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Exploring genetic diversity in blackgram (*vigna mungo* (l.) Hepper) through D² statistics and principal component analysis

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ABSTRACT

Genetic diversity is an essential prerequisite for crop improvement, enabling the identification of divergent parents for hybridization. The present study evaluated fifty-five blackgram genotypes for yield and quality traits using multivariate analysis. Mahalanobis D² statistics grouped the genotypes into six clusters, with Cluster I containing the maximum of 35 genotypes, followed by Cluster II with nine genotypes and Cluster III with eight genotypes, while Clusters IV, V and VI were solitary. The distribution of genotypes across clusters was random, suggesting distinct genetic backgrounds. The highest inter-cluster distance was observed between Clusters III and VI, followed by Clusters III and V, indicating wide genetic divergence. Principal component analysis identified five components with eigenvalues greater than one, explaining 76.71% cumulative variance. The first two PCs alone accounted for 56.04% of total variability. Seed yield per plant, test weight, protein content, and number of pods per plant were the major contributors to divergence. The results suggest that hybridization involving genotypes from distantly related clusters could yield superior segregants for yield and quality improvement.

Keywords: *Blackgram, Genetic diversity, Mahalanobis D² Statistics and Principal component analysis*

Blackgram (*Vigna mungo* (L.) Hepper) is an important pulse crop in India, contributing significantly to dietary protein intake. India is the world's largest producer of Blackgram, contributing 70% of the global production, followed by Myanmar and Pakistan. Blackgram grows best in well-drained loamy or sandy loam soils, especially black cotton soils, and is particularly suited for rice fallows. The country produced about 2.05 million tonnes from 3.2 million hectares of land, with an average yield of 640 kg per hectare (Annual report 2023-24). Andhra Pradesh emerged as a notable contributor, having production of 3.84 lakh tonnes of blackgram from 3.08 lakh hectares with average productivity of 1247 kg/ha, during 2023-2024 (Annual report 2023-2024). Despite its economic and nutritional importance, productivity has remained stagnant due to limited genetic variability and vulnerability to diseases and stress conditions. Broadening the genetic base through the identification of diverse parental lines is critical for effective breeding. Multivariate techniques such as Mahalanobis D² statistics and Principal Component Analysis (PCA) are widely used to assess genetic divergence. D² statistics provide information on genetic distance between genotypes, while PCA identifies the most variable traits contributing to diversity. Together, these tools help in classifying genotypes into distinct clusters and identifying traits that account for maximum variability, thereby guiding the selection of parents for hybridization.

MATERIAL AND METHODS

The study was conducted during *rabi*, 2024-25 at Regional Agricultural Research Station, Lam, Guntur, Andhra Pradesh, with 55 blackgram genotypes in alpha lattice design. Observations were recorded on five randomly selected plants from each genotype in each replication. Data was recorded on 11 quantitative traits *viz*., days to 50% flowering, days to maturity, plant height, number of branches per plant, number of clusters per plant, number of pods per plant, number of pods per cluster, pod length, seeds per pod, test weight, seed yield per plant and two bio chemical traits *viz*., protein content and carbohydrate content. Genetic divergence was estimated using Mahalanobis D² statistics (Rao, 1952), and genotypes

were grouped into clusters following Tocher's method. Principal Component Analysis (PCA) was carried out to identify the main traits contributing to genetic variation.

RESULTS AND DISCUSSION

The success of any plant breeding programme largely depends on the existence of diversity among the genotypes (Allard. 1960). The choice of parents is of greater importance in breeding programme. Assessment of a large number of accessions for genetic diversity is of immense importance in selection of diverse genotypes for hybridization programme to develop superior heterotic hybrids.

Multivariate analysis of variance revealed the significant difference for all the thirteen characters under study in the fifty-five blackgram genotypes. This significance of difference among fifty-five genotypes of blackgram for majority of characters justify further calculation of D^2 values. To estimate the D^2 values, correlated means of characters were transformed into uncorrelated variables using Pivotal Condensation Method. It measures the degree of diversification and determines the relative contribution of each component character to total divergence.

