MOLECULAR CHARACTERIZATION OF IMPROVED CASSAVA CULTIVARS AND COMMONLY GROWN NIGERIAN LANDRACES USING SIMPLE SEQUENCE REPEAT MARKERS

BY

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ABSTRACT

Cassava is a starchy staple food of tropical Africa whose yield is affected by several biotic stresses. Improved cassava cultivars that are resistant to biotic stresses were developed to boost cassava production. Also, some Nigerian landraces of cassava that are resistant to some of these stresses have been discovered. Simple sequence repeats (SSR) are molecular markers with high discriminatory power and technical and analytical simplicity. SSR markers have been applied successfully to crops such as rice, cowpea, sorghum, and sunflower but scarcely used on cassava. Based on this, the study evaluated the genetic diversity between improved cassava cultivars and commonly grown Nigerian landraces using simple sequence repeat (SSR) markers and also determined the SSR markers that could readily be used for genotype identification of cultivated cassava in Nigeria.

For the evaluation of genetic diversity, 31 improved cultivars and 5 Nigerian landraces of cassava were assessed at genomic deoxyribonucleic acid (DNA) level with SSR markers. Polymerase chain reaction (PCR) amplification of the genomic DNA of the cultivars were carried out with 16 polymorphic SSR primers. A total of 38 distinct and scorable DNA bands generated were used for data analysis by Numerical Taxonomy and Multivariate Analysis System (NTSYS). Principal component analysis, which revealed the major underlying sources of variation, was also carried out using Statistical Analysis System

(SAS) For genotype identification study, 16 SSR markers were assessed using 36 genotypes of cassava. Data from each primer were analyzed by NTSYS and primers that generated between 6 and 9 cluster groups at 0.70 similarity coefficients were selected. Combinations of data from selected primers were also analyzed by NTSYS to select minimum number of SSR markers for genotype identification of cultivated cassava in Nigeria.

The results of genetic diversity study identified 12 distinct DNA cluster groups at 0.70 similarity coefficient, the similarity indices ranged from 0.42 to 0.84. The closest genetic relationship between improved cultivars and Nigerian landraces was observed at 0.82 similarity coefficient, while the most distant relationship was at 0.55 similarity coefficient. Ten principal components that contributed 70.59 % of the variation observed among the cassava cultivars were revealed. The first and tenth principal components contributed 11.70 and 4.03 % of the variance of genetic distance, respectively. The results of the determination of SSR markers for genotype identification study revealed five polymorphic SSR markers that could readily be used for genotype identification of cultivated cassava, because they were able to distinguish the 36 cassava genotypes at 0.95 similarity coefficient. Furthermore, the results of this study revealed that SSR primers that amplified DNA from improved cassava successfully did so in Nigerian landraces.

SSR markers detected polymorphisms among improved cultivars and Nigerian landraces of cassava and are therefore ideal molecular tools for genetic and genotype identification of cultivated cassava in Nigeria that could be exploited in cassava breeding programs

Key words: Genetic diversity, SSR markers, Improved cassava cultivars, Landraces, *Manihot esculenta* Crantz.

CERTIFICATION

Oluwasayo Kehinde **MOYIB** truly carried out this project work at IITA, Ibadan

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DEDICATION

To Almighty Allah and all good Muslims all over the world who gave out of what they had to the less privileged

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I thank almighty Allah for his protection, guidance, and provision throughout this program He made this dream a reality

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CHAPTER ONE

INTRODUCTION

There is need to achieve food self-sufficiency in order to ensure the overall security of Africa The crops to be targeted to solve this problem of food inadequacy should be staple crops that sustain local culture and communities such as rice, sorghum, maize, potatoes, tomatoes and tropical fruits, beans, yam, cassava and plantain Cassava, a root crop, is a crop of choice because it plays an important role in food security, particularly in drought- prone areas and during the times of famine.

Food and Agriculture Organization (FAO, 1997) reported that cassava accounts for over 60% of the daily calorie intake of some 500 million people in the sub-Saharan Africa. Nores (1992) ranked cassava among the ten most important crops in the developing world and the most important crop in sub-Saharan Africa, while Nweke (2001) classified it as the second most important food in Africa.

In contrast to many other crops that are highly vulnerable to environmental stresses during the critical stages of plant development, cassava has the advantages of being well adapted to a wide range of environmental stresses and growing very well in a less fertile soil and these make cassava a crop of choice for many farmers in Africa.

Cassava, *Manihot esculenta* Crantz is an important starchy staple of the low tropics Its storage roots are a valuable source of energy calories and the main nutritional content is carbohydrate It has a small amount of protein, ranging from one to two percent on a fresh weight basis. It is rich in vitamin C and calcium, with acceptable levels of vitamin A and B and other nutrients, but deficient in essential amino acids such as methionine and tryptophan (Bomierbale *et al.* 1997)

In Nigeria, it is the main source of energy-rich food The root is processed and prepared as gari and to a lesser extent as *fufu*, for home consumption and sale in local markets Leaves and tendrils contain much higher protein content and some people consume them as a vegetable (Fregene, 1996) Also, a sweet variety of cassava is boiled and eaten as *goro* with pepper sauce in Northern Nigeria Cassava is also used as an animal feed and as starch locally

In Ghana, cassava is cultivated as a reserve crop against lean periods, because it can survive all weather conditions It is the last resort for food, when there is a severe shortage of other food crops Several food items are produced from cassava in this country; examples are *fufu*, cassava flour (*kenkey*), bread, *kuri*, *tapioca*, cassava dough, starch, and biscuit (Kumah, 2001)

Cassava also finds uses in industries for example, as a component in the manufacture of drugs, a binding material in construction, and as a raw material in the distillation of alcoholic beverages, and starch in textiles industries (Kulata, 2001). Dung (2001) reported that VEDAN-VIETNAM, a company in Vietnam collected and processed cassava at a large scale for many industrial uses such as paper, textiles, manufacture of adhesive, for oil drilling, making different chemicals such as glutamic acid, lysine and modified starch. Derived sugar products from cassava are mannitol, glucose, fructose and maltose syrup. By products of cassava used in industries include ethanol glucose paste and glue (Mataya *et al*., 2001).

Recent economic advancement has turned cassava into a cash crop, since several items are processed from it in some part of Africa for various uses (Benesi *et al*., 2001). These products could be sold to interested industries or exported for income generation. In fact, Benesi *et al*. (2001) reported that many industries in Malawi are showing interest in the use of cassava but the limitation is in the quality of cassava needed for flour, starch, and glucose making. Also, they need to be assured of the regular supply of cassava throughout the year. Many countries, therefore, stand a chance of improving their economy through cassava if the constraints to its production are addressed and solved.

There are many constraints to cassava production in Africa, which limit its role as a food security crop and also as a cash crop (i) It is produced by resource poor farmers. (ii) it is also being neglected by many African governments and international donor agencies, because of the myth about its nutritional value. Cassava was categorized as one of undeserved group of minor crops with negligible support for its research and development (Nores, 1992). (iii) Diseases, pests, weeds, poor soils, and agronomic and socio-economic factors affect its yield. (iv) The roots of some cassava species have a high content of cyanogenic glycosides, which necessitate extensive processing before cassava is edible (Ugoji, 1998). All these constraints, together with post-harvest deterioration are major targets for the cassava breeding scheme.

The best solution to problems of increasing cassava production so it can serve as the main food security crop and also as a cash crop in Africa and developing countries is by increasing yield per hectare through a combination of improved farming systems and the development of better varieties to boost production through resistance to diseases, pests, and drought. Successful use of biotechnology for plant propagation and breeding can dramatically raise cassava production.

So, a need for cultivar selection becomes paramount. The major goal of selection in breeding is the identification of desirable genotypes. Effective selection in breeding is based upon the existence of genetic variability in the gene pools accessible to cassava breeders.

The extent of genetic variability depends on the germplasm included in it. However success in cassava improvement is highly reliant on the efficient manipulation of the genetic variability in its gene pool. The larger the genetic variability within the cassava gene pool, the higher the number of new varieties that can be produced and the lower the susceptibility of new varieties to diseases and pests prevalent in the targeted areas.

The genetic resource of cassava consists of landraces, improved varieties, mutants, and related wild species (Ng, 1995; Bonierbale *et al.*, 1997). Many researchers have proved that these relatives of cassava are sources of resistance to some of the biotic stresses of cassava. These useful traits could be an additional variation to enlarge the existing cassava gene pool. Chavez (1990) reported the potential of wild relatives of cassava as sources of genes resistant to certain pests and diseases, and tolerant to the most common stresses. They provide high genetic variability for important biochemical characteristics such as low hydrocyanic acid content and high protein content.

Improved varieties of cassava have been developed based on some of these stresses and they exhibit low hydrocyanogen content, high yielding capabilities, high carotene content (yellow roots), high carbohydrate levels for starch production, resistance to cassava mosaic virus and other pests and diseases. For example, scientists at the International Institute of Tropical Agriculture (IITA) developed high yielding Tropical Manihot Species (TMS) varieties that have transformed cassava from a low yielding famine reserve crop to a high yielding cash crop for both rural and urban consumers (Nweke, 2001).

Several improved genotypes and their seed populations have been developed for a range of ecologies that include wetland, valleys, moist and dry savannas, and the mid altitudes. These incorporate multiple pest and disease resistance, desired traits of early vigor in plant growth for high foliage yield, appropriate plant horticulture and early bulking of storage roots, with high dry matter nd carotene content, low cyanide content, ease of peeling, acceptable root shape and food quality (Dixon *et al.*, 2001). The improved cassava varieties are useful in sub-Saharan Africa, as a major source of income from industrial uses and in the animal feed sector as well as many non-traditional food uses (Dixon *et al.*, 2001).

Cassava landraces have been shown to have specific environmental adaptivity that could be used to boost cassava production. Recently, significant advances have been made in broadening the genetic base of cassava and diversifying resistance to the major biotic and abiotic stresses in sub-Saharan Africa. One of these has been through the introgression of Latin American landraces with African landraces to confer resistance or reduce the susceptibility of cassava germplasms to cassava mosaic disease. Currently, several Nigerian landraces have been identified that show extreme resistance to cassava mosaic disease (Akano *et al.*, 2001). It was also reported that the additional variability of the previously untapped African landraces has 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).

A prerequisite for any genetic improvement program of cassava is the knowledge of the extent of genetic variation present within *Manihot* species, and also, the genetic distance between cassava and closely related species. These are important in the identification of close relatives of cassava, from which good hybrids could be produced, and through which introgression of desirable traits into cassava is possible. These are useful in broadening the genetic base of cassava to produce new stable varieties and hence increase cassava production to perform its two targeted roles, food security and cash crop.

Objectives of the project

International Institute of Tropical Agriculture (IITA), Ibadan, released some improved cultivars of cassava that are high yielding, resistant to diseases and pests and with low cyanogen content, to Nigerian farmers in 2000, through national programs. 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. Molecular marker(s) is / are therefore needed to identify the cassava genotypes and also to assess the genetic diversity between the improved cultivars and Nigerian landraces.

Specific objectives

1. To evaluate the genetic diversity among improved cassava cultivars and commonly grown Nigerian landraces using Simple Sequence Repeat (SSR) markers, and
2. To determine SSR marker(s) that could be used for genotype identification of cultivated cassava in Nigeria.

CHAPTER TWO

LITERATURE REVIEW

2. 1. Manihot species and its taxonomy

Manihot species are members of the family *Euphorbiacea*, sub-family *Crotonoidae* and tribe *Manihot*. They occur as herbs, shrubs, and trees, among which production of latex and cyanogenic glucosides occurs. Rogers and Appan (1973) reported that Bauhis in 1651 was the first European botanist to describe a plant from the genus *Manihot*, from studies of a species collected in Brazil and that in 1753, Linnaeus placed the only species of *Manihot* known at that time in the genus *Jatropha* and designated it *Jatropha manihot*. In 1753, Adanson (quoted in Rogers and Appan, 1973) recognized and described *Manihot* as a distinct genus while Crantz in 1776 (Rogers and Appan, 1973) provided the first published record of the cultigen, *M. esculenta*. There remained considerable confusion over the classification of cassava cultivars for a long time. Classification based on the level of HCN concentration divided the cultivars into high HCN content types, i.e., bitter cassava and types with low HCN content, i.e., sweet cassava.

The most elaborate classification in the genus *Manihot* today is the description of 98 species, grouped into 19 sections and the separation of one species into a new genus called *manihotoides* based on 44 descriptors from plant morphology and growth habit traits by Rogers and Appan, (1973). The genus *Manihot* occurs naturally only in the

Western hemisphere between the south west United States of America (33 ^{0}S) and Argentina (33 ^{0}S) (Jennings, 1995). Earliest workers believed that Latin America was the most likely area of origin of cassava, because of the immense variability found in many cassava cultivars in Latin America. The area is home to the largest forms of cassava landraces and *Manihot* species. The presence of members in Africa and Asia can be attributed to introductions through international programs beginning from the 16th century (Fregene, 1996). Cassava, *Manihot esculenta Crantz*, includes all known cultivars. Common names are *yucca* (Spanish), *manioc* (French), *mandioca* (Portuguese), and cassava (English) (Bormiebale *et al.*, 1997).

Early domestication of cassava began with selection for large roots, more erect plant type with less branched growth, and the ability to establish easily from stem cuttings. Current selections for cassava are high yielding varieties with resistance to cassava mosaic disease (CMD), cassava bacteria blight (CBB), cassava mealy bug (CBB), cassava green mites (CGM), and cassava anthracnose disease (CAD). Yellow root flesh that has high carotene content and low cyanogenic potential, good *gari* (grated, fermented, and roasted), fresh cassava quality, and good pounding quality are also needed. All these are included in the improvement scheme for cassava.

