Genetic Diversity in the Semi-Arid Grown Cowpea (*Vigna unguiculata* L. Walp) Accessions Using Morphological and Molecular Characterization

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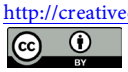

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Abstract

Assessment of genetic diversity of the indigenous crop accessions is extremely important for breeders to identify potential parents in cross-breeding programs. Fourteen cowpea accessions collected from different parts of Sudan were used for characterization at morphological and molecular levels. The seeds of the accessions were sown in the field using a randomized complete block design with three replicates. Sixteen morphological descriptors (9 qualitative and 7 quantitative) and 20 Random Amplified Polymorphic DNA (RAPD) markers were used for characterization of the accessions. The results of morphological data revealed considerable variability within and between state's accessions. Some morphological traits revealed similarity between accessions from different states. Among the 20 RAPD markers used, 18 were polymorphic. A total of 379 polymorphic patterns were generated; polymorphic information content (PIC) ranged from 0.63 to 0.98 with an average of 0.9. The number of fragment detected ranged from 2 for OPL-11 to 51 for OPY-2 with an average of 26.06/primer and 27.07/genotype. One to five (1 - 5) unique fragments of different sizes were detected for particular accessions, which may provide a valuable resource for breeding superior cowpea cultivars in Sudan and other semi-arid zones. Genetic similarity was ranged from 0.02 to 0.47 with an average of 0.25. Highest genetic similarity was between genotypes HSD-2966 and HSD-2967 and between genotypes HSD-5131 and HSD-5627 and the lowest was between HSD-5131 and HSD-5861 followed by that between HSD-2976 and HSD-29130 accessions. The study recommends the combination of morphological and molecular data for more efficient genetic diversity assessment and management.

Keywords

Cowpea Accessions, Genetic Diversity, Characterization, Morphological Markers, RADP Markers, Polymorphism

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is important food and feed legume in sub-Saharan Africa, Asia, and Central and South America due to its high protein contains (22% - 33%) in the seed [1]. This is in addition to its capacity to fix atmospheric nitrogen through symbiosis with root nodule bacteria, ability to grow in soils of low fertility as well as its tolerance to high temperatures and drought, which makes cowpea a key crop in the context of global climate change and food security [2].

Knowledge of genetic diversity in available landrace collections is very important in promoting the efficient use of genetic variations in breeding programs through supporting proper selection of cross combination among large sets of parental genotypes [3]. Such knowledge could be used by a cowpea breeder for identifying new genes for further germplasm improvement and for solving production constraints [4].

Although cowpea has large *ex-situ* collection of diverse cultivated and wild germplasm at the International Crops Research Institute for the Semi-Arid Tropics (ICRIST) and the International Institute of Tropical Agriculture (IITA) that made great contribution to cowpea breeding in West Africa and Asia, limited information on the genetic diversity of the indigenous cowpea germplasm is available in Sudan. Understanding the genetic diversity in the available germplasm is the base of all plant improvement programs as it is a source of variation that constitutes the raw material for genetic improvement of a crop species. Moreover, genetic diversity is essential to decrease crop vulnerability to abiotic and biotic stress, ensure long-term selection gain in genetic improvement, promote rational use of genetic resources [4] [5] and may help breeding programs in the development of more productive cultivars [2]. Therefore, the knowledge of the genetic diversity of the present germplasm could be useful for development of cowpea genotypes suitable for arid and semi-arid environment as they are still facing climate variability and need to develop more climate-resilient cowpea cultivars [5]. To study the genetic diversity of a crop species, and as the morphological markers/descriptors are affected by environment and reducing the fine resolution that needed to ascertain phylogenetic relationships, the combination of morphological and molecular characterization is essential to exploit the potential of the given genotypes [3]. Among the molecular markers, RAPD, although nonspecific, has low cost, being more rapid and technically ease compared to other molecular markers and successfully used in the

studies to evaluate the diversity of the genotypes of the species [6]. Therefore, the objective of this study was to assess the genetic variability and relationships among different cowpea accessions collected from different parts of Sudan using morphological and molecular (RAPD) markers.

