Electronic Journal of Plant Breeding

Research Article

Estimation of genetic distances among various genotypes of blackgram (*Vigna mungo* (L.) Hepper) using D² statistics

Md. Ayesha[1], D. Ratna Babu[1]*, A. Prasanna Rajesh[2], Lal Ahmed Md[1] and V. Manoj Kumar[3]

[1]Department of Genetics and Plant Breeding, Agricultural College, Bapatla
[2]Department of Genetics and Plant Breeding, Agricultural College, Mahanandi
[3]Department of Plant Pathology, Agricultural College, Bapatla
*E-Mail: didlaratnababu@gmail.com

Abstract
The genetic distance assessment of forty blackgram genotypes was conducted employing Mahalanobis' D² statistics considering ten quantitative characters. All the studied genotypes were grouped in ten discrete clusters, among which cluster I was indeed the largest with thirteen genotypes followed by cluster V with eight genotypes, cluster IV with seven genotypes and cluster II with five genotypes. Cluster IX was digenotypic, other clusters III, VI, VII, VIII and X were all solitary. Cluster IV and IX had the greatest inter-cluster gap followed by cluster VIII and IX, clusters VII and IX, clusters II and IX, clusters III and IX, clusters V and IV, clusters IX and Cluster X and between cluster VI and IX, implying that a successful breeding programme can be begun by selecting diverse lines to improve productivity and other yield-related traits. In the current investigation, cluster X, cluster VI, cluster IV and cluster VIII were considered divergent enough and found to be the best. Hence, genotypes from these clusters *viz.*, TU 94-2 from cluster IX, PU 31 from cluster VI, IPU 94-1 from cluster IV and LBG 623 from cluster VIII can be used in the crossing programme for additional genetic enhancement. Cluster IX had the highest mean score for traits *viz.*, the number of clusters per plant, the number of pods per plant, pod length, test weight and grain yield per plant. The number of pods per plant provided the most to genetic diversity succeeded by the number of clusters per plant, days to maturity, plant height, pod length and test weight. As a result, in a crossing programme, selecting and utilising genotypes based on these traits will be more rewarding. Furthermore, taking into account the genotype disposition in various clusters, one can perform different possible crosses to produce heterotic hybrids or transgressive segregants, based on the nature of gene action regulating the traits.

Key words: Blackgram, Genetic distance, Genetic divergence, D², *Vigna mungo*

INTRODUCTION

Blackgram (*Vigna mungo* (L.) Hepper) is a self-pollinating grain legume with chromosome number 2n = 2x=22 and is a widely grown crop in the Fabaceae family (Naga *et al*., 2006). It is mainly cultivated in Asia since ancient times (Paroda and Thomas, 1987). In India, it is cultivated under wide agro-ecological zones. The country that produces and consumes the most blackgram is India. In India, blackgram is grown on 56.02 lakh hectares with average production and productivity of 30.60 lakh tonnes and 546 kg/ha, while it is grown on 3.18 lakh hectares in Andhra Pradesh with an output of 3.10 lakh tonnes and productivity of around 977 kg/ha. (Ministry of Agriculture, 2018). Blackgram is the ultimate source of protein for vegetarians. Protein (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids, and vitamins all contribute to its high nutritional value. (Singh and Singh, 2013). In any hybridization programme, genetic variation is an essential consideration and a requirement.

The involvement of a variety of parents in a hybridization programme helps in obtaining desirable recombination (Jayamani and Sathya, 2013). Therefore, characterization and divergence analysis of the available genotypes is needed for modern crop improvement programmes. The limited amount of blackgram potential is utilized for varietal development programmes, (Roy *et al*., 2016). Further, only a few studies were performed on the extent of genetic variability and divergence analysis using diverse blackgram genotypes (Ghafoor *et al*., 2001).

Any breeding programme needs a thorough understanding of genetic divergence. Genetic diversity analysis information aids in the identification of genetically diverse genotypes for use in breeding programmes. For effective hybridization programmes, a variety of biometrical approaches have been used to select parents. D^2 analysis has proven to be the most efficient and commonly used method for classifying parental lines for the production of high yielding genotypes in blackgram (Dasgupta and Das, 1984; Elangaimannan *et al*., 2008; Neelavathi and Govindarasu, 2010; Rao *et al*., 2019).

It is important to identify genetic materials that contain desirable traits when developing populations for various breeding programmes or varietal selection. As a result, an effort was made in the current investigation to classify and understand the nature and magnitude of the genetic diversity of 40 blackgram genotypes obtained from different research stations for various yields and its related traits.

