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ORIGINAL RESEARCH ARTICLE

Agronomic and Molecular Characterization of Sweet Potato (*Ipomoea batatas* [L.] Lam) Varieties in Nigeria

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ABSTRACT
Sweet potato (*Ipomoea batatas* [L.] Lam.); 2n = 6x = 90) is a significant root crop globally. However, breeding and cross-breeding of the crop are difficult due to its enormous, complex genome and highly heterozygous hexaploid genetic makeup. This study used data on agronomic traits and morphological and molecular characterization to determine the best parental combinations for improved breeding and cross-breeding of the crop. Agronomic traits analysis and morphological characterization were carried out in the research farm while molecular analysis was done in the molecular biology laboratory. Results of the study show significant variations (p-value = 0.000) and a strong positive correlation among the majority of the agronomic traits and morphological characters, principal component analysis shows 100% variability at the 5th principal component, a dendrogram of agronomic traits analysis shows that TIS 8164 and TIS 0087/087 varieties are about 76% similar and Dixon variety is approximately 66.67% similar to the Umuspo 1 and the Butter Milk varieties, morphological characterization shows 37% similarity between the Umuspo 1 variety and the TIS 8164 variety, polymorphic information content shows polymorphism of 42.86% for IBS166 primer, 28.5% for IB02 primer and 14.29% for IBS199 and Ibu4 primers respectively, a dendrogram of molecular characterization shows no similarity between the Umuspo 1 variety and the other variety and 100% similarity between the TIS 8164 and the TIS 0087/087 varieties. The study concluded that the Nwaoyinma and the Umuspo 1 varieties are good parental combinations.

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Agronomic traits, dendrogram, molecular markers, morphological markers, parental combination

INTRODUCTION
As reported by the United Nations Food and Agriculture Organization (FAO, 2019), Sweet potato (*Ipomoea batatas* [L.] Lam.), 2n = 6x = 90 is one of the most significant root crops grown globally, with significant cultivation, marketing, and usage in practically every ecological zone in Nigeria (FAOSTAT, 2020). Nigeria leads in sweet potato production in Africa with 3.46 million metric tons; and is surpassed only by China worldwide (Afolabi *et al.*, 2023).

In developing nations in particular, sweet potatoes have emerged as an inexpensive source of flavonoids, fibre, calories in diets, protein, vitamins, and minerals due to their high yield potential, dry matter content and starch content, and adaptability to a variety of environmental circumstances (Kurabachew, 2015; Kitahara *et al.*, 2017; Gemenet *et al.*, 2020; Yan *et al.*, 2022). Additionally, to being grown for human consumption (storing the roots and leaves) and to feed animals, sweet potato is also used as a raw material in industries to make natural colours, starch, and alcohol (Katayama *et al.*, 2017; Yan *et al.*, 2022).

Agronomic characteristics, of which the most significant is storage root, account for the wide-range applications of sweet potato (Yan *et al.*, 2022), and numerous Quantitative Trait Loci related to yield and/or the numbers of storage roots in individual plants have been reported by Li *et al.* (2014), Yada *et al.* (2017), Okada *et al.* (2019), da Silva Pereira *et al.* (2020), Chen *et al.* (2021) and Suematsu *et al.* (2021). Apart from storage root characteristics, other agronomic traits, such as leaf length, internode length, internode diameter, etc., have been used in sweet potato diversity studies to eliminate duplicate accessions (Alfred *et al.*, 2019).

Apart from the use of agronomic characters, sweet potato diversity has traditionally been described using

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morphological indicators reported by Koussao *et al.* (2014), Liu *et al.* (2020), Nawaz and Chung (2020) and Mores *et al.* (2021). Morphological characterization has been effective in analyzing genetic diversity required to protect sweet potato germplasm, reducing the number of accessions by identifying and removing duplicates, and improving crop breeding (Koussao *et al.*, 2014; Alfred *et al.*, 2019). While morphological data (descriptors) offer valuable information, they have modest levels of polymorphism (Yan *et al.*, 2022), and different environmental conditions tend to affect the expression of morphological features (Nadeem *et al.*, 2018), prompting the use of molecular markers.

Unlike morphological markers, molecular markers have been employed in studies of population structure and gene pools of sweet potatoes that originate in Africa, Latin America, Central America, South America, Asia, and Oceania, as well as to classify and define accessions in sweet potato germplasm (Koussao *et al.*, 2014; Alfred *et al.*, 2019; Yan *et al.*, 2021) and estimate genetic diversity in sweet potatoes (Anglin *et al.*, 2021). A variety of molecular markers as reported by Som *et al.* (2014), Yang *et al.* (2015), Zawedde *et al.* (2015), Marian *et al.* (2018), Feng *et al.* (2018), Lee *et al.* (2019) and Palumbo *et al.* (2019) include; random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLPs), inter-simple sequence repeats (ISSRs), endogenous transcripts (ESTs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), and single nucleotide polymorphism (SNP) which is far more effective than other classes of molecular markers in detecting polymorphisms, and SNP have been widely used to certify sweet potato cultivars and breeding lines (Su *et al.*, 2017; Wadl *et al.*, 2018; Wu *et al.*, 2018; Yan *et al.*, 2022).

Despite the use of agronomic traits, morphological and molecular markers in sweet potato diversity studies, the enormous, complicated genome (roughly 2-3 Gb in size) and highly heterozygous hexaploid (2n = 6x = 90) genetic makeup of this plant (Feng *et al.*, 2020; Yan *et al.*, 2022) have made it difficult for breeders to determine best parental combination for breeding of new varieties of the plant resulting in few high-yielding sweet potato varieties in Nigeria. Therefore, to meet the rising demand for high-quality food and raw materials for industries and support Nigeria's food security initiatives, it is necessary to develop sweet potato cultivars that will be resistant to pests and diseases with improved nutritional content and yield (Alfred *et al.*, 2019; Yan *et al.*, 2021). Therefore, The present study aimed to use data on agronomic traits and sweet potatoes' morphological and molecular characterization to determine the best parental combinations for improved crop breeding.

MATERIALS AND METHODS

Collection of Sweet Potato Varieties

The six (6) sweet potato varieties that were used in this research (Table 1) were collected from the National Root Crop Research Institute in Umudike, Abia State, Nigeria. Vines of the different varieties were harvested in sets of 5 and transported from the research institute to the Department of Horticulture and Landscape Technology research farm, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria, for planting.

Table 1: Sweet Potato Varieties Collected

S/N	Name of Variety	Collection Site
1	Nwaoyinma	NRCRI Umudike
2	Umuspo 1	NRCRI Umudike
3	Butter Milk	NRCRI Umudike
4	TIS 8164	NRCRI Umudike
5	Dixon	NRCRI Umudike
6	TIS 0087/087	NRCRI Umudike

Planting

The 6 sweet potato varieties were planted on ridges in an already prepared portion of land in the Department of Horticulture and Landscape Technology research farm, Akanu Ibiam Federal Polytechnic in Unwana, Ebonyi State, Nigeria. Planting out in the field was done to enable field data collection for agronomic trait analysis and morphological characterization, while for DNA extraction for molecular characterization, the 6 varieties of sweet potato were planted in perforated buckets at the greenhouse of the molecular laboratory of Joseph Sarwarn Tarka University, Makurdi, Benue State, Nigeria.