2.2 Molecular genetic diversity studies in cassava

Genetic diversity studies, either in understanding the inter-relationship in *Manihot* species mapping the genome, or cloning specific genes, are necessary and important for any genetic improvement scheme of cassava. The major interest lies in developing and adopting efficient methods for assessing diversity that can be used for the definition of the representative subset of *Manihot* germplasm for conservation and utilization. Bonierbale *et al*., (1997) reported that further efforts in the study and use of genetic diversity are needed in order to confirm possible new sources of resistance from close relatives of species for a breeding program. Knowledge of genetic diversity in cassava is needed for its genetic improvement program, to confirm possible new sources of resistance to diseases, pests, and other ecological factors that constrain its productivity.

Genetic diversity studies in *Manihot* species were very scanty in the past decades. Among the few were the studies by Carvalho *et al*., (1992), that assessed polymorphism in *Manihot esculenta* Crantz using mitochondria DNA via recombinant DNA (Restriction Fragment Length Polymorphism, RFLP) and also nuclear DNA via Polymerase Chain Reaction (PCR). The results established the species relationship and distinguished particular individuals in a germplasm bank. Angel *et al*., (1992) also carried out polymorphism studies on cassava using genomic DNA based on RFLP and Random Amplified Polymorphic DNA (RAPD). low genetic diversity was observed among the cultivated cassava used.

Fregene (1996) first elucidated the phylogeny and taxonomy of the genus *Manihot* using RFLP as a first step towards the development of cassava breeding program. He suggested that cassava originated from the domestication of some tuberous accessions of wild *Manihot* species closely related to cassava. The most likely wild progenitor is *M. esculenta* subspp. *flabellifolia*, *M. tristis* and *M. irwinii*.

There has been great improvement in the study of genetic diversity in cassava since the elucidation of the phylogeny of cassava by Fregene (1996). Tonukari et al. (1997) studied the genetic diversity in cassava using RAPD, also Fregene *et al.*, (2000) did a study that assessed genetic differentiation in cassava landraces from Tanzania, Nigeria, Brazil, Colombia, Guatemala, Mexico, Peru, Argentina, and Venezuela using Simple Sequence Repeat (SSR) markers. Low level of genetic differentiation was observed overall in cassava landraces studied.

The focus of study in cassava now is to improve the resistance of cassava to diseases and pests (Dixon *et al.*, 2001). Resistance is being sought in closely related species of cassava. This has led to the genetic diversity studies among cassava cultivars and related species such as landraces, improved cultivars, wild species, and so on (Bonierbale *et al.*, 1997). In recent times some Nigerian landraces were discovered to be very resistant to cassava mosaic disease. Virtually, no work has been done on the genetic diversity among Nigerian landraces and improved cultivars of cassava. A study on this genetic diversity would be useful in the hybridization and introgression of useful genes into cassava germplasm.

2.3 Molecular markers tools used in genetic diversity studies

Genetic diversity study was formerly based on morphological characteristics cytogenic description, natural occurrence of inter-specific hybrids, facilitated gene exchange among species of *Manihot*, as well as the categorization of individuals based on computer-aided analysis (Rogers and Appan, 1973). However, further research on the characterization of the cassava gene pool is needed. The advance in the development of methods using DNA polymorphisms as molecular markers is having a significant impact on studies such as species evolution, molecular taxonomy, genetic diagnosis as well as breeding (Carvalho *et al*., 1992).

DNA molecular markers are of two types, PCR-based and non-PCR-based. An example of non PCR-based marker is Restricted Fragment Length Polymorphism (RFLP) (Beckmann and Sollers, 1983), which involves the use of southern blotting and hence is time consuming, and technically difficult to use in species with a large genome. Analysis is also very costly. pCR-based molecular markers are currently in use now, because of their advantages over non-PCR-based markers. Few of the advantages are as follws: they require a small quantity of DNA and a short time for experiment, and hence they are the most commonly used for crops. Reagents used for the amplification of DNA using PCR technique include the following, PCR buffer (20 mM Tris HCl (pH 9.0) 25 mM KCl), 50uM deoxynucloside triphosphates (dNTPs), 1.5M $MgCl_2$, 2 units of *Thermus aquaticus* polymerase, double deionized water, and sample DNA.

Most commonly used PCR based markers that have been developed over the past few years and are available for studies of genetic diversity in plants include Random Amplified Polymorphic DNA (RAPD) (Williams *et al.* 1990) otherwise known as Arbitrarily Primed PCR (AP-PCR, Welsh *et al.*, 1991) Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.*, 1995), and Microsatellites / Simple Sequence Repeat (SSR) markers (Tautz, 1989) Simple Sequence Repeat (SSR) markers have been confirmed to be the most informative and appropriate for cassava (Mba et *al.*, 2000) Perara *et al* (2000) also supported SSR markers as the most informative for plants. Their main advantage is that they are highly variable dominant loci and therefore are ideal tools for many genetic applications.

2.4. Simple Sequence Repeat / Microsatellite Markers

Microsatellites markers / Simple Sequence Repeats (SSRs), also called Simple Tandem Repeats (STRs) (Tautz, 1989), consist of tandem repeats of relatively short DNA sequences (1-7 bases long) that are scattered throughout the genome of *eukaryotes* cells and make up 10 to 80% of genome content (Perera *et al.*, 2000) Because of the simplicity of assay of co-dominance, and strong discriminatory power due to high allelic diversity, SSR markers can provide information more easily than previous DNA-based genetic markers such as RFLP and RAPD

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 10Kb[3] Microsatellites are becoming popular tools in molecular genetic analysis Microsatellite length polymorphism has been applied to forensic analysis (Khasa *et al* 2000), the analysis of genetic structure, parentage, and gene flow of plant and animal populations (Kent *et al*., 2000).

Genetic analysis using microsatellites involves PCR amplification of DNA using oligonucleotide primers complementary to a flanking region of a given microsatellite locus, size fractionation of the amplified product by polyacrylamide gel electrophoresis (PAGE) or metaphor agarose electrophoresis, and detection of DNA fragments on the gel through staining or via automated systems The interpretation of the single-locus marker by one of the numerous statistical analyses (e g , Nei unbiased genetics H, 1987 Genepop v and NTSYS v 2.0) is useful in studies of population genetics (Perera *et al*, 2000), and for map construction (Kent *et al*, 2000) Current research has suggested that the length variation between alleles on SSR locus are created by slippage of DNA polymerase during the replication of the tandem repeats followed by a failure of DNA mismatch repair enzymes to restore the original recurrence

Ahmad (2002) assessed the genomic diversity among wheat (*Triticum aestivum L.*) based on Simple Sequence Repeats and detected a high level of polymorphism among the cultivars used. Yu *et al*, (2002) studied allelic diversity among elite inbred lines of cultivated sunflower (*Helianthus annuus L.*) using SSR markers. Ehlers *et al* (2001) determined the genetic similarities and relationships among cowpea breeding lines and cultivars using simple sequence repeat markers. Also, Smith *et al* (2000)

was able to detect genetic diversity among elite sorghum inbred lines with SSR markers

SSR markers have been shown by many scientists to detect more polymorphism than other markers in some crops. Bligh *et al*., (1999) in their study detected more polymorphism in long grain rice cultivars using simple sequence length polymorphism than amplified fragment length polymorphism (AFLP produced 375 polymorphic products with a mean number of 15 polymorpbic bands per primer, the mean number of polymorphic products obtained per inter-SSR PCR was 18.7 per primer) Also, Joobeur *et al*., (2000) used 54 RAPD markers and 6 SSR markers on a molecular marker map with 120 RFLP and 7 Isozyme, and created more groups with SSR than with RAPD.

Ajay *et al* (1999) in their study on the evaluation of genetic diversity and genome fingerprinting of *Pandorea* (Bignoniacea) *pandorana* using RAPD and inter-SSR PCR, indicated that inter-SSRs revealed higher genetic variation among the cultivars of *P. pandorana* than RAPD. They concluded that SSR markers appeared to be optimal for mapping and map transference to different populations, due to characteristics of co-dominance and a high level of polymorphism

Mba *et al* (2000) cloned and developed 172 new SSR markers for the cassava genome and placed 36 of these markers on the existing RFLP frame work map of cassava studied by Fregene (1996), this led to the joining of a few small groups and the

creation of one new group. He reported that the abundance of allelic bridges as shown by SSR markers would lead to the development of a consensus map of the male and female - derived linkage bridges, 30% as against 10% for RFLP, in cassava.

SSR markers have also found applications in human genetics. Brown *et al.* (1996) reported that 100 SSR loci were mapped in the human genome by Hudson and coworkers. They also reported that a novel mechanism for the amplification of tri-nucleotide SSR sequences, either within or proximal to hereditary disease genes, seems to be the root cause of these genetic abnormalities found in *Kennedy's disease* and *Huntington's disease*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant samples

This research work was sponsored and carried out at IITA-Ibadan.

Thirty-one improved cassava cultivars and 5 Nigerian landraces were selected randomly from IITA collections on the field. The names of the cultivars and their pedigrees are listed in Table 1. The stems of the plants were collected and labeled 1-36. and were grouped into two, improved cultivars (IC) and Nigerian landraces (LR) (Table 1). The plants were grown from stem cuttings in two rows in a bed. Plant spacing was 1metre by 1 metre.

The young leaflets were collected in an ice bucket, 8 days after planting, 0.1 gram of each sample was quickly weighed, transferred into eppendorf tubes and stored at -80 °C for further usage.

Table 1. The list of 36 cassava genotypes used for genetic diversity and genotype identification studies

Cassava genotypes	Pedigree	Cassava genotypes	Pedigree
1. NR8212 (IC)	Unknown	20. 30337 (IC)	58308 OP
2. NR8208 (IC)	Unknown	21. 920057 (IC)	30555 • TME 1⅓ sib
3. 90257 (IC)	58308 *Oyarugbo dudu	22. W1095.D (IC)	Parent stock 1971
4. 91934 (IC)	58308 • Oyarugbo dudu	23. W8202.49 (IC)	58308*Branca de Santa Catarina
5. 30001 (IC)	Lost pedigree	24. 088401304 (IC)	Nwikka-2 OP
6. 30572 (IC)	58308*Branca de Santa Catarina	25. NR89107 (IC)	Unknown
7. 84537 (IC)	58308 * Oyarugbo dudu	26. W1092 (IC)	58308*Branca de Santa Catarina
8. 920061 (IC)	58308 • branca de Santa Catarina	27. TME 51	Landrace
9. 50395 (IC)	58308 • 58198	28. TME 2 (LR)	Landrace
10. 81/00110 (IC)	58308*Branca de Santa Catarina	29. TME 1 (LR)	Landrace
11. NR8082 (IC)	Unknown	30. TME 7 (LR)	Landrace
12. 4(2)1425 (IC)	58308 • Oyarugbo dudu	31. TME 59 (LR)	Landrace
13. 30555 (IC) 14. 920326 (IC)	58308 • Oyarugbo dudu 91934 • TME 1	32. 089/00023	58308*Branca de Santa Catarina •very low cyanide population
15. 30555[F1-2](IC)	58308 • Oyarugbo dudu	33. 518 (IC)	Unknown
16. 91/02327 (IC)	TME 1 X sibling	34. 60444 (IC)	Nigeria ex Moor plantation
17. 30004(IC)	58308 • Oyarugbo dudu	35. 60447 (IC)	Nigeria ex Moor plantation
18. 91/02324 (IC	58308* branca de Santa Catarina	36. 60502,	Nigeria ex Moor plantation
19. W8204.22 (IC)	58308 • branca de Santa Catarina		

IC - Improved Cultivars and

LR-Landraces

3.2 DNA extraction

PRINCIPLE:

The basic steps in DNA extraction include: Breaking open the cell (wall) to release the nucleus. The nucleus (if present) is then opened to release the DNA. The DNA should however be protected from enzymes that causes degradation by using proteinases. The released DNA is then precipitated in alcohol. Cell walls are opened up using detergent and salt solutions. Centrifugation is then used to break down the cell walls, cell membranes, and nuclear membranes using a centrifuge or a blender. DNA is then selectively precipitated using ethanol, air-dried, and finally resuspend in water to solubilize it and is ready for use as stock.

PROCEDURE:

Total genomic DNA was extracted from fresh and young leaves using DNeasy mini plant kit purchased from Qiagen International. The kit contains the following reagents, lysis buffer, precipitant buffer, binding buffer, washing buffer, elution buffer, and RNAse A (names and composition of the buffers are listed in appendix A). The plant material was mechanically disrupted in liquid nitrogen and then lysed with the lyses buffer, AP1 that had been previously mixed with RNAse and incubated at 65°C. After lyses, proteins and polysaccharides were salt precipitated using buffer AP2. Cell debris and precipitates were removed in a single step by a brief spin through a shredder, a unique filtration and homogenization unit, supplied. The lysate was transferred to a new tube, binding buffer, AP3, previously mixed with ethanol was added to promote binding of DNA to the DNeasy membrane, supplied. The sample was then applied to a

DNeasy spin column and spun briefly in a microcentrifuge. Contaminants such as proteins and polysaccharides were removed from bond DNA by two washing steps using buffer AW. Pure DNA was eluted in a small volume of low salt buffer AE that had been previously mixed with ethanol and centrifuged. The final DNA was dissolved in Tris EDTA (TE) buffer (10 mM Tris HCl and 1mM EDTA) and made into a concentration of 20ng/ul.

Qiagen mini plant DNA extraction protocol

The weighed young leaves were ground in liquid nitrogen into fine powder. The DNA was quickly extracted using Qiagen mini plant kit, according to its protocol as follows

1. Cell was lysed by addition of 400 µl of buffer AP1 and 4 µl of RNAse A stock solution (100 mg/ml) to 100 mg of ground net weight plant and vortexed vigorously.

