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Original scientific article

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF COWPEA (*Vigna unguiculata* L. Walp.) GENOTYPES

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Cowpea [*Vigna unguiculata* (L.) Walp.], is a legume and one of the most ancient crops known to man and grown in tropical and subtropical regions. Cowpea seeds have a high nutritional value containing high amount of protein (20–25%). Despite of its importance, the utilization of genetic diversity and germplasm characterization in cowpea breeding program has not been fully exploited. Therefore, twelve morphological characters and six polymorphic microsatellite/simple sequence repeat (SSRs) markers were used to analyze genetic diversity in thirty-eight cowpea genotypes. The dendrogram was constructed using UPGMA algorithm and Gower's dissimilarity values (ranged from 0.0601 to 0.5589) derived from twelve morphological characters. It was grouped in seven clusters showing the most diverse genotypes were CGD 1246 and CGD 1311 (Gower's distance: 0.5589) and the most similar genotypes were GC 1501 and GC 1601 (Gower's distance: 0.0601). In molecular characterization, a total of 14 amplicons were detected with a ranged from two to three with an average 2.33 alleles per loci. The mean values of polymorphic information content (PIC) and heterozygosity was 0.319 and 0.399, respectively which are measures of the efficiency of markers for studying polymorphism level available in the cowpea genotypes. Total 224 amplicons were considered for to derive Jaccard's similarity matrix for the construction of dendrogram (having six clusters) and 2-D PCA (Principal Component Analysis) plot. The morphological characters and SSR markers can be used in diversity analysis and characterization of cowpea genotypes.

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The *per se* performing genotypes for individual character can be exploited in population/genotype development of cowpea for the improvement of that particular character. This will provide information to plant breeders for selection of parents to develop populations in cowpea breeding programs.

Key words: Cowpea, genetic diversity, germplasm characterization, microsatellite markers, *Vigna unguiculata* (L.) Walp.,

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a legume crop originated in Africa and is extensively grown in Africa, Southeast Asia, Latin America, and in the southern United States (KABAS *et al*., 2007). It plays an important role in human nutrition not only because of its good protein quality with a high nutritive value but also because cowpea hay is critical for feeding animals during the dry season (KABAS *et al*., 2007). In India, cowpea is one of the important legume crops because of its short duration, high yield potential and quick growing habit along with high protein content and as cover crop which helps in conservation of soil (SINGH *et al*., 2003). Cowpea has a great possibility to increase its growers' and traders' incomes (DIOUF, 2011). Although it is an important crop in developing countries, it remains one of the orphan grain legumes due to relatively less attention (GUPTA and GOPALAKRISHNA, 2013).

Genetic diversity plays an important part in the success of any breeding programs (SARR *et al*., 2020; DHARAJIYA *et al*., 2022). Knowledge of genetic diversity in available germplasm and genotypes is very applicable for crop improvement, assisting the effective use of genetic variations in breeding programs through encouraging proper selection of cross combination among large sets of parental genotypes (CHAUDHARI *et al*., 2019). It is also important in improving effective conservation and management approaches. Genetic diversity is evaluated by estimating variations in quantitative and qualitative characters, although sometimes it is limited to characterization of quantitative traits affected by environmental situations. Hence, molecular diversity is also utilized for the estimation of variability among genotypes and germplasms (DHARAJIYA *et al*., 2021). Good amount of diversity has been reported in cowpea for various morphological characters (GHALMI *et al*., 2010; LAZARIDI *et al*., 2017).

The advancements of molecular markers technology have broadened the area of genotyping and genetic diversity analysis by potentially revealing a large amount of genetic variation even between closely related taxa (GELOTAR *et al*., 2019). Molecular markers are quicker and far more precise in the species and genotypes identification (TIWARI *et al*., 2021). Simple sequence repeat (SSR) or microsatellite markers have proved to be polymorphic robust, multi-allelic in nature, highly reproducible but require nucleotide information for primer design (KALIA *et al*., 2011; PARITA *et al*., 2018; KAPURIA *et al*., 2019). Microsatellite markers have also been extensively used in genotype identification, seed purity evaluation and variety protection, pedigree analysis, genetic mapping of simple and quantitative traits and marker assisted selection (MAS) (BROWN *et al*., 1996; KALIA *et al*., 2011; DHARAJIYA *et al*., 2020). Microsatellite markers are one of the most frequently used markers in the genetic diversity analysis of cowpea (LI *et al*., 2001; OGUNKANMI *et al*., 2008; LEE *et al*., 2009; ASARE *et al*., 2010; BADIANE *et al*., 2012; OGUNKANMI *et al*., 2014; ALI *et al*., 2015; CHEN *et al*., 2017; RAINA *et al*., 2020; SARR *et al*., 2020).

The utilization of genetic diversity and germplasm characterization in breeding program has not been fully exploited. It is therefore, felt necessary to study the genetic diversity in cowpea for improvement, by utilizing desired traits in breeding programme. Therefore, the present investigation was carried out to access the genetic diversity among thirty-eight genotypes of cowpea using morphological characters and SSR markers.

