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Full Length Research Paper

SSR markers reveal genetic variation between improved cassava cultivars and landraces within a collection of Nigerian cassava germplasm

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Thirty-one improved cultivars and five Nigerian landraces of cassava were assessed at genomic DNA level with 16 SSR primers for genetic diversity study. The minimum number of SSR primers that could readily be used for identification of the 36 cassava genotypes was also determined. For the genetic diversity study, the similarity coefficients generated between improved cultivars and Nigerian landraces ranged from 0.42 to 0.84, and 12 distinct DNA cluster groups were identified at 0.70 coefficients using Numerical Taxonomy and Multivariate Analysis System software package. For the genotype identification study, the 16 SSR primers were screened by their polymorphic information content (PIC) values. Five SSR primers that have PIC values between 0.50 and 0.67 were selected and further assessed using simple arithmetic progression combination method. The results obtained revealed a combination of these 5 primers from SSR primers collection at IITA that could readily distinguish the 36 cassava genotypes at 0.93 similarity coefficient. These five primers clustered the 36 cassavas into 16 groups at 0.70 similarity coefficient. Application of this few SSR primers would ultimately reduce the cost and time of research for genetic diversity and genotype identification studies for the genetic improvement program of cassava.

Key words: Genetic Improvement, genomic DNA, genotype identification, polymorphic information content, SSR primers.

INTRODUCTION

In Nigeria, cassava (Manihot *esculenta* Crantz) serves as the main source of energy-rich food. The root is processed and prepared as “garri” and, to a lesser extent as “akpu/fufu”, as added values to raw cassava. Leaves and tendrils contain much higher protein contents and some people consume them as vegetables (Fregene, 1996). Also, a sweet variety of cassava is boiled and eaten as “rogo” with pepper sauce in Northern Nigeria. Cassava is also used as an animal feed and locally, as a source of starch. Recently, cassava is used for the preparation of bread, chips, salad cream and confectionary such as cake and chin-chin.

Cassava has the advantage of being well adapted to a wide range of environmental stresses. It grows very well in a less fertile soil in contrast to many other crops that are highly vulnerable to environmental stresses during the critical stages of plant development (Ugorji, 1998). These values and advantages make cassava a crop of choice for many farmers in Nigeria. Current economy advancement has also turned cassava into a cash crop, since several items are processed from it, which find various end uses. These products could be sold to interested industries in the vicinity or exported for income generation (Benesi et al., 2001)

One of the best methods to increase cassava production to serve as the main food security and cash crop in Africa and developing countries is by the development of better varieties that are resistant to diseases, pests, and drought (Ugorji, 1998). Several improved genotypes and their seed populations have been developed at IITA for a range of ecologies that incorporate multiple pest and dis-

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ease resistance. Desirable traits in cassava also include early vigour in plant growth for high foliage yield, and early bulking of storage roots, with high dry matter and carotene content, low cyanide content, ease of peeling, acceptable root shape, and food quality (Dixon et al., 2001). Research shows that the additional variability of the previously untapped African landraces has also proven useful for improving the breeding population for desirable traits, especially for resistance to the cassava mosaic disease and green mite, as well as preferred food quality, and canopy characteristics (Dixon et al., 2001; Fregene et al., 2001). Currently, a classical genetic and molecular genetic mapping study revealed a major dominant gene, CMD2, which confers resistance to cassava mosaic disease, in Nigerian landraces (Akano et al., 2002; Lokko et al., 2005).

International Institute of Tropical Agriculture (IITA), Ibadan, released a total number of thirty-one improved cultivars of cassava that are high yielding, resistant to diseases and pests, and with low level of HCN content, to Nigerian farmers in 2000 and 2005, through national programs (unpublished). The farmers failed to label them and mixed them with their local breeds. IITA could neither identify the improved cultivars from the Nigerian landraces with the farmers nor in their local markets, to verify if the improved cassava satisfied the above-mentioned qualities.

Identification of desirable genotypes for various end uses is a major goal of selection in breeding. The "best" cassava is no longer the highest yielding cassava, or the cassava with the greatest resistance to pests and diseases, unless it is the "best" cassava to use for the production of a specific product (FAO, 2003). Therefore, identification of genotypes within Nigerian cassava collection for proper labeling is a necessity so as to have adequate record on the breeding pedigree and good passport data for various end uses. This may also go a long way to developing a core collection (from the germplasm) that is easier to manage and utilize for development of better varieties for genetic improvement program of cassava in Nigeria.

