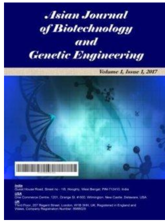

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Diversity Analysis of Sweet Potato (*Ipomoea batatas* [L.] lam) Accessions from North Central Nigeria Using Morphological and Simple Sequence Repeats Markers

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Authors' contributions

This work was done in collaboration among the Authors. Author UJA designed the study, did literature searches, carried out the field and laboratory experiments, wrote the protocol, performed statistical analysis, managed the analyses of the study and wrote the first draft of the manuscript. Authors CCI and CUA supervised the study while all the authors read and approved the final manuscript.

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ABSTRACT

Aims: Genetic diversity analysis was carried out with the aim of assessing the genetic similarities and variability that existed 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

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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 were significant variation for 18 out of the 21 morphological characters studied among the sweet potato accessions, with the first 4 Principal Components accounting for 72.1% of the total variations 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 clusters based on their average linkages 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 characterization. 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 in 1 (IB02) and 4 (IBS 199). For cluster analysis, three distinct clusters were observed with all the accessions in cluster I and II which were approximately 30% similar while accession NC 10 which stood alone in cluster III shared no similarity with any of the other accessions 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 indicated that a very low diversity existed among the accessions.
Conclusion: This study established that a lot of duplicates existed among the sweet potato accessions indicating that there is a very low level of sweet potato genetic diversity in the North Central region of Nigeria.

Keywords: Accessions; diversity analysis; morphological characterization; molecular characterization; simple sequence repeats; sweet potato.

1. INTRODUCTION

Sweet potato (*Ipomea batatas* [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 [1]. 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 [1]. Generally, sweet potatoes are consumed because their nutrients contain beta carotene that prevents vitamin A deficiency in many developing countries [2].

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 while in South America; Peruvian sweet potato remnants dating as far back as 8000 BC have been found [3]. Sweet potatoes are cultivated throughout tropical and warm temperate regions wherever there is sufficient water to support their growth [3].

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 by [4] 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 [4].

As a scientific discipline, morphological characterization was originated by Goethe in 1790 as reported by [5]. The characterization is carried out on a representative population of an accession using a list of descriptors for the species [5]. Principal Component Analysis (PCA) of the characterization results which identifies a few key or minimum descriptors that effectively accounts for the majority of diversity observed is usually performed with this analytical method. This saves time and effort for future characterization [6]. 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 [6,5]. However, there are limitations that are associated with morphological markers [5]. 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 [5]. Furthermore, 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 [7] and the first step in the assessment of plant diversity [8].

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 [9]. They are often referred to as short tandem repeats (STRs) by forensic geneticists, or as simple sequence repeats (SSRs) by plant geneticists [10]. They are a crucial tool in the field of population genetics [11] and have been proposed for use as markers to assist plant breeders in marker assisted selection [12]. 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 [13]. It can be described as a variation (which may arise due to mutation or alteration in the genomic loci) that can be observed [13]. 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 [13]. The molecular marker techniques are based on naturally occurring polymorphisms in DNA sequences [13]. Its concept was started in the nineteenth century by Gregor Mendel who employed phenotype based genetic markers in his experiments [5]. Later on, phenotype based genetic markers for Drosophila melanogaster led to the founding of the theory of genetic linkage [5]. In General, Molecular markers are well established and their applications as well as limitations have been realized [5]. They offer numerous advantages over conventional phenotype characterization because they are stable and detectable in all tissues regardless of growth differentiation, development, pleiotropic effect, epistatic effects and not confounded to environment where they grow [5].

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 [7,14,15], and apart from its usage on the sweet potato plant, morphological characterization has also has been used extensively on various crop plants diversity assessments in various parts of the world [7,16]. Also, during the last decade, a lot of molecular information has been gathered on the sweet potato plant and this information has also been used as a means of molecular characterization of the genetic diversity assessment of the plant germplasm [17]. 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 [18,19,7]. These markers are highly polymorphic, co-dominant, and can easily be detected on high-resolution gels [8]. Of all the molecular makers employed, Expressed Sequence Tags-Simple Sequence Repeats (EST-SSR) and Simple Sequence Repeats (SSR) markers have been reported to be efficient in evaluating genetic diversity and potato germplasm characterization among different geographical regions [20]. According to [21] extensive diversity exists among cultivated sweet potato both inter or intra variety accessions in morphological, physiological, and agronomic traits and a wide range of diversity in cultivated sweet potato at the molecular level among accessions can be accessed using Simple Sequence Repeats primers which are the bases for modern cultivar improvements by hybridization.

