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Full Length Research Paper

Morphological and molecular based diversity studies of some cassava (*Manihot esculenta* crantz) germplasm in Ghana

P. A. Asare[1]*, I. K. A. Galyuon[2], J. K. Sarfo[3] and J. P. Tetteh[1]

[1]Department of Crop Science, University of Cape Coast, Ghana.
[2]Department of Molecular Biology and Biotechnology, University of Cape Coast, Ghana.
[3]Department of Biochemistry, University of Cape Coast, Ghana.

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Cassava (*Manihot esculenta* Crantz) is an important food crop in many tropical countries in Africa, South America and Asia. However, yields are below the productivity of the crop. This requires breeding and selection for improved varieties. The current study therefore investigated genetic diversity among some Ghanaian preferred accessions which could be used for breeding purposes. Genetic diversity of 43 cassava accessions was determined using 14 morphological descriptors and 20 SSR primer pairs. Principal component analysis (PCA) indicated that the first three principal components accounted for 72.7% of the total variation with PCA1, PCA2 and PCA contributing 46.6, 14.7 and 11.4%, respectively. The size of amplified alleles ranged from 75 to 350 bp, most of which were closed to the published values. The polymorphic information content (PIC) values ranged from 0.07 for SSRY181 to 0.75 for SSRY175, with an average of 0.52. Gene diversity was high and the average observed heterozygosity was 0.77. Both morphological descriptors and SSR markers were able to group the accessions into distinct clusters independent of locality of collection. However, where the morphological descriptors indicated some accessions were the same, SSRs markers were able to distinguish them into distinct genotypes with some located in different clusters. The wider genetic diversity observed using SSR markers would be valuable for efficient management of germplasm and for effective utilization of materials in breeding programmes to produce hybrids of desirable characteristics. Therefore, the application of morphological descriptors in management of germplasm should be backed by the use of molecular markers.

Key words: Cassava germplasm, simple sequence repeat (SSR), morphological descriptors, genetic diversity.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), currently is the sixth world food crop (FAO, 2008). The genus *Manihot* belongs to family Euphorbiaceae, subfamily Crotonoideae, tribe Manihoteae (Webster, 1994). It is highly heterozygous due to its out-crossing nature (Nassar, 2005). The DNA content is estimated around 1.7pg per cell nucleus with 2n = 36 (Bennet et al., 1992).

Cassava has an edible starchy root tuber, which provides more than half of the calories consumed by more than 800 million people in Sub-Saharan Africa (SSA), Latin America and Asia (Shore, 2002). It has become the most important source of dietary energy in SSA (Scott et al., 2000) as it provides more dietary energy per hectare and working hours than any other staple crop (Akoroda, 1995; Fregene et al., 2000; Nassar, 2005). Other advantages of cassava include flexibility in planting time, harvesting time, and its drought tolerance ability. Moreover, it is also able to grow and produce on low nutrient soils, where cereals and other crops do not grow well, and is well suited for incorporation in various cropping systems (Onwueme, 1978; Fregene et al., 2000; Nassar, 2005). The leaves of cassava are also used as a vegetable in Africa and are a cheap but rich source of proteins, vitamins A, B and C, and other minerals (Hahn, 1988; FAO, 1993; Fregene et al., 2000; IITA, 2001)

In Ghana, cassava is grown across all agro-ecological zones and ranks first in the area under cultivation and

*Corresponding author. E-mail: syasare@yahoo.com. Tel: 0244734191.

utilization; and contributes 22% of Agricultural Gross Domestic Product (AGDP) (Parkes, 2009). Cassava is produced by over 70% of Ghanaian farmers and consumed by more than 80% of the population (Parkes, 2009), indicating it is very important in food security.

However, the average crop yield in Ghana is 12.42 mt ha^{-1} against an achievable yield of 28.0 mt ha^{-1} (MOFA, 2005). The low yield of the crop is attributable to many factors. Chief among them is that farmers still use unimproved planting materials alongside few improved varieties which are seldom planted in pure stand. Secondly, farmers also use unimproved agronomic practices, such as late planting, random planting, weed control at the wrong time as well as apathy in controlling diseases and pests. Furthermore, there is also little or lack of knowledge of the genetic diversity among the various accessions available for crop improvement (Amenorpe, 2002).

