

Original Research Article
**GENETIC DIVERSITY OF COWPEA GENOTYPES BASED ON PHENOTYPIC
MARKERS**

Comment [AA1]: Usage of phenotypic markers is not correct. Revise title to include divergence studies in title

ABSTRACT

Aim: Assessment and characterization of genotypes is essential for successful crop breeding efforts. This study was undertaken at the Department of Vegetable crops, GBPUAT, Pantnagar, Uttarakhand, India to assess and characterize the 40 cowpea germplasms from indigenous and exotic collections.

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Methodology: To analyze the genetic diversity, Mahalanobis D^2 ~~static~~ statistic analysis is employed.

Results: Based on the present study, it can be concluded that genotypes EC-572715 for plant height, EC-390216 for days to first flowering, days to first pod emergence and days to first pod edible maturity, WB-9, IC- 628899 for number of primary branches. COPBVAR-3 and EC-97306 for number of pods per cluster, EC-390216, IC-628899 for pod length, EC-472272 and COPBVAR-3 for number of seeds per pod, COPBVAR-3, EC-390241, EC-390216 for number of pods per plant and green pod yield per plot can be recommended for large scale farming after ~~multiplication~~ multi-location testing and can be used as a ~~donar~~ donor parent in breeding programmes.

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Comment [AA2]: Maybe rewritten as it is similar to the conclusion in the manuscript. Other relevant points may also be included. Only a few of these characters mentioned here are directly related to improve yield and can be released as varieties while others can be used as parents for breeding programmes. This has to be clearly mentioned.

KEYWORDS: Cowpea, genetic diversity, Mahalanobis D^2 analysis, pod yield.

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the important annual, autogamous leguminous vegetable crop mainly grown both in kharif and ~~spring~~ summer seasons ~~crop~~ in most parts of India. It is native to West Africa (Vavilov, 1951). It is a versatile legume for hot and dry conditions. Cowpea is an essential component of sustainable cropping systems in the sub- humid tropics and, generally, dry regions across the globe. It is tolerant to drought as well as water logging conditions. Cowpea is adapted to warm weather and requires less rainfall than most crops. Therefore, it is cultivated in the semi-arid regions of lowland tropics and subtropics, where soils are poor and rainfall is limited (Mortimore *et al.*, 1997). It is commonly known as black-eyed pea, lobia, barbatti, southern pea, long yard bean, asparagus bean, snake bean and china bean. It is one of the most important legume vegetable crops commonly grown throughout India for its long, tender green pods as vegetable and seeds as pulse. Cowpea is particularly important as a rotation crop with cereals. Like other legumes, it also has a unique ability to fix atmospheric nitrogen into nitrate. Cowpea is cultivated in an area of 12.5 million hectares with

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the production of 7.3 million tonnes. In India, cowpea is an underutilized pulse crop cultivated in an area of 0.5 million ha with an average productivity of 600-750 kg grains per ha.

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Cowpea is strictly an autogamous species and hence yield improvement has come through hybridization and irradiation. Hybridization is the most commonly used approach for creating variability since the variation created is not random like in irradiation but directed one. But, selection of parents for generating variability is rather restricted to only few genotypes. During the process of domestication in common bean, several morphological changes have occurred. The change under domestication are typically loss of seed dormancy and pod dehiscence mechanism, a change from perennial to annual life form and great change in seed size correlated with modified shoot architecture. Stems tend to be thicker, leaves larger, branches fewer, the nodes may reduced and internode length is shortened. This process culminates in evaluation of self supporting plants well adapted to monocrop husbandry systems (Smart, 1976). Further with the ideotype concept (Donald, 1968) the productivity in any legume species can be improved by tailoring the plant type using well planned recombination breeding with carefully selected parents. Breeding objective of cowpea is based on production problem and consumer preference. The main objectives are compact and erect plant type, high yielding varieties having greater number of pods per plant, earliness, non fibrous flesh at edible stage, resistance to trips, pod borer and maruca (Kumar, 2009).

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Genetic diversity in a population of crop species is a key to ~~successful hybridization for obtaining individuals~~ develop recombinants with desired horticultural traits. Mahalanobis (1936) set the ground rules for study of variability in a population when he proposed the D² statistic analysis. This invariably strengthened the concept of breeding for superior genotypes by defining the levels of exploitable variability and by predicting the results of a breeding programme D² analysis permits precise comparison among all possible pairs of populations before effecting actual cross in modeling the cultivars in a desired genetic architecture. Mahalanobis D² statistic analysis is a numerical approach that is used for the assessment of genetic diversity in plant breeding. It helps in the selection of genetically divergent parents in hybridization programmes. This technique can evaluate a large number of germplasm lines at a time for the presence of genetic diversity.

