Received: 13 Dec 2022 | Revised: 04 Feb 2023 | Accepted: 11 Mar 2023

Electronic Journal of Plant Breeding

Research Note

Genetic divergence study in greengram [*Vigna radiata* (L.) Wilczek]

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Abstract
The present investigation was undertaken to obtain information on the nature and extent of genetic diversity among 60 greengram genotypes for yield related traits and quality traits by using Mahalanobis's D^2 statistics. The genotypes were grouped into eleven clusters. Cluster I was found to be the largest with 38 genotypes followed by cluster V with 13 genotypes and all the other clusters were found to be solitary, each containing a single genotype. Clusters VIII and XI had the maximum inter-cluster distance, followed by clusters IV and XI. Cluster XI had the highest mean values for yield and other yield attributing traits. Iron content contributed high towards total genetic diversity followed by protein content and test weight. Based on the mean performance and diversity studies, the genotypes COGG 18-17, LGG 460, Daftri vikas and IPM 1603-3 were found to be the best for further yield improvement in greengram. Utilizing the genotypes from the more divergent clusters as parents in breeding programmes will yield relatively good amount of heterosis in F_1 and high frequency of transgressive segregants and genetic variability in subsequent generations can be acquired.

Keywords: D^2 statistic, Cluster analysis, Genetic Divergence, Greengram, Tocher's method.

Pulses are the key source of dietary proteins, of which greengram is one of the important annual legumes (Sandhiya and Saravanan , 2018). Greengram [*Vigna radiata* (L.) Wilczek], also called as mungbean, belongs to the family Leguminosae, is a main pulse crop in Asia. Itm is a self-pollinated crop with a chromosome number of 2n=2x=22. In India, it is cultivataedin a total area of 5.13 million hectares with a production of 3.9 million tonnes and productivity of 601 kg/ha. In Andhra Pradesh, itis cultivated in an area of 1.05 lakh hectares, with a production of 0.81 lakh tonnes and productivity of 772 kg/ha (Indiastat, 2020-21).

Development of high yielding varieties that are rich in proteins and essential micronutrients like zinc and iron are the need of the hour in greengram. (Nagrale *et al*., 2018; Renu *et al*., 2018).

The favourable accomplishment of any successful plant breeding programme chiefly depends on the incidence of diversity among the genotypes. Genetic diversity analysis is an influential means in enumerating the extent of divergence between biological populations and to evaluate the comparative contribution of various components for total divergence. From the point of selecting the parents for hybridization, estimation of the genetic distance among the population for the trait of interest is most important. Mahalanobis D^2 statistic is a method for quantifying genetic divergence among the existing genotypes based on morphological traits.. The parents identified for hybridization on the basis of genetic divergence analysis provide scope for generation of more promising recombinants. By keeping all these considerations in view, the current examination was undertaken to study genetic divergence among 60

greengram genotypes for 11 quantitative and three grain quality characters.

The experimental material comprised of 60 greengram genotypes (**Table 1**) which were gathered from all over India and assessed in alpha lattice design with two replications at Regional Agricultural Research Station (RARS), Lam, Guntur during 2021-22. Each genotype was sown in two rows of four meters length with a spacing of 30 cm between the rows and 10 cm between the plants within the row. Observations on 11 quantitative traits *viz.,* days to 50% flowering, days to maturity, plant height, branches per plant, clusters per plant, pods per cluster, pods per plant, pod length, seeds per pod, test weight and yield per plant were recorded. Five competitive random plants from each replication were marked for all recording data pertaining to the traits under study excluding days to 50% flowering and days to maturity which were recorded replication wise on plot basis. The seed protein content (%) of each sample was assessed by procedure described by Sadasivam and Manickam (1996). For the estimation of seed zinc and iron content, seeds were crushed into flour and estimated as per the procedure of Tandon,1999.

Windostat ver. 9.3 software was used for statistical analysis. The documented data on various characters was subjected to D^2 analysis (Mahalanobis, 1928) for evaluating the genetic divergence amongst the genotypes. The cluster means, average intra and inter cluster distances and involvement of different traits towards the total divergence was estimated by as per the procedure proposed by Singh and Choudhary (1977).