Experimental Design and Layout

Randomized Complete Block Design (RCBD) was adopted as the experimental design in this study. Ridges 3 meters long were created for the experimental layout, and 2 vines of the same variety were planted on each ridge at a distance of 1.5 meters. For every ridge, an intra-ridge gap of 0.5 meters and an inter-ridge gap of 1 meter were given. For 3 months (March to June), 3 replicates of each variety were kept in an RCBD experimental design.

Cultural Practices

Cultural practices adopted in this research are fertilizer application and weeding. Nitrogen, phosphate, and potassium (N.P.K.) fertilizer based on the ratio 5:10:10 was applied 30 Days After Planting (DAP) by disseminating 2 kg per 100 square meters. Before applying N.P.K. fertilizer, the farm was first weeded every 14 days. Later, weeding was done more frequently as needed to maintain a weed-free farm.

Agronomic Traits Analysis

Agronomic traits evaluated in this study are leaf length, leaf diameter, vine length, number of vine branches, node

length, internode length, tuber length, tuber weight, and tuber diameter. Apart from tuber weight measured in grams using a digital weighing scale, all the other agronomic traits were measured in centimeters using a measuring tape. Agronomic traits were measured at 90 DAP.

MORPHOLOGICAL DISCRIPTORS	CIP ASSIGNED VALUES
Plant Type	Erect (<75 cm) = 3; Semi-compact (75-150 cm) = 5; Spreading (151-250cm) = 7; Extremely spreading (>250 cm) = 9
Vine Internode Diameter	Very thin (<4mm) = 1; Thin (4-6 mm) = 3; Intermediate (7-9 mm) = 5; Thick (10-12 mm) = 7; Very thick (> 12 mm) = 9
Vine Internode Length	Very short (< 3 cm) = 1; Short (3-5 cm) = 3; Intermediate (6-9 cm) = 5; Long (10-12 cm) = 7; Very long (> 12 cm) = 9
Vine Pigmentation (Predominant Color)	Green = 1; Green + few purple spots = 3; Green + many purple spots = 4; Green + many dark purple spots = 5; Mostly purple = 6; Mostly dark purple = 7; Totally purple = 8; Totally dark purple = 9
Vine Pigmentation (Secondary Color)	Absent = 0; Green base = 1; Green tip = 2; Green nodes = 3; Purple base = 4; Purple tip = 5; Purple nodes = 6; Other = 7
Vine Tip Pubescence	None = 0; Sparse = 3; Moderate = 5; Heavy = 7; Very heavy = 9
General Leaf Outline	Rounded = 1; Reniform = 2; Cordate = 3; Triangular = 4; Hastate = 5; Lobed = 6; Almost divided = 7
Mature Leaf Shape (Types of Leaf Lobes)	No lateral lobes (entire) = 0; Very slight (teeth) = 1; Slight = 3; Moderate = 5; Deep = 7; Very deep = 9
Shape of Central Lobe	Absent = 0; Teeth = 1; Triangular = 2; Semi-circular = 3; Semi-elliptic = 4; Elliptic = 5; Lanceolate = 6; Oblanceolate = 7; Linear (broad) = 8; Linear (narrow) = 9
Abaxial Leaf Vein Pigmentation	Yellow = 1; Green = 2; Purple spot at base of main rib = 3; Purple spots in several veins = 4; Main rib partially purple = 5; Main rib mostly or totally purple = 6; All veins partially purple = 7; All veins mostly or totally purple = 8; Lower surface and veins totally purple = 9
Mature Leaf Size	Small (< 8 cm) = 3; Medium (8-15 cm) = 5; Large (16-25 cm) = 7; Very large (> 25 cm) = 9
Foliage Color (Mature Leaf Color)	Yellow-green = 1; Green = 2; Green with purple edge = 3; Greyish (heavy pubescence) = 4; Green with purple veins on upper surface = 5; Slightly purple = 6; Moderately purple = 7; Mostly purple = 8; Totally purple = 9
Foliage Color (Immature Leaf Color)	Yellow-green = 1; Green = 2; Green with purple edge = 3; Greyish (heavy pubescence) = 4; Green with purple veins on upper surface = 5; Slightly purple = 6; Moderately purple = 7; Mostly purple = 8; Totally purple = 9
Petiole Pigmentation	Green = 1; Green with purple near stem = 2; Green with purple near leaf = 3; Green with purple at both sides = 4; Green with purple spots = 5; Green with purple stripes = 6; Purple with green near leaf = 7; Some petioles purple, others green = 8; Totally or mostly purple = 9
Petiole Length	Very short (less than 10 cm) = 1; Short (10-20 cm) = 3; Intermediate (21-30 cm) = 5; Long (31-40 cm) = 7; Very long (more than 40 cm) = 9

Figure 1: Morphological Descriptors and their Assigned CIP Values

Morphological Characterization

Morphological characterization was carried out utilizing descriptors created by the International Potato Center (CIP) and reported by Alfred *et al.* (2019). In this study, 21 descriptors, of which 15 are captured in Figure 1, were used for morphological characterization, and based on these descriptors, data were collected 90 DAP by taking the average of 3 rounds of measurements of the center and tip of the main vine using a tape and meter rule.

Molecular Characterization

Molecular characterization was carried out in the Molecular Biology laboratory of Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria.

DNA Isolation

Utilizing the DNA Zol extraction methodology reported by Alfred *et al.* (2018) and Alfred *et al.* (2019), DNA was isolated from tender leaves at the tip of the main potato vine at 20 DAP. After weighing 1 gram of the harvested leaves from each variety, 5 mL of 100% ethanol was added, and the leaf tissues were allowed to be submerged for 30 minutes. After decanting the excess ethanol, the leaves were grounded in a mortar and poured into 1.5 mL labelled micro-centrifuge tubes with codes designating the various sweet potato varieties. After that, 750 µL of DNA zol reagent was added to the tubes, and they were left to stand for 5 minutes. Then 750 µL of chloroform was added, leaving them to stand for another 5 minutes. After centrifuging the tubes for 10 minutes at 10,000 × g, the supernatant was re-filled into tubes with new labels, and 750 µL of absolute ethanol was added to the tubes containing the supernatant and were centrifuged at 5000 × g for 5 minutes. Then, 750 µL of 70% ethanol was added to re-suspend the pelletized DNA, and it was left to stand for 5 minutes following a 5-minute centrifugation at 5000 × g. The liquid component was carefully decanted, revealing pure and pelletized DNA in the tubes. After an hour of air drying, the tubes were frozen at minus 20°C for later use.

Amplification of DNA

Amplification of DNA of the 6 sweet potato varieties was carried out as follows: Five primers (Table 2) were used for DNA amplification via Polymerase Chain Reaction (PCR). Each of the 5 primers came in two tubes containing the forward and the reverse sequence, respectively. To constitute each of the primers for use, a micropipette was used to transfer 50 µL of the forward sequence and 50 µL of the reverse sequence into a new tube that had been labeled. The new tube (a mixture of 50 µL of the forward sequence and 50 µL of the reverse sequence) was vortexed for ten seconds and refrigerated for later use.

The following touch-down method of PCR was adopted using an Applied Biosystems version thermocycler: (i) Initiation: This was carried out for 5 minutes at 94°C. (ii) Denaturation: This process was performed at 94°C for 1 minute. (iii) Annealing: Depending on the primer's annealing temperature, this was done between 50.0 and 66.0°C. (iv) Polymerization: This took place for 2 minutes at 72°C. (v) The first 3 steps were repeated 30 times. (vi) Final extension: This was done for 5 minutes at a temperature of 72°C.