Before the advent of molecular markers, plant breeders relied on phenotypic traits as markers for cultivar identification (Elias et al., 2001; Zacarias et al., 2004). These markers are still used in Africa because they are readily available for use; particularly where the capacity to use molecular markers is not yet fully developed (Fregene, 2000). Indeed many released cultivars in Ghana were developed based on morphological descriptors. With the development and application of molecular (DNA) markers, the estimation of plant genetic diversity has become much more simple and reliable. This is because, in contrast to morphological or biochemical marker techniques, DNA-based methods are independent of environmental factors and highly polymorphic for each loci (Karp et al., 1997).

A number of DNA marker techniques are available and are important tools for genetic identification in plant breeding and germplasm management (Mba et al., 2001). These DNA markers used in diversity analysis include random amplified polymorphic DNA (RAPDs), restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs). Molecular characterization of agronomically important traits within the cassava germplasm, using different markers will be useful in the molecular breeding programmes. Among the recently developed molecular markers, SSR markers are being considered as the markers of choice as they are able to detect variation in allele frequency at many unlinked loci (Moyib et al., 2007). SSR markers are particularly attractive to study because they are abundant in plants; they have high level of polymorphism, and are adaptable to automation (Tauz and Renz, 1984; Gupta et al., 1996; Powel et al., 1996; Mba et al., 2001). In cassava, SSR markers have been used to search for duplicates at the International Centre for Tropical Agriculture (CIAT), Cali, Colombia core collection (Chavarriaga-Aguiree et al., 1999) and to analyze variation in natural populations of putative progenitors of cassava (Carvalho and Schaal, 2001).

Morphological descriptors are still used, particularly in situations where molecular markers are not readily available. There is the need to assess the suitability of these descriptors in germplasm management and diversity studies in comparison with available molecular markers, such as SSRs. With the limitation of morphological descriptors, due to their response to changes in environmental factors, there might be mislabeling or wrong naming of some accessions as a result of close semblance to some others. This study was therefore designed to assess the genetic diversity of some Ghanaian cassava germplasm based on morphological descriptors and SSR markers in order to provide information that could be used to improve upon the current accessions through breeding and selection for desirable characteristics.

MATERIALS AND METHODS

43 accessions were used for the work. Thirty-one (31) of them were obtained from the Plant Genetic Resource Research Institute at Bunso in the Eastern Region of Ghana, while the remaining materials were obtained from the University of Cape Coast cassava germplasm stock. Ten healthy cuttings were obtained for each accession and planted in a single row to sprout.

DNA extraction and purification

The youngest leaves were harvested when they were 14 days old for DNA extraction. DNA extraction was carried out at the Crop Research Institute (CRI) Molecular Biology Laboratory, Fumensua, Ashanti Region, Ghana. DNA was extracted by Dellaporta et al. (1983) method. This protocol consisted of cell lysis, DNA extraction, precipitation and purification.

PCR amplification of SSRs

36 highly polymorphic SSR markers, procured form Integrated DNA Technologies (Crolville, Iowa, USA) which are widely distributed in the cassava genome (Chavarriaga-Aguirre et al., 1998; Mba et al., 2001) were used in genotyping the accessions. PCR kits were obtained from Fermantas (IndustrialCol, South Africa). PCR reactions were conducted in a TECHNE Thermocycler (TC-512) in a 10 µl reaction mixture in 96-well plates. The mixture contained 1 µl 10x PCR Buffer (Fermentas, Germany), 1 µl 5mM dNTPs, 0.75 µl each of the forward and reverse primers, 0.1 µl of Super-Therm Taq polymerase (JMR-801, Fermentas, Germany), 3.4 µl sterile distilled water (SDW) and 20 ng genomic DNA. The PCR programme consisted of an initial denaturation for 2 min at 94°C and then 30 cycles of denaturation for 30 s at 94°C, annealing at the appropriate temperature for each pair of primers for 30 s, and extension at 72°C and then put on hold at 4°C at infinity (∞). The amplified products were stored at -20°C until they were needed to run gels.

