academicJournals

Vol. 9(7), pp. 293-304, July 2015
DOI: 10.5897/AJPS2015.1313
Article Number: 21E708B54366
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Assessing the genetic diversity of cowpea [*Vigna unguiculata* (L.) Walp.] accessions from Sudan using simple sequence repeat (SSR) markers

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Received 5 May, 2015; Accepted 14 July, 2015

Genetic diversity and phylogenetic relationships among 252 cowpea (*Vigna unguiculata* (L.)Walp) accessions collected throughout the six geographical regions of Sudan were evaluated using simple sequence repeat (SSR) molecular markers. Eighteen (18) published primer pairs were selected based on their informativeness, out of which 16 primer pairs gave reproducible results among all of the cowpea accessions tested. A total of 129 alleles were detected from the 16 loci with an average of 8.1 alleles per locus. Heterozygosity values ranged from 0.01 to 0.13 with an average occurrence of 0.05 while the gene diversity ranged from 0.34 to 0.85 with an average of 0.60. The polymorphism information content (PIC) varied from 0.33 to 0.83 with an average of 0.56. Sudanese Cowpea germplasm clustered into three main groups with control germplasm obtained from the International Institute for Tropical Agriculture (IITA) showing distribution along two groups. This study confirms earlier suggestions that cowpea was first introduced into Sudan from West African countries into western Sudan (Kordofan and Darfur) regions. Accession TVU 8812-IITA Benin was found to be the most divergent cowpea accession within the individuals followed by accession HSD 5738 Sudan-Blue Nile and HSD 6782 Sudan-South Kordofan.

Key words: Simple sequence repeat, microsatellites, genetic diversity, cowpea.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is a tropical grain legume widely distributed in sub-Saharan Africa, Asia, Central and South America as well as parts of southern Europe and the United States (Singh et al., 1997). Domestication of cowpea is presumed to have occurred in Africa given the exclusive presence of wild cowpea (Steele, 1976) although knowledge about the general region or regions of origin and number of domestication events within Africa is fragmented (Faris, 1965; Purseglove, 1968; Steele, 1976; Ng and Padulosi, 1988;

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Coulibaly et al., 2002). It is very important, widely adapted, and versatile grain legume of high nutritional value. Cowpea is mainly produced and consumed in Africa, where it provides a major low-cost dietary protein for millions of smallholder farmers and consumers, who cannot afford high protein foods, such as fish and meat. The s eed protein content is reported to range from 23 - 32% of seed weight (Nielson, 1993) and therefore is often referred to as a “poor man’s meat” (Diouf et al., 2005). In many parts of West Africa, cowpea hay is also critical as livestock feed, especially during the dry season (Wests et al., 1982).

Being a legume, cowpea is nitrogen-fixing (Sanginga, 2003) and fits perfectly in the traditional intercropping systems that are common in Africa, especially given its ability to tolerate shade. The total area under cowpea cultivation is more than 12.5 million hectares worldwide, with an annual production of around 4.5 million metric tons (Singh et al., 2002).

Cowpea remains one of the most important summer adapted food grain legumes grown under rained conditions in Sudan. Despite its importance in Sudan, the yields remain extremely low at an average of 0.26 tons/ha FAO STAT (2010). This production is mainly limited by a wide range of biotic and abiotic constraints.

Cowpea is believed to have been introduced into the western regions of Sudan from West Africa from where it spread to other regions. A national effort to conserve Sudanese cowpea collections resulted in the conservation of more than 250 accessions from different agro-ecological zones at the central gene bank in Wad Medani. These cowpea accessions have been morphologically characterized using the International descriptor list for cowpea published by International Board for Plant Genetic Resources (IBPGR). Traditional selection methods in cowpea depended mainly on the observed morphological variations even though morphological characteristics are easily influenced by the environment (Meglic et al., 1996). The genetic diversity information is extremely important, accurate assessment of genetic variability is important for the preservation and utilization of germplasm resources (Huaqiang et al., 2012).

There is an urgent need to undertake more detailed genetic characterization of cowpea germplasm in order to optimally exploit the resources for improved cowpea production in Sudan. Such analysis would also reveal the true origin of Sudanese cowpea germplasm and establish the extent at which the global cowpea collection at IITA would benefit the cowpea breeding programs in Sudan.

Simple sequence repeat (SSR) 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). The earliest cowpea SSR research is conducted by Li et al. (2001) and 27 SSR primers have been developed. Comparative studies in plants have shown that SSR markers, which are single locus markers with multiple alleles, provide an effective means for discriminating between genotypes (Powell et al., 1996; Li et al., 2001). This study assessed the genetic diversity of 245 Sudanese cowpea accessions alongside 22 global accessions obtained from IITA, Nigeria using SSR markers. The main objectives were to understand the extent of genetic variation and likely origin of Sudanese germplasm as well as create a mini-core collection based on.

MATERIALS AND METHODS

Plant materials

Seeds of 231 Cowpea (*V. unguiculata* L. Walp.) accessions obtained from Plant Genetic Resources Unit of the Agricultural Research Corporation of Sudan representing six different agro-ecological zones of Sudan that is, Northern, River Nile, South Kordofan, North Kordofan, Blue Nile and Bahr Eljabel State (Figure 1) in addition to 36 global cowpea accessions obtained from International Institute of Tropical Agriculture (IITA)-Ibadan-Nigeria (Table 1) were used in the present study. These materials (267 accessions) were planted in the greenhouse of Bioscience Eastern Central Africa BecA-ILRI Hub-Kenya for seedling establishment.15 accessions failed to germinate in the green house; a rest of 252 accessions was successfully grown and used.

