African Journal of Biotechnology Vol. 10(6), pp. 913-921, 7 February, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1113
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Genetic relationships between some released and elite Ghanaian cassava cultivars based on distance matrices

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Accepted 21 December, 2010

Eleven (11) released and two local Ghanaian cassava cultivars were fingerprinted to estimate the genetic diversity among them using 35 SSR markers. Genomic DNA of thirteen cassava cultivars (*UCC, IFAD, Agelifiaa, Nyerikobga, Nkabom, Essam Bankye, Akosua Tumtum, Debor, Filindiakong, Afisiafi, Doku Duade, Bankye Hemaa* and *Bankye Botan)* were isolated and used as template for PCR amplification involving 35 SSR markers. The recorded gel bands (163 polymorphic bands) were subjected to NTSYSpc Version 2.1 software for cluster analysis and development of dendrogram to show the corresponding similarity coefficients. Genetic relationships between *Bankye Hemaa* and *Filindiakoh* and that between *Bankye Hemaa* and *Afisiafi* recorded 1.2% similarity coefficients respectively. The local cultivars, *Debor* and *Akosua Tumtum* were distantly related at 52.31% similarity. *Filindiakoh* was found to be the closest relative to *Akosua Tumtum* and *Debor* at 17.9 and 29.1% similarity, respectively. *Bankye Botan* and *Bankye Hemaa,* however, were distantly related to most of the cultivars, including the local varieties. *Bankye Hemaa* was found to be closest relative of *Filindiakoh* and Afisiafi (1.2 and 1.1% similarity, respectively) and suggesting that they could serve as very good candidates in breeding programs in Ghana. *Bankye Botan* and *Bankye Hemaa* are distant relatives to most of the cultivars, including the local varieties which could however make these cultivars also very useful in breeding.

Key words: *Maninhot esculenta*, cassava, molecular diversity, fingerprint, selection of genotype, DNA, SSR markers.

INTRODUCTION

Cassava (*Maninhot esculenta*), also known as yucca (spanish), manoic (french) or mandioca (portuguse), belongs to the Division Magnoliophyta, Class Magnoliopsida, Order Euphorbiales, Family Euphorbiaceae and subfamily Crotonoideae (Fauguet and Fargette, 1990).

The crop is diploid, with a chromosome number of 2n = 36. Cassava, a perennial woody shrub, grows in both tropical and subtropical parts of the world (Shore, 2002) and it is cultivated as food crop. South America, probably the Amazon region, is the place of origin and domestication (Gibbons, 1990). The Portuguese first brought cassava to Africa in the form of flour (Ross, 1975) and was first cultivated in Africa in 1558. Although, cassava was absent along the Gold Coast (now Ghana) in the 18th century its cultivation was reported in Accra in 1985.

The Ghana Living Standard Survey (GLSS) shows that 83% out of 1.73 million households engage in cassava production in Ghana (Okai et al., 2003) and the bulk of the nation's cassava is produced in the south and middle of Ghana, accounting for 78% of the total national production (Stump, 1998). In Ghana, cassava ranks first

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Abbreviations: PAGE, Polyacrylamide gel electrophoresis; **EDTA,** ethylene diamine tetraacetic acid; **PVP,** polyvinyl pyrrolidone; **PCR,** polymerase chain reaction; **RAPD,** random amplified polymorphism DNA; **AFLP,** amplified fragment length polymorphism; **RFLP,** restriction fragment length polymorphism; **SSR,** simple sequence repeat.

Table 1. Names and description of released and local cultivars used for genetic diversity study.

S/N	Name of cultivar	Description
1	*UCC*	Improved cultivar
2	*IFAD*	Improved cultivar
3	*Agelifiaa*	Improved cultivar
4	*Nyerikobga*	Improved cultivar
5	*Nkabom*	Improved cultivar
6	*Essam Bankye*	Improved cultivar
7	*Akosua Tumtum*	Local cultivar
8	*Debor*	Local cultivar
9	*Filindiakong*	Improved cultivar
10	*Afisiafi*	Improved cultivar
11	*Doku Duade*	Improved cultivar
12	*Bankye Hemaa*	Improved cultivar
13	*Bankye Botan*	Improved cultivar

in the area under cultivation and utilization and is the most important staple food crop (Moses et al., 2005). Nweke et al. (1999) reported in Collaborative Study of Cassava in Africa (COSCA) that villages that did not experience the famine of 1983 in Ghana were those that cultivated cassava as the most important and dominant staple crop. Cassava therefore plays famine preventive role in Ghana. This could be inferred for its Ewe (dialect spoken in the Volta region of Ghana) name, “agbeli”, meaning “there is life”.

Though in recent years, various research groups have taken up the challenge to breed for improved varieties, the increase in domestic and industrial demand for cassava however suggests the need to increase cassava production. There are also new emerging and diversified markets demand for cassava in Ghana (Ampong et al., 2004) which further suggests breeding for improved cultivars that will meet specific domestic and industrial needs. The possible disappearance of desired traits, the improved varieties in the country and subsequent vulnerability to diseases and pests that were formally resistant to calls for an immediate release of new varieties to replace varieties are losing their desirable traits.

