Received: 19 Nov 2022 | Accepted: 21 Apr 2023 | Online: 29 Apr 2023

Electronic Journal of Plant Breeding

Research Note

Assessment of genetic diversity in blackgram [*Vigna mungo* (L.) Hepper] germplasm

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Abstract
Genetic diversity analysis was carried out with 50 black gram genotypes by using Mahalanobis D^2 statistics for ten quantitative traits. All the genotypes were grouped into ten non-overlapping clusters. Cluster I was the largest with 19 genotypes, followed by cluster II with 14 genotypes, cluster III with 8 genotypes, clusters VII and IX were digenotypic, while the remaining clusters IV, V, VI, VII and X were monogenotypic. The maximum inter-cluster distance was observed between cluster VII and IX. Cluster IX had recorded high mean values for most of the traits. Selection of genotypes from these diverse clusters with high mean performance and more per cent contribution as parents in the crossing may result in superior combinations along with maximum variability in the segregating generations. Hence, crosses among the genotypes *viz*., LBG 623 (cluster I), LBG 787 (cluster III), DKU 87 (cluster II) and VBN 8 (cluster VII) may result in production of superior pureline varieties in black gram. Among the traits, pods/plant contributed maximum towards the total divergence.

Keywords: Genetic diversity, Black gram, Mahalanobis D^2, Pods/plant

Black gram [*Vigna mungo* (L.) Hepper] also known as urd bean, is one of the important short duration legume crop in India. It belongs to the *Fabaceae* family having diploid chromosome number 2n=2x=22 with an estimated genome size of 574 Mbp (Arumuganathan and Earle, 1991; Debbarma *et al*., 2022). Black gram seeds are nutritious and consists of 25-26 % of easily digestible protein, 60 % of carbohydrates, 1.5 % fats and also a good source for minerals, vitamins and amino acids (Jeberson *et al*., 2018). It enriches the soil fertility and soil properties through its biological nitrogen fixation as other legumes.

It was originated in Indian subcontinent (Vavilov, 1926) and globally cultivated widely in India, Bangladesh, Philippines, Myanmar, Pakistan, Thailand, Indonesia and China (Poehlman, 1991). It adapts well to different cropping systems and also succeeds well as sole crop under rice fallows with residual moisture conditions. Among various black gram growing countries, India is the largest producer with a production of 2.22 million tonnes from 4.14 million hectares and with an average productivity of 538 kg/ha (Ministry of Agriculture, 2020-2021). Even though India is the largest producer of black gram, its average productivity and per cent contribution towards the total pulse production is less due to the lack of genetic variability, unavailability of potential ideotype, poor harvest index and lack of resistances to biotic and abiotic stresses (Devi *et al*., 2020). These constraints are due to ingeminate usage of related parents in hybridization programmes (Jayamani and Sathya, 2013)

and breeding for few economic traits to reach the targets of market requirements (Naing *et al*., 2022). Hence, to develop the cultivars with wide genetic base, there is a need to estimate the genetic divergence among the available black gram germplam.

Assessment of genetic divergence helps in recognition of genotypes with diverse gene complexes and hybridization between them will be responsive for genetic improvement (Arunachalam, 1981). Among different biometrical techniques available for divergence studies, Mahalanobis' D^2 statistic (1936) explained by Rao (1952) is a robust technique in quantifying the degree of divergence at species and sub-species level in classifying the genotypes. In view of the above facts in consideration, present study was conducted to classify and to understand the nature and magnitude of divergence in the 50 black gram genotypes based on D^2 analysis.

Fifty blackgram germplasm lines were evaluated for their genetic diversity. All the genotypes were raised experimentally by following the statistical design, ARCBD-II (Augmented Randomized Complete Block Design-II) with four checks and five blocks at RARS, Lam, Guntur during *kharif*, 2021. Each test genotype and checks were accommodated in two rows of four meters length with 30 cm between rows and 10 cm within rows spacing. All agronomic and plant protection measures were taken up as per recommendation to raise healthy crop. Data was recorded on 10 traits of which eight traits (plant height (cm), branches/plant, clusters/plant, pods/plant, pod length (cm), seeds/pod, test weight and grain yield/plant) data was recorded on per plant basis, where, the remaining two traits (number of days taken to attain 50 % flowering and maturity) data was recorded per plot.
The data on the recorded observations for all ten traits were subjected to statistical analysis. ANOVA was taken up to know the genetic variability and significance of variation for each trait among the studied genotypes as per Federer (1956) and Fisher and Yates (1967), respectively. The magnitude and nature of genetic divergence among the 50 genotypes was obtained by following Mahalanobis D^2 statistics (Mahalanobis, 1936) and clustering was done by employing Tocher's method (Rao, 1952).