DNA extraction

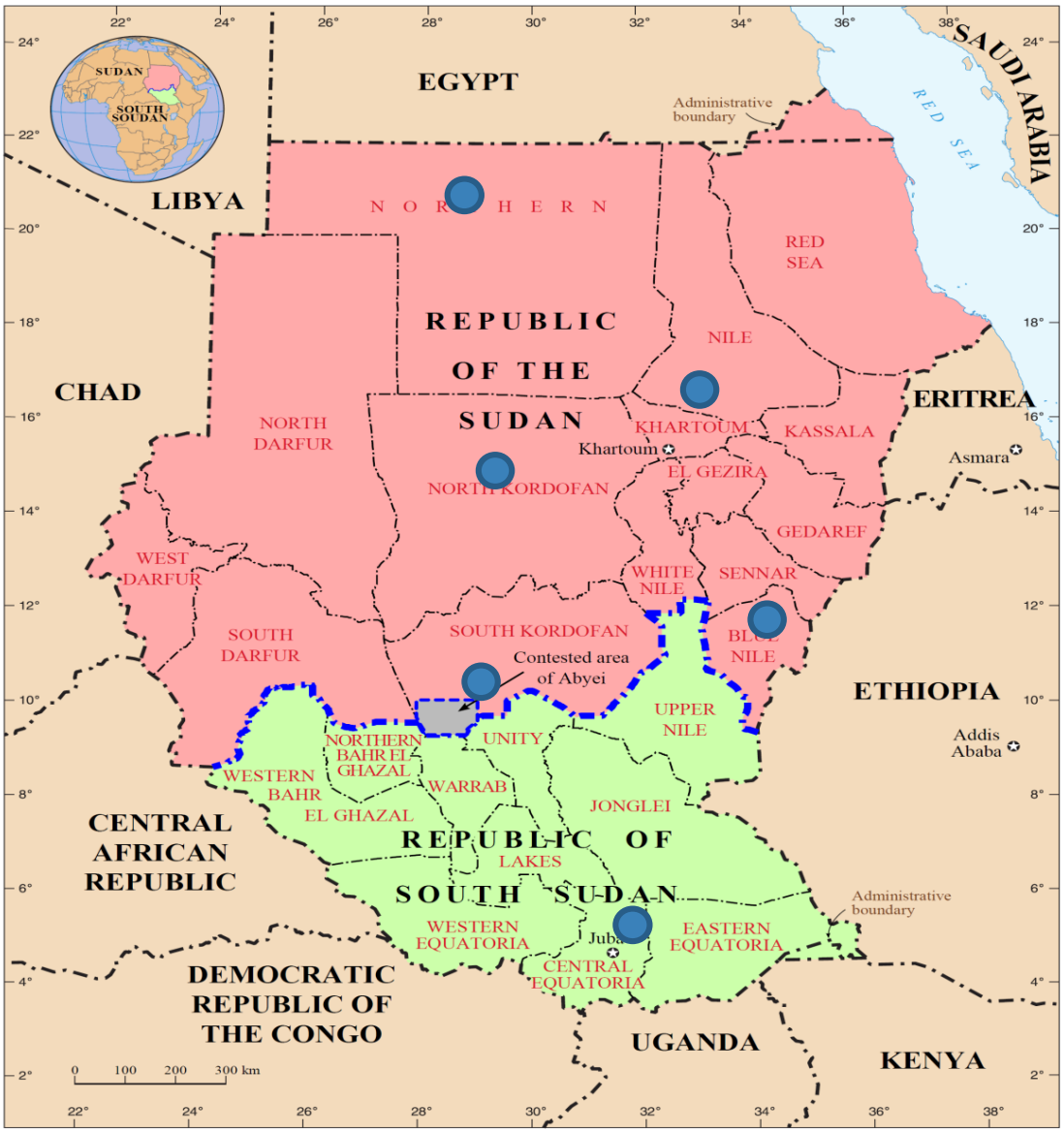
Young leaves sampling were taken eight days after sowing in 1.5 mL Eppendorf tube, frozen immediately in liquid nitrogen and stored in -80°C, then leaves samples were manually grinded using micropestle. Genomic DNA isolated from young seedlings leaves following ZR plant/seed DNA protocol. DNA quality and quantity check done using Nano-drop spectrophotometer and 1% Agarose gel electrophoresis stained with Gel red was used to run the gel. The DNA was normalized by adjusting its concentration to 25 ng μL^{-1} in an optical 96-well Reaction plates using sterile de-ionized water.

Microsatlite amplification

A total of 18 polymorphic SSR markers were used to screen 252 cowpea DNA samples (Table 2). The forward and reverse primers for each of the 18 SSR markers were labeled at the 5´ end of the oligonucleotide using fluorescent dyes to enable detection. PCR reaction were performed in 10 µL final volume in a mixture containing (Tag DNA polymerase1U, dNTPs 1 mM and Reaction buffer 1x) in bulk Polymerase chain reaction (PCR) premix, 5 mM reverse and forward primers, 2 µL DNA, 0.2 µL of 25 mM $MgCl_2$ and 7.2 µL of double distilled water. The optimal annealing temperature varied according to the T_m of the primer pairs and was determined using gradient PCR. For each amplification process, an initial denaturation of DNA at 95°C for 3min was followed by 30 cycles consisting of 30 sec at 94°C, 30 s at 50 to 60°C for annealing temperature (Table 2) 2 min at 72°C extensions a final extension of 15 min at 72°C was performed and the amplification products analyzed on 2% agarose gels in Tris Borate buffer stained with Gel red for visualization to establish polymorphism (Figure 2).