MATERIALS AND METHODS

Morphological characterization

Total thirty-eight diverse genotypes of cowpea (Supplementary Table 1) employed in the present study were procured from Pulses Research Station, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India and used for the diversity analysis. The seeds were sown in a Randomized Block Design (RBD) with four replications in July, 2019. Each genotype was accommodated in a single row of 4 m length with a spacing 45 cm between row and 15 cm between plants. All the recommended crop production and protection practices were followed to raise the good crop. The land selected for the experiment is sandy loam soil which brings to fine tilth. Five competitive plants per genotype were randomly selected for recording observations on twelve characters, namely days to flowering, days to maturity, plant height (cm), number of branches per plant, number of pods per plant, number of seeds per pod, pod length (cm), test weight/100 seed weight (g), leaf area per plant (cm^2) (using leaf area meter (LI-3100C; LI-COR, Lincoln, Nebraska, USA)), harvest index (%), protein content (%), and seed yield per plant (g) in each replication and averages were worked out for statistical analysis. The mean values for all the characters and genotypes were used for the construction of dendrogram. The Unweighted Pair Group Method with Arithmetic Average (UPGMA) based dendrogram was constructed using Gower's distance matrix in PAST software version 3.23 (HAMMER *et al*. 2001).

Molecular Characterization

Extraction of genomic DNA

Total genomic DNA was extracted from tender leaves of all thirty-eight cowpea genotypes (Supplementary Table 1) using CTAB (Cetyl Trimethyl Ammonium Bromide) method as described by (DOYLE and DOYLE, 1990) with minor modifications. Purity of extracted genomic DNA samples was confirmed by agarose gel electrophoresis using 0.8 % agarose gel. The quality and quantity of DNA samples were confirmed by spectrophotometer (BioSpectrometer, Eppendorf, Germany). Based on the quantification data, a portion of DNA samples were diluted to yield a working concentration of 50 ng/µl and stored at −20°C for further molecular analysis.

PCR amplification

A set of eight SSR primer pairs (LI *et al*., 2001) were utilized for diversity analysis (Table 1). PCR master mix contained 1.5 µl Taq buffer B (10x) (Genei, Bangalore, India), 0.3 µl dNTP mix (10mM) (Thermo Fisher Scientific, USA), 1.5 µl primer pairs (5 pmol) (Eurofins Genomics India Pvt. Ltd., Bengaluru), 0.1 µl Taq DNA polymerase (3U/µl) (Genei, Bangalore, India), 1.0 µl template DNA (50ng/µl) and 10.6 µl nuclease free water to make final reaction

volume of 15 µl. PCR amplification were carried out in a thermal cycler (Eppendorf, Germany) programmed for 35 cycles with an initial denaturation at 94°C for 4 minutes, followed by denaturation at 94°C for 1 minute, annealing at 56°C for 1:30 minutes, and extension at 72°C for 1 minute. Final extension step was performed at 72°C for 7 minutes. Amplified DNA products were mixed with 2 µl of 6X gel loading dye and loaded on 3% agarose gel. The standard DNA marker (100 bp or 50 bp) was also run along with the samples. The electrophoresis was carried out at 80 V for about 4-5 hr. DNA bands were visualized and images were captured using gel documentation system (Alpha Innotech Corporation, USA).

Table 1. List of SSR primers used in the present study

Sr. no.	Primer	Sequence (5'→3')	Repeat motifs	Expected product size (bp)	Tm value (ºC)
1	VM22	F: GCGGGTAGTGTATACAATTTG	$(AG)_{12}$	217	55.92
		R: GTACTGTTCCATGGAAGATCT			55.92
2	VM28	F: GAATGAGAGAAGTTACGGTG	$(TC)_{20}$	250	55.25
		R: GAGCACGATAATATTTGGAG			53.20
3	VM31	F: CGCTCTTCGTTGATGGTTATG	$(CT)_{16}$	200	57.87
		R: GTGTTCTAGAGGGTGTGATGGTA			60.65
4	VM36	F: ACTTTCTGTTTTACTCGACAACTC	$(CT)_{13}$	160	57.59
		R: GTCGCTGGGGGTGGCTTATT			61.40
5	VM37	F: TGTCCGCGTTCTATAAATCAGC	$(AG)_5.(CCT)_3.(CT)_{13}$	289	58.39
		R: CGAGGATGAAGTAACAGATGATC			58.87
6	VM68	F: CAAGGCATGGAAAGAAGTAAGAT	$(GA)_{15}$	254	57.08
		R: TCGAAGCAACAAATGGTCACAC			58.39
7	VM70	F: AAAATCGGGGAAGGAAACC	$(AG)_{20}$	186	54.51
		R: GAAGGCAAAATACATGGAGTCAC			58.87
8	VM71	F: TCGTGGCAGAGAATCAAAGACAC	$(AG)_{12}.(AAAG)_3$	225	60.65
		R: TGGGTGGAGGCAAAAACAAAAC			58.39

Scoring and data analysis

The SSR amplicons/bands were scored as 1 (present) or 0 (absent). Faint or unclear bands were not considered. Band size was estimated by comparing with standard DNA marker. Genetic similarity among cultivars was calculated according to Jaccard's similarity coefficients (JACCARD, 1908) and a dendrogram using UPGMA was constructed using PAST software version 3.23 (HAMMER *et al*., 2001). Principal Component Analysis (PCA) was also performed by considering first two components to construct 2-D PCA plot using PAST software (Version 3.23). Polymorphic information content (PIC) and heterozygosity were calculated using Gene-Calc online software (BINKOWSKI and MIKS, 2018). The polymorphism percentage was calculated using formula given by CHAUDHARI *et al*. (2019).

RESULTS AND DISCUSSION

Morphological characterization

The data for twelve characters were recorded and used for the analysis. Mean values for each characters and genotypes are shown in Table 2 and Table 3. On the bases of their mean value, best six per se performing genotypes are shown in Table 4. These per se performing genotypes can be utilized for population development and other breeding programs for cowpea improvement. The mean values were used for the construction of dendrogram. The comparison between cophenetic correlation coefficients of different algorithms and similarity/dissimilarity indices for dendrogram construction using morphological data is shown in Table 5. In the present study, UPGMA algorithm and Gower's dissimilarity index were used for the construction of dendrogram as the combination showed the maximum value of cophenetic correlation coefficients among three indices (Gower, Manhattan, and Euclidean).

