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GENETIC VARIABILITY STUDIES FOR YIELD AND YIELD-ATTRIBUTING TRAITS IN COWPEA GERMPLASM ACCESSIONS (VIGNA UNGUICULATA L. WALP.)

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A set of 153 cowpea germplasm accessions were evaluated under the augmented design at Zonal Agricultural Research Station, Gandhi Krishi Vignana Kendra (GKVK), Bengaluru during *Kharif* 2022 to study genetic variability for yield-related traits. High phenotypic coefficient of variation (PCV) for pods plant⁻¹, plant height, primary branches plant⁻¹, clusters plant⁻¹, pods cluster⁻¹, seed yield plant⁻¹ and 100 seed weight, high genotypic coefficient of variation (GCV) for pods plant⁻¹, plant height, primary branches plant⁻¹, clusters plant⁻¹, plant height, primary branches plant⁻¹, clusters plant⁻¹, pods cluster⁻¹, seed yield plant⁻¹ and 100 seed weight were observed. PCV was relatively higher than GCV and the difference between the phenotypic and genotypic coefficient of variation was narrow for all the traits under consideration indicating higher genetic variability. The plant height, primary branches plant⁻¹, pods cluster⁻¹, pods plant⁻¹, 100 seed weight and seed yield plant⁻¹ had high heritability implying that these traits are highly inherited and environmental factors have little influence on trait expression. Selection practiced based on these characters is inherited efficiently by the next generation. k- means clustering analysis conducted for genetic divergence assessment categorized the accessions into eleven clusterswith the number of accessions per cluster varying from 4 to 29 indicating substantial genetic diversity among the accessions.

Key words : Cowpea, Germplasm accessions, Yield, Genetic variability, k-means clustering.

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of the most important annual herbaceous self-pollinated pulse crop belonging to the family Fabaceae and subfamily Faboideae (Verdcourt, 1970). Cowpea was domesticated in Africa (D'Andrea *et al.*, 2007), from where it spread into all continents and now is commonly grown in many areas of Asia, Europe, Central and South America. Vavilov (1951) recognized India and Africa as the origin of cowpea, while China is considered a secondary center of origin. Cowpea have a nutritional profile similar to other pulses with a relatively low fat content and a 25 percent total protein level, which is two to four times higher than cereal and tuber crops. The same as other pulses, the protein in cowpea grain is rich in lysine and tryptophan which are limiting amino acids in cereal crops. Besides being a rich source of protein, cowpea grain has a good profile of vitamins and minerals (Timko *et al.*, 2007). Along with a high amount of quality protein, cowpea contains carbohydrate (60.3%), fat (1.8%), sufficient amount of calcium (76 mg / 100 gm), iron (57 mg / 100 gm) and vitamins such as thiamine (0.92 mg / 100 g), riboflavin (0.18 mg / 100 g) and nicotinic acid (1.9 mg / 100 g) (Chatterjee and Bhattacharya, 1986). It is often called "black-eyed pea" due to its black or brown ringed hilum and as "vegetable meat" or "poor man's meat" as it serves a major role in human nutrition. In India, cowpea is grown over an area of 4 mha with a production of 2.7 mt and with productivity of 567 kg/ha (FAOSTAT, 2020).

According to the estimates, India's population will increase to 1.69 billion people by 2050, requiring a total of 32.0 million tons of pulse. A growth rate of 2.2% per year is needed to reach this level (Mousumi and Jayita, 2020). Therefore, increasing yield through the development of varieties that are responsive to inputs, possess multiple disease and insect pest resistances, shortduration varieties that perform well across various cropping systems and climate-resilient varieties of pulses will be incredibly beneficial in increasing pulse production in the nation. The poor average yields of cowpea speak of the need for adopting the appropriate method of handling populations to ensure quick progress in the plant breeding programs. India is diverse in terms of environment, there is also diversity in terms of using cowpea. Hence, the varietal requirements also vary in terms of plant type, pod type, days to maturity, pattern of use and growth to suit the diverse regions of the country.