Notably, each tube loaded in the wells of the PCR machine contained pure and pelletized DNA of the different varieties of sweet potato and 10 µL each of the constituted primers (forward and reversed sequence mixed).

Table 2: Primers used for Amplification of DNA

Primer	Forward Sequence	Reverse Sequence
IBS 166	TCCGTCTTTCTTC TTCTTCTTC	ATACACTAACTGC ATCCAAACG
Ibu 4	GGCTGGATTCTT CATATTTAGC	GCTTAATGGATC AGTAACACGA
IB02	CTGTGGATCTGT TCTTTGAACC	TTCCATGTGGAG TGTGAAGTAT
IBS 199	TAACTAGGTTGC AGTGGTTTGT	ATAGGTCCATATA CAATGCCAG
IBS 139	CTATGACACTTCT GAGAGGCAA	AGCCTTCTTGTTA GTTTCAAGC

Agarose gel electrophoresis

Agarose gel electrophoresis was carried out: Tris base Acetic acid (500 mL) and EDTA (TAE) were added to an Erlenmeyer flask with 5 grams of agarose powder. The flask's top was covered with a paper towel and after swirling the contents inside, the agarose powder was microwaved in the flask until it was fully dissolved and the contents were crystal clear. After letting it cool down, 1.5 µL of ethidium bromide (EtBr) was added, and the flask's contents were poured onto a casting tray with combs already in it. After the combs were removed and the mixture had cooled, the casting tray was placed in a gel tank that contained enough 10X Tris base acetic acid and EDTA (TAE) buffer.

Following PCR amplification, 1 µL of DNA loading dye was added to each tube, and a centrifuge was used to spin the tubes for 10 seconds. Afterwards, a micropipette was used to carefully transfer 10 µL of the tube contents into several wells in the casting tray. Using a micropipette, 10 µL of a 100 kb DNA ladder was subsequently put into wells before each set of wells holding a set of DNA for each primer. After that, the gel tank was correctly covered, and for 60 minutes, a steady voltage of 120 volts was applied.

Following the DNA's separation, the gel was moved to a bench-top transilluminator, and a digital camera was used to take a picture of the gel for scoring and analysis.

Data Analysis

Data analysis was done using Minitab statistical software version 21. Analysis of variance, correlation analysis, principal component analysis, and cluster analysis were performed for agronomic traits and morphological characterization. Polymorphic information content and cluster analysis were then carried out after the gel image was assessed for molecular characterization based on the intensity of bands generated; the formation of a band for each variety per primer was recorded as 1 and no formation as 0.

RESULTS

Agronomic Traits

The result for agronomic traits shows that; the highest value for leaf length (16cm) was recorded for the Dixon variety and the least (10cm) for the Nwaoyinma variety, the highest value for leaf diameter (16cm) was recorded for the Dixon variety and the least (9cm) for Nwaoyinma; the highest value for vine length (820cm) was recorded for the Nwaoyinma variety while the least value (35cm) was recorded for Butter Milk variety, the highest value for number of vine branches (10) was recorded for TIS 0087/087 variety and the least (1) for Butter Milk variety, the highest value for tuber length (30cm) was recorded for Dixon variety and the least (14cm) for TIS 0087/087 variety among others (Figure 2). This shows that morphological variations exist among the different sweet potato varieties, implying that they can be useful for breeding and crossing-breeding programs to develop new sweet potato varieties.

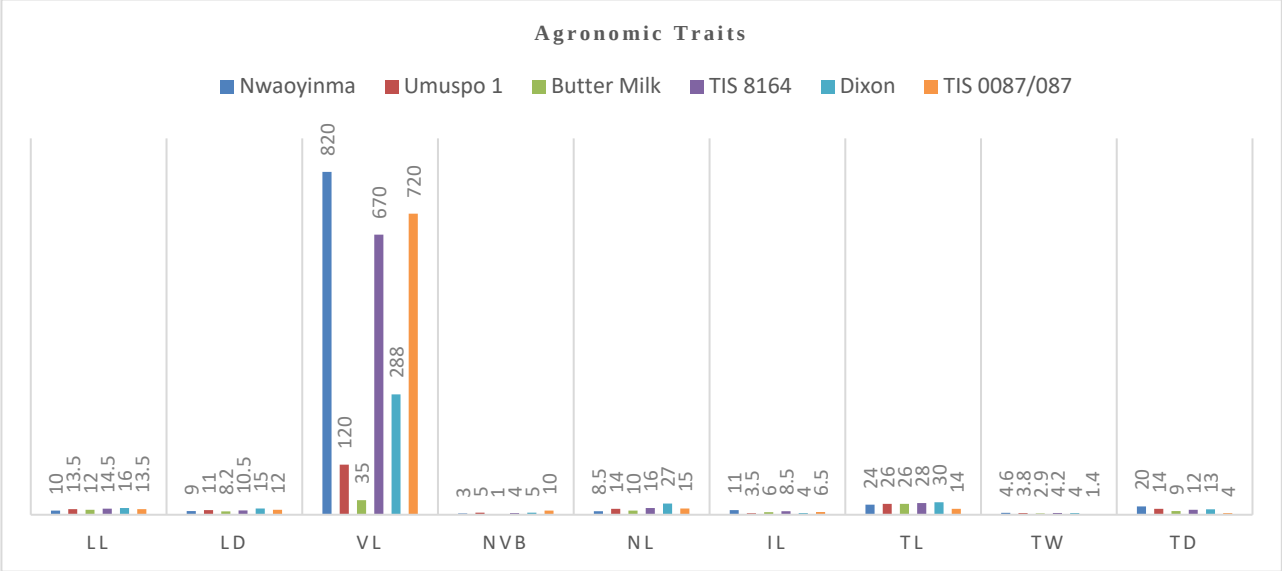

Figure 2: Agronomic Traits

Key: LL = Leaf Length, LD = Leaf Diameter, VL = Vine Length, NVB = Number of Vine Branches, NL = Node Length, IL = Internode Length, TL = Tuber Length, TW = Tuber Weight, TD = Tuber Diameter.

Analysis of Variance

The analysis of variance result shows significant variations (p-value = 0.000) among the different agronomic traits with standard deviation values ranging between 1.167 for tuber weight and 6.53 for node length (Table 3). This implies that the agronomic characters in Table 3 are different and are useful in the agronomic characterization and breeding of sweet potatoes.

Correlation Analysis

The result of the correlation analysis of agronomic traits is shown in Table 4. The table showed a strong positive correlation between leaf diameter and number of leaves (0.947), tuber weight and tuber diameter (0.917), leaf length and number of leaves (0.909), leaf length and leaf diameter (0.827), tuber length and tuber weight (0.803), vine length and internode length (0.783), leaf diameter and number of vine branches (0.567) and tuber length and tuber diameter (0.562); and a weak positive correlation exists between leaf length and number of vine branches (0.369), number of leaf and tuber length (0.348), number of leaf and number of vine branches (0.327) among others. When breeding or cross-breeding sweet potatoes, leaf characteristics such as leaf diameter, length, and number of leaves are important because they affect tuber characteristics such as length and weight.