Table 2. Mean values of morphological characters (days to flowering, days to maturity, plant height, number of branches per plant, number of pods per plant, and number of seeds per pod) of cowpea

Genotype	Character					
	DF	DM	PH (cm)	NBPP	NPPP	NSPP
GC 2	41.50±0.58	63.75±1.26	57.90±4.71	8.80±0.43	20.70±1.16	12.25±0.38
GC 3	44.25±1.26	68.25±1.26	58.00±4.45	7.45±0.72	9.70±0.96	14.75±0.72
GC 4	43.25±1.26	65.25±1.71	55.00±6.20	9.15±0.53	18.15±3.59	10.40±0.78
GC 5	44.50±0.58	65.25±2.22	51.05±5.25	6.40±0.59	18.45±1.12	11.70±0.48
GC 6	46.00±0.82	63.00±1.15	54.55±2.14	8.20±0.71	13.35±1.32	11.20±0.67
GC 1506	44.75±0.50	61.25±1.26	62.90±6.43	9.15±0.53	15.45±0.53	12.20±2.18
GC 1203	41.50±0.58	57.50±1.29	64.45±2.89	7.95±0.41	11.40±0.69	10.50±0.35
GC 1501	41.50±1.73	62.75±1.50	55.00±3.00	9.15±0.87	16.35±1.11	13.30±1.09
GC 1601	43.50±1.29	65.25±1.50	46.50±4.81	8.60±0.82	15.85±1.14	13.25±0.64
GC 1602	41.75±0.50	64.50±1.29	59.40±6.27	7.65±1.18	15.70±0.26	13.40±0.37
GC 1603	39.25±0.50	58.50±2.08	51.40±6.60	9.30±1.33	13.75±1.05	10.20±0.97
GC 1612	40.75±1.71	60.50±1.91	62.20±4.57	8.90±0.53	18.20±3.15	12.30±0.53
GC 1712	45.00±0.82	67.75±0.50	48.90±4.51	9.70±1.29	8.85±0.50	12.05±0.64
GC 1801	42.25±1.26	65.75±2.06	49.90±2.00	9.20±0.49	14.45±0.68	12.30±0.2
GC 1805	41.50±0.58	64.50±2.38	61.50±3.06	7.00±1.32	16.95±1.02	11.80±0.63
CGD 987	43.50±1.29	65.50±1.00	49.35±3.28	9.15±0.57	14.60±1.12	12.30±1.47
CGD 997	42.50±0.58	65.25±0.50	56.45±4.52	8.35±0.34	14.65±0.79	11.95±0.72
CGD 1032	44.50±1.29	67.00±0.82	65.10±2.86	6.60±1.02	14.70±0.66	13.30±0.58
CGD 1116	45.75±1.26	65.50±1.00	46.50±2.46	7.20±0.37	8.15±0.66	10.80±0.85
CGD 1123	43.25±1.50	67.75±0.50	36.95±1.59	10.10±0.50	13.75±1.24	12.20±0.52
CGD 1246	57.50±1.29	75.25±0.96	26.75±3.36	7.15±0.30	7.65±0.34	10.45±0.66
CGD 1254	57.25±0.50	76.75±0.96	50.50±2.09	8.35±0.53	15.30±1.16	13.35±0.53
CGD 1264	51.00±0.82	71.50±1.00	48.00±4.19	8.70±0.42	12.65±1.05	12.55±1.39

Table 2 con. Mean values of morphological characters (days to flowering, days to maturity, plant height, number of branches per plant, number of pods per plant, and number of seeds per pod) of cowpea

Genotype	Character					
	DF	DM	PH (cm)	NBPP	NPPP	NSPP
GDVC 2	43.00±0	70.00±1.15	44.15±1.69	6.65±0.77	5.15±0.62	10.25±1.36
CGD 1287	57.25±1.26	76.75±1.71	69.65±2.42	10.20±0.37	8.20±0.73	11.50±0.48
CGD 1290	57.50±0.58	78.00±1.41	57.75±2.91	10.15±0.62	3.55±0.68	14.40±0.91
PL 4	44.00±1.83	66.75±0.96	27.25±3.53	8.00±0.54	13.25±1.02	15.05±0.57
CGD 1311	41.50±0.58	64.25±1.26	41.35±4.48	10.55±0.50	16.00±1.49	15.75±0.34
CGD 1315	41.50±0.58	65.75±2.22	27.15±2.95	9.30±0.62	13.10±0.35	8.75±0.68
CGD 1320	50.25±1.50	70.00±1.15	48.60±2.76	9.00±0.54	14.45±0.96	13.85±1.44
CGD 1326	48.75±0.96	70.75±1.50	54.55±2.46	7.70±0.26	10.95±0.64	13.25±1.41
CGD 1331	47.75±2.06	69.00±1.41	56.80±4.87	10.10±0.38	7.55±0.19	12.30±1.41
CGD 1383	41.00±1.41	63.75±1.71	52.55±3.82	8.50±0.70	6.55±0.25	16.60±1.32
CGD 1385	41.50±0.58	63.75±0.96	60.10±3.59	9.90±0.68	5.60±0.63	12.90±2.91
CGD 1393	42.25±1.50	65.25±0.50	61.00±4.45	9.15±0.44	13.00±2.44	9.65±2.51
CGD 1399	48.00±0.82	68.25±0.96	59.20±5.71	7.45±0.44	7.20±0.57	12.50±2.55
CGD 1401	44.75±1.50	64.75±0.96	60.05±2.59	8.90±0.50	11.85±1.95	12.90±1.21
CGD 1402	42.75±1.71	64.25±1.26	59.70±2.47	10.35±1.06	10.05±0.44	13.45±1.66
General mean	45.22±1.04	66.57±1.30	52.58±3.74	8.63±0.64	12.51±1.01	12.41±1.00
Range	39.25±0.50 to 57.50±1.29	57.50±1.29 to 78.00±1.41	26.75±3.36 to 69.65±2.42	6.40±0.59 to 10.55±0.50	3.55±0.68 to 20.70±1.16	8.75±0.68 to 16.60±1.32
S.Em.±	0.57	0.68	1.98	0.35	0.61	0.60
C.D. at 5%	1.60	1.90	5.54	0.98	1.70	1.68
C.V.%	2.52	2.04	7.51	8.14	9.69	9.66