However, cowpea cultivars are known to be well adapted to the low input conditions, but are generally poor in yield and highly susceptible to the major diseases and pests. These production constraints are the main targets of cowpea breeding programs. The plant breeding programme will depend on the variability existing in the population. Hence, this problem could be addressed by screening and evaluating selected germplasm accessions to ascertain genetic stability in yield and yield components. Hence study of genetic variability, heritability and genetic advance become important for the effective selection and utilization of genotypes in breeding programme as in the autogamous crops like cowpea, the existence of variability is limited. In this study, a set of 153 cowpea germplasm accessions was evaluated under the augmented design at the Zonal agricultural research station, GKVK, University of Agricultural Sciences, Bengaluru, during Kharif 2022 to study genetic variability for yield and its attributing traits.

Materials and Methods

The experimental material consisted of 153 cowpea [*Vigna unguiculata* (L.) Walp.] germplasm accessions with two checks, KBC-9 and C-152. The germplasm accessions were collected from the All-India Network Research Project on Arid Legumes (AINP on Arid Legumes) at the Zonal Agricultural Research Station, GKVK, Bengaluru. These accessions were evaluated in augmented design with two checks in 20 blocks with 10 entries in each block following spacing of 0.15 m within the rows and 0.45 m between the rows for assessment of genetic variability for yield and yield-related parameters during *Kharif* 2022 at Zonal Agriculture Research Station, GKVK, Bengaluru. A standard package of practice was followed to ensure a uniform and healthy crop stand.

Observations on the yield and yield-attributing traits were recorded on five randomly selected plants for each of the accessions and the mean was computed viz, Plant height (cm), primary branches plant⁻¹, clusters plant⁻¹, pods cluster⁻¹, pods plant⁻¹, pod length (cm), seeds pod⁻¹, 100 seeds weight (g), seed yield plant⁻¹ (g). Statistical analysis was performed using the Agricolae package of R environment. Phenotypic and genotypic coefficients of variation for all the characters were estimated using the formulae of (Burton and Devane, 1953). Heritability in broad sense was estimated as the ratio of genotypic variance to the total phenotypic variance as suggested by Hanson (1956). Genetic advance and Genetic advance over mean was estimated by using the formula given by Johnson et al. (1955). The genotypes were classified following the model-based 'k-means' clustering approach, an algorithm that is a powerful genetic diversity assessment technique that generates genetically diverse clusters based on genetic distances between germplasm accessions (Mac Queen, 1967) to unravel the organization of variability using the 'Factoextra' package in R software Ver 4.1.3.

Results and Discussion

The presence of genetic variability within and between genotypes aids the breeders in the selection of superior genotypes that further contribute towards crop improvement. The analysis of variance revealed significant genotypic differences for all yield and yieldattributing traits under consideration. Simple measures of variability for yield and yield-attributing traits studied among cowpea germplasm accessions viz, mean, standard error and the range of nine traits for which observations were recorded in this study is depicted in Table 1. The initial step in any breeding program is the evaluation of the variability present in the population. If the population exhibits a significant amount of variability, it can be categorized into heritable and non heritable components using genetic indicators such as the genotypic co-efficient of variation (GCV), phenotypic co-efficient of variation (PCV), heritability (h_{bs}^2) and genetic advance as percent of mean (GAM), enabling the efficient selection of elite genotypes.

The results showed that the phenotypic coefficient of variation was relatively higher than the genotypic coefficient of variation and the difference between the phenotypic and genotypic coefficient of variation was narrow for all the traits under consideration indicating the higher genetic variability. It was found that all the characters under study had high heritability, implying that these traits are highly inherited and that environmental