2. Materials and Methods

2.1. Plant Material

The plant material used in this study consisted of 14 accessions of cowpea collected from different States in Sudan (Table 1). The seeds of the accessions were collected and *ex-situ* conserved by the Plant Genetic Resources Unit, Agricultural Research Corporation (ARC), Sudan. The experiment was conducted at the Demonstration Farm of the Faculty of Agriculture, University of Khartoum, Shambat (lat. 15˚40'N, long. 32˚32'E, 380 m above sea level). A randomized complete block design with three replicates was used to execute the experiment. The gross plot size was 4 × 4 m, consisting of five ridges, 80 cm apart. Three seeds were planted per hole (October, 2015/2016-2016/2017 seasons) on the shoulder of the ridge, 40 cm between holes along the ridge. Three weeks later, the plants were thinned to two per hole. The experimental plots were irrigated at an interval of 14 days. Weeding was carried out by hand hoeing three times during the season.

2.2. Morphological Characterization

Sixteen descriptors (9 qualitative and 7 quantitative) were evaluated based on the

Table 1. List of the studied genotypes along with their States/region of collection in Sudan.

Code	State/region of collection
HSD-2966	Bahreljibal
HSD-2967	Bahreljibal
HSD-2976	Bahreljibal
HSD-29130	River Nile
DSH-5130	Northern
HSD-5131	Northern
HSD-5132	Northern
HSD-5670	Blue Nile
HSD-5671	Blue Nile
HSD-5672	Blue Nile
HSD-5674	Blue Nile
HSD-5859	Kordofan
HSD-5861	Kordofan
HSD-5864	Kordofan

International Board for Plant Genetic Resources (IBPGR) for cowpea descriptors of 1983. The qualitative characters included: growth habit, terminal leaflet shape, raceme position, pod curvature, seed shape, seed colour, plant vigour, flower colour and colour of mature pod. The quantitative characters corresponded to: pod length, pod width, peduncle length, number of locules/pod, seed length, seed width and seed thickness. Data were collected from 10 randomly selected plants per accession.

2.3. Molecular Characterization

Primer Selection, DNA Extraction and PCR Amplification

Twenty arbitrary RAPD markers were used to investigate the genetic diversity of 14 cowpea genotypes. The random primers used for DNA amplification were 10 base sequences obtained from OPERON Technologies as listed in **Table 4**.

Fresh leaves sample from young plants were collected and put in zip log bags with ice and ground with a mortar and pestle and used for DNA extraction. The DNA was extracted using a modified [7] method. Fifty (50) mg of ground leaf tissue was transferred to 15 ml centrifuge tubes, mixed with 500 µl of 2% CTAB extraction buffer and incubated in a 60˚C water bath with frequent agitation for 1 hour. The tubes were removed from the water bath and allowed to cool until room temperature before 500 µl of Chloroform-Isomyl Alcohol (24:1) was added and mixed thoroughly. The mixture was centrifuged at 3000 rpm for 10 min and the upper supernatant phase collected in a new 15 ml tubes. A second extraction was performed with 3 ml of cold isopropanol (2-propanol) and left overnight. The samples were then centrifuged at 5000 rpm for 10 min and the supernatant was carefully discarded. The pellets formed were then washed by ethanol and centrifuged at 3000 rpm for 5 minutes. One ml of TE buffer (10 mM Tris + 1 mM EDTA, ph 8.0) was added and the tube was incubated at 65˚C water bath for 5 minutes. After incubation, the supernatant was treated with RNase (10 ml/mg). The upper phase was transferred into a new tube and DNA was precipitated with equal volumes of 2 ml cold ethanol (95% - 100%) and 8 M ammonium acetate. Then the contents were mixed gently by inversion. The DNA pellet was kept overnight with 3 ml TE buffer (pH 7.5). Then the DNA was centrifuged at 2000 rpm for 10 minutes and transferred to 1.5 ml Eppendorf tubes and dried for 5 min in air room. The resulting DNA pellet was dissolved in 100 µl of distilled and sterilized water for working stock.

PCR reactions were performed in 20 µl volume in a mixture containing 2 mM $MgCl_2$, 1 × PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0), 0.1 mM of each dNTPs, 0.1 µM of random decamer primer, 50 ng of DNA and 1 unit of Taq DNA polymerase. The PCR amplification process was conducted in T3 Thermocycler Biometra. For each amplification process, an initial heat denaturation of DNA at 94˚C for 3 min was followed by 36 cycles consisting of 1 min at 94˚C, 1 min at 35˚C, and 2 min at 72˚C. A final incubation for 7 min at 72˚C was performed. The amplification products were analyzed by electrophoresis in

2% agarose in TAE buffer stained with 2 μg/ml ethidium bromide and photographed under UV light. The buffer was added to agarose then heated in microwave till milting; cooling to 60˚C then the ethidium bromide was added. Sample was prepared by using 10 μl PCR-product and 2 μl loading buffer; DNA ladder 1 Kb was used as molecular-weight size markers.