Easy identification and selection of genenotype is an essential step (Moyib et al., 2007) for any breeding program. In Ghana, there are several local and improved cultivars of cassava which share the same or similar morphology. Cultivars which show close genetic relationships cannot be easily identified using morphological techniques (Kaemmer et al., 1992). For instance, the International Institute of Tropical Agriculture (IITA) released 31 improved cultivars of cassava to farmers in Nigeria in 2000 and 2005. The farmers however failed to label them and they got mixed up with their local breeds hence, IITA could not identify their improved cultivars from the Nigerian landraces (Moyib et al., 2007). Taking a clue from this unfortunate experience in Nigeria, there is the need therefore to fingerprint the released Ghanaian cultivars to avoid mislabeling and mix up with other varieties.

The extent of genetic diversity among cassava cultivars to be used in future breeding programs is necessary. Phenotypic markers, isozymes and SDS-PAGE (protein based) and molecular (DNA) markers are techniques mostly used for genetic diversity studies (Prakash and He, 1996). The molecular methods include the use of restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphism DNA (RAPD) and simple sequence repeats (SSR). DNA markers have been used to gain insight into genetic diversity within crops and establishment of fingerprints for many crops (Caetana-Anolles, 1994) including cassava (Palaniswami and Peter, 2008). Among the DNA markers, the SSR marker remain the most rapid, reliable, abundant, co-dominant and allows all possible genotypes to be distinguished in any segregating generation. It is also highly polymorphic and requires smaller amounts of genomic DNA for polymerase chain reaction (PCR) and gel electrophoretic analyses (Akkaya et al., 1995).

In the present study, we used 36 SSR markers to fingerprint 11 released and two local Ghanaian cassava cultivars for the first time and also to estimate their genetic relationships.

MATERIALS AND METHODS

Plant materials

Thirteen (13) cassava cultivars including 11 released and two local varieties were evaluated in this study (Table 1). These cultivars were established for a period of five weeks in plastic pots at the CSIR-Crops Research Institute screen house, free from insects attack and exposed to external temperature, rainfall and humidity conditions averaging at 25.58°C (78.05F), 123.67 mm (4.87 in) and 80.167% respectively.

Genomic DNA isolation and amplification

Genomic DNA was isolated as described by Egnin et al. (1998) and adopted by the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) laboratory with some modifications. 0.2 g of tender cassava leaves was crushed in liquid nitrogen and 800 µl of lysis buffer (50 mM Tris HCl (pH 8.0), 300 mM NaCl, 20 mM EDTA, 20% PVP, 1.5% sarkocine and 0.1 g/L $Na_2S_2O_5$) was used to lyses the nuclear membrane. Protein contaminates from the cell lysate were removed using 400 µl of 5 M potassium acetate (instead of 800 µl of phenol chloroform isoamyl alcohol as used by Egnin et al., 1998) and centrifugation at 1300 rpm for 15 min. The RNA component was also removed by adding 4µl RNase A and incubated at 37°C for 30 min as DNA purification and recovery was carried out by 700 µl isopropanol and 80% alcohol. DNA pellets were also dried at room temperature and dissolved in 200 µl of 1 X TBE Buffer after which the DNA quality was determined on 0.8% agrose gel.

The DNA from the 13 cultivars were fingerprinted (using 35 SSR makers, Table 2a) in a 10 µl PCR reaction mixture containing 1.0 µl of buffer (10X), 0.9 µl of $MgCl_2$ (25 mM), 0.4 µl of dNTPs (10 mM),

Table 2a. SSR sequences and their thermocycler programs.

SSR Locus	Left primer sequence	Right primer sequence	Thermocycler programme
SSRY 4	ATAGAGCAGAAGTGCAGGCG	CTAACGCACACGACTACGGA	MicroBC1
SSRY 9	ACAATTCATCATGAGTCATCAACT	CCGTTATTGTTCCTGGTCCT	MicroBC1
SSRY 12	AACTGTCAAACCATTCTACTTGC	GCCAGCAAGGTTTGCTACAT	MicroBC1
SSRY 19	TGTAAGGCATTCCAAGAATTATCA	TCTCCTGTGAAAAGTGCATGA	MicroBC1
SSRY 20	CATGGACTTCCTACAAATATGAAT	TGATGGAAAGTGGTTATGTCCTT	MicroBC1
SSRY 21	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA	MicroBC1
SSRY 34	TTCCAGACCTGTTCCACCAT	ATTGCAGGGATTATTGCTCG	MicroBC1
SSRY 38	GGCTGTTCGTGATCCTTATTAAC	GTAGTTGAGAAAACTTTGCATGAG	MicroBC1/ NewBC1
SSRY 51	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT	MicroBC1/Yucadiv (Yu)
SSRY 52	GCCAGCAAGGTTTGCTACAT	AACTGTCAAACCATTCTACTTGA	MicroBC1/Yu
SSRY 59	GCAATGCAGTGAACCATCTTT	CGTTTGTCCTTTCTGATGTTC	MicroBC1
SSRY 63	TCCAGAATCATCTACCTTGGCA	AAGACAATCATTTTGTGCTCCA	MicroBC1/Yu
SSRY 64	CGACAAGTCGTATATGTAGTATTCAG	GCAGAGGTGGCTAACGAGAC	MicroBC1/Yu
SSRY 69	AGATCTCAGTCGATACCCAAG	ACATCCGTTGCAGGCATTA	NewBC1(Ne)
SSRY 82	TGTGACAATTTTCAGATAGCTTCA	CACCATCGGCATTAAACTTG	MicroBC1/Yu
SSRY 100	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG	NewBC1
SSRY 102	TTGGCTGCTTTCACTAATGC	TTGAACACGTTGAACAACCA	NewBC1

Table 2b. SSR sequences and thermocycler programs continuation.