S.	Traits	Mean ± SE	Ra	nge	PCV	GCV	h ² _{bs}	Expected
no.			Minimum	Maximum	(%)	(%)	(%)	GAM(%)
1	Plant height (cm)	35.13 ± 0.64	15.09	56.99	22.13	21.21	91.82	41.93
2	Primary branches plant ⁻¹	1.80 ± 0.06	1.00	4.89	39.74	36.17	82.85	67.92
3	Clusters plant ⁻¹	4.08 ± 0.13	1.41	12.54	33.83	21.80	41.51	28.97
4	Pods cluster ⁻¹	1.44 ± 0.04	1.00	4.73	39.22	36.37	85.96	69.56
5	Pods plant ⁻¹	5.40 ± 0.18	1.18	13.77	42.51	37.33	77.13	67.63
6	Pod length (cm)	12.35 ± 0.14	8.03	16.78	12.68	9.68	58.33	15.26
7	Seeds pod ⁻¹	10.41 ± 0.14	6.39	14.32	16.36	14.96	83.64	28.22
8	Average grain yield plant ⁻¹ (g)	7.99 ± 0.22	4.09	15.93	33.93	31.77	87.70	61.38
9	Test weight (g)	10.36 ± 0.21	4.11	23.12	25.06	23.97	91.43	47.27

Table 1: Simple measures of variability and genetic variability parameters among cowpea germplasm accessions.

SE: Standard Error PCV: Phenotypic coefficient of variation GCV: Genotypic coefficient of variation: Heritability (Broad sense) GAM: Genetic advance as *per cent* of mean.

Table 2 : k-means clustering ba	used grouping of cov	wpea germplasm accessions.
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Clusters	No. of germplasm accessions	Germplasm accessions
Ι	5	IC-1070, IC-202711(58), EC-170604, IC-219489, NBC-4716
Π	29	KM-5, EC-458442, IC-402175, EC-458417, IC-202290, NBC-39, GW-HOPE, IC-202325, EC-394838, EC-271040, V-585, NBC-51, 2574422(7), EC-458470, NBC-6, NBC-210, NBC-44, EC-458440, IC-402101, NBC-24, NBC-36, IC-2591054, NBC-21, EC-472250, EC-458473, PCD-1124-1, IC-1061, GC-1602, IC-603187
Ш	4	NBC-29, EC-472257, EC-472252, NBC-23
IV	4	*C-152, IC-402154, IC-202792(72), DC-15
V	13	IC-58905, EC-492292, C-157, NBC-98, EC-458480, TOME-774, GC-3, CB-10, IC-202867(99), NBC-12, NBC-27, IC-206240, C-33
VI	5	PCP-0306-01, SKUA-WCP-49, IC-402162, NBC-30, IC-402166
VII	25	EC-472267, CPD-340, CPD-15, EC-075180, C-720, 202329-89, V-16, VCP-18-032, NBC-19, 202804(83), NBC-40, IT-9715499, EC-458505, NBC-43, NBC-16, GC-810, IC-1071, EC-458418, IC-249593, IC-422174, NBC-41, IT-97K499-38, V-578, EC-394779, IC-402090
VIII	19	GENOTYPE-36, 201095(32), IC-402164, IC-237422, PMCP-1016, EC-458438, C-24-1, NBC-14, EC-170574-6, EC-458483, IC-402106, EC-394708, EC-472271, KBC-2, IC-458430, EC-458489, 198355(45), 202521(93), GC-810-1
IX	19	IC-402172, CP-98, IC-1983-26, IC-330996, PMCP-1131, NBC-68, IC-202781, IC-402161, TPTC-29, EC-458485, 202854(97), IC-402048, EC-458511, NBC-25, C-457, IC-402159, IC-25105, IC-402135, IC-45061
Х	15	*KBC-9, NBC-18, 97767(10), CPD-331, IC-4506, EC-394839, VC-458492, V-240, IC-402098, IC-402180, IC-253251, NBC-38, NBC-33, IC-402182, GC-1801
XI	18	EC-458411, VCP-17-019, V-604-7-29-3, EC-390287, SUPER-30, GP-154, NBC-32, 27749(20), IC-249588, IC-202777, NBC-7-W, IC-402114, IC-402125, NBC-8, IC-402104, GC-1805, IC-107120, IC-462099

*Checks.

factors have little influence on trait expression. Selection practiced based on these characters is inherited efficiently by the next generation. The PCV values ranged from 12.68 to 42.51 %. The highest PCV was observed for pods plant⁻¹. High PCV values were also observed for traits including plant height,