Information on the diversity of sweet potato in Nigeria is scanty. It is possible that the North Central region of Nigeria has diverse varieties of sweet potatoes that the analysis of their genetic make-up will contribute immensely to the food security program of the country by providing

baseline information for plant breeders to utilize in developing better varieties with high yielding capacities and disease resistance. Hence, the present study was aimed at employing Morphological and Simple Sequence Repeats markers to determine the genetic diversity among Sweet potato accessions in the North Central region of the country to provide the necessary information that is largely lacking at present for improved sweet potato production that will guarantee food sufficiency and security.

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 obtained from the National Root Crop Research Institute (NRCRI) Abuja while the rest were collected 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 [22]. Twenty-one (21) descriptors as shown in Table 2 were used in this study.

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 1 meter and intra-ridge distance of 0.5 meters was allowed for all the ridges constructed. Five (5) replicates for all the accessions were 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 2 kg 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.

2.2.2 Data collection

Data for 21 characters (Table 2) used for morphological characterization 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 [23] and reported by [22] using a meter rule and tape. The

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	Dankalin Lafia 1	Lafia	Nasarawa
NC 16	Dankalin Lafia 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

Table 2. List of morphological descriptors and their assigned values

S/N	Morphological Characters	CIP Assigned Values
1	Plant Type	3-Erect (<75 cm); 5-Semi-compact (75-150 cm); 7-Spreading (151-250cm); 9-Extremely spreading (>250 cm)
2	Vine Internode Diameter	1-Very thin (<4mm); 3-Thin (4-6 mm); 5-Intermediate (7-9 mm); 7-Thick (10-12 mm); 9-Very thick (> 12 mm)
3	Vine Internode Length	1-Very short (< 3 cm); 3-Short (3-5 cm); 5-Intermediate (6-9 cm); 7-Long (10-12 cm); 9-Very long (> 12 cm)
4	Vine Pigmentation (Predominant Color)	1-Green; 3-Green + few purple spots; 4-Green + many purple spots; 5-Green + many dark purple spots; 6-Mostly purple; 7-Mostly dark purple; 8-Totally purple; 9-Totally dark purple
5	Vine Pigmentation (Secondary Color)	0-Absent; 1-Green base; 2-Green tip; 3-Green nodes; 4-Purple base; 5-Purple tip; 6-Purple nodes; 7-Other
6	Vine Tip Pubescence	0-None; 3-Sparse; 5-Moderate; 7-Heavy; 9-Very heavy
7	General Leaf Outline	1-Rounded; 2-Reniform; 3-Cordate; 4-Triangular; 5-Hastate; 6-Lobed; 7-Almost divided
8	Mature Leaf Shape (Types of Leaf Lobes)	0-No lateral lobes (entire); 1-Very slight (teeth); 3-Slight; 5-Moderate; 7-Deep; 9-Very deep
9	Mature Leaf Shape (Number of Leaf Lobes)	0; 1; 3; 5; 7; 9
10	Shape of Central Lobe	0-Absent; 1-Teeth; 2-Triangular; 3-Semi-circular; 4-Semi-elliptic; 5-Elliptic; 6-Lanceolate; 7-Oblanceolate; 8-Linear (broad); 9-Linear (narrow)
11	Abaxial Leaf Vein Pigmentation	1-Yellow; 2-Green; 3-Purple spot at base of main rib; 4-Purple spots in several veins; 5-Main rib partially purple; 6-Main rib mostly or totally purple; 7-All veins partially purple; 8-All veins mostly or totally purple; 9-Lower surface and veins totally purple
12	Mature Leaf Size	3-Small (< 8 cm); 5-Medium (8-15 cm); 7-Large (16-25 cm); 9-Very large (> 25 cm)
13	Foliage Color (Mature Leaf Color)	1-Yellow-green; 2-Green; 3-Green with purple edge; 4-Greyish (heavy pubescence); 5-Green with purple veins on upper surface; 6-Slightly purple; 7-Moderately purple; 8-Mostly purple; 9-Totally purple
14	Foliage Color (Immature Leaf Color)	1-Yellow-green; 2-Green; 3-Green with purple edge; 4-Greyish (heavy pubescence); 5-Green with purple veins on upper surface; 6-Slightly purple; 7-Moderately purple; 8-Mostly purple; 9-Totally purple
15	Petiole Pigmentation	1-Green; 2-Green with purple near stem; 3-Green with purple near leaf; 4-Green with purple at both sides; 5-Green with purple spots; 6-Green with purple stripes; 7-Purple with green near leaf; 8-Some petioles purple, others green; 9-Totally or mostly purple
16	Petiole Length	1-Very short (less than 10 cm); 3-Short (10-20 cm); 5-Intermediate (21-30 cm); 7-Long (31-40 cm); 9-Very long (more than 40 cm)
17	Storage Root Shape	1-Round; 2-Round elliptic; 3-Elliptic; 4-Ovate; 5-Obovate; 6-Oblong; 7-Long oblong; 8-Long elliptic; 9-Long irregular or curved
18	Storage Root Arrangement	1-Closed cluster; 3-Open cluster; 5-Disperse; 7-Very disperse
19	Storage Root (Predominant color)	1-White; 2-Cream; 3-Yellow; 4-Orange; 5-Brownish orange; 6-Pink; 7-Red; 8-Purple-red; 9-Dark purple
20	Storage Root (Skin Color Intensity)	1-Pale; 2-Intermediate; 3-Dark
21	Storage Root Defects	0-None; 1-Alligator-like skin; 2-Veins; 3-Shallow horizontal constrictions; 4-Deep horizontal constriction; 5-Shallow longitudinal groove; 6-Deep longitudinal groove; 7-Deep constrictions, deep grooves; 8-Others

