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Multivariate analysis in blackgram (*Vigna mungo* (L) hepper) genotypes

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Abstract
A study was carried out to determine the relationship and genetic diversity among twenty-one blackgram genotypes using Mahalanobis's D^2 and principal component analysis for various quantitative traits over three seasons. Analysis of variance revealed significant differences among the blackgram genotypes. Twenty-one genotypes were grouped into six clusters. The maximum intra cluster distance was observed in cluster II. The maximum inter cluster distance was found between cluster III and V (D^2 = 3402.97). The clusters II and V showed high mean values for seed yield per plant. In principal component analysis, first component had contribution from the traits *viz.*, number of pods per plant, pod length, number of seeds per pod, seed yield per plant which accounted 35.44% to the total variability. The remaining variability of 20.06%, 12.39% and 11.23% was accounted by second, third and fourth principal components by various traits *viz.*, number of branches, pod weight and 100 seed weight. The cumulative variance of 79.12% of total variation among eleven characters was explained by the first four axes.

Keywords: Blackgram, Mahalanobis's D^2, principal component analysis

Introduction
Blackgram (*Vigna mungo* (L.) Hepper) is one of the important pulse crops in India. Blackgram is a rich source of protein (20.80 to 30.50 percent) and also a good source of Phosphoric acid and calcium. It is contributing 12 percent of the total pulse production in India. In spite of its importance, the productivity of this crop is relatively low. The major constrains in achieving higher productivity are lack of exploitable genetic variability, absence of suitable ideotype, poor harvest index, susceptibility to biotic and abiotic stresses, non-availability of quality seeds of improved varieties and narrow genetic base occur due to repeated usage of few parents with high degree of relatedness in crossing programmes (Hadimani *et al.*, 2016) [1]. An assessment of the genetic diversity of pulses is an important first step in a research programme to improve crop yield. In order to improve yield, new blackgram varieties must be developed. Genetic distance estimates for grouping can be estimated by different methods.
Multivariate statistical tools include Principal Component Analysis (PCA), Cluster analysis and Discriminate analysis (Oyelola, 2004) [2]. Principal component analysis (PCA) can be used to uncover similarities between variable and classify the cases (genotypes), while cluster analysis on the other hand is concerned with classifying previously unclassified materials (Leonard and Peter, 2009). Mahalanobis D^2 statistic is a powerful tool in quantifying the degree of divergence at phenotypic level. PCA is very helpful for identification of plant characters that categorize the distinctiveness among promising genotypes (Chakravorty *et al.*, 2013) [4]. In view of these, twenty-one blackgram genotypes were evaluated by different multivariate analysis to identify genetically diverse genotypes and to identify traits that contribute to variability in the population.

Materials and Methods
For this experiment, twenty-one blackgram genotypes were grown in Plant Breeding Farm, Department of Genetics & Plant Breeding, Annamalai University in three seasons (Jan-2017, Jul-2017 & Jan-2018). The experiments were conducted in Randomized Block Design with two replications. Standard agronomical practices were followed to raise the crop. The mean value of two replications over seasons were used for statistical analysis. The observations were recorded for eleven quantitative characters *viz.*, days to first flowering, plant height, number of branches, number of clusters per plant, number of pods per plant, pod length, pod weight, number of seeds per pod, seed size, 100 seed weight and seed yield per plant. The data was subjected to statistical analysis using Mahalanobis D^2 statistic and Principal Component Analysis (PCA).

Results and Discussion

Based on the pooled analysis, the analysis of variance showed significant differences among the genotypes with respect to all the characters and indicated high genetic variability (Table 1). Multivariate analyses to be a valid system to deal with germplasm collections (Felcinelli *et al.*, 1988).

