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Genetic diversity studies in blackgram

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Abstract
The present investigation was undertaken with 40 blackgram genotypes to estimate genetic diversity for seed yield, yield components and seed protein content. The diversity was evaluated using multivariate technique of Mahalanobis D^2. The genotypes studied were grouped into seven clusters. Cluster I was the largest, comprising of 14 genotypes followed by Cluster IV with seven genotypes, Cluster V with six genotypes, Cluster II and III with five genotypes each, while Cluster VI and VII comprised of one and two genotypes, respectively. The distribution of genotypes was random and genotypes from different geographic regions were grouped in the same cluster, while genotypes from the same geographic region were scattered in different clusters, indicating that there is no parallelism between geographical diversity and genetic diversity. Results on inter-cluster distance revealed maximum diversity between genotypes of Cluster VI and Cluster VII, while intra-cluster distance was noticed to be maximum for Cluster V. Cluster VI recorded maximum seed yield per plant, in addition to plant height, number of clusters per plant and hundred seed weight. This cluster had also recorded protein content more than 20 per cent, number of seeds per pod more than six and number of pods per plant more than 50 and hence, the genotype, LBG 20 belonging to the cluster may be utilized in hybridization programmes aimed at improvement of these traits. Further, days to maturity (64%) was noticed to contribute maximum for genetic divergence, followed by number of pods per plant (44.36%) and minimum contribution was observed for number of seeds per pod (0.26%).

Keywords: Mahalanobis D^2, blackgram, genetic diversity, seed yield per plant

1. Introduction
Blackgram [*Vigna mungo* (L.) Hepper], is one of the important pulse crops, grown throughout the country. The crop is resistant to adverse climatic conditions and improves the soil fertility by fixing atmospheric nitrogen in the soil. It has been reported that the crop produces equivalent to 22.10 kg of N/ha., which has been estimated to be supplement of 59 thousand tonnes of urea annually. The pulse 'Black gram' plays an important role in Indian diet, as it contains vegetable protein and is an important supplement to cereal based diet. It contains about 26% protein, which is almost three times that of cereals and other minerals and vitamins. Besides, it is also used as nutritive fodder, especially for milch animals. Seed yield of black gram is however, low, being about 450–800 kg/ha (Gupta *et al.*, 2013) [1]. One of the major constraints in achieving higher productivity is lack of exploitable genetic variability coupled with narrow genetic base due to repeated usage of few parents with high degree of relatedness in crossing programmes (Jayamani and Sathya, 2013) [2]. In this context, the present study was undertaken to assess the genetic divergence of few blackgram genotypes for seed yield, yield components and seed protein content using Mahalanobis D^2 statistic and Tocher's method towards identification of suitable parents for use in hybridization programmes for improvement of seed yield and protein content in blackgram.

2. Material and Methods
The experimental material consisted of 40 blackgram genotypes (Table 1) obtained from Agricultural Research Station, Ghantasala (27 Nos.); Regional Agricultural Research Station, Lam Farm, Guntur (4 Nos.); and Regional Agricultural Research Station, Tirupati (2 Nos.), Andhra Pradesh State in addition to collections from G.B Pant University of Agricultural Sciences and Technology, Pantnagar (2 Nos.) and National Pulses Research Centre, Vamban (5 Nos.). All the 40 blackgram genotypes were sown at Agricultural College Farm, Bapatla during *Rabi* 2020-21 in Randomized Complete Block Design in three replications. Each genotype was sown in six rows of four meters length with spacing of 30 cm × 10 cm. All the recommended package of practices were followed to raise a good crop.

Observations were recorded for days to 50 per cent flowering, days to maturity, plant height, number of clusters per plant, number of pods per plant, pod length, number of seeds per pod, 100 seed weight, seed protein content and seed yield per plant. Data for quantitative traits were recorded from five randomly selected plants and the mean was taken for analysis. For protein content, the nitrogen content in each sample was estimated by Microkjeldahl method and seed protein content of each sample was estimated as per the procedure described by Sadasivam and Manickam (1992) [7]. The data collected was subjected to statistical procedures. Genetic diversity in the material was analyzed using Mahalanobis D^2 statistic (Rao, 1952) and the varieties were grouped into different clusters according to Tocher' method.