2.4. Statistical Analysis

For morphological analysis, the computer program PLABSTAT [8] was used for statistical analysis of the morphological data. The data (quantitative morphological data) were analyzed according to the complete randomized block design as described by [9].

For DNA analysis, and after electrophoresis separation, amplified DNA fragments detected in each accession were scored for presence (1) or absence (0) of a particular DNA fragment of a similar length. Faint fragments were omitted and only reproducible fragments were considered for the analysis. The binary data then processed with the SPSS v.16.0 software to create data matrix to analyze genetic similarity. A dendrogram of 14 cowpea accessions was constructed based on Jaccard's dissimilarity coefficient using each marker data for all cowpea accessions following weighted pair group mean arithmetic method (UPGMA).

Polymorphism information content (PIC) values were calculated as described by [10] as follows:

$$PIC = 1 - \sum P_{ij}^2$$

where, P_{ij} is the relative frequency of the i^{th} allele of the j^{th} locus, summed over all alleles for individual marker locus over all lines.

3. Results

3.1. Morphological Characterization

For qualitative traits, the analyses of nine qualitative morphological characters of the 14 cowpea accessions are presented in **Table 2**. All accessions characterized themselves by having determinate growth pattern. For growth habit, 4 accessions (HSD-2966, HSD-2967, HSD-5859 and HSD-5861) had erect growth habit, 6 accession (HSD-2976, HSD-5131, HSD-5670, HSD-5671, HSD-5672, and HSD-5130) had semi-erect growth habit, and 3 accessions (HSD-29130, HSD-5131 and HSD-5864) had semi-prostrate growth habit. However, HSD-5674 was the only accession that had climbing growth habit. The terminal leaflet shape of 6 accessions (HSD-2976, HSD-29130, HSD-5132, HSD-5670, HSD-5671, and HSD-5130) were sub-hastate, 8 accessions (HSD-2966, HSD-2967, HSD-5131, HSD-5672, HSD-5859, HSD-5861, HSD-5674 and HSD-5864) were sub-globose. However none of the accessions had a globose or hastate terminal leaflet shape. While 6 accessions (HSD-2976, HSD-5131, HSD-5671, HSD-5672, HSD-5861 and HSD-5674) had raceme position mostly above canopy, another 6 accessions (HSD-2966, HSD-29130, HSD-5132, HSD-5859, HSD-5130 and HSD-5864) had

Table 2. Mean score of fourteen qualitative morphological descriptors of fourteen cowpea accessions.

Accession/ descriptor*	HSD-2966	HSD-2967	HSD-2976	HSD-29130	HSD-5131	HSD-5132	HSD-5670	HSD-5671	HSD-5672	HSD-5859	HSD-5861	HSD-5674	HSD-5130	HSD-5864
Growth habit	2	2	3	5	3	5	3	3	3	2	2	7	3	5
Terminal leaflet shape	2	2	3	3	2	3	3	3	2	2	2	2	3	2
Raceme position	2	3	1	2	1	2	3	1	1	2	1	1	2	2
Pod curvature	3	3	3	0	3	3	0	0	0	0	3	0	0	3
Seed shape	1	4	2	4	5	1	2	5	5	2	1	5	2	4
Seed colour	2	2	3	3	2	5	1	3	3	1	3	5	6	2
Plant vigor	5	7	7	7	5	7	7	7	7	7	9	7	3	5
Flower colour	2	1	1	1	2	1	2	2	2	2	1	2	2	2
Mature pod color	4	4	4	1	1	1	4	1	1	3	1	1	4	1

*Growth habit: 2 = erect, 3 = semi erect, 4 = intermediate, 5 = semi-prostate and 7 = climbing. *Terminal leaf shape: 2 = Sub-globose, 3 = Sub-hastate. *Raceme position: 1 = mostly above canopy, 2 = in upper canopy 3 = throughout canopy. *Pod curvature: 0 = Straight and 3 = slightly curved. *Seed shape: 1 = Kidney, 2 = ovoid, 4 = globose and 5 = rhomboid. *Seed colour: 1 = White 2 = Cream, 3 = Brown, 5 = Purple, 6 = Black. *Plant vigour: 3 = non-vigorous, 5 = intermediate, 7 = vigorous and 9 = very vigorous. *Flower colour: 1 = white, 2 = violet. *Mature pod colour: 1 = pale tan or straw, 3 = dark brown and 4 = black or dark purple.