Traditional characterization of local varieties in Nigeria was based on the level of HCN content which distinguished the cultivars into sweet (non-poisonous) and bitter (poisonous) varieties. Current advance in the development of methods using DNA polymorphisms as molecular markers provides alternative methods for characterization of cultivars and hold a promise for revealing genetic variation and resolving the ambiguities in the Nigerian cassava collection. Recently, various research studies on cassava genetic variation were based on molecular markers such as Restriction Fragment Length Polymorphsms (RFLP, Angel et al., 1992; Fregene et al., 1997); Random Amplified Polymorphic DNA (RAPD, Angel et al., 1992; Carvalho and Schaal, 2001; Colombo et al., 2000a; Tonukari et al. 1997; Ugorji, 1998); Amplified Fragment Length Polymorphisms (AFLP, Colombo et al., 2000a, b; Elias et al., 2000; Lokko et al., 2005; Fregene et al., 2000); and Simple Sequence Repeats (SSRs, Chavarriaga et al., 1999; Fregene et al., 2003; Fregene et al., 2001; Lokko et al., 2005; Mba et al., 2000).

A prerequisite to any genetic improvement programme for cassava is the knowledge of the extent of genetic variations present within *Manihot* species. These are important and have been applied using diverse number of molecular markers to the following genetic studies in cassava; in the identification of close relatives of cassava with which good hybrids could be produced and from where introgression of desirable traits into cassava is possible (SSR and RAPD, Carvalho and Schaal, 2001; RAPD, Tonukari et al., 1997), characterization of germplasm to develop large number of new varieties with diverse agronomic traits (RAPD and RFLP, Angel et al., 1992; AFLP and RAPD Colombo et al., 2000a; AFLP, Elias et al., 2000; SSR, Fregene et al., 2001), identification of duplicates and elimination of dormancy (AFLP and SSRs, Chavarriage et al., 1999; AFLP, Fregene et al., 2000; RAPD, Ugorji, 1998), identification of representative set for development core collection (RAPD and SSR, Carvalho and Schaal, 2001; RAPD, Colombo et al., 2000b), and identification of source of resistance to diseases and pests and other agronomic traits (SSRs, Fregene et al., 2001; AFLP Fregene et al., 2000; AFLP and SSR Lokko et al., 2005; Okogbenin et al., 2006).

RAPD markers are not used routinely any more because of their inconsistency from laboratory to laboratory. Simple Sequence Repeats (SSRs) markers have been confirmed to be the most informative and appropriate for cassava (Mba et al., 2000). Perera et al. (2000) also supported SSR markers as the most informative for plants. Valuable attributes of all SSR markers are co-dominance (many alleles are found among closely related individuals), technical simplicity, sensitivity, analytical simplicity (data are unambiguously scored, and highly reproducible) and are high abundance (markers are uniformly dispersed throughout genome as frequently as every 10 Kb^3 and therefore are ideal tools for many genetic applications.

Various research studies have indicated that the number of DNA markers used for genetic studies in plants varies with the total number of accessions assessed (Thotttapilly et al., 1996). Hence, the larger the number of plant accession assessed the larger the number of DNA markers utilized. Generally, large numbers of SSR primers are being utilized for genetic diversity and genotype identification studies; that ranged from 6 for almond (Joobeur et al., 2000) to 136 for sunflower (Yu et al., 2000). Ahmad (2002) used 43 SSR primers on 13 genotypes of wheat and proposed that a collection of polymorphic SSR primers could be used for genotype identification, genetic diversity, and map linkage studies

The objectives of this study, therefore, are to (1) assess the genetic diversity present between improved cassava and commonly grown Nigerian landraces within Nigerian

Table 1. The list of thirty-six Cassava genotypes used for genetic diversity study.

Cassava genotypes	Pedigree	Cassava genotypes	Pedigree
1. NR8212 (IC)	Unknown	20. 30337 (IC)	58308 OP
2. NR8208 (IC)	Unknown	21. 92/0057 (IC)	30555 * TME 1½ sib
3. 90257 (IC)	58308 x Oyarugba dudu	22.. W1095-D (IC)	Parent stock 1971
4. 91934 (IC)	58308 x Oyarugba dudu	23. W820249 (IC)	58308 x BSC
5. 30001 (IC)	Lost pedigree	24. 088/01504 (IC)	Nsukka-2 OP
6. 30572 (IC)	58308 x Branca de santa Catarina (BSC)	25. NR89107 (IC)	Unknown
7. 84537 (IC)	58308 x Oyarugba dudu	26. W4092 (IC)	58308 x BSC
8. 82/00661 (IC)	58308 x BSC	27. TME 51 (LR)	Landrace
9. 50395 (IC)	58308 x 58198	28. TME 2 (LR)	Landrace
10. 81/00110 (IC)	58308 x BSC	29. TME 1 (LR)	Landrace
11. NR8082 (IC)	Unknown	30.TME 7 (LR)	Landrace
12. 4(2)1425 (IC)	58308 x Oyarugba funfun	31. TME 59 (LR)	Landrace
13. 30555 (IC)	58308 x Oyarugba dudu	32. 089/00023	58308 x BSC x very low cyanide population
14. 92/0326 (IC)	91939 x TME 1		
15. 30555P_{3-2}(IC)	58308 x Oyarugba dudu	33. 518 (IC)	Unknown
16. 91/02327 (IC)	TME 1 ½ sibling	34. 60444 (IC)	Nigeria e x Moor plantation
17. 30040(IC)	58308 x Oyarugba dudu	35. 60447 (IC)	Nigeria e x Moor plantation
18. 91/02324 (IC	58308 x BSC	36. 60506	Nigeria e x Moor plantation
19. W820422 (IC)	58308 x BSC		

collection, so as to obtain a good passport data and also, to enlarge the existing Nigerian cassava gene pool and (2) characterize commonly cultivated cassava in Nigeria with few number of SSR markers to reduce the time and cost of research studies.