PCR analysis

PCR products of 4 primers with different dyes coloaded together in 96-well working plate vortexes and spined then a sub sample of 1.4



Map compiled from United Nations data, for information purposes only. Borders are approximate.

Figure 1. Different ecologica Zones where sample collected.

Table 1. List of cowpea accessions used; names, and origin.

S/N	Accession	Origin	S/N	Accession	Origin
1	HSD 10295	Sudan	127	HSD 5695	Sudan
2	HSD 10297	Sudan	128	HSD 5698	Sudan
3	HSD 10311	Sudan	129	HSD 5699	Sudan
4	HSD 10323	Sudan	130	HSD 5701	Sudan
5	HSD 10355	Sudan	131	HSD 5702	Sudan
6	HSD 10368	Sudan	132	HSD 5703	Sudan
7	HSD 10393	Sudan	133	HSD 4845	Sudan
8	HSD 10394	Sudan	134	HSD 4846	Sudan
9	HSD 10402	Sudan	135	HSD 4847	Sudan
10	HSD 10438	Sudan	136	HSD 4848	Sudan
11	HSD 10439	Sudan	137	HSD 4849	Sudan

Table 1. Contd.

S/N	Accession	Origin	S/N	Accession	Origin
12	HSD 10474	Sudan	138	HSD 4850	Sudan
13	HSD 10491	Sudan	139	HSD 4851	Sudan
14	HSD 10502	Sudan	140	HSD 4853	Sudan
15	HSD 10506	Sudan	141	HSD 4854	Sudan
16	HSD 10513	Sudan	142	HSD 4855	Sudan
17	HSD 10531	Sudan	143	HSD 4856	Sudan
18	HSD 10550	Sudan	144	HSD 4857	Sudan
19	HSD 10551	Sudan	145	HSD 4858	Sudan
20	HSD 10666	Sudan	146	HSD 4859	Sudan
21	HSD 10698	Sudan	147	HSD 4860	Sudan
22	HSD 10699	Sudan	148	HSD 4861	Sudan
23	HSD 1310	IITA	149	HSD 4862	Sudan
24	HSD 1311	IITA	150	HSD 4863	Sudan
25	HSD 1313	IITA	151	HSD 4864	Sudan
26	HSD 1314	IITA	152	HSD 4865	Sudan
27	HSD 2968	Sudan	153	HSD 5130	Sudan
28	HSD 2976	Sudan	154	HSD 5131	Sudan
29	HSD 2979	Sudan	155	HSD 5133	Sudan
30	HSD 2984	Sudan	156	HSD 5134	Sudan
31	HSD 3347	Sudan	157	HSD 5665	Sudan
32	HSD 3586	Sudan	158	HSD 5666	Sudan
33	HSD 3587	Sudan	159	HSD 5668	Sudan
34	HSD 3588	Sudan	160	HSD 5669	Sudan
35	HSD 3589	Sudan	161	HSD 5670	Sudan
36	HSD 3590	Sudan	162	HSD 5672	Sudan
37	HSD 3591	Sudan	163	HSD 5673	Sudan
38	HSD 3592	Sudan	164	HSD 5674	Sudan
39	HSD 3593	Sudan	165	HSD 5675	Sudan
40	HSD 3594	Sudan	166	HSD 5676	Sudan
41	HSD 3595	Sudan	167	HSD 5677	Sudan
42	HSD 3596	Sudan	168	HSD 5704	Sudan
43	HSD 3598	Sudan	169	HSD 5706	Sudan
44	HSD 3600	Sudan	170	HSD 5707	Sudan
45	HSD 3602	Sudan	171	HSD 5708	Sudan
46	HSD 4356	Sudan	172	HSD 5710	Sudan
47	HSD 4357	Sudan	173	HSD 5711	Sudan
48	HSD 4358	Sudan	174	HSD 5724	Sudan
49	HSD 4359	Sudan	175	HSD 5729	Sudan
50	HSD 4360	Sudan	176	HSD 5737	Sudan
51	HSD 4361	Sudan	177	HSD 5738	Sudan
52	HSD 4362	Sudan	178	HSD 5838	Sudan
53	HSD 4363	Sudan	179	HSD 5839	Sudan
54	HSD 4364	Sudan	180	HSD 5840	Sudan
55	HSD 2845	Sudan	181	HSD 5841	Sudan
56	HSD 2846	Sudan	182	HSD 5843	Sudan
57	HSD 2963	Sudan	183	HSD 5844	Sudan
58	HSD 2964	Sudan	184	HSD 5845	Sudan
59	HSD 2966	Sudan	185	HSD 5846	Sudan
60	HSD 2967	Sudan	186	HSD 5847	Sudan
61	HSD 4406	Sudan	187	HSD 5848	Sudan
62	HSD 4410	Sudan	188	HSD 5850	Sudan

Table 1. Contd.