3. Results and Discussion

Analysis of variance (Table 2) revealed the significant differences among the genotypes studied, for all the characters indicating the existence of sufficient variability for effective selection. This significant differences among 40 genotypes for all the characters justified further calculation of D^2 values. The results on genetic diversity of the 40 blackgram genotypes for seed yield, yield components and seed protein content are presented in Tables 2-6 and Figs. 1-3.

The 40 blackgram genotypes were grouped into seven clusters using Tocher's method based on D^2 value such that the genotypes belonging to the same cluster (Intra-cluster) had an average smaller D^2value than those belonging to different clusters (Inter cluster). The distribution of genotypes grouped into seven clusters is presented in Table 3 and Fig. 1. A perusal of these results revealed Cluster I to be the largest comprising of 14 genotypes (TBG-106, GKB-4, SRI, MARUTHI, TGBG-143, TUTIMINUMU, T-9, VBG 12-110, NANDHI, VBG 13-3, GBG-45, VBG 14-16, TGBG-401, GBG-1), followed by Cluster IV with seven genotypes (LBG-645, TBG-104, TU-94-2, TGBG-26, GBG-47, WBG-108, ADT-6), Cluster V with six genotypes (PU-40, TU-18, KUG 216 X PU 40, PU-31, TGBG-136, TGBG-258), Cluster II (IPU 2-43, LBG-788, KUG 216 X SPS 5, LBG-752, VBG 12-034) and Cluster III (TGBG-281, KUG 216 X BG 018-2, TGBG-74, DPU 8831 X VBG 4-088, TGBG-344) with five genotypes, each and Cluster VII with two genotypes (IPU 11-2, VBG 17-026). Cluster VI was observed to be a monogenotypic cluster comprising of only one genotype (LBG-20). The distribution of genotypes in different clusters revealed grouping of genotypes from different geographic regions into the same cluster. Similarly genotypes from the same geographic region were scattered into different clusters at random, indicating that there is no parallelism between geographical diversity and genetic diversity. Similar results were reported earlier by Sagar *et al.* (2001) [8], Reddy *et al.* (2011) [5], Rolaniya *et al.* (2017) [6] and Partap *et al.* (2020) [3].

The results on inter and intra-cluster distances are presented in Table 4 and Fig. 2. A perusal of these results revealed maximum inter-cluster distance between Cluster VI and VII (622.88). Greater the distance between two clusters, wider is the diversity expected between genotypes of the two clusters. Therefore, hybridization between genotypes of Cluster VII (IPU 11-2, VBG 17-026) with LBG-20 of the cluster VI is expected to result in greater variability and transgressive segregants. Further, minimum inter-cluster distance was observed between cluster I and cluster II (58.09), indicating their relatively closer relationship and similarity with regards to the characters studied for most of the genotypes in the two clusters. An examination of intra-cluster distances, indicative of the diversity among the genotypes grouped in a cluster revealed D^2 values ranging from 0.00 (Cluster VI) to 79.72 (Cluster V). Maximum intra-cluster distance was observed for cluster V (79.72), followed by cluster IV (67.85), cluster VII (46.36) and cluster III (45.60), indicating that genotypes from these clusters were highly divergent meriting their consideration in selection of parents for hybridization. However, the intra-cluster distance was zero for the mono-genotypic cluster VI.