MATERIALS AND METHODS

DNA extraction and polymerase chain reaction with SSR markers

Thirty-one improved cassava cultivars that were released to Nigerian farmers in 2000 and 2005 and five Nigerian landraces that were commonly cultivated in Nigeria were selected from IITA's collections. The names and pedigree of the cultivars are listed (Table 1). One hundred mg of young leaves, harvested 8 days after planting, were ground in liquid nitrogen into fine powder. The total genomic DNA of each plant sample was extracted using DNeasy mini plant kit (QIAGEN International, USA) according to the manufacturer's protocol. SSR primers designed from *M. esculenta* Crantz by Mba et al. (2000) and purchased from Research Genetics USA were used for the study. A total number of 64 primers were screened for polymorphism on 8 DNA samples randomly selected amplification process, an initial denaturation of DNA at 94°C for 5 min was followed by 10 cycles of touchdown PCR, with from the 36 DNA samples. Twenty-two SSR primers that showed polymorphism from the primer screening test were used for the denaturation for 45 amplification of the 36 DNA samples. The PCR reactions were carried out in a Themolyne Amplitron 11 Thermocycler. For each s at 94°C, annealing at 65°C for 1 min -1°C/cycle, and an extension for 1 min at 72°C. A final 26 cycles consisting of 45 s at 94°C, 1 min at 55°C, and 1 min at 72°C. The DNA fragments were separated on 4% agarose gel run at 100V for 2 h using 0.55 M trimas base EDTA (TBE) buffer. The DNA fragments in gel were visualized by staining in 0.5 ug/mg ethidium bromide and observed under a fluorescent lamp.

Genetic diversity study

Sixteen SSR primers that showed distinct and scorable DNA bands were considered for analysis (Table 2). Generated DNA bands were scored as 1 for the presence or 0 for the absence of a particular DNA fragment of a similar length. The data generated was analyzed using Numeric Taxonomy Statistics System (NTSYS, Roulph, 2000) software package. A binary matrix data was generated, by selecting similarity coefficient method of Jaccard 1908 in NTSYS. The similarity index of Jaccard between plant i and j is given by

$S_{ij} = a / (a + b + c)$
$D_{ij} = 1 - S_{ij}$

Where a is the number of DNA band(s) present in both plants i and j, b is the number of DNA band(s) present in i and not in j and c is the number of DNA band(s) present in j and not in i and D is the distant coefficient. The similarity data matrix was then used for clustering of the genotypes based on unweighted pair clustering group arithmetic average (UPCGMA) by selecting Sequential and Hierarchial Numeric option (SAHN) in clustering. The clustering was used to construct a dendrogram that revealed the relationship between the improved cultivars and Nigerian landraces by selecting the tree plot option in the same software package. Principal Component Analysis (PCA) which underlies the major contributor to the variation observed among the improved cassava.And Nigerian landraces was also carried out using Statistics Analysis System (SAS, Version 8, 1999). A three dimension scatter diagram was plotted for the 36 genotypes using the scores obtained from the first three principal components (i.e. Principal component 1* Principal component 2 * Principal component 3).

Characterization of commonly cultivated cassava in Nigeria with few number of SSR markers

Polymorphic Information Content (PIC) values that revealed the ability of each primer to distinguish the 36 cassava clones were calculated for each of the 16 SSR primers assessed. The formula

Table 2. SSR primers` names, repeat types, and sequences used for the study.