SSR locus	Left primer sequence	Right primer sequence	Thermocyler programme
SSRY 103	TGAGAAGGAAACTGCTTGCAC	CAGCAAGACCATCACCAGTTT	NewBC1
SSRY 105	CAAACATCTGCACTTTTGGC	TCGAGTGGCTTCTGGTCTTC	NewBC1
SSRY 106	GGAAACTGCTTGCACCAAAGA	CAGGCAAGACCATCACCAGTTT	NewBC1
SSRY 108	ACCCTATGATGTCCAAAGGC	CATGCCACATAGTTCGTGCT	MiccroBC1/ Yu
SSRY 110	TTGAGTGGTGAATGCGAAAG	AGTGCCACCTTGAAAGAGCA	NewBC1
SSRY 135	CCAGAAACTGAAATGCATCG	AACATGTGCGACAGTGATTG	Yucdiv
SSRY 147	GTACATCACCACCAACGGGC	AGAGCGGTGGGCGAAGAGC	Yucadiv
SSRY 148	GGCTTCATCATGGAAAAACC	CAATGCTTTACGGAAGAGCC	Yucadiv
SSRY 151	AGTGGAAATAAGCCATGTGATG	CCCATAATTGATGCCAGGTT	NewBC1
SSRY 155	CGTTGATAAAGTGGAAAGAGCA	ACTCCACTCCCGATGCTCGC	Yucadiv
SSRY 161	AAGGAACACCTCTCCTAGAATCA	CCAGCTGTATGTTGAGTGAGC	Yucadiv
SSRY 164	TCAAACAAGAATTAGCAGAACTGG	TGAGATTTCGTAATATTCATTTCACTT	NewBC1
SSRY 169	ACAGCTTAAAAACTGCAGCC	AACGTAGGCCCTAACTAACCC	Yucadiv
SSRY 171	ACTGTGCCAAAATAGCCAAATAGT	TCATGAGTGTGGGATGTTTTTATG	NewBC1
SSRY 177	ACCACAAACATAGGCACGAG	CACCCAATTCACCAATTACCA	Yucadiv
SSRY 179	CAGGCTCAGGTGAAGTAAAGG	GCGAAAGTAAGTCTACAACTTTTCTAA	MicroBC1
SSRY 180	CCTTGGCAGAGATGAATTAGAG	GGGGCATTCTACATGATCAATAA	MicroBC1
SSRY 181	GGTAGATCTGGATGGAGGAGG	CAATCGAAACCGACGATACA	Yucadiv
SSRY 182	GGAATTCTTTGCTTATGATGCC	TTCCTTTACAATTCTGGACGC	Yucadiv

0.25 µl of both forward and reverse primer (10 µM), 0.125 µl Taq polymerase (5 U), 1.0 µl 0f genomic DNA template (10 ng/ µl) all together with 6.075 µl of nuclease free PCR water.

The reaction was carried out in Bio RAD MyCycler^{Tm} thermal cycler with heated lid to reduce evaporation. The 35 SSR markers had three different cycling programmes. The programmes Yucadiv, MicroBC1 and NewBC1 are shown in Table 2b. The cycling profiles are as follows: Yucadiv [95°C for 2 min (initial denaturation), 30 cycles of the following steps; 94°C for 30 s (denaturation), 55°C for 1 min (primer annealing), 72°C for 1 min (extension) then 72°C for 5 min (final extension) and storage at 4°C]; MicroBC1 [94°C for 2 min (initial denaturation), 30 cycles of the following steps; 94°C for 1 min (denaturation), 55°C for 1 min (primer annealing), 72 °C for 1 min (extension) then 72°C for 5 min (final extension) and storage at 4°C]; and NewBC1 [95°C for 2 min (initial denaturation), 30 cycles of the following steps; 94°C for 30 s (denaturation), 55°C for 30 s (primer annealing), 72°C for 1 min (extension) then 72°C for 5 min (final extension), 25 cycles of the following steps; 94°C for 30 s, 65°C for 30 s (-) 1°C/ cycle, 72°C for 1 min then 94°C for 30 s (final denaturation), 55°C for 30 s (final primer annealing), 72°C for 1 min