2. The mixture was incubated for 10 min at 65^{0}C, mixed 2-3 times, during incubation by inverting the tubes.

3. 100 ul of buffer AP2 was added to the lysate, mixed, and incubated for 5 min on ice.

4. The lysate was applied to the QIA shredder spin column sitting in a 2 ml collection tube and centrifuged for 2 min at 14000 r.p.m.

5. The flow through was transferred to a new tube and 1.5 volume of buffer AP3/E was added and mixed by pipeting.

6. 650 ul of the mixture from above was applied to the DNeasy spin column and centrifuged for 1min at 8000 rpm and the flow through was discarded. This step was carried out twice.

7. 500 ul of Buffer AW was added to the DNeasy Column and centrifuged for 1 min at 8000 rpm.

8 500 ul of Buffer AW was again added to the DNeasy spin column and centrifuged for 2 min at 14000 rpm

9 The DNeasy column was transferred to a 1.5 ml microcentrifuge tube and 100 ul of preheated Buffer AE at 65 ^{0}C was added and incubated for 5 min at room temperature and then centrifuged for 1min at 8000 rpm to elute the DNA

A second elution was performed as in (9) above. the DNA samples were air dried for 1 hr

3.2.1. DNA quantification

The DNA of each plant sample was quantified using Hoechst dye33258 with Flourometer TD-700 Rutner Designs

PRINCIPLE

Hoechst 33258 is a class of bis-benzemididazole fluorescent dye, which binds non-intercalatively with high specificity into the minor groove of double stranded DNA. Binding of Hoechst dye to DNA increases the fluorescent yield from 0.01 to 0.60. The dye alone absorbs light maximally at 354nm and emits maximally at 492nm, when bound, it absorbs maximally at 365nm and emits at 458nm

PROCEDURE

Three standards (500 ng/ul, 250 ng/ul and 100 ng/ul) of DNA solutions were prepared from the calf thymus DNA stock supplied and also a blank containing only x1TNE. The machine was calibrated by putting in the highest concentration followed by a lower and the least respectively and a blank last of all. Readings of the DNA samples

were taken after completion of calibration and results were printed out. The genotypes and their DNA concentrations are listed in appendix C.

3.2.2. DNA quality

DNA quality was checked by running the DNA samples on 1% agarose gel. in 0.5M TBE buffer at 100Volts for 2 hours. Visualization of the DNA bands was done by staining in ethidium bromide and destaining in distilled water and the photograph was taken. Smearing or Discrete bands were checked for. Smearing indicated the presence of shredded DNA.

3.3. Primer screening test

SSR Primers from *Manihot esculenta* Crantz cloned by Mba R.E.C and purchased from Reseach Genetics USA were used. Their names, repeat types, repeat number and right and left sequences and sizes are listed in Table 2. A total number of 65 SSR primers and their reverse were randomly selected and used for amplification of genonuc DNA of 8 samples, randomly selected from the 36 genotypes

3.4 Polymerase Chain Reaction amplification of DNA from the 36 cassava cultivars

PRINCIPLE

Polymerase chain reaction (PCR) is a method of nucleic acid synthesis by which a particular DNA can be specifically replicated. It involves two oligonucleotide primers that flank the DNA fragment to be amplified and repeated cycles of heat denaturation

of the DNA, annealing of the primers to their complementary sequences and extension of the annealed primers with DNA polymerase. The enzyme for DNA synthesis is obtained from a bacterium, *Thermus aquaticus* and hence named *Taq polymerase*. It can survive a very high temperature which is needed for DNA synthesis. The primers hybridize to the opposite strands of the target sequence and are oriented so that DNA synthesis by the polymerase proceeds across the region between the primers. The extension products themselves are also complementary to and capable of binding primers. Successive cycles of amplification essentially double the amount of the target DNA synthesized in the previous cycle. The result is an exponential accumulation of the specific target fragments.

PROCEDURE

Twenty-two microsatellite markers that showed polymorphism from primer screening tests were used. Their names, repeats and numbers are listed in Table 2. The PCR reactions were carried out in a Themolyne Amplitron II Thermocycler. It involved three steps that were repeated a number of times (26 cycles)

1. DNA denaturation: The template DNA was dissociated into single strand by heating the sample at 92 ^{0}C briefly

2. Primer annealing: The temperature was lowered to 55 ^{0}C. to allow binding of the primers to the complementary sequence on single DNA

3. Primer extension: The temperature was increased to 72 ^{0}C. The three ends of the oligonucleotide primers were extended towards each other with the newly synthesized DNA

The PCR program used was SSRTD-55 and consisted of the following profiles

Step1 94 ^{0}C - 5.00 min denaturing

Step2 94 ^{0}C - 0.45 min

Step3 65 ^{0}C - 1.00 min annealing

Step 4 72 ^{0}C - 1.00 min Extension (repeat steps 2 to 4 (10 times with progressive decreases in temperature from 65 ^{0}C to 55 ^{0}C at the rate of 0.5 ^{0}C per cycle)

Step 6 94 ^{0}C - 0.45 min

Step 7 55 ^{0}C - 1.00 min

Step 8 72 ^{0}C - 1.00 min repeat steps 6 to 8, 26 times and

Keep at 4 ^{0}C for for futher usage

Table 2. List of SSR markers, repeat types and primers used for the study

SSR LOCUS	REPEAT TYPES	LEFT PRIMERS	RIGHT PRIMER
SSRY 3	$(CA)_{17}$	TTAGCCAGGCCACTGTTCTT	CCAAGAGATTGCACTAGCGA
SSRY 9	$(GT)_{15}$	ACAATTCATCATGAGTCATC AACT	CCGTTATTGTTCCTGGTCCT
SSRY 45	$(CT)_{27}$	TGAAACTGTTTGCAAATTAC GA	TCCAGTTCACATGTAGTTGGCT
SSRY 48	$(GA)_{24}$	TGAAAATCTCACTGGCATTA TTT	TCATAAAGCTCGTGATTTCCA
SSRY 51	$(CT)_{11}CG(CT)_{11}(CA)_{16}$	AGGTTGGATGCTTGAAGCAA	GGATGCAGGAGTGCTCAACT
SSRY 50	$(CA)_{16}(N)_4(GA)_{31}$	CCGCTTAACTCCTTGCTGTC	CAAGTGGATGAGCTACGCAA
SSRY 13	$(CT)_{29}$	GCAAGAATTCCACCAGGAAG ATCTCAGCTTCCAACTCTT	CAATGATGGTAAGATGGTGCG CGAAATGCTTGGAGACAGGT
SSRY 66	$(GA)_{10}AAGA$	TCAT	AAG
SSRY 78	$(CT)_{22}$	TGCACACGTTCTGTTTCCA T	ATGCCTCCACGTCCAGATAC
SSRY 100	$(CT)_{17}TT(CT)_7$	ATCCTTGCCTGACATTTTG C	TTCGCAGAGTCCAATTGTTG
SSRY 101	$(GCT)_{11}$	GGAGAATACCACCGACAG GA	ACAGCAGCAATCACCATTTC
SSRY 175	(GA)38	TGACTAGCAGACACCGGT TTA	GCTTAACAGTCCAATAACGA TAAGG
SSRY 111	$(GA)_{28}$	GCATCTTACATCCAGAAT ACTGT	GAAGGAATGCCTGGCTTAAA
SSRY 106	$(CT)_{24}$	GGAAACTGCTTGCACAAA GA	CAGCAAGACCATCACCAGTT T
SSRY 35	$(GT)_3GC(GT)_{11}(CA)_{19}$	GCAGTAAACCATTCCTCCAA	CTGATCAGCAGGATGCATGT

Table 3. The PCR reaction mixture for 25ng/ul of DNA in 20ul

No. of sample	UpH2O	X10PCR Buffer	$MgCl_2$	dNTPs 5mM	P-1	P-2	Taq	DNA 25ng/ul
1	9.04	2.0	2.0	0.8	2.0	2.0	0.16	2.0
36	325.44	72.0	72.0	28.8	72.0	72.0	5.76	Each

P-1 - Right primer.

P-2 - Left primer.

Taq - Taq polymerase. and

UpH2O - Ultra pure water

3.5 Gel electrophoresis of the amplified products

PRINCIPLE

Electrophoresis is movement of molecules in an electric field and the separation of DNA fragments is based on the number of nucleotide units they contain. DNA is negatively charged and therefore would migrate to the anode. Short DNA fragments would migrate faster than the long fragments. This method is sensitive to the extent that DNA fragments up to the 200 nucleotides long can be separated from each other, even if they differ by only one nucleotide (Damell *et al* 1986).

PROCEDURE:

Metaphor agarose gel was first cast by sifting 4g of the gel into 100ml of Trisma base buffer (TBE) and the solution was heated in an oven. The solution was allowed to cool for 20-30 minutes and poured in a prepared gel box. Gel combs with 20 teeth were quickly and meticulously immersed in the hot solution and allowed to solidify. Amplified DNA was mixed with cresol red dye in ratio one to one. The mixture was loaded into the gel well and run in an electric field at 120V for 2 to 3 hours using an E-C Midi cell Primo EC 330 electrophoresis gel system.

3.6 Visualization and documentation

PRINCIPLE:

DNA forms a red fluoresce-complex with ethidium bromide that can be visualized under a fluorescent lamp

PROCEDURE

The gels were stained in 10mg/ml ethidium bromide for 5 mins and destained in distilled water for ten mins and the photographs were taken using Polaroid film 667 black and white color

3.7 Scoring of DNA bands on gel

The gels were scored on a scoring sheet for each SSR primer used, a binary number was used, and presence of a DNA band was scored as 1 and absence as 0

3.8 DATA ANALYSIS

3.8.1 Polymorphism of SSR markers in cassava cultivars

Numbers of alleles for each SSR primer pairs were calculated from the above data

Polymorphic information content was calculated for each SSR markers using the formular $PIC = 1 - \sum_{n} P^2$

3.8.2 Evaluation of genetic diversity

Generated data from 3.7 was assembled into a data matrix. This data matrix was then subjected to Numeric Taxonomy System of Statistics v. 2.0j (NTSYS, Roulph, 2000) and Principal Component Analysis (PCA) in Statistics Analysis System V8 (SAS, 2000).

3.8.2.1 Numeric Taxanomy System of Statistic (NTSYS) Analysis

Similarity indices between pairs of genotypes were calculated for the combination of data from the 16 primers by selecting Similarity for Qualitative Analysis (SIMQUAL) using method of Jaccard (1908) Similarity Coefficient (Table 4). The similarity data matrix was then used for clustering of the genotypes based on Unweighted Pair Clustering Group Arithmetic Average (UPCGMA), which does not consider the joint absence of a DNA band as an indication of similarity, by selecting Sequential and Hierarchial Numeric option (SAHN) in clustering and the clustering was used to generate dendrograms (Fig 1, 2, and 3) by selecting tree plot option.

The similarity index of Jaccard between plant i and j is given by

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

$D_{ij} = 1 - Sij$

Where a is the number of characters present in both plants i and j, b is the number of characters present in i and not in j and c is the number of characters present in j and not in i and D is the distant coefficient.

3.8.2.2 Principal Components Analysis

Principal Component Analysis (PCA) which operates on an n*p units by variables data array (matrix) was also carried out using SAS v8 (Statistical Analysis System SAS, Institute, Inc., Cary, NC). PCA reveals the major underlying sources of variation, i.e., eigenvectors/latent vectors/principal component, in such a way that the first component has maximum variation, the second component is uncorrelated with the first, and has the maximum of the remaining variation, the third component is uncorrelated with the previous components, and has the maximum of the remaining variation, and so on. Scatter diagrams of the scores for different components (PRIN2 * PRIN1, PRIN3 * PRIN2 and PRIN3 * PRIN1), which are useful in revealing a grouping or ordination of the units, were plotted. SAS procedure for Principal Component Analysis is PROC PRINCOMP.

3.8.3 Determination of SSR markers for genotype identification of cultivated cassava in Nigeria using NTSYS

Sixteen SSR markers were assessed for genotype identification of the 36 genotypes of cassava cultivars. Data of each primer were analyzed by NTSYS, primers that generated between 6 and 9 cluster groups were selected. The selected primers' data were combined in various numbers and analyzed on NTSYS to select the minimum numbers of primers that could be used for genotype identification of cultivated cassava in Nigeria.

CHAPTER FOUR

EXPERIMENTS AND RESULTS

4.1 Experiment 1: Polymorphism of SSR markers in cassava cultivars

Introduction

The advance in the development of methods using DNA polymorphisms as molecular markers is making a significant impact on studies such as species evolution, molecular taxonomy, genetic diversity, genetic diagnosis, as well as breeding (Carvalho *et al* 1992) SSR are new PCR-based DNA markers that are currently used for genetic studies The main advantage of SSR markers is that they are highly variable dominant loci and therefore an ideal tool for many genetic applications

Procedure

Genomic DNA from 36 accessions of cassava cultivars were amplified using PCR technique with a set of 65 SSR primer pairs The DNA amplification products were scored and the generated data were analyzed using NTSYS software packaged by Roulph. 2000. polymorphic information content was caculated sing the formula

PIC (P) =1- $\sum np^2$, where np is the allele number

Results

Twenty-two of these primers have two or three alleles that showed polymorphism among the cassava cultivars used Examples are shown in Plates 1 and 2, while 43 SSR primer pairs yielded monomorphic amplified products or no PCR fragment at all 16 SSR primers pairs that amplified clear polymorphic bands on 4% metaphor agarose gel were used to analyze the 36 cassava cultivars These primers and their repeat types and repeat numbers are listed in Table 3

The allele number varied from 2 to 4 with an average of 2.4, primer pairs of SSRY (45) only, amplified four alleles , the maximum number , while primer pairs of SSRY (13), SSRY (100), and SSRY (51) amplified three alleles, and the rest amplified two alleles each. The polymorphic information content ranged from 0.21 to 0.66 (Appendx E).