raceme positions in upper canopy. On the other hand, raceme position of 2 accessions (HSD-2967 and HSD-5670) was localized throughout the canopy. For pod curvature, 7 accessions (HSD-2966 ,HSD-2967, HSD-2976, HSD-5131, HSD-5861) were kidney shape, 4 accessions (HSD-2976, HSD-5670, HSD-5859 and HSD-5130) were ovoid shape and 3 accessions (HSD-2967, HSD-29130 and HSD-5864) were globose shape. On the other hand, 4 accessions (HSD-5131, HSD-5671, HSD-5672 and HSD-5674) had rhomboid seed shape. None of the accessions had crowder seed shape. The seed colour of two of the accessions (HSD-5670 and HSD-5859) was white, four accessions (HSD-2966, HSD-2967, HSD-5131 and HSD-5864) were crème, 5 accessions (HSD-2976, HSD-29130, HSD-4671 and HSD-5672) were brown, and two accessions (HSD-5132 and HSD-5674) had purple seed. Only HSD-513030 had black seed colour. For plant vigour, 9 accessions (HSD-2967, HSD-2976, HSD-29130, HSD-5132, HSD-5670, HSD-5671, HSD-5672, HSD-5859 and HSD-5674) were classified as vigorous and 3 accessions (HSD-2966, HSD-5131 and HSD-5864) were intermediate between vigorous and non-vigorous. However, HSD-5861 and HSD-5130 accession were characterized as non-vigorous and very vigorous, respectively. Two flower colours were observed for the studied accessions, white color in 5 accessions (HSD-2967, HSD-2976, HSD-29130, HSD-5132 and HSD-5861) and violet-pink colour in 9 accessions (HSD-2966, HSD-5131, HSD-5670, HSD-5671, HSD-5672, HSD-5859, HSD-5674, HSD-5130 and HSD-5864). However, no other flower color as documented by IBPGR (1983) was observed. For mature pod color, 8 accessions (HSD-29130, HSD-5131, HSD-5132, HSD-5671, HSD-5672, HSD-5674, HYDOOB and HSD-5864) had pale tan pods and 5 accessions (HSD-2966, HSD-2967HSD-2976, HSD-5670 and HSD-5130) had black

or dark purple mature pod. However, HSD-5861 was the only genotype that had dark brown mature pods.

For qualitative traits (Table 3), while pod length was in the range from 11 cm (HSD-5674) to 17 cm (HSD-5861 and HSD-5859), pod width was in the range from 0.7 cm (HSD-5674) to 1.6 cm (HSD-2966). The highest peduncle length (32.80 cm) was obtained for HSD-5131 and the lowest (10.30 cm) for HSD-5670. On the other hand, number of locules/pod ranged from 10.7 for HSD-5674 to 15.1 for HSD-5859 with an average of 12.5. For seed length, HSD-5861 had the highest value (89.7 mm) and HSD-5674 had the lowest one (52.7 mm). Seed width was ranged from 49.7 mm (HSD-5131) to 30.8 mm (HSD-5674) with an average of 41.8 mm. The highest seed thickness (65 mm) was registered for HSD-5861 and the lowest (29.2) for HSD-5674.

3.2. Molecular Characterization

Among the 20 RAPD primers that used to characterize the genetic diversity of 14 cowpea accessions, 18 primers were polymorphic and two were non-polymorphic (Table 4); therefore, they were excluded in the analysis. A total of 379 polymorphic patterns were generated. A representative of the RAPD banding profile of the accessions was shown in Figure 1. The size of amplified fragments was

Table 3. Mean of eight morphological quantitative descriptors of 14 cowpea genotypes.

Genotype/ descriptor	PL (cm)	PW (cm)	PDL (cm)	NL/P	SL (mm)	SW (mm)	ST (mm)
HSD-2966	16.6	1.6	15.4	13.6	77.7	40.8	49.2
HSD-2967	14.8	1.0	21.5	14.5	55.3	35.5	41.8
HSD-2976	15.7	1.1	27.8	11.1	76.3	47.5	53.0
HSD-29130	14.7	1.0	21.0	12.5	69.3	43.2	52.5
HSD-5130	14.9	1.2	31.5	11.2	73.3	40.6	55.8
HSD-5131	14.3	1.1	32.8	11.9	68.0	41.5	58.3
HSD-5132	15.5	1.2	10.9	11.9	84.7	49.7	52.0
HSD-5670	15.0	1.1	10.3	14.0	72.7	46.5	52.3
HSD-5671	12.8	0.8	22.5	11.7	61.7	32.0	38.0
HSD-5672	12.8	0.9	21.7	12.0	73.7	41.8	38.2
HSD-5674	11.1	0.7	23.6	10.7	52.7	30.8	29.2
HSD-5859	17.0	1.1	16.8	15.1	73.0	45.5	52.8
HSD-5861	17.2	1.1	22.7	11.1	89.7	45.0	65.0
HSD-5864	16.1	1.7	17.6	14.0	77.7	45.2	47.3
Overall mean	14.9	1.1	21.1	12.5	71.8	41.8	49.0
LSD (5%)	1.7	0.5	7.4	2.4	11.0	8.2	9.9
CV%	6.8	26.9	20.9	11.3	9.1	11.6	12.0