S/N	Accession	Origin	S/N	Accession	Origin
63	HSD 4411	Sudan	189	HSD 5852	Sudan
64	HSD 4412	Sudan	190	HSD 5853	Sudan
65	HSD 4414	Sudan	191	HSD 5854	Sudan
66	HSD 4480	Sudan	192	HSD 5855	Sudan
67	HSD 6782	Sudan	193	HSD 5856	Sudan
68	HSD 4836	Sudan	194	HSD 5858	Sudan
69	HSD 4837	Sudan	195	HSD 5859	Sudan
70	HSD 4838	Sudan	196	HSD 5861	Sudan
71	HSD 4839	Sudan	197	HSD 5862	Sudan
72	HSD 4840	Sudan	198	HSD 5864	Sudan
73	HSD 4842	Sudan	199	HSD 5865	Sudan
74	HSD 4843	Sudan	200	HSD 5678	Sudan
75	HSD 4844	Sudan	201	HSD 5679	Sudan
76	HSD 4373	Sudan	202	HSD 5680	Sudan
77	HSD 4374	Sudan	203	HSD 5681	Sudan
78	HSD 4375	Sudan	204	HSD 5683	Sudan
79	HSD 4376	Sudan	205	HSD 5684	Sudan
80	HSD 4377	Sudan	206	HSD 5685	Sudan
81	HSD 4378	Sudan	207	HSD 5686	Sudan
82	HSD 4379	Sudan	208	HSD 5687	Sudan
83	HSD 4380	Sudan	209	HSD 5688	Sudan
84	HSD 4381	Sudan	210	HSD 5689	Sudan
85	HSD 4382	Sudan	211	HSD 5690	Sudan
86	HSD 4383	Sudan	212	HSD 4568	Sudan
87	HSD 4384	Sudan	213	HSD 6560	Sudan
88	HSD 4385	Sudan	214	HSD 6561	Sudan
89	HSD 4386	Sudan	215	HSD 6612	Sudan
90	HSD 4387	Sudan	216	HSD 6628	Sudan
91	HSD 4388	Sudan	217	HSD 6629	Sudan
92	HSD 4389	Sudan	218	HSD 6659	Sudan
93	HSD 0306	Sudan	219	HSD 6671	Sudan
94	HSD 0311	Sudan	220	HSD 6698	Sudan
95	HSD 0576	Sudan	221	HSD 6706	Sudan
96	HSD 0806	Sudan	222	HSD 6715	Sudan
97	HSD 0836	Sudan	223	HSD 6732	Sudan
98	HSD 10296	Sudan	224	HSD 6744	Sudan
99	HSD 4365	Sudan	225	HSD 6759	Sudan
100	HSD 4366	Sudan	226	HSD 6760	Sudan
101	HSD 4367	Sudan	227	HSD 4370	Sudan
102	HSD 4369	Sudan	228	HSD 1317	IITA
103	HSD 4371	Sudan	229	HSD 1320	IITA
104	HSD 4372	Sudan	230	TVU 8779	Benin
105	HSD 1315	IITA	231	TVU 8812	Benin
106	HSD 1316	IITA	232	TVU 8834	Benin
107	HSD 1318	IITA	233	TVU 10281	Benin
108	HSD 1319	IITA	234	TVU 10754	Benin
109	HSD 1321	IITA	235	TVU 8262	BorkinaFaso
110	HSD 1323	IITA	236	TVU 8516	BorkinaFaso
111	HSD 1324	IITA	237	TVU 9257	BorkinaFaso
112	HSD 2844	Sudan	238	TVU 10835	Cameron
113	HSD 4390	Sudan	239	TVU 8082	Cotedivoire

Table 1. Contd.

S/N	Accession	Origin	S/N	Accession	Origin
114	HSD 4391	Sudan	240	TVU 7325	Ghana
115	HSD 4392	Sudan	241	TVU 7614	Mali
116	HSD 4395	Sudan	242	TVU 7625	Mali
117	HSD 4396	Sudan	243	TVU 7647	Mali
118	HSD 5691	Sudan	244	TVU 10362	Mali
119	HSD 5694	Sudan	245	TVU 467	Mauritania
120	HSD 5696	Sudan	246	TVU 43	Nigeria
121	HSD 5697	Sudan	247	TVU 889	Nigeria
122	HSD 4368	Sudan	248	TVU 4669	Nigeria
123	HSD 1325	IITA	249	TVU 4783	Nigeria
124	HSD 4394	Sudan	250	TVU 6966	Nigeria
125	HSD 5692	Sudan	251	TVU 8923	Nigeria
126	HSD 5693	Sudan	252	HSD 5700	Sudan

Table 2. List of polymorphic primers.

SSR primer	Primer sequence	Annealing temperature range
VM30_F	5'**NED**_CTC TTT CGC GTT CCA CAC TT	59-62
VM35_F	5'**PET**_GGT CAA TAG AAT AAT GGA	48-53
VM37_F	5'**VIC**_TGT CCG CGT TCT ATA AAT	49-56
VM39_F	5'**NED**_GAT GGT TGT AAT GGG	48-53
VM40_F	5'**6FAM**_TAT TAC GAG AGG CTA TTT	48-52
VM51_F	5'**PET**_CAT TGC CAC TGG TTT CAC TTA	48-62
VM53_F	5'**VIC**_GAG TTC CGT TCG TTG TGA GTA GAG	48-62
VM54_F	5'**NED**_CAC ACA CAC ACA TAG ATA TAG	53-60
VM57_F	5'**6FAM**_GGA AGG GGT AGA GGA AAA GTG AA	57-62
VM70_F	5'**PET**_AAA ATC GGG GAA GGA AAC C	48-62
VM74_F	5'**NED**_CTG CTA CAC CTT CCA TCA TTC	48-62
VM94_F	**5'6FAM**_TCG AAC TTT GGC TTG AGG	**48-62**
Bmd17_F	5'**VIC**_GTT AGA TCC CGC CCA ATA GTC	48-62
SSR-6569_F	5'**PET**-GTTAACATCAGTCCCTTTCA	52-56
SSR-6573_F	5'**VIC**-TGTATGTAATGGAATCGTAA	48-54
SSR-6577_F	5'**6FAM**-GAACTTGATAGGATCCTAGA	57-62

ml from the mixture added to 8 ml Hi-Di (Formamide) and 500 LIZ mixture in 96 PCR plate then the reaction vortexes spines and loaded on PCR machine at 95°C as Denaturation temperature for 3 min the product fast cooled in ice for 10 min before analysis. Fragment analysis was performed on the ABI 3730 sequencer machine, and peaks were sized and the alleles classified using the Gene mapper software (Applied biosystems). The informativeness of each primer pairs was realized using the polymorphic information content (PIC) using the Power Marker software program. The genetic structure of the accessions was investigated by Analysis of Molecular Variance (AMOVA) (Input as Allelic Distance Matrix for F-Statistics using GenAlEx software program; whereas the principal coordinate analysis (PCoA) was performed to identify genetic variation patterns among the cowpea genotypes.