Conclusion

SSR markers detected polymorphisms among the cassava cultivars used for the study and hence could be used for genetic applications in cassava

Plate 1. Polymorphisms of DNA alleles among the 36 cassava cultivars used for genetic diversity study using SSRY 100
1 to 36 are the genotypes numbers

Plate 2. PCR amplification products of the 36 cassava cultivars in Nigeria by SSRY 9

4.2 Experiment 2: Evaluation of genetic diversity between improved cassava and commonly grown Nigerian landraces

Introduction

Evaluation of genetic diversity in cassava is a prerequisite to any genetic improvement program. The higher the genetic diversity within cassava gene pool, the larger the number of new varieties that could be produced and the less susceptible the new varieties will be to the diseases and pests prevalent in the targeted area. The lower the genetic diversity presents within cassava species, the easier the formation of hybrids and introgession of useful genes among the cassava cultivars.

Procedure

Thirty-one improved cultivars and 5 Nigerian landraces of cassava were assessed at genomic deoxyribonucleic acid (DNA) level with SSR markers. Polymerase chain reaction (PCR) amplification of the genomic DNA of the cultivars were carried out with 16 polymorphic SSR primers that produced clear and scorable bands (see detail in page 29). The data was analyzed using NTSYS and also dendrograms (Figs. 1, 2, and 3) that showed the genetic relationship among the cultivars were constructed. The same data was also analyzed using Principal Components Procedure in SAS to revealed the major source of variation.

Results

4.2.1 NTSYS Analysis

In total 38 clear and scorable DNA bands were detected among the 36 cassava cultivars The SSR primers were able to distinguish all the 36 cultivars, none of the primer pairs was able to distinguish all cultivars when treated alone When all the primer pairs were considered together, the 36 genotypes were distinguished A dendrogram was generated by complete cluster analysis based on similarity indices, which showed the genetic similarity among the 36 cassava cultivars used for the study The similarity indices ranged from 0.42 to 0.84 (Table 4) Twelve distinct cluster groups were identified at 0 70 similarity index The dendrogram is shown in Fig 1 The clustering was as follows from left to right

Cluster I: It contains only accession TME 59

Cluster II: Two genotypes, TME 7 and TME 2, were present in this cluster and the similarity index between them is 0 76

Cluster III: There were two subgroups 'a' and 'b', subgroup 'a' had genotypes 089/00023 and TME 1 and subgroup 'b' had genotype 50395 only Similarity index between genotypes in subgroup 'a' is 0 76 and interpolated with subgroup 'b' at a similarity index of 0 72

Cluster IV: It had only genotype 088/01504

Cluster V: There were two subgroups in this cluster, subgroup 'a' had genotype 92/0057 and subgroup 'b' is further divided into two 'b1' and 'b2'. 'b1' had genotypes

60506 and 60447 with similarity indices of 0.84 and 'b_2' had genotype 90257. The similarity index between the genotypes in subgroup 'a' and 'b' is 0.71 and between the subgroups 'b_1' and 'b_2' is 0.75.

Cluster VI: Comprised subgroup 'a' and 'b', subgroup 'a' had genotypes TME 51 and 92/0326 and subgroup 'b' had genotype NR8082, only. The similarity index between the genotypes in 'a' is 0.82 and similarity index between subgroup 'a' and 'b' is 0.73.

Cluster VII: It contained subgroup 'a' and 'b', subgroup 'a' was further divided into a_1 and a_2, a_1 had genotype NR89107 and a_2 had genotypes W820249 and 30040. The similarity index between the two genotypes in subgroup 'a_2' is 0.82. The similarity index between the two sub -groups 'a_1' and 'a_2' is 0.75. Sub - group b had two genotypes only, W1095-D and 84537, with similarity index of 0.82 between them.

Cluster VIII: It had genotype 518 only.

Cluster IX: This cluster had genotypes 60444 and W4092. The similarity index between them is 0.74.

Cluster X: This contained two sub- groups 'a' and 'b', subgroup 'a' contained genotypes 81/00110, 82/00661, 91/02324, 30553P3-2, 91/02327 and 30572 while subgroup 'b' had W820422, 4(2)1425, 91934, 30555, 30337, and NR8208. The highest genetic similarity found in this cluster is between genotypes 81/00110 and 82/00661 (0.82), 91/02327 and 30572 (0.82), and 30337and NR8208 (0.82).

Cluster XI: Only genotype 30001 was present.

Cluster XII This contained genotype NR8212 only

In general, the closest relatives were genotypes 60506 and 60447 at similarity index of 0.84 and the most distant relatives were genotypes TME 59 and NR82121, TME 2 and 30555, NR8208, 50395, and 84537, TME 7 and 4(2) 1425, 82/00661, and 518 and 60444, all at 0.42 similarity index, and related to others at 0.56 similarity. The highest genetic relationship between improved cassava and Nigerian landraces was at similarity level of 79% and was between genotype NR89107 (IC) and TME 51(LR) (Table 5)

Table 4. Genetic similarity index matrix data among the 36 cassava genotypes used for genetic diversity the study

NR8212	1.00
NR8206	0.66 1.00
90257	0.58 0.71 1.00
91934	0.61 0.79 0.66 1.00
30001	0.66 0.47 0.66 0.53 1.00
30572	0.50 0.68 0.61 0.63 0.58 1.00
84537	0.55 0.68 0.66 0.63 0.58 0.74 1.00
82/00881	0.47 0.68 0.53 0.66 0.50 0.71 0.66 1.00
30395	0.66 0.68 0.55 0.74 0.47 0.53 0.42 0.61 1.00
81/0011	0.55 0.74 0.61 0.68 0.53 0.68 0.53 0.82 0.74 1.00
NR8082	0.61 0.63 0.66 0.58 0.63 0.53 0.63 0.45 0.58 0.58 1.00
4(2)1425	0.58 0.66 0.63 0.71 0.50 0.71 0.61 0.68 0.55 0.71 0.61 1.00
30555	0.55 0.74 0.76 0.74 0.53 0.68 0.68 0.61 0.63 0.63 0.63 0.68 1.00
92/0329	0.61 0.66 0.71 0.68 0.58 0.58 0.68 0.61 0.63 0.63 0.74 0.71 0.63 1.00
30553P3-2	0.45 0.68 0.61 0.68 0.53 0.79 0.68 0.76 0.53 0.74 0.58 0.71 0.74 0.63 1.00
91/02327	0.53 0.71 0.63 0.76 0.68 0.82 0.71 0.74 0.61 0.76 0.61 0.74 0.71 0.66 0.82 1.00
30040	0.58 0.66 0.58 0.61 0.55 0.68 0.71 0.58 0.50 0.61 0.61 0.63 0.61 0.66 0.71 0.68 1.00
91/02324	0.55 0.68 0.61 0.74 0.58 0.74 0.68 0.71 0.63 0.74 0.63 0.66 0.74 0.63 0.74 0.82 0.71 1.00
W820422	0.68 0.76 0.74 0.76 0.61 0.71 0.61 0.74 0.66 0.76 0.61 0.79 0.71 0.66 0.68 0.74 0.63 0.79 1.00
30337	0.63 0.82 0.74 0.76 0.55 0.71 0.71 0.68 0.61 0.76 0.61 0.79 0.82 0.71 0.76 0.74 0.74 0.71 0.74 1.00
92/0057	0.61 0.53 0.66 0.63 0.58 0.47 0.63 0.68 0.63 0.53 0.63 0.45 0.63 0.63 0.58 0.61 0.55 0.63 0.58 0.55 1.00
W1085D	0.58 0.61 0.55 0.63 0.55 0.55 1.00 0.74 0.61 0.68 0.68 0.68 0.68 0.76 0.71 0.79 0.63 0.76 0.68 0.68 0.66 1.00
W820249	0.61 0.68 0.66 0.68 0.63 0.68 0.79 0.71 0.53 0.68 0.58 0.68 0.63 0.63 0.79 0.76 0.82 0.74 0.71 0.71 0.58 0.71 1.00
088/01504	0.53 0.68 0.74 0.66 0.58 0.66 0.68 0.53 0.61 0.58 0.68 0.58 0.76 0.71 0.68 0.63 0.63 0.61 0.63 0.68 0.71 0.63 0.58 1.00
NR89107	0.68 0.68 0.68 0.61 0.61 0.61 0.71 0.47 0.61 0.58 0.71 0.58 0.71 0.68 0.58 0.58 0.79 0.71 0.58 0.68 0.68 0.63 0.71 0.68 1.00
W4092	0.58 0.66 0.63 0.61 0.58 0.66 0.66 0.58 0.68 0.76 0.61 0.58 0.61 0.61 0.61 0.74 0.63 0.76 0.63 0.68 0.58 0.74 0.66 0.63 0.74 1.00
TME51	0.68 0.71 0.63 0.61 0.66 0.71 0.71 0.53 0.61 0.68 0.71 0.63 0.66 0.82 0.61 0.68 0.74 0.68 0.63 0.74 0.58 0.68 0.66 0.68 0.76 0.68 1.00
TME2	0.58 0.42 0.58 0.53 0.53 0.47 0.47 0.45 0.63 0.53 0.53 0.45 0.42 0.58 0.47 0.58 0.61 0.58 0.45 0.58 0.53 0.61 0.53 0.61 0.61 0.71 0.58 1.00
TME1	0.58 0.63 0.61 0.68 0.47 0.63 0.63 0.66 0.74 0.63 0.63 0.58 0.66 0.74 0.68 0.74 0.63 0.63 0.58 0.68 0.74 0.71 0.63 0.78 0.71 0.61 0.71 0.68 1.00
TME7	0.53 0.58 0.58 0.55 0.55 0.61 0.55 0.42 0.68 0.58 0.61 0.42 0.58 0.61 0.55 0.63 0.68 0.61 0.53 0.47 0.61 0.58 0.61 0.74 0.68 0.68 0.68 0.76 0.79 1.00
TME58	0.42 0.58 0.58 0.55 0.58 0.71 0.61 0.63 0.61 0.61 0.55 0.58 0.68 0.58 0.68 0.68 0.58 0.71 0.58 0.68 0.61 0.68 0.61 0.58 0.63 0.68 0.58 0.68 0.71 0.68 1.00
88/00021	0.47 0.58 0.63 0.58 0.45 0.58 0.45 0.58 0.71 0.61 0.61 0.63 0.58 0.68 0.53 0.61 0.63 0.58 0.68 0.68 0.45 0.63 0.68 0.68 0.68 0.71 0.47 0.68 0.45 0.61 0.58 0.58 0.63 0.58 0.68 0.61 1.00
518	0.58 0.58 0.58 0.53 0.58 0.79 0.58 0.76 0.58 0.68 0.53 0.61 0.63 0.58 0.68 0.68 0.45 0.63 0.68 0.68 0.68 0.71 0.47 0.68 0.45 0.61 0.58 0.58 0.63 0.58 0.68 0.61 1.00

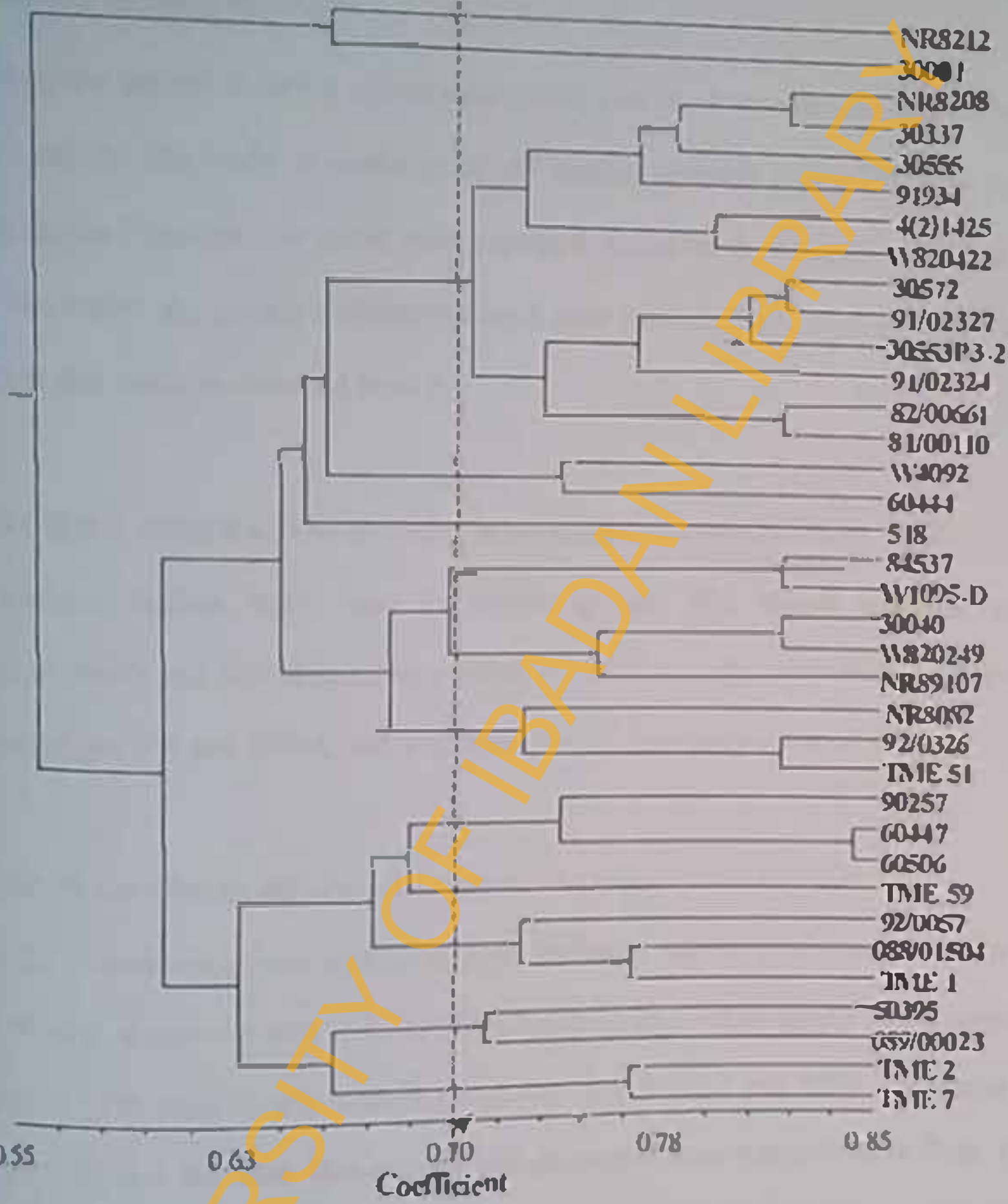

Fig 1. A dendrogram showing the genetic diversity among the 36 cassava genotypes based on UPCGMA using NTSYS

Intra-variety genetic diversity

This shows the genetic diversity within each variety, i.e. the improved variety and local variety, used for this study. Knowledge of the genetic diversity within the gene pools of each variety indicates how many new genotypes could be developed from the gene pools. The higher the genetic diversity within a gene pool the lrger the number of new genotypes that could be obtained from it.