2. MATERIALS AND METHODS

As per the ecological and geographical conditions, the Vegetable Research Centre of Pantnagar, University is situated between latitude 29°5' and longitudes 79°2'E. The altitude measured of the site is 243.83 meters above the mean sea level. The research center lies in the foothills of Shivalik range of Himalayan region. It is located in a narrow, geographical and fertile belt called Tarai and falls under the humid subtropical climate zone. The Tarai region of Pantnagar and other adjoining areas have been categorized under the Humidhumid-subtropical climate region. The present study consists of experiments conducted during Kharif season in

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2021 ~~and consisted of~~with forty genotypes of cowpea obtained from various sources including two check varieties Pusa Komal and Kashi Kanchan.

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For the given combination of *i*th and *j*th genotype, the mean deviation are computed and the D^2 values were calculated as $D^2 = \sum (Y^i - Y^j)^2$. The D^2 value obtained for a pair of population was taken as calculated value of χ^2 and was tested against the tabulated value of χ^2 for *P* degrees of freedom where *P* is the number of characters considered. The average intra- and inter-cluster distances were calculated by the formula given by Singh and Chaudhary (1977). The character contribution towards genetic divergence was computed using the method given by the same authors. Cluster analysis classifies a set of observations into two or more mutually exclusive unknown groups based on combinations of interval variables. In this agglomerative hierarchical clustering technique was followed. The method is agglomerative and hierarchical because the strategy starts from all the individuals as single member groups and forms a complete hierarchy joining single pairs of groups till the process is completed with all members in one group. It is the incremental sum of squares as the strategy fuses the pair of groups at each level of the hierarchy that increases the within group sum of squares the least. Distance matrix was converted into dendrogram by using Ward's method where the distance between two clusters is the sum of squares between two clusters summed over all variables. At each stage in the clustering procedure, the within cluster sum of squares was minimized over all partitions obtained by combining 2 clusters from previous stage.

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3. RESULTS AND DISCUSSION

3.1. Grouping of genotypes into different clusters (D^2 analysis)

The D^2 values between any two genotypes was calculated as sum of squares of the differences between the mean values of all the sixteen characters and used for final grouping of the genotypes. Procedure suggested by Tocher (Rao, 1952) was used to group 40 genotypes into six clusters by treating the estimated D^2 Values as the square of the generalized distance. Based on D^2 values, the 40 genotypes were grouped into six highly divergent clusters (Fig. 1) some of the genotypes were so divergent in all the characters studied; hence each single of these genotype found were placed in a separate clusters. Thus, four clusters viz. III (COPBVAR-3), IV (COPBVAR-4), V (COPBVAR-2) and VI (COPBVAR-6) were solitary with one genotype in each cluster. The remaining two clusters out of six clusters were having maximum number of genotypes. Cluster I was biggest with 26 genotypes viz., (EC-390223), (EC-422272), (IC-331250), (IC-628900), (IC-628895), (IC-201098), (IC-202790), (EC-97738), (EC572715), (IC-202718), (IC-202826), (IC-3379320), (EC-390241), (IC-536635), (IC-201095), (IC-202824), (EC-19736), (IC-559405), (IC-628894), (IC-209711), (IC-628897), (EC-97306), (EC-37588), (EC-390216), (EC-202858), (EC-528382) followed by cluster II with (EC-528382), (WB-9), (EC-390268), (Kashi Unnati), (IC-51154), (Kashi Gauri), (PVCP-21), (IC-628893), (Pusa Komal) and (Kashi Kanchan). Singh et al. (2018) classified the genotypes into 10 clusters and cluster II contained highest number of genotypes while cluster III, IV, V, VI, VIII, IX, X

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contained only one genotype. Similarly, Patel *et al.* (2017) classified thirty-two cowpea genotypes into eight clusters. He reported, Cluster - II contained as many as 12 genotypes. Out of 8 clusters, five had only one genotype. The results also resemble with Rai *et al.* (2020), Patel *et al.* (2017) and Chandrakaret *al.* (2016).