Characters were scored using a scale of 0 to 9 and used for characterization based on the standard descriptors in CIP guide 36 as shown in Table 2.

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 [24] with slight modifications. The protocol was carried out as follows: One gram of fresh and healthy leaf 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. Excess ethanol was then decanted and the leaves pulverized in the mortar and transferred into labelled microcentrifuge tubes (1.5 ml) bearing numbers representing the accession codes. DNA zol reagent (750 µl) was then dispensed into the tubes and allowed to stand for five minutes. Chloroform (750 µl) was then added to the tubes and allowed to stand for 5 minutes. The tubes were then centrifuged at 10,000 ×g for 10 minutes and the supernatant transferred into a new labelled tubes. Absolute ethanol (750 µl) was then added to the tubes containing the supernatant and allowed for 5 minutes after which the tubes were centrifuged at 5000 × g for 5 minutes. Seventy per cent ethanol (750 µl) was then added to re-suspend the pelletized DNA and allowed to stand for 5 minutes. The tubes were then centrifuged at 5000 × g for 5 minutes and the liquid portion was gently decanted leaving the pelletized and pure DNA. The tubes were 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 3 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.

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: (i) Initiation: This was done at a temperature of 94°C for 5 minutes. (ii) Denaturation: This was carried out at a temperature of 94°C for 1 minute. (iii) Annealing: This was carried out at between 50.0 and 66.0°C (depending on the annealing temperature of the primer). (iv) Polymerization: This was done at a temperature of 72°C for 2 minutes. (v) Steps 2 to 4 were repeated for 30 cycles. (vi) Final extension: This was carried out at a temperature of 72°C for 5 minutes.

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 weighed into an Erlenmeyer flask containing 500 mL of Tris base 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. The flask content 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.

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 using a centrifuge. Ten µl of the content of the tubes was then carefully loaded into separate wells using a micropipette.

