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Genetic Diversity Analysis and Nutritional Profiling of Black Fonio Millet (*Digitaria iburua* Stapf) Revealed Genotypes with High Nutritional Value

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Abstract

Black fonio millet (*Digitaria iburua*) is an underutilized African crop that is significant in promoting food and nutrition security in West Africa. Despite its benefits, the potential for improvement of this crop remains largely untapped, and there is a paucity of knowledge about its genetic diversity and nutritional properties. This study evaluated the variability of forty-one (41) *D. iburua* accessions collected from farmers in Abuja, Nasarawa, Plateau, and Kaduna in Nigeria using nutritional traits as well as molecular markers. The descriptive statistics results showed a broad range of variations in the nutritional traits examined. Among the collection sources, Nasarawa accessions had the highest crude protein content, while Plateau accessions had the highest carbohydrate and crude lipid content. Also, sulfur, phosphorus, and potassium are among the most abundant mineral contents found in *D. iburua.* Furthermore, the molecular marker analysis revealed that the genetic distance between pairs of sources of collection varied from 0.61 to 0.79 with higher similarity between Nasarawa and Kaduna accession. The molecular variance analysis depicted 11% of the total variation existed among populations, whereas 89% was distributed within populations. Also, we identified four populations or major sub-clusters from the studied germplasm. Finally, these findings have shown the presence of high genetic diversity for Nigeria *D. iburua* accessions and present an opportunity for the crop's genetic improvement. Thus, accessions from Nasarawa State with higher protein content should be harnessed for use in areas with nutritional deficiency to aid in nutrition security.

Keywords: *Digitaria iburua*, Genetic Diversity, Genetic Analysis, Polymerase Chain Reaction, Nutrition Analysis

Introduction

Achieving food security is one of the most critical global challenges, especially in Africa. This could be due to the neglect of indigenous crops, which have traditionally and significantly sustained the continent [1]. Muyonga et al. [2] noted that African native crops, including cereals like fonio, significantly help in

addressing the challenge of food and nutrition security across the continent.

Fonio (*Digitaria exilis* (Kipp.) Stapf and *Digitaria iburua* Stapf) is an ancient cereal which originated from the West African region, particularly Mali and Guinea [4], and has been largely neglected despite its nutritional, socio-cultural, and economic significance [3]. It is often called the "Grains of life" due to the fact that it matures faster than other grains and belongs to Poaceae family [3]. It is a plant that completes its life cycle in a year, and grows above 150cm as in the case of the *D. iburua*. While both cultivated species are widely referred to as fonio and 'Acha' in various communities in West Africa, they can be morphologically distinguished. The seed of *D. exilis* is brown in colour while that of the *D. iburua* is black hence they are commonly referred to as the white and black fonio respectively.

Black fonio is currently cultivated only in three countries in West Africa—Nigeria, Togo, and Benin—and particularly in the northern regions [5]. In contrast, *D. exilis* is widely cultivated across the West African regions [6]. Due to its high demand, particularly in the West African region, the crop has a significant yearly production volume. According to FAOSTAT [7], global production reaches 658,707.96 metric tons, with Guinea being the highest producing country, contributing 487,535.12 tons that account for 74.01% of the world's fonio production. Nigeria ranks second with a total production of 83,372 metric tons, while Mali is the third highest producing country, producing 33,641 metric tons.

Digitaria species are recognized for their unique ability to cope favourably in diverse environment, including areas with water scarcity, which makes these crops essential food sources for people in rural communities [8;9]. In spite of its agro-ecological potential, fonio's production remains significantly lower compared to sorghum, maize and millet.