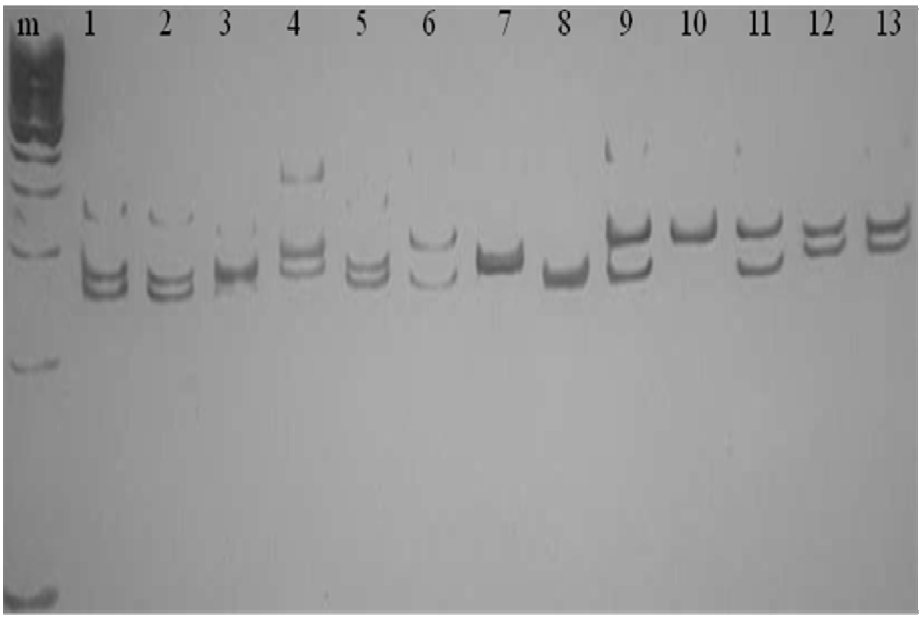

Figure 1a. 6% polyacrylamide gel showing silver stained PCR amplified allelic fragments of locus SSRY 59 for 11 released and two local Ghanaian cassava cultivars. The gel was scored for the presence (1) or absence (0) for all the amplified alleles at their respective length starting from least to the highest allelic length using 100 bp marker as the standard. At 275 bp, cultivar 1, 2, 8, 9 and 11 were scored present (1) whiles cultivar 3, 4, 5, 6, 7, 10, 12 and 13 were scored absent (0).

(final extension) and storage at 4°C]. The amplified DNA fragments were separated on 6% PAGE gel at 200 v for 35 min in TBE (1X) using a mini-protean 3 cell electrophoretic apparatus.

A 100 bp DNA marker (0.05 µg/µl, 25 µg) (Invitrogen) was used as a standard and the DNA amplified fragments were visualized in gel by silver nitrate staining.

Genetic diversity determination

Generated DNA bands were scored as 1 for the presence or 0 for the absence of a particular DNA fragment of a similar size. The data generated was analyzed using Numeric Taxonomy Statistics System (NTSYS) (Roulph, 2000) software package, version 2.1. A binary matrix data was generated, by selecting similarity coefficient method of Jaccard 1908 in NTSYSpc. Binary data generated from the analysis of scoring was used to construct similarity matrix using Jaccards similarity coefficient (Jaccard, 1908) and the information obtained from the similarity matrix was used to construct a dendrogram using unweighted pair group method using averages (UPGMA) and sequential and hierarchical numeric (SHAN) option. The same data used in NTSYS was used in genestat to generate the percentage variation accumulated by the first two principal components analysis (PCA) and also to plot the two-dimensional dispersion of the cultivars using the correlation matrix option.

RESULTS

DNA isolation, PCR and gel electrophoresis

Genetic diversity in cassava was evaluated using 11 improved and two local Ghanaian cassava cultivars with 36 SSR primers. 35 out of 36 primers representing 97.2% produced clear and scorable bands in electrophoretic gels (Figures 1a and b). However, there was no successful amplification using primer SSRY 177 in all the triplicate reactions. Most of the primers (80%) amplified the target sequences across all cultivars tested but *Afisiafi* cultivar generated no products at locus SSRY 38, 106 and 164. Similarly, with locus SSRY 179 and 180 no PCR products were observed for *Agbelifia* cultivar. No successful PCR product was also observed for *Nkabom* cultivar using locus SSRY 52. For a total of 13 cultivars analyzed, all the 35 microsatellite markers used were found to be polymorphic, recording 163 polymorphic bands**.**

Genetic relationships

The genetic similarity revealed among the cultivars ranged from 0.012 to 0.523 (Table 3). The highest similarity occurred between the two local cultivars, *Debor* and A*kosua Tumtum* (52.3%). This was followed by 31.4% between *Afisiafi* and *Filindiahoh* and 31.3% between *Nkabom* and *Nyerikobga*. On the other hand, the lowest percentage of similarity was obtained between *Bankye Hemaa* and *Filindiahoh* and between *Bankye Hemaa* and *Afisiafi* at 1.2 and 1.1% similarity, respectively. This was followed closely by the relationship between *Bankye Hemaa* and *Abgelifaa* at 5.0%. The dendrogram

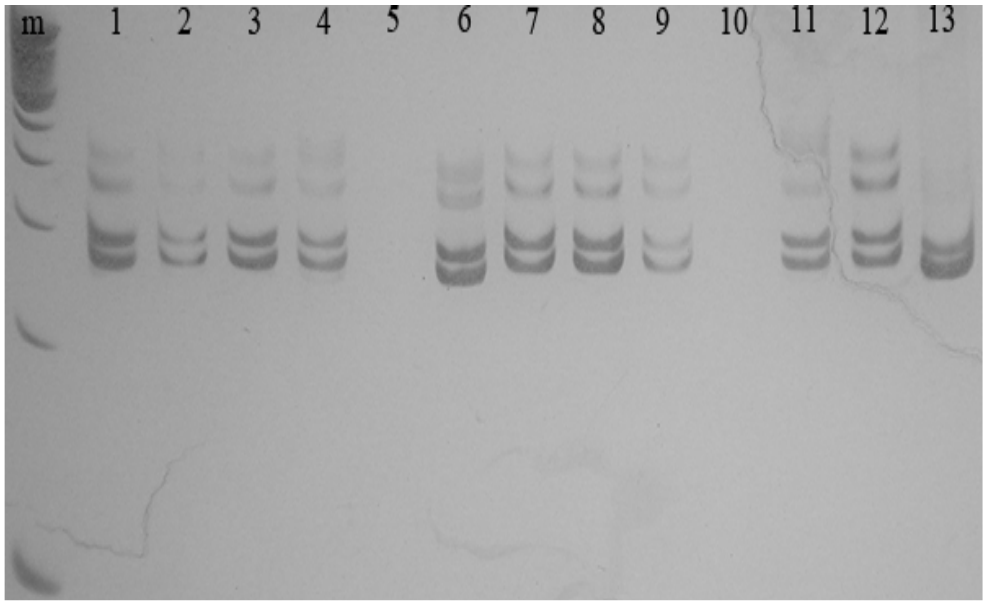

Figure 1b. 6% polyacrylamide gel showing no successful PCR amplified fragment observation for Nkabom and *Afisiafi* after a triplicate reaction using locus SSRY 52.