MATERIALS AND METHODS
The current investigation was conducted using 40 genotypes of blackgram collected from various research stations *viz*., RARS Lam, ARS Gantasala and Baba Atomic Research Centre (BARC). During *Kharif*, 2019-20 these 40 genotypes were raised in a Randomised Complete Block Design with three replications at APGC, Lam, Guntur. Each genotype was represented by two lines of three metre length, with a 30 cm x l0 cm spacing between and within the rows. All the recommended packages of practices and need-based crop protection measures were also followed. For eight quantitative traits *viz*., plant height (cm), the number of branches per plant, the number of clusters per plant, the number of pods per plant, pod length (cm), the number of seeds per pod, test weight (g) and grain yield per plant (g) observations were taken on five randomly selected plants in each replication, and their mean values were statistically analysed. However, data on days to 50% flowering as well as days to maturity were obtained on plot basis.

The Mahalanobis D^2 statistic (Mahalanobis, 1936) was used to assess the genetic divergence in forty genotypes

for ten characters. For each of the ten characters under investigation, variances were calculated and a test of significance was performed. The mean values were used to approximate the analysis of covariance (ANCOVA) for the character pairs and a dispersion table was prepared using these estimates. Following the testing of genotype differences for each character, a simultaneous test of significance of the difference between the mean values of a number of correlated variables was performed (Rao, 1952) using the 'V statistic, which employs Wilk's criterion (Wilk, 1932). For this, the sum of squares and sum of products of error and error plus variety, variance - covariance matrix, was used (Panse and Sukhatme, 1978).

RESULTS AND DISCUSSION
For all the characters studied, analysis of variance revealed highly significant variations among the accessions, indicating a significant amount of variability in the experimental material. D^2 values calculated from the means of 40 blackgram genotypes for 10 quantitative characters were used to conduct genetic divergence analysis, and these genotypes were clustered into ten distinct nonoverlapping clusters (**Table 1**), indicating the existence of maximum divergence for further crop improvement. Cluster I had the highest number of genotypes (13), succeeded by cluster V with 8 genotypes. Cluster IV, with seven genotypes, was the third largest cluster, followed by Cluster II which had five genotypes. Cluster IX was digenotypic, while the remaining five clusters; III, VI, VII, VIII and X were solitary. Geographic barriers preventing gene flow or intense natural and human selection for diverse and adaptable gene complexes may be responsible for the development of distinct solitary clusters (Arunachalam and Ram, 1967).

Table 2 displays the average intra- and inter-cluster D^2 distances and the distance within the clusters varied from 0.00 to 28.00. Cluster V recorded the maximum intra cluster distance of 28.00, followed by cluster IV (20.32), cluster IX (17.04), and cluster II (16.97), suggesting that within these clusters, there exist a considerable amount of genetic variability. Clusters III, VI, VII, VIII, and X have one genotype each and therefore the intra cluster gap for these clusters was found to be (0.00).

The maximum inter-cluster gap was found between clusters IV and IX (172.09), followed by clusters VIII and IX (148.19), clusters VII and IX (144.29), clusters II and IX (134.44), clusters III and IX (129.37), clusters V and IV (94.44), clusters IX and cluster X (85.96) and between clusters VI and IX (82.62) indicating that the genotypes of these clusters are diverse (**Fig. 1**), and thus the selection of parents for hybridization programmes between these clusters produces a wide range of variability in the segregating population.

Table 1. Clustering pattern of 40 blackgram genotypes by Tocher's method

Cluster Number	Number of genotypes	Name of genotypes
I	13	GKB 2, GBG 1, TBG 104, MBG 1050, VBG 4-008, VBG 12-110, GKB 3, GKB 4, MBG 1053, VBG 4-14, LBG 20, MBG 1069, VBG 13-003
II	05	PU 212, LBG 771, LBG 726, GKB 1, LBG 752
III	01	LBG 787
IV	07	MBG 207, VAMBAN 8, UAHS BG 6, KPU 26, IPU 94-1, UAHS BG 2, IPU 7-3
V	08	UAHS BG 7, IPU 2-43, UAHS BG 8, UAHS BG 3, VBG 14-016, MBG 1046, TBG 106, GBG 12
VI	01	PU 31
VII	01	UAHS BG 1
VIII	01	LBG 623
IX	02	GAVT 12, GAVT 7
X	01	TU 94-2

Table 2. Average intra and inter-cluster distances (D^2 values) among ten clusters of 40 blackgram genotypes