Running of gel and scoring of bands

Horizontal polyacrylamide gel electrophoresis (hPAGE) was used for running the samples. 6X Orange DNA loading dye (10 mM Tris-HCl (pH 7.6), 0.15% orange G, 0.03% xylene cyanol FF, 60%

glycerol, 60 mM EDTA) was used for visual tracking of DNA migration during electrophoresis. The presence of glycerol in the solution was to ensure that the samples were properly laid at the bottom of the well, while the EDTA was to bind any divalent metal ions and inhibit metal dependent nucleases from degrading the DNA samples. One volume of the dye was added to 5 volumes of DNA sample. After initial denaturing at 95°C for 5 min using the PCR machine, 6 µl of sample (or DNA ladder in the first well) was loaded in each well of a 41-well 5% polyacrylamide gel. The DNA ladder used was a 50 bp ladder (Fermentas, Germany). Gels were run at 300V for 2 h using a Baid and Tatlock Nucleic Acid Electrophoresis Cell and powerpack and 1x TBE as running buffer. The products were visualized by ethidium bromide staining method. The gel (attached to the back glass plate using bind-Silane) was stained in 500 ml TE buffer containing 25 µl of ethidium bromide for 50 min. The stained gel was washed in 500 ml of TE buffer to get rid of excess ethidium bromide on the gel, viewed using a UV transilluminator (Uvitech, UK) and photographed with a digital camera (Canon, Power Shoot A620). The bands for each gel were scored and used for diversity analysis. The data were scored in binary form - presence of a band was scored as 1 and absence as 0.

Data analysis

19 morphological descriptors were recorded and used. Descriptive statistics and correlation coefficients analysis were done using excel programme. Factor analysis was performed to determine which trait contributed the highest variability. Principal component analysis of the traits was employed to examine the percentage contribution of each trait to total genetic variation using GenStat Discovery version. The chosen variables were used to construct a dendrogram (Figure 1). Genetic similarity between pairs was estimated using Euclidean method. The similarity matrix was run on sequential, agglomerative, hierarchical and nested clustering using the unweighted pair-group method with arithmetic average (UPGMA) according to Nei (1973) using PowerMarker version 3.5 program (Liu and Muse, 2005) and MEGA version 4 software (Tamura et al., 2007) was used to generate the dendrograms (Figure 2). Cophenetic correlation coefficient (CCC) which measures the goodness-of-fit of the dendrogram was calculated using MATLAB version 7. For SSR analysis, based on the allelic profile, the PowerMarker program (Liu and Muse, 2005) was used to calculate the number of alleles per locus, gene diversity (He), heterozygosity (Ho) and the polymorphic information content (PIC). The gene diversity was calculated as:

$$D = \left(1 - \sum_{u=1}^{k} Plu^2\right) / \left(1 - \frac{1+f}{n}\right)$$

Where, Plu^2 is the population frequency at the l^{th} locus; f is inbreeding coefficient and n is the number of alleles present. The heterozygosity was calculated as:

$$H = 1 - \sum_{u=1}^{k} Plu^2$$

Where, Plu^2 is the population frequency at the l^{th} locus. The polymorphic information content is (PIC) was calculated as:

$$PIC = 1 \quad \sum n\,(Pi)^2$$

Where, P is the proportion of number of alleles present in genotypes, and n is the total number of alleles present at a marker locus.

RESULTS

Genetic diversity analysis based on morphological data

Cluster analysis

The cassava accessions were genetically diverse for the nineteen morphological traits assessed. All the morphological descriptors recorded were subjected to factor and principal component analysis. Principal component 1, 2 and 3 accounted for 46.6, 14.7 and 11.4% of total variance, respectively, that is, 72.7% of the total variation (Table 1). Central leaf lobe length and petiole color were the most important in distinguishing between the accessions. These were followed by color of root cortex, yield/plant and petiole length. The other morphological characters seemed not important.

The Euclidean dissimilarity coefficient calculated ranged from 0.0 to 0.40, while the cophenetic correlation coefficient (CCC) was 0.843. The resultant dendrogram grouped the forty-three cassava accessions into two main clusters (Figure 1). The smallest cluster was I with just 5, followed by clusters II and III with 10 members each while cluster IV was the largest with 18 members. AFS131 and OFF025 were morphologically the same (cluster II), Adehye and Capevars cassava accessions were also morphologically the same (cluster III) as well as ADW053 and DMA066 in the same cluster. Similarly, in cluster IV, OFF023, OFF066 and KW161 were the same, Beserebema and Nkabom also the same and AFS041, AFS048, OFF063, OFF136, and UCC153 were classified as being the same.