Table 3. 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

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 using a micropipette. The gel tank was then 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 package. 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 software 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 4) indicated strongly that traits with *P*-values (<.001) were discriminatory and were 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 included: 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 5. Principal Component Analysis was able to capture 100% characters of the plants at the 16th principal component. The first four principal components identified accounted for 72.1% of the total variations among the accessions. The first Principal Component accounted for 26.8%, the second principal component accounted for 18.4%, the third principal component accounted for 15.2% while the forth Principal Component accounted for 11.7%. The first Principal Component with reference to its high value (26.8%) was positively associated with traits related to mature leaf such as Shape of General Outline, Type of Leaf Lobes, Numbers of Leaf Lobes and Shape of Central Lobes. The second Principal Component was positively associated with petiole and storage root characteristics such as Petiole Length and Skin Color Intensity. The third Principal Component was positively associated with storage root characteristics only (Storage Root Defects), while the forth Principal Component was positively associated with traits related to the vines (Internode Diameter). Positive Eigen values accounted for similarities across the components while negative Eigen Values accounted for dissimilarities across the components. In the first three Principal Components, Plant Type (PT) and Internode Length (IL) were dissimilar and accounted for variation among the accessions while in the first four Principal Components, Pigment Color (PC) and Vine Tip Pubescence (VTP) were dissimilar and also accounted for variation among the accessions (Table 5).

Table 4. Variability of measured traits as revealed by analysis of variance

Variable	Count	Mean	SE Mean	St dev	Variance	Coef Var	Sum of square	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	0.215	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
SCI	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*

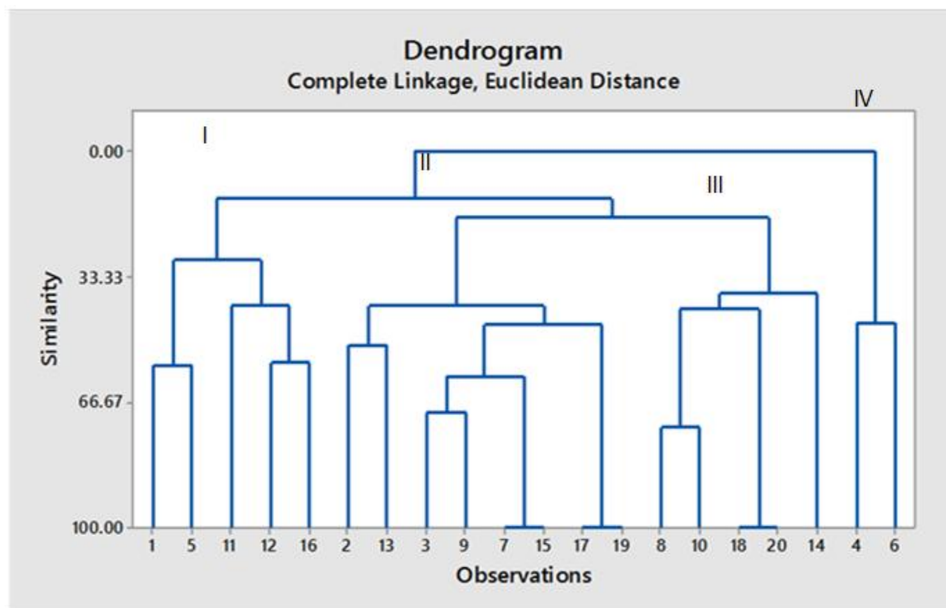

Fig. 1. Cluster analysis

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

Table 5. Magnitude of variability revealed by principal components analysis

Variable	PC 1	PC 2	PC 3	PC 4
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 General Outline of Matured Leaf	0.374	0.112	-0.017	-0.177
Types of Leaf Lobes	0.372	0.144	-0.025	-0.186
Number of Leaf Lobes	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
Abaxial Leaf Vein Pigmentation	0.252	-0.025	-0.017	0.201
Foliage color of Matured Leaf	0.062	0.295	-0.345	-0.067
Foliage Color 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 Predominant Color	-0.280	0.262	0.069	-0.208
Skin Color Intensity	-0.207	0.347	-0.045	0.060
Arrangement of Storage Root	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	42.2	60.4	72.2