The analysis of variance for alpha lattice design revealed highly significant differences among the greengram genotypes for all the characters under investigation indicating the presence of a considerable magnitude of genetic variability (**Table 2**). Mahalanobis' D^2 analysis utilizing Tocher's method grouped the 60 greengram genotypes into 11 non-overlapping clusters (**Table 3**) evidently showing that there was noteworthy variability among the genotypes studied, which was directed to genetic diversity.

From divergence analysis, average intra and inter cluster D^2 values were calculated (**Table 4**) and found significant level of genetic variation among the genotypes, as evidenced by the fact that the inter-cluster distances were greater than the intra-cluster distances. Genotypes grouped within the same cluster likely differ from one another only slightly in terms of the totality of the assessed traits.

In the present investigation the distribution of genotypes in different clusters was at random with maximum number of 38 genotypes in cluster I followed by cluster V with 13 genotypes and the rest of the clusters, *i.e.* cluster II, III, IV, VI, VII, VIII, IX, X and XI were found to constitute solitary clusters (hence no intra-cluster distances / D^2 values). The genotypes belonging to different geographical origin were grouped in the same cluster which indicated that geographical diversity is not a measure of genetic diversity. Similar findings were also reported by Muthuswamy *et al.* (2019) in blackgram. Intra-cluster D^2 values ranged from 0.00 (cluster II, III, IV, VI, VII, VIII, IX, X and IX) to 11.2 (cluster V). Cluster V had the maximum intra-cluster distance indicating presence of substantial divergence between genotypes followed by cluster I. Earlier researchers like Singh *et al.* (2009), Gokulakrishnan *et al.* (2012), Gadakh *et al.* (2013), Panigrahi and Baisakh (2014) and Ahmad *et al.* (2016) observedmono clusters in their finding.

The maximum inter-cluster distance was observed between cluster VIII and XI (24.93), followed by cluster IV and XI (23.72) and cluster VII and XI (22.52) which suggested that there was high degree of genetic diversity between genotypes of these clusters. Crosses between parents from these more divergent clusters may produce valuable recombinants in later generations as well as relatively good heterosis in F_1 and a high frequency of transgressive segregants.

The minimum inter cluster distance was observed between cluster II and cluster VII (7.35) indicating that they are closely related, and that common parents might be involved in development of these genotypes. The genotypes falling in the same cluster have little diversity and selection of parents from within the same cluster might not be promising to use in a hybridization programme for the development of good segregants.

The choice of parents is largely influenced by the contribution of different traits to the total diversity. In the present study, the maximum contribution to genetic diversity was observed to be by iron content (36.67%), followed by protein content (19.15%), test weight (14.41%), days to 50% flowering (8.76%), zinc content (8.25%), days to maturity (6.27%), seeds per pod (2.26%), seed yield (1.86%), pods per plant (0.68%), clusters per plant (0.62%), plant height (0.45%), pods per cluster (0.06%) and branches per plant (0.06%) (**Table 5**) . This is in tune with the results of Joshi *et al*. (2022).

Cluster means describe the average trait performance of all genotypes found in a specific cluster. Cluster VIII (**Table 6**) showed the highest mean value for days to fifty percent flowering (48.50) and plant height (36.20). Cluster XI recorded highest mean value for number of branches per plant(5.40), pods per plant (28.40), pods per cluster (6.75), pod length (8.67), test weight (4.99) and zinc content (26.80) and seed yield (10.24). Cluster IV showed the highest mean value (72.00) for days