PL = Pod Length; PW = Pod Width; PDL = Peduncle Length; NL/P = Number of Locules/Pod; SL = Seed Length; SW = Seed Width; ST = Seed Thickness.

Table 4. RAPD primers used for molecular characterization and Percentage of polymorphism and polymorphism information content (PIC) calculated for each primer.

Primer	Nucleotide sequence (5′-3′)	Bands/primer	Polymorphic Bands/accession	PIC
OPA18	AGGTGACCGT	28	28	0.89
OPB10	CTGCTGGGAC	25	25	0.87
OPC1	TTCGAGCCAG	18	18	0.93
OPC9	CTCACCGTCC	30	30	0.71
OPD7	TTGGCACGGG	4	4	0.98
OPG5	CTGAGACGGA	34	34	0.93
OPH4	GGAAGTCGCC	22	22	0.98
OPK9	CCCTACCGAC	3	3	0.98
OPL11	ACGATGAGCC	2	2	0.98
OPL16	AGGTTGCAGG	17	17	0.63
OPK16	GAGCGTCGAA	24	24	0.92
OPK17	CCCAGCTGTG	10	10	0.97
OPR5	GACCTAGTGG	14	14	0.96
OPY1	GTGGCATCTC	26	26	0.93
OPY2	CATCGCCGCA	51	51	0.84
OPY7	AGAGCCGTCA	16	16	0.97
OPY14	GGTCGATCTG	17	17	0.96
UBC4	CCTGGGCTGG	38	38	0.88
Total	-	379	379	-
Average	-	21.06	27.07	0.91

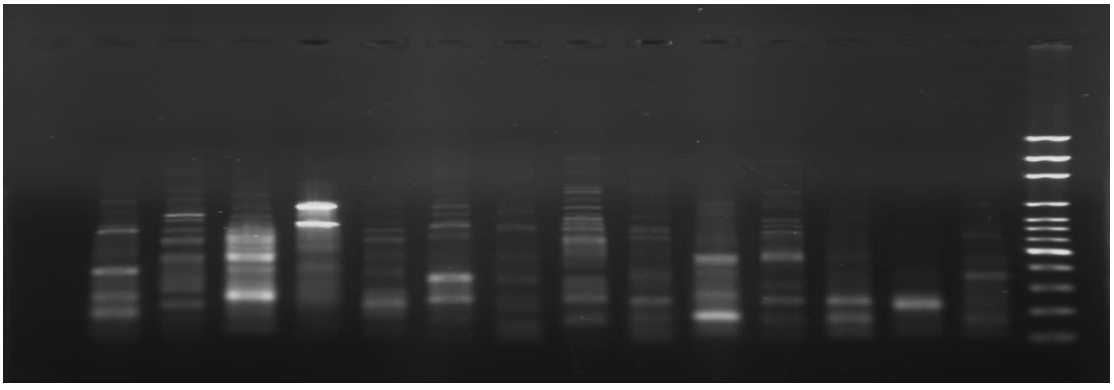

Figure 1. Representative of RAPD banding profile of 14 cowpea (*Vigna unguiculata* L.) accessions.

ranged in size from 100 to 3000 bp (Table 5). The average number of amplified fragments (RAPD bands) was 27.07 alleles per accession whereas the average number of amplified DNA per primer was 21.06 bands. The PIC value derived from the allelic diversity calculated to estimate the information of each primer

among the accessions varied from 0.63 for primer OPL16 to 0.98 for OPD7, OPH4, OPK9 and OPL11 primers, with an average of 0.91 (Table 4). Moreover, one to five unique fragments with different sizes were detected for particular accessions. For example, HSD-5861 and HSD-5674 had five unique fragments, HSD-5670 had three, and HSD-5131 had two unique fragments. On the other hand, five accessions (HSD-2967, HSD-5671, HSD-5672, HSD-5130 and HSD-5864) showed just one unique fragment (Table 5).