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 (Fig. 1). Clusters I and II had a total of 13 accessions which were white and orange fleshed, while cluster III and IV consisted of 7 accessions which are all pink fleshed. Cluster I, II and IV had two accessions each from the National Root Crop Research Institute Abuja while cluster III consist entirely of accessions obtained from farmers. Cluster I was made up of 5 accessions (NC 1, NC 5, NC 11, NC 12 and NC 16) that had 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) had the same characteristic trait for Petiole Length and apart from accession NC 14 in this cluster; others shared 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) shared 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) shared no specific uniform trait across the cluster. Accessions NC 1 and NC 5 shared approximately 50% similarities as well as accessions NC 12 and NC 16. Accessions NC 2 and NC 13 were approximately 45% similar while accessions NC 3 and NC 9 were approximately 70% similar. The dendrogram also revealed that accessions NC 4 and NC 6 were approximately 40% similar but entirely dissimilar with all the other accessions (Fig. 1).

3.1.4 Identification and elimination of duplicates

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

3.2 Molecular 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 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 are shown in Table 6. A total of 46 monomorphic and 22 polymorphic bands were formed thereby bringing the total number of bands formed (both monomorphic and polymorphic bands) to 80 (Table 6).

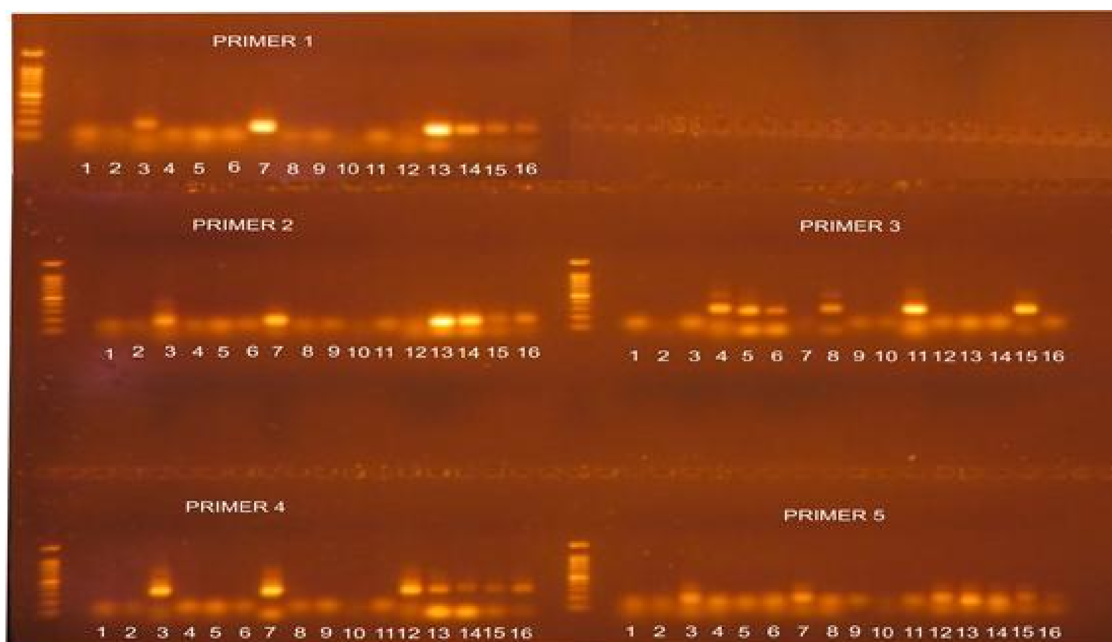

Plate 1. Gel Image showing amplification of DNA of 16 accessions of sweet potato wit SSR primers

Table 6. 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**

3.2.4 Polymorphic information content

Polymorphic Information Content (PIC) for the DNA bands formed showed the usefulness of the primers used in revealing genetic diversity among the accessions (Table 7). Table 7, showed that all the primers used were very informative as primer 1 (IB02) showed 31.818% polymorphism which is the same value with primer 4 (IBS 199), followed by primer 3 (IBS 166) with 27.273% polymorphism and then primer 2 (IBS 139) and primer 5 (Ibu 4) with the same value of 4.545% polymorphism each.