Table 1. Details of parentage and origin of sixty greengram genotypes

S.No.	Genotype	Parentage	Origin
1	RM 03-71	TM 96-2 x VC 6370	Chattisgarh
2	ML 2506	SML 668 x V 270	Punjab
3	NVL 1143	NVL 857 x NVL 824	Maharasthra
4	MI 98-64	JM 45 x ML 131	Madhya Pradesh
5	COGG 18-17	SML 668 x Pusa Vishal	Tamil Nadu
6	PM 1711	PM 5 x Pusa 0672	Uttarakhand
7	OUM 11-5	Mutant of Dhauli	Odisha
8	VGG 18-021	VBN (Gg) 2 x MH 421	Tamil Nadu
9	OBGG 105	NM 94 x ML 1628	Odisha
10	BCM-20-9	IPM 02-14 x TMB 37	West Bengal
11	BM 2019-10	BPMR 145 x BPMR 75	Maharasthra
12	Pusa M 2171	MH 318 x Mash 114	New Delhi
13	IPM 1603-3	MH 03-18 x EC 369223	Uttar Pradesh
14	COGG 912	MGG 336 x COGG 902	Tamil Nadu
15	VGG 17-106	CO 8 x Cinnamung	Tamil Nadu
16	Pusa BM 9	Mutant of Pusa Vishal	Rajasthan
17	RMG 1132	RMG 62 x PDM 219	Rajasthan
18	GJM 1701	GM 4 x BPMR 145	Gujarat
19	Daftri Vikas	D-Gaurav X D-6372	Maharasthra
20	SKNM 1904	GM 2 x HUM 1	Gujarat
21	RMG 1166	IPM 02-3 x COGG 912	Rajasthan
22	IPM 13-6	IPM 6-5 x IPM 409-4	Uttar Pradesh
23	MH 1857	Satya X MH 318	Haryana
24	VBN 4	PDM 139 x BB 2664	Tamil Nadu
25	MH 1830	KM 2241 x MH 521	Haryana
26	LGG 610	MGG 295 x LGG 486	Andhra Pradesh
27	ML 2500	PAU 911 x ML131	Punjab
28	KM 2421	KM 2328 x K 851	Uttar Pradesh
29	Pusa M 2172	Pusa 0672 x Pant Mung 5	New Delhi
30	JLPM 702-1	Vaibhav x Samrat	Maharasthra
31	MHBC-20-2	MH 96-1 x BDYR 1	West Bengal
32	IPM 2-14	IPM 99-125 x Pusa bold 2	Uttar Pradesh
33	OBGG 104	NM 94 x Harsha	Odisha
34	IPMD 1603-7	MH 03-18 x EC 369233	karnataka
35	MH 1772	VGG-rt-1 x MH 2-15	Haryana
36	PM 1723	PM 6 x PM 4	Uttarakhand
37	AKM 12-28	PKV Green Gold x BM 2003-2	Maharasthra
38	IIPM20-1	NM 94 x ML 1628	Uttar Pradesh
39	VGG 15-013	VBN (Gg) 2 x ML 1451	Tamil Nadu
40	PUSA BM -11	Mutant of Pusa Vishal	Bihar
41	MGG 453	Madhirmung x RM 8-666	Telangana
42	IIPM 20-2	NM 94 x ML 1628	Uttar Pradesh
43	TMB127	Samarat x Kopergaon	Maharasthra
44	OBGG 110	NM 94 x ML 1628	Odisha
45	COGG 16-10	CO 6 x SML 668	Tamil Nadu

Table 1. Continued..

S.No.	Genotype	Parentage	Origin
46	VGG 17-049	VBN (Gg) 2 x Pusa M 1402	Tamil Nadu
47	LGG 600	MGG 295 x P 109	Andhra Pradesh
48	LGG 604	MGG 295 x COGG 912	Andhra Pradesh
49	LGG 606	MGG 295 x COGG 912	Andhra Pradesh
50	LGG 625	Sipai x P115	Andhra Pradesh
51	LGG 645	LGG 460 x COGG 912	Andhra Pradesh
52	LGG 649	Sipai x COGG 912	Andhra Pradesh
53	GGG 4	TM 96-2 x LGG 407	Andhra Pradesh
54	LGG 460	Lam M2 x ML 267	Andhra Pradesh
55	LGG-574	LGG 460 x P 101	Andhra Pradesh
56	LGG-607	MGG 295 x COGG 912	Andhra Pradesh
57	LGG-609	MGG 295 x LGG 486	Andhra Pradesh
58	LGG-630	LGG 460 x P 109	Andhra Pradesh
59	TM-96-2	Kopergaon x TARM-2	Maharasthra
60	WGG-42	Local selection	Telangana

Table 2. Analysis of variance for seed yield and other characters in greengram genotypes