Cluster means indicate the average performance of genotypes present in a particular cluster. Estimate of cluster means provides information on suitable donors for improvement of particular traits. The cluster means for seed yield per plant, yield components and seed protein content for the 40 genotypes studied in the present investigation are presented in Table 5. A perusal of the results on cluster means for seed yield, yield components and seed protein content revealed considerable differences between the clusters for all characters under study. The cluster means ranged from 30.83 days (Cluster VII) to 39.87 days (Cluster II) for days to fifty per cent flowering; 62.17 days (Cluster VII) to 72.93 days (Cluster II) for days to maturity; 36 cm (Cluster VII) to 53.53 (Cluster VI) for plant height; 6.23 (Cluster VII) to 14.2 (Cluster VI) for number of clusters per plant; 31.28 (Cluster III) to 52.87 (Cluster II) for number of pods per plant; 2.37 (Cluster VI) to 4.75 (Cluster III) for pod length; 6.55 (Cluster VII) to 8.34 (Cluster III) for number of seeds per pod; 2.52g (Cluster VII) to 6.19g (Cluster VI) for hundred seed weight; 19.01% (Cluster V) to 23.92% (Cluster VII) for seed protein content; 5.64g (Cluster III) to 10.3g (Cluster VI) for seed yield per plant. Further, Cluster VI had recorded maximum seed yield per plant, in addition to plant height and number of clusters per plant along with hundred seed weight, while cluster II had recorded maximum cluster means for days to fifty per cent flowering, days to maturity and number of pods per plant. Cluster III recorded maximum cluster means for pod length and number of seeds per pod, while, Cluster VII had recorded maximum seed protein content. There was no single cluster with all the desirable traits, which ruled out the possibility of direct selection off genotypes for immediate use. Selection of genotypes from clusters with high mean for the respective traits is suggested for utilization in hybridization programmes aimed at improvement of the respective traits. Further, hybridization between the selected genotypes from divergent clusters is suggested for judicious combination of all the targeted traits. In this direction, selection of genotypes from the clusters, VI and VII is suggested for utilization in hybridization programmes aimed at the development of genetically diverse and high yielding genotypes with high seed protein content in blackgram.

Information on the relative contribution of various plant characters towards the divergence was also reported to aid the breeder in the choice of parents for hybridization and effective selections in the advance generations (Suneetha *et al.* 2012) [9]. A perusal of the results of the present investigation (Table 6 and Fig. 3) on number of times each of the characters appeared first and their per cent contribution towards genetic diversity revealed maximum contribution towards genetic diversity by days to maturity (64%), followed by number of pods per plant (44.36%), number of clusters per plant (11.28%), days to fifty per cent flowering (10.77%), seed protein content (9.62%), pod length (8.59%), seed yield per plant (6.28%), plant height (4.87%), hundred seed weight

(3.33%) and number of seeds per pod (0.26%). Hence, selection for divergent parents based on days to maturity and number of pods per plant would be useful for increasing the scope of isolating desirable recombinants in breeding of high yielding blackgram genotypes.

