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Research Article

Comparative diversity analysis of cowpea genotypes using multivariate approaches

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Abstract
Cowpea improvement is possible if the information on genetic diversity among existing genotypes is available. Principal Component Analysis and Mahalanobis D^2 analysis were used to analyse 30 cowpea genotypes based on eleven quantifiable traits. The whole variance was divided into eleven major principal components using PCA, with the top three PCs with eigenvalues >1 accounting for 69.47 per cent of the total variation. From the analysis of the first two PCs and Mahalanobis D^2 analysis, it was confirmed that genotypes *viz.,* AVCP-1, PGCP-1, PGCP-13, CGD-1406, and CGD-1439 were scattered apart in all four quadrates of the bi-plot and fall in clusters with high inter-cluster distance representing maximum genetic variation. As a result, 30 cowpea genotypes had genetic and phenotypic differences that could be used to improve the cowpea by simple selection and crossing potential parents.

Key words: Cowpea, Principal Component Analysis, D^2 analysis, Genetic divergence.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) walp.] is a self-pollinated crop that belongs to the family Fabaceae and subfamily Papillonaceae and has a chromosome number 2n=2x=22. It is a significant grain legume crop in tropical and subtropical countries, especially in Sub-Saharan Africa, Asia, Central and South America (Singh *et al.*, 1997). According to Vedcourt (1970), *Vigna unguiculata* (L.) Walp is divided into five different subspecies. Among these, two subspecies are wild *viz.*, *dekinditiana* and *mensensis.* The remaining three subspecies, on the other hand, are cultivated and widely distributed throughout India (Steel, 1976) such as *unguiculata*, *sinensis* (common cowpea), and *sesquipedalis* (asparagus bean or yardlong bean). Vavilov (1949) identified India and Africa as the primary centres of cowpea, with China serving as a secondary centre. In comparison to other crop species, cowpea is one of the most highly adaptable and flexible crops and able to withstand extreme temperatures and drought. Cowpea is mostly grown in India for fodder, green manure, and as a soil-improvement cover crop. Around 240 kg/ha of atmospheric nitrogen can be fixed by the crop, leaving around 60-70 kg/ha accessible for the next crops in the cycle (Aikins and Afuakwa, 2008; Kamau and Weru, 2001).

The rapid accumulation of several favourable characteristics from various genotypes into a single genotype is critical for any crop's yield boost. The more the diversity between parents, the greater the heterosis in the progeny and the greater the likelihood of getting transgressive segregants. A breeder must recognize various parents with significant genetic diversity for combining desirable features in order to produce enhanced crop varieties over existing farmed varieties. To choose the parents for the hybridization with wide genetic divergence, it is necessary to classify all the accessible germplasm into clusters based on genetic divergence and estimate the amount of genetic diversity between them to initiate the hybridization programme

(Pandey, 2007). Multivariate analysis through Principal Component Analysis and Mahalanobis D^2 statistics is a vital tool to study morphologically complex individuals as well as for determining the degree of divergence across the populations. It is widely employed in genetic diversity study, whether morphological, molecular or biochemical. Using Principal Component Analysis and Mahalanobis D^2 statistics, the current work was attempted to estimate the genetic diversity for seed yield and its component characteristics in cowpea genotypes.

MATERIALS AND METHODS

The present investigation was conducted in a Randomized Block Design with three replications at the Agronomy Instructional Farm, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar. The experimental material was sourced from the Pulses Research Station, S. D. Agricultural University, Sardarkrushinagar and the Vegetable Research Station, Anand Agricultural University, Anand, Gujarat, which included 30 genotypes of cowpea. The genotypes were assessed for the traits *viz.,* days to flowering, days to maturity, plant height (cm), the number of branches per plant, the number of pods per plant, the number of seeds per pod, pod length (cm), test weight (g), seed yield per plant, protein content (%) and leaf area (cm^2). An experiment's plot size was 432 m^2, with 45 cm inter-row spacing and 15 cm intra-row spacing. Data was collected from five plants chosen at random from each entry in each replication, and mean values were computed for statistical analysis. Principal Component Analysis and Mahalanobis D^2 statistics were used to analyse the data for eleven characteristics in order to explore genetic diversity. INDOSTAT v8.1 was used for D^2 analysis, while Past 3.23 was used to perform Principal Component Analysis.