The basic pre-requisite for clustering of genotypes through multivariate analysis is existence of significant differences among the genotypes for multivariate traits (Punithavathy *et al*., 2020). The univariate analysis of variance unfolds the existence of significant differences for all the ten characters. The pooled differences determined by the Wilk's criterion were also significant among the 50 genotypes and justifying to proceed for the calculation of D^2 values. Similar results have been reported by Rajalakshmi *et al*. (2020) and Punithavathy *et al*. (2020).

Means of all the ten characters of 50 black gram genotypes were analyzed by Mahalanobis D^2 analysis. Further, the distance between all pairs of genotypes (n(n-1)/2 pairs) was calculated based on the relative magnitudes of D^2 estimates and the genotypes were categorized into ten clusters by following Tocher's clustering method (**Table 1, Fig.1**). Out of the ten clusters, cluster I was the largest with 19 genotypes followed by 14 genotypes in cluster II and 8 genotypes in cluster III. Clusters VII and IX had two genotypes each. While, the remaining five clusters *ie*., IV, V, VI, VII and X were solitary consisting of one genotype with unique characters. The grouping of genotypes into different non-overlapping discrete clusters confirmed the existence of broad genetic diversity in the studied experimental material. The clustering of genotypes from same geographical region into different clusters and genotypes from different geographic regions into same cluster revealed that the geographical diversity may not necessarily represent the genetic diversity

Table 1. Distribution of 50 black gram genotypes in various clusters using Tocher's method

Cluster Number	Number of genotypes	Name of genotype (S)
I	19	LBG 808, GBG 12, LBG 889, LBG 972, PU 1815, PU 1527, ADT 5, PU 19, IPU 16-9, LBG 806, LBG 941, LBG 922, MBG 1037, LBG 623, WBU 108, SPS-5, LBG 818, Uttara, LBG 852
II	14	PBG 221, OBG 39, OBG 103, LBG 867, SUG 11-37, TBG 125, LBG 884, TBG 141, TBG 129, TU-68, DKU 87, PU 14-28, MBG 1050, LBG 851
III	08	LBG 824, CoBG 13-04, ABG 12, LBG 809, T 9, VBN-10, PU 1805, LBG 787
IV	01	ACM 14-001
V	01	GBG 45
VI	01	VBN 6
VII	02	PU 31, VBN-8
VIII	01	VBN-11
IX	02	LBG 904, LBG 932
X	01	Sekhar-2