Similarity index values obtained from the polymorphic data were used to estimate the genetic relatedness among the accessions. As shown in Table (6) the genetic similarity coefficient for all accessions based on RAPD markers varied from 0.02 to 0.47 with an average of 0.25; *i.e.*, the dissimilarity started from 0.53 - 0.98 genetic distances. The highest pairwise similarity (0.47) was between HSD-2966 and HSD-2967 and between HSD-5131 and HSD-5672 accessions and the lowest pairwise similarity association (0.02) was between HSD-5131 and HSD-5861 followed by that of 0.03 registered between HSD-2976 and HSD-29130 accessions. Also, the lowest pairwise similarity of 0.05 was exhibited

Table 5. Specific fragments (bands) for cowpea accessions generated by different RAPD primers.

Genotypes	Primer	Specific fragments size
	OPR5	3000
		500
HSD-5674	OPY2	700
	OPY7	900
	OPK9	800
	OPH4	3000
		1000
HSD-5861		200
	OPY2	3000
		600
HSD-5670	OPH4	600
		300
		100
HSD-5131	OPL11	200
	OPY1	700
HSD-2967	OPA18	900
HSD-5671	OPY7	1000
HSD-5672	OPY7	500
HSD-5130	OPG5	800
HSD-5864	OPR5	800

Table 6. Genetic similarity matrix for cowpea accessions as assessed by RAPD markers.

Accession	HSD-2966	HSD-2967	HSD-2976	HSD-29130	HSD-5131	HSD-5132	HSD-5670	HSD-5671	HSD-5672	HSD-5859	HSD-5861	HSD-5674	HSD-5130	HSD-5864
HSD-2966	1.00													
HSD-2967	0.47	1.00												
HSD-2976	0.32	0.39	1.00											
HSD-29130	0.17	0.09	0.03	1.00										
HSD-5131	0.20	0.19	0.14	0.36	1.00									
HSD-5132	0.33	0.35	0.34	0.24	0.20	1.00								
HSD-5670	0.20	0.19	0.21	0.14	0.23	0.31	1.00							
HSD-5671	0.22	0.32	0.24	0.23	0.42	0.38	0.32	1.00						
HSD-5672	0.22	0.24	0.12	0.27	0.47	0.21	0.25	0.40	1.00					
HSD-5859	0.20	0.29	0.24	0.13	0.18	0.37	0.20	0.42	0.44	1.00				
HSD-5861	0.11	0.11	0.13	0.05	0.02	0.15	0.16	0.10	0.14	0.20	1.00			
HSD-5674	0.18	0.17	0.18	0.20	0.30	0.27	0.23	0.30	0.33	0.27	0.10	1.00		
HSD-5130	0.09	0.09	0.09	0.13	0.16	0.15	0.16	0.15	0.22	0.25	0.16	0.15	1.00	
HSD-5864	0.22	0.27	0.23	0.16	0.13	0.17	0.05	0.14	0.16	0.24	0.07	0.15	0.32	1.00

between DSD-5864 and HSD-5670, and between HSD-5861 and HSD-29130. In addition, the lowest pairwise similarity of 0.09 were registered between each of HSD-2966, HSD-2967 and HSD-2976 with HSD-5130.

A dendrogram based on the similarity values produced from the RAPD was constructed using the UPGMA cluster to illustrate the association between the accessions (Figure 2). The dendrogram revealed six major clusters of cowpea accessions: Cluster I consisted of two accessions namely, HSD-5130 and HSD-5864; cluster II composed of six accessions namely, HSD-5131, HSD-5672, HSD-5674, HSD-5132, HSD-5671, and HSD-5859. The accessions HSD-2976, HSD-2967 and HSD-2966 were isolated from all other accessions in a separate group (cluster IV). However cluster III, V and VI each had one accession: HSD-5670 (cluster III), HSD-29130 (cluster V) and HSD-5861 (cluster VI).