Principal Component Analysis (PCA)

The result of PCA (Table 5) shows that 100% variability among the agronomic traits studied was recorded at the 5th principal component, the 1st principal component (PC) accounted for 42.8% of the whole variations and is positively associated with vine length, internode length, tuber length and tuber diameter, the 2nd PC accounted for 33.4% of the total variations and is positively associated with vine length, number of vine branches and internode length, the 3rd principal component accounted for 18% of the whole variations and is positively associated with leaf length, leaf diameter, vine length, among others, the 4th principal component accounted for 4.4% of the whole variations and it is positively associated with leaf length, vine length, tuber length among others while the 5th principal component accounted for 1.3% of the whole variations and it is positively associated with leaf length, vine length, number of vine branches, among others.

Cluster Analysis

The result of cluster analysis for agronomic traits (Figure 3) shows two distinct clusters with varieties Nwaoyinma,

TIS 8164, and TIS 0087/087 belonging to the first cluster and varieties Umuspo 1, Butter Milk, and Dixon belonging to the second cluster. Varieties Nwaoyinma and TIS 8164 are approximately 90% similar and about 76% similar to variety TIS 0087/087, while variety Umuspo 1 and Butter Milk are approximately 88% similar and about 60% similar to variety Dixon. These findings imply that low agronomic diversity exists among the sweet potato varieties studied highlighting the need for sweet potato characterization from far apart regions.

Table 3: Analysis of Variance for Agronomic Traits

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	8	991898	123987	9.86	0.000
Error	45	565856	12575		
Total	53	1557754			
Factor	**Mean**	**StDev**	**95% CI**		
Leaf Length	13.250	2.068	(-78.955, 105.455)		
Leaf Diameter	10.950	2.411	(-81.255, 103.155)		
Vine Length	442	336	(350, 534)		
Number of Vine Branches	4.67	3.01	(-87.54, 96.87)		
Node Length	15.08	6.53	(-77.12, 107.29)		
Internode Length	6.58	2.82	(-85.62, 98.79)		
Tuber Length	24.67	5.61	(-67.54, 116.87)		
Tuber Weight	3.483	1.167	(-88.721, 95.688)		
Tuber Diameter	12.00	5.33	(-80.20, 104.20)		

Table 4: Correlation Analysis for Agronomic Traits

	LL	LD	VL	NVB	NL	IL	TL	TW
LD	0.827							
VL	-0.250	-0.017						
NVB	0.369	0.567	0.402					
NL	0.909	0.947	-0.157	0.327				
IL	-0.656	-0.547	0.783	-0.208	-0.563			
TL	0.293	0.098	-0.451	-0.718	0.348	-0.181		
TW	-0.085	-0.069	0.032	-0.628	0.059	0.274	0.803	
TD	-0.381	-0.181	0.13	-0.548	-0.155	0.386	0.562	0.917

Key: LL = Leaf Length, LD = Leaf Diameter, VL = Vine Length, NVB = Number of Vine Branches, NL = Node Length, IL = Internode Length, TL = Tuber Length, TW = Tuber Weight, TD = Tuber Diameter.

Table 5: Principal Component Analysis for Agronomic Traits

Variable	PC1	PC2	PC3	PC4	PC5
Leaf Length	-0.44	-0.21	0.11	0.39	0.51
Leaf Diameter	-0.43	-0.14	0.34	-0.23	-0.40
Vine Length	0.13	0.29	0.64	0.21	0.10
Number of Vine Branches	-0.33	0.35	0.29	-0.36	0.43
Node Length	-0.41	-0.26	0.26	0.14	-0.44
Internode Length	0.390	0.180	0.380	0.439	-0.227
Tuber Length	0.060	-0.562	-0.025	0.287	0.097
Tuber Weight	0.247	-0.452	0.284	-0.103	0.360
Tuber Diameter	0.323	-0.333	0.292	-0.562	-0.028
Eigenvalue	3.8557	3.0066	1.6186	0.4001	0.1190
Proportion	42.8	33.4	18.0	4.4	1.3
Cumulative (%)	42.8	76.2	94.2	98.7	100

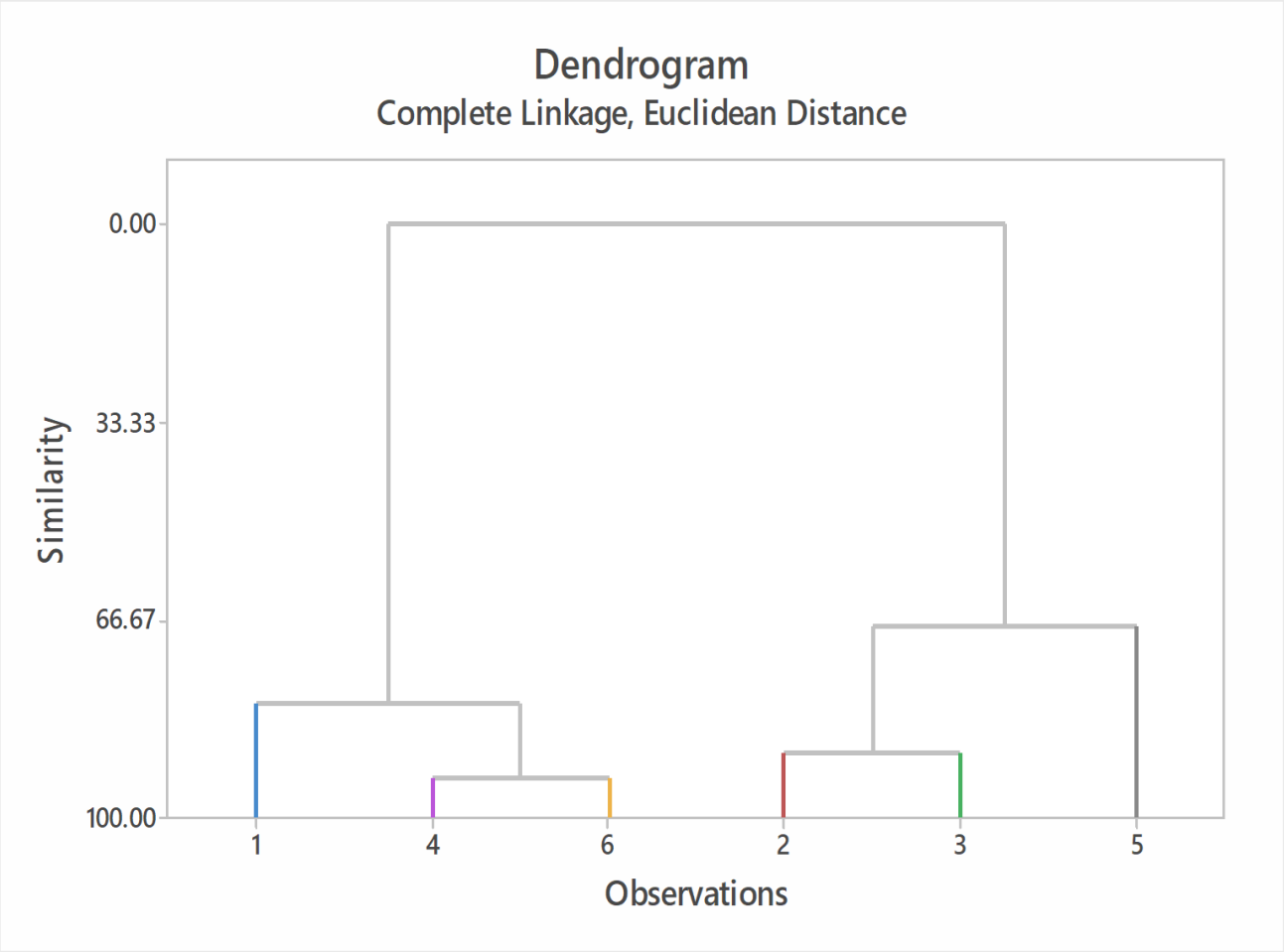

Figure 3: Cluster Analysis based on Agronomic Traits

Key: 1 = Nwaoyinma, 2 = Umuspo 1, 3 = Butter Milk, 4 = TIS 8164, 5 = Dixon, 6 = TIS 0087/087

Morphological Characterization

Analysis of Variance

The analysis of variance results shows significant variations (p-value = 0.000) among the different morphological traits studied, with standard deviation values ranging between 0.000 for internode diameter and 4.08 for storage root shape (Table 6). This implies that the morphological characters are useful as morphological descriptors in morphological characterization studies and can play a significant role in sweet potato breeding programs.