Table 1: Details of blackgram genotypes studied in the present investigation

S. No	Germplasm No.	Genotype	Source
1.	GP9	TU-94-2	ARS, Ghantasala, Andhra Pradesh
2.	GP11	TGBG-26	ARS, Ghantasala, Andhra Pradesh
3.	GP12	TGBG-344	ARS, Ghantasala, Andhra Pradesh
4.	GP13	TGBG-74	ARS, Ghantasala, Andhra Pradesh
5.	GP14	TGBG-143	ARS, Ghantasala, Andhra Pradesh
6.	GP15	TGBG-281	ARS, Ghantasala, Andhra Pradesh
7.	GP16	KUG 216 × BG 018-2	ARS, Ghantasala, Andhra Pradesh
8.	GP17	DPU 8831 × VBG 4-088	ARS, Ghantasala, Andhra Pradesh
9.	GP18	LBG-645	RARS, Lam Farm, Guntur, Andhra Pradesh
10.	GP19	TBG-104	RARS, Tirupati, Andhra Pradesh
11.	GP20	WBG-108	ARS, Ghantasala, Andhra Pradesh
12.	GP21	KUG-216 × PU 40	ARS, Ghantasala, Andhra Pradesh
13.	GP23	PU-40	G.B Pant University of Agricultural Sciences and Technology, Pantnagar, Uttarakhand
14.	GP24	TU-18	ARS, Ghantasala, Andhra Pradesh
15.	GP26	PU 31	G.B Pant University of Agricultural Sciences and Technology, Pantnagar, Uttarakhand
16.	GP27	TGBG 258	ARS, Ghantasala, Andhra Pradesh
17.	GP28	IPU 2-43	ARS, Ghantasala, Andhra Pradesh
18.	GP29	LBG 788	RARS, Lam Farm, Guntur, Andhra Pradesh
19.	GP30	KUG 216 × SPS 5	ARS, Ghantasala, Andhra Pradesh
20.	GP31	TGBG 136	ARS, Ghantasala, Andhra Pradesh
21.	GP32	T9	ARS, Ghantasala, Andhra Pradesh
22.	GP33	TGBG-401	ARS, Ghantasala, Andhra Pradesh
23.	GP34	LBG-752	RARS, Lam Farm, Guntur, Andhra Pradesh
24.	GP36	LBG-20	RARS, Lam Farm,Guntur, Andhra Pradesh
25.	GP37	Tutiminumu	ARS, Ghantasala, Andhra Pradesh
26.	GP39	TBG 106	RARS, Tirupati, Andhra Pradesh
27.	GP40	SRI	ARS, Ghantasala, Andhra Pradesh
28.	GP41	Maruthi	ARS, Ghantasala, Andhra Pradesh
29.	GP42	Nandhi	ARS, Ghantasala, Andhra Pradesh
30.	GP43	GBG 47	ARS, Ghantasala, Andhra Pradesh
31.	GP44	GBG-45	ARS, Ghantasala, Andhra Pradesh
32.	GP46	GBG 1	ARS, Ghantasala, Andhra Pradesh
33.	GP50	GKB 4	ARS, Ghantasala, Andhra Pradesh
34.	GP51	VBG 12-110	NRPC, Vamban, Tamil Nadu
35.	GP52	VBG 13-3	NRPC, Vamban, Tamil Nadu
36.	GP53	VBG 14-16	NRPC, Vamban, Tamil Nadu
37.	GP54	VBG 12-034	NRPC, Vamban, Tamil Nadu
38.	GP56	IPU 11-2	ARS, Ghantasala, Andhra Pradesh
39.	GP58	VBG 17-026	NRPC, Vamban, Tamil Nadu
40.	GP59	ADT 6	ARS, Ghantasala, Andhra Pradesh

Table 2: Analysis of variance for yield, yield components and seed protein content in blackgram

Source of variation	Degrees of freedom	Days to 50 per cent flowering	Days to maturity	Plant height	Number of clusters per plant	Number of Pods per plant	Pod length	Number of Seeds per pod	Hundred seed weight	Seed protein content	Seed yield per plant
		Mean sum of squares									
Replications	2	5.83	15.46	2.54	0.07	0.07	0.04	0.15	0.01	0.30	0.07
Treatments	39	99.38**	187.39**	110.94**	15.40**	188.68**	1.44**	1.63**	2.34**	9.83**	5.09**
Error	78	2.40	8.77	2.23	0.16	0.98	0.04	0.10	0.07	0.17	0.07

****** Significant at 1 per cent level

Table 3: Clustering pattern of 40 genotypes for yield, yield components and seed protein content in blackgram

Clusters	No. of genotype(s)	Name of genotype(s)	Source
I	14	TBG-106, GKB-4, SRI, MARUTHI, TGBG-143, TUTIMINUMU, T-9, VBG 12-110, NANDHI, VBG 13-3, GBG-45, VBG 14-16, TGBG-401, GBG-1	Ghantasala, Tirupati and Vamban
II	5	IPU 2-43, LBG-788, KUG 216 X SPS 5, LBG-752, VBG 12-034	Ghantasala, Guntur and Vamban
III	5	TGBG-281, KUG 216 X BG 018-2, TGBG-74, DPU 8831 X VBG 4-088, TGBG-344	Ghantasala
IV	7	LBG-645, TBG-104, TU-94-2, TGBG-26, GBG-47, WBG-108, ADT-6	Guntur and Tirupati
V	6	PU-40, TU-18, KUG 216 X PU 40, PU-31, TGBG-136, TGBG-258	Pantnagar and Ghantasala
VI	1	LBG-20	Guntur
VII	2	IPU 11-2, VBG 17-026	Ghantasala and Vamban