RESULTS AND DISCUSSION

The PCA technique is employed to reduce a large set of variables into a manageable number of uncorrelated components. Principal Component Analysis indicated that only the first three principal components (PCs) exhibited eigenvalue >1 and collectively explicated 69.47 per cent of the overall variability **(Table 1)**. Principal component 1 explicated 34.56 per cent of the total variation and the remaining ten principal components explicated 19.50, 15.40, 8.35, 7.33, 6.20, 4.28, 2.39, 0.89, 0.72 and 0.32 per cent variation, respectively. The PC1 possessed the highest per cent variance (34.56%) and was the most prominent one, thus the selection of lines for the characters under PC1 might be desirable. Jaime and Anita (2019) and Vijaykumar *et al.* (2020) reported a similar result with the per cent variance when the eigenvalue is more than one.

The first two PCs were used to create the biplot, which showed the scattering pattern of 30 cowpea genotypes (**Fig. 1**). More similar genotypes are those that are dispersed closer to the origin and closer to one other, whereas genotypes that are scattered further apart are more divergent. (Sharma *et al.,* 2016). Genotypes AVCP-1, PGCP-1, PGCP-13, CGD-1406, and CGD-1439 were scattered across apart in all four quadrates of the biplot indicating high genetic divergence across the genotypes. Genotypes such as PGCP-4, GC-5, GC-2, and GC-1203 were found to be closer to the origin and each other, indicating minimal genetic divergence.

Table 2 showed the factor loadings of various variables acquired. PC1 attributed for four variables and designated as yield factor as it enabled high loadings of seed yield with three of its vital component characters *viz*., the number of pods per plant, pod length and the number of seeds per pod. PC2 possessed high loadings of days to maturity, leaf area and plant height. Test weight was loaded on PC3. PC4 could be designated as the component for branches per plant. The sixth principal component was designated as the factor for days to flowering and protein content. Loadings with a score of more than 0.3 are thought to be a significant contributor to the divergence (Walle *et al.,* 2019).

The PC scores of each component had positive and negative values. The greatest PC score in PC1 was achieved by genotype CGD-1406, followed by the genotypes CGD-1439 and CGD-1441, indicating that these genotypes had high values for the characteristics number of pods per plant, the number of seeds per pod, pod length, and seed yield per plant. By choosing lines from principal component 1, an intense selection method can be devised for the rapid improvement of dependent characteristics, such as yield characters in cowpea. Thus, the selection of these lines can assist in the further development of high yielding and good quality cowpea varieties.

Table 1. Eigen values, % variance and cumulative variability (%) of germplasm

PC	Eigen value	% Variance	Cumulative variability (%)
1	3.802	34.562	34.562
2	2.146	19.507	54.069
3	1.695	15.409	69.478