Genetic diversity analysis using SSR markers

20 out of the 36 primers gave polymorphic bands; the remaining primers were either monomorphic or failed to amplify any product and therefore were not considered for further analysis. The size of amplified alleles ranged from 75 to 350 bp as shown in Table 2 and Plate 1. A total number of 100 alleles were detected with the 20 SSR primer pairs among the 43 cassava accessions. The number of alleles ranged from 2 (in SSRY181) to 9 (in SSRY20 and SSRY175) per locus with a mean of 5 alleles per locus. The polymorphic information content (PIC) values also ranged from 0.07 in SSRY181 to 0.75 in SSRY175 with an average of 0.52. The most polymorphic primers were SSRY175, SSRY101, SSRY20 and SSRY100 based on PIC values (Table 2).

The allele frequency of all the primers was generally below 0.95 indicating that they were all polymorphic in character. Gene diversity was high ranging from 0.07 in

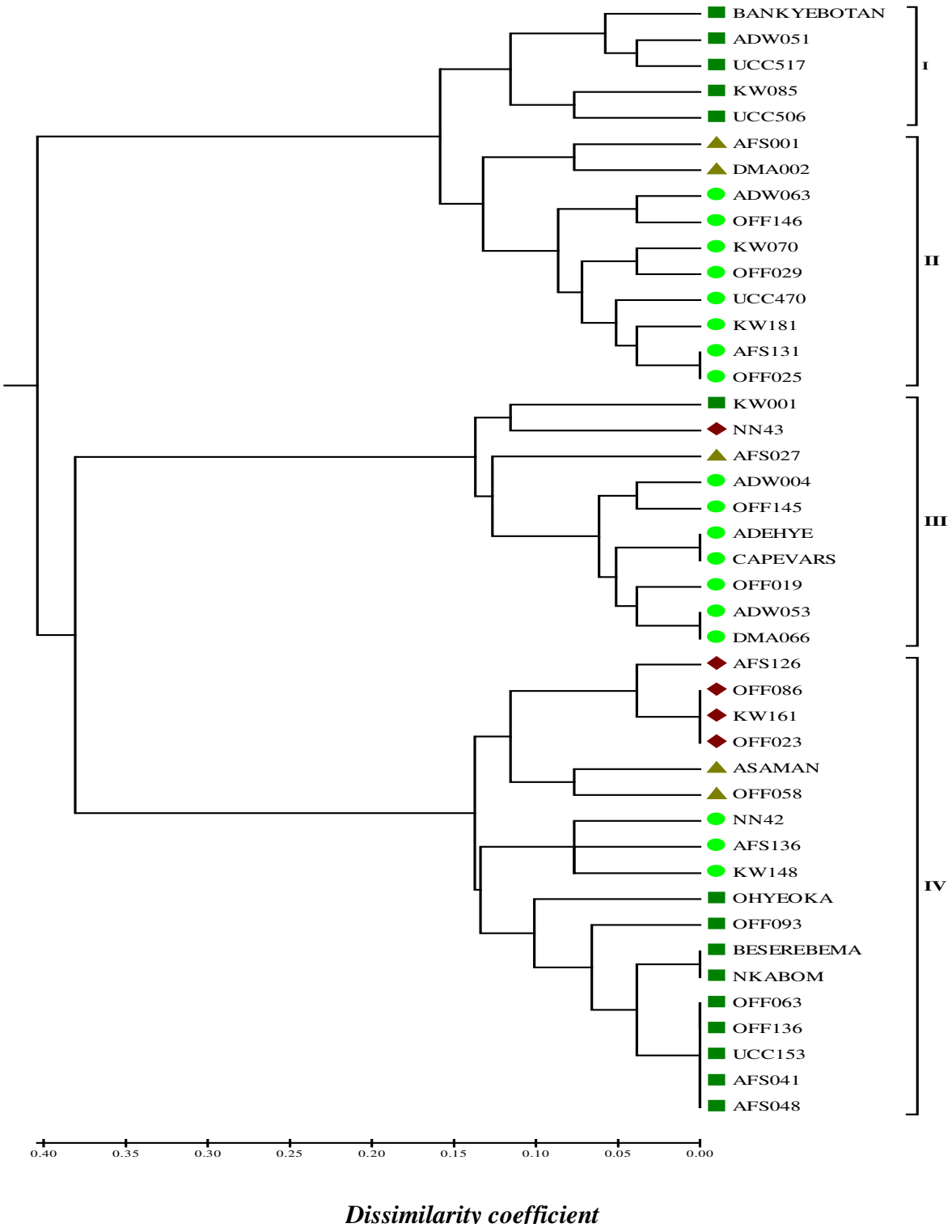

Figure 1. Dendrogram showing genetic dissimilarity among the cassava accessions based on morphological descriptors.