Table 4: Average intra-and-inter-cluster D² values among seven clusters of blackgram genotypes for yield, yield components and seed protein content

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Cluster I	36.45	58.09	307.8	86.79	88.74	120.2	399.01
Cluster II		31.79	448.45	153.7	116.13	65.34	517.49
Cluster III			45.6	192.47	304.68	567.1	121.34
Cluster IV				67.85	138.65	234.46	272.52
Cluster V					79.72	220.29	337.32
Cluster VI						0	622.88
Cluster VII							46.36

Note: Diagonal values are intra-cluster distances. Off-diagonal values are inter-cluster distances.

Table 5: Cluster means of 40 blackgram genotypes for yield, yield components and seed protein content

Clusters	Days to 50 per cent flowering	Days to maturity	Plant height	Number of clusters per plant	Number of pods per plant	Pod length	Number of seeds per pod	Hundred seed weight	Seed protein content	Seed yield per plant
I	37.02	68.64	51.07	11.67	48.44	4.18	7.59	5.3	19.55	8.86
II	39.87	72.93	52.45	13.46	52.87	3.14	6.89	4.89	20.27	8.92
III	38.73	68.2	40.31	7.88	31.28	4.75	8.34	5.41	22.35	5.64
IV	36.62	70.2	48.5	10.74	38.48	4.24	7.5	5.03	19.05	8.58
V	34.5	66.11	45.58	9.45	50.64	3.47	7.88	4.28	19.01	7.59
VI	38	65.67	53.53	14.2	52.6	2.37	6.57	6.19	23.43	10.3
VII	30.83	62.17	36	6.23	31.5	3.87	6.55	2.52	23.92	6.58

Table 6: Relative contribution of characters towards genetic divergence in blackgram

Source	Contribution %	Times ranked 1st
Days to 50 per cent flowering	10.77%	84
Days to maturity	64%	5
Plant height	4.87%	38
Number of clusters per plant	11.28%	88
Number of pods per plant	44.36%	346
Pod length	8.59%	67
Number of seeds per pod	0.26%	2
Hundred seed weight	3.33%	26
Seed protein content	9.62%	75
Seed yield per plant	6.28%	49