Table 3. Similarity matrix generated from NTSYSpc version 2.1 for Cassava using SSR (codominant maker) IFA (*IFAD*), AGB (*Agbelifia*), NYE (*Nyerikobga*), ESS (*Essam Bankye*), AKO (*Akosua Tumtum*), DEB (*Debor*) FIL (*Filindiakoh*), AFI (*Afisiafi*), DOK (*Doku Duade*), HEM (*Bankye Hemaa*), BOT (*Bankye Botan*).

	UCC	IFA	AGB	NYE	NKA	ESS	AKO	DEB	FIL	AFI	DOK	HEM	BOT
UCC	1.000												
IFA	0.268	1.000											
AGB	0.212	0.184	1.000										
NYE	0.182	0.169	0.184	1.000									
NKA	0.130	0.235	0.095	0.313	1.000								
ESS	0.106	0.130	0.184	0.209	0.280	1.000							
AKO	0.149	0.124	0.088	0.136	0.202	0.149	1.000						
DEB	0.132	0.132	0.085	0.120	0.195	0.157	0.523	1.000					
FIL	0.132	0.144	0.085	0.120	0.182	0.184	0.179	0.291	1.000				
AFI	0.057	0.094	0.082	0.107	0.080	0.192	0.141	0.211	0.314	1.000			
DOK	0.108	0.108	0.097	0.132	0.118	0.096	0.112	0.133	0.200	0.211	1.000		
HEM	0.060	0.060	0.050	0.071	0.103	0.104	0.074	0.071	0.012	0.012	0.265	1.000	
BOT	0.072	0.051	0.051	0.106	0.061	0.083	0.053	0.073	0.051	0.069	0.132	0.278	1.000

clustered the 13 cassava cultivars into three main clusters (Figure 2). The first cluster comprised six individuals (*UCC*, *IFAD*, *Agbelifia*, *Nyerikobga*, Nkabom, *Essam Bankye*) whereas the second cluster included four individuals (the two local cultivars, *Akosua Tumtum* and *Debor,* and two released cultivars, *Filindiakoh* and *Afisiafi)*. The third cluster however constituted of three members namely *Doku Duade, Bankye Hemaa* and *Bankye Botan*. Each clone of the 13 cassava was an entity at 0.53 similarity coefficient.

When the improved and the local varieties were considered separately, the similarity coefficient observed for the released varieties ranged from 0.15 to 0.42% whiles the dendrogram was constituted of two main clusters; cluster one included six individuals (*UCC*, *IFAD*, *Agbelifia*, *Nyerikobga*, Nkabom, *Essam Bankye*) and cluster two comprised five individuals (*Afisiafi, Doku Duade*, *Bankye Hemaa* and *Banjye Botan*) (Figure 3). The similarity coefficient of the local varieties was 0.49%. The first and second principal components of the 13 cassava cultivars comprised 19.95 and 12.62% of the total variation (32.62%) respectively whereas the analysis for only the improved cultivars yielded a total of 35.77% variation; 22.44 and 13.33% for the first and second