Values are represented as Mean±SD. DF: Days to flowering; DM: Days to maturity; PH: Plant height; NBPP: Number of branches per plant; NPPP: Number of pods per plant; NSPP: Number of seeds per pod

Table 3. Mean values of morphological characters (pod length, test weight (100-seed weight), seed yield per plant, leaf area per plant, harvest index, and protein content) of cowpea genotypes

Genotype	Character					
	PL (cm)	TW (g)	SYPP (g)	LAPP (cm^2)	HI (%)	PC (%)
GC 2	12.61±0.99	7.90±0.68	20.02±1.16	1747.59±81.20	56.28±1.49	21.43±0.33
GC 3	13.28±0.83	9.76±0.26	13.97±1.62	1873.55±93.40	34.78±2.25	21.13±0.39
GC 4	14.53±0.36	13.50±1.05	25.48±5.26	1644.19±61.60	70.35±1.09	20.45±0.44
GC 5	13.02±0.18	8.14±1.70	17.88±1.22	1727.26±113.80	57.95±4.93	21.44±0.35
GC 6	12.83±1.34	9.84±0.23	14.77±1.52	1763.83±166.07	51.29±3.47	20.36±0.34
GC 1506	13.95±0.12	10.64±0.43	20.28±2.56	1889.57±233.17	70.01±3.3	21.94±0.35
GC 1203	15.12±0.51	15.02±0.99	18.23±2.83	1543.69±135.69	69.21±1.97	21.24±0.51
GC 1501	12.79±0.31	7.85±0.85	17.06±2.06	1360.42±216.46	64.96±2.93	21.74±0.75
GC 1601	12.86±0.8	7.72±0.31	16.37±1.88	1208.81±87.76	64.97±3.44	22.15±0.18
GC 1602	12.55±1.46	12.76±1.07	26.84±1.09	1805.06±211.77	68.5±3.42	20.08±0.42
GC 1603	14.41±0.08	8.82±1.22	12.51±1.38	1224.05±148.84	55.71±3.92	21.76±0.78
GC 1612	12.18±0.49	9.71±1.45	21.82±4.13	1914.8±205.58	71.19±1.06	21.79±0.18
GC 1712	13.22±0.90	9.18±1.60	9.80±0.59	1461.89±223.43	49.74±2.84	21.82±0.42
GC 1801	12.65±0.86	8.29±0.62	14.70±1.24	1647.69±114.70	66.91±2.66	22.70±1.34
GC 1805	13.11±0.27	8.57±1.00	17.17±1.15	1373.71±210.43	64.09±3.06	20.66±0.96

Genotype	Character					
	PL (cm)	TW (g)	SYPP (g)	LAPP (cm^2)	HI (%)	PC (%)
CGD 987	12.69±0.27	9.75±0.23	17.40±1.14	1402.91±95.95	67.13±3.41	21.74±0.45
CGD 997	15.46±0.86	12.83±0.83	22.64±2.47	1093.02±24.54	70.21±2.74	22.40±0.99
CGD 1032	12.92±1.37	7.90±0.22	15.52±1.09	1102.69±15.01	69.84±3.27	22.29±0.36
CGD 1116	14.97±0.27	8.72±0.32	6.96±2.41	2522.92±218.63	26.8±2.81	20.82±0.19
CGD 1123	15.85±0.98	8.71±0.87	14.78±1.46	1135.29±96.63	56.71±2.01	20.56±1.74
CGD 1246	15.11±0.21	10.52±2.06	8.49±1.19	2116.31±80.08	29.46±4.42	21.83±0.40
CGD 1254	14.24±0.26	11.57±0.74	23.76±1.39	1658.83±232.64	56.65±2.03	21.53±1.52
CGD 1264	15.72±0.15	11.94±1.51	19.21±1.12	918.79±18.13	37.84±2.98	23.93±0.44
GDVC 2	13.19±0.87	10.68±1.64	5.67±0.69	709.45±14.96	27.34±1.57	23.72±0.92
CGD 1287	15.35±0.88	11.69±0.19	11.10±0.96	1046.69±27.78	24.85±2.84	21.59±0.79
CGD 1290	18.89±0.31	11.88±0.14	6.11±1.22	2306.33±215.17	23.61±2.55	22.61±0.88
PL 4	19.21±0.26	11.28±1.14	22.60±1.82	2590.84±191.06	70.12±1.07	20.64±0.63
CGD 1311	13.68±0.65	14.27±0.35	35.96±2.51	904.45±7.38	71.08±1.05	21.03±0.27
CGD 1315	13.75±0.43	14.41±1.14	16.70±1.59	2938.78±89.42	44.36±3.9	19.80±1.43
CGD 1320	14.54±0.88	11.43±0.31	22.84±2.01	3228.03±184.79	60.45±3.37	21.52±0.76
CGD 1326	14.05±0.22	12.99±0.53	18.95±0.76	2475.27±114.43	52.58±2.92	21.32±1.40
CGD 1331	15.78±0.18	9.48±1.12	8.77±0.59	2782.34±160.31	24.19±2.55	22.69±0.40
CGD 1383	27.82±0.12	9.55±0.43	10.14±1.01	1799.81±162.48	36.72±3.50	22.40±0.79
CGD 1385	15.71±0.28	13.82±0.39	10.10±1.58	2525.55±119.55	41.16±3.18	21.87±0.20
CGD 1393	15.88±0.83	11.75±0.19	15.58±1.03	2208.98±161.70	38.35±3.25	21.37±0.28
CGD 1399	14.94±0.13	8.90±0.16	7.79±1.27	2358.13±72.20	23.8±2.50	21.56±0.87
CGD 1401	12.12±0.52	13.96±0.18	23.40±0.99	2271.69±189.59	52.85±3.75	19.76±0.15
CGD 1402	10.64±1.14	9.65±0.29	13.35±1.25	2183.92±84.86	46.87±3.20	22.75±0.74
General mean	14.52±0.57	10.67±0.75	16.44±1.61	1801.77±128.45	51.81±2.81	21.59±0.64
Range	10.64±1.14 to	7.72±0.31 to	5.67±0.69 to	709.45±14.96 to	23.61±2.55 to	19.76±0.15 to
	27.82±0.12	15.02±0.99	35.96±2.51	3228.03±184.79	71.19±1.06	23.93±0.44
S.Em.±	0.35	0.45	0.92	72.18	1.47	0.37
C.D. at 5%	0.97	1.26	2.57	202.28	4.11	1.05
C.V.%	4.76	8.43	11.15	8.01	5.66	3.46

