Diversity Analysis of Sweet Potato (*Ipomoea batatas* [L.] lam) Accessions from North Central Nigeria using Morphological and Simple Sequence Repeats Markers

ABSTRACT

Aims: Genetic diversity analysis was carried out with the aim of assessing the genetic similarities and variability that exist among sweet potato accessions that are grown in North Central Nigeria using Morphological and Simple Sequence Repeats makers.

Study Design: The field experiment was laid out in a Randomized Complete Block design (RCB) with 5 replications. Morphological characterization was done in the field while Molecular characterization was carried out in the Molecular laboratory.

Place and Duration of Study: Field experiment was carried out at the Teaching and Research farm of the Federal University of Agriculture Makurdi while the laboratory experiment was done at the Molecular Biology laboratory of the Federal University of Agriculture Makurdi. The experiment was carried out between May and August 2018.

Methodology:A total of 20 potato accessions collected from six states (Benue, Kogi, Nasarawa, Niger, Plateau and Abuja) in north central Nigeria were planted in the field for morphological characterization and Observations were made on 21 morphological characters at 90 days after planting (DAP). Genomic DNA for molecular characterization was extracted from young leaves (20 DAP) located at the tip of the main vine of the sweet potato plant using DNA Zol extraction protocol. The extracted DNA was amplified using five SSR primers via Polymerase Chain Reaction in a thermocycler. The amplified DNA was then subjected to 5% Agarose gel electrophoresis and the products were viewed under U-V light. The bands formed as a result of amplification were scored in a binary pattern for analysis.

Results: ANOVA of the of morphological characters revealed that there was significant variation for 18 out of the 21 morphological characters studied among the sweet potato accessions, and the first 4 Principal Components accounted for 72.1% of the total variation among the accessions. The 18 characters were thus useful as morphological markers for diversity analysis and based on them, cluster analysis grouped the sweet potato accessions into 4 clustersbased on their average linkage and the Euclidean test. Four pairs of duplicates (NC 7 and NC 15, NC 8 and NC 16, NC 17 and NC 19, NC 18 and NC 20) were identified to be similar accessions based on the morphological data obtained. For molecular characterization, Polymorphic Information Content (PIC) for the DNA bands formed showed the usefulness of the primers used in revealing genetic diversity among the accessions with primer 1 (IB02) and 4 (IBS 199) showing 31.818% polymorphism respectively. For cluster analysis, three distinct clusters were observed with all the accessions in cluster I and cluster II being approximately 30% similar while accession NC 10 which stood alone in cluster III shared no similarity with any other accession and could possibly be a hybrid. The cluster analysis also revealed a total of 4 sets of duplicates thereby further reducing the total number of accessions to 6 which indicates that a very low diversity exist among the accessions.

Conclusion: A lot of duplicates exist among the sweet potato accessions proving that there is a very low level of sweet potato genetic diversity existing in the North Central region of Nigeria.

Keywords: Accessions, Diversity analysis, Morphological characterization, Molecular characterization, Simple Sequence Repeats, Sweet Potato

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1. INTRODUCTION

Sweet potato (*Ipomeabatatas* [L] Lam), is a dicotyledonous plant that belongs to the family Convolvulaceae. It has large, starchy, sweet-tasting, tuberous roots hence the plant is seen as a root vegetable. Sweet potato is only distantly related to the potato (*Solanum tuberosum* L) and does not belong to the family Solanaceae commonly known as the nightshade family, but both families belong to the same taxonomic order which is the Solanales. The plant is an herbaceous perennial vine, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. Sweet potato cultivars with white or pale yellow flesh are less sweet and moist than those with red, pink or orange flesh [7]. Generally, sweet potatoes are consumed because their nutrients contain beta carotene that prevents vitamin A deficiency in many developing countries.

The origin and domestication of sweet potato is thought to be in either Central America or South America.In Central America, sweet potatoes were domesticated at least 5,000 years ago.In South America, Peruvian sweet potato remnants dating as far back as 8000 BC have been found [8]. Sweet potatoes are cultivated throughout tropical and warm temperate regions wherever there is sufficient water to support their growth.

A study published in 2015 by scientists from Ghent University and the International Potato Centre revealed that the genome of cultivated sweet potatoes contains sequences of DNA from Agrobacterium, with genes being actively expressed by the plant. The discovery of the transgenes was made while performing metagenomic analysis of the sweet potato genome for viral diseases. Transgenes were observed both in the sweet potatoes closely related wild relatives, and also were found in more distantly related wild species. This observation makes cultivated sweet potatoes the first known example of a naturally transgenic food crop [16]

As a scientific discipline, morphological characterization is originated by Goethe in 1790 as reported by [4]. It is carried out on a representative population of an accession using a list of descriptors for the species [4]. Principal Component Analysis (PCA) of the characterization results which identifies a few key or minimum descriptors that effectively account for the majority of diversity observed is usually performed. This saves time and effort for future characterization. This approach of using a list of descriptors and carrying out Principal Component Analysis has been used successfully for different types of crops in different countries [18]; [4]. However, there are limitations that are associated with morphological markers. Its major drawback being its high dependency on environmental factors and this limitation of phenotype based genetic markers has given rise to the development of molecular markers which may or may not correlate with phenotypic expression of a trait [4].Despite the environmental influences on plant morphology, this direct inexpensive and easy to use method of estimations of similarities and variability among plants was perceived as the strongest determinant of the agronomic value and taxonomic classification of plants [17] and the first step in the assessment of plant diversity [15].