RESULTS

Polymorphism of SSRs in cowpea germplasm

A set of 18 primer pairs pre-selected by their ability to PCR amplify SSRs in cowpea germplasm were used to examine the genetic diversity and phylogenetic relationships among 252 cowpea accessions Sixteen of the primer pairs gave polymorphic DNA fragments following fragment analysis of PCR amplification products. One primer pair generated monomorphic allelic amplification profile across all cowpea genotypes tested, (Figure 3)

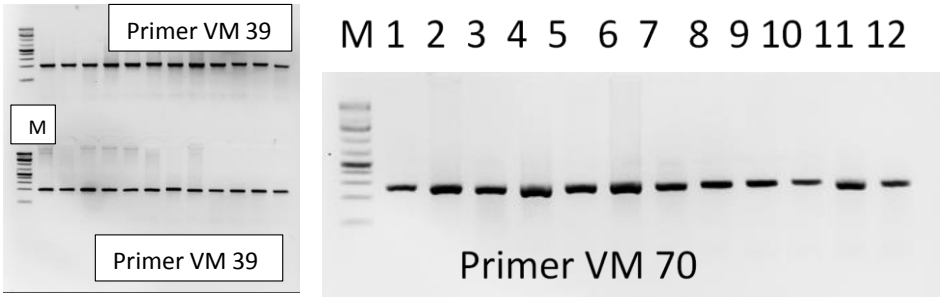


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Figure 2. PCR Ampification of cowpea genomic DNA from 252 accessions.

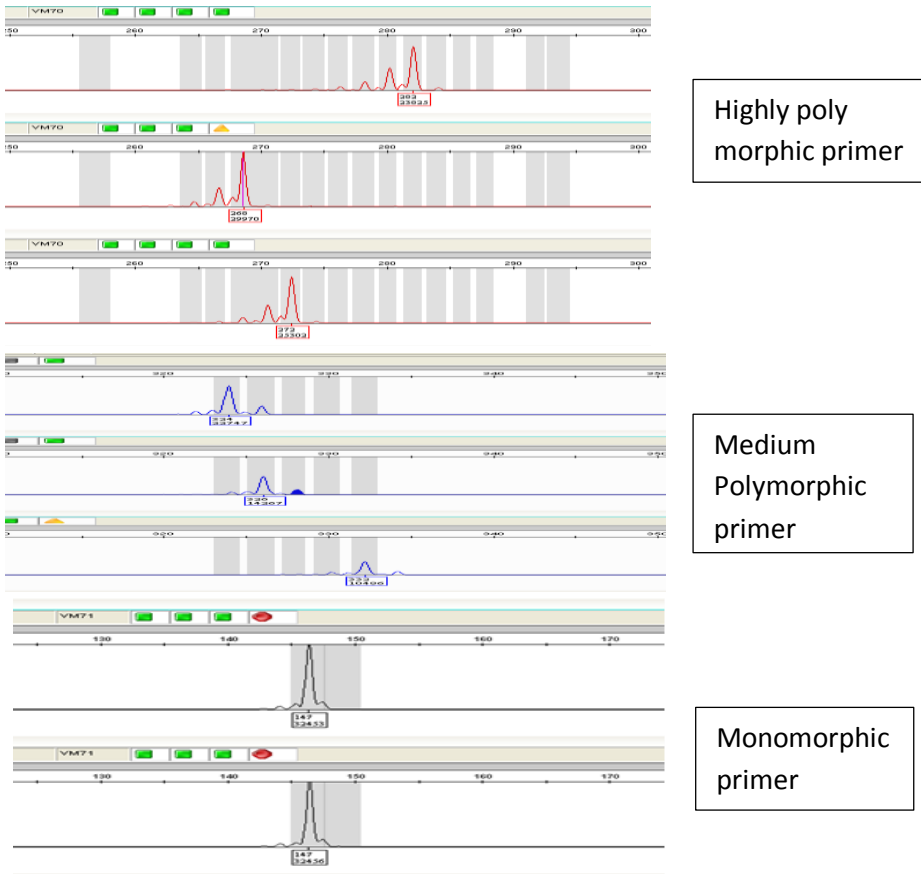

Figure 3. Capillary electrophorese product detection and band scoring.

and the other one showed an inconsistent fragment band therefore the two pairs were excluded. The informative (sixteen) SSRs were able to distinguish the whole accessions of the cowpea used in this study. A total of 129 alleles at 16 loci could be scored. The number of alleles detected per primer pairs varied from 2 to 17 with an average of 8.1 alleles. Polymorphic Information Content (PIC) ranging from 0.33 to 0.83 with a mean of 0.56 (Table 3).