IMPROVED CASSAVA: The similarity indxe ranged from 0.42 to 0.84, (Table 4), at 0.70 similarity indices, there were 11 cluster groups. The closest relatives were genotypes 60447 and 60506 (similarity index of 0.84) and the most distant relatives were genotypes 518 and 60444, and 50395 and 84537 (similarity index of 0.42).

NIGERIAN LANDRACES: The similarity indices ranged from 0.55 to 0.76 (Table 3). There were three groups at 0.70 similarity indices, group one made up of TME 59 only, group two had TME 1, TME 2 and TME 7, while group three consisted of TME 51. The most closely related genotypes were TME 2 and TME 7 at similarity index of 0.76 and the most distantly related genotypes were TME 51 and TME 2 at similarity index of 0.55 and were related to others at 0.63 similarity index.

Conclusion

Genetic diversity between improved cassava and commonly grown Nigerian races is medium and this therefore will facilitate the formation of hybrids and introgression of useful genes between them. More new genotypes could be developed from the improved cassava than the Nigerian landraces.

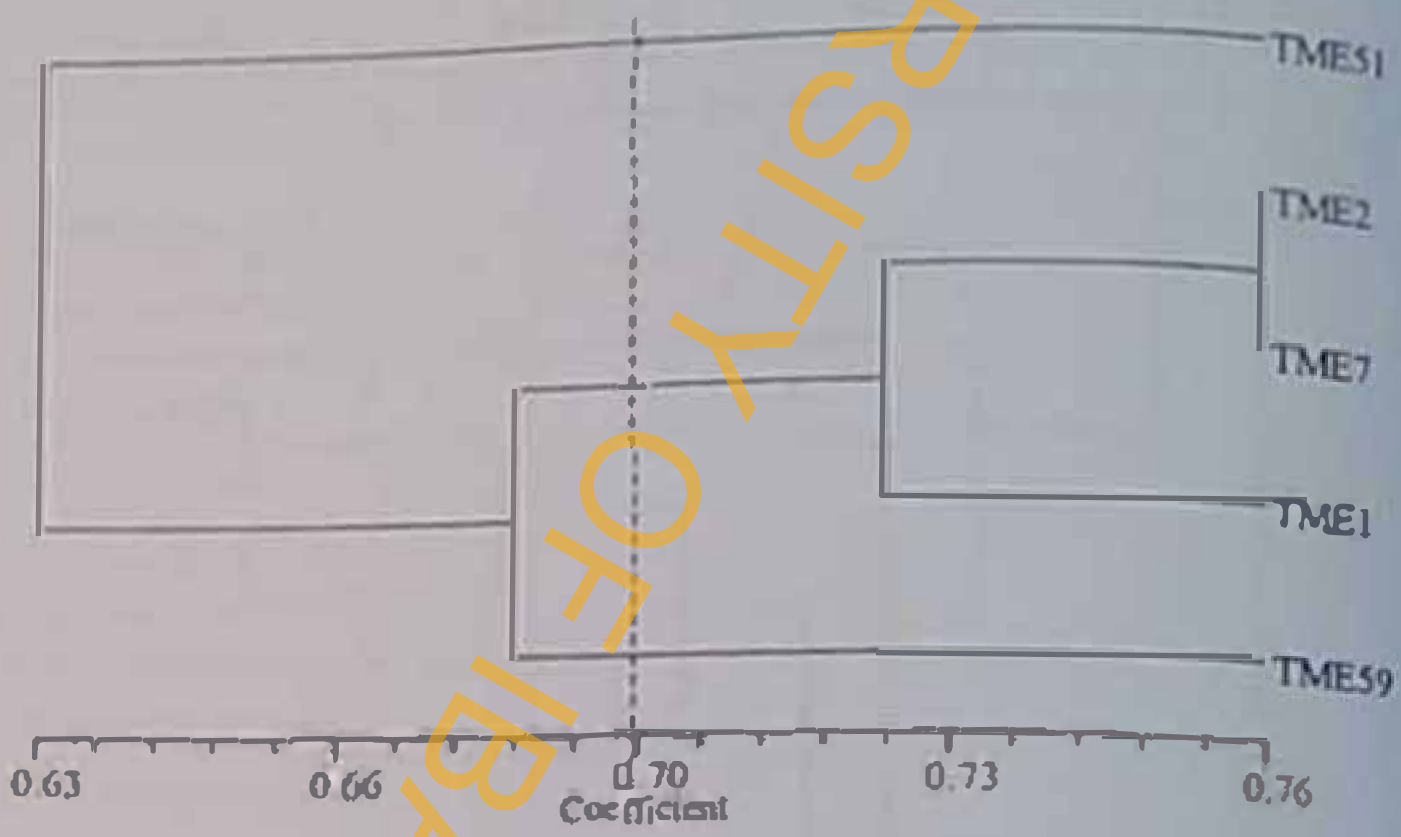

Fig. 2 A dendrogram showing the genetic diversity among the Nigerian landraces

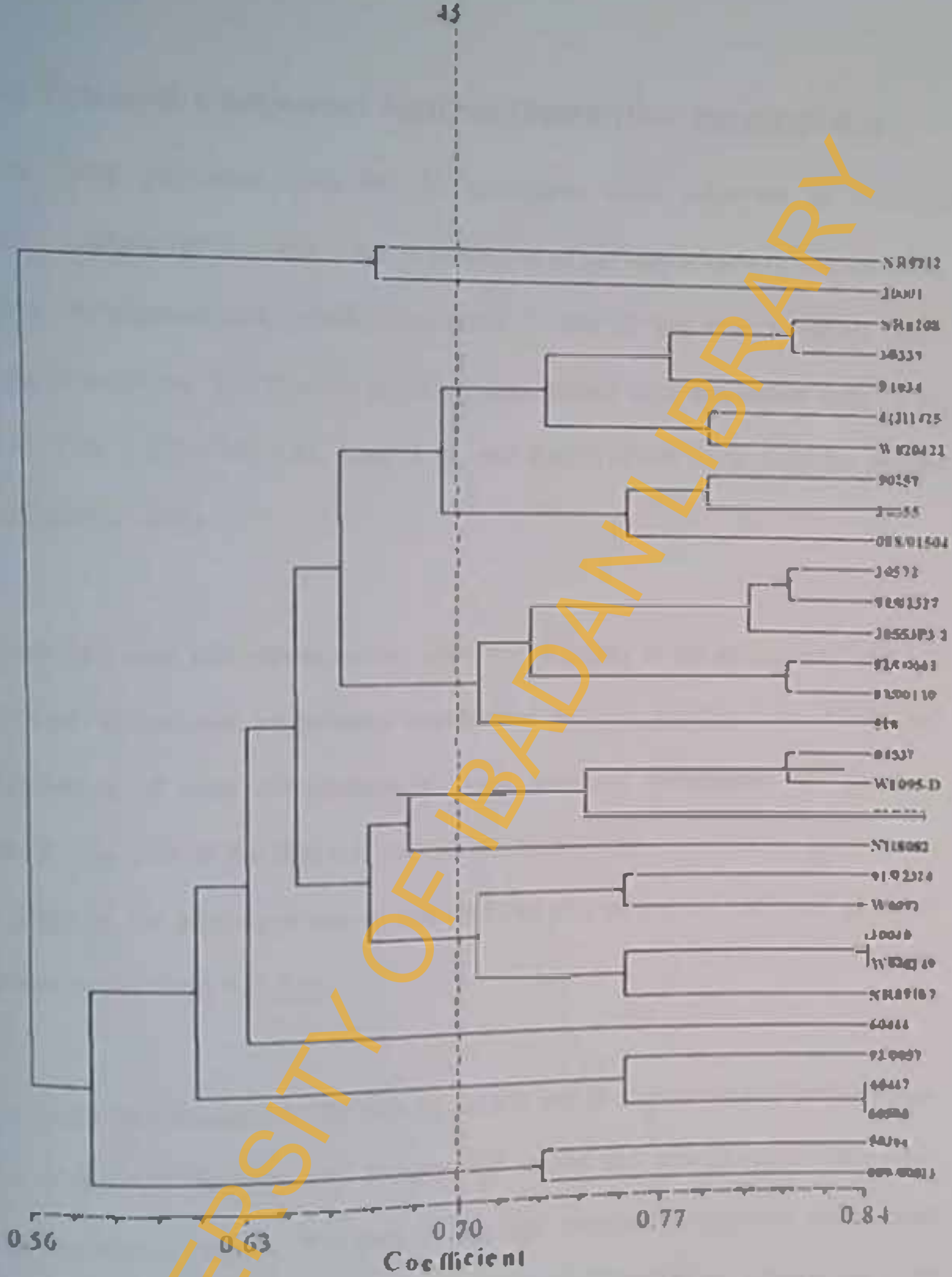

Fig 3 A dendrogram showing the genetic diversity among the improved cassava cultivars

4.2.2 The Principal Component Analysis (PRINCOMP PROCEDURE)

The binary data generated from the 36 genotypes were subjected to Principal Component Analysis (PCA) using SAS. it made use of the matrix data of the variables, to produce ten eigenvectors, which contributed 70.4% of the total variation found among the 36 cultivars. The first ten principal components were associated with 11.70, 9.23, 7.95, 7.76, 7.29, 6.69, 5.84, 5.44, 4.46, and 4.03% of the variance of the generic distance (70.59% total).

There were also main alleles/primers that contributed highly to the proportion found in each principal component, proportions contributed by each principal component and the contribution of each allele/primer to each principal component are listed in appendix E. The plot of the first and second principal components scores generated a scatter graph of the genotypes and so also did the plot of second and third principal components scores (Figs 4, 5 & 6).

More variation was observed in the plot of second and first principal components than the plots of first and third principal components' scores and also the second and third principal components' scores. The plot of the first principal component and second principal components' scores showed interesting relationships among the 36 genotypes. The 36 genotypes distributed themselves into four different quadrants. The lower left quadrant was composed solely of the improved cultivars, W820422,

W820249, 088/01504, 60444, NR8208, 4(2)1425, and NR8212, the upper left quadrant was also composed of improved cultivars only 82/00661, 30555P3-2, 30572, 91/02327, 81/00110, 91/02324, and W1095-D, while 84537 and 91934 were at the 0 0 score level. The lower right quadrant was also composed of seven improved cultivars, 90257, 50395, 30001, 30040, 92/0326, NR89107, and NR8083 and two landraces TME 7 and TME 51. The upper right quadrant contained three Nigerian landraces TME 1, TME 2, and TME 59, and five improved cultivars, 60506, 518, 60447, 92/0057, and 30572.