Values are represented as Mean±SD. PL: Pod length; TW: Test weight (100-seed weight); SYPP: Seed yield per plant; LAPP: Leaf area per plant; HI: Harvest index; PC: Protein content

The data of morphological characters were compared among genotypes by using a distance matrix based on Gower's distance (Supplementary Table 2) to construct a dendrogram (Fig. 1). The dendrogram can be divided into seven clusters among which cluster-I contained one genotype (CGD 1383), cluster-II contained two genotypes (CGD 1287 and CGD 1290), cluster-III contained five genotypes (CGD 1116, CGD 1399, CGD 1331, CGD 1246, and GDVC 2), cluster-IV contained one genotype (CGD 1311), cluster-V contained five genotypes (CGD 1315, CGD 1393, CGD 1401, CGD 1385, and CGD 1402), cluster-VI contained 19 genotypes (GC 1603, GC 1712, CGD 1123, GC 5, GC 1805, CGD 1032, GC 1505, GC 1612, GC 2, GC 1501,

GC 1601, GC 1801, CGD 987, GC 6, GC 3, GC 1203, GC 4, GC 1602, and CGD 997), and cluster-VII contained five genotypes (CGD 1254, CGD 1320, CGD 1326, CGD 1264, and PL 4). The Gower's distance ranged from 0.0601 to 0.5589. Based on Gower's distance matrix, the most diverse genotypes were CGD 1246 and CGD 1311 with 0.5589 Gower's distance and the most similar genotypes were GC 1501 and GC 1601 with 0.0601 Gower's distance. The approach to study diversity using data from morphological characters has already been utilized. Diversity analysis using Gower's distance matrix was also studied in twenty landraces of Algeria and the dendrogram was divided them in six different clusters (GHALMI *et al*., 2010). Plant breeders can maximize the utilization of cowpea genetic resources by keeping in mind these genetic differences among genotypes.

Table 4. Best six per se performing cowpea genotypes

Characters	*per se* performing genotypes
Days to flowering	GC 1603 (39.25), GC 1612 (40.75), CGD 1383 (41.0), GC 2 (41.5), GC 1203 (41.5), GC 1501 (41.5)
Days to maturity	GC 1203 (57.5), GC 1603 (58.5), GC 1612 (60.5), GC 1506 (61.25), GC 1501 (62.75), GC 6 (63.0)
Plant height (cm)	Lowest: CGD 1246 (26.75), CGD 1315 (27.15), PL 4 (27.25), CGD 1123 (36.95), CGD 1311 (41.35), GDVC 2 (44.15) Highest: CGD 1287 (69.65), CGD 1032 (65.10), GC 1203 (64.45), GC 1506 (62.90), GC 1612 (62.20), GC 1805 (61.50)
Number of branches per plant	CGD 1311 (10.55), CGD 1402 (10.35), CGD 1287 (10.20), CGD 1290 (10.15), CGD 1123 (10.10), CGD 1331 (10.10)
Number of pods per plant	GC 2 (20.70), GC 5 (18.45), GC 1612 (18.20), GC 4 (18.15), GC 1805 (16.95), GC 1501 (16.35)
Number of seeds per pod	CGD 1383 (16.60), CGD 1311 (15.75), PL 4 (15.05), GC 3 (14.75), CGD 1290 (14.40), CGD 1320 (13.85)
Pod length (cm)	CGD 1383 (27.82), PL 4 (19.21), CGD 1290 (18.89), CGD 1393 (15.88), CGD 1123 (15.85), CGD 1331 (15.78)
100 seed weight (g)	GC 1203 (15.02 g), CGD 1315 (14.41 g), CGD 1311 (14.27 g), CGD 1401 (13.96 g), CGD 1385 (13.82 g), GC 4 (13.50 g)
Leaf area per plant (cm^2)	CGD 1320 (3228.03), CGD 1315 (2938.78), CGD 1331 (2782.34), PL 4 (2590.84), CGD 1385 (2525.55), CGD 1116 (2522.92)
Harvest index (%)	GC 1612 (71.19 %), CGD 1311 (71.08 %), GC 4 (70.35 %), CGD 997 (70.21 %), PL 4 (70.12 %), GC 1506 (70.01 %)
Protein content (%)	CGD 1264 (23.93 %), GDVC 2 (23.72 %), CGD 1402 (22.75 %), GC 1801 (22.70 %), CGD 1331 (22.68 %), CGD 1290 (22.61 %)
Seed yield per plant (g)	CGD 1311 (35.96 g), GC 1602 (26.84 g), GC 4 (25.48 g), CGD 1254 (23.76 g), CGD 1401 (23.40 g), CGD 1320 (22.84 g)

Values in parenthesis are mean values of genotypes for particular character.