Fifty-five genotypes were grouped into six clusters using the Tocher's method Table 1 and, with the criterion that the intra cluster average D^2 values

Table 1. Clustering pattern by Tocher's method of 55 blackgram genotypes

Cluster No.	No. of Genotypes	List of Genotypes
I	35	PU 18-11, VBG 17-29, OBG 102, VBG 17-7, ABG 03, PU 15-04, OBG 141, TU 130, LBG 826, LBG 854, SB 428, GBG 10, VBN 14-06, DKU 95, GBG 66, GBG 47, GBG 7, LBG 787, ABG 06, VBN 11-031, LBG 924, LBG 818, PU 13-14, LBG 880, NDKU 17-05, TJU 111, LBG 976, PU 18-02, GBG 80, VBN 7, GBG 14, ABG 05, LBG 791, GBG 88, TBG 104
II	9	GBG 94, MBU 10-37, PU 19, GBG 91, PU 18-07, PU 9, LBG 968, ABG 12, ADBG 13-02
III	8	ABG 14-001, LBG 752, LBG 852, LBG 883, VBG 12-08, PU 18-10, NDKU 17-16, GBG 84
IV	1	PU 15-27
V	1	VBG 12-034
VI	1	DKU 87

Table 2. Average intra (bold) and inter cluster D^2 and D values among six clusters in 55 blackgram genotypes

Cluster Distances						
	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**	**Cluster 6**
Cluster 1	**39.75**	64.13	80.11	57.35	81.65	87.85
	-6.3	-8.01	-8.95	-7.57	-9.04	-9.37
Cluster 2		**44.42**	106.57	73.25	116.84	98.58
		-6.66	-10.32	-8.56	-10.81	-9.93
Cluster 3			**68.44**	102.92	123.24	158.33
			-8.27	-10.14	-11.1	-12.58
Cluster 4				**0**	95.71	112.52
				0	-9.78	-10.61
Cluster 5					**0**	155.17
					0	-12.46
Cluster 6						**0**
						0