A microsatellite is a tract of repetitive DNA in which certain DNA motifs (ranging in length from 1–6 or more base pairs) are repeated, typically 5–50 times [10].They are often referred to as short tandem repeats (STRs) by forensic geneticists, or as simple sequence repeats (SSRs) by plant geneticists [20].They are a crucial tool in the field of population genetics [2] and have been proposed to be used as markers to assist plant breeders in marker assisted selection [9]. A genetic marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. It can be described as a variation (which may arise due to mutation or alteration in the genomic loci) that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like minisatellites [5]. The molecular marker techniques are based on naturally occurring polymorphisms in DNA sequences. Its concept was started in the nineteenth century by Gregor Mendel who employed phenotype based genetic markers in his experiments. Later on, phenotype based genetic markers for *Drosophila melanogaster* led to the founding of the theory of genetic linkage. In General, Molecular markers are well established and their applications as well as limitations have been realized. They offer numerous advantages over conventional phenotype characterization because they are stable and detectable in all tissues regardless

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of growth differentiation, development, pleiotropic effect, epistatic effects and not confounded to environment where they grow [4].

On sweet potato, Morphological characterization has been used successfully to analyse genetic diversity necessary for its germplasm conservation, to reduce accession number by identification and elimination of duplicates and to enhance crop breeding [17]; [14]; [21], it also has been used extensively on various crop plants diversity assessments in various parts of the world [17]; [12].During the last decade, a lot of molecular information has been accumulated and used for genetic diversity assessment on sweet potato germplasm [19]. The most widely used molecular marker procedures for population genetic analysis of both animals and plants during the past few years is the simple sequence repeat (SSR) markers or microsatellites [13]; [22]; [17]. These markers are highly polymorphic, co-dominant, and can easily be detected on high-resolution gels [15].

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2 MATERIALS AND METHODS

2.1 POTATO PLANT MATERIALS

A total of twenty (20) sweet potato vines collected from six states (Benue, Kogi, Nasarawa, Niger, Plateau and Abuja) in North Central Nigeria as shown in table 1 were used as plant materials (accessions). Six of the accessions (vines) were gotten from the National Root Crop Research Institute (NRCRI) Abuja while the rest were gotten directly from farmers.

2.2 MORPHOLOGICAL MARKER ANALYSIS

Morphological markers analysis was done based on the morphological descriptors developed by Centro Internacional de la papa (CIP) as reported by [6].

2.2.1 Experimental Design and Layout for Morphological Characterization

The experiment was conducted at the Teaching and Research Farm of the Federal University of Agriculture Makurdi. The experimental design adopted was the Randomized Complete Block Design (RCB). Ridges of 3 meters long where constructed and 3 vines (for the same accession) where planted on each of the ridges at 1 meter apart. An inter-ridge distance of 1meter and intra-ridge distance of 0.5 meters was allowed for all the ridges constructed. Five (5) replicates for all the accession was maintained in a RCB experimental design for a period of 3 months. Nitrogen, Phosphate and Potassium (N.P.K) fertilizer using the formula 5-10-10 was applied at 40 Days After Planting (DAP) using the broadcasting method at 2kg per 100 square meters. Weeding of the farm was initially done at 2 weeks intervals before the application of fertilizers and later done more frequently as much as required to keep the farm weed free.

Table 1: List of accessions collected in North Central Nigeria

Accession Code	Name	Site	State
NC 1	Mothers Delight	NRCRI	Abuja

NC 2	King J	NRCRI	Abuja
NC 3	Butter Milk	NRCRI	Abuja
NC 4	NR 8164	NRCRI	Abuja
NC 5	DanZaria	NRCRI	Abuja
NC 6	87/TIS0087	NRCRI	Abuja
NC 7	Atsaka 1	Makurdi	Benue
NC 8	Atsaka 2	Makurdi	Benue
NC 9	Arigenge 1	Otukpo	Benue
NC 10	Arigenge 2	Otukpo	Benue
NC 11	Odumu 1	Idah	Kogi
NC 12	Odumu 2	Idah	Kogi
NC 13	Dankalin Mina 1	Mina	Niger
NC 14	Dankalin Mina 2	Mina	Niger
NC 15	DankalinLafia 1	Lafia	Nasarawa
NC 16	DankalinLafia 2	Lafia	Nasarawa
NC 17	Dankalin Jos 1	Jos	Plateau
NC 18	Dankalin Jos 2	Jos	Plateau
NC 19	Dankalin Jos 3	Pada Bali	Plateau
NC 20	Dankalin Jos 4	Pada Bali	Plateau

2.2.2 Data collection

Morphological data for 21 characters were collected 90 days after planting based on the average of three measurements from the middle portion and tip of the main vine as recommended by [11] and reported by [6] using a meter rule and tap. The Characters were scored using a scale of 0 to 9 and used for characterization based on the standard descriptors in CIP guide 36.