Correlation Analysis

The result of correlation analysis of morphological character (Table 7) shows strong positive correlation between; internode length and storage root defects (0.93), predominant colour for pigmentation and petiole pigmentation (0.88), internode length and tip pubescence (0.81), plant type and internode length (0.80), plant type and storage root defect (0.77), secondary colour for pigmentation and general outline of matured leaf (0.77), internode length and petiole pigmentation (0.72), secondary colour for pigmentation and number of lobes for mature leaf (0.72), internode length and storage root predominant skin colour (0.67), secondary colour for pigmentation and type of leaf lobe for mature leaf (0.66), secondary colour for pigmentation and shape of central lobe of mature leaf (0.65), internode length and predominant colour for pigmentation (0.61), internode length and storage root secondary colour intensity (0.59), plant type and storage root predominant skin colour (0.57), plant type and tip pubescence (0.54), secondary colour for pigmentation and abaxial leaf vine pigmentation (0.54), among others while weak positive correlation exist between tip pubescence and immature leaf colour (0.49), number of lobes for mature leaf and storage root shape (0.47), tip pubescence and storage root secondary colour intensity (0.46), general outline of mature leaf and mature leaf colour (0.46), size of mature leaf and immature leaf colour (0.45), size of mature leaf and storage root shape (0.40), type of leaf lobe and storage root shape (0.38), general outline of mature leaf and storage root shape (0.22), tip pubescence and abaxial leaf vine pigmentation (0.14), among others. These results

imply that the type of storage root (tuber) defects are tied to the vines' internode length and the vines' colour and petioles. This knowledge can be useful in cross-breeding programs to determine possible parental combinations, especially if the cross-breeding program aims to develop sweet potatoes without tuber defects.

Principal Components Analysis

The principal components analysis of the morphological characters revealed 100% variability among the characters used to differentiate sweet potato varieties at the 5th PC (Table 8). The 1st principal component accounted for 44.7% variability and is associated with plant type (0.136), internode length (0.265), and predominant colour for pigmentation (0.102), among others. The second principal component accounted for 27.1% variability and is associated with the size of the mature leaf (0.391), mature leaf colour (0.113), and immature leaf colour (0.290), among others. The third principal component accounted for 17.0% variability and is associated with abaxial leaf vine pigmentation (0.273) and petiole pigmentation (0.183), among others. The fourth principal component accounted for 7.7% variability and is associated with internode length (0.131), mature leaf colour (0.393), and petiole pigmentation (0.171), among others, while the fifth principal component accounted for 3.6% variability and is associated with the size of the mature leaf (0.504), storage root defects (0.371), storage root secondary colour intensity (0.373) among others (Table 8).

Cluster Analysis

Figure 4 shows the result of cluster analysis of morphological characterization with three distinct clusters. Varieties Nwaoyinma and Butter Milk were clustered together in cluster 1 with approximately 33% similarities, varieties Dixon and TIS 0087/087 were clustered together in cluster 2 with approximately 50% similarities, while varieties Umuspo 1 and TIS 8164 were clustered together in cluster 3 with approximately 37% similarities. This implies average morphological diversity among the varieties of sweet potatoes, and morphological characterization is vital in sweet potato diversity studies and breeding programs.

Table 6: Analysis of Variance for Morphological Characters

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	19	333.2	17.535	3.10	0.000
Error	100	566.0	5.660		
Total	119	899.2			
Factor	**Mean**	**StDev**	**95% CI**		
Plant Type	5.67	2.73	(3.74, 7.59)		
Internode Diameter	7.000	0.000	(5.073, 8.927)		
Internode Length	5.000	2.191	(3.073, 6.927)		
Predominant Colour for pigmentation	5.00	3.58	(3.07, 6.93)		
Secondary Colour for pigmentation	2.833	1.835	(0.906, 4.760)		
Tip Pubescence	4.83	3.37	(2.91, 6.76)		
General Outline of Mature Leaf	4.333	1.033	(2.406, 6.260)		
Type of Leaf Lobe of mature leaf	1.83	2.79	(-0.09, 3.76)		
Number of Lobes of mature leaf	2.000	1.673	(0.073, 3.927)		
Shape of Central Lobe of mature leaf	2.167	2.041	(0.240, 4.094)		
Size of mature leaf	5.333	0.816	(3.406, 7.260)		
Abaxial Leaf Vine Pigmentation	3.83	3.37	(1.91, 5.76)		
Mature Leaf Colour	1.500	0.837	(-0.427, 3.427)		
Immature Leaf Colour	2.000	1.095	(0.073, 3.927)		
Petiole Pigmentation	4.17	3.54	(2.24, 6.09)		
Petiole Length	3.000	1.265	(1.073, 4.927)		
Storage Root Shape	5.67	4.08	(3.74, 7.59)		
Storage Root Defects	1.333	1.966	(-0.594, 3.260)		
Storage Root Predominant Skin Colour	2.67	2.73	(0.74, 4.59)		
Storage Root Skin Colour Intensity	1.500	1.225	(-0.427, 3.427)		

Table 7: Correlation Analysis of Morphological Characters

	PT	IL	PC	SC	TP	GO	TLL	NL	SCL	S
IL	0.80									
PC	0.49	0.61								
SC	0.35	-0.10	0.12							
TP	0.54	0.81	0.39	-0.49						
GO	0.331	-0.18	0.22	0.77	-0.56					
TLL	0.07	-0.33	0.08	0.66	-0.75	0.93				
NL	0.00	-0.44	0.00	0.72	-0.82	0.93	0.99			
SCL	-0.02	-0.45	0.00	0.65	-0.81	0.92	0.99	0.99		
S	-0.84	-0.45	-0.59	-0.48	-0.27	-0.63	-0.32	-0.29	-0.28	
ALVP	0.32	0.11	0.46	0.54	0.14	0.25	-0.05	0.04	-0.02	-0.56
MLC	-0.53	-0.66	-0.27	0.07	-0.82	0.46	0.73	0.71	0.76	0.29
ILC	0.00	0.33	-0.41	-0.69	0.49	-0.71	-0.59	-0.66	-0.63	0.45
PP	0.48	0.72	0.88	-0.12	0.72	-0.18	-0.38	-0.44	-0.45	-0.44
PL	0.00	-0.28	-0.35	0.35	-0.66	0.61	0.79	0.76	0.78	0.00
SRS	-0.41	-0.67	-0.82	0.39	-0.76	0.22	0.38	0.47	0.44	0.40
SRD	0.77	0.93	0.46	0.07	0.58	-0.07	-0.13	-0.24	-0.27	-0.33
SRPSC	0.57	0.67	-0.16	-0.29	0.67	-0.38	-0.46	-0.53	-0.53	-0.12
SRSCI	0.36	0.59	-0.18	-0.13	0.46	-0.47	-0.44	-0.49	-0.52	0.20