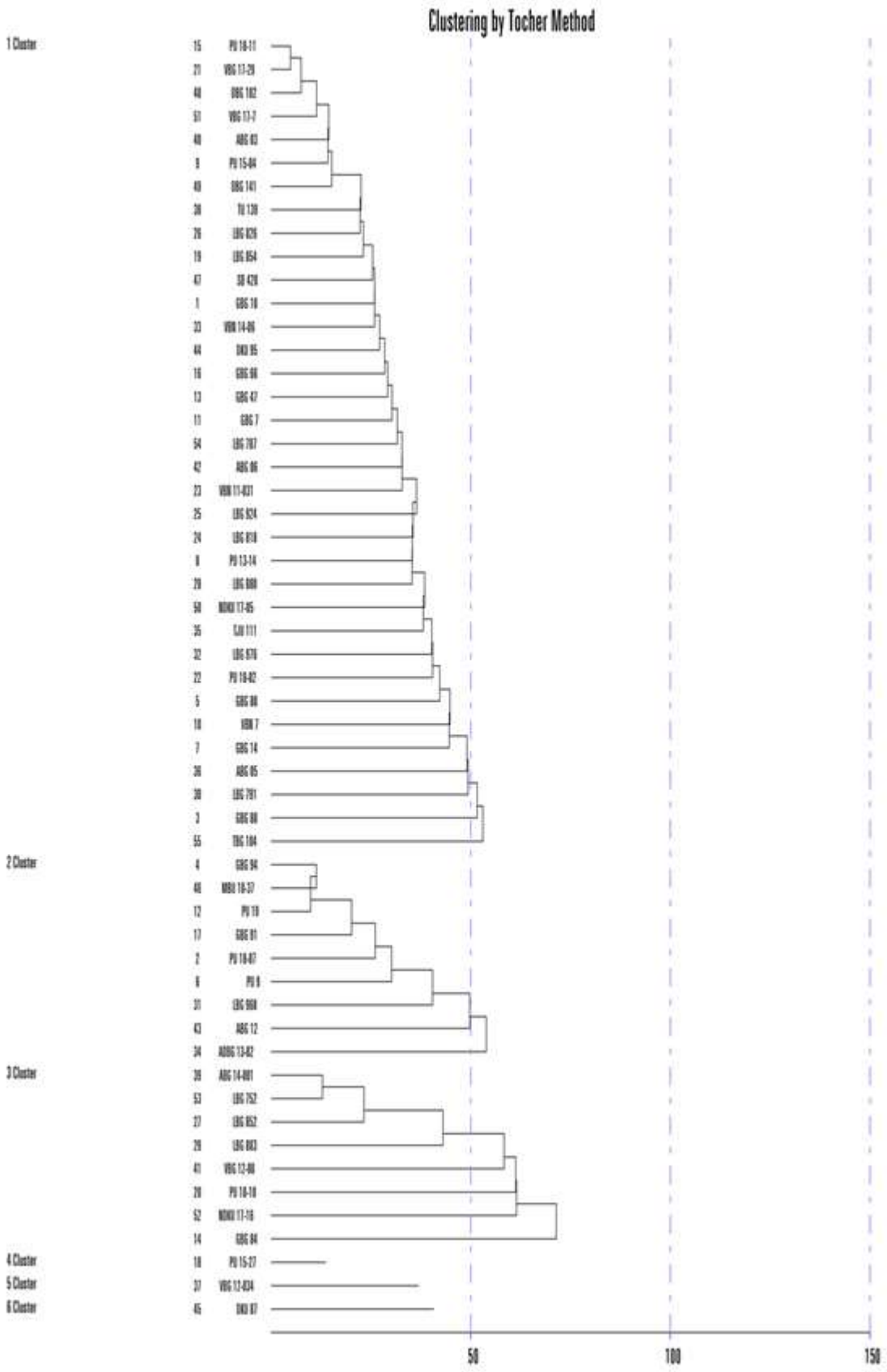

Fig 1. Dendrogram showing relationship based on Tochers method

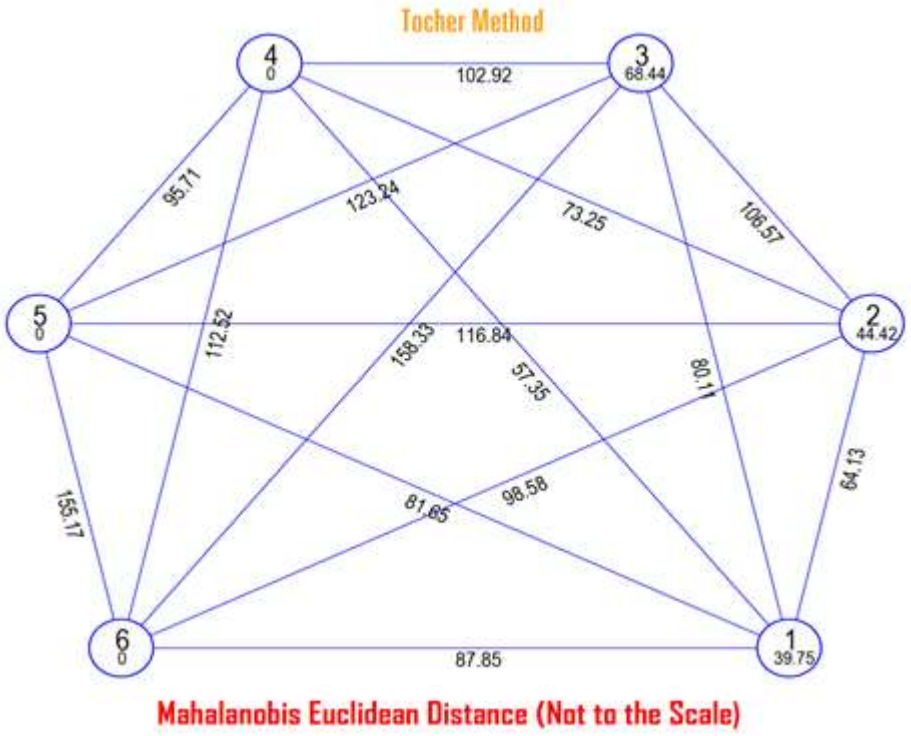

Fig 2. Intra and inter cluster distances of Blackgram genotypes in six clusters based on euclidean distances