Source of variations	Mean sum of squares							
	D.F	Days to 50% flowering	Days to maturity	Plant height (cm)	Branches per plant	Clusters per plant	Pods per plant	Pods per cluster
Replications	1	221.41	667.41	31.31	1.26	0.00	135.26	0.11
Treatments (unadjusted)	59	2.87**	12.97**	24.23**	0.91**	0.46**	26.23**	0.81**
Blocks within Replicated (adj)	6	0.45	0.45	16.23	1.21	0.08	2.52	0.16
Intrablock error	53	0.19	0.20	8.00	0.43	0.16	5.42	0.38

Source of variations	Mean sum of squares							
	D.F	Pod length (cm)	Seeds	Test weight (g)	Protein Content	Zinc (mg/kg)	Iron (mg/kg)	Seed yield per plant (g)
Replications	1	2.49	18.10	0.00	0.12	38.86	75.21	0.47
Treatments (unadjusted)	59	1.02**	2.26**	0.35**	9.33**	30.39**	579.27**	2.89**
Blocks within Replicated (adj)	6	1.26	0.45	0.00	0.80	1.69	20.44	1.13
Intrablock error	53	0.36	0.24	0.02	0.38	4.70	15.13	0.56

** Significant at 1% level

to maturity. Cluster X recorded highest mean value for iron content (95.50) and clusters per plant (4.40). Cluster II showed highest mean value (28.00) for protein content. Cluster III showed lowest mean value for days to fifty percent flowering (44.50) and protein content (19.95). For a breeding programme to be successful, it is a vital pre-requisite to select genetically diverse parents so as to obtain better and desirable recombinants. The usefulness and success of Mahalanobis' D^2 analysis in quantifying the genetic divergence was already indicated in greengram by Jeeva and Saravanan (2017), Mahalingam *et al*. (2018), Sneha *et al*. (2020), Priya *et al*. (2020), Sheena *et al*. (2021), Joshi *et al*. (2022) and Kumar *et al*. (2022).

Table 3. Clustering pattern of 60 greengram genotypes by Tocher's method

Cluster number	Number of genotypes	Name of genotype (S)
I	38	VBN 4, LGG 610, LGG 600, LGG 625, ML 2500, LGG 606, LGG 604, IPM 13-6, RMG 1132, BCM-20-9, VGG 17-106, MHBC-20-2, PUSA BM -11, VGG 15-013, OBGG 104, COGG 16-10, WGG-42, Pusa M 2171, LGG-574, PM 1723, Pusa M 2172, VGG17-049, OBGG 105, AKM 12-28, LGG-607, LGG-630, GGG 4, PM 1711, OUM 11-5, LGG 645, MH 1830, MH 1772, MI 98-64, GJM 1701, MH 1857, TM-96-2, LGG-610, IPM 2-14
II	01	LGG 649
III	01	NVL 1143
IV	01	IPM 1603-3
V	13	RMG 1166, OBGG 110, SKNM 1904, RM 03-71, IIPM20-1, IIPM 20-2, TMB127, MGG 453, ML 2506, BM 2019-10, COGG 912, KM 2421, Pusa BM 9
VI	01	IPMD 1603-7
VII	01	Daftri Vikas
VIII	01	LGG 460
IX	01	JLPM 702-1
X	01	VGG 18-021
XI	01	COGG 18-17

Table 4. Average intra and inter-cluster distances (D^2 values) among 11 clusters in 60 greengram genotypes

Cluster number	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	**10.27**	11.67	12.65	11.79	14.23	12.66	11.96	11.57	14.39	17.26	21.62
II		**0.00**	17.48	16.29	16.44	10.63	7.35	9.34	14.87	16.9	20.99
III			**0.00**	10.04	10.81	14.67	17.22	18.45	15.43	19.03	20.09
IV				**0.00**	14.47	15.71	16.47	16.26	16.71	18.68	23.72
V					**11.20**	13.33	16.99	18.39	15.36	20.28	19.33
VI						**0.00**	12.84	13.96	11.77	15.31	17.52
VII							**0.00**	7.63	14.78	18.04	22.52
VIII								**0.00**	15.29	18.5	24.93
IX									**0.00**	9.05	12.58
X										**0.00**	12.94
XI											**0.00**

Table 5. Contribution of different characters towards genetic divergence in 60 greengram genotypes