SSRY181 to 0.78 in SSRY175 with a mean value of 0.58. The observed heterozygosity (H_o) calculated for each primer ranged from 0.07 to 1.0 with the mean being 0.77 (Table 2). The lowest H_o value was recorded for SSRY181 while 8 primers recorded the highest value of 1.0. These high observed heterozygosity values were

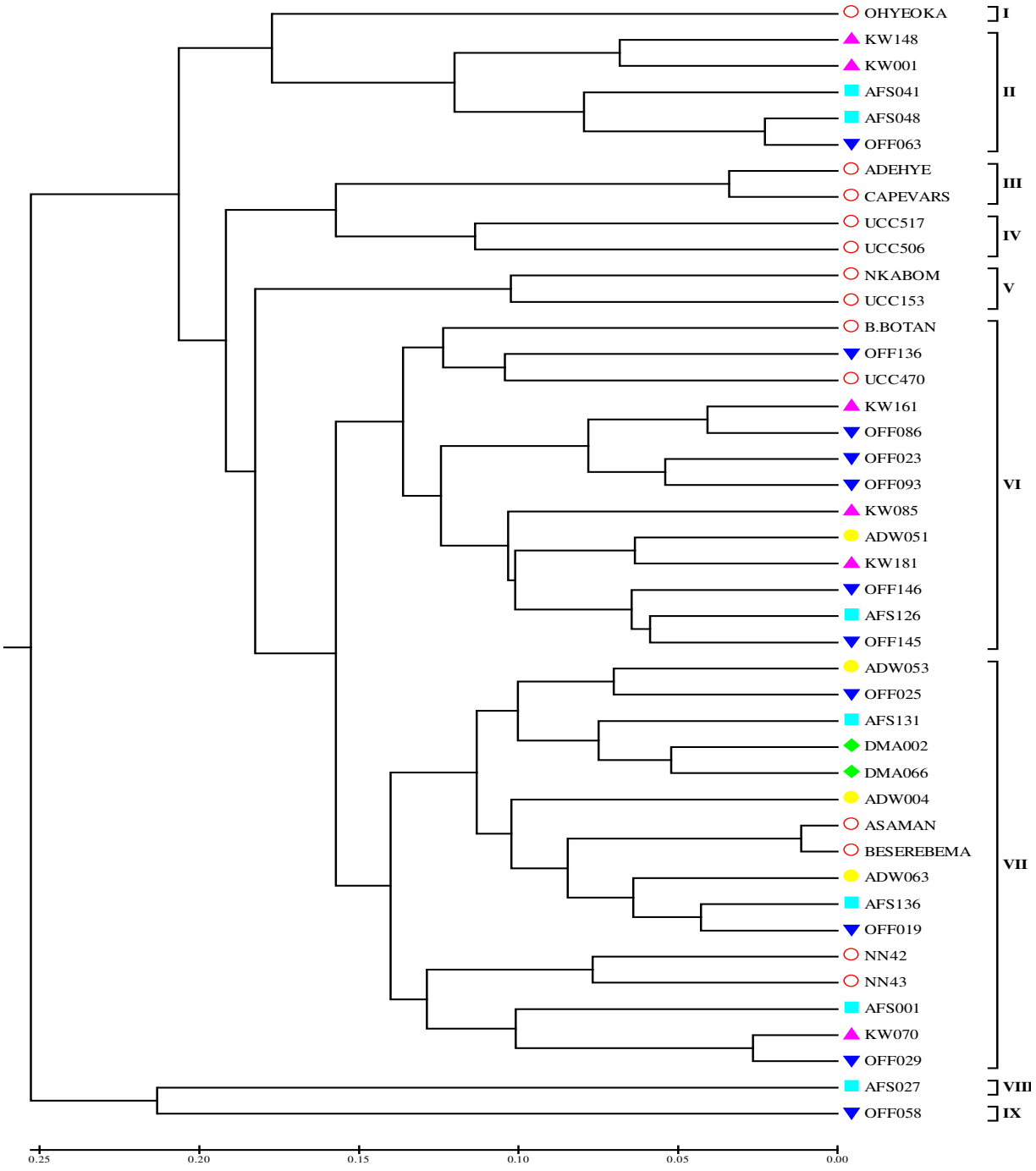

Figure 2. Dendrogram showing genetic dissimilarity among the cassava accessions based on SSR data.