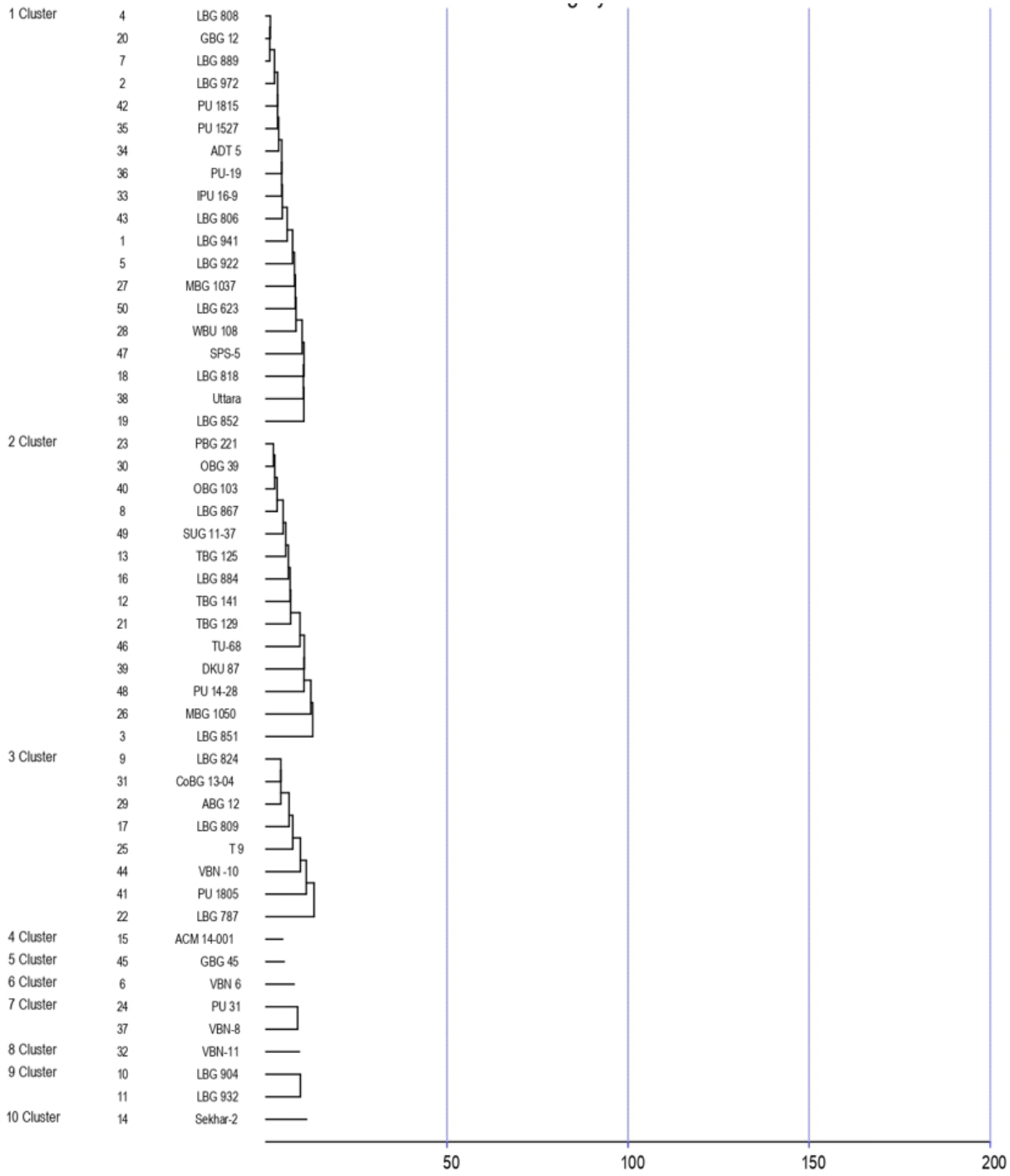

Fig. 1. Clustering pattern of 50 black gram genotypes based on Tocher's method.

(Murty and Arunachalam, 1966). Monogenotypic clusters were obtained may be due to the geographic barriers preventing gene flow or intense natural and artificial selection for diverse and adaptable gene complexes (Arunachalam and Ram, 1967). The clustering of genotypes into discrete clusters by using Mahalanobis D^2 statistics were earlier done in black gram by various scientists (Panigrahi *et al*., 2014; Ayesha *et al*., 2021; Chippy *et al*., 2021).

The distance between the clusters represents the degree of divergence among the genotypes. The inter and intra-cluster average distances were obtained from the means of euclidian D^2 values of each cluster. The average inter and intra-cluster divergences among the ten clusters were presented in **Table 2**. Intra-cluster distance was varied from 0.00 to 30.61. The maximum intra-cluster distance was recorded by Cluster III, followed by, cluster II (26.91), cluster IX (25.45), cluster VII (23.38) and cluster I (22.04), suggesting that, the genotypes grouped under these clusters have substantial amount of genetic divergence among them. Regarding inter-cluster distances, the maximum inter-cluster divergence was found between clusters VII and IX (580.85), followed by clusters VII and VIII (336.57), clusters IX and X (326.23). This designates that, the genotypes in these clusters are more divergent. Hence, for hybridization, genotypes selection from these clusters may generate desirable transgressive segregants in segregating generations. The inter-cluster divergence was minimum between clusters I and IV (33.32), followed by clusters I and V (34.20), clusters IV and V (38.39) (**Fig.2**). This reveals that, the genotypes specified under these clusters may have similar proximity of genetic makeup.