Cluster Number	I	II	III	IV	V	VI	VII	VIII	IX	X
I	**11.61**	32.15	40.22	55.96	37.13	24.06	49.81	40.09	64.53	44.44
II		**16.97**	26.39	40.26	69.29	48.83	39.64	23.49	134.44	50.68
III			**0.00**	47.93	47.37	61.06	14.87	16.72	129.37	57.61
IV				**20.32**	94.44	37.92	30.73	51.40	172.09	42.56
V					**28.00**	58.01	54.22	56.54	53.37	60.51
VI						**0.00**	55.22	64.23	82.62	28.78
VII							**0.00**	25.07	144.29	45.54
VIII								**0.00**	148.19	73.86
IX									**17.04**	85.96
X										**0.00**

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

Cluster III and VII (14.87), cluster III and VIII (16.72) and cluster II and VIII (23.49) showed lower inter cluster distances, indicating that the genotypes of these clusters are less diverse and have a similar genetic makeup. For the purposes of further selection and the choice of parents for hybridization, the clusters that contribute the most to the divergence should be given more attention. For ten characters related to yield in blackgram, the cluster mean values were calculated over genotype and this revealed a wide range of variation between the clusters (**Table 3**). The number of days taken to attain 50% flowering was minimum in genotypes of cluster VI (37.00 days) followed by genotypes of cluster I (42.67), while the maximum number of days taken by the genotypes to attain 50% flowering was recorded in cluster VII (51.33). The highest

mean for plant height was recorded by cluster II (88.30 cm) and the lowest by cluster VII (29.80 cm). The number of branches per plant had a range of 1.97 in cluster IV to 3.44 in cluster IX, the number of clusters per plant varied from 4.95 (cluster IV) to 20.50 (cluster IX). Cluster IX recorded the highest mean (57.04) for the number of pods per plant, while cluster VIII recorded the lowest mean (10.52). Pod length had a range of 4.35 cm (cluster VII & cluster X) to 5.19 cm (cluster IX), the number of seeds per pod had a range of 5.47 (cluster X) to 7.38 (cluster I), test weight varied from 3.92 g (cluster VIII) to 4.88 g (cluster IX), days to maturity varied from 73.00 days (cluster VI) to 89.00 days (cluster III) and grain yield per plant ranged from 4.42 g (cluster IV) to 14.76 g (cluster IX). As a result, crosses between members
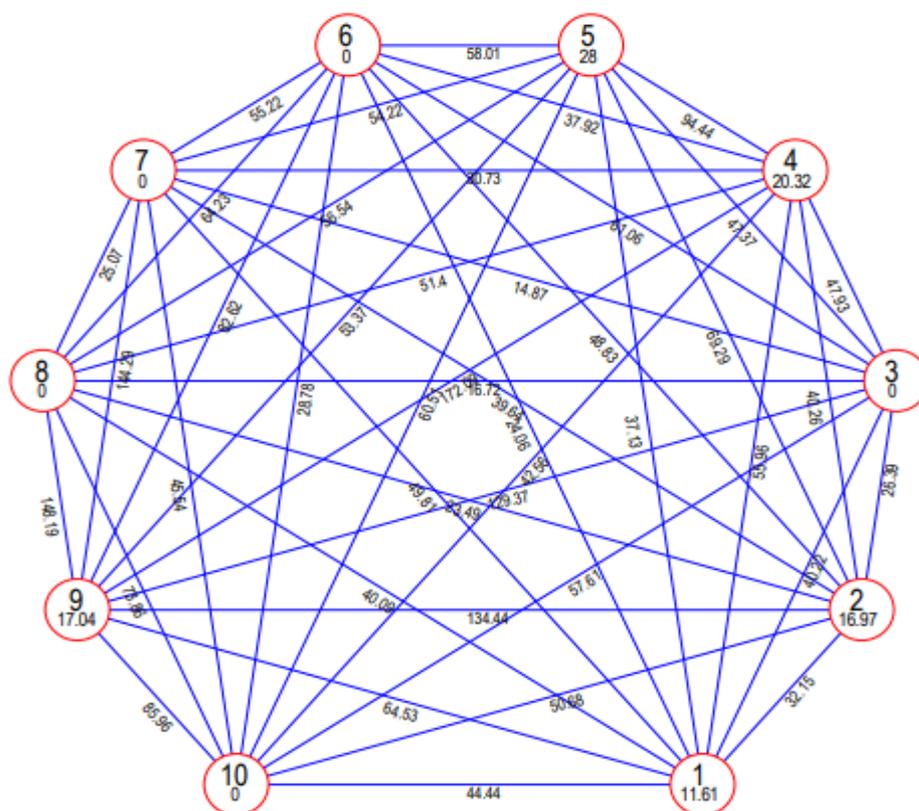
Fig. 1. Intra and inter-cluster distance of 40 blackgram genotypes in ten clusters based on Tocher's method