significant, since they tend to substantiate the heterozygote nature of most of the accessions and the fact that cassava is largely cross-pollinated. The cophenetic correlation coefficient (CCC) was 0.857 using Euclidean similarity matrix.

The SSR primers were able to distinguish all the 43 accessions used for the study. The dendrogram revealed two main clusters with 9 distinct sub-clusters (Figure 2). Sub-clusters I-VII comprised one of the main clusters while the other consisted of sub-clusters VIII and IX. The sub-cluster size varied from 1 accession to 16 accessions. The largest sub-cluster, VII had 16 accessions, and

Table 1. Principal component analysis showing the contribution of morphological traits to total variation among the cassava genotypes.

Variable	PC1	PC2	PC3
Colour of young leaf	0.045	-0.412	0.073
Pubesences on young leaf	-0.003	-0.133	-0.289
Leaf vein color	0.071	-0.867	-0.116
Central lobe length	0.85	0.063	0.319
Petiole length	0.194	-0.042	0.062
Stem color	-0.025	0.112	-0.204
Root length	0.183	-0.043	-0.373
Root diameter	0.026	0.135	-0.589
Root surface color	-0.023	0.095	-0.178
Root pulp color	0.016	0.027	-0.021
Colour of root cortex	0.339	0.081	-0.192
Petiole color	0.78	0.069	0.056
Plant height	0.03	-0.022	-0.011
Yield/plant	0.272	0.037	-0.437
Eigen value	26.002	8.21	6.352
% Variance	46.6	14.7	11.4
Cumulative variance	46.6	61.3	72.9

followed by sub-cluster VI with 13 accessions. Sub-clusters III, IV and V had 2 accessions each while sub-clusters I, VIII, and IX were made up of only one accession each.

Adehye and Capevars accessions again clustered together (sub-cluster III); however, they were distinguished as separate genotypes unlike the morphological data which showed they were same. Similarly, AFS041, AFS048 and OFF063 also grouped together in sub-cluster II with KW001 and KW048 which were morphologically different from the former. UCC153 and OFF136, which were the same morphologically as AFS041, AFS048 and OFF063, were in sub-clusters V and VI, respectively. Indeed, UCC153 clustered with Nkabom which was also morphologically the same as Beserebema. With the SSR data, Beserebema was in the same sub-cluster as Asaman. These two even though in the same sub-cluster based on morphological descriptors were about 90% similar. Other accessions which were morphologically the same, such ADW053 and DMA066, and AFS131 and OFF025 were clearly separated as different genotypes based on SSR data.

Ohyeoka, AFS027 and OFF058 were genotypically different from the other accessions they clustered with based on morphological descriptors. Furthermore, AFS027 and OFF058 seemed more related genotypically than morphologically.

DISCUSSION

An efficient breeding programme requires information concerning the extent and nature of genetic diversity within the gemplasm for characterizing and determination of the breeding potential of cultivars. Phenotypic identification of plants has been used in the classification of genotypes and study of taxonomic status (Bonierbale et al., 1997; Nassar, 2005). The basic identification of cultivar diversity by local farmers depends on botanical traits. In the absence of DNA markers, the most available tool for genetic diversity studies is morphological descriptors. These morphological traits sometimes are quite variable, and have been used by local farmers for basic identification of plant material. Morphological characterization has been used for various purposes including identification of duplicates, studies of genetic variation patterns, and correlation with characteristics of agronomic importance (CIAT, 1984). Morphological traits are useful for preliminary evaluation, because they offer a fast and easy approach for assessing the extent of diversity. The 19 morphological descriptors used in the present study were able to distinguish the 43 cassava accessions into distinct groups. The current findings are similar to other findings (Ampong-Mensah, 2000; Fregene et al., 2000; Carvalho and Schaal, 2001; Elias et al., 2001; Raghu et al., 2007) who used stem girth, tuber length, tuber girth, yield per plant, tuber attachment on parent cuttings, tuber inner skin color, tuber outer skin color, tuber surface texture and pulp color and yield for genetic diversity studies in cassava. From PCA, central lobe length, petiole color, color of root cortex and yield per plant were the most discriminating descriptors. Since morphological descriptors are influenced by the environment and subject to environment x cultivar

Table 2. Size range, number and frequency of alleles, gene diversity, heterozygosity and polymorphic information content (PIC) values generated from SSR data.

