	5.90	4.40
Pods/ Plant	30.67	12.31	48.12	20.43	41.32	9.10	34.20	17.60
Pod Length	7.08	6.64	7.20	7.20	7.43	6.00	6.80	7.65
No. Seeds/ pod	8.73	7.82	9.28	8.15	9.10	6.63	8.50	9.80
Pod Yield/ Plant	14.48	4.85	25.29	8.52	19.51	1.89	14.58	8.93
Threshing	73.19	74.27	77.56	72.53	84.31	48.12	83.05	135.89
Biological Yield	22.47	7.87	38.76	13.56	29.93	3.49	22.63	21.06
Harvest Index	46.43	46.05	51.92	45.98	50.89	26.11	55.15	55.16
Test Weight	4.12	4.32	4.52	4.73	4.28	4.05	3.84	4.11
Leaf Area	18.99	10.75	21.21	14.21	19.71	9.41	18.91	14.72
Specific Leaf Weight	5.79	5.73	5.67	6.00	6.60	6.33	9.00	8.00
Leaf Area Ratio	0.19	0.19	0.19	0.18	0.15	0.17	0.11	0.13
Seed Yield/ Plant	9.77	3.53	19.61	6.14	14.75	0.88	12.11	12.31
Seed Yield/ Plot	293.25	105.97	588.25	184.32	442.47	26.32	363.28	369.23

In conclusion the results indicate the presence of high genetic variability among the green gram genotypes. D² analysis is very much useful in assessment of green gram diversity and also to develop core collection of germplasm to be utilised in crop improvement programmes (Muthusamy *et al.*, 2008; Arpita *et al.*, 2010; Ghulam *et al.*, 2010; Singh *et al.*, 2013; Vyas *et al.*, 2018). Breeding strategies to improve traits for yield, biotic and abiotic stress are largely dependent upon presence of genetic variability among parental lines. Hence assessment and characterization of genetic diversity among germplasm lines is of great importance. In this study, we have successfully assessed the levels of genetic diversity, intra and inter cluster diversity and genetic relatedness that existed among germplasm lines of green gram representing different eco-geographical / agro-climatic zone.

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