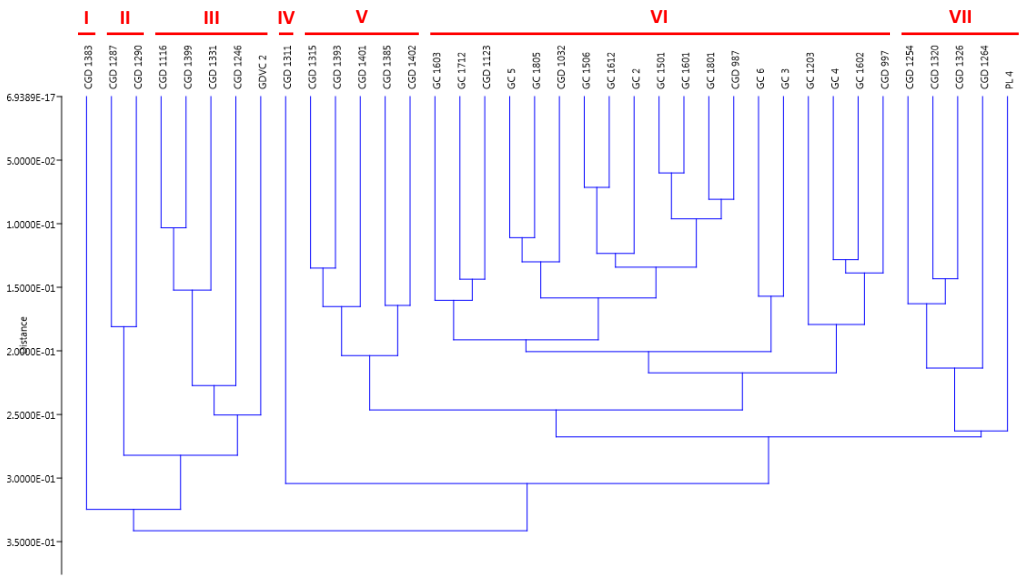

Fig. 1. Dendrogram of cowpea genotypes based on Gower's distance matrix derived from morphological characters

Table 5. Comparison of cophenetic correlation coefficients of different algorithms and similarity/dissimilarity indices for dendrogram construction using morphological data

Similarity/dissimilarity index	Algorithm	
	Single	UPGMA
Gower	0.6879	0.7597
Manhattan	0.7203	0.7349
Euclidean	0.7338	0.7301

Molecular characterization

Out of eight SSR markers, six were polymorphic and two were monomorphic. Six polymorphic SSR primers *viz.*, VM22, VM28, VM31, VM36, VM68, and VM71 generated total numbers of 224 amplicons throughout 38 genotypes. The minimum (152 bp) sized amplicon was amplified by the primer VM36, whereas maximum (302 bp) sized amplicon was amplified by primer VM28. The PIC values were determined for different SSR loci based on the number of alleles and allele distribution. The PIC value ranged from 0.206 to 0.412 with an average of 0.319 (Table 6). The highest PIC value was exhibited by the primer VM68 (0.412), whereas the lowest PIC value was exhibited by the primer VM71 (0.206). Number of amplicons per primer ranged from two to three with an average 2.33. The highest number of amplified amplicons (03)

was exhibited by the primer VM28 and VM68, whereas the lowest numbers of amplified amplicons (02) were exhibited by the primer *viz.*, VM22, VM31, VM36, and VM71. The heterozygosity values ranged from 0.233 to 0.523 (Table 5) with an average of 0.399. The highest heterozygosity value was exhibited by the primer VM68 (0.523), whereas the lowest value was exhibited by the primer VM71 (0.233). OGUNKANMI *et al*. (2014) recorded number of amplicons ranged from two to five and PIC values ranged from 0.075 to 0.603. Primer VM36 exhibited two amplified alleles having PIC value 0.33 which was in agreement with ADETILOYE *et al*. (2013). Primer VM28 exhibited three amplified alleles, which was similar result as recorded by LAL *et al*. (2016).

Table 6. Particulars of polymorphic SSR primers

Sr. no.	Primer	Amplicon size range (bp)	TA	PA	TAG	AF	PIC	H
1.	VM22	223-234	2	2	32	1	0.366	0.482
2.	VM28	228-302	3	3	41	1	0.233	0.255
3.	VM31	209-241	2	2	41	1	0.367	0.485
4.	VM36	152-174	2	2	37	1	0.330	0.417
5.	VM68	250-296	3	3	36	1	0.412	0.523
6.	VM71	226-288	2	2	37	1	0.206	0.233
Total		-	14	14	224	-	-	-
Average		152-302	2.33	2.33	37.33	1	0.319	0.399

TA: Total no. of amplicons; PA: No. of polymorphic amplicons; TAG: Total no. of amplicons among all the genotypes; PIC: Polymorphic Information Content; H: Heterozygosity: AF: Allele Frequency