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2.2.3. Data analysis

The data obtained were entered into Microsoft Excel package and then imported into Minitab 17 software for further analysis.

2.3 MOLECULAR MARKER ANALYSIS

Molecular markers analysis was carried out in the Molecular Biology laboratory of the Federal University of Agriculture Makurdi, Benue State.

2.3.1 DNA Extraction from the leaves

Genomic DNA was extracted from young leaves located at the tip of the main vine of the potato plant at 20 Days after Planting (DAP) using DNA Zol extraction protocol as reported by [1] with slight modifications as follows: One gram of fresh and healthy leave sample from the tip of the main vine of a 20 day old plant was weighed and placed in a mortar in which 5 ml of absolute ethanol was added to submerge the leaf tissue for 30 minutes. The Excess ethanol was decanted and the leaves pulverized and transferred into labelled microcentrifuge tubes (1.5 ml) bearing numbers representing the accession codes. DNA zol reagent (750 µl) was then dispense into the tube and allowed to stand for five minutes. Chloroform (750 µl) added to the tube and allowed to stand for 5 minutes. The tube was then centrifuge at 10,000 ×g for 10 minutes and the supernatant transferred into a new labelled tube. Absolute ethanol (750 µl) was then added to the tube containing the supernatant and allowed for 5 minutes after which the tube was centrifuge at 5000 × g for 5 minutes. Seventy per cent ethanol (750 µl) added to re-suspend the pelletized DNA and allowed to stand for 5 minutes. The tube was centrifuge at 5000 × g for 5 minutes and the liquid portion was gently decanted leaving the pelletized and pure DNA. The tube was then air dried for 1 hour and stored in a freezer at -20°C for further use

2.3.2 DNA AMPLIFICATION

DNA Amplification of twenty (20) sweet potato accessions was carried out using the under listed procedures:

2.3.2.1 Selection of Primers

Five (5) primers listed in table 2 were used to amplify total genomic DNA by PCR.

2.3.2.2 Preparation of Primer working solution

The forward and reverse primers were constituted by transferring 50 µl of the forward and revers primers respectively into a new labeled tube using a micropipette. The mixture was then vortex for 10 seconds and stored in a refrigerator for further use.

Table 2: List of Primers and their Sequence

S/N	Primer Name	Forward Sequence	Reverse Sequence
1	IB02	CTGTGGATCTGTTCTTTGAACC	TTCCATGTGGAGTGTGAAGTAT
2	IBS139	CTATGACACTTCTGAGAGGCAA	AGCCTTCTTGTTAGTTTCAAGC
3	IBS166	TCCGTCTTTCTTCTTCTTCTTC	ATACACTAACTGCATCCAAACG
4	IBS199	TAACTAGGTTGCAGTGGTTTGT	ATAGGTCCATATACAATGCCAG
5	Ibu4	GGCTGGATTCTTCATATTTAGC	GCTTAATGGATCAGTAACACGA

2.3.2.3 Polymerase Chain Reaction

Polymerase Chain Reaction was performed using a thermocycler (Applied Biosystem version) in a touch down fashion and covered the following steps:Initiation: This was done at a temperature of 94°C for 5 minutes.Denaturation: This was carried out at a temperature of 94°C for 1 minute.Annealing: This was carried out at between 50.0 and 66.0°C (depending on the annealing temperature of the primer).Polymerization: This was done at a temperature of 72°C for 2 minutes.Step 2 to 4 was repeated for 30 cycles.Final extension: This was carried out at a temperature of 72°C for 5 minutes. Each tube

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loaded into the P.C.R machine temperature blocks contained G.E Healthcare premix (13µl), 1 µl of primers (forward and revers primers that have been constituted) and 1 µl of genomic DNA.

2.3.3 Agarose gel electrophoresis

Agarose gel electrophoresis was done using the following steps:

2.3.3.1 Preparation of gel

Five grams of agarose powder was weighted into an Erlenmeyer flask containing 500 mL of Trisbase Acetic acid and EDTA (TAE). The content of the flask was then swirled and the top covered with a paper towel. The flask was microwaved till the agarose powder was completely dissolved and the content of the flask crystal clear. This was allowed to cool a little and then 1.5 µl of Ethidium bromide (EtBr) was added to intercalate with the DNA and makes it visible as a band under UV- light.The content of the flask was emptied into a casting tray with combs already inserted. After cooling, the combs where removed and the casting tray was then placed in a gel thank containing adequate 10X Tris base Acetic acid and EDTA (TAE) buffer.