SSR marker	Allele size range (bp)	Published allele size (bp)*	Number of allele	Allele frequency	Gene diversity	Heterozygosity (Ho)	PIC
SSRY59	150 - 170	158	5	0.59	0.59	0.51	0.54
SSRY20	130 - 160	143	9	0.37	0.73	0.98	0.68
SSRY 21	160 - 230	192	8	0.73	0.45	0.28	0.44
SSRY51	240 - 320	298	5	0.50	0.63	1.00	0.57
SSRY64	198 - 250	194	5	0.47	0.62	1.00	0.55
SSRY100	195 - 230	210	6	0.48	0.67	0.58	0.61
SSRY101	200 - 250	213	7	0.36	0.76	1.00	0.72
SSRY103	255 - 350	272	8	0.40	0.72	1.00	0.68
SSRY182	220 - 255	253	7	0.45	0.64	1.00	0.57
SSRY175	75 - 110	136	9	0.35	0.78	0.91	0.75
SSRY4	280 - 300	287	3	0.60	0.50	0.42	0.39
SSRY19	200 - 250	214	4	0.45	0.65	1.00	0.58
SSRY9	270 - 290	278	4	0.42	0.69	1.00	0.63
SSRY47	249 - 295	244	3	0.60	0.55	0.79	0.48
SSRY69	228 - 239	239	3	0.76	0.40	0.49	0.36
SSRY151	190 - 210	182	3	0.58	0.54	0.67	0.46
SSRY155	145 - 200	158	3	0.69	0.44	0.60	0.35
SSRY179	195 - 226	226	3	0.42	0.62	1.00	0.54
SSRY181	165 - 195	199	2	0.97	0.07	0.07	0.07
NS911	120 - 140	-	3	0.48	0.63	0.95	0.56
Mean			5.0	0.53	0.58	0.78	0.53

*Published sizes for the various SSR products were obtained from Mba et al. (2001).

interaction (Kaemmer et al., 1992; Gepts, 1993) molecular markers are preferred for such studies since these are not influenced by changes in environment.

Genetic characterisation based on SSR markers

The number of alleles produced by different primers ranged from 2 to 9 with an average of 5.0 alleles per locus. Similarly, Raghu et al. (2007) studying Indian cassava accessions with 15 SSR primers recorded mean number of 4 alleles with a range of 2 to 6 alleles. Moyib et al. (2007) also obtained a range of 2 to 4 alleles among 31 improved cassava cultivars and landraces. Thus the highest number of alleles in our study was higher than those reported by Moyib et al. (2007) and Raghu et al. (2007).

SSRs generally have high levels of polymorphism in many important crops including *M. esculenta* (Chavarriaga-Aguirre et al., 1999; Mba et al., 2001; Zacarias et al., 2004; Raghu et al., 2007). The mean PIC value recorded in the current study compared favourably with results obtained from another study by Tams et al. (2004), where a study of 128 accessions of Triticale with 28 SSR markers gave a mean PIC value of 0.54. Similarly, Moyib et al. (2007) have reported PIC values ranging from 0.19 to 0.66 with a mean of 0.42 compared to a range of 0.07 to 0.75 with a mean of 0.52 in the current study. PIC values greater than or equal to 0.7 were 10%, those greater than or equal to 0.6 were 30% while those from 0.5 were 65% in the current study. This suggests that most of the SSRs used were highly informative and can be used for genetic diversity studies and the study of phylogenetic relationships.