Key: PT = Plant Type, ID = Internode Diameter, IL = Internode Length, Pigmentation: PC = Predominant Colour, Pigmentation: SC = Secondary Colour, Pigmentation: TP = Tip Pubescence, Mature Leaf: GO = General Outline, Mature Leaf: TLL = Type of Leaf Lobe, Mature Leaf: NL = Number of Lobes, Mature Leaf: SCL = Shape of Central Lobe, Mature Leaf: S = Size, ALVP = Abaxial Leaf Vine Pigmentation, Foliage Colour: MLC = Mature Leaf Colour, Foliage Colour: ILC = Immature Leaf Colour, PP = Pitiole Pigmentation, PL = Pitiole Length, SRS = Storage Root Shape, SRD = Storage Root Defects, SRPSC = Storage Root Predominant Skin Colour, SRSCI = Storage Root Skin Colour Intensity.

Table 8: Principal Components Analysis of Morphological Characters

Variable	PC 1	PC 2	PC 3	PC4	PC 5
Plant Type	0.136	-0.316	-0.295	-0.084	-0.234
Internode Length	0.265	-0.202	-0.218	0.131	0.148
Predominant Colour for pigmentation	0.102	-0.351	0.113	0.345	0.303
Secondary Colour for pigmentation	-0.180	-0.265	-0.120	-0.427	0.271
Tip Pubescence	0.331	-0.072	-0.007	0.066	-0.236
General Outline of Mature Leaf	-0.246	-0.281	-0.140	0.015	-0.161
Type of Leaf Lobe of mature leaf	-0.292	-0.169	-0.168	0.154	0.060
Number of Lobe of mature leaf	-0.311	-0.156	-0.124	0.047	0.048
Shape of Central Lobe of mature leaf	-0.312	-0.144	-0.121	0.113	-0.005
Size of mature leaf	-0.015	0.391	0.099	0.058	0.504
Abaxial Leaf Vine Pigmentation	0.028	-0.306	0.273	-0.433	0.029
Mature Leaf Colour	-0.288	0.113	-0.046	0.393	0.029
Immature Leaf Colour	0.199	0.290	-0.251	0.115	-0.114
Petiole Pigmentation	0.228	-0.272	0.183	0.171	0.192
Petiole Length	-0.239	0.044	-0.381	0.161	-0.035
Storage Root Shape	-0.225	0.211	-0.173	-0.404	0.100
Storage Root Defect	0.203	-0.163	-0.358	0.075	0.371
Storage Root Predominant Skin Colour	0.230	0.067	-0.379	-0.092	-0.276
Storage Root Skin Colour Intensity	0.205	0.114	-0.362	-0.202	0.373
Eigenvalue	8.4864	5.1463	3.2211	1.4542	0.6921
Proportion	0.447	0.271	0.170	0.077	0.036
Cumulative %	44.7	71.8	88.7	96.4	100

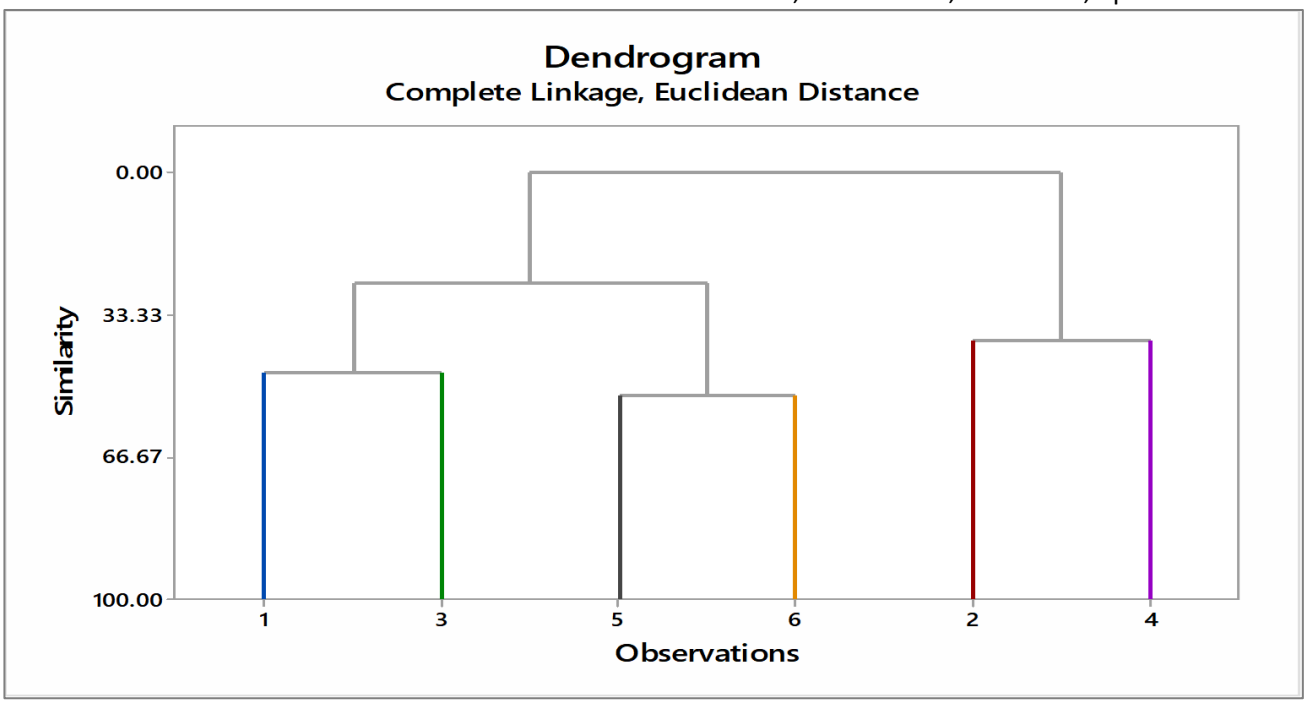

Figure 4: Dendrogram based on Morphological Characterization

Key: 1 = Nwaoyinma, 2 = Umuspo 1, 3 = Butter Milk, 4 = TIS 8164, 5 = Dixon, 6 = TIS 0087/087

Molecular Characterization

Gel Image

Molecular characterization gave rise to the gel image (Figure 5), which shows the presence of monomorphic and polymorphic bands with the different primers used showing a high amplification rate.

Polymorphic Information Content

The result of polymorphic information content is shown in Table 9. The table shows that the IBS166 primers have the highest polymorphism percentage (42.86%), followed by the IB02 primer (28.57%) and IBS199 and Ibu4 primers with 14.29%, respectively. This shows that these primers are useful in differentiating the sweet potato varieties.