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2.3.3.2 Loading into wells

After PCR amplification, 1 µl of DNA loading dye was added into each of the tubes and then made to spin for 10 seconds. Ten µl of the content of the tubes was carefully loaded into separate wells using a micropipette. Ten µl of DNA ladder (100 kb) was then loaded into wells before each set of wells containing a set of DNA for each primer. The gel tank was covered properly and a constant volt (120 volts) was applied for 60 minutes

2.3.4 Gel Visualization Using U-V Light

After the DNA had separated, the gel was transferred to a Bench top transilluminator and the gel image was captured using a digital camera for scoring and analysis.

2.3.5 Data Analysis

The gel image formed was scored based on the intensity of bands formed using Microsoft Excel. Presence of band for each accession per primer was scored as 1 and absence as 0. The Excel file was then imported into Minitab 17 for further analysis

3 RESULTS AND DISCUSSION

3.1 MORPHOLOGICAL CHARACTERIZATION

3.1.1 Analysis of Variance

The results for the variability of measured traits as revealed by analysis of variance (Table 3) indicates strongly that traits with *P*-Values (<.001) are discriminatory and are important in distinguishing the accessions. Out of the 21 traits considered in this study and used as standard descriptors, 18 were useful as morphological markers. These 18 traits include: Plant Type, Internode Length, Predominant Color, Secondary Color, Vine Tip Pubescence, Shape of General Outline, Type of Leaf Lobes, Numbers of Leaf Lobe, Shape of Central Lobe, Size, Abaxial Leaf Vine Pigmentation, Foliage Color of Immature Leaf, Petiole Pigmentation, Petiole Length, Storage Root Shape, Storage Root Defects, Skin Color Predominant Color and Arrangement of Storage Roots.

3.1.2 Principal Components Analysis

The results for the magnitude of variability among the morphological characters scored as revealed by Principal Components Analysis are shown in table 4. Principal Component Analysis was able to capture 100% characters of the plants at the 16^{th} principal component. The first four principal components identified accounted for 72.1% of the total variation among the accessions. The first Principal Component accounted for 26.8% whereas the second, the third and the forth Principal Component axes accounted for 18.4, 15.2 and 11.7% respectively.

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Table 3: Variability of measured traits as revealed by analysis of variance

Variable	Count	Mean	SE Mean	Stdev	Variance	CoefVar	Sumofsquare	P. value
PT	20	6.200	0.551	2.462	6.063	39.72	884.000	<0.001
ID	20	7.500	0.199	0.889	0.789	11.85	1140.000	0.104
IL	20	4.700	0.442	1.976	3.905	42.05	516.000	<0.001
PC	20	4.900	0.552	2.469	6.095	50.38	596.000	<0.001
SC	20	3.750	0.446	1.997	3.987	53.25	357.000	<0.001
VTP	20	3.000	0.665	2.974	8.842	99.12	348.000	<0.001
SGO	20	4.850	0.302	1.348	1.818	27.80	505.000	<0.001
TLL	20	3.200	0.713	3.189	10.168	99.65	398.000	<0.001
NLL	20	2.700	0.482	2.155	4.642	79.80	234.000	<0.001
SCL	20	2.800	0.439	1.963	3.853	70.10	230.000	<0.001
SIZE	20	5.000	0.251	1.124	1.263	22.48	524.000	<0.001
ALP	20	4.850	0.758	3.392	11.503	69.93	689.000	<0.001
FCML	20	1.700	0.219	0.979	0.958	57.57	76.000	0.190
FCIL	20	4.100	0.684	3.059	9.358	74.61	514.000	<0.001
PP	20	4.450	0.545	2.438	5.945	54.79	509.000	<0.001
PL	20	2.900	0.270	1.210	1.463	41.71	196.000	<0.001
SRS	20	5.350	0.708	3.167	10.029	59.19	763.000	<0.001
SRD	20	1.200	0.296	1.322	1.747	110.16	24.0000	<0.001

SCPC	**20**	**3.600**	**4.260**	**1.903**	**3.621**	**52.86**	**72.0000**	**<0.001**
SC1	**20**	**2.050**	**1.700**	**0.759**	**0.576**	**37.03**	**41.0000**	**0.579**
ARR	**20**	**4.300**	**4.870**	**2.179**	**4.747**	**50.67**	**86.0000**	**<0.001**