3.2. Cluster mean of characters

The cluster mean for the 40 genotypes of cowpea studied in cowpea genotypes revealed considerable differences among all the clusters (Table 1). For the present data it is evident that plant height was highest in cluster V (398.33) cm and lowest in cluster II (69.57). For the days to first flowering the maximum days was taken by cluster VI (57.13) days, whereas minimum was recorded in the cluster III (39.7) days. For attaining the stage of 50 % flowering maximum days was taken by the genotypes in cluster VI (63.87) and minimum days was observed in the cluster III (44.33). The cluster VI (59.90) had taken the maximum days for the first pod emergence and minimum days was taken in cluster III i.e. (38.20) days. The cluster III had taken minimum days to pod to reach its edible maturity i.e., (46.20) days and maximum days was taken by cluster VI (67.90). Maximum number of primary branches per vine was recorded in cluster V (7.00), whereas minimum was recorded in cluster III. The minimum number of pods per cluster was observed in cluster II (1.75) whereas the maximum was found in cluster VI (2.33). For pod length the minimum value was found in cluster III (16.50) and the maximum value is recorded in cluster VI (41.67). The cluster I has the lowest number of seeds per pod (8.74) followed by cluster II (8.93) and cluster III (8.96) whereas, cluster VI has the highest number of seeds per pod (20.83). The 100 seed weight was found to be highest in cluster IV (13.07), followed by cluster V (12.83) and cluster II (12.39) and lowest 100 seed weight is observed in cluster III (8.07). The genotype in cluster III has maximum number of pods per plant (66.67) and minimum was recorded in cluster I (34.94). For the number of pods per plot the maximum value was observed in cluster V (694.17) followed by cluster III (693.83), whereas, minimum number of pods per plot was observed in cluster I (372.10). Average green pod weight was recorded highest in the cluster IV (13.80) followed by cluster VI (13.30) whereas the lowest was recorded in cluster III (6.67). Green pod yield per plant was found to be highest in cluster IV (778.27) whereas lowest was recorded in the cluster I (317.90). Similarly for the green pod yield per plot highest yield was observed in cluster IV (8.11kg) and lowest was recorded in cluster I (3.63). Green pod yield per hectare was highest in cluster IV (135.23) and lowest was observed in cluster I (64.71). Singh *et al.* (2018) concluded Cluster VII had the genotype with the highest mean value for number of seed yield per plant, number of pods per plant and number of clusters

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3.3. Average intra- and inter-cluster distance

The mean intra and inter cluster D^2 values among the six clusters are given in the Table 2. The intra cluster D^2 Values ranged from 0.00 (cluster III, IV, V, VI) to 32.91. (Cluster I). The cluster I had the maximum D^2 value (32.91) followed by cluster II (32.4). The inter cluster D^2 values of the VI cluster revealed that the highest inter cluster generalized distance (106.23) was

between cluster VI and II followed by cluster V and II (105.22), while the lowest (30.65) was between the cluster IV and cluster III followed by (32.38) in cluster V and cluster III. Cluster VI followed by Cluster V is most diverse as all other cluster showed maximum inter cluster distance from it showed in Table 2). Singh *et al.* (2018) reported maximum inter-cluster distance (D=41.97) was found between cluster VII and X, followed by that between VI and VII (D=40.84). The minimum inter-cluster distance was observed between cluster III and IV (D=5.38). These results of genetic diversity study were in accordance with the finding of Valarmathiet *al.* (2007), Vavilapalliet *al.* (2014), Aswathiet *al.* (2015) and Patel *et al.* (2017).

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3.4. Nearest and farthest clusters

The nearest and distant clusters from each of the following clusters based on the D^2 values presented in Table 3 Cluster I was nearest to the cluster III (51.32) and distant from cluster VI (85.76). Cluster II exhibit close proximity with cluster I (56.05) and maximum divergence with cluster VI (106.23). Cluster III was nearest to cluster IV (30.65), while it was farthest from cluster VI (96.74). Cluster IV exhibited intimate relation with cluster III (30.65) and wide distribution with the cluster II (85.49). Cluster V was nearest to cluster III (32.38) and showed maximum divergence with cluster II (105.22). Cluster VI exhibit close proximity with cluster IV (79.20). While, it was farthest from cluster II (106.23). Similar work is done by Nancee *et al.* (2013) and reported the nearest inter-cluster distance was found between cluster-III and IV (3.85) followed by cluster-IV and VII (4.26), cluster-III and VII (4.43). The widest inter cluster distance was found between cluster-V and VIII (8.63) followed by cluster-II and VIII (8.49). Similar results were also reported by Venkatesan *et al.* (2004) and Bertiniet *al.* (2009).