Clusters	Cluster means									
Crusters	Plant height	Primary branches plant ⁻¹	Clusters plant ¹	Pods cluster ⁻¹	Pods plant ⁻¹	Pod length (cm)	Seeds pod ⁻¹	Average grain yield plant ¹ (g)	Test weight (g)	
Ι	38.67 ^{abc}	1.62 cd	2.11 °	1.24 °	4.18 ^{bc}	12.23 bcd	8.77 ^d	6.28 ^d	18.90ª	
П	27.67 ^d	1.39 cd	3.19 ^{de}	1.61 °	3.76°	10.59 ^d	9.01 ^d	6.43 ^d	9.55 ^{bc}	
Ш	34.55 bcd	1.00 ^d	3.25 de	4.00 ª	4.90 ^{bc}	12.84 ^{abc}	11.09 abc	8.60 bcd	8.76 ^{bc}	
IV	49.09 ª	2.29 ^{bc}	9.68 ª	1.36 ^{bc}	9.72 ª	12.73 abcd	11.37 ^{abc}	10.92 ^{ab}	9.30 ^{bc}	
V	42.74 ª	1.69 cd	5.61 ^b	1.43 ^{bc}	7.91 ª	14.54 ª	12.78ª	9.05 ^{bc}	11.69 ^b	
VI	27.12 ^d	3.54 ª	5.50 bc	1.54 ^{bc}	8.52 ª	11.11 ^{cd}	10.97 ^{abc}	8.35 bcd	7.55 °	
VII	38.80 ^{ab}	1.21 ^d	3.58 de	1.28 °	4.53 bc	12.71 bcd	10.47 ^{bc}	6.76 ^d	9.53 ^{bc}	
VIII	33.47 bcd	2.53 ^b	3.56 de	1.45 ^{bc}	4.77 ^{bc}	11.63 ^{cd}	9.30 ^{cd}	7.82 bcd	10.83 bc	
IX	30.33 cd	2.03 bc	3.86 cd	1.26 °	4.25 bc	14.20ª	11.95ª	7.13 ^{cd}	9.79 ^{bc}	
Х	34.81 bc	1.59 cd	5.26 bc	1.71 ^b	8.32 ª	11.15 ^{cd}	8.83 ^d	7.27 bcd	10.00 bc	
XI	41.90 ^a	2.10 ^{bc}	3.94 ^{cd}	1.46 ^{bc}	5.65 ^b	13.07 ^{ab}	11.64 ^{ab}	12.66 ª	11.18 ^b	
F-value	15.524	17.292	24.102	26.047	19.299	18.221	22.109	15.362	12.712	
Prob	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	

Table 3 : Cluster means of yield and yield-related traits based on k-means clustering.

primary branches plant⁻¹, clusters plant⁻¹, pods cluster⁻¹, seed yield plant⁻¹ and 100 seed weight. Moderate PCV was recorded for seeds pod⁻¹ and pod length. The lowest value of PCV was found for pod length. The GCV values ranged from 9.68 to 37.33%. The highest GCV was recorded for pods plant⁻¹. High GCV was also observed for plant height (cm), primary branches plant⁻¹, clusters plant⁻¹, pods cluster⁻¹, seed yield plant⁻¹ and 100 seed weight. Moderate GCV was recorded for seeds pod-1 and low GCV was observed for pod length. High PCV and GCV for plant height and clusters plant⁻¹ was observed by Vinay et al. (2022), for primary branches plant⁻¹ by Sharma et al. (2017), for pods cluster⁻¹ by Verma et al. (2019), for pods plant⁻¹ by Ajayi et al. (2014) for seed yield plant⁻¹ and 100 seed weight by Singh et al. (2022).

GCV values serve as an estimate of genetic variability, but it is also important to consider heritability and genetic progress expressed as a per cent of mean when selecting genotypes for a trait of interest. The broad sense heritability values, in the present study, ranged from 41.51% to 91.82%. The heritability values were high for all the traits except clusters plant⁻¹ and pod length. Similarly, the genetic advance as per cent of mean ranged from 15.26% to 69.56%. GAM values were high for all the traits except pod length.