Marker SSR 6569 detected the highest alleles number which was 17 while the lowest one 2 showed by BMD17.Two primers VM70 and SSR6577 detected 16 alleles each whereas four primers; VM30, VM 39, VM 51 and VM 94 had 8 alleles each (Table 3). Marker SSR6569 exhibited highest gene diversity with 0.85 while the least 0.34 was detected by Marker VM74. All primers studied were able to detect the levels of heterozygosity which was observed ranging from 0.01 to 0.13 with a

Table 3. Number of alleles, gene diversity, heterozygosity and polymorphism information content for the primers used in this study.

Marker	Major allele frequency	Allele no.	Gene diversity	Heterozygosity	PIC
VM30	0.61	8	0.60	0.08	0.57
VM 39	0.73	8	0.45	0.04	0.43
VM51	0.59	8	0.56	0.01	0.50
VM 53	0.60	7	0.56	0.05	0.50
VM57	0.63	5	0.54	0.05	0.49
VM70	0.36	16	0.82	0.07	0.80
VM 35	0.60	11	0.58	0.07	0.54
VM40	0.66	5	0.53	0.03	0.49
VM54	0.71	3	0.42	0.02	0.34
VM37	0.40	6	0.67	0.06	0.61
VM 94	0.39	8	0.75	0.03	0.71
BMD 17	0.46	2	0.60	0.13	0.51
SSR 6569	0.24	17	0.85	0.08	0.83
VM 74	0.80	6	0.34	0.02	0.33
SSR 6573	0.51	3	0.58	0.06	0.50
SSR 6577	0.35	16	0.82	0.05	0.80
Mean	0.54	8.1	0.60	0.05	0.56

Table 4. Private alleles, average observed (Ho), expected (He) Heterozygosis and percentage of polymorphic Loci among cowpea accessions studied.

Population	Number of private alleles	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Percentage of polymorphic loci (%)
Bahr Eljabel	2	0.000	0.339	81.25
Blue Nile	5	0.062	0.521	100.00
IITA	8	0.055	0.531	100.00
North Kordofan	5	0.057	0.550	100.00
Northern	2	0.163	0.507	93.75
River Nile	1	0.074	0.445	81.25
South Kordofan	10	0.046	0.526	100.00
Grand Mean	4.7	0.065	0.488	93.75
SE	0.107	0.009	0.020	3.34

mean of 0.05 (Table 3).

The highest number of private alleles was 10 observed by South Kordofan while the lowest was 1 revealed by River Nile Region, the average Observed (Ho) and Expected (He) Heterozygosity among Sudanese cowpea Accessions varied among the different Agro ecological Regions; The highest average of (Ho) 0.163 was recorded by Northern while the lowest 0.000 observed by Bahr Eljabel Region. Highest average of (He) was 0.550 detected by North Kordofan whereas the lowest 0.339 observed by Bahr Eljabel Region, four regions (Blue Nile, IITA, North Kordofan and South Kordofan) revealed 100% polymorphic loci (Table 4).

Phylogenetic analysis

Analysis of Molecular variances (AMOVA) Input as Allelic Distance Matrix for F-Statistics showed that the genetic variation of the total accessions among the geographical

Table 5. Summary of Analysis of Molecular Variance (AMOVA) for Sudanese cowpea Input as Allelic Distance Matrix for F-statistics analysis.

Source	Df	SS	MS	Est. Var.	Genetic variation (%)
Among Pops	6	193.840	32.307	0.388	8%
Among Individuals	245	2133.670	8.709	4.141	83%
Within Individuals	252	107.500	0.427	0.427	9%
Total	503	2435.010		4.956	100%