Table 1: Analysis of variance for eleven morphological characters in 21 blackgram genotypes for pooled analysis

Source	Df	Days to first flowering	Plant height (cm)	Number of branches	Number of clusters per plant	Number of pods per plant	Pod length (cm)	Pod weight (g)	Number of seeds per pod	Seed size (mm)	100 seed weight (g)	Seed yield per plant (g)
Replication	1	0.36	37.49	0.04	0.23	0.51	0.005	0.0001	0.17	0.0001	0.003	0.027
Genotype	20	26.19**	820.59**	1.72**	3.10**	37.71**	0.17**	0.003**	0.48**	0.0007**	0.37**	1.83**
Error	20	0.31	37.47	0.09	0.27	1.05	0.009	0.0003	0.08	0.0001	0.007	0.02

* Significant at 5 percent level; ** Significant at 1 percent level

Based on Mahalanobis D^2, the twenty-one genotypes were grouped into six clusters by the application of the clustering technique Cluster I comprised largest number of twelve genotypes followed by cluster II with five genotypes, while cluster III, IV, V and VI were solitary clusters (Table 2). It shows that the selection of parents for hybridization based on geographical origin would be arbitrary. The distribution of genotypes from different eco-geographical regions into these clusters was apparently random (Jeena and Singh, 2002). Genotypes from similar origin were grouped into different clusters. This tendency of genotypes to occur in clusters cutting across geographical boundaries demonstrates that geographical is not only factor causing genetic diversity (Sihag *et al.*, 2004) [8].

Table 2: Composition of D^2 clusters for 21 blackgram genotypes in pooled analysis

Clusters	Number of genotypes	Name of Genotypes
I	12	G1, G2, G6, G11, G14, G15, G16, G17, G18, G19, G20, G21.
II	5	G4, G5, G8, G10, G12.
III	1	G3.
IV	1	G13.
V	1	G7.
VI	1	G9.

The intra cluster distance varied from 0.00 (Cluster III, IV, V and VI) to 17.80 (Cluster II) (Table 3 & Fig. 1). Cluster II showed maximum intra cluster distance. It is reported that genotypes would produce more desirable breeding materials for achieving maximum genetic distance with regard to yield *per se*, provided that there is adequate complementation of gene effects of parental lines (Rahman *et al.*, 1997) [9].

Table 3: Average inter (D^2) and intra (D) cluster distance for 21 blackgram genotypes in Pooled analysis

Clusters	I	II	III	IV	V	VI
I	271.42 (16.47)	767.0684 (27.696)	534.71 (23.12)	748.46 (27.35)	2194.82 (46.84)	681.52 (26.10)
II		316.9112 (17.80)	1322.55 (36.36)	803.26 (28.34)	782.60 (27.97)	1339.48 (36.59)
III			0.00 (0.00)	1353.43 (36.78)	3402.97 (58.33)	719.41 (26.82)
IV				0.00 (0.00)	1457.10 (38.17)	789.72 (28.10)
V					0.00 (0.00)	3070.71 (55.41)
VI						0.00 (0.00)

Diagonal values (bold) – Intra cluster distance

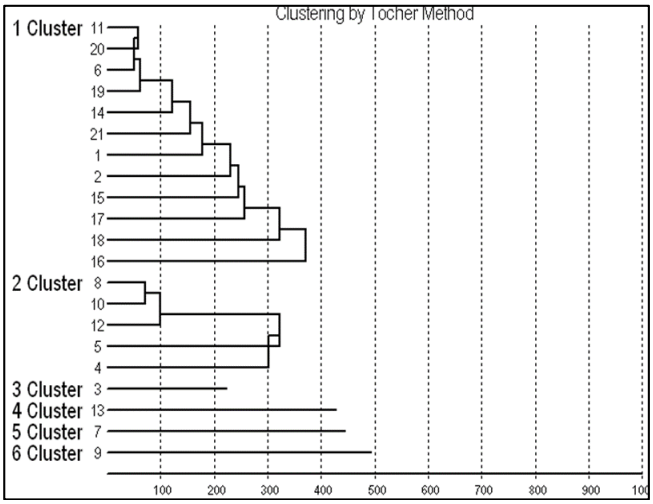

Fig 1: Dendrogram for morphological data using toucher method

Inter cluster distance was minimum between cluster I and II (23.12) followed by clusters I and VI (26.10) suggesting closed relationship. Such genotypes can also be used in breeding programmes for developing biparental crosses between the most diverse and closet groups to break the undesirable linkages between yield and its associated traits (Haddad *et al.*, 2004) [10].