Key: PT=Plant Type, ID= Internode Diameter, IL= Internode length, PC=Pigment Color, SC=Secondary Color, VTP=Vine Tip Pubescence, SGO=Shape of General Outline of matured leaves, TLL=Types of Leaf Lobes, NLL=Number of Leaf Lobes, SCL=Shape of Central Lobe, SIZE=Size of Mature Leaf, ALP=Abaxial Leaf Vein Pigmentation, FCML=Foliage color of Matured Leaf, FCIL=Foliage Color of Immature Leaf, PP=Petiole Pigmentation, PL=Petiole Length, SRS=Storage Root Shape, SRD=Storage Root Defects, SCPC=Skin Color Predominant Color, SCI=Skin Color Intensity, ARR=Arrangement of Storage Root

3.1.3 Cluster Analysis

From the hierarchical cluster analysis, the accessions were grouped into four (4) clusters based on their average linkage and the Euclidean test (Figure 1). Clusters I and II has a total of 13 accessions which are white and orange fleshed, while cluster III and IV consisted of 7 accessions which are all pink fleshed. Cluster I, II and IV has two accessions each from the National Root Crop Research Institute Abuja while cluster III consist entirely of accessions gotten from farmers. Cluster I is made up of 5 accessions (NC 1, NC 5, NC 11, NC 12 and NC 16) that have the same traits related to mature leaf shape and size such as: General Outline, Type of Leaf Lobes, Number of Leaf Lobes, Shape of Central Lobe and Size of Matured Leaf. Accessions in cluster III (NC 8, NC 10, NC 18, NC 20 and NC 14) have the same characteristic trait for Petiole Length and apart from accession NC 14 in this cluster; others share similar traits such as: Plant Type, Internode Diameter, Internode Length, Predominant Color of the vine, Foliage Color of Matured Leaf and Foliage Color of Immature Leaf. Accessions in cluster IV (NC 4 and NC 6) share same characteristics like Predominant Color of Storage Root while in contrast; accessions in cluster II (NC 2, NC 13, NC 3, NC 9, NC 7, NC 15, NC 17 and NC 19) share no specific uniform trait across the cluster. Accessions NC 1 and NC 5 share approximately 50% similarities as well as accessions NC 12 and NC 16. Accessions NC 2 and NC 13 are approximately 45% similar while accessions NC 3 and NC 9 are approximately 70% similar. The dendrogram also revealed that accessions NC 4 and NC 6 are approximately 40% similar but entirely dissimilar with all the other accessions.

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Table 4: Magnitude of variability revealed by Principal Components Analysis

3.1.4 Identification and Elimination of Duplicates

From the hierarchical cluster analysis (Figure 1), three pairs of duplicates were identified. Accessions NC

Variable	PC1	PC2	PC3	PC4
Plant Type	-0.091	-0.048	-0.447	0.101
Internode Diameter	0.043	0.102	-0.331	0.360
Internode Length	-0.098	-0.329	-0.196	0.167
Pigment Color	-0.041	-0.285	-0.317	-0.146
Secondary Color	0.265	0.124	-0.232	0.186
Vine Tip Pubescence		-0.174	-0.126	-0.262 -0.050
Shape of Gen. Outline of leaf	0.374	0.112	-0.017	-0.177
Types of Leaf Lobe	0.372	0.144	-0.025	-0.186
Num. of Leaf Lobe	0.387	0.120	0.039	-0.120
Shape of Central Lobe	0.335	0.166	-0.007	-0.225
Size of Matured Leaf	0.081	0.107	0.115	0.472
AbaxialLeaf Vine Pigmentation	0.252	-0.025	-0.017	0.201
FolliageCol. of Mat. Leaf	0.062	0.295	-0.345	-0.067
FolliageColor of Immature Leaf	-0.114	0.153	0.262	-0.306
Petiole Pigmentation		0.067	-0.207	-0.289 -0.273
Petiole Length	-0.001	0.326	0.109	0.271
Storage Root Shape	0.239	0.298	0.093	0.262
Storage Root Defects	-0.221	0.133	0.302	-0.051
Skin Color	-0.280	0.262	0.069	-0.208
Skin Color Intensity -	0.207	0.347	-0.045	0.060
Arrangement of storage roots		0.135	-0.352	0.154 -0.128
Eigen value	5.6318	3.8680	3.1898	2.4468
Proportion	0.268	0.184	0.152	0.117
Cumulative %	26.8	45.2	60.4	72.1

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7 (White fleshed from Benue State) and NC 15 (White fleshed from Nasarawa State) from two close states were found to be identical. Also, accessions NC 17 and NC 19 both white fleshed from Jos and Pada Bali respectively both in Plateau State were also identical. Furthermore, accession NC 18 (Pink fleshed from Jos) was found to be identical with accession NC 20 (Pink fleshed from Pada Bali) all in Plateau State. These duplicates were further subjected to a separate molecular characterization and the results obtained correlated with the results of the morphological characterization proving that these accessions were the same in all ramifications hence, the accessions were eliminated thereby reducing the accession number for final molecular characterization to sixteen (16)