Table 3. Mean values of ten clusters estimated by Tocher's method in 40 blackgram genotypes

Cluster Number	Days to 50% flowering	Plant height (cm)	Number of branches per plant	Number of clusters per plant	Number of pods per plant	Pod length (cm)	Number of seeds per pod	Test weight (g)	Days to maturity	Seed yield per plant (g)
I	42.67	61.29	2.90	11.56	27.85	5.05	7.38	4.39	74.75	10.20
II	44.40	88.30	2.37	5.97	17.32	4.82	6.40	4.21	79.29	5.53
III	49.00	71.23	2.27	8.07	17.80	4.39	6.93	4.80	89.00	10.51
IV	43.76	35.72	1.97	4.95	15.22	4.35	5.69	3.99	76.86	4.42
V	46.71	52.91	3.00	15.54	35.50	5.11	7.14	4.46	85.58	12.86
VI	37.00	30.18	3.07	9.80	27.87	4.41	6.73	4.34	73.00	8.41
VII	51.33	29.80	2.33	7.47	18.87	4.61	6.87	4.33	86.67	7.66
VIII	47.00	75.33	3.29	8.13	10.52	5.08	7.07	3.92	88.00	5.70
IX	43.00	57.08	3.44	20.50	57.04	5.19	7.37	4.88	75.67	14.76
X	45.00	50.43	2.87	6.86	41.47	4.35	5.47	4.01	76.33	6.19

of the cluster with a high inter-cluster gap and a high mean value for key traits are likely to be more promising. The best donor for hybridization can be picked from a suitable cluster and used in breeding programmes to improve any specific trait (Chauhan *et al*., 2008; Elangaimannan *et al*., 2008). Cluster IX has the maximum

mean value for traits *viz*., the number of clusters per plant, the number of pods per plant, pod length, test weight and grain yield per plant.

Similar usage of genetic distance analysis through Mahalanobis' D^2 statistics (Tocher's method) in obtaining

Table 4. Contribution of different characters towards genetic divergence in 40 blackgram genotypes

S. No.	Source	Number of times ranked first	Per cent contribution
1	Days to 50% flowering	27	3.46
2	Plant height	80	10.26
3	Number of branches per plant	8	1.02
4	Number of clusters per plant	158	20.26
5	Number of pods per plant	211	27.04
6	Pod length	56	7.18
7	Number of seeds per pod	23	2.95
8	Test weight	46	5.9
9	Days to maturity	152	19.49
10	Seed yield per plant	19	2.44

the respective cluster diagram and dendrogram to understand the genetic diversity was earlier employed in various crops (Naik *et al*., 2016 in American cotton; Gowsalya *et al*., 2017 in blackgram; Shivani *et al*., 2018 in rice; Ayesha and Babu., 2019 in foxtail millet and Mohan *et al*., 2019 in greengram) to indicate the successful hybrid combination to obtain superior hybrids or transgressive segregants depending on the gene action guiding for the inheritance of different traits.

When deciding the clusters for the purpose of selecting parents for further hybridization, the characters that contribute to maximum divergence should be given more weight. The per cent contribution of all the ten characters in the 40 genotypes towards genetic divergence is presented in **Table 4**. The number of pods per plant (27.04%) contributed the most to genetic diversity among the different traits, followed by the number of clusters per plant (20.26%), days to maturity (19.49%), plant height (10.26%), pod length (7.18%) and test weight (5.9%), while the number of seeds per pod (2.95%), grain yield per plant (2.44%), days to 50% flowering (3.46%) and the number of branches per plant (1.02%) contributed least towards the genetic divergence. As a result, genotype selection and use based on traits that contribute the most to diversity will be more useful in selection for hybridization programmes.

It is not mandatory that genotypes should only be selected from more diverse clusters, instead genotypes belonging to any two different clusters could also be diverse, with a considerable amount of genetic distance between them and such genotypes can also be preferred for conducting crosses because they have a fair chance of generating heterotic hybrids or transgressive segregants, provided that they report maximum per se values (data not presented for brevity) for the majority of yield contributing traits. In light of the above, crosses can be made among TU 94-2 (cluster X), PU 31 (cluster VI), IPU-94-1

(cluster IV) and LBG 623 (cluster VIII) in all possible ways to create more variation in the segregating population and to develop superior high yielding blackgram varieties.