Table 3. The nearest and the farthest cluster from each cluster based on D^2 values using Tocher's method in 55 blackgram genotypes

Cluster No.	Nearest cluster	Farthest cluster
I	IV (57.35)	VI (87.85)
II	I (64.13)	V (116.84)
III	I (80.11)	VI (158.33)
IV	I (57.35)	VI (112.52)
V	I (81.65)	VI (155.17)
VI	I (87.85)	III (158.33)

should be less than the inter cluster D^2 values. The dendrogram showing relationship among all the genotypes is present in Fig 1. The distribution of 55 genotypes into six clusters was at random with maximum number of 35 genotypes in cluster I followed by cluster II having 9 genotypes and cluster III with 8 genotypes, whereas clusters IV, V & VI were solitary clusters. The formation of distinct solitary cluster may be due to the fact that geographic barriers preventing gene flow or intensive natural and human selection for diverse and adaptable gene complexes must be responsible for this genetic diversity (Arunachalam and Ram., 1967).

The highest inter-cluster distance was observed between Clusters III and VI, followed by Clusters III and V Table 2. and is present in Fig 2. The nearest and farthest cluster for each of the five clusters are indicated in Table 3., suggesting that crosses between these clusters could result in maximum heterosis and transgressive segregants. The intra-cluster distance was highest in Cluster VI, reflecting considerable variability within the group.

Table 4. Canonical vectors for 13 characters in 55 genotypes of Blackgram (*Vigna mungo* (L.) Hepper)

Canonical Roots Analysis (P. C. A.)							
S.No.	Parameter	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6
	Eigene Value (Root)	3.1616	2.4273	1.6963	1.4971	1.1903	0.9465
	% Var. Exp.	24.32	18.67	13.05	11.52	9.16	7.28
	Cum. Var. Exp.	24.32	42.99	56.04	67.56	76.71	83.99
1	Days to 50% flowering	0.4008	0.0266	0.3979	0.0745	0.0624	0.2281
2	Days to maturity	0.4315	-0.0211	0.2983	0.0275	0.0562	0.4223
3	Plant height (cm)	0.2376	0.3069	0.0004	0.4935	-0.0587	0.1623
4	Number of Branches per plant	0.0474	0.3	0.3087	-0.461	0.2779	-0.355
5	Number of clusters per plant	0.4036	0.2217	-0.1709	0.0109	0.1284	-0.2968
6	Number of pods per plant	0.0512	0.4412	-0.363	-0.2257	0.1421	0.1035
7	Number of pods per cluster	-0.3389	0.2263	0.1183	-0.3323	0.0211	0.4491
8	Pod length (cm)	0.2524	-0.3256	-0.2533	-0.4428	-0.0314	0.1354
9	Number of seeds per pod	0.1194	-0.542	-0.2458	-0.0072	-0.0336	0.0247
10	Test weight	0.3857	-0.1593	0.1741	-0.2982	0.0012	-0.2045
11	Seed yield per plant	0.1839	0.2224	-0.2934	-0.2598	-0.4869	0.3344
12	Protein	-0.213	-0.13	0.4837	-0.1537	-0.3155	0.0678
13	Carbohydrate	-0.091	-0.1676	-0.0861	-0.0003	0.7324	0.3736

Table 5. Mean values of canonical vectors for 55 genotypes of blackgram (*Vigna mungo* (L.) Hepper)