S.No.	Traits	Times Ranked 1st	Contribution %
1	Days to 50% flowering	155	8.76
2	Days to maturity	111	6.27
3	Plant height	8	0.45
4	Branches per plant	1	0.06
5	Clusters per plant	11	0.62
6	Pods per plant	12	0.68
7	Pods per cluster	1	0.06
8	Pod length	9	0.51
9	Seeds per pod	40	2.26
10	Test weight	255	14.41
11	Protein content	339	19.15
12	Zinc content	146	8.25
13	Iron content	649	36.67
14	Seed yield per plant	33	1.86

Table 6. Cluster mean value of eleven clusters estimated by Tocher's method in 60 greengram genotypes

	DFF	DM	PH (cm)	BPP	CPP	PPP	PPC	PL (cm)	SPP	TW (g)	PC (%)	ZINC (mg/kg)	IRON (mg/kg)	YIELD (g)
Cluster I	46.36	67.88	32.77	4.42	3.61	15.59	4.28	7.35	11.45	3.51	23.25	20.51	77.66	5.80
Cluster II	46.00	66.00	29.60	4.00	3.50	14.30	3.60	**7.24**	11.20	3.62	**28.00**	20.90	80.50	7.20
Cluster III	**44.50**	65.50	**28.70**	4.60	3.50	14.40	5.10	7.56	10.60	3.47	**19.95**	23.20	55.50	6.89
Cluster IV	45.00	**72.00**	29.60	4.80	3.00	12.50	4.10	7.98	10.80	**3.08**	21.00	16.27	71.50	6.80
Cluster V	45.73	67.12	32.79	4.64	3.43	14.75	4.36	7.57	11.48	3.63	22.66	21.88	**44.72**	6.37
Cluster VI	45.00	65.00	33.95	3.80	3.60	16.30	4.90	7.27	11.50	4.19	26.69	**12.75**	60.50	**5.61**
Cluster VII	47.50	66.50	29.30	**3.40**	**2.40**	**7.80**	**3.55**	8.17	10.80	3.74	25.46	23.69	86.00	7.58
Cluster VIII	**48.50**	66.50	**36.20**	5.20	3.20	15.40	4.40	8.02	11.50	3.40	25.29	15.45	**95.50**	5.74
Cluster IX	47.50	66.50	31.90	5.10	4.35	20.90	5.70	8.14	14.40	4.41	22.05	14.40	77.50	9.47
Cluster X	45.00	65.00	34.20	5.30	**4.40**	22.60	5.80	7.70	15.30	4.63	21.61	17.38	**95.50**	10.15
Cluster XI	**44.50**	**64.50**	32.20	**5.40**	**4.40**	**28.40**	**6.75**	**8.67**	16.10	**4.99**	22.31	**26.80**	64.00	**10.24**

DFF-Days to 50% flowering, DM-Days to maturity, PH-Plant height (cm), CPP- Clusters per plant, PPP- Pods per plant, PPC- Pods per cluster, PL-Pod length (cm), SPP- Seeds per pod, TW-Test weight (g), PC-Protein content (%), ZINC- Zinc content (mg/kg), IRON- Iron content (mg/kg), SYPP-Seed yield per plant (g).

Considering the highest inter-cluster distance, COGG 18-17 (belonging to cluster XI) was genetically divergent with LGG 460 (belonging to cluster VIII), with IPM 1603-3 (belonging to cluster IV) and with Daftri vikas (belonging to cluster VII) .. Intra-cluster value was highest for cluster V followed by cluster I which indicated that genotypes of these clusters were most heterogeneous and these clusters were the best for within group hybridization. Cluster mean data revealed that genotypes in cluster XI (COGG 18-17) had medium pod (8.67 cm), maximum test weight (4.99 g), maximum number of pods per plant (28.40) and maximum seed yield per plant (10.24 g); genotypes in cluster VIII (LGG 460) had maximum iron content (95.50); cluster II (LGG 649) had the highest mean value for protein content (28.00). These clusters could be directly selected and utilized for breeding programme. Based on the results obtained from the present study, it could be concluded that hybridization between genotyoes in cluster VIII and XI; cluster IV and XI; and cluster VII and XI could result in desirable trangressive segregants with earliness coupled with higher pods per plant, test weight, zinc, iron and protein contents and seed yield in greengram.

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