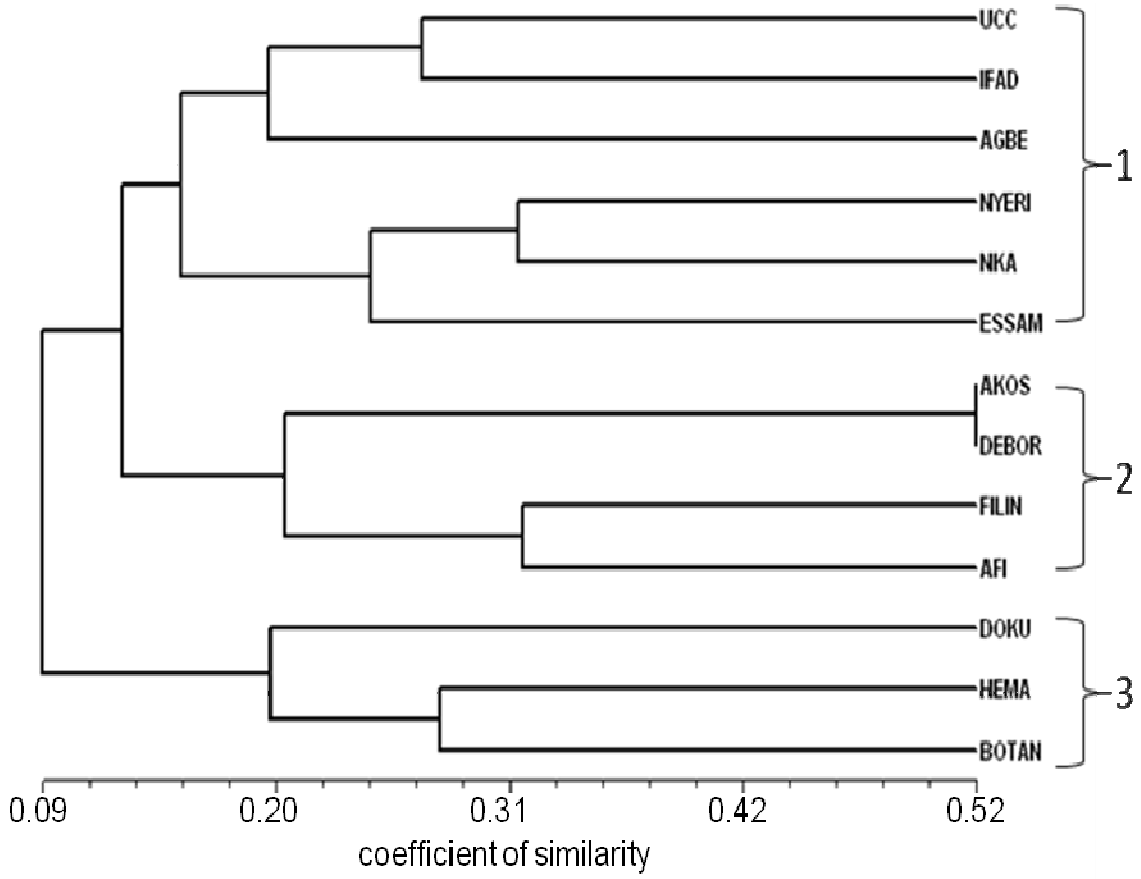

Figure 2. Dendogram generated, by NTSYSpc version 2.1, using 35 SSR markers for 11 released and two local Ghanaian cassava cultivars. Three major clusters were obtained and the most distantly releated cultivars, *Bankye Hemaa* and *Agbelifia*, were grouped in the first and the third clusters whereas *Akosua Tumtum* and *Debor* were clustered in the second major cluster. AGBE (*Agbelifia*), NYERI (*Nyerikobga*), NKA (*Nkabom*), ESSAM (*Essam Bankye*), AKOS (*Akosua Tumtum*), FILIN (*Filindiakoh*), AFI (*Afisiafi*), Doku (*Doku Duade*), HEMA (*Bankye Hema*), BOTAN (*Bankye Botan*).

principal components. The PCA in both cases (Figures 4 and 5) were however comparable to the cluster analysis.

DISCUSSION

Genetic diversity among 11 released and two local cassava cultivars in Ghana was evaluated using cluster analysis method because it has the ability to identify cultivar varieties with the highest level of similarity (Aliyu et al., 2000). The principal component analysis was also carried out to visualize the genetic relationships between cultivars, in a two dimensional plot. PCA results in all cases in this study confirmed the results of the cluster analysis, showing clear genetic relationships between the cassava cultivars. None of the cultivars released by the same research groups were found forming a sub-cluster within the main clusters. This shows that the released materials are genetically diverse and indicating that their source could be from diverse ancestral parents and also possibly indicates that they were released for specific domestic and industrial purposes.

Genetic diversity in cassava has been previously studied using DNA molecular primers such as isozyme markers (Sarria et al., 1992), RFLP (Angel et al., 1992), RAPD (Tonukari et al., 1997; Ugorji, 1998) and SSR (Fregene et al., 2001) with either low or medium observable genetic diversity (Moyib et al., 2007). In Nigeria, a medium genetic diversity was observed between improved cassava cultivars and commonly grown Nigerian landraces (Moyib et al., 2007). However, the study showed high diversity between the improved cultivars (0.15 to 0.42% coefficient of similarities) and the two local varieties (0.49% coefficient similarities). This suggests that the improved and the local cultivars may have distantly related parents. With the help of marker assisted selection, specific desirable traits in the improved cultivars which may be absent in the local cultivars or vice versa may be introgressed into either cultivar thereby transforming them into newly improved cultivars. A