SSR locus	Repeat types	Left primers sequence	Right primer sequence
SSRY 3	$(CA)_{17}$	TTAGCCAGGCCACTGTTCTT	CCAAGAGATTGCACTAGCGA
SSRY 9	$(GT)_{15}$	ACAATTCATCATGAGTCATCAAC	CCGTTATTGTTCCTGGTCCT
SSRY 45	$(CT)_{27}$	TGAAACTGTTTGCAAATTACGA	TCCAGTTCACATGTAGTTGGCT
SSRY 48	$(GA)_{25}$	TGAAAATCTCACTGGCATTATTT	TCATAAAGCTCGTGATTTCCA
SSRY 51	$(CT)_{11}CG(CT)_{11}(CA)_{18}$	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT
SSRY 50	$(CA)_6(N)_6(GA)_{31}$	CCGCTTAACTCCTTGCTGTC	CAAGTGGATGAGCTACGCAA
SSRY 13	$(CT)_{29}$	GCAAGAATTCCACCAGGAAG	CAATGATGGTAAGATGGTGCG
SSRY 66	$(GA)_{19}AAGA$	ATCTCAGCTTCCAACTCTTTCAT	CGAAATGCTTGGAGACAGGTAAG
SSRY 78	$(CT)_{22}$	TGCACACGTTCTGTTTCCAT	ATGCCTCCACGTCCAGATAC
SSRY 100	$(CT)_{17}TT\ (CT)_7$	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG
SSRY 101	$(GCT)_{13}$	GGAGAATACCACCGACAGGA	ACAGCAGCAATCACCATTTC
SSRY 175	$(GA)_{38}$	TGACTAGCAGACACCGGTTTA	GCTTAACAGTCCAATAACGATAAG
SSRY 111	$(GA)_{29}$	GCATCTTACATCCAGAATACTGT	GAAGGAATGCCTGGCTTAAA
SSRY 106	$(CT)_{24}$	GGAAACTGCTTGCACAAAGA	CAGCAAGACCATCACCAGTTT
SSRY 35	$(GT)_3GC(GT)_{11(}GA)_{19}$	GCAGTAAACCATTCCTCCAA	CTGATCAGCAGGATGCATGT
SSRY 230	Not available	Not available	Not available

used for the calculation of the polymorphic information content is PIC = 1 - $\sum n(Pi)^2$, where P is the proportion of number of alleles present in genotypes, and n = the total number of alleles present in a primer. Primers that have PIC value falling between 0.50 to 0.70 were selected. The selected primers' data were analyzed using simple progression combination method of arithmetic (2, 3, 4, and 5), in accordance with increasing PIC values, and analyzed on NTSYS to select the minimum numbers of primers that could be used for genotypic identification of the 36 cassava samples.

RESULTS

Genetic variation between improved cassava and Nigerian landraces

Genetic diversity in cassava was evaluated using thirty-one improved cultivars and 5 Nigerian landraces of cassava with SSR primers. Sixteen polymorphic SSR primers that produced clear and scorable bands were analysed for the study. The number of SSR primer loci detected among the 36 cassava ranged from 2 alleles for primer SSRY 66 to 4 for SSRY 45 with an average of ≅ 2.4 (Table 3). A total of 38 clear and scorable DNA fragments were detected among the 36 cassava cultivars using the 16 SSR primers and used for analysis on NTSYS software package by Rolph, 2000. The similarity matrix coefficient generated by the 38 SSR loci based on the NTSYS analysis ranged from 0.42 to 0.84 coefficients; the dendrogram obtained using unweighted pair clustering group arithmetic average (UPCGMA) analysis method in NTSYS software package revealed 12 distinct DNA cluster groups at 0.70 similarity coefficient (Figure 1, grouping is from right to the left). Nigerian landrace TME 59 formed a distinct group (group 12), TME 7 and TME 2 jointly formed a group (group 11) TME 51 was found among the improved cultivars in group 7 and TME 1 in group 10. Each clone of the 36 cassava was an entity at 0.85 similarity coefficient.

Table 3. SSR primers, their allele numbers, and polymorphic information content.

S/N	Primers	Allele number	PIC values
1	ssry45	4	0.66
2	ssry13	3	0.63
3	ssry100	3	0.62
4	ssry51	3	0.61
5	ssry50	3	0.52
6	ssry101	2	0.44
7	ssry3	2	0.42
8	ssry175	2	0.40
9	ssry230	2	0.39
10	ssry111	2	0.36
11	ssry35	2	0.36
12	ssry78	2	0.35
13	ssry48	2	0.30
14	ssry106	2	0.27
15	ssry9	2	0.21
16	ssry66	2	0.19

The binary data generated from the 36 cassava cultivars were also subjected to Principal Component Analysis (PCA) using SAS. The first three principal components Contri- buted 11.70, 9.23, and 7.95, respectively, 28.88% of the total variation observed among the 36 genotypes of cassava. The plot of the first three principal components scores generated a 3-dimension scatter graph that showed the relationship between the 31 improved lines and the 5 Nigerian landraces (Figure 2).