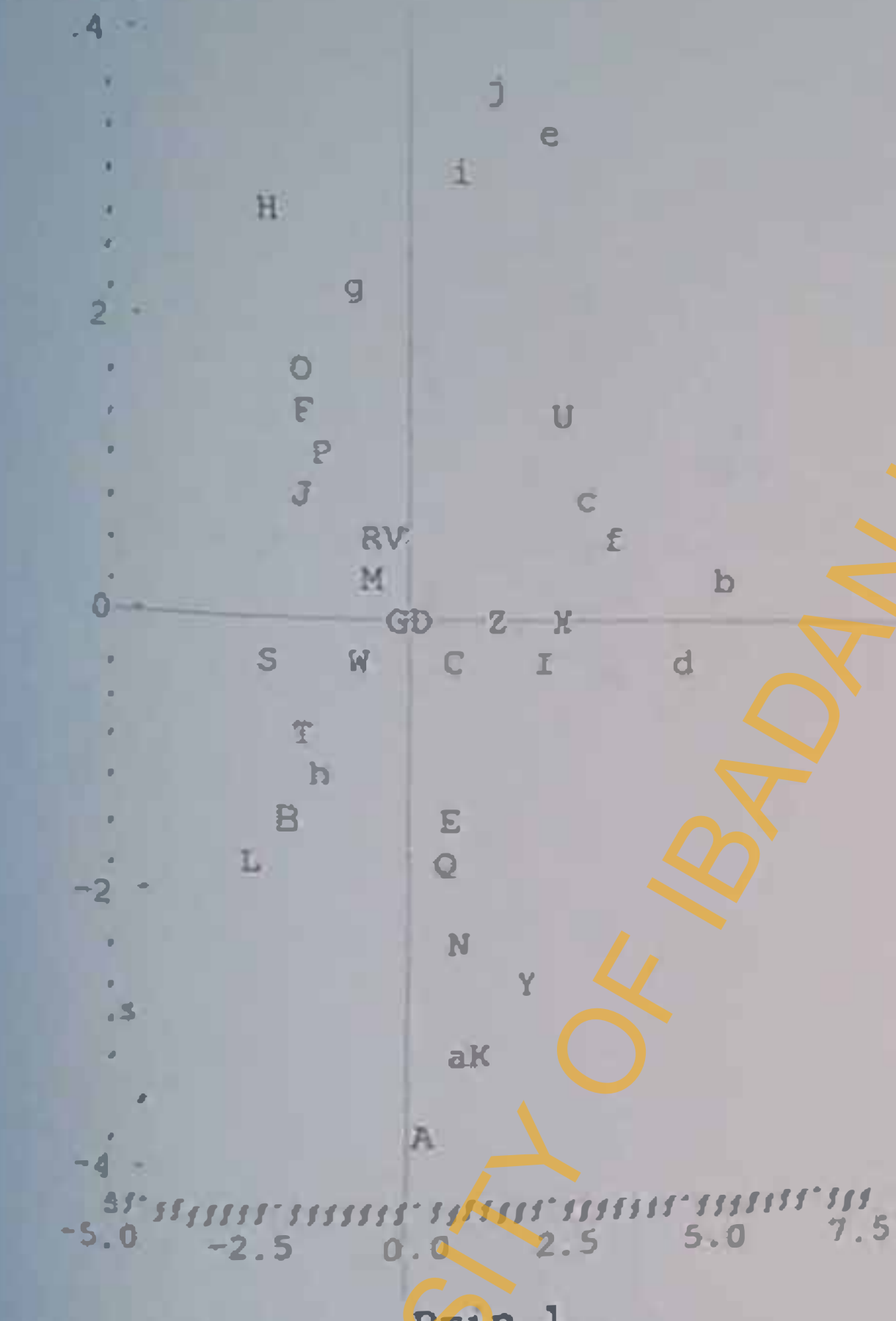

Fig. 4. Plot of second Principal components' scores against first Principal components' scores

Symbol rep. Genotypes A to Z, f to j rep. improved cultivars, and a to e represent Nigerian landraces Prin 2 and Prin 1 are principal components that revealed the major source of variation observed among the cassava cultivars

Fig.5 Plot of third principal components scores against second Principal components scores

Symbol represents Genotypes Letter A to Z, f to j represent improved cultivars, and a to e represent Nigerian landraces of Cassava.

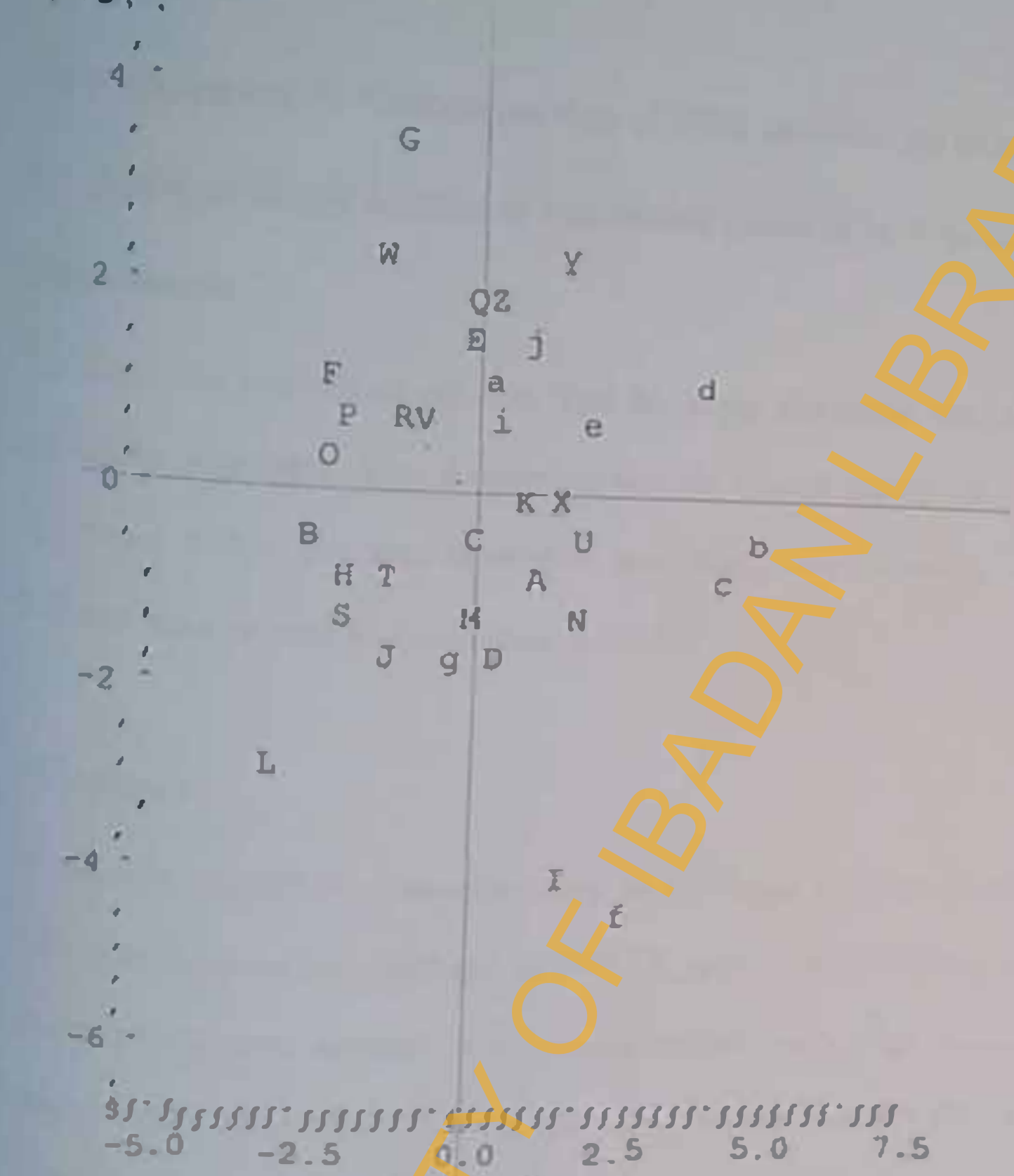

Fig.6 Plot of third Principal components' scores against first Principal components' scores

Symbol represents Genotypes A to Z, f to j represent improved cultivars, and a to e represent Nigerian landraces.

4.3 Experiment 3: Determination of SSR marker (s) that could be used for genotype identification of cultivated cassava in Nigeria

Introduction

The larger the numbers of cultivars used for study, the higher the number of markers needed for assessment. Use of many markers for a small number of sampling materials is a waste of time and there is need to save time during laboratory work in order to facilitate research work and encourage scientists.

Procedure

Sixteen SSR markers were assessed using 36 genotypes of cassava cultivars in Nigeria. Data of each primer were analyzed by NTSYS; primers that generated between 6 and 9 cluster groups were selected. The selected primers' data were combined in various numbers and analyzed on NTSYS to select the minimum numbers of primers that could be used for genotype identification of cultivated cassava in Nigeria.

Results

None of the SSR markers could solely identify the 36 cassava cultivars used in this study. They clustered many cassava genotypes together at 70% and 100% similarity indices. Combination of data of primers detected five polymorphic SSR markers that were the minimum number of SSR markers that could readily distinguish the 36

genotypes of cassava. The similarity indices ranged from 0.19 to 0.94 (Table 6), the primers differentiated the genotypes into 16 cluster groups at 0.70 similarity index and distinguished them at 0.95 similarity index. The clustering was as follow from left to right (Fig. 7):

Cluster group I: TME 59

Cluster group II: 91/02324

Cluster group III: W1095-D

Cluster group IV: 84537 and W820249

Cluster group V: 90257 and 60506

Cluster group VI: There were two subgroups, 'a' and 'b' subgroup 'a' had genotype W820422 and 'b' had 518, 81/00110 and 82/00661

Cluster group VII: had genotype 91934

Cluster group VIII: had subgroup 'a' and 'b', 'a' contained 60444, W4092, 91/02327, 4(2)1425, 30572 and 'b' had 30555Psa, 30555, 60447, 30337 and NR8208

Cluster group IX: had genotypes TME 2 and TME 7

Cluster group X: NR89107 and 30040

Cluster group XI: 92/0057

Cluster group XII: had subgroups 'a' and 'b', a' had TME 1 and 088/01504 and 'b' had genotypes NR8082 and 92/0326

Cluster group XIII: genotypes 50395 and 089/00023 were present

Cluster group XIV: contained only genotype 3001

Cluster group XV only genotype TME 51 and

Cluster group XVI only genotype NR8212

Conclusion

Five SSR markers out of the 16 markers used for evaluation of this genetic diversity study could be readily used for genotype identification of cultivated cassava in Nigeria.

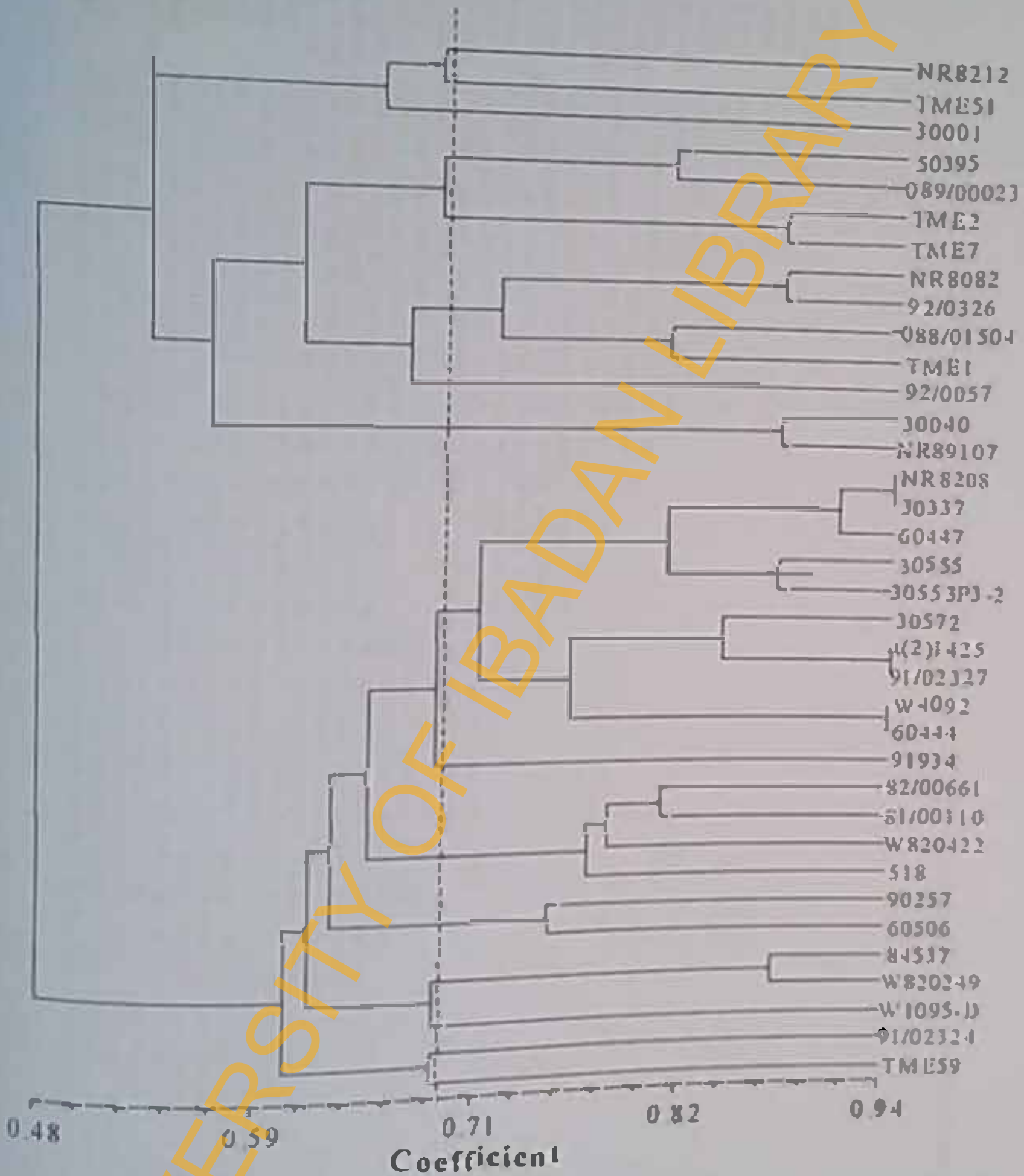

Fig. 7 A dendrogram showing the genotype identification by five polymorphic SSR markers

Table 5 Genetic similarity matrix data of the 36 cultivated cassava for genotype identification