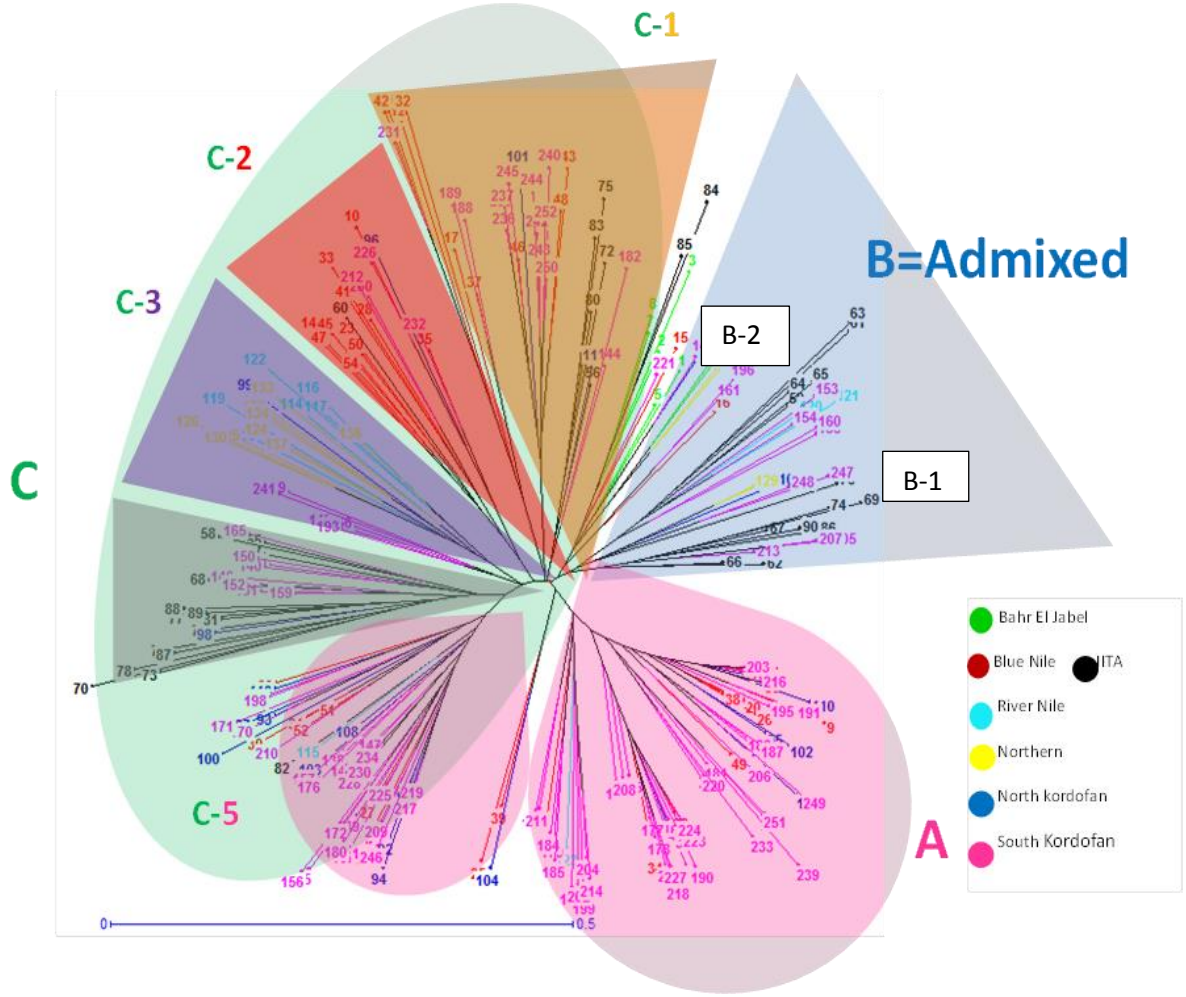

Figure 4. Phylogenetic tree among 252 Cowpea Accessions studied revealed by neighboring joining analysis.

regions was 8%, variation within individuals of sub-population was 9% while the variation among individuals of total population was 84% these results revealed that there was low differentiation among population studied with great diversity among individuals of Sudanese cowpea (Table 5).

Based on their molecular profiles resolved using informative SSRs, the 252 cowpea accessions used in this study clustered into three main groups, which they designated as groups A, B and C (Figure 4). Groups A and B were almost the same in size. Group B divided into two main sub-groups, sub group 1 and sub group 2, the main constituents of sub-group 1 are the out group IITA and South Kordofan germplasm, all Bahr Eljbel germplasm clustered together in sub-group 2 with some of South Kordofan (Figure 4). While group C which

Table 6. Pairwise Population Matrix of Nei Unbiased Genetic Distance among Population.

Agro-ecological zones	Bahr Eljabel	Blue Nile	IITA	North Kordofan	Northern	River Nile
Bahr Eljabel	0.000					
Blue Nile	0.264	0.000				
IITA	0.303	0.240	0.000			
North Kordofan	0.272	0.071	0.131	0.000		
Northern Sudan	0.303	0.170	0.190	0.115	0.000	
Rive Nile	0.261	0.247	0.267	0.194	0.087	0.000
South Kordofan	0.233	0.034	0.165	0.031	0.135	0.218

considered as a largest group comprised the different accessions from different Ecological zones and it contains the main accessions that are the most diverged in the collection that is, TVU 8812 IITA-Benin, followed by accession HSD 5738 –Sudan Blue Nile and HSD 6782-Sudan South Kordofan, Group C also divided into five sub groups where South and North Kordofan were clustered together, Northern and River Nile, South Kordofan and IITA, and South Kordofan and Blue Nile. (Figure 4).

Genetic distance

Generally, genetic distances among cowpea genotypes are low, reflecting the initial bottleneck during domestication, and maintained by the inherent self-pollination mechanism in the crop (Asare et al., 2010). On the whole, the genetic difference observed among the different ecological zones was varied, the least GD 0.031 observed between North and South Kordofan while the greater distance 0.303 was observed by two pairs; IITA; Bahr Eljabel and Northern; Bahr Eljabel state. The lowest GD between Sudanese regions and the out-group was observed by North Kordofan 0.131 then South Kordofan with 0.165, this closely related Genetic Distance to the out group germplasm can confirm the earlier suggestion that cowpea crop introduced to Sudan from West African Countries to the Western part of Sudan (Darfur and Kordofan) and from there it spread to the rest of the county, never the less this result showed a light bar of possibility of other sources of Sudanese cowpea a part from West African countries this can be clear from phylogenetic tree where Group A is mostly dominated by South Kordofan while IITA cluster together with Sudanese germplasm in other two groups (Figure 4). Blue Nile germplasm was observed to be more closely related to South Kodofan with GD of 0.034 then to North Kordofan with 0.071 GD (Table 6).

Three pairs of accessions (HSD 5700 Blue Nile, HSD 4854 South Kordofan; HSD 5701 Blue Nile, HSD 4854 South Kordofan and HSD 4568 South Kordofan, HSD 6560 South Kordofan) possessed genetic distances 0.000, suggesting that the members of these pairs may in fact be either separately collected with different names in the same Region or gene flow between Regions.

DISCUSSION

Variability in SSR markers

The delineation of cowpea germplasm into groups of genetic relatedness will be valuable for guiding introgression efforts in breeding programs and for improving the efficiency of germplasm management (Bao-Lam Huynh et al., 2013).