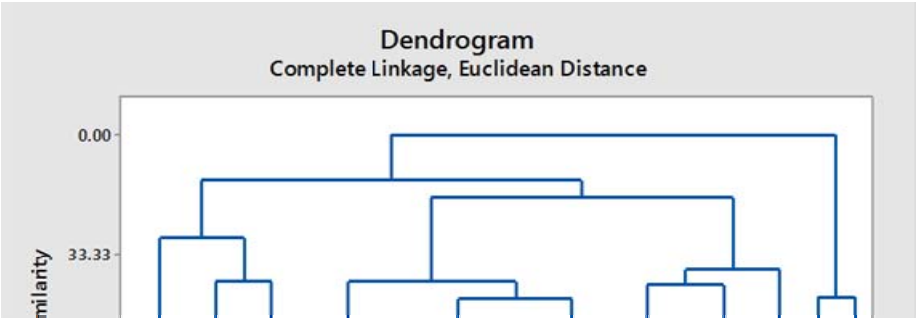

Figure 1: Cluster analysis

Key: 1=NC1, 2=NC2, 3=NC3, 4=NC4, 5=NC5, 6=NC6, 7=NC7, 8=NC8, 9=NC9, 10=NC10, 11=NC11, 12=NC12, 13=NC13, 14=NC14, 15=NC15, 16=NC16, 17=NC17, 18=NC18, 19=NC19 and 20=NC20

3.2MOLECULAR CHARACTERIZATION

3.2.1 Screening of sweet potato accessions with SSR markers

The gel image gotten for molecular markers showed the formation of monomorphic and polymorphic bands (Plate 1). The five primers used were able to show a high degree of amplification for all the accessions.

3.2.2 Scoring of bands from the gel image

Bands formed as seen in the gel image (Plate 1) where counted and scored using binary format (1 for presence of bands and 0 for absence of bands). Only clear and intense bands where scored.

3.2.3 Monomorphic and Polymorphic DNA bands

The number of monomorphic and polymorphic bands formed as a result of the amplification of DNA fragments is shown in table 5. A total of 46 monomorphic and 22 polymorphic bands were formed respectively thereby bringing the total number of bands formed (both monomorphic and polymorphic bands) to 80.

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3.2.4 Polymorphic Information Content

Polymorphic Information Content (PIC) for the DNA bands formed shows the usefulness of the primers used in revealing genetic diversity among the accessions. Table 6 shows that all the primers used were very informative as primer 1 (IB02) and 4 (IBS 199) shows 31.818% polymorphism respectively followed by primer 3 (IBS 166) with 27.273% polymorphism and then primer 2 (IBS 139) and primer 5 (Ibu 4) with

4.545% polymorphism respectively.

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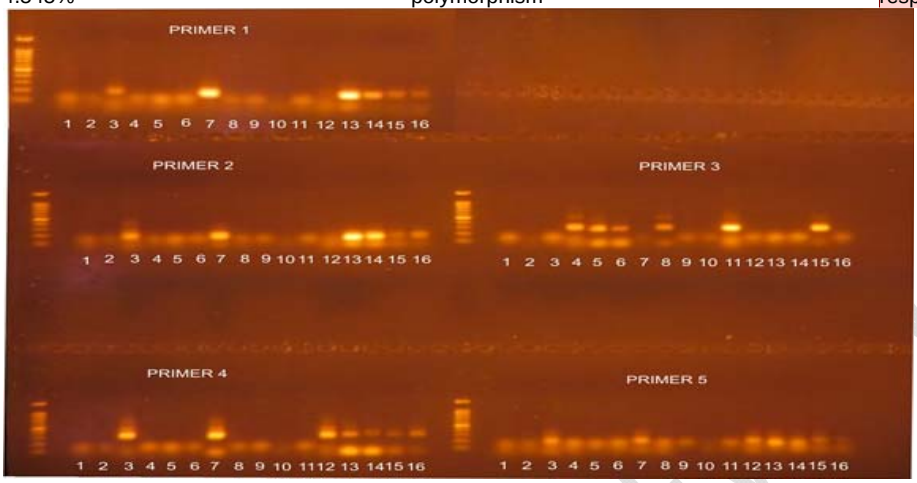

Plate 1: Gel Image showing amplification of DNA of 16 accessions of sweet potato wit SSR primers

Table 5: DNA Monomorphic and Ploymorphic bands

	No. of Amplified Fragments		
Primer	**Monomorphic bands**	**Polymorphic bands**	**Total**
SSR1	9	7	16
SSR 2	15	1	16
SSR 3	10	6	16
SSR 4	9	7	16
SSR 5	3	1	16
Total	**46**	**22**	**80**