Indeed, the current study has shown that morphological descriptors can be limited in their use for genetic diversity studies. The dendrogram based on morphological descriptors grouped AFS131 and OFF025 as the same accession; Adehye and Capevars as the same; OFF023, OFF086 and KW161 as the same; ADW053 and DMA 066 as same and as many as five other accessions (AFS041, AFS048, OFF063, OFF136 and UCC153) as the same accession. SSR data on the other hand were able to separate these into different genotypes; some were not even in the same sub-cluster. Those in the same sub-cluster were clearly distinguished as different genotypes. These findings are similar to those by Carvalho and Schaal (2001) who identified a high degree

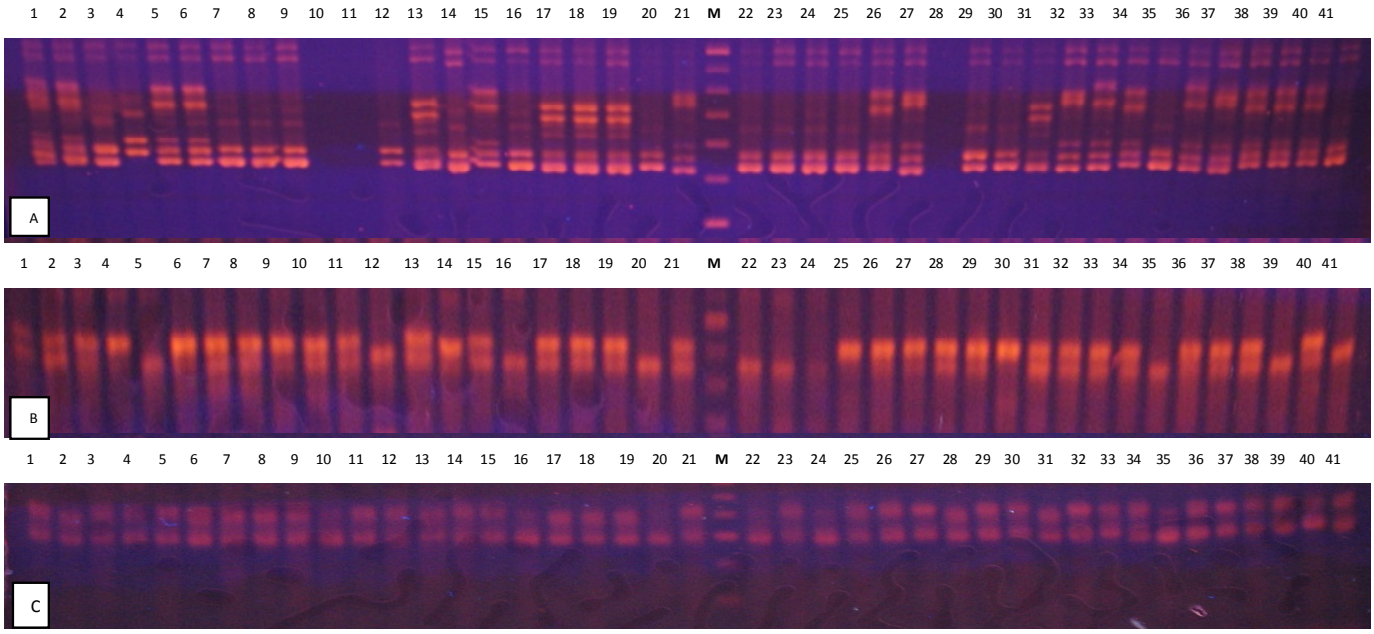

Plate 1. SSR markers profile of cassava accessions generated by primers SSRY9 (A), SSRY181 (B) and SSRY64 (C).

of genetic variability among 94 cassava accessions of Brazilian origin using PCR-based markers.

The results from this study indicate that there is enough genetic diversity among Ghanaian cassava germplasm which could be exploited for breeding and selection programmes for improved genotypes for cassava production. The current findings also suggest that morphological descriptors, even though easy to use and readily available, may lead to mislabelling particularly in the case here where certain genotypes were identified based on morphological descriptors as the same accessions.

Conclusions

Both morphological descriptors and SSR markers were able to group cassava accessions into distinct groups, but the SSRs were more discriminating. The SSRs were able to separate accessions which were identified as morphologically the same into distinct genotypes or groups different from the associations derived from morphological descriptors. Therefore, when possible, use of morphological descriptors should be backed with DNA markers for efficient and reliable genetic diversity studies and germplasm management. Otherwise, morphological descriptors should be used with caution.

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