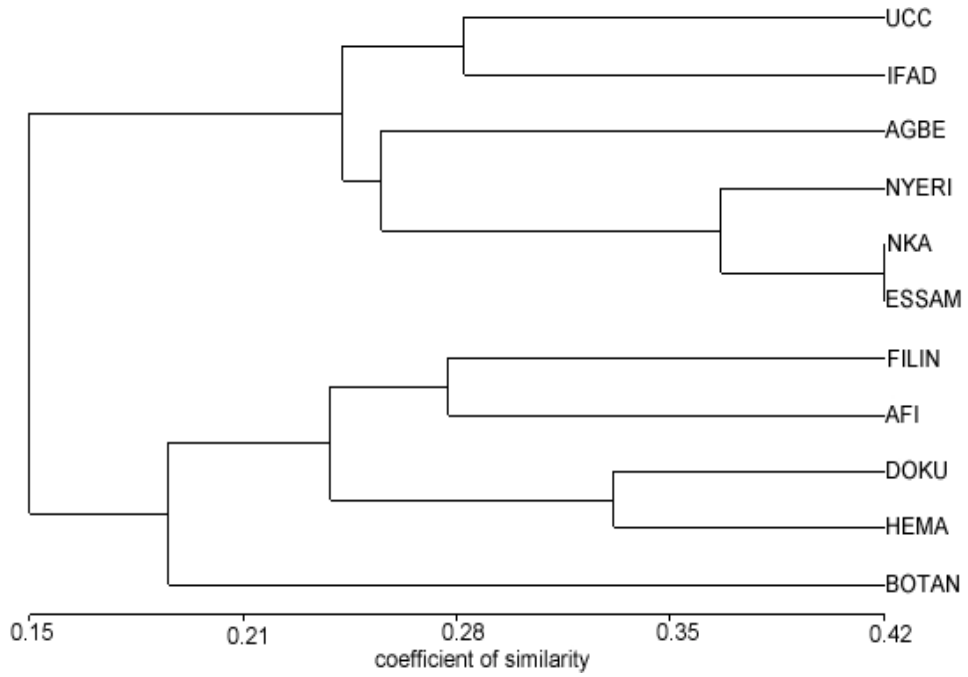

Figure 3. Dendogram showing similarity coefficient range of 0.15 to 0.42 for 11 released Ghanaian cassava varieties using NTSYSpc version 2.1.

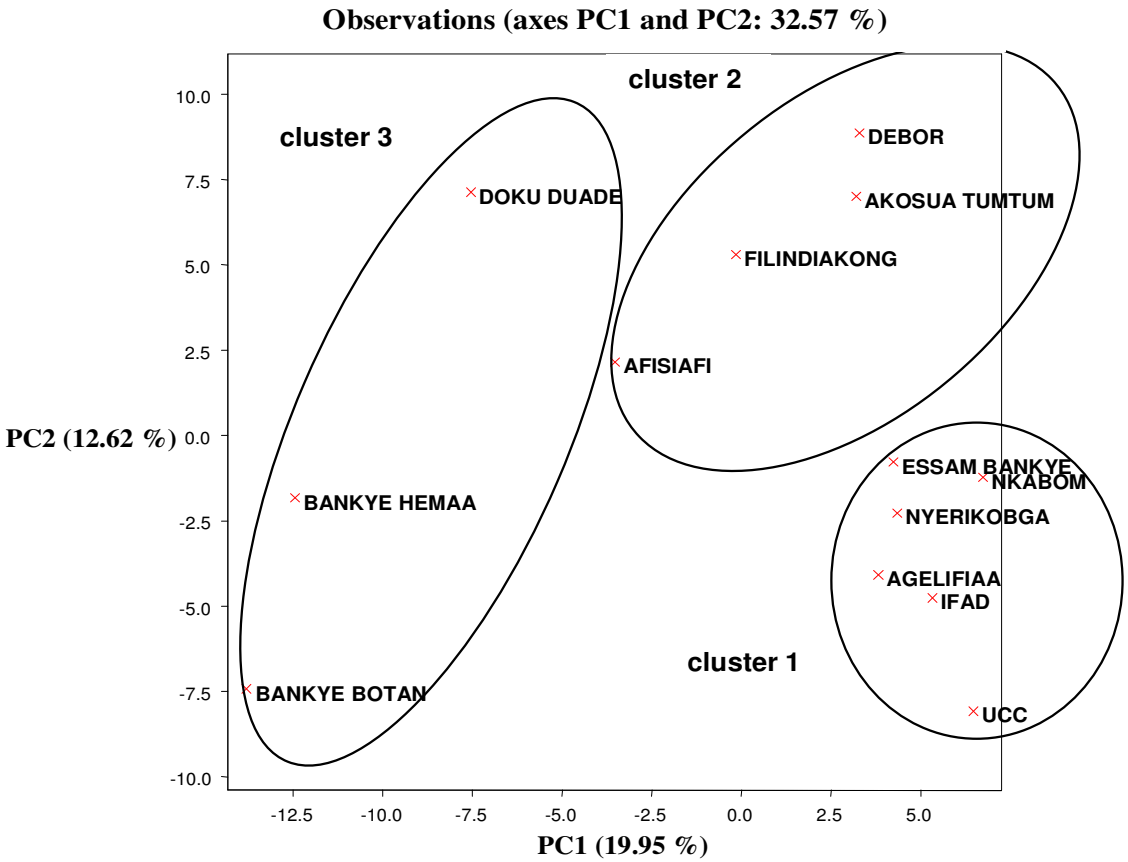

Figure 4. Principal component analysis (2 dimensional plots) of 13 cassava cultivars based on correlation matrix using genestat discovery (edition 3). The PCA result was comparable to the cluster analysis in Figure 2.