3.2.5 Cluster analysis

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

3.2.6 Identification of duplicates

From the dendrogram (Fig. 2), accessions NC 1(Mothers Delight), NC 2 (King J) and NC 9 (Arigenge 1) were all identical. Similarly, accession NC 4 (NR 8164), NC 5 (DanZaria), NC 6 (87/TIS 0087), NC 8 (Atsaka 2) and NC 11 (Odumu 1) were all identical. Furthermore, accessions NC 3 (Butter Milk), NC 7 (Atsaka 1), NC 12 (Odumu 2), and NC 13 (Dankalin Mina 1) were all identical and accessions NC 14 (Dankalin Mina 2) and NC 16 (Dankalin Lafia 2) were also duplicates.

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. [15] 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 study, although [7] in his own Morphological and agronomical characterization of different accessions of sweet potato (I*pomoea batatas*) in Cameroon, reported that even though the above morphological descriptors were discriminatory, only a few of them were 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 [8] and also reported to be discriminatory by [15] was also found to be useful in distinguishing between the accessions in this present study although this is contrary to the findings of [8] who reported that predominant skin colour was not discriminatory and hence cannot be used in distinguishing between accessions. In Kenya, [18] showed that general outline of leaf and the shapes of central leaf lobe were two morphological descriptors that differentiated among 89 accessions and separated them into two clusters. This is in line with the findings of this study which also demonstrated that the two descriptors identified were part of the 18 descriptors that have been reported as discriminatory in this study. Also, [22] 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) which also agrees with the findings in this study in that the P values for these characters were also significant. In agreement with the findings of [15,18,22,8,25], the results from Cluster 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 study, low genetic diversity was observed among the accessions which could probably be due to the fact that all the accessions used in this study were obtained in the same geographical region and few Simple Sequence Repeats markers (5) were used. This

is in tandem with the findings of [17] who reported similarity values ranging from 15 to 78% between Indonesian accessions and attributed the low diversity observed in their study to narrow geographic zone of collection of the cultivars. In contrast to the low diversity observed in this current study, moderate genetic diversity values were reported among 192 accessions in Uganda using 10 Simple Sequence Repeats markers [19]. Similarly, [8] in their own study revealed that sweet potato germplasm in Burkina Faso presented moderate to high diversity values based on molecular and phenotypic assessment approaches. This finding is in tandem with those of [7] who reported a very high diversity value among sweet potato accessions in China where the Jaccard's coefficient of similarity ranged from 0.400 to 0.938 using Simple Sequence Repeats-based markers. Also, [23] reported a high level of polymorphism among 40 sweet potato accessions tested with 10 Simple Sequence Repeats primer pairs in India.

Based on the 21 morphological characters that were considered in this current study, the 20 accessions were grouped into 4 clusters with discriminatory significant *P* values of <.001 while molecular characterization grouped the accessions into 3 clusters only. The use of morphological characterization in the identification of duplicates and further subjecting the duplicates to separate molecular analysis reduced the number of accessions in this study from 20 to 16. However, considering the dendrogram obtained from molecular characterization, the 16 accessions were further