4. Discussion

4.1. Morphological Characterization

Knowledge of genetic diversity of cowpea germplasm is extremely essential for cowpea breeders to produce cowpea cultivars with high yield and better quality. Morphological traits were used by many researchers [11] [12] and found to be of great importance to distinguish genetic variability and led to a better classification and characterization of cowpea genotypes. In the present study, about two-third of growth habit variation used by IBPGR for cowpea descriptor were observed in the studied accessions (cf. Table 2). In West African cowpea genotypes [13] reported the association of the highest pod yield and possibility of

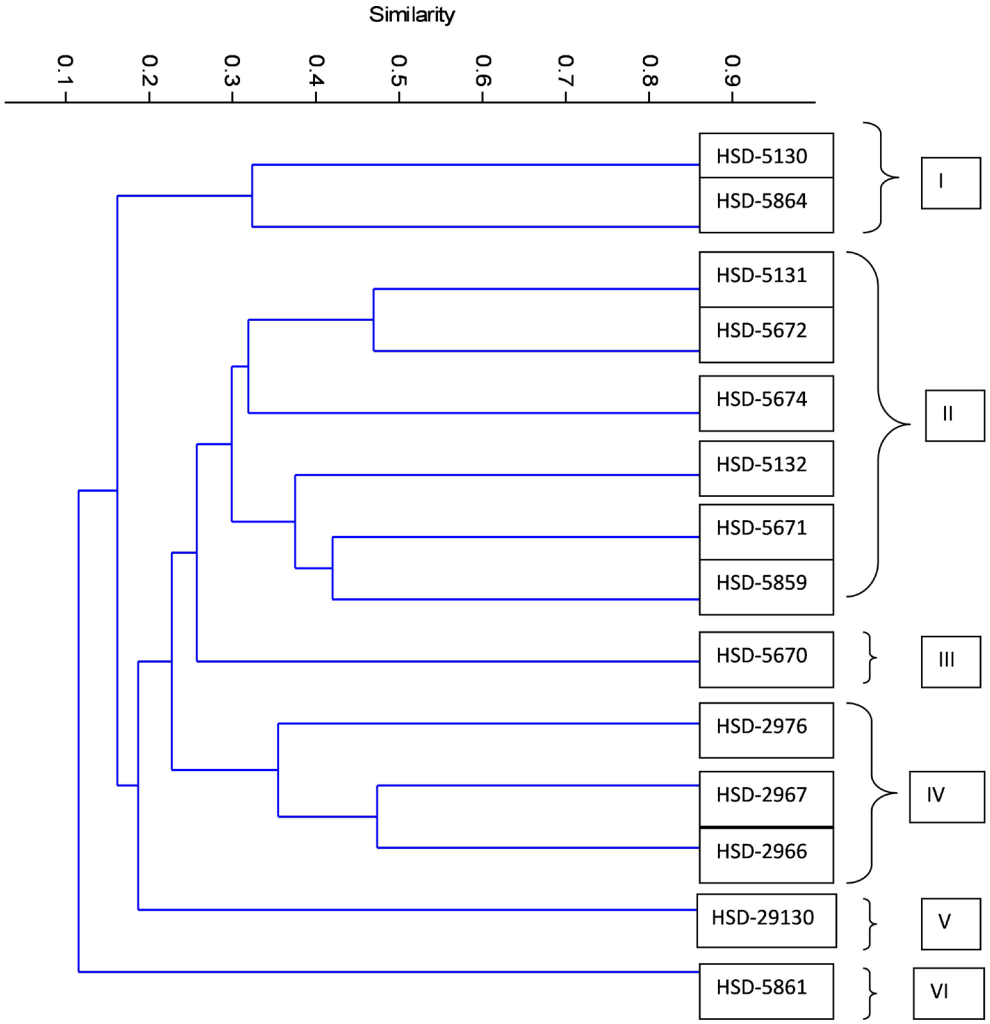

Figure 2. Dendogram showing the 14 cowpea accessions based on RAPD markers values in X-axis corresponds to Jaccard's coefficients of similarity.

mechanical harvesting with the semi-erect growth habit and the association of high reproductive efficiency with the erect growth habit. Moreover, the variations in two out of four of IBPGR cowpea descriptors were observed for terminal leaflet shape, pod curvature and flower colour of the accessions. These variations may be attributed to the genetic makeup of the tested accessions. On the other hand, all variations of IBPGR of cowpea descriptors for plant vigour, raceme position, and seed colour, and almost most of the variations in descriptors for mature pod colour and seed shape were observed in the present investigation. These wide variations among the accessions for these traits could be used as selection criteria in breeding programs for improvement of agronomic performance of the current cowpea accessions. Similar results were obtained by [14] and [15] in cowpea genotypes having similar variations. Although seed coat was reported to have no value in protecting cowpea seed against cowpea seed beetle (bruchid) [16], the black seed coat accession (HSD-5130) was observed not to be attacked by bruchid.