Table 6: DNA Polymorphic Information Content

Primer	Polymorphic bands	% Polymorphism	Mean PIC
SSR1	7	31.81818182	0.398
SSR 2	1	4.545454545	0.0454
SSR 3	6	27.27272727	0.273
SSR 4	7	31.81818182	0.398
SSR 5	1	4.545454545	0.0454
Total	**22**	**100**	**1.124**

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3.2.5 Cluster Analysis

The dendrogram showing complete linkage and Euclidean distance for cluster analysis, showed three distinct clusters (Figure 2). Cluster I has a total of 8 accessions with 5 out of the 8 accessions originating from the National Root Crop Research Institute Abuja. Cluster II has a total of 7 accessions with one out of the 7 accessions also originating from the National Root Crop Research Institute Abuja while cluster III has just a single accession which originated from Otukpo in Benue State. The accessions in all the clusters share no specific morphological characteristics in common. Accessions NC 1, NC 2, NC 9, NC 4, NC 5, NC 6, NC 8 and NC 11 are approximately 65 % similar as well as accessions NC 3, NC 7, NC 12, NC 13 and NC 15. All the accessions in cluster I and cluster II are approximately 30% similar while accession NC 10 is not similar to any other accession and could possibly be a hybrid.

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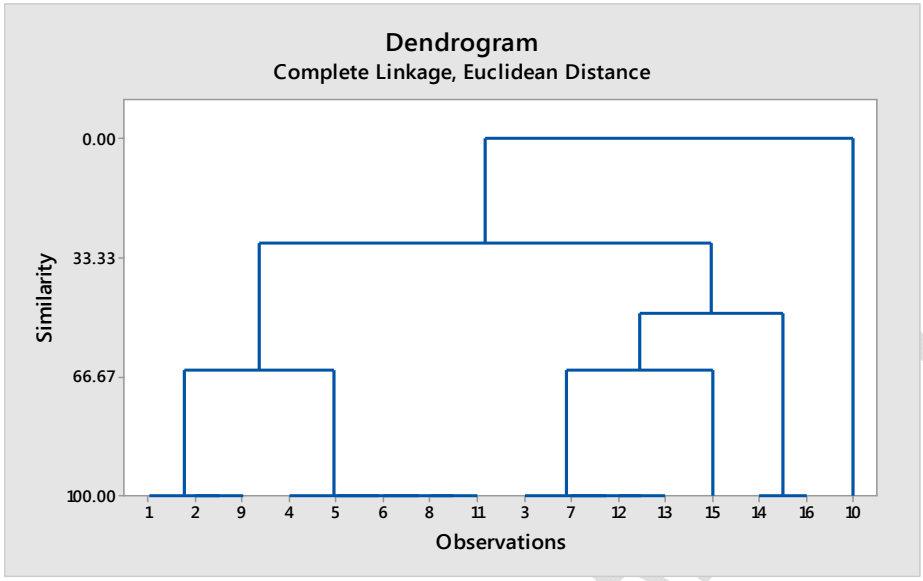

III

I II

Figure 2: Dendrogram for molecular analysis

3.2.6 Identification of Duplicates

From the dendrogram (Figure 2), accessions NC 1(Mothers Delight), NC 2 (King J) and NC 9 (Arigenge 1) are all identical. Also, accession NC 4 (NR 8164), NC 5 (DanZaria), NC 6 (87/TIS 0087), NC 8 (Atsaka 2) and NC 11 (Odumu 1) are all identical. Furthermore, accessions NC 3 (Butter Milk), NC 7 (Atsaka 1), NC 12 (Odumu 2), and NC 13 (Dankalin Mina 1) are all identical and accessions NC 14 (Dankalin Mina 2) and NC 16 (DankalinLafia 2) are also duplicates. This therefore reduces the accession number from 16 to 6.

Comment [HS23]: Replace with were

Comment [HS24]: Replace with were

Comment [HS25]: Replace with were

Comment [HS26]: Replace with were

3.3 COMPARISON BETWEEN MORPHOLOGICAL AND MOLECULAR DATA

Based on the morphological characters studied, the 20 accessions were grouped into 4 clusters with discriminatory significant *P*values of <.001 while molecular characterization gave rise to only 3 clusters. The use of morphological data in the identification of duplicates and further subjecting the duplicates to separate molecular analysis reduced the number of accessions from 20 to 16. However, considering the dendrogram obtained from molecular data, the 16 accessions was further reduced to 6 which indicates that a very low diversity exist among the accessions. Accessions NC 1 and NC 5 which shows an approximately 50% similarities with morphological characterization, however shows an approximately 66.67% similarities with molecular characterization. Accessions NC 1, NC 2, NC 9, NC 4, NC 5, NC 6, NC 8 and NC 11 which are scattered across four clusters based on morphological data, were however clustered together based on molecular data. Accessions NC 1, NC 2 and NC 9 which according to morphological data share no similarities and even belong to different clusters are however 100% similar and considered duplicates based on molecular data. Accessions NC 4, NC 5, NC 6, NC 8 and NC 11, NC 3, NC 7, NC 12 and NC 13, NC 4 and NC 16 which belongs to different clusters as revealed by morphological data are however different sets of duplicates respectively as revealed by molecular data. Accession NC 15 which according to morphological data is approximately 50% similar to accession NC 3 appears to be approximately 66.67% similar according to molecular data. Accession NC 10 which shares approximately 80% similarities with accession NC 8 as revealed by morphological data however shares no similarities with accession NC 8 or any of the other accessions characterized as revealed by molecular data.