S. No.	Genotype	PCA I X Vector	PCA II Y Vector	PCA III Z Vector
1	GBG 10	28.137	-6.321	11.098
2	PU 18-07	26.266	-0.079	10.522
3	GBG 88	25.663	-5.935	7.114
4	GBG 94	29.798	-0.848	9.932
5	GBG 80	25.291	-8.077	13.209
6	PU 9	27.637	-0.927	7.846
7	GBG 14	29.826	-5.276	10.355
8	PU 13-14	26.315	-3.748	11.607
9	PU 15-04	25.593	-4.876	9.928
10	VBN 7	27.255	-2.731	11.478
11	GBG 7	24.709	-3.298	10.339
12	PU 19	28.992	-1.795	10.001
13	GBG 47	25.687	-2.783	10.868
14	GBG 84	27.726	-8.382	9.11
15	PU 18-11	25.053	-7.519	9.407
16	GBG 66	27.667	-3.438	10.005
17	GBG 91	29.205	-0.025	9.554
18	PU 15-27	24.202	-3.418	13.713
19	LBG 854	26.398	-4.818	10.787
20	PU 18-10	22.651	-6.685	11.875
21	VBG 17-29	26.326	-7.413	9.099
22	PU 18-02	23.832	-6.332	10.951
23	VBN 11-031	28.834	-5.126	10.452
24	LBG 818	28.462	-2.006	10.589

S. No.	Genotype	PCA I X Vector	PCA II Y Vector	PCA III Z Vector
25	LBG 924	27.841	-1.862	10.992
26	LBG 826	27.418	-5.439	11.219
27	LBG 852	27.591	-4.502	7.849
28	LBG 880	26.571	-2.704	9.483
29	LBG 883	26.044	-3.894	10.7
30	LBG 791	22.145	-7.064	10.349
31	LBG 968	26.565	-2.276	12.226
32	LBG 976	23.781	-5.442	12.037
33	VBN 14-06	24.962	-6.837	13.257
34	ADBG 13-02	29.5	-3.568	8.465
35	TJU 111	27.021	-6.02	9.857
36	ABG 05	26.363	-8.545	10.4
37	VBG 12-034	22.189	-5.262	12.485
38	TU 139	25.95	-4.938	10.287
39	ABG 14-001	26.531	-4.407	8.531
40	ABG 03	25.69	-6.673	11.607
41	VBG 12-08	23.952	-6.933	13.298
42	ABG 06	26.84	-2.302	9.036
43	ABG 12	25.089	-1.906	14.502
44	DKU 95	26.858	-5.774	10.028
45	DKU 87	28.018	-1.695	7.615
46	MBU 10-37	30.297	-2.714	9.194
47	SB 428	27.103	-3.897	11.318
48	OBG 102	26.16	-6.32	8.124
49	OBG 141	23.7	-6.996	10.432
50	NDKU 17-05	26.084	-5.529	8.141
51	VBG 17-7	25.071	-7.05	10.258
52	NDKU 17-16	23.59	-4.923	11.193
53	LBG 752	27.213	-5.716	8.155
54	LBG 787	27.686	-6.719	8.934
55	TBG 104	26.465	-6.293	7.538

Principal Component Analysis (PCA)

PCA identified five components with eigenvalues >1, cumulatively explaining 76.71% of variability from Table 4. The first principal component (PC1) accounted for 28.31% of variation, mainly due to seed yield per plant, number of pods per plant, and test weight. The second component (PC2) contributed 27.73% and was associated with protein content, plant height and number of clusters per plant. The first two PCs together explained 56.04% of total variation, suggesting that these traits are the primary contributors to genetic diversity in blackgram and the mean values of the canonical vectors for 55 genotypes were present in Table 5.

Similar results were reported by Mallikarjuna *et al*. (2021), Yadav *et al*. (2023) and Gangadhar *et al.* (2023), highlighting the role of yield and seed quality traits in shaping diversity.

CONCLUSION

D² statistics and PCA revealed substantial genetic diversity among blackgram genotypes, with wide inter-cluster distances indicating potential for heterotic hybridization. Seed yield per plant, number of pods per plant, 100-seed weight, and protein content were the most influential traits contributing to divergence. Genotypes from distant clusters, such as those in Clusters IV and VI, may serve as promising

parents for developing high-yielding and nutritionally improved cultivars.

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