The range of cluster mean performances for the studied 10 characters revealed the existence of wide variability (**Table 3**). The least number of days to attain 50% flowering was recorded by clusters VI, VII and X (40 days). Hence, genotypes from these three clusters can be used as a source for earliness. Cluster IX recorded the maximum mean values for most of the traits like plant height (89.27 cm), branches/plant (6.45), clusters/plant (14.81), pods/plant (67.00), pod length (6.45cm), seeds per pod (8.11), test weight (5.17) and grain yield/plant (10.41). The preferable least mean performance for days to maturity was noticed for cluster VII (71 days).

Selection of more divergent genotypes based on the estimates of genetic distances along with the consideration of maximum percentage of character contribution to the total diversity would result in more variability in segregating generations. The character contribution towards total diversity has been estimated based on the number of times each trait appeared in the first rank among the total pairs of genotypes. The per cent contribution of the characters under study towards the total diversity was presented in **table 4**. The maximum trait contribution was due to pods/plant (38.45%), followed by plant height (18.37%) and days to maturity (14.94%). This implies that the parental selection from the clusters with highest mean value for pods/plant will generate more variation during segregation in further generations.

However, genotypes selection based on the more divergent clusters is not mandatory, instead genotypes grouped under any two clusters will have ample amount of genetic diversity (Ayesha *et al*., 2021). Hence crossing between genotypes with high performance grouped under any two different clusters may have a fair chance of generating heterotic hybrids or transgressive segregants. It can be concluded from this present investigation that

Table 2. Average intra and inter-cluster distances (D^2 values) among ten clusters (obtained by Tocher's method) of 50 black gram genotypes

Cluster Number	I	II	III	IV	V	VI	VII	VIII	IX	X
I	**22.04**	50.35	43.99	33.32	34.20	53.59	182.09	119.21	254.38	83.93
II		**26.91**	52.20	57.73	45.62	42.87	134.99	89.62	207.07	52.21
III			**30.61**	76.39	51.85	61.28	245.59	63.56	150.36	94.46
IV				**0.00**	74.23	38.39	103.45	182.08	361.16	59.16
V					**0.00**	83.40	213.79	90.23	200.23	118.15
VI						**0.00**	123.67	129.96	273.58	43.42
VII							**23.38**	336.57	580.85	72.88
VIII								**0.00**	66.78	159.23
IX									**25.45**	326.23
X										**0.00**