3.5. Percent contribution of each characters towards genetic divergence

The percent contribution of each character towards the genetic divergence is presented in Table 4. It was observed that plant height contributed maximum (65.38 %) towards genetic divergence followed by days to first pod emergence (22.82 %), green pod yield per plot (3.72 %), green pod yield per hectare (3.08 %), number of primary branches (1.54 %), green pod weight (1.28 %), green pod yield per plant (1.03 %), number of pods per plant (1.03 %), number of pods per plot (0.13 %). The remaining characters viz. days to first flowering, days to 50% flowering, days to first pod edible maturity, number of pods per cluster, pod length, number of seeds per pod and 100 seed weight did not contribute to the total divergence. Patel *et al.* (2017) reported that plant height at final harvest (51.81 %) contributes maximum towards genetic divergence of cowpea followed by number of pods per plant (44.15 %), green pod yield per plant (1.21 %). Similarly, Nancee *et al.* (2013) observed the highest contribution to the divergence was through number of seeds per pod followed by plant height at final harvest.

4. CONCLUSION

Based on the findings of this study, it can be inferred that certain genotypes exhibit desirable traits suitable for large-scale farming and could serve as valuable donor parents in breeding programs. Specifically, genotype EC-572715 displays favorable characteristics for plant height, EC-390216 for traits related to flowering, pod emergence, and maturity timing, WB-9 and IC-628899 for the number of primary branches, and COPBVAR-3 and EC-97306 for pod cluster density. Additionally, EC-390216 and IC-628899 are recommended for pod length, while EC-472272 and COPBVAR-3 show promise for seed production per pod. Moreover, COPBVAR-3, EC-390241, and EC-390216 demonstrate potential for maximizing the number of pods per plant and green pod yield per plot. These genotypes warrant further evaluation through ~~multiplication~~ multi-location testing before being deployed for large-scale cultivation and could significantly contribute to breeding programs aimed at enhancing ~~crop cowpea~~ productivity.