NR8212	1.00																																	
NR8208	0.56	1.00																																
90257	0.44	0.75	1.00																															
91934	0.31	0.75	0.63	1.00																														
30001	0.63	0.31	0.56	0.31	1.00																													
30572	0.56	0.75	0.63	0.63	0.56	1.00																												
84537	0.56	0.75	0.50	0.63	0.44	0.75	1.00																											
82/00681	0.38	0.56	0.56	0.56	0.50	0.56	0.56	1.00																										
50395	0.63	0.56	0.56	0.69	0.38	0.44	0.31	0.38	1.00																									
81/00770	0.44	0.75	0.75	0.63	0.44	0.75	0.50	0.81	0.56	1.00																								
NR8082	0.63	0.56	0.44	0.56	0.50	0.44	0.56	0.38	0.63	0.44	1.00																							
4(Z)1405	0.50	0.69	0.56	0.69	0.50	0.81	0.69	0.75	0.50	0.81	0.63	1.00																						
30555	0.38	0.81	0.56	0.69	0.25	0.69	0.69	0.63	0.50	0.69	0.50	0.63	1.00																					
82/00318	0.63	0.69	0.56	0.56	0.50	0.44	0.56	0.50	0.63	0.56	0.69	0.50	0.63	1.00																				
30555(P3-2	0.38	0.81	0.56	0.69	0.38	0.69	0.81	0.75	0.38	0.69	0.50	0.75	0.88	0.69	1.00																			
91/02327	0.44	0.75	0.63	0.75	0.56	0.88	0.75	0.69	0.44	0.75	0.56	0.94	0.69	0.56	0.81	1.00																		
30040	0.56	0.63	0.50	0.38	0.44	0.63	0.75	0.31	0.31	0.38	0.56	0.44	0.56	0.56	0.56	0.50	1.00																	
91/02324	0.50	0.69	0.56	0.69	0.38	0.69	0.69	0.63	0.50	0.56	0.50	0.63	0.75	0.38	0.63	0.69	0.56	1.00																
W820422	0.63	0.69	0.69	0.56	0.50	0.69	0.56	0.75	0.50	0.81	0.50	0.75	0.50	0.50	0.50	0.69	0.44	0.75	1.00															
30337	0.50	0.94	0.69	0.69	0.38	0.81	0.69	0.63	0.50	0.81	0.50	0.75	0.88	0.63	0.88	0.81	0.58	0.63	0.63	1.00														
92/00057	0.56	0.50	0.50	0.50	0.44	0.25	0.50	0.56	0.56	0.38	0.69	0.44	0.56	0.69	0.56	0.38	0.50	0.56	0.44	0.44	1.00													
W1086-D	0.56	0.63	0.50	0.63	0.44	0.63	0.75	0.69	0.56	0.63	0.69	0.81	0.56	0.69	0.69	0.75	0.50	0.69	0.69	0.56	0.50	1.00												
W82024-9	0.56	0.63	0.63	0.50	0.56	0.63	0.88	0.56	0.31	0.50	0.44	0.56	0.56	0.44	0.69	0.63	0.63	0.56	0.56	0.56	0.50	0.63	1.00											
088/01504	0.50	0.81	0.69	0.69	0.38	0.56	0.56	0.38	0.63	0.56	0.75	0.50	0.75	0.75	0.63	0.56	0.69	0.63	0.50	0.75	0.69	0.44	0.44	1.00										
NR891 07	0.56	0.50	0.50	0.38	0.44	0.50	0.63	0.19	0.44	0.25	0.56	0.31	0.56	0.44	0.44	0.38	0.88	0.56	0.31	0.44	0.63	0.38	0.63	0.69	1.00									
W4092	0.56	0.75	0.75	0.63	0.44	0.75	0.63	0.44	0.56	0.63	0.44	0.69	0.56	0.44	0.56	0.75	0.63	0.69	0.69	0.69	0.38	0.63	0.63	0.56	0.63	1.00								
TME51	0.69	0.63	0.50	0.38	0.69	0.75	0.63	0.31	0.44	0.50	0.69	0.56	0.56	0.69	0.56	0.63	0.75	0.44	0.44	0.69	0.38	0.50	0.50	0.69	0.63	0.50	1.00							
TME2	0.50	0.44	0.56	0.56	0.50	0.44	0.31	0.13	0.75	0.31	0.63	0.38	0.38	0.50	0.25	0.44	0.56	0.50	0.38	0.38	0.44	0.44	0.31	0.63	0.69	0.69	0.56	1.00						
TME1	0.44	0.63	0.50	0.63	0.31	0.38	0.50	0.31	0.69	0.38	0.69	0.31	0.69	0.69	0.56	0.38	0.63	0.56	0.31	0.56	0.63	0.50	0.38	0.81	0.63	0.38	0.63	0.69	1.00					
TME7	0.63	0.44	0.44	0.44	0.50	0.44	0.44	0.00	0.63	0.19	0.63	0.25	0.38	0.50	0.25	0.31	0.69	0.38	0.25	0.38	0.44	0.31	0.44	0.63	0.81	0.56	0.69	0.69	0.69	1.00				
TME59	0.44	0.50	0.50	0.50	0.44	0.63	0.50	0.44	0.56	0.50	0.44	0.56	0.69	0.31	0.56	0.63	0.50	0.69	0.44	0.56	0.38	0.63	0.50	0.44	0.63	0.75	0.50	0.69	0.50	0.56	1.00			
088/00023	0.56	0.50	0.50	0.50	0.44	0.38	0.25	0.31	0.81	0.50	0.69	0.44	0.38	0.69	0.44	0.38	0.38	0.31	0.31	0.56	0.63	0.38	0.25	0.69	0.50	0.38	0.63	0.69	0.75	0.69	0.50	1.00		
518	0.63	0.69	0.56	0.56	0.50	0.69	0.44	0.75	0.63	0.81	0.50	0.75	0.63	0.63	0.63	0.69	0.31	0.63	0.75	0.75	0.44	0.69	0.31	0.50	0.19	0.56	0.56	0.38	0.44	0.25	0.56	0.56	1.00	
60444	0.50	0.81	0.69	0.69	0.38	0.81	0.69	0.50	0.50	0.69	0.50	0.75	0.63	0.50	0.63	0.81	0.69	0.75	0.75	0.75	0.31	0.69	0.56	0.63	0.56	0.94	0.56	0.63	0.44	0.50	0.69	0.31	0.63	1.00
60447	0.63	0.94	0.81	0.69	0.38	0.69	0.69	0.50	0.63	0.69	0.50	0.63	0.75	0.63	0.75	0.69	0.56	0.63	0.63	0.69	0.56	0.56	0.69	0.75	0.56	0.81	0.56	0.50	0.56	0.50	0.56	0.56	0.63	0.75

CHAPTER FIVE

DISCUSSION

5.1 Microsatellites markers polymorphism

Microsatellites markers have shown high levels of polymorphism in many important crops including rice (*Oryza sativa L.*, Bligh *et al.*, 1999), Sorghum (*Sorghum bicolor*, Smith *et al.*, 2000), grapevine (*Vitis vinifera*, Di Gaspero *et al.*, 2000), cowpea (*Vigna unguiculata L.*Chen-Dao *et al.*, 2001), almond (*Prunus pandorea* and *P. amygdalus*, Ajay *et al.*, 1999), wheat (*Triticum aestivum L.*, Alunad, 2002), sun flower (*Helianthus annus L.*, Yu *et al.*, 2002) and melon (*Cucumis melo l.*, Danin-Poleg *et al.*, 2001).

The present study showed that SSR markers were also polymorphic in cassava. The number of allele per primer in sorghum is one to ten with mean of 5.9 and in cowpea, it is two to seven with mean of 4.7. In this study, only two to four alleles with mean of 2.4 were generated from the 36 cassava cultivars used. All the SSR primer pairs that amplified DNA from improved cassava successfully did so in Nigerian landraces, in the present study. Furthermore, the results of this study showed that SSR were reproducible in cassava.

Though the level of polymorphism in cassava is relatively high, it is much lower than in other crops. One possible reason is that most materials used in the present study were from the IITA breeding program while others were clones from some other institutes and two, the Nigerian landraces with desirable traits, could be one of the exotic sources of the improved cassava. Hence, the cultivars had a relatively low genetic base.

Another reason for the low level of polymorphism is that cultivated cassava is relatively low in genetic diversity compared with other crops. The following researchers have reported this, Sarria *et al.*, 1992 (isozyme markers), Angel *et al.*, 1992 (RFLP), Tonukari *et. al.*, 1997, Ugoji, 1998 (RAPD) and Fregene *et al.* 2000 (SSR).

5.2 Genetic diversity/polymorphism of cassava cultivars

SSR markers have been used to study genetic diversity in large number of plants including rice, sorghum, wheat, sunflower, and many other crops. They could also distinguish cassava cultivars in this study. In fact, five polymorphic SSR markers (SSRY 100, SSRY 45, SSRY 48, SSRY 51, SSRY 13) were able to distinguish the 36 cassava genotypes studied.

Genetic diversity in cassava has been previously studied using some other molecular markers and low or medium genetic diversity was observed (Sarria *et al.*, 1992 (isozyme markers), Angel *et al.*, 1992 (RFLP), Tonukari *et. al.*, 1997, Ugorji, 1998 (RAPD) and Fregene *et al.*, 2000 (SSR). In the present study, also, there was moderately low genetic diversity in cassava cultivars studied.

The dendrogram constructed on NTSYS using similarity index based on UPGMA showed that at 0.70 similarity index, the 31 improved cultivars and 5 Nigerian landraces were clustered into 12 main distinct groups. Nigerian landrace TME 59 formed a distinct group (group I), two Nigerian landraces (TME 2 and TME 7) also formed a group (group II), while the remaining landraces (TME 1 and TME 51) were found among the improved cultivars in separate groups (Group III and VI respectively). These close Nigerian landraces could be easily hybridized with the improved cultivars.

The scatter plot of the first two principal components and first and third principal components in SAS also showed a close relationship among the cassava cultivars. The relationships showed by the scatter graph of the first two principal components scores were almost similar to the ones observed in the dendrogram by NTSYS. Many of the genotypes found in the same quadrant were also in the same cluster group or near cluster groups. None of the principal components was able to demarcate the improved

cultivars from the Nigerian landraces in the scatter graphs, although PRIN1 grouped all Nigerian landraces into the right half of the plot but with inclusion of some improved cultivars, which did not give a clear-cut division between the improved and the Nigrian landraces.

There was great similarity between NTSYS and PCA results, therefore, useful information in PCA could be applied to NTSYS result, the first ten principal components could be used to represent variables that contributed the variation found among the cultivars because they contributed 70.59% of the total variation. The remaining could be ignored as minimal. Main alleles that contributed greatly to each principal components' proportion could be taken as the main alleles that contributed to the variation found among the 36 cultivars.

The genetic diversity in Nigerian landraces is lower than that observed in improved cassava. The similarity indices in the Nigerian landraces ranged from 0.55 to 0.76 while that of the improved is from 0.42 to 0.84. This indicated that the lowest relationship in Nigerian landraces was at 55% similarity indices level while that of the improved cassava was at 42% similarity level. This might stem from the fact that the Nigerian landraces were domesticated in the same ecological zones while the improved were obtained from different exotic sources that might have had diverse ranges of ecological zones. Comparison of the dendrogram produced by RAPD

(Tonukari et *al.*, 1997 and Ugorji, 1998) showed consistency only in the large groupings. This lack of consistency between different marker techniques was also observed in cowpea (Chen-Dao *et al.*, 2001). This may be due to the fact that different marker techniques detect different components of DNA variation, subject to different evolutionary mechanisms.

5.3 Determination of SSR marker(s) that could be used for genotype identification of cultivated cassava in Nigeria

SSR markers were polymorphic in the cassava cultivars used in this study to the extent that five polymorphic SSR markers were able to identify the 36 cassava genotypes studied at 95% similarity level. This shows that one need not undergo the stress of primers screening or the application of too many SSR markers for the identification of cassava cultivars. So, the minimum number of highly polymorphic SSR primers for the 36 cassava genotypes identification was five and they were SSRY 100, SSRY 45, SSRY 48, SSRY 51, and SSRY 13. The larger the number of genotypes, the higher the number of markers required for identification. This result further confirmed the PCA results of the major underlying sources of variation observed among the cassava cultivars. These primers were found among the few major primers in principal components 1, 2, and 3, and also have highest polymorphic information content values.

CHAPTER SIX

SUMMARY AND CONCLUSION

6.1. Summary

1. SSR markers were polymorphic in cassava; the degree of polymorphism was however lower than for other crops. Two to four alleles were amplified per primer pair, which is lower than in crops such as rice and sorghum. This might be due to the low genetic base of cassava.

2. SSR markers that amplified DNA from improved cassava successfully did so in Nigerian landraces and they were also reproducible in the cassava genotypes studied

3. The genetic diversity between the improved cultivars and the Nigerian landraces of cassava was moderately low. The highest genetic relationship was at 79% and lowest was at 42%. This therefore facilitates the formation of hybrids and introgression of useful genes among the improved cassava and the Nigerian landraces

4. There was lower genetic diversity in Nigerian landraces (0.55 to 0.76 similarity indices) than in improved cassava cultivars (0.42 to 0.84 similarity indices), possibly because improved cassava cultivars might have a diverse range of ecological zones that incorporate multiple traits

5 No varieties demarcation was observed in this study, in fact there was a close genetic relationship between Nigerian landraces and the improved cultivars assessed which indicated that Nigerian landraces could be exotic sources for improved cassava

6 Five SSR markers could easily be used for cassava genotypes identification of cultivated cassava in Nigeria, they are SSRY 51, SSRY 13, SSRY 100, SSRY 45, and SSRY 48 because they were able to distinguish the 36 cassava genotypes at 0.95 similarity index

6.2 Conclusion

SSR detected polymorphisms among cassava cultivars used in this study and is therefore an ideal molecular tool for genetic diversity and genotypes' identification studies. It could also be used for the collection and conservation of cultivated cassava in Nigeria that could be exploited in cassava breeding programs

Recommendation

This study revealed a close genetic relationship between improved cassava cultivars and commonly grown Nigerian landraces. The Nigerian landraces could therefore be developed easily through hybridization with improved cassava and introgression of desirable genes from improved cassava. Hence, Nigerian cassava could serve its two targeted roles, as both a food security crop and a cash crop.