Cluster Analysis

The dendrogram generated from the cluster analysis (Figure 6) grouped the varieties of sweat potatoes into four distinct clusters. Cluster 1 has only the Nwaoyinma variety, cluster 2 has varieties TIS 8164, TIS 0087/087 and Dixon, cluster 3 has only the Butter Milk variety, and Cluster 4 has only the Umuspo 1 variety. Varieties TIS 8164 and TIS 0087/087 are 100% similar and approximately 70% similar to the Dixon variety, all the varieties in cluster 2 are approximately 48% similar to the Nwaoyinma variety in cluster 1, the Butter Milk variety in cluster 3 is 33.33% similar with all the varieties in cluster 1 and 2 while Umuspo 1variety in cluster 4 shares no similarity with all the varieties studied. This implies that the genetic diversity among the sweet potato varieties is high.

Table 9: Polymorphic Information Content

Primer Name	Number of Polymorphic Bands	% Polymorphism	Mean PIC
IB02	2	28.57	0.2857
IBS139	0	0	0
IBS166	3	42.86	0.4286
IBS199	1	14.29	0.1429
Ibu4	1	14.29	0.1429
Total	7	100	1.0001

Determination of Parental Combination

The dendrogram of molecular characterization shows that the Umuspo 1 variety is not similar to any other variety, implying that it can form a good parental combination with others. However, based on morphological characterization, the Umuspo 1 variety is approximately 37% similar to TIS 8164 variety and since molecular characterization shows 100% similarities between the TIS 8164 and TIS 0087/087 varieties, it, therefore, implies that the Umuspo 1variety will not form a good parental combination with TIS 8164 and TIS 0087/087 varieties, and this is also supported by results of agronomic traits which shows that TIS 8164 and TIS 0087/087 varieties are about 76% similar. Also, agronomic characterization shows that the Dixon variety is approximately 66.67% similar to the Umuspo 1 and Butter Milk varieties; hence, they will not form a good parental combination. Based on these, the suggested parental combination will be the Nwaoyinma and Umuspo varieties. This suggested parental combination is further supported by the results of agronomic traits which show that the Nwaoyinma

variety has good vine length (820cm), good internode length (11cm), good tuber length (24cm) and good tuber diameter (20cm) while Umuspo 1variety has good leaf length (13.5cm), good leaf diameter (11cm), good number of vine branches (5), good node length (14cm), good tuber length (26cm) and good tuber diameter (14cm). Cross-breeding the Nwaoyinma and Umuspo varieties will give a sweet potato variety with improved leaf traits, vine traits, and tuber yield.

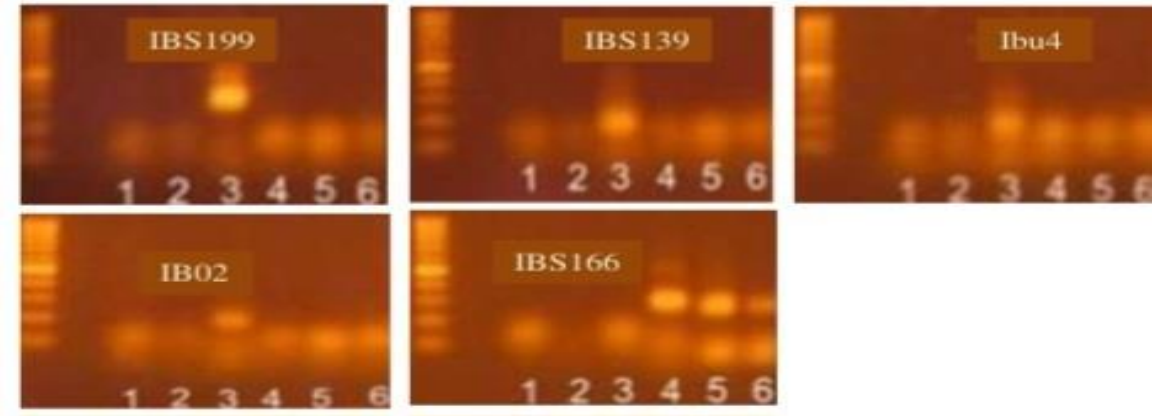

Figure 5: Gel Image

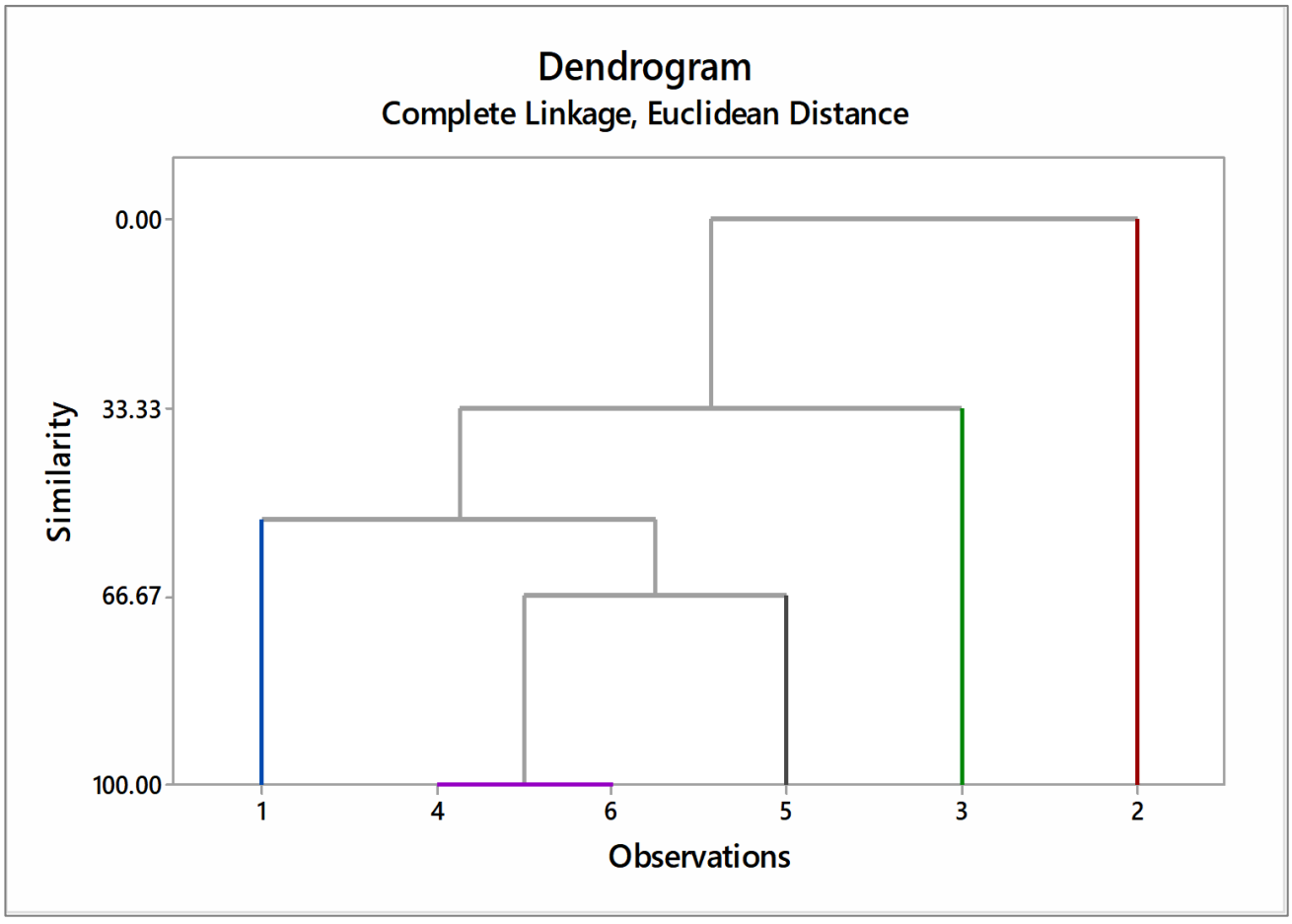

Figure 6: Dendrogram based on Molecular Characterization

Key: 1 = Nwaoyinma, 2 = Umuspo 1, 3 = Butter Milk, 4 = TIS 8164, 5 = Dixon, 6 = TIS 0087/087