Maximum inter cluster distance existed between clusters III and V (58.33) followed by clusters V and VI (55.41). This is clearly indicated that the genotypes included in these clusters are have broad spectrum of genetic diversity. It is therefore suggested that the superior genotypes from different clusters, may be used as parents for the hybridization programme.

Perusal of data on cluster mean for various traits revealed wide difference in mean values under study. The clusters II and V showed high mean values for seed yield per plant, cluster II exhibited high mean value for number of branches, number of clusters to plant, number of pods per plant, number of seeds per plant and 100 seed weight and the cluster V registered maximum mean value for plant height, number of branches, number of clusters per plant, number of pods per plant, number of seeds per plant. The minimum mean value for days to first flowering was observed in cluster V, which may be a suitable source for earliness (Table 4).

Table 4: Cluster means of 21 blackgram genotypes for various characters in pooled analysis

Clusters	Days to first flowering	Plant height (cm)	Number of branches	Number of clusters per plant	Number of pods per plant	Pod length (cm)	Pod weight (g)	Number of seeds per pod	Seed size (mm)	100 seed weight (g)	Seed yield per plant (g)
I	38.05	71.73	5.81	6.09	13.15	4.18	0.35	5.34	3.70	4.13	2.50
II	34.70	94.22	6.28	7.28	19.93	4.46	0.34	6.13	3.60	4.35	4.15
III	43.59	109.92	6.09	5.17	11.67	4.38	0.37	5.39	3.90	5.00	2.66
IV	33.59	110.02	5.83	5.50	13.50	4.52	0.37	5.44	4.30	3.95	2.69
V	32.34	114.09	6.50	7.94	25.67	4.30	0.37	5.78	3.60	3.59	5.47
VI	38.34	91.50	2.50	6.17	12.00	4.97	0.45	6.00	3.80	4.71	2.71
General mean	36.76	98.58	5.50	6.36	15.99	4.47	0.37	5.68	3.80	4.29	3.36

In Principal component analysis, the first four components with eigen values >1 contributed 79.12% of the variability amongst 21 genotypes evaluated for 11 quantitative traits (Table 5). A scatter plot was drawn between first and second principal component depicted a clear pattern of grouping genotypes in the factor plane (Fig 2). The distribution of genotypes based on PC 1 and PC2 exhibits the phenotypic variation among the population and its explains how they widely dispersed along the both axes (Fig 3).

Table 5: Eigen value, factor scores and contribution of the first four principal component axes to variation in Blackgram genotypes

Principal components	1	2	3	4
Eigen values	3.89	2.20	1.36	1.23
% of Variance	35.44	20.06	12.39	11.23
Cumulative %	35.44	55.5	67.9	79.12
Days to first flowering	-0.3255	-0.0274	-0.4475	0.1293
Plant height (cm)	0.2755	-0.1362	-0.4524	-0.3531
Number of branches	0.0355	0.5451	-0.1261	-0.0404
Number of clusters per plant	0.289	0.258	0.2349	0.1988
Number of pods per plant	0.4579	0.2132	0.076	-0.0802
Pod length (cm)	0.3049	-0.4541	0.0017	-0.0233
Pod weight (g)	0.0566	-0.3449	0.6117	0.0215
Number of seeds per pod	0.4246	-0.2054	-0.1698	-0.0057
Seed size (cm)	-0.1664	-0.1686	0.0286	-0.7659
100 seed weight (g)	0.0436	-0.3904	-0.3091	0.4708
Seed yield per plant (g)	0.4666	0.1527	-0.1373	-0.0421