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APPENDICES

Appendix A

Materials, Reagents, and Equipment

Materials and Reagents

Eppendorf tubes (0.5, 1.5 and 2mls)

Micro-Pipettes (2.5-1000 µl, manual and computerized)

PCR tubes

Foil sheet

Cellophane paper

Scissors

Scoring sheets

TBE buffer

Agarose powder

Metaphor Agarose

Cresol red dye

Distilled water

TE (Tris-EDTA)

TNE buffer

Absolute ethanol

Qiagen Mini-plant DNA extraction kit:

Lysis buffer, AP1 - 0.5 M EDTA, Sodium dodecylsulphate and 1 M trisHCl
Precipitant buffer, AP2 - Phenol : Chloroform : Isoamyl alcohol (v/v) 24 : 25 : 1
Binding buffer, AP3 - 3 M Potassium acetate (pH 5.5)
Washing buffer, AW - 50 mM MOP(N-morpholino) propane sulphonic acid (pH 7.5-7.6)
Elution Buffer - 50 mM trisHCl (pH 8.1-8.2), 1.4 M NaCl, 15 % (v/v) ethanol

Deoxyribonucleoside triphosphates (dNTPs)

PCR buffer (Tris HCl, KCl and Bovine serum albumin)

$MgCl_2$

Thermus aquaticus polymerase

Calfthymus DNA

EQUIPMENT

Water bath

Autoclave machine

Microwaves

Oven

Microcentrifuge

Electrophoresis box and tools

Polaroid camera and film

Fluorometer

Computer wares and scanning machine

PCR machine

Appendix B

Preparation of Solutions

1. 100 ml of 0.5M EDTA: 18g of disodium ethylenediamine tetraacetate was added to 90 ml of distilled H_2O and the pH was adjusted to 8.0 and the solution was made up to100 ml with distilled H_2O.

2. 1M Tris: 1000 ml was prepared by dissolving 121.1g of Tris base in 800 ml of distilled H_2O and the pH was adjusted to desired pH and made up to 1000 ml with distilled water.

3. TRISMA BASE EDTA (TBE): To prepare 1000 ml of 10M TBE, the following mixture were put into 900 ml of distilled water.

Tris base	108 g
Boric acid	55 g
EDTA	40 ml of 0.5M or 7.2 g

and made up to 1000 ml. To make 0.5 M TBE, dilution factor ($C_1V_1 = C_2V_2$) was used

4. TRIS EDTA (TE) (10mM Tris HCl (1.21g) and 1mM EDTA (0.36g) in 1000ml): 100 ml was prepared with the following mixture

Tris HCl (1M)	1 ml
EDTA (0.5M)	200 µl

and made up with distilled H_2O to 100 ml

TE*(10 mM TrisHCl (1.21g) and 0.1mM EDTA (0.036 g). 100 ml

Tris HCl (1 M)	1ml
EDTA (0.5 M)	2.0 µl

and made up with distilled H_2O to 100 ml

5. 10M TRIS - Sodium EDTA (TNE)-Dye To prepare 500 ml of the dye solution

Tris base (100 mM)	6.05 g
EDTA-Na_2 (10 mM)	1.9 g
NaCl (1M)	29.2 g

The above mixtures were added together and the pH adjusted to 7.4 with concentrated HCl and made up to 500 ml with distilled water

6 Deoxynucleotide triphospates (dNTPs): Preparation of 100 µl of 2.5 mM dNTPs from the stock solution,

dG, dT and dC (100 mM): dilution factor was used to prepare the amount needed.

C1V1 =C2V2

2.5 mM * 100 l = 100mM *V

V = 2.5 mM *100 ul / 100 mM =2.5 µl

2.5 µl of each stock was added to 90 µl of 0.1TE

7. 1% Agarose gel: 1g of agarose powder in 100 ml of distilled water and brought to the boil in microwaves. The solution was allowed to cool to 55-60°C and poured into gel tray for electrophoresis.

8. 4% MetaPhor agarose: 10g of agarose was added to 250ml pre-chilled distilled water and stirred continuously until the agarose was well dissolved. The solution was boiled in microwaves and allowed to cool to 55°C and poured into gel tray for electrophoresis.

9. 50 mls Calf thymus standards 500ng/ul, 250ng/ul and 100ng/ul: Calf thymus DNA was supplied as stock 1mg. This was diluted with 1ml of x1 TNE buffer and dilution factor was used for preparation of the three standards

C1V1 = C2V2

For 500ng/ul

500ng/ul X 50 ul = 1000 ng/ul X V2

500ng/ul x 50 ul / 1000 = V2

V2 = 25 ul

25 ul of 1mg/ml of stock was taken and made up to 50 ul with x1 TNE buffer

250ng/ul

250ng/ul x 50 ul / 1000ng/ul = v2

V2 = 12.5ul

12.5 ul of the stock was made to 50 ul with 37.5 ul of x1 TNE

100ng/ul

100ng/ul x 50 ul / 1000 ng/ul = V2

V2 = 5 ul

5ul of the stock solution was made up to 50ul with x1 TNE

Appendix C

Concentration of DNA extracted from the 36 cassava cultivars as measured by Fluorometer

Lab No	Genotypes	DNA conc ng/ul	Lab No	Genotypes	DNA conc ng/ul
1	NR 8212	56.69	19	W820442	129.20
2	NR8208	453.10	20	30337	163.10
3	90257	423.20	21	92/0057	84.10
4	91934	574.10	22	W1095-D	232.20
5	30001	677.50	23	W820249	45.30
6	30572	317.90	24	088/01504	402.60
7	84537	205.10	25	NR89107	323.00
8	82/00661	221.50	26	W4092	319.30
9	50395	298.10	27	TME51	20230
10	81/00110	104.40	28	TME2	327.40
11	NR8082	285.30	29	TME1	336.70
12	4(2)1425	180.50	30	TME7	267.70
13	30555	220.20	31	TME59	57.60
14	92/0326	313.60	32	089/00023	65.70
15	30555P$_{3-2}$	79.00	33	518	286.70
16	91/0327	289.10	34	60444	106.00
17	30040	372.80	35	60447	123.00
18	91/02324	50.90	36	60506	29.20

Appendix D

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	4.32854248	0.91426657	0.1170	0.1170
2	3.41427590	0.47155928	0.0923	0.2093
3	2.94271663	0.07206303	0.0795	0.2888
4	2.87065360	0.17489952	0.0776	0.3664
5	2.69575408	0.22063527	0.0729	0.4392
6	2.47511881	0.31484303	0.0669	0.5061
7	2.16027577	0.14678692	0.0584	0.5645
8	2.01348885	0.36202225	0.0544	0.6189
9	1.65146661	0.15903352	0.0446	0.6636
10	1.49243309		0.0403	0.7039

Eigenvectors		Prin1	Prin2	Prin3	Prin4	Prin5
Z113A	Z113A	0.132822	-.136709	0.103471	-.003281	0.167843
Z113B	Z113B	0.005457	-.204197	0.313724	0.083434	-.093967
Z113C	Z113C	-.041732	0.224921	-.325942	-.092367	0.130926
Z230A	Z230A	-.087852	0.074546	0.025006	0.054110	0.483822
Z230B	Z230B	0.069622	-.099767	0.128565	-.183534	-.202112
Z119A	Z119A	0.000000	0.000000	0.000000	0.000000	0.000000
Z119B	Z119B	0.179457	-.105172	0.162334	0.151287	0.019277
Z164A	Z164A	0.088512	0.174690	-.013807	0.231427	-.124442
Z164B	Z164B	0.001270	0.061962	-.157860	0.203885	-.047718
Z52A	Z52A	-.271245	0.114488	0.149393	-.113623	0.225590
Z52B	Z52B	0.341082	-.267867	-.068742	0.067429	-.101328
Z52C	Z52C	0.325313	-.208768	0.019568	0.170302	-.051162
Z114A	Z114A	-.233286	-.289645	0.135145	0.155704	0.015014
Z114B	Z114B	0.135020	0.398163	-.069524	0.058555	-.015756
Z73A	Z73A	0.192278	0.279671	0.091359	-.152201	0.007877
Z73B	Z73B	-.026532	-.001197	0.220767	0.103462	-.137240
Z88A	Z88A	0.146378	0.133492	0.067201	-.097750	-.132716
Z88B	Z88B	0.015388	0.283485	0.010173	0.278713	-.079168

Z168A	Z168A	-.108538	-.327038	-.156511	-.105904	0.017601
Z168B	Z168B	0.068994	-.092401	-.048606	-.185223	0.310606
Z167A	Z167A	-.132606	-.024355	0.044868	0.164082	-.127117
Z167B	Z167B	0.311468	0.015306	-.150516	0.000466	0.132615
Z167C	Z167C	0.005642	0.195702	0.227838	0.067308	0.124074
Z9A	Z9A	0.117407	-.085500	-.245973	0.174184	-.004644
Z9B	Z9B	0.119858	0.201273	0.097381	0.196957	0.081155
Z49A	Z49A	-.168869	0.127870	-.143257	0.275285	-.130899
Z49B	Z49B	0.290854	0.020382	-.262248	0.038006	0.112392
Z49C	Z49C	0.089140	-.033019	0.056033	0.096823	0.446221
Z46A	Z46A	0.054415	0.057518	0.363353	-.006139	0.210888
Z46B	Z46B	-.113731	0.106551	0.033210	-.067788	-.171425
Z46C	Z46C	0.219224	0.033089	0.234243	-.115749	0.140786
Z46D	Z46D	-.301845	-.055973	-.048156	0.196225	0.133782
ZAA	ZAA	-.024886	0.000932	0.063666	0.385163	0.058286
ZAB	ZAB	-.054866	-.029574	0.041254	0.309519	0.114190
Z166A	Z166A	-.037014	-.096363	-.180779	0.218953	0.142269
Z166B	Z166B	-.148125	-.086779	-.255488	-.061164	0.094167
Z125A	Z125A	0.051365	-.045127	0.189446	0.173455	-.043352
Z125B	Z125B	0.187692	-.148016	-.089503	0.139517	0.058702

Eigenvectors	Prin6	Prin7	Prin8	Prin9	Prin10
Z113A	0.387423	-.068246	0.158878	-.017446	-.198038
Z113B	-.283188	0.133800	-.000522	0.015523	0.031855
Z113C	-.000872	-.097111	0.044446	-.150454	0.322386
Z230A	0.163732	-.006209	-.034299	-.091333	0.109121
Z230B	0.075575	-.263150	0.146330	-.014709	0.068450
Z119A	0.000000	0.000000	0.000000	0.000000	0.000000
Z119B	-.112813	-.161327	-.029693	-.245623	0.189477
Z164A	0.112707	0.239865	-.129841	0.152444	-.106181
Z164B	0.067822	-.252192	0.183528	0.285756	0.007720
Z52A	0.136270	0.089557	0.071445	0.136324	-.031190
Z52B	0.064019	-.011020	-.082280	-.064422	-.167381
Z52C	0.174475	-.050799	0.085144	-.018642	0.030841
Z114A	0.201503	0.079946	-.042023	0.083071	0.026633
Z114B	-.207757	0.018958	-.157898	-.111088	0.031923
Z73A	-.098492	0.089822	0.244429	0.060158	0.117487
Z73B	-.063050	0.131816	0.298446	-.253235	0.038399
Z88A	0.291507	0.010545	0.298074	-.019091	0.200620
Z88B	0.223844	0.100514	0.128838	0.004827	-.110139
Z168A	0.071467	0.016454	-.118668	0.223408	0.310667

Z168B	-.011615	0.306978	0.020530	-.217893	0.179806
Z167A	0.192743	0.154244	-.068425	-.384207	0.135575
Z167B	0.074378	-.085249	-.219333	0.034455	-.091415
Z167C	0.191057	0.182872	-.169698	-.169797	-.217380
Z9A	0.009748	0.115243	0.391668	-.040928	0.238728
Z9B	0.198072	-.087724	-.256134	0.083178	0.236214
Z49A	-.072214	-.052869	0.059118	0.243419	-.066849
Z49B	-.119265	0.066529	-.128017	-.000530	-.135621
Z49C	-.063232	-.106753	0.169848	0.060100	0.113094
Z46A	-.000784	-.078730	0.038537	0.338542	-.092330
Z46B	0.133268	0.301054	0.007202	0.205853	0.300744
Z46C	-.291917	0.123220	0.136921	0.116041	-.045337
Z46D	-.021082	-.217887	-.100343	-.204055	-.071902
Z4A	-.187551	0.065776	0.044345	-.227499	0.020369
Z4B	-.208608	-.249576	0.043440	0.136202	0.231601
Z166A	-.013379	0.204077	0.350016	0.067447	-.248060
Z166B	-.194509	0.306683	0.086332	0.034453	-.202719
Z125A	-.191241	0.197692	-.177817	0.153701	0.223312
Z125B	0.131132	0.311534	-.176197	0.180434	0.169279

Appendix E

SSR markers and their polymorphic information content

Primers	Polymorphic information content
SSRY113	0.62
SSRY230	0.39
SSRY119	0.27
SSRY164	0.36
SSRY52	0.61
SSRY114	0.44
SSRY73	0.19
SSRY88	0.35
SSRY168	0.4
SSRY167	0.63
SSRY9	0.21
SSRY49	0.52
SSRY46	0.66
SSRY4	0.42
SSRY166	0.3
SSRY125	0.36