Jaccard's similarity co-efficient for all thirty-eight genotypes were calculated for thirty-eight genotypes (Supplementary Table 3) and were utilized in the construction of dendrogram. Similarity coefficients were estimated on the basis of six primers ranged from zero to one. The maximum similarity value (1) was observed between GC 1601 and GC 1203, GC 1602 and GC 1501, GC 1805 and GC 1712, CGD 1123 and GC 1501, CGD 1123 and GC 1602, CGD 1287 and GC 5, CGD 1311 and GC 1203, CGD 1311 and CGD 1601, CGD 1315 and GC 1203, CGD 1315 and GC 1601, CGD 1326 and GC 1203, CGD 1326 and GC 1601, CGD 1326 and CGD 1311, CGD 1326 and CGD 1315, CGD 1385 and CGD 1264, CGD 1393 and PL 4, CGD 1401 and GC 1501, CGD 1401 and GC 1602, CGD 1401 and CGD 1123, and CGD 1402 and GC 4. The minimum similarity value (0) was observed between CGD 1246 and CGD 987 as well as CGD 1246 and CGD 1116, indicating them as the most diverse genotypes.

Generally, genetic distances among the cowpea genotypes are low, reflecting the initial obstruction during domestication process, and maintained by the inherent self-pollination system in the crop (ASARE *et al*., 2010). The overall range of the similarity indices among thirty-eight genotypes of cowpea was found to be very wide ranging from 0.00 to 1.00 which indicated that there was high variability among the cowpea genotype under study. The range of similarity indices was observed as 0.55 to 0.97 (SONKER *et al*., 2019) and 0.52 to 0.83 (SAXENA and

TOMAR, 2020). However, a greater number of primers for the diversity analysis will give clear picture of the diversity.

Clustering pattern of dendrogram generated by SSR data showed six clusters (Fig. 2). Nine genotypes, namely CGD 997, CGD 1254, CGD 1320, CGD 1290, CGD 1246, CGD 1264, CGD 1385, GDVC 2, and CGD 1331 were placed in Cluster-I. Cluster-II comprised of one genotype, CGD 1032. Genotype GC 1603 was placed in cluster-III. Cluster-IV comprised of 10 genotypes, namely GC 1801, GC 5, CGD 1287, GC 4, CGD 1402, GC 2, PL 4, CGD 1393, GC 6, and GC 3. Cluster-V consisted 14 genotypes, namely GC 1203, GC 1601, CGD 1311, CGD 1315, CGD 1326, GC 1612, GC 1501, GC 1602, CGD 1123, CGD 1401, GC 1712, GC 1805, CGD 987, and GC 1506. Cluster-VI contained three genotypes, namely CGD 1383, CGD 1116, and CGD 1399.

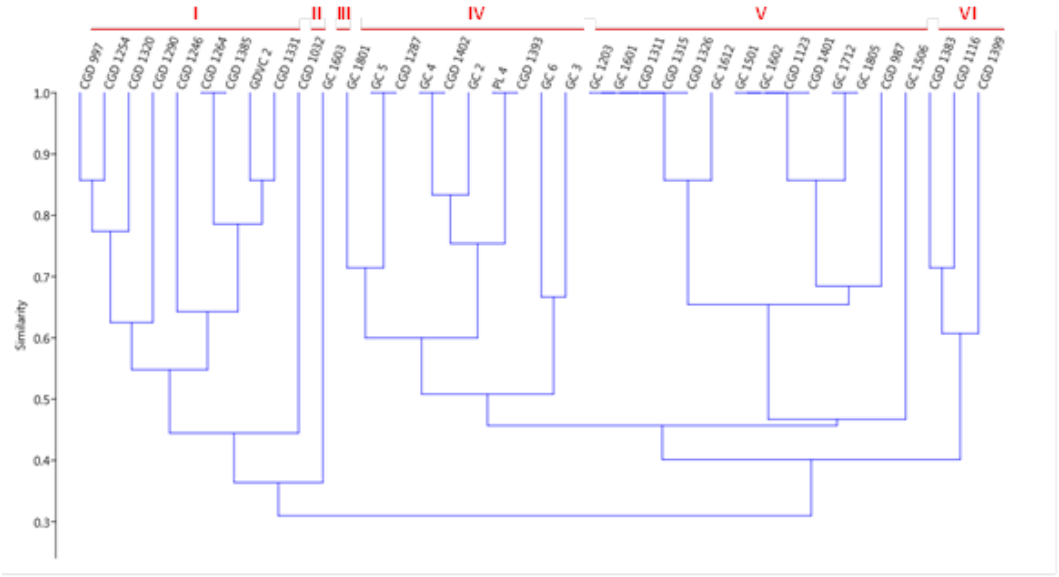

Fig. 2. Dendrogram of cowpea genotypes based on Jaccard's similarity matrix derived from SSR markers

Grouping of genotypes also assessed by PCA method and the results of PCA were in agreement with the results of cluster analysis. From the 2-D plot of PCA, it can be observed that CGD-1331 and CGD 987 were placed farthest in the 1st component (X-axis), while GC 1603 and CGD 1402 placed farthest in the 2nd component (Y-axis) (Fig. 3). Scattering of genotypes on 2-D plot resembled with the pattern of their grouping in the dendrogram although some genotypes have been diverted on the PCA plot. Some genotypes namely, CGD 1290, CGD 997, CGD 1032, CGD 1254, and CGD 1320 placed adjacent on 2-D plot which also appeared similarity among different morphological traits like number of seed per pod and protein content. Some genotypes namely, CGD 1331, CGD 1385, CGD 1246, and GDVC 2 fall under the same group which also

showed similarity at morphological level for the characters like number of seed per pod, number of pods per plant, pod length, and protein content. Protein contents were observed nearly similar for GC 2, GC 4, GC 6, CGD 1402, PL 4, and CGD 1393 and these genotypes were grouped in the same cluster in PCA. Genotypes CGD 1311, CGD 1315, and CGD 1326 were grouped in the same cluster in PCA which showed similarity for morphological characters like pod length, 100 seed weight, and protein content.