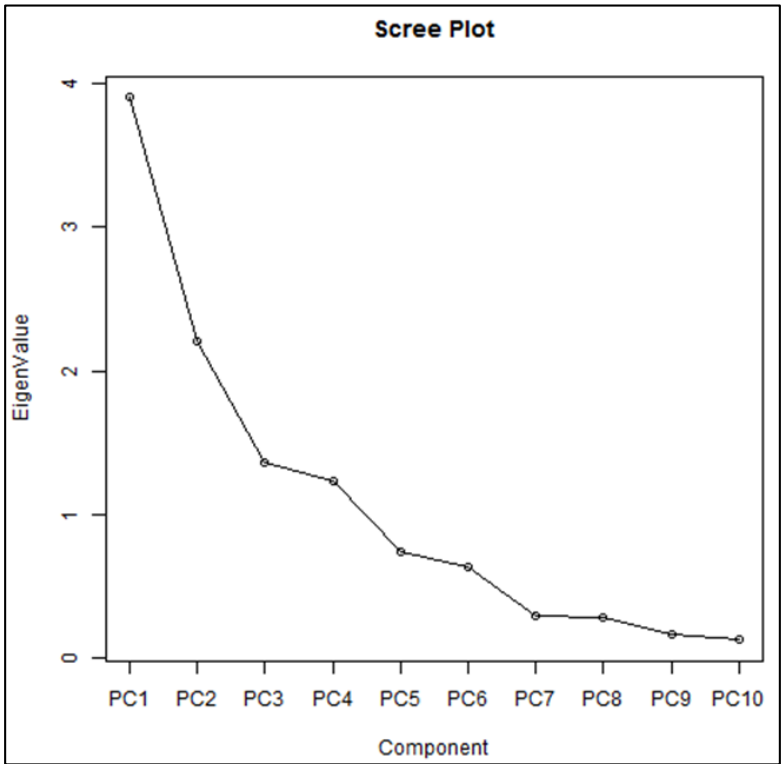

Fig 2: Scree plot showing Eigen value variation.

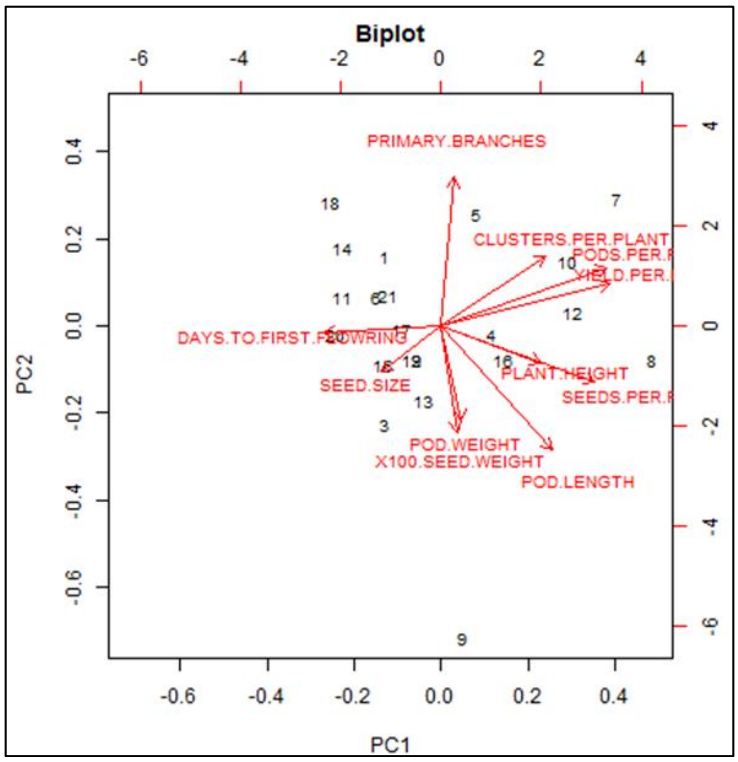

Fig 3: Distribution of various traits and genotypes across two components

PCA of quantitative traits found that, the first principal component accounted 35.44% to the total variability, where by number of pods per plant (0.45), pod length (0.30), number of seeds per pod (0.42) and seed yield per plant (0.46) were contributed positively (Table 5).

The second principal component accounted 20.06% to the total variability. The variables contributing most positively was number of branches (0.54). The third component accounted 12.39% to the variance, in which the variable pod weight (0.61) contributed positively. Fourth principal component accounted 11.23% of variance in the total variability by 100 seed weight (0.47). Thus, the prominent characters coming together in different principal components and contributing towards explaining the variability and have the tendency to remain together. This may be kept into consideration during utilization of these characters in breeding programme.

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