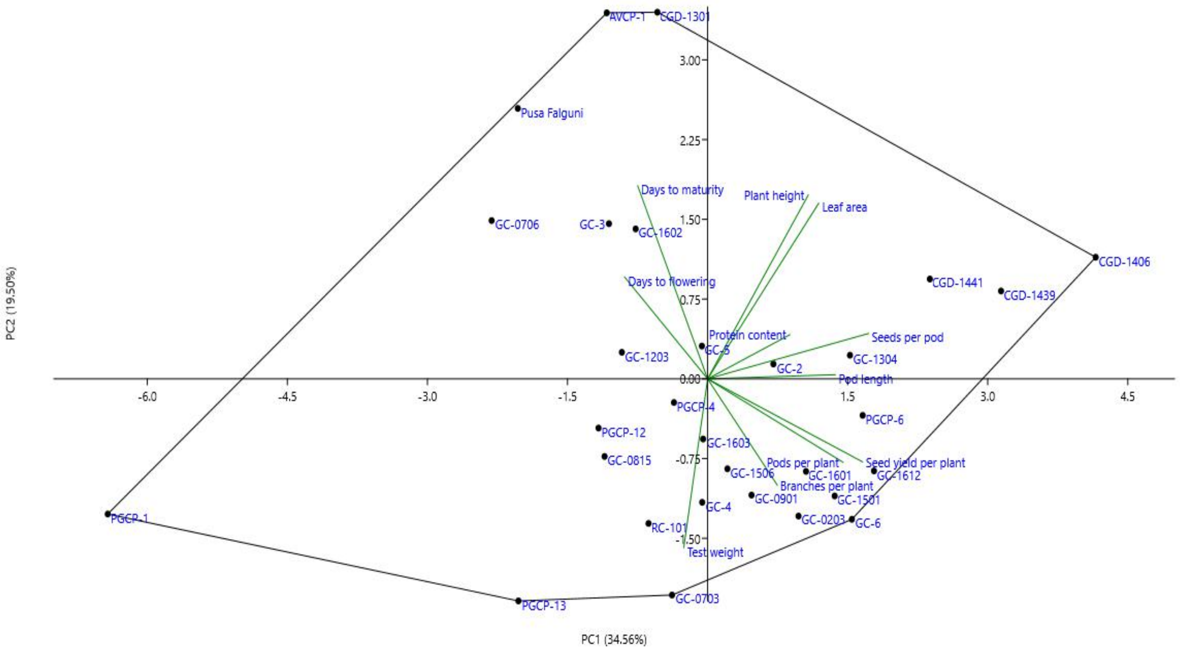

Fig. 1. Variation among 30 cowpea genotypes along with 11 quantitative characteristics, in a biplot

Table 2. Factor loadings of characteristics in cowpea for various principal components

	PC 1	PC 2	PC 3
Days to flowering	-0.447	0.362	0.475
Days to maturity	-0.376	0.686*	-0.035
Plant height	0.542	0.654*	0.354
Leaf area	0.598	0.624*	0.184
Number of branches per plant	0.375	-0.377	0.244
Number of pods per plant	0.730*	-0.296	-0.393
Number of seeds per pod	0.867*	0.161	0.128
Pod length	0.687*	0.015	0.433
Test weight	-0.128	-0.599	0.729*
Seed yield per plant	0.835*	-0.295	0.050
Protein content	0.443	0.157	-0.597

Tocher's method (Rao, 1952) was used to group the genotypes **(Fig. 2)**, with the premise that genotypes within a cluster had a lower D^2 value among themselves than genotypes from other clusters and five clusters were constructed from 30 cowpea genotypes. Cluster I was the largest, with 26 genotypes, followed by the four smaller clusters, each with one genotype. These genotypes may be evaluated under different environmental conditions compared to prior studies, which may be the reason for monotypic clusters due to confounded environmental effects in phenotypic performance. So, for more clarity, there is a further need to evaluate the genotypes through molecular marker-based genetic diversity. Similarly, Vavilapalli *et al.* (2014) performed a genetic divergence analysis in 26 cowpea germplasm and observed the grouping of all genotypes into six clusters. Aswathi *et al*. (2015) grouped 10 cowpea verities into four different clusters, Chandrakar *et al*. (2016a) grouped 21 genotypes in five different clusters and Patel *et al*. (2017) grouped 22 cowpea genotypes in eight different clusters.