Nevertheless, fonio significantly contributes in enhancing food security in Africa, particularly in countries that prioritize its cultivation [10]. The cereal is a valuable source of cysteine and methionine [11]. These two amino acids are indispensable for human health, and exist in low quantities in major cereals like wheat and sorghum [11]. Additionally, fonio is endowed with minerals; however, its nutrient bioavailability is compromised by antinutritional factors like phytates [12]. Therefore, enhancing the nutritional quality of fonio grains is crucial. Fonio has traditional usage such as porridge, couscous, and alcoholic beverages [13]. In recent times, there has been a huge increase in the utilization of fonio for various food applications. Aside from the fonio grains, other parts of the crop serve various purposes [14-15]. To give an instance, the straw can be used as livestock feed as well as the grain. [16]. Additionally, fonio straw has potential applications in adobe formulations [15], and fonio husk ash has been evaluated as a pozzolan in concrete [14]. Medically, fonio is ideal for diabetic patients [17].

Despite its socioeconomic and nutritional value, fonio has been largely overlooked in scientific research and development programs in Nigeria. Compared to globally cultivated cereals, it has received minimal attention, notwithstanding its role in improving the economy and food security of the local population. Consequently, fonio remains underdeveloped and encounters numerous agronomic challenges that hinder its production [4], and yield potential [18].
There is limited understanding of the genetic diversity of fonio in literature, and this limits its selection for improvement and conservation. As a result, its potential remains underexploited and underutilized, increasing the danger of extinction through genetic erosion, which may additionally reduce development opportunities for impoverished communities [19]. Being an orphan crop, understanding the genetic diversity within fonio accessions is crucial for identifying superior landraces that can be bred for higher yields and better nutritional quality. Genetic diversity in plants allows breeders to develop superior varieties that are high in quality, resilient to stresses, and suitable to diverse environments [20]. Molecular markers which are specific sequences of DNA used to identify genetic variation among individuals like Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSRs) and Single nucleotide polymorphism (SNP), offer a valuable tool for assessing genetic diversity and can greatly assist in the selection of desirable traits for breeding programs [21].

Additionally, a lot of studies have shown improved nutritional and functional properties in globally grown cereals like rice, sorghum, oats and wheat after they develop into seedlings [22-24]. Thus, more research is needed to investigate innovative and underutilized cereal grains, such as fonio. While some studies have documented the phytochemical properties and nutrient composition of white fonio (*D. exilis*) [25;26], Information on the nutritional composition and phytochemical traits of black fonio (*D. iburua*). is limited. Conducting a nutritional analysis of fonio accessions will help identify varieties with superior nutritional profiles. This will contribute to combating malnutrition and improving health in populations that depend on this crop as a staple in their diet [27].

Against this backdrop, the study aimed to enhance genetic improvement, efficient Preservation and sustainable utilization of fonio's genetic resources. Specifically, the objectives of the study were to 1) evaluate the genetic variation and population structure of the fonio accessions; 2) select or identify more nutritious cultivars from the germplasm; 3) determine the variability of total mineral and phytochemical content of the genotypes; and 4) determine interrelationship between nutritional and phytochemical traits.

Materials and Methods

Germplasm collection and DNA extraction

Forty-one black fonio genotypes sourced from Abuja, Nasarawa, Plateau, and Kaduna— all in the northern part of Nigeria —were phenotyped using an alpha lattice design at the experimental site of Abomey-Calavi University (UAC), Benin. The experiment was replicated three (3) timesDNA was extracted from freshly collected leaves of each of the accessions for DNA extraction after four weeks, and this was performed in the Laboratory of Genetics, Biotechnology, and Seed Science, UAC, Benin. Genomic DNA from the 41 *D. iburua* accessions was isolated using the mixed alkyltrimethylammonium bromide (MATAB) method [28] without any modifications. Leaf samples (0.2 g each) were finely ground using a pestle and mortar. During the grinding process, 2 mL of Tris-EDTA-Sorbitol solution was added.

The mixture was poured into an eppendorf tube and was then subjected to centrifugation at 10,000 rpm for ten (10) minutes. After removing the liquid portion of the sample, 750 µL of pre-incubated 4% MATAB buffer (65°C) was introduced. The precipitate was extracted, and the remaining solution Was maintained at 65°C in a water bath for 1.5 hours, with gentle shaking every 10 minutes interval. A volume of 750 µL of isoamyl chloroform (24:1) was introduced, