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DNA Fingerprinting and Assessment of Genetic Diversity among 22 Cowpea [*Vigna unguiculata* (L.) Walp] Varieties Grown in Ghana

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Abstract

Identification of varieties based on only morphological traits is limited by the influence of environment on such morphological traits. Deoxyribonucleic acid (DNA) fingerprinting offers an efficient system of identifying varieties at the DNA level without any environmental interference. This work used 20 Simple Sequence Repeat (SSR) markers to characterise twenty-two cowpea [*Vigna unguiculata* (L.) Walp] varieties for the purposes of varietal protection and further assessed for genetic diversity. This study was conducted at the Biotechnology laboratory of Council for Scientific and Industrial Research-Crops Research Institute, Ghana (CSIR-CRI). The varieties were made up of 15 cultivars released by CSIR-CRI, Ghana, 5 cultivars released by CSIR-Savannah Agricultural Research Institute, Ghana (SARI), 1 advanced line, 1 landrace and 1 exotic variety. Nineteen out of the 20 SSR markers used in this study were polymorphic. These polymorphic primers generated a range of 1 to 6 alleles per primer with polymorphic information content (PIC) varying from 0.107 (SSR-6608) to 0.656 (SSR-6613). Allele frequency ranged from 0.136 (SSR-6371) to 0.841 (SSR-6608) with mean of 0.445. With the aid of Darwin software, dissimilarity matrix and a dendrogram were generated from the molecular data to evaluate and group the varieties based on genetic resemblance. Three pairs of varieties (Agyenkwa and Adom; Hewale and Ayiyi; Zamzam and Hewale) recorded the highest genetic distance of 0.652 each. The genetic information gathered for each variety has been made available to the breeding institutions. The genetic diversity detected among the varieties fingerprinted will be helpful to plant breeders in selecting parents for future cowpea improvement programmes.

Subject Areas

Agricultural Science, Biotechnology, Genetics

Keywords

Cowpea, Characterization, Diversity, Simple Sequence Repeats

1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is an early maturing crop that tolerates poor soil fertility and water stress [1]. This makes it an important food crop in areas hard hit with climate change, famine, poor soil fertility, poverty and high population growth rate [1] [2]. Cowpea has evolved from native wild types and its genetic diversity is greater than that of any other crop in the dry African savannah [3]. However, the cultivated cowpea has been shown to have a narrow genetic base suggesting the crop went through a "genetic bottleneck" during domestication [4]. Awareness of existing plant genetic diversity in the cowpea germplasm is therefore fundamental for effective management of cowpea genetic resources [5]. The establishment of the forensic identity of crop varieties has become vital for protecting plant breeders' and farmers' right following the enactment of the Convention of Biodiversity Conservation particularly in developing countries [6]. DNA fingerprinting which is also known as molecular characterization is a useful tool for genotype identification, diversity studies and associating molecular markers to phenotypic traits [7]. Fingerprinting with molecular markers allows precise, objective and rapid variety identification of plant varieties [5]. Unlike phenotypic based markers, molecular markers are stable and detectable in all tissues regardless of growth differentiation, development, pleitropic effect, epistatic effects and not confounded to environment where they grow [6] [8]. SSRs, also known as Microsatellites, are co-dominant markers that are routinely used to study genetic diversity in different crop species [6]. These markers occur at high frequency and appear to be distributed throughout the genome of higher plants [8].

Several successful molecular characterization and genetic diversity study works have been conducted on many cowpea accessions in the past using SSR markers in Ghana and the world at large [6] [9] [10] [11] [12]. However, new varieties have been released by the CSIR-Crops Research Institute of Ghana (CRI) and CSIR-Savannah Research Institute of Ghana (SARI) in recent times and there is therefore the need to record a forensic fingerprint of these newly released varieties together with old varieties using SSR markers for purposes of conservation, patent rights and genetic diversity studies. The aim of this study was therefore to fingerprint and evaluate genetic diversity among 22 cowpea varieties grown in Ghana, including four newly released varieties with the aid of 20 SSR markers.

2. Materials and Methods

2.1. Planting Materials

Seeds of 22 cowpea varieties were collected from the gene banks of CSIR-SARI

and CSIR-CRI, sowed and nurtured in sterile soil in a greenhouse. Germplasm for this study was composed of 15 cultivars released by CSIR-CRI, 4 cultivars released by CSIR-SARI, 1 advanced line from CSIR-SARI, 1 landrace and 1 exotic variety grown in Ghana (Table 1).

2.2. Deoxyribonucleic Acid (DNA) Extraction

The laboratory studies were conducted in the Biotechnology laboratory of CSIR-CRI, Kumasi. Leaf explants were harvested from the 22 cowpea varieties two weeks after planting and kept in liquid nitrogen. Total DNA was extracted according to the prescribed protocol of the DNeasy™ Plant Mini extraction kit (Qiagen, Germany). DNA quality was checked using 0.8% (w/v) agarose gel electrophoresis. The concentration of the DNA was determined on a Nanodrop (spectrophotometer 2000C). The genomic DNA samples were diluted to a final concentration of 20 ng/μL with 1× TE buffer (10 mM Tris-HC1, pH 8.0; 1 mM EDTA) and stored at −20˚C for further use.

Table 1. Cowpea varieties used for the study.

No.	Local Name	Accession Name	Source of material	Status	Year of Release in Ghana
1	Hewale	IT93K-192-4	CSIR-CRI	Released variety	2012
2	Asomdwoe	IT94K-410-2	CSIR-CRI	Released variety	2012
3	Videza	IT95K-142-20	CSIR-CRI	Released variety	2012
4	Nhyira	IT87D-611-3	CSIR-CRI	Released variety	2005
5	Tona	IT87D-2075	CSIR-CRI	Released variety	2005
6	Asetenapa	IT32D-1951	CSIR-CRI	Released variety	1999
7	Adom	CR-06-07	CSIR-CRI	Released variety	1999
8	Ayiyi	IT83S-728-13	CSIR-CRI	Released variety	1992
9	Bengpla	IT83S-818	CSIR-CRI	Released variety	1992
10	Asontem	IT82D-32	CSIR-CRI	Released variety	1999
11	Soronko	TVX2724-OIF	CSIR-CRI	Released variety	1999
12	Agyenkwa	11(8)-1	CSIR-CRI	Released variety	2015
13	Zamzam	11(9)-5	CSIR-CRI	Released variety	2015
14	Hansadua	11(9)-2	CSIR-CRI	Released variety	2015
15	Nketewade	11(9)-3	CSIR-CRI	Released variety	2015
16	Zaayura	SARC4-75	CSIR-SARI	Released variety	2008
17	Songotra	IT97K-499-35	CSIR-SARI	Released variety	2008
18	Padi-Tuya	SARC3-122-2	CSIR-SARI	Released variety	2008
19	Apabgaala	ITXP48-2	CSIR-SARI	Released variety	2003
20		SARC-1-57-2	CSIR-SARI	Advanced line	
21	Sanzi		Northern Ghana	Landrace	
22	Bra-01		Brazil	Exotic	

2.3. Polymerase Chain Reaction Using SSR Markers

A total of 20 SSR primers (Table 2) were used to fingerprint the 22 cowpea varieties. Information about the primers was obtained from the SSR panel reported by Timko [13]. The primers were synthesized by Inqaba Biotechnical Industries Ltd., Pretoria, South Africa. Polymerase chain reaction (PCR) amplification was conducted in 20 µl volume tubes. Each PCR reaction contained 6 µl "One Taq Quick-Load 2x Master Mix" (composed of 20 mM Tris-HCl, 1.8 mM $MgCl_2$, 22 mM NH_4Cl, 22 mM KCl, 0.2 mM dNTPS and 25 units/ml One Taq DNA Polymerase), 2.0 µl Molecular Grade Distilled Water (MGDW), 0.5 µl of each primer pair and 2 ng/µL of genomic DNA sample to make a total volume of 10 µl. The PCR amplifications were performed in a thermal cycler C1000 (Seegene, Korea). The thermal cycler was set to initial denaturation at 94˚C for 1 minute followed by 35 cycles of denaturation for 30 seconds at 94˚C, annealing at 55˚C for 30 seconds, extension at 72˚C for 1 minute and ended with final extension at 72˚C for 10 minutes.

Table 2. SSR primers used for molecular characterization and their sequences

No.	SSR Name	Original Name	Left Sequence	Right Sequence	Annealing Temperature (˚C)	Product Size (bp)
1	SSR-6265	CP215/CP216	CAGAAGCGGTGAAAATTCAAC	GCATGTTGCTTTGACAATGG	55	239
2	SSR-6258	CP201/CP202	GGTTTCCTAGTTGGGAAGGAA	ATTATGCCATGGAGGGTTCA	55	260
3	SSR-6243	CP171/CP172	GTAGGGAGTTGGCCACGATA	CAACCGATGTAAAAAGTGGACA	55	176
4	SSR-6218	CP117/CP118	GTGGAAGGAATGGGTCCAG	AGGAAATTTGCATTCCCTTGT	55	287
5	SSR-6217	CP115/CP116	GGGAGTGCTCCGGAAAGT	TTCCCTATGAACTGGGAGATCTAT	55	294
6	SSR-6353	CP397/CP398	TCATGGGTTAAATTTGCTTCAA	AAACCATGTGGTTGTTGCAC	50.9	109
7	SSR-6352	CP395/CP396	GTTGTGAGCTTCCCCAGATG	AATTTTGAACCCACCACCAG	55	127
8	SSR-6336	CP359/CP360	TGAAAAACAACGATATGCAGAAG	TCAGTCTTAGAATTGAGTTTTCTTCG	55	247
9	SSR-6323	CP333/CP334	CAAAGGGTCATCAGGATTGG	TTTAAGCAGCCAAGCAGTTGT	55	218
10	SSR-6277	CP239/CP240	CACCCCCGTACACACACAC	CACTTAAATTTTCACCAGGCATT	50.9	157
11	SSR-6436	CP573/CP574	GCAGAATCCTTGTGAACCTG	TTTCGCAATATGCCCTTTTC	50.9	280
12	SSR-6375	CP443/CP444	GCTCGGATATGGTCCTGAAA	TCAGTGTCAGCACCATACCC	55	293
13	SSR-6371	CP435/CP436	TGCTCATCGTGCTTTGTCTT	CACTTCAGACTTAGAGCGAAGAAA	55	189
14	SSR-6370	CP433/CP434	CAACTTCACAGCCCTCACAA	TTGAAGGTATGGCCTTTTGTTT	55	253
15	SSR-6356	CP403/CP404	TGCAATATGGACCAGAAGAAA	ATGCCCCAACAACAACATTT	55	158
16	SSR-6613	Y31	CTATTGGAATCTTGCCGTTG	CTTTACCTTTATGCAAACCAATTC	55	333
17	SSR-6608	Y26	CTAAATTATAATATTCGTCGGTC	GGTTAAGGAAAAGAGGGTAGG	55	299
18	SSR-6603	Y21	GAGAACTTCACGCACAATAG	CGCGGTAGCATGATTGAATTTTG	55	330
19	SSR-6587	Y1	GATATAGAATAGCATATTTAACATATTAG	GTTGAAAGTTTGATAGTAAAGTGG	55	319
20	SSR-6451	CP605/CP606	AAAGAGATACACATGCCTAACA	GACCAACAGCGACTTTGAGC	55	142

2.4. Gel Electrophoresis

PCR products were resolved on 6% (w/v) polyacrylamide gel electrophoresis (PAGE) in 1 X Tris/borate/EDTA buffer stained with 0.5% Ethidium bromide (Criterion cell model, vertical centrion tank) for 1 hour 30 minutes at a voltage of 80 V. The gel was photographed under Ultraviolet light with the aid of Alpha Innotech Multimage™ Light Cabinet for further analysis.

2.5. Scoring of Bands and Data Analysis

The bands observed on the gels after staining were scored on the basis of presence/absence (1/0) with the aid of the Alpha Imager version 3.41 software along with a 100-bp DNA ladder (Invitrogen®) to identify the molecular-weight of the DNA samples. Following the gel scoring, the molecular data was fed into Darwin software [14] to generate a dissimilarity matrix and a dendrogram.

3. Results

The primers generated a total of 481 bands across the selected varieties out of which 428 (88.98%) were polymorphic. Only 1 (SSR 6336) out of the 20 primers did not show polymorphism among the varieties, and therefore was excluded from the analysis. A representative picture of the amplification product of primer SSR6243 showing pattern of allelic bands across the 22 cowpea varieties on 6% polyacrylamide gel is presented in **Figure 1**.

The size of amplified alleles ranged from 90 bp to 391 bp (**Table 3**). The primers SSR-6613 and SSR-6608 recorded the highest and lowest number of polymorphic bands of 79 and 5 respectively. The number of alleles varied from 1 to 6. The allele frequency ranged from 0.136 (SSR-6371 to 0.841 (SSR-6608) with mean of 0.445 among the varieties. The polymorphic information content (PIC) representing the allele diversity for a specific locus ranged from 0.107 (SSR-6608) to 0.656 (SSR-6613) with a mean of 0.293.

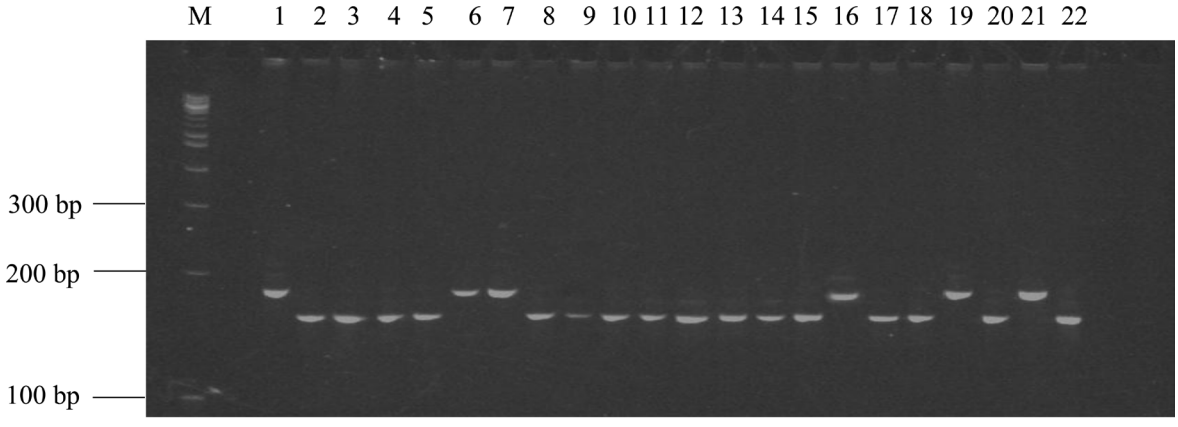

Figure 1. SSR 6243 marker scored on ethidium bromide stained PAGE gel (6%) for 22 cowpea varieties M = 100bp ladder; 1 = SARC-1-57-2; 2 = Apabgaala; 3 = Nhyira; 4 = Hewale; 5 = Asomdwee; 6 = Adom; 7 = Soronko; 8 = Bengpla; 9 = Hansadua; 10 = Agyenkwa; 11 = Padi-Tuya; 12 = Zaayura; 13 = Nketewade;14 = Zamzam;15 = Ayiyi; 16 = Songotra; 17 = Videza; 18 = Asontem; 19 = Tona; 20 = Asetenapa; 21 = Sanzi; 22 = Bra 01.

Table 3. Resolving power analysis of SSR primers.

Primer	Allele size range (bp)	No. of alleles	Allele frequency	Number of bands	Number of Polymorphic bands	Polymorphic Information Content (PIC)
SSR-6265	217 - 282	5	0.445	49	49	0.631
SSR-6258	195 - 269	2	0.523	23	21	0.242
SSR-6243	165 - 187	2	0.5	22	20	0.202
SSR-6218	149 - 287	2	0.409	18	16	0.266
SSR-6217	230 - 294	2	0.341	15	15	0.242
SSR-6353	90 - 115	2	0.523	23	22	0.199
SSR-6352	113 - 141	3	0.257	17	17	0.246
SSR-6323	220 - 285	3	0.348	23	22	0.331
SSR-6277	114	1	0.318	7	7	0.34
SSR-6436	266 - 370	3	0.454	30	29	0.405
SSR-6375	296 - 333	2	0.522	23	22	0.405
SSR-6371	164 - 195	2	0.136	6	6	0.185
SSR-6370	254 - 275	2	0.386	17	14	0.091
SSR-6356	127 - 147	2	0.5	22	22	0.223
SSR-6613	250 - 391	6	0.598	79	79	0.656
SSR-6608	233 - 300	2	0.841	37	5	0.107
SSR-6603	358 - 386	2	0.477	21	20	0.261
SSR-6587	336 - 352	2	0.409	18	14	0.204
SSR-6451	110 - 155	3	0.469	31	28	0.325
Mean		2.526	0.445	25.32	22.53	0.293

Dissimilarity Matrix and Cluster Analysis Based on SSR Markers

The genetic distances within pairs of the 22 varieties were evaluated in a dissimilarity matrix generated from the molecular data using Darwin software. From the dissimilarity matrix (Table 4), there was no distance between Videza and Asomdwoe which implies that the two varieties are very similar. A short distance of 0.109 was found between Zamzam and Agyenkwa while the highest distance (most divergent) was found among three pairs of varieties: Agyenkwa and Adom; Hewale and Ayiyi; Zamzam and Helwale at genetic distance of 0.652. The dendrogram grouped the 22 varieties into four major clusters (A, B, C and D) at genetic distance of 0.20 (Figure 2). Cluster A was made up of only Hewale. Cluster B had two sub-clusters diverging at genetic distance of 0.125. Sub-cluster I consisted of only Zaayura while subcluster II consisted of Asomdwoe and Videza. The third major cluster was cluster C which consisted of 9 varieties grouped into two subclusters diverging at genetic distance of 0.15. Cluster D also consisted of two sub-clusters. The first sub-cluster comprised of 5 varieties (Adom, Asontem, Tona, Nhyira and Soronko) while the second sub-cluster consisted of 3 varieties (Hasnsadua, Bengpla and Songotra).

Table 4. Dissimilarity matrix based on molecular data.

Variety	Adom	Agyenk	Apabg	Aseten	Asomd	Asonte	Ayiyi	Bengp	Bra-01	Hansa	Hewal	Nhyira	Nket	Padi-Tu	S1-57-2	Sanzi	Songo	Soronko	Tona	Videza	Zamzam
Adom																					
Agyenk	***0.652***																				
Apabgaala	0.565	0.304																			
Asetenapa	0.326	0.543	0.457																		
Asomdwoe	0.326	0.500	0.413	0.130																	
Asontem	0.217	0.478	0.391	0.413	0.370																
Ayiyi	0.587	0.283	0.196	0.478	0.435	0.457															
Bengpla	0.478	0.435	0.478	0.500	0.457	0.304	0.413														
Bra-01	0.413	0.370	0.326	0.435	0.391	0.326	0.348	0.500													
Hansadua	0.522	0.304	0.391	0.587	0.500	0.391	0.413	0.348	0.457												
Hewale	0.413	0.630	0.587	0.391	0.435	0.370	***0.652***	0.543	0.565	0.543											
Nhyira	0.457	0.413	0.413	0.522	0.522	0.239	0.522	0.370	0.391	0.370	0.435										
Nketewade	0.630	0.152	0.370	0.435	0.435	0.543	0.304	0.413	0.261	0.326	0.609	0.565									
Padi-Tuya	0.565	0.261	0.261	0.500	0.457	0.348	0.326	0.391	0.370	0.348	0.587	0.283	0.370								
S1-57-2	0.522	0.261	0.348	0.587	0.500	0.391	0.457	0.522	0.370	0.391	0.587	0.413	0.370	0.348							
Sanzi	0.370	0.457	0.413	0.391	0.391	0.326	0.391	0.500	0.304	0.587	0.565	0.478	0.391	0.457	0.283						
Songotra	0.457	0.413	0.370	0.391	0.348	0.413	0.391	0.326	0.522	0.370	0.565	0.391	0.435	0.326	0.413	0.478					
Soronko	0.391	0.522	0.435	0.630	0.630	0.304	0.587	0.391	0.457	0.391	0.543	0.196	0.587	0.391	0.391	0.457	0.370				
Tona	0.413	0.457	0.413	0.478	0.478	0.283	0.435	0.326	0.478	0.370	0.435	0.217	0.565	0.370	0.413	0.478	0.304	0.283			
Videza	0.326	0.500	0.413	0.130	***0.000***	0.370	0.435	0.457	0.391	0.500	0.435	0.522	0.435	0.457	0.500	0.391	0.348	0.630	0.478		
Zamzam	0.587	***0.109***	0.239	0.478	0.435	0.457	0.217	0.413	0.261	0.283	***0.652***	0.435	0.130	0.239	0.326	0.478	0.348	0.500	0.435	0.435	
Zaayura	0.413	0.413	0.326	0.261	0.261	0.326	0.391	0.457	0.391	0.457	0.435	0.348	0.478	0.370	0.543	0.435	0.391	0.500	0.391	0.261	0.391
Min	0.217	0.109	0.196	0.130	0.000	0.239	0.217	0.326	0.261	0.283	0.435	0.196	0.130	0.239	0.283	0.391	0.304	0.283	0.391	0.261	0.391
Max	0.652	0.630	0.587	0.630	0.630	0.543	0.652	0.543	0.565	0.587	0.652	0.565	0.587	0.457	0.543	0.478	0.391	0.630	0.478	0.435	0.391

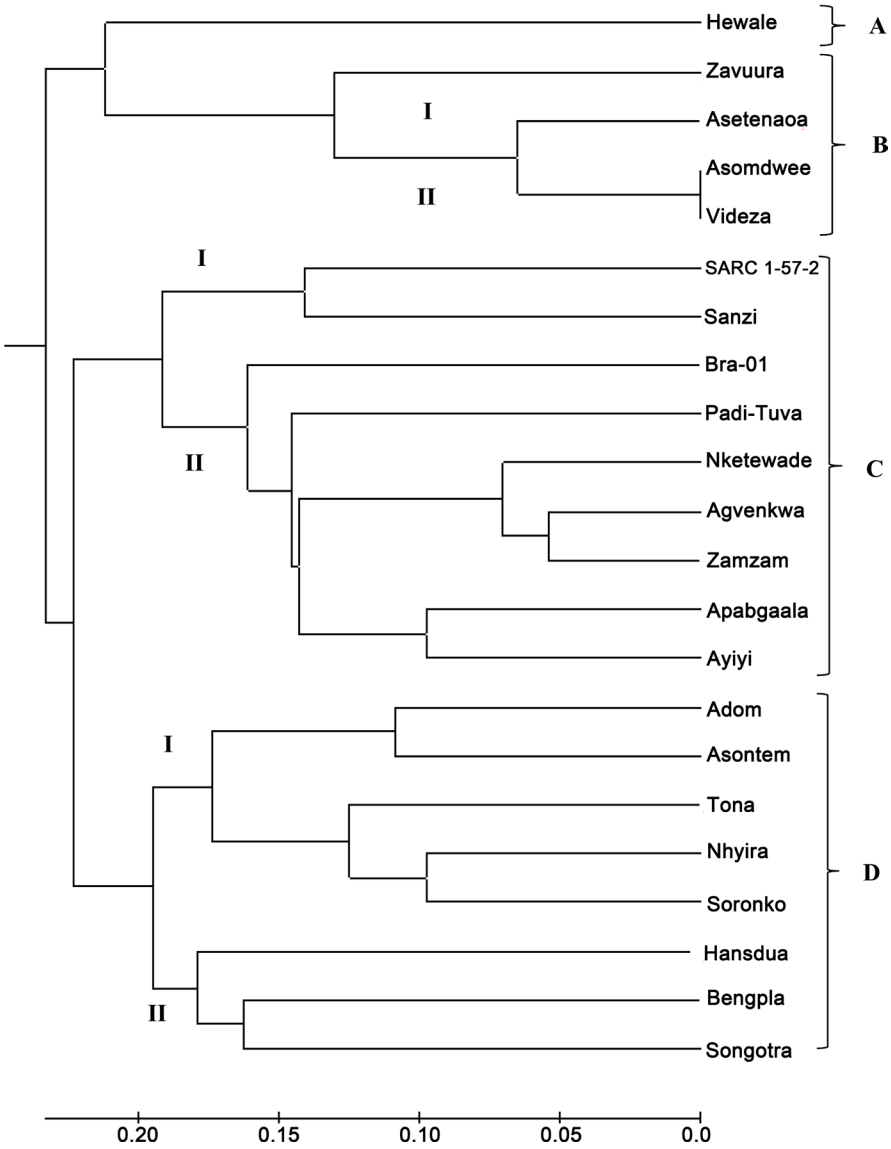

Figure 2. Dendrogram of 22 cowpea varieties using 19 SSR primers based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) generated by Darwin 6.0.010 software.