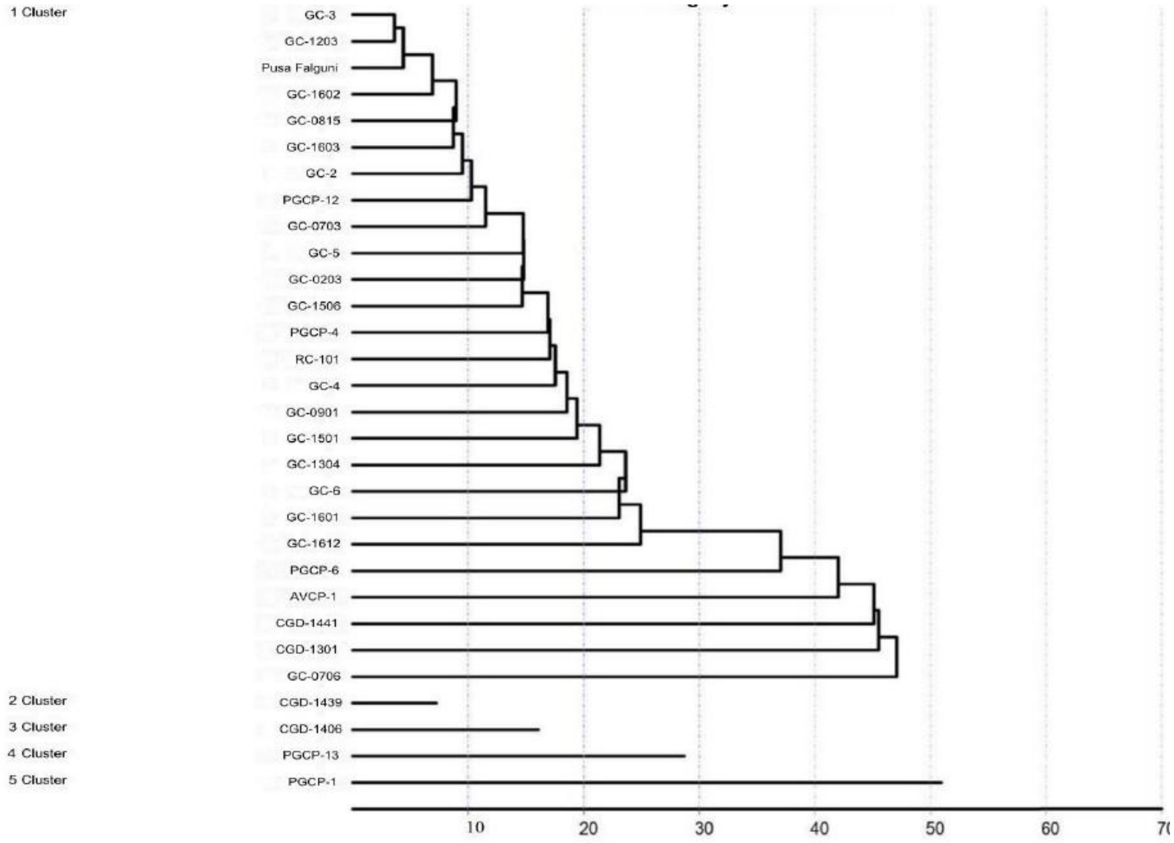

Fig. 2. Clustering 30 cowpea genotypes by Tocher's method

Inter-cluster and intra-cluster distances are presented in **Table 3**. Cluster III and V had the greatest inter-cluster distance (D=266.39), indicating that they had the most varied genotypes and are best suited for transgressive breeding, followed by Cluster II and V (D=212.09). Cluster II and III were found to have the shortest inter-cluster distance (D=18.09). Within Cluster I, the intra-cluster distance was determined to be 28.53.

The four clusters (II, III, IV, and V) with a single genotype had zero intra-cluster distances. The genotypes grouped in a similar cluster had the least amount of divergence. The transgressive segregants cannot be expected from a cross between genotypes from the same cluster. So, the parents belonging to the different clusters with extreme divergence could be used to get a desirable transgressive segregant. In any of the clusters, the genotypes with high values of seed yield and its component traits can be used for hybridization or direct adoption, followed by selection. Valarmathi *et al.* (2007) found a significant degree of divergence across clusters and concluded that hybridizing genotypes from diverse clusters should result in a greater number of useful segregants and high hybrid vigour. Dalsaniya *et al.* (2009) found a significant intra-cluster distance, indicating a greater genetic diversity between genotypes that may be exploited to increase cowpea output.