Molecular diversity in cowpea genotypes has been studied by UMA *et al.* (2009), ISEGHOHI *et al*. (2016), SONKER *et al*. (2019), and SOUFRAMANIEN *et al.* (2017). Genetic diversity using morphological characters and molecular markers can be well evaluated and utilized in the cowpea improvement.

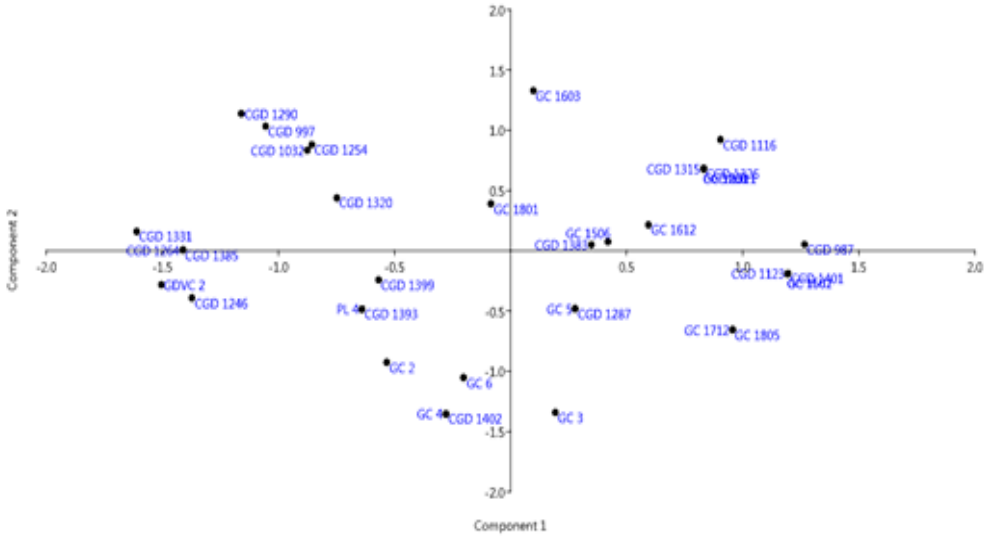

Fig. 3. PCA plot of cowpea genotypes based on SSR markers

Molecular diversity in cowpea genotypes has been studied by UMA *et al.* (2009), ISEGHOHI *et al*. (2016), SONKER *et al*. (2019), and SOUFRAMANIEN *et al.* (2017). Genetic diversity using morphological characters and molecular markers can be well evaluated and utilized in the cowpea improvement.

CONCLUSION

The morphological characters can be used in characterization of cowpea genotypes and assessment of genetic diversity. The *per se* performing genotypes for individual character can be exploited in population/genotype development of cowpea for the improvement of that particular character. The microsatellite markers can be used for genetic diversity analysis which can

ultimately be informative in the selection of parents for the development of population. The genetic diversity will provide information to plant breeders for selection of parents to develop populations in cowpea breeding programs.

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MORFOLOŠKA I MOLEKUALRNA KARAKTERIZACIJA GENOTIPOVA *Vigna unguiculata* L. Walp.

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Izvod

Stočni grašak (*Vigna unguiculata* (L.) Valp.) je mahunarka i jedna od najstarijih kultura poznatih čoveku koja se uzgaja u tropskim i suptropskim regionima. Seme ima visoku hranljivu vrednost i sadrži veliku količinu proteina (20-25%). Uprkos svom značaju, korišćenje genetičkog diverziteta i karakterizacije germplazme u programu oplemenjivanja nije u potpunosti iskorišćeno. Prema tome, dvanaest morfoloških karaktera i šest polimorfnih markera mikrosatelita/ponavljanja jednostavne sekvence (SSR) korišćeno je za analizu genetske raznovrsnosti u trideset osam genotipova. Dendrogram je konstruisan korišćenjem UPGMA algoritma i Gauerovih vrednosti različitosti (u rasponu od 0,0601 do 0,5589) izvedenih iz dvanaest morfoloških karaktera. Grupisan je u sedam klastera koji pokazuju najraznovrsnije genotipove CGD 1246 i CGD 1311 (Gauerovo rastojanje: 0,5589), a najsličniji genotipovi su GC 1501 i GC 1601 (Gauerovo rastojanje: 0,0601). U molekularnoj karakterizaciji, otkriveno je ukupno 14 amplikona u rasponu od dva do tri sa prosečno 2,33 alela po lokusu. Srednje vrednosti polimorfnog informacionog sadržaja (PIC) i heterozigotnosti bile su 0,319, odnosno 0,399, što su mere efikasnosti markera za proučavanje nivoa polimorfizma dostupnog u genotipovima. Ukupno 224 amplikona su uzeta u obzir da bi se izvela Jaccardova matrica sličnosti za konstrukciju dendrograma (koji ima šest klastera) i 2-D PCA (analiza glavnih komponenti). Morfološki karakteri i SSR markeri se mogu koristiti u analizi diverziteta i karakterizaciji genotipova. Genotipovi per se za individualni karakter mogu se iskoristiti u razvoju populacije/genotipa za poboljšanje tog specifičnog karaktera. Ovo će pružiti informacije oplemenjivačima biljaka za odabir roditelja za razvoj populacije u programima oplemenjivanja.

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