High heritability coupled with high genetic advance as percent of mean was recorded for plant height, primary branches plant⁻¹, pods cluster⁻¹, pods plant⁻¹, seeds pod⁻¹, seed yield plant⁻¹ and 100 seed weight revealing the influence of additive gene action for these traits. Hence the improvement of the traits can made through direct phenotypic selection. These results are in accordance with Darshana *et al.* (2021) and Singh *et al.* (2022).

Clustering of genotypes using k-means clustering

153 cowpea germplasm accessions along with two checks were grouped into eleven distinct clusters following 'k-means' clustering algorithm for genetic divergence assessment with number of accessions per cluster varying from 4 to 29 (Table 2, Fig. 1). The genotypes were grouped using non-hierarchical clustering based on the homogeneity of variances within the clusters and the non-significance of Levene's test. In contrast to Kanavi *et al.* (2020), the pattern of genotype distribution into 11 clusters was based on all traits taken into account and was not geographically based. Results revealed that Cluster II has the highest number of germplasm accessions (29) and Cluster III and IV have lowest number of germplasm accessions (4).

For plant height, cluster IV consisted of genotypes with higher mean value of 49.09 cm (Table 3). Similarly, for primary branches plant⁻¹, cluster VI with higher mean of 2.29, clusters plant⁻¹ (cluster IV), pods cluster⁻¹ (cluster III), pods plant⁻¹ (cluster IV), pod length (cluster V), seeds pod⁻¹ (cluster IX), average grain yield plant⁻¹ (cluster XI) and test weight (cluster I). Therefore, we can use genotypes for breeding from the relevant clusters with the highest mean value to improve the corresponding attributes. The mean sum of squares between clusters was highly significant, indicating that genotypes performed differently in clusters formed.



Fig. 1: k-means clustering of cowpea germplasm accessions based on yield and yield-attributing traits.

Clusters	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
C1	0.00	4.76	7.53	7.68	5.70	6.82	4.64	4.46	5.09	5.37	5.26
C2		0.00	6.51	6.77	5.25	4.96	3.15	3.23	3.64	3.94	4.65
C3			0.00	8.01	6.55	7.19	6.01	6.27	6.21	6.19	6.32
C4				0.00	4.61	5.39	5.77	5.88	5.87	5.15	5.08
C5					0.00	5.14	3.91	4.51	3.77	4.45	3.69
C6						0.00	5.02	4.07	4.46	4.40	4.87
C7							0.00	3.25	2.96	3.73	3.71
C8								0.00	3.27	3.69	3.75
C9									0.00	4.27	3.63
C10										0.00	4.47
C11											0.00

Table 4 : Inter-cluster distances among eleven clusters.

For inter-cluster distance (D^2) , statistics showed that there is high genetic distance and highly significant variation at P \leq 0.01 and P \leq 0.05 among the eleven clusters, indicating wide diversity among cowpea germplasm accessions (Table 4). The highest average inter-cluster distance was recorded between the cluster III and IV $(D^2 = 8.01 \text{ units})$ followed by cluster I and IV $(D^2 = 7.68 \text{ units})$, cluster I and III $(D^2 = 7.53 \text{ units})$, indicating genotypes in the clusters that are distantly related. While the lowest was between cluster VII and IX $(D^2 = 2.96 \text{ units})$, which are distinctly less related. Thus, the germplasm accessions belonging to the distant clusters could be used for cowpea hybridization program.

Conclusion

In the present study, high PCV and GCV were observed for plant height, primary branches plant ⁻¹, cluster

plant⁻¹, pods cluster⁻¹, pods plant⁻¹, average grain yield plant⁻¹ and test weight. The GCV and PCV for average pod length were found to be low and medium, respectively. The GCV and PCV values for seeds pod-1 were found to be moderate. These characters have also exhibited high heritability coupled with high GAM indicating the presence of additive genetic effects and thus selection can be practiced. Results from k-means clustering analysis indicated substantial genetic diversity among the genotypes. The analysis of variance between the clusters was significant indicating that the genotypes performed differentially in the clusters formed. The highest average inter-cluster distance was recorded between the clusters III and IV indicating that the genotypes in these clusters are distantly related. Therefore, accessions from these clusters can be used in hybridization programmes for cowpea improvement.

Conflict of interest

The authors declare no conflicts of interest.

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