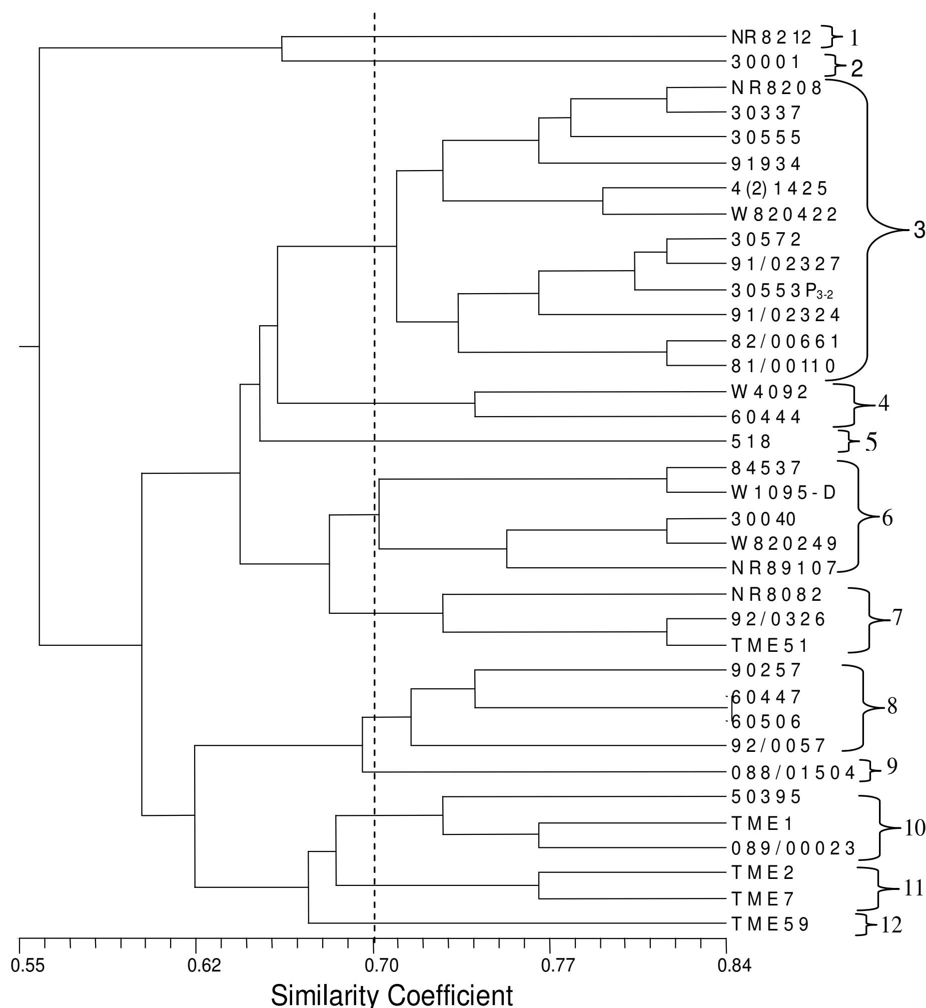

Figure 1. A dendrogram showing the genetic similarity of 36 cassava genotypes based on UPGMA using NTSYS. The basic shape encloses the genotypes in each cluster and also gives the cluster's number from right to left.

Intra-variety genetic diversity

When the binary data from the thirty-one improved genotypes were treated alone in NTSYS, eleven DNA cluster groups were generated among the thirty - one improved cultivars (Figure 3). The closest relatives were clones 60447 and 60506 (similarity coefficient of 0.84) and the most distant relatives were clones 518 and 60444, and 50395 and 84537 (similarity coefficient of 0.42). Similarity coefficient among the five Nigerian landraces ranged from 0.55 to 0.76. There were three groups generated at 0.70 similarity coefficient (Figure 4).

Characterization of commonly cultivated cassava in Nigeria with few number of SSR markers

None of the primer pairs was able to distinguish the 36 clones when treated alone. DNA analysis of each primer's data on NTSYS revealed DNA cluster groups that ranged from 2 for SSRY 66 to 9 groups for SSRY45. Only 5 SSR primers that generated cluster groups within the range of 6 to 9 at 1.00 similarity coefficients were selected for further analysis. The polymorphic information content values of the 16 primers ranged from 0.19 for SSRY66 to 0.66 for SSRY45 with an average of 0.42 (Table 3). The PIC result also revealed the 5 primers as SSR primers with highest PIC values that ranged from 0.52 to 0.66 with an average of 0.61.A minimum of one highly polymorphic SSR primer could detect polymorphisms among the 36 Nigerian cassava varieties and assign them into cluster groups. Analysis of the combinations of data of primers detected a combination of these five polymorphic SSR primers as the minimum number of SSR primers that could distinguish the 36 Nigerian cassavas studied. The 5 SSR primers generated a similarity matrix coefficient that ranged from 0.19 to 0.94 and clustered the 36 cassava into 16 cluster groups at 0.70 simi-