4. Discussion

From the study it was observed that, 19 informative SSR primer pairs produced 1 to 6 alleles per primer pair with an average of 2.53. This collaborates with the 1 to 6 alleles per primer reported by Asare *et al.* [9] when they assessed the genetic diversity in cowpea germplasm from Ghana using SSR primers including some of the primers used in this study. There is however, reports of number of allele per locus ranging from 1 to 9 [15], 5 to 12 [16], 1 - 16 [11], 2 to 5 [17], 5 to 12 [18] and 2 to 17 [15] in previous cowpea variability studies. According to Ali *et al.* [19], such 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.

According to the Bostein *et al.* [20] scale of informativeness, PIC value ≥ 0.5 is highly informative, 0.25 - 0.5 reasonably informative and ≤0.25 is slightly informative, and marker loci with many alleles and a PIC value near 1 are most desirable. In this study, the polymorphic information content (PIC) ranged from 0.107 to 0.631 with an average of 0.293. Asare *et al.* [9] also reported PIC range from 0.07 to 0.66 in a variability study of cowpea germplasm from Ghana. However, other researchers have reported PIC values in the range of 0.02 to 0.73 [21], 0.08 to 0.33 [11], 0.61 to 0.92 [18] and 0.33 to 0.83 [19]. Based on the Bostein *et al.* [20] scale of informativeness, the most desirable markers used in this study were SSR-6265 and SSR-6613.

Three of the primers used in this study (*i.e.* SSR-6258, SSR-6243 and SSR-6323) were also used by Badiane *et al.* [11] to study the genetic relationship among cowpea varieties from Senegal. The three primers showed polymorphism just as was reported by Badiane *et al.* [11]. Badiane *et al.* [11] further observed that the primer that gave the highest allele frequency also recorded the lowest genetic diversity as well as lowest polymorphic information content (PIC). A similar situation was observed in this current study where SSR primer 6608 scored the highest allele frequency but the lowest PIC value. Previous studies conducted by Badiane *et al.* [11] and Doumbia *et al.* [18] reported low levels of polymorphism among SSR primers. This present work reports high level of polymorphism among cowpea varieties as reported also by other authors [9] [22] [23].

Dissimilarity Matrix and Cluster Analysis Based on SSR-Markers

The delineation of cowpea germplasm into groups of genetic relatedness is a valuable resource for guiding introgression efforts in breeding programmes and for improving the efficiency of germplasm management [24]. The results from the dissimilarity matrix showed that there was no distance between Videza and Asomdwoe (**Table 4**) implying that the two varieties are very similar. The two varieties were released by CSIR-CRI, Ghana in the year 2012. There is however enough evidence in the Catalogue of Crop varieties released and registered in Ghana [25] to prove morphological variation among the two varieties. Future fingerprinting involving these varieties should include more informative primers to detect differences within the varieties.

The dendrogram generated from the molecular data grouped the varieties into four clusters. Nketewade, Agyenkwa and Zamzam (three out of four newly released varieties by CSIR-CRI) were in the same subcluster CII (**Figure 2**). Adom and Asontem were found in the same subcluster DI in this study. In terms of morphology, these two varieties share several morphological traits [25]. The trend re-enforces the closeness of the two varieties. They share several characteristics in common and this may be due to the fact that they were developed from a common ancestor. Three pairs of varieties (Agyenkwa and Adom; Hewale and Ayiyi; Zamzam and Hewale) recorded high genetic distance of 0.652

each. The varieties in each pair have different ancestry, different agro-ecological zones of production and different release times [25]. These varieties will serve as good materials from which suitable parental lines could be selected for hybridization programme.

5. Conclusion

This present work has provided additional molecular information about 22 cowpea varieties grown in Ghana including four newly released varieties (Agyenkwa, Zamzam, Hansadua and Nketewade). The information has been made available to CSIR-CRI and CSIR-SARI in Ghana for the purpose of varietal protection and patent right. The 19 polymorphic SSR cowpea primers were able to detect genetic diversity among cowpea germplasm grown in Ghana except for Videza and Asomdwoe. Future diversity studies involving these two varieties should include additional polymorphic primers in other to detect the differences between the two varieties.

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Compliance with Ethical Standards

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Datasets

The datasets generated and analysed in this current study are available from the corresponding author on reasonable request.

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