DISCUSSION

Sweet potato remains an important root crop in Nigeria, and its genetic improvement will significantly aid food security programs in the country. Efforts towards providing the necessary data for the improvement of this important root crop have been made in the last decade (Koussao *et al.*, 2014; Som *et al.*, 2014; Alfred *et al.*, 2019) and this study is one such effort further demonstrate the potentials the different varieties of the crop in Nigeria holds in genetic improvement programs. But unlike other studies that utilized morphological characters and

molecular markers in the diversity study of sweet potatoes, this study used these methods in addition to agronomic traits in determining parental combination for better breeding of sweet potatoes.

The ability of the different agronomic traits and morphological characters to differentiate sweet potato varieties is evident in this study. By implication, plant breeders and geneticists may use these agronomic traits and morphological characteristics for diversity studies, breeding, and cross-breeding of sweet potatoes. At the 5th principal component, 100% variability was recorded for all agronomic characters (Table 5) and morphological (Table 8) characters studied. This is consistent with different diversity studies of sweet potatoes, which have reported 100% variability between the 4th and 6th principal components (Koussao *et al.*, 2014; Som *et al.*, 2014; Elisângela *et al.*, 2017). However, this finding is inconsistent with that of Alfred *et al.* (2019), who reported 100% variability at the 16th PC, with the first 4 PC accounting for just 72.1% variability. This inconsistency could be due to the difference in the number of varieties or accessions used; Alfred et al. (2019) used 20 accessions in their study in contrast with the 6 varieties used.

Also, in this study, the 21 morphological descriptors used were all essential in differentiating sweet potato varieties (Table 6). By implication, these morphological descriptors can be used in diversity studies and characterization of sweet potato varieties in breeding programs. This finding aligns with those of Kouassi *et al.* (2023) but not with the study of Alfred *et al.* (2019), which showed that only 18 of these 21 morphological descriptors were essential in differentiating sweet potato accessions. In Alfred *et al.* (2019) study, the foliage colour of immature leaves and storage root secondary colour intensity were not identified as being essential which is inconsistent with the findings in this study. This is probably because these 2 morphological descriptors have previously been reported by Koussao *et al.* (2014) to be indiscriminate when used in differentiating accessions, especially accessions collected within the same geographical region. Another morphological descriptor reported to be discriminatory in this study is predominant skin colour. This morphological descriptor was earlier reported by Koussao *et al.* (2014) to be widely used in identifying and differentiating sweet potato cultivars among farmers in Burkina Faso. Although in tandem with findings in this study, Koussao *et al.* (2014) maintained that predominant skin colour was not discriminatory and, hence, not important in the taxonomic differentiation of sweet potato accessions.

Furthermore, correlation analysis (Tables 4 and 7) shows a strong positive correlation between characters associated with leaf, vine, and tuber of sweet potato. These findings imply that leaf characters such as leaf length and leaf diameter and vine characters such as vine length, internode length, and internode diameter affect the tuber length, weight, and diameter of sweet potato, and a good vine length will translate to a good tuber length and weight. Hence, in breeding programs or diversity studies and characterization for determination of parental combinations, leaf and vine characters must be considered if improved tuber yield is to be achieved. Similarly, Regessa *et al.* (2023) reported a positive correlation between above-ground fresh biomass weight and plant height, vine length, number of branches per plant, and unmarketable tuber yield. However, Regessa *et al.* (2023) reported a negative correlation between leaf diameter and root length and root diameter. By implication, a sweet potato variety with a vigorous vegetative will produce fewer storage roots, indicating competition for photosynthesis between the shoots and roots. Notably, findings in this study demonstrate that important agronomic traits of sweet potatoes that must be taken into cognizance in breeding programs are those relating to leaf, vine, and tuber, and this agrees with the assertions of Chen *et al.* (2021) and Yan *et al.* (2022) that storage root is the most significant agronomic character in sweet potato.

Molecular markers have remained an important tool in diversity studies of sweet potatoes and necessary data provision for breeding and crossing breeding of the plant. This study demonstrates the importance of SSR markers in differentiating sweet potato varieties and subsequent plant breeding. The primers used in the present study showed a high level of polymorphism and have been previously used in studies by Alfred *et al.* (2018), Alfred *et al.* (2019), and Anglin *et al.* (2021). However, the polymorphic information content reported in this study for the different primers where higher than those previously reported by Alfred *et al.* (2019). This could be due to differences in the number of varieties and accessions used and the method employed in scoring the gel image formed from the amplification of genomic DNA. By implication, the high polymorphism reported for the different primers may account for leaf, vine, and tuber characteristics. However, since quantitative trait loci analysis was not performed in this study, the genes responsible for these characteristics were not identified.

Furthermore, contrary to the reports of Alfred *et al.* (2019), which showed low genetic diversity among sweet potato accessions in north central Nigeria and attributed it to the same geographical region of collection, a high level of genetic diversity was reported among the sweet potato varieties in this study as four distinct clusters were formed (Figure 6). Similarly, Koussao *et al.* (2014) reported moderate to high diversity values for sweet potato germplasm in Burkina based on molecular and phenotypic assessment in their genetic diversity study.

Additionally, the result of cluster analysis for agronomic traits analysis (Figure 3), morphological characterization (Figure 4), and molecular characterization (Figure 6) shows significant variation in the clustering of the different sweet potato varieties for the different methods. This variation in dendrogram, especially in morphological and molecular characterization, has also been reported by Koussao *et al.* (2014). The major reason for this variation is that morphological and molecular characterization are independent. By implication, agronomic traits analysis,

morphological characterization, and molecular characterization can be used in diversity studies and determining parental combinations for breeding and cross-breeding improved sweet potato varieties.

Determination of parental combination (s) remains integral to crop breeding and improvement programs. This is true as parents (genotype) in every breeding program and the environment (phenotype) determines the traits of their offspring; hence, parental selection and combination are crucial for a successful breeding program. One of the early successful attempts at determining parental combinations for sweet potatoes was by Dai-fu and others in 2009, who utilized agronomic characters and ISSR and RAPD markers, as reported by Barb (2016). This approach was adopted in this study, and the results reiterate the importance of this approach in breeding programs.

CONCLUSION

Combining agronomic traits and morphological and molecular markers effectively determines parental combination among sweet potato varieties. This can be applied to other crops of importance. This study reveals the importance of different agronomic traits and morphological descriptors in differentiating sweet potato varieties; shows a strong positive correlation between numerous agronomic important traits relating to the leaf, vine, and tuber of sweet potato and concludes that the Nwaoyinma and the Umuspo 1 variety are a good parental combination. To boost food availability and security in Nigeria, it is recommended that more efforts be channelled toward agronomic, morphological, and molecular screening of available sweet potato varieties to obtain good parental combinations for improved breeding and cross-breeding programs.

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