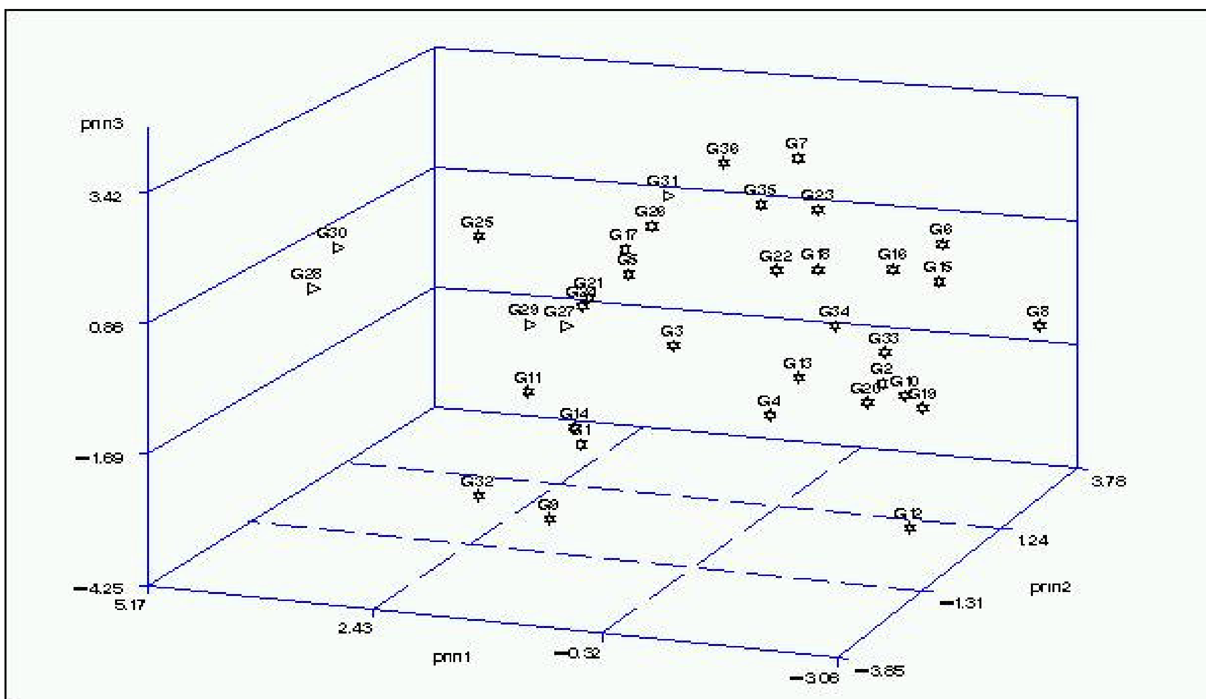

Figure 2. A 3-dimension scatter plot of the 36 cassava genotypes based on the scores of the first three principal components using SAS software package. G1 to G36 represent the names of the according to Table 1. Star shapes are the 31 improved lines and the triangles are the 5 Nigerian landraces.

larity coefficient (Figure 5). All the 36 cassava varieties were distinguished at 0.95 similarity coefficient.

DISCUSSION

Genetic diversity in cassava has been previously studied using DNA molecular primers, isozyme markers (Sarria et al., 1992), RFLP (Angel et al., 1992), RAPD (Tonukari et al., 1997; Ugorji, 1998) and SSR (Fregene et al., 2001), and low or medium genetic diversity has been observed. In the present study, also, there was generally medium genetic diversity between improved cassava cultivars and the commonly grown Nigerian landraces of cassava studied, as shown by the dendrogram. The relationships between the improved cultivars and Nigerian landraces showed by the scatter graph are based on ≈ 29% of the total variation observed in the dendrogram generated by NTSYS. The Nigerian landrace TME 2 and TME 7 were still found to be very close, so also, were the improved cultivars 60447 and 60506 in the scatter diagram (Figures 1 and 2). TME 2 and TME 7 formed a distinct group from all other genotypes including the remaining 3 Nigerian landraces as it was in the dendrogram. Nigerian landraces TME 1, TME 51 and TME 55 were found close to the improved cultivars. Generally, many of the genotypes were clustered together and very few were distinct (NR89107, 4(2)1425, 82/00661 and 90527) in the scatter diagram.

These findings, both the dendrogram and the scatter plot, indicated medium genetic diversity among the improved lines and Nigerian landraces assessed in this study. This might be as a result of a common source of collection (IITA) from which Nigerian farmers choose their common desirable traits of cassava, such as high yielding and resistance to pest and diseases. This result showed that there would be easy formation of hybrids, and introgression of useful genes among the improved cassava cultivars and Nigerian landraces assessed.

When the improved cultivars and the Nigerian landraces are considered separately, the similarity matrix coefficient of the Nigerian landraces ranged from 0.55 to 0.76 and that of the improved cultivars ranged from 0.42 to 0.84. This indicated that the genetic diversity in Nigerian landraces is lower than that observed in improved cassava. This might stem from the fact that the Nigerian landraces were domesticated in the same ecological zones with narrow genetic base while the improved were obtained from different exotic sources that might have diverse ecological ranges. Comparison of the dendrogram produced by RAPD (Tonukari et al., 1997; Ugorji, 1998) and SSR primers in the present study showed consistency only in the large groupings. This lack of consistency between different primer techniques was also observed in cowpea (Chen-Dao et al*.,* 2001). This might be due to the fact that different primer techniques detect different components of DNA variation when sub-