Comment [HS27]: Replace with results

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Comment [HS30]: Replace with were

Comment [HS31]: Replace with were

Diversity analysis of sweet potato accessions using Morphological descriptors have been done in various parts of the world and the observed similarities or differences have been attributed to various factors like sample size, number and type of descriptors used, the origin of accessions and the method of analysis. [21]using 40 morphological descriptors in Uganda on 1256 accessions, reported that 20 out of the 40 morphological descriptors used were discriminatory. These 20 descriptors were also found to be among the 18 descriptors that have been reported to be discriminatory and useful in distinguishing between the accessions in this analysis, although [6] in his own Morphological and agronomical characterization of different accessions of sweet potato (*Ipomoea batatas*) in Cameroon, reported that even though the above morphological descriptors are discriminatory, only a few of them are important in taxonomic differentiation of accessions. In agreement with this study, predominant skin colour, commonly used in the identification of cultivars in farmers' fields in Burkina Faso [15] and also reported to be discriminatory by [21] was also found to be useful in distinguishing between the accessions in this present analysis although this is contrary to the findings of [15]. In Kenya, [13] identified general outline of leaf and the shape of central leaf lobe as two morphological descriptors that differentiated among 89 accessions and separated them into two clusters. This is in line with the findings of this analysis as the two descriptors identified are part of the 18 descriptors that are reported as discriminatory. Also, [6] further reported that there was a significant variation among sweet potato accessions in Cameroon for morphological characters such as petiole length, internode diameter, leaf area, leaf size and internode length (*P*<.001). This observation is in line with the findings in this study as the *P* values for these characters were significant. In agreement with the findings of [21], [13], [6] and [15], the results from Clusters Analysis and Principal Component Analysis for this current analysis also showed a high level of variability among the different accessions.

Results from similar studies using Simple Sequence Repeats markers in sweet potato diversity analysis have been reported and most of the differences in results have been ascribed to sample size, the number of Simple Sequence Repeats markers used and the source of materials. In this current analysis, low

genetic diversity was observed among the accessions. This is in line with the findings of [19] in Java who reported similarity ranging from 15 to 78% between Indonesian accessions. The reason for the low diversity has been attributed to narrow geographic zone of collection of the cultivars. In contrast to the findings of this current analysis, moderate genetic diversity values have been reported in Uganda by [22] among 192 accessions, using 10 Simple Sequence Repeats markers, [15] in his own study revealed that sweet potato germplasm in Burkina Faso presented moderate to high diversity based on molecular and phenotypic assessment approaches, high Simple Sequence Repeats-based diversity has also been reported in China by [17] where the Jaccard's coefficient of similarity ranging from 0.400 to 0.938 was observed and [3] reported a high level of polymorphism which indicated a wide genetic diversity among the 40 sweet potato accessions tested with the 10 Simple Sequence Repeats primer pairs in India. The weak agreement between the morphological based data and the Simple Sequence Repeats based data was also confirmed by different duplicates identified by each of these approaches. The findings of the present study are in agreement with those of [13] in Kenya who compared morphological and SSR-based evaluation of diversity. The reason for this weak agreement could be as a result of the independent nature of morphological and molecular variations. From the molecular characterization, accession NC 10 showed distinct characteristics from the other accessions and occupied a different cluster all by itself. This shows that this accession may be a hybrid with distinct genetic characters. The results from this study has shown that Simple Sequence Repeats markers as one of the most widely used molecular markers in recent years are more advantageous over morphological markers as they are highly polymorphic, highly abundant, genetically co-dominant, and analytically simple.

4 CONLUSION

A lot of duplicates exist among the sweet potato accessions proving that there is a very low level of sweet potato genetic diversity existing in the North Central region of Nigeria because out of the 20 accessions, genetic characterization gave rise to only 6 distinct genetic accessions among which is a hybrid. It is therefore necessary to recommend that high priority be given to further collect and/or introduce divergent sweet potato materials in the region and also in Research Institute, since variation in the collections is needed for a successful breeding program. Also, more primers should be used for molecular characterization of sweet potato in North Central Nigeria in order to have more comprehensive details of the variability and similarities that exist among the accessions in the region.

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