Table 7. 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**

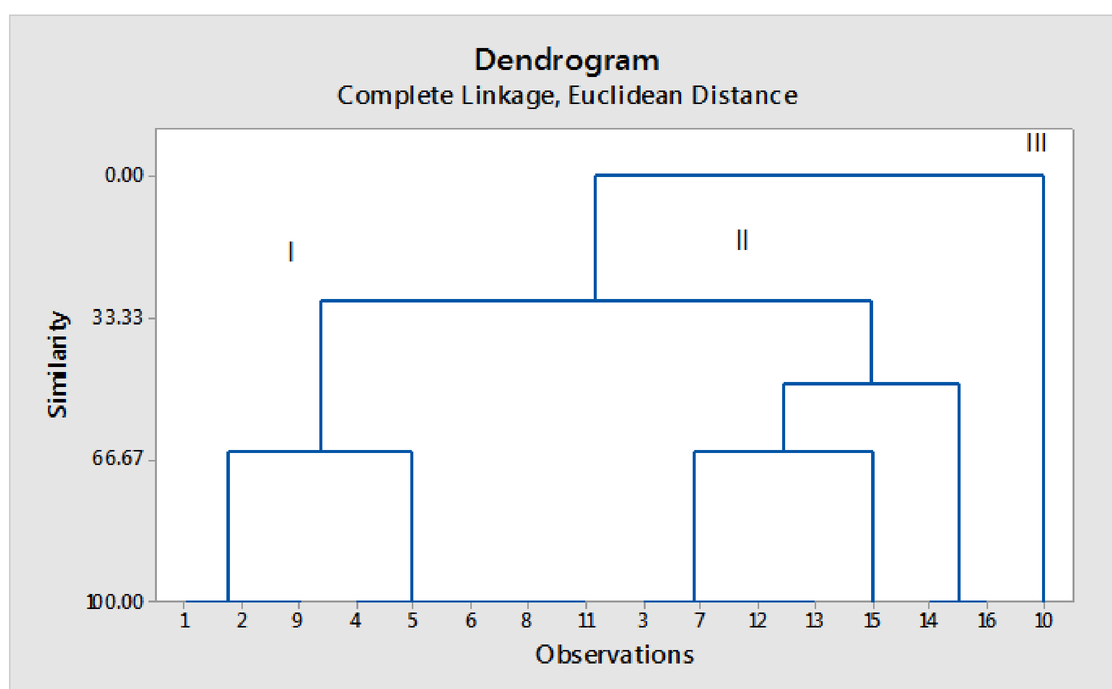

Fig. 2. Dendrogram for molecular analysis

reduced to 6 which indicated that a very low level of diversity existed among the accessions. Accessions NC 1 and NC 5 which showed an approximately 50% similarities with morphological characterization, were however approximately 66.67% similar based on molecular characterization. Accessions NC 1, NC 2, NC 9, NC 4, NC 5, NC 6, NC 8 and NC 11 which were scattered across four clusters based on morphological characterization, were however clustered together based on molecular characterization. Accessions NC 1, NC 2 and NC 9 which according to morphological characterization shared no similarities and even belonged to different clusters were however 100% similar and were therefore considered duplicates based on molecular characterization. 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 belonged to different clusters as revealed by morphological analysis were however different sets of duplicates respectively as revealed by molecular analysis. Accession NC 15 which according to morphological analysis was approximately 50% similar to accession NC 3 appeared to be approximately 66.67% similar according to molecular analysis. Accession NC 10 which shared approximately 80% similarities with accession NC 8 as revealed by morphological analysis, however shared no similarities with accession NC 8 or any of the other accessions characterized as revealed by molecular analysis. The poor correlation between the morphological based characterization and the Simple Sequence Repeats based characterization as seen in this study was also confirmed by different duplicates identified by each of these approaches as reported by [18] in Kenya who compared morphological and SSR-based characterization in the evaluation of sweet potato diversity and concluded that the reason for this poor correlation between the two characterization methods could be as a result of the independent nature of morphological and molecular variations.

From the information gotten in this study from molecular characterization, accession NC 10 showed distinct characteristics from the other accessions and occupied a different cluster all by itself. This showed that this accession may be a hybrid with distinct genetic characteristics. Also, the results from this study has shown that Simple Sequence Repeats markers which is 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 codominant, and analytically simple [18].

4. CONLUSION

This study established that a lot of duplicates existed among the sweet potato accessions indicating that there is a very low level of sweet potato genetic diversity in the North Central region of Nigeria. This is because out of the 20 accessions, genetic characterization gave rise to only 6 distinct genetic accessions among which is a possible hybrid. However, the results obtained in this analysis will serve as a guide for germplasm management and improvement of the current breeding strategies and for the release of new cultivars and commercial exploitation of sweet potato in Nigeria especially in the North Central Region. The usefulness of the 18 morphological descriptors and 5 Simple Sequence Repeats markers in differentiating the accessions in this study could serve as a guide in subsequent studies. Despite the poor correlation between morphological and molecular markers characterization, both techniques can be used effectively in sweet potato characterization. This can be achieved by constituting core collections based on Simple Sequence Repeats data and using the 18 phenotypic characters in distinguishing the cultivars in the field.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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