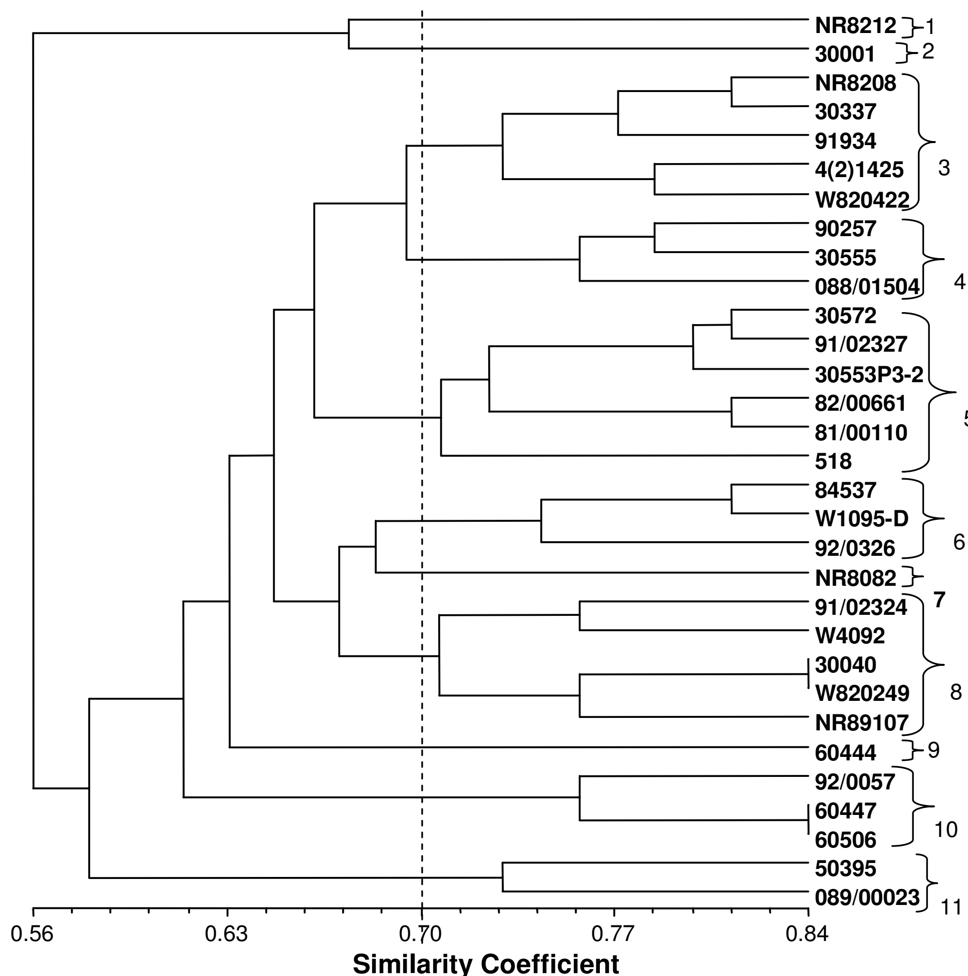

Figure 3. A dendrogram showing the genetic diversity among the thirty-one improved cassava cultivars based on NTSYS analysis.

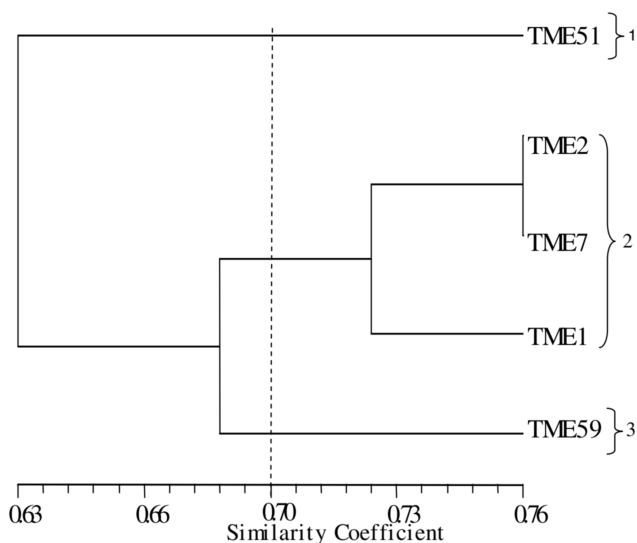

Figure 4. A dendrogram showing the genetic diversity among the five Nigerian landraces.

jected to different evolutionary mechanisms.