Table 3. Average distance between clusters and within clusters (diagonal)

Cluster	I	II	III	IV	V
I	28.53	62.72	79.92	77.42	94.05
II		0	18.09	161.00	212.09
III			0	196.79	266.39
IV				0	67.11
V					0

Table 4 shows the wide range of mean values among the clusters for distinct characteristics. Cluster II had a higher number of branches per plant (9.96) and a larger leaf area (3192.83). Plant height (68.13), the number of pods per plant (30.67), the number of seeds per pod (14.43), pod length (17.35), seed yield per plant (51.32), and protein content (22.74) were all given favourable ratings in Cluster III. In terms of test weight (15.77), days to flowering (52.33) and days to maturity (66.00), Cluster IV received a favourable grade. So, the improvement for a particular character can be done by selecting a genotype giving the best performance in a particular cluster for a hybridization programme. The findings of Vavilapalli *et al.* (2014), Chandrakar *et al.* (2016b) and Patel *et al.* (2017) are all in agreement with the findings of the current study.

The components of D^2 due to each trait variable were ranked I being allotted to the highest value. The total of these ranks over all conceivable combinations [n (n-1)/2 = 435] would give indirect information about the trait's importance in terms of its percentage contribution to total divergence (**Table 5).** The significant contributors to overall genetic diversity were leaf area (29.66), seed yield per plant (26.67), the number of pods per plant (25.98), test weight (7.59), and pod length (5.29). A small contribution was seen in the number of branches per plant (2.53), days to maturity (0.92), the number of seeds per pod (0.92), and protein content (0.46). Characters like days to flowering and plant height, on the other hand, had no effect on the overall genetic difference.

On the basis of the maximum genetic distance, it is advisable to go for the crossing of the genotypes in between clusters III × V (CGD-1406 × PGCP-1) for plant height, the number of pods per plant, the number of seeds per pod, pod length, seed yield per plant and protein content, clusters II × V (CGD-1439 × PGCP-1) for the number of branches per plant and leaf area and clusters III × IV (CGD-1406 × PGCP-13) for plant height, the number of branches per plant, the number of pods per plant, the number of seeds per pod, pod length, seed

Table 4. Cluster mean value of eleven characters in 30 cowpea genotypes

	DF	DM	PH	NBP	NPP	NSP	PL	TW	SYP	PC	LAP
Cluster I	53.59	68.41	56.08	8.53	24.13	9.39	12.25	10.07	22.84	22.24	1618.88
Cluster II	53.33	67.33	67.67	9.96	27.67	12.27	15.67	12.02	37.14	21.46	3192.83
Cluster III	55.33	68.13	68.13	9.07	30.67	14.43	17.35	11.72	51.32	22.74	2636.53
Cluster IV	52.33	66.00	49.00	8.33	4.87	6.68	15.24	15.77	4.89	22.09	687.44
Cluster V	57.67	70.33	43.13	8.60	3.20	2.07	5.40	13.13	0.94	19.88	691.97

Where, **DF =** Days to flowering, **DM =** Days to maturity, **PH =** Plant height (cm), **NBP =** Number of branches per plant, **NPP =** Number of pods per plant, **NSP =** Number of seeds per pod, **PL =** Pod length (cm), **TW =** Test weight (g), **SYP =** Seed yield per plant (g), **PC** = Protein content (%), **LAP =** Leaf area (cm^2)

Table 5. Different characteristics' relative contributions to genetic diversity in cowpea genotypes

S. No.	Characters	Time Ranked 1st	Contribution to divergence (%)
1	Days to flowering	0	0
2	Days to maturity	4	0.92
3	Plant height	0	0
4	Number of branches per plant	11	2.53
5	Number of pods per plant	113	25.98
6	Number of seeds per pod	4	0.92
7	Pod length	23	5.29
8	Test weight	33	7.59
9	Seed yield per plant	116	26.67
10	Protein content	2	0.46
11	Leaf area	129	29.66

yield per plant and protein content, which might lead to the creation of a wide range of beneficial genetic diversity for improving cowpea yields.

The current study revealed phenotypic and genetic variation across 30 cowpea genotypes. The conclusions of Principal Component Analysis and D^2 analysis were both confirmed by each other. This genetic diversity opens up plenty of possibilities for improving cowpea by easy selection based on unique characteristics and crossing potential parents.

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