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FIGURES & TABLES

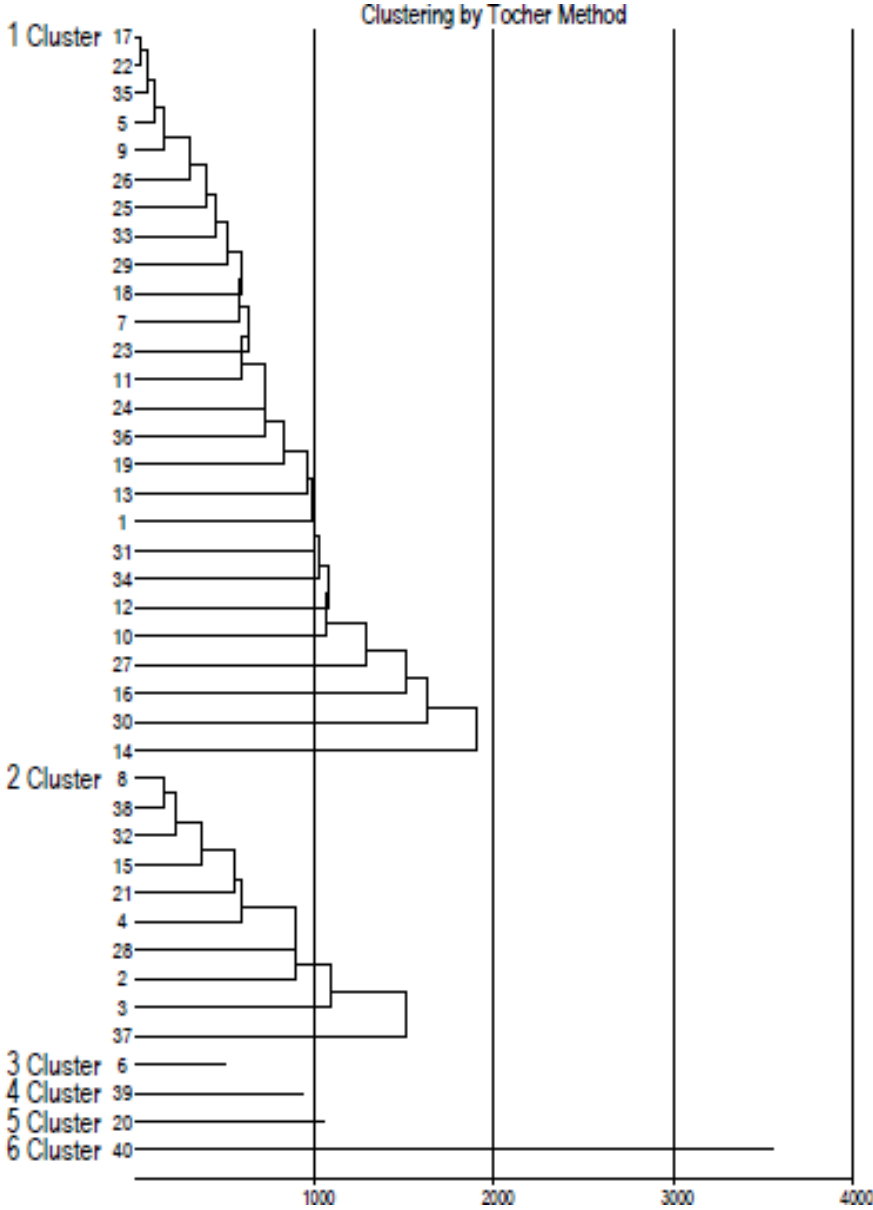


Fig. 1. Dendrogram showing clustering of 40 genotypes using Ward method

Table 1. Cluster mean analysis

Clusters	PH	DF	DFP	DFPE	DFPM	NPB	PC	PL	SP	HSW	PP	PW	YP
I	208.55	40.87	48.09	42.55	50.55	6.10	2.01	16.50	8.74	11.45	34.94	8.78	317.90
II	69.57	40.87	48.28	42.83	50.83	5.90	1.75	22.42	8.96	12.39	41.80	12.96	539.08
III	315.67	36.97	44.33	38.20	46.20	3.00	1.93	16.50	8.93	8.07	66.67	6.67	443.33
IV	331.67	39.43	46.83	41.87	49.87	4.33	1.93	23.00	14.67	13.07	56.33	13.80	778.27
V	398.33	40.23	47.03	42.77	50.77	7.00	1.87	20.17	10.27	12.83	65.67	6.57	431.10
VI	334.00	57.13	63.87	59.90	67.90	6.90	2.33	41.67	20.83	12.03	57.33	13.30	456.00

PH – Plant height (cm); DF – Days to flowering; DFP – days to 50% flowering; DFPE – Days to first pod emergence; DFPM – Days to first pod maturity; NPB – Number of primary branches; PC – pods cluster⁻¹; PL – pod length (cm); SP – seeds pod⁻¹; HSW – Hundred seed weight (g); PP – pods plant⁻¹; PW – pod weight (g); YP – yield plant⁻¹ (g)

Table 2. Average intra- and inter-cluster D² values for six clusters in 40 genotypes of cowpea

Clusters	I	II	III	IV	V	VI
I	32.91	56.05	51.32	53.78	64.65	85.76
II		32.4	86.95	85.49	105.22	106.23
III			0	30.65	32.38	96.74
IV				0	33.61	79.4
V					0	82.61
VI						0

Table 3. The nearest and farthest clusters from each cluster based on D² values in forty genotypes

Cluster No.	Nearest cluster with D ² values	Farthest cluster with D ² value
I	51.32 (III)	85.76 (VI)
II	56.05 (I)	106.23 (VI)
III	30.65 (IV)	96.74 (VI)
IV	30.65 (III)	85.49 (II)
V	32.38 (III)	105.22 (II)
VI	79.40 (IV)	106.23 (II)

Table 4. Percent contribution of plant traits

S. No.	Characters	Times ranked 1st	Per cent contribution
1	Plant height	510	6538.00
2	Days to first flowering	0	0.00
3	Days to 50 % flowering	0	0.00
4	Days to first pod emergence	178	2282.00

5	Days to first pod edible maturity	0	0.00
6	Number of primary branches	12	1.54
7	Number of pods cluster ⁻¹	0	0.00
8	Pod length	0	0.00
9	Number of seeds pod ⁻¹	0	0.00
10	100 seed weight	0	0.00
11	Number of pods plant ⁻¹	8	1.03
12	Green pod weight	10	1.28
13	Green pod yield plant ⁻¹	8	1.03

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