For quantitative descriptors, as presented in **Table 3**, the wide variations among the accessions for pod length and width, peduncle length and number locules/pod could be due to the genetic factors including those control time-factor for assimilates [15]. Such variations are very important to the framer, consumer and breeder; for example, peduncle length determines the position of the pods on the plant and ease the visibility of pods on crop canopy, hence becomes an important character with respect to harvesting [17]. Also, plant with extra-long peduncle may easily be lodged by strong winds [17], thus causing the problems of rotting and rodent attack. Moreover, Pod length, number of locules/pods were found to affect seed yield and reported by [13] as genetically controlled traits. For the traits related to seed size (seed length, width and thickness), usually breeder selects plants with big seeds to satisfy consumer's needs. Therefore, the variation between the present accessions in qualitative and quantitative traits could be used for improvement of yield and quality of cowpea.

4.2. Molecular Characterization and Genetic Relatedness among the Accessions

From the result of RAPD data, the high range of PIC value from 0.63 to 0.98 with an average of 0.91 indicates the presence of high polymorphism in the studied cowpea accessions. The PIC value of the present results (cf. **Table 4**) is higher than the range of PIC value of 0.00 - 0.69 that reported by [18] for within country's cowpea accession from Benin, Egypt, India, Nigeria, Philippines, USA and Zambia using RAPD markers. This implies that the present cowpea accessions are highly diverse and the RAPD markers could be used for detecting polymorphism and differentiating closely related cowpea genotypes as well as genetically distant *Vigna* subspecies. The success of RAPD analysis in distinguishing *Vigna unguiculata* accessions as reported by other workers [19] [20] [21], strongly supports the result of the present work. Such polymorphism could be useful in making decision for improvement of the present cowpea accessions. Moreover, the 1 to 5 unique fragments amplified by some RAPD primers for some accessions as shown in **Table 5** could be used as marker assisted selection for identification of these accessions in cross-breeding programs and to track the transfer of superior alleles that governing the inheritance of economic important traits [6]. Therefore, they may provide a valuable resource for breeding superior cowpea cultivars in Sudan and other semi-arid zones. The presence of one unique fragment detected for some of the accessions could likely be expanded if more primers were tested.

Genetic diversity is commonly measured by the genetic distance or genetic similarity, both of which imply either differences or similarities at the genetic level [22]. In the present study, the arbitrary RAPD markers significantly differentiated the cowpea accessions and clustered them into six groups (cf. **Figure 2**). The low level of similarity was in the range from 0.47 to 0.20, *i.e.*, the

level of dissimilarity started from 0.53 - 0.98 genetic distance (Table 6). The close genetic distance between HSD-5672 (Blue Nile collection) and HSD-5131 (Northern collection) in one hand and that between HSD-5131 (Northern collection) and HSD-5771 (Blue Nile collection) in the other hand could be attributed to the exchange of plant genetic materials of a common genetic make-up between farmers for cultivation and among population for food uses. Similarly, the same justification could be applied to the close genetic distance between HSD-5859 of Kordofan collection and each of HSD-5671 and HSD-5672 of Blue Nile collection. On the other hand, the low genetic similarity between Kordofan (HSD-5864) and Blue Nile (HSD567) collection, between Kordofan (HSD-5861) and River Nile (HSD-29130) collection, as well as the low genetic similarity between Northern collection (HSD-3150) and each of Bahreljibal collection, indicating that northern collection is genetically dissimilar from Bahreljibal collection as they grouped in different clusters. Moreover, the grouping of HSD-5670 (Blue Nile collection), HSD-29130 (River Nile collection) and HSD-5861 (Kordofan collection) in different cluster each, also indicate that they are genetically different from each other. Such vitiations could be of potential to cowpea breeding in Sudan and other semi-arid regions. On the other hand, the grouping of HSD-2966, HSD-2967 and HSD-2976 in one cluster is not unexpected as they were collected from one state (Bahreljibal), which also reveals the efficiency of RAPD in the estimation of the extent of genetic diversity and to ascertain the genetic relationship between different accessions of *Vigna unguiculata* L. Walp.

5. Conclusion

Based on the results of the present study, it could be concluded from morphological and molecular (RAPD) data that there is enough genetic diversity within and between state's accessions, which could be useful in cowpea improvement in Sudan. However, some morphological descriptors identified some accessions as similar, although they appeared genetically dissimilar on the basis of molecular data. Therefore, accurate and reliable genetic diversity could be achieved through the combination of morphological characterization and molecular markers. Moreover, RAPD markers are shown to be useful to ascertain unique bands in cowpea accessions that could be used as marker assisted selection for identification of superior alleles governing important agronomic traits in future breeding programs in arid zones.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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