SSR primers have shown high levels of polymorphism in many important crops including *Oryza sativa* L. (Bligh et al., 1999), *Sorghum bicolor* (Smith et al., 2000), *Vitis vinifera*, (Di Gaspero et al., 2000), *Vigna unguiculata* L.,(Chen-Dao et al., 2001), *Prunus pandorea* and *P. amygdalus* (Ajay et al., 1999), *Triticum aestivum* L. (Ahmad, 2002), *Helianthus annuus* L., (Yu et al., 2000), *Cucumis melon* L. (Danin-Poleg et al., 2001). SSR primers were also polymorphic in the Nigerian cassava cultivars assessed in this study. The results of this study showed that each of the 16 primers detected polymorphisms among the 36 cassava studied. Eleven of these primers were SSR markers that have been mapped in cassava genome (Fregene et al., 2003; Okogbenin et al., 2006). The results also revealed a combination of 5 highly polymorphic SSR primers that detected more polymorphisms in the 36 cassava studied than did the combination of the 16 primers. The higher number of cluster groups generated by the 5 highly polymorphic

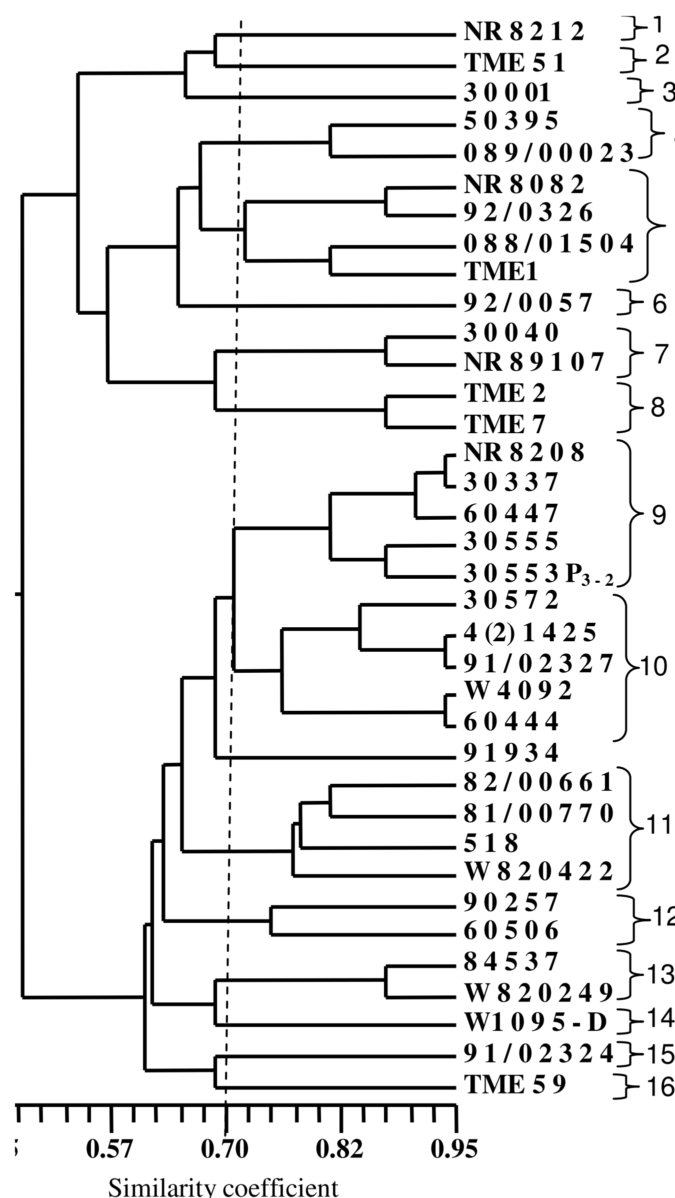

Figure 5. A dendrogram showing the characterization of the Nigerian cultivated cassava by 5 highly polymorphic SSR markers.

SSR primers was due to the fact that a large number of less polymorphic SSR primers were added to the 5 primers to make up the 16 primers. This reduced their overall polymorphism information content with an average of 0.42, as compared to an average of 0.61 obtained from the combination of the 5 primers. This study also revealed that the 5 highly polymorphic SSR primers, apart from generating more cluster groups among the Nigerian cassava, were able to distinguish all the 36 cultivated cassava at 0.94 similarity coefficient. However, no duplicate was detected by either of the combinations of 5 and 16 SSR primers. Each clone of the 36 cassava was an entity at 0.95 and 0.84 similarity coefficients using combinations of 5 and 16 SSR primers. This indicated that each of the 36 Nigerian cassava cultivars is complete genotype on its own, but with some good similarities.

Results of this study, thereby, established a collection of these 5 highly polymorphic SSR primers, SRY51, SSRY45, SSRY50, SSRY100 and SRRY13, that could readily be used for genotype identification and genetic diversity studies in Nigerian cultivated cassava following the earlier proposition of Ahmad (2002). Three of these SSR markers, SSRY 100, SSRY 51, and SSRY 50 were located at L, N and B, respectively, on genetic linkage map of cassava (Fregene et al., 2003). Therefore, application of few number of highly polymorphic SSR markers is possible for genetic variation studies in cassava. This reduces the stress of applying many SSR primers for the identification of cassava cultivars in Nigeria and, hence, saves time, ambiguous data, and also cuts the cost of research studies for genotype identification and genetic diversity studies.

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