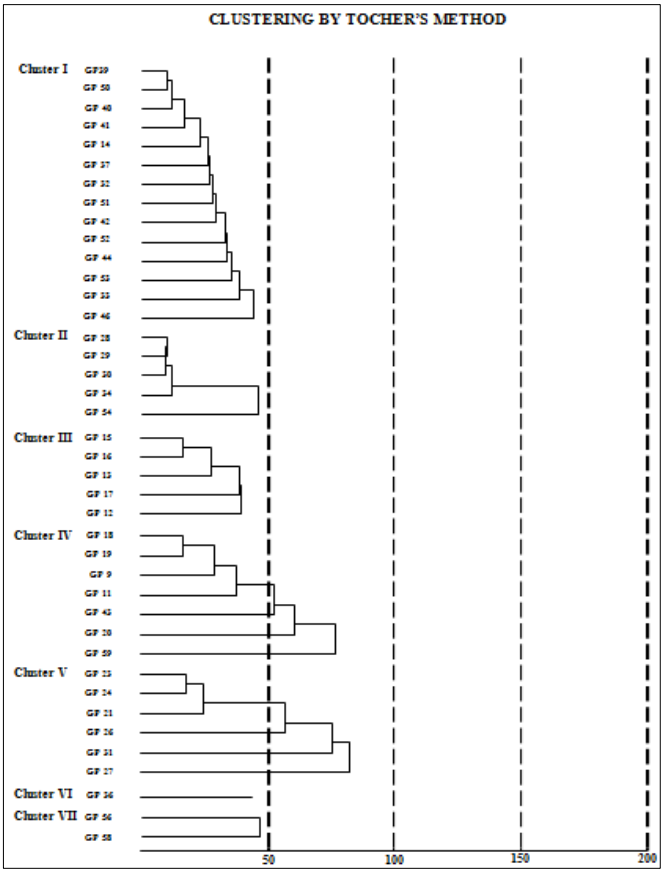

Fig 1: Grouping of 40 blackgram genotypes into different clusters

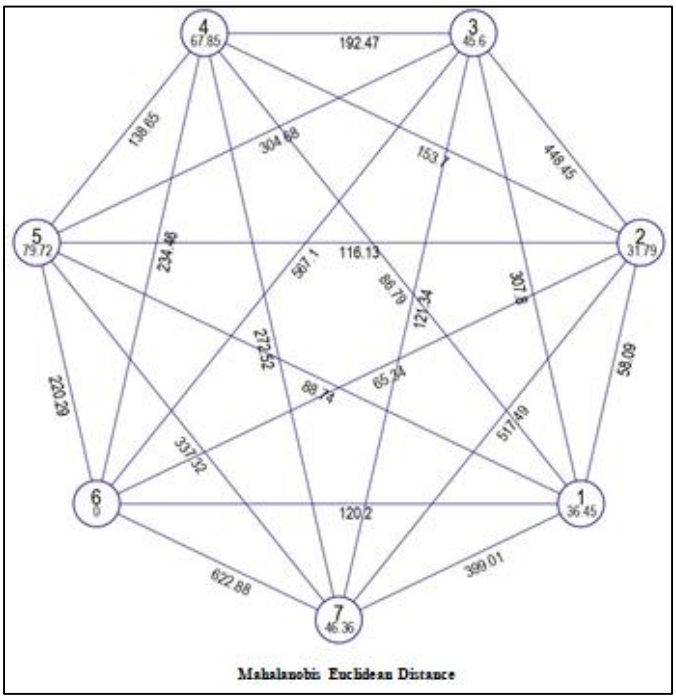

Fig 2: Inter-and-intra-cluster distance of 40 blackgram genotypes in seven clusters

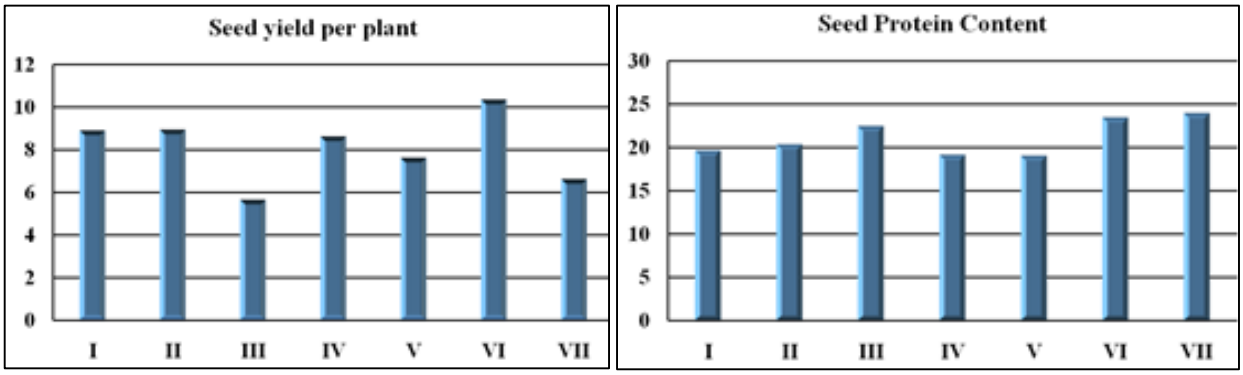

Fig 3: Seed yield per plant and seed protein content of different clusters obtained in the present study

4. Conclusion

The results suggest hybridization between the genotypes of the highly divergent clusters, namely, Cluster VI (LBG 20) and Cluster VII (IPU 11-2 and VBG 17-026) for judicious combination of seed yield and seed protein content, in addition to other important yield component traits for the development of high yielding blackgram varieties with superior levels of seed protein content.

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