REFERENCES

Arunachalam, V. and Ram, J. 1967. Geographical diversity in relation to genetic divergence in cultivated sorghum. *Indian J. Genet*., **27**: 369-380.

Ayesha, Md. and Babu, D.R. 2019. Genetic divergence studies in foxtail millet [*Setaria italica* (L.) Beauv.]. *Electronic Journal of Plant Breeding,* **10**(1): 18-29. [Cross Ref]

Chauhan, M.P., Mishra, A.C and Singh, A.K. 2008. Genetic divergence studies in urd bean (*Vigna mungo* L.). *Legum. Res.,* **31**(1): 63-67.

Dasgupta, T. and Das, P. 1984. Multivariate analysis and selection of parents for hybridization in blackgram. *Philippine Agriculturist*., **67**(1): 86-92.

Elangaimannan, R., Anbuselvam, Y. and Karthikeyan, P. 2008. Genetic diversity in blackgram [*Vigna mungo* (L.) Hepper]. *Legum. Res.,* **31**(1): 57-59.

Ghafoor, A., Sharif, A., Ahmad, Z., Zahid, M.A. and Rabbani, M.A. 2001. Genetic diversity in blackgram [*Vigna mungo* (L). Hepper]. *Field Crops Res*., **69**: 183-190. [Cross Ref]

Gowsalya, P., Kumaresan, D., Packiaraj, D. and Bapu, J.R.K. 2017. Genetic divergence in blackgram [*Vigna mungo* (L) Hepper]. *Indian J. Agric. Res.,* **51**(2): 184-187.

Jayamani, P. and Sathya, M. 2013. Genetic diversity in pod characters of blackgram (*Vigna mungo* L. Hepper). *Legum. Res.,* **36**(3): 220-223.

Mahalanobis, P.C. 1936. On the generalized distance in statistics. Proceedings of National Institute of Sciences (India). pp. 49-55.

Ministry of Agriculture. 2018. Government of India. http://www.indiastat.com

Mohan, S., Sheeba, A. and Kalaimagal, T. 2019. Genetic diversity and association studies in greengram [*Vigna Radiata* (L.) Wilczek]. *Legum. Res.* DOI: 10.18805/LR-4176. [Cross Ref]

Naga, N., Sharma, S.K. and Kant, S.K. 2006. Agronomic evaluation of some induced mutants of urd bean [*Vigna mungo* (L.) Hepper]. *SABRAO J Breed Genet.,* **38**: 29-38

Naik, B.M., Satish, Y. and Babu, D.R. 2016. Genetic diversity analysis in American cotton (*Gossypium hirsutum* L.). *Electronic Journal of Plant Breeding,* **7**(4): 1002-1006. [Cross Ref]

Neelavathi, S. and Govindarasu, R. 2010. Analysis of variability and diversity in rice fallow blackgram [*Vigna mungo* (L.) Hepper]. *Legum. Res.,* **33**: 206-210.

Panse, V.G and Sukhatme, P.V. 1978. *Statistical Methods for Agricultural Workers*. ICAR, New Delhi. 103-108.

Paroda, R.S. and Thomas, T.A. 1987. Genetic resources of mungbean [*Vigna radiata* (L.) Wilczek] in India. *In: mungbean, Proceeding of the Second International Symposium*. 16-20 November, Bangkok, Thailand.

Rao, C.R. 1952. *Advanced Statistical Methods in Biometrical Research.* John Wiley and Sons INC., New York. 357-363.

Rao, K.N., Bandi, H.R.K., Srinivasulu, K., Padmavathi, J and Krishna, K.V. 2019. Genetic diversity for yield and yield component characters in rice fallow blackgram [*Vigna mungo* (L.) Hepper]. *Journal of Food Legumes*., **32**: 1, 57-59.

Roy, D., Kishore, A., Joshi, A.P.K and Mishra, B. 2016. Pulses for food and nutritional security: The technology perspective. *Indian J. Genet.,* **76**(4): 375-387. [Cross Ref]

Shivani, D.K., Dwivedi., Husain, R., Gyanendra, K. and Khan, N.A. 2018. Genetic divergence for yield and other quantitative traits in rice (*Oryza sativa* L.). *Int J Curr Microbiol Appl Sci.,* **7**(1): 1201-1207. [Cross Ref]

Singh, S.K. and Singh, G.C. 2013. Effect of phosphorus, sulphur and zinc on nutrient composition in Blackgram . *The Journal of Rural and Agricultural Research*., **13**(2): 63-64.

Wilk, S.S. 1932. Criterion of generalization in the analysis of variance. *Biometrika*. **24**: 471-484. [Cross Ref]