In the present study, the 16 informative SSR primer pairs used to analyze the 252 cowpea germplasm from Sudan and IITA-Nigeria resulted in 2 to 17 alleles per primer pairs with an average of 8.1. This result is in agreement with Badiane et al. (2012), who found number of alleles in Senegal cowpea varied from 1 to 16. Fatokun et al. (2008) detected alleles ranging from 4 to 13 alleles among 48 wild cowpea lines collected from different Agro- ecological zones in Africa with an average of 7.5 alleles per primer. However, previous works in Ghana, Burkina Faso, Senegal and Nigeria have revealed detections of allels ranging from 1 to 6, 5 to 12, 1 to 9 and 2 to 5 respectively (Asare et al (2010), Sawadogo et al. (2010) Diouf and Hilu (2005) and Adetiloye et al. (2013)). These variations in numbers of alleles can be attributed to the types of primers used in each study and/or the rate of polymorphism of each primer pairs.

In this study the polymorphic information content (PIC) ranged from 0.33 to 0.83 with a mean of 0.56. Fatokun et al. (2008) observed PIC ranging from 0.29 to 0.87 with a mean of 0.68 among the 48 wild cowpea lines. However other cowpea Researchers (Li et al. (2001), Badiane et al. (2012) and Asare et al. (2010) reported PIC ranging from (0.02 to 0.73, 0.08 to 0.33 and 0.07 to 0.66) respectively. The informativeness of PIC value measured by Botstein et al. (1980) scale revealed that the mean PIC value ≥ 0.5 is highly informative, 0.25~0.50 reasonably

informative and < 0.25 is slightly informative, and Loci (Marker) with many alleles and a PIC value near 1 are most desirable (Botstein et al., 1980).

Gene diversity in this study was 0.60 on average ranging from 0.34 to 0.85. In Senegal cowpea gene diversity varied from 0.08 to 0.42 with mean of 0.28 (Badiane et al., 2012), whereas In Ghana cowpea germplasm gene diversity ranged from 0.12 to 0.68 with an average of 0.44 (Asare et al., 2010). The results of gene diversity reflect the proportion of polymorphic loci across the genome. Therfore according to the result of this study the markers used were highly polymorphic compared to those used by the Badiane et al. (2012), and Asare et al. (2010).

Heterozygosity in this study was observed ranging from 0.01 to 0.13 with a mean of 0.05, Asare et al. (2010) revealed Variation in heterozygosity among Ghanaian cowpea SSRs increasing from 0.01 to 0.84 with an average occurrence of 0.19. The low value of Heterozygosity agrees with previous series reported by several cowpea researchers who documented that cowpea in general has a narrow genetic base due to the result of a bottleneck induced by a single domestication event which involved in the origin of this crop, where the proportion of heterozygozity is likely to be low, and likely its inherent nature of self-pollination mechanism (Pasquet, 2000; Coulibaly et al., 2002; Ba et al., 2004).

Genetic relationship among population

Generally, genetic distances among cowpea genotypes are low, reflecting the initial bottleneck during domestication, which was maintained by the inherent self-pollination mechanism in the crop (Asare et al., 2010). In this study the shorter genetic distance of 0.031, which was found between North and South Kordofan states, might be due to gene flow resulting from the seed exchange practiced by farmers particularly within and between these two neighbouring states. The same phenomenon was observed in Northern and River Nile states, which are also neighbouring states in the northern region of the country, showing a shorter genetic distance of 0.087 mostly due to the same reasons.

The greatest genetic distance of 0.303, which was found among the accessions obtained from the IITA compared to the accessions collected from Bahr Eljabel state, as well as among accessions collected from Northern state compared to those collected from Bahr Eljabel state, can be attributed to the far distances between those non-neighbouring geographical regions, where the possibility of gene flow due to seed exchange is almost lacking. Accessions obtained from the IITA were representing materials that were originally collected from other countries in west Africa. Northern state is located in the far north of Sudan whereas Bahr Eljabel is within republic of South Sudan. On the other hand, the shorter genetic distance found between the IITA and North Kordofan accessions as well as IITA and South Kordofan accessions can interpreted by the earlier suggestion that cowpea crop was introduced to Sudan from West African Countries to the western part of Sudan (Kordofan and Darfur), from where it spread to the rest of the country. The closely genetic distance between Blue Nile state and North and South Kordofan states can be attributed also to seed and or grain exchange between farmers and inhabitants of these states for cultivation and food uses. Moreover, an ethnic relationship with similar cultural background exists between some groups of populations in these three states, which reflecting in similar nutritional and cultivation habits and practices within them.

Three pairs of accessions in this study were found to have genetic distances of 0.00, which indicate the common genetic make-up within each pair of accessions, though they were observed to have different farmers' variety names. The same variety with the same morphological characters can have different names following the locality or ethnic groups. Consequently, a better understanding of the genetic variation and strong sound footing system of classification of the cowpea collection in Sudan with molecular markers is urgent need.

Conclusions and recommendations

The current study suggested low level of genetic diversity among Sudanese Cowpea population with great diversity with individuals. Therefore broadening the genetic base of Sudanese cowpea population may be achieved through introgression of new alleles either from wild cowpea germplsam or by out breeding with more closely related species to Cowpea *V. triphylla* and *V. reticulate*.

A total of 11 markers (SSR 6569, VM 70, SSR 6577, VM 94, VM 37, VM 30, VM 35 BMD 17, VM 53, VM 51 and SSR 6573) were highly informative (0.5 <PIC<0.83) and can be used in future diversity studies as marker core set in cowpea; IITA germplasm grouped together with Sudanese germplasm revealing the similarity of Sudanese cowpea to West African countries. Sudanese Cowpea Core collection can be created from South Kordofan, River Nile and Bahr Eljabel germplasm.

Conflict of interest

The authors have not declared any conflict of interest.

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