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

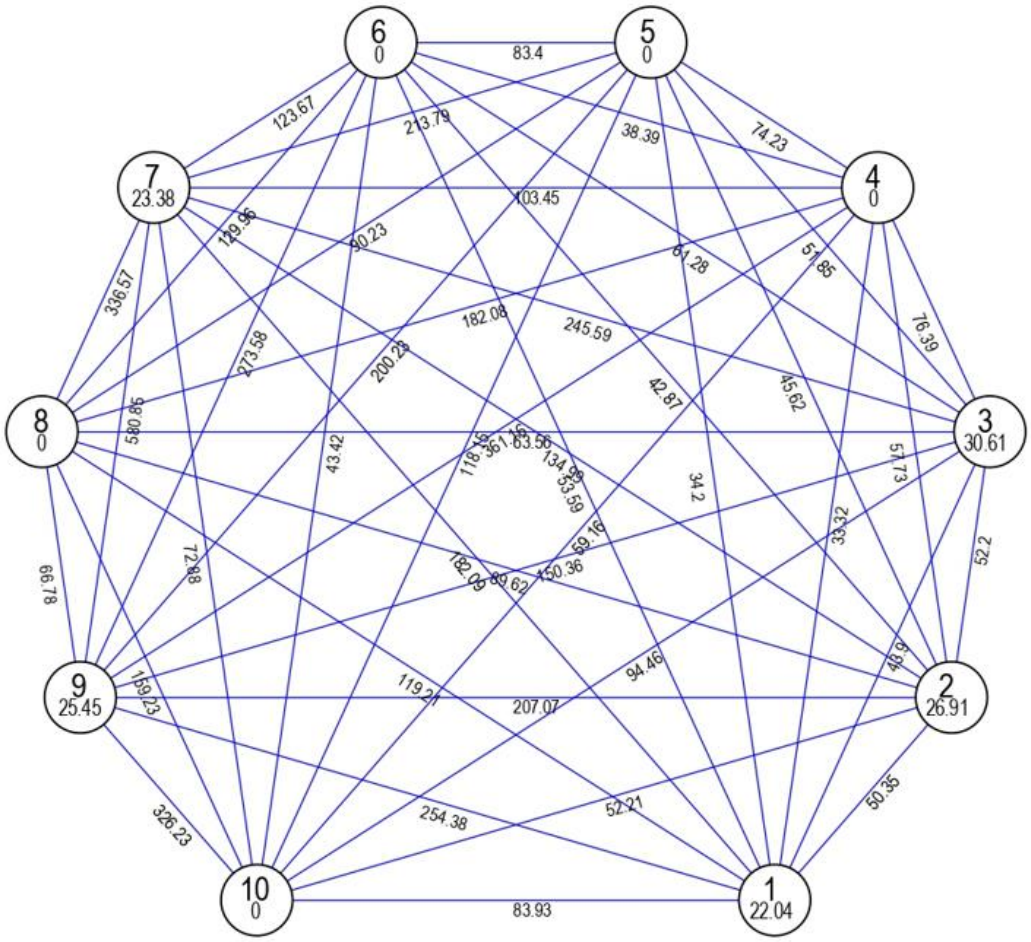

Fig. 2. Intra and inter-cluster distances of 50 blackgram genotypes among the ten clusters based on Tocher's method

Table 3. Cluster mean values for ten traits in 50 black gram genotypes

Cluster number	Days to 50% flowering	Plant height (cm)	Branches per plant	Clusters per plant	Pods per plant	Pod length (cm)	Seeds per pod	Test weight (g)	Days to maturity	Grain yield per plant (g)
I	45.34	69.41	2.53	8.00	19.73	5.33	7.00	4.32	86.84	4.46
II	43.46	59.70	2.80	10.32	33.86	5.82	7.14	4.65	83.39	6.89
III	44.75	80.25	3.32	7.81	34.44	5.52	7.17	4.47	87.50	6.22
IV	43.00	71.61	1.50	8.83	**14.59**	4.59	**6.07**	**3.98**	79.50	**3.69**
V	47.50	55.66	2.50	11.56	24.53	6.13	6.79	4.95	**92.50**	5.36
VI	**40.00**	65.74	**0.63**	6.80	33.69	**3.77**	7.57	4.69	82.00	5.13
VII	**40.00**	**34.09**	3.16	8.39	20.97	4.29	6.08	4.15	**71.00**	4.62
VIII	**49.00**	73.81	4.19	9.46	55.63	4.90	6.41	4.19	90.50	6.12
IX	47.25	**89.27**	**6.45**	**14.81**	**67.00**	**6.45**	**8.11**	**5.17**	92.25	**10.41**
X	**40.00**	55.38	4.52	**3.26**	35.10	5.61	6.99	4.40	79.00	4.12

Note: Bold figures indicate minimum and maximum values for each character

Table 4. Per cent contribution of different characters towards genetic divergence in 50 black gram genotypes

S. No.	Source	Number of times ranked first	Per cent contribution
1	Days to 50% flowering	20	1.63%
2	Plant height (cm)	225	18.37%
3	Branches per plant	7	0.57%
4	Clusters per plant	79	6.45%
5	Pods per plant	471	38.45%
6	Pod length (cm)	62	5.06%
7	Seed per pod	114	9.31%
8	Test weight (g)	27	2.2%
9	Days to maturity	183	14.94%
10	Grain yield per plant (g)	37	3.02%

the crosses between LBG 623 (cluster I), LBG 787 (cluster III), DKU 87(cluster II) and VBN 8 (cluster VII) in all possible cross combinations may generate more variation in segregating generations which fecilitate in developing superior high yielding pureline varieties.

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