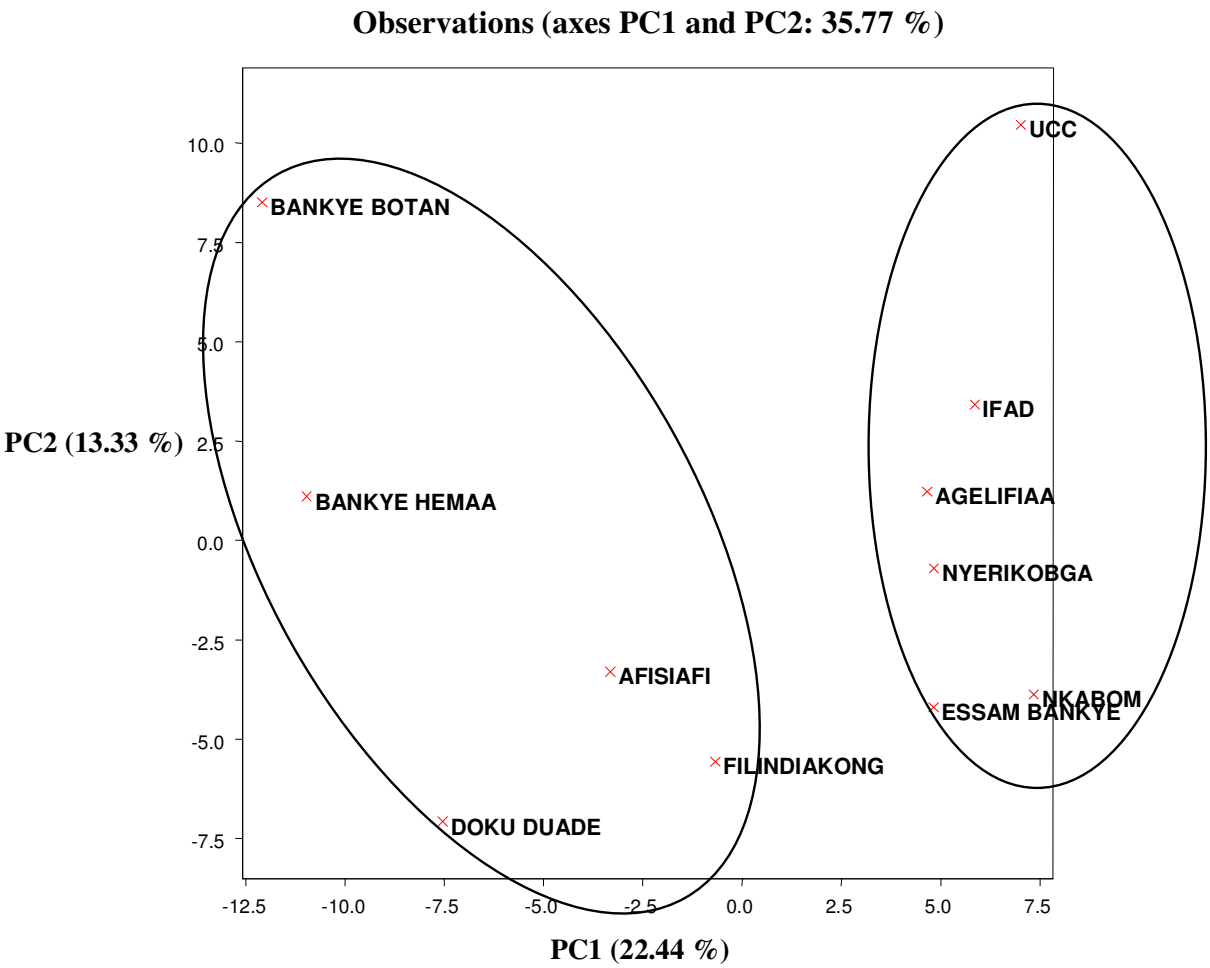

Figure 5. Principal component analysis (2 dimensional plot) of released cassava cultivars based on correlation matrix using genestat discovery (edition 3). The PCA result was comparable to the cluster analysis in Figure 3.

breeding programme between the released varieties and local cultivars and among the released cultivars therefore has the potential to widen the genetic base of Ghanaian cassava germplasm and also provide new varieties with higher agronomic value.

The released varieties employed in this study had a common source of collection, IITA- Ibadan, Nigeria, and yet highly diversified with a narrow genetic similarity range of 0.050 to 0.314%. This narrow genetic similarity range reveals that the released cultivars have diverse genetic traits as a result of their distantly related parents. The distant relations between *Bankye Botan* and *IFAD*, *Bankye Botan* and *Agbelifia* and *Bankye Hemaa* and *Agbelifia*, were 0.051 and 0.050% similarity respectively. The distantly related improved cultivars may serve as representatives for the parents which may have been lost from the IITA germplasm collections.

Their DNA fingerprints may also be used to discover their parents from germplasm collections for further genetic analysis. The narrow genetic similarity range found between the released cultivars further suggests that there could be several parental options to choose from during breeding programmes and therefore the use and conservation of the two local cultivars may not be essential. Planting space management, reduction in duplication of cultivars and reduction in the cost of conservation of germplasm could further be enhanced by the use of targeted insertion of useful and unique genes into the genome of closely related cultivars.

By this, it would be possible to carry out genetic modifications at specific points in the plant genome, especially in the case of closely related cultivars, such as *Akosua Tumtum* and *Debor.* Thus, the parents of the all close relatives may be discarded to make way for enough planting space and also avoid duplication.

In conclusion, the study has shown *Bankye Hemaa* to be the most distant relative of *Filindiakoh* and *Afisiafi* (1.2 and 1.1% similarity, respectively) and therefore could be used in breeding programs in Ghana as it could increase the chances of heterosis. Two other cultivars, *Bankye Botan* and *Bankye Hemaa* are relatively distant relatives of the cultivars studied and therefore could have a high

hybrid vigor and also be appropriate for breeding.

ACKNOWLEDGEMENT

We acknowledge Dr. Elizabeth parks, Dr. Joe Manu-Aduening and Ruth Thompson of Crop Research Institute for the diverse support provided to support this work. We also thank the technicians of the Molecular and Tissue Culture laboratories. Finally, we appreciate the support of the Ghana's Ministry of Food and Agriculture (MOFA) Roots and Tuber Multiplication Station at Ashanti Mampong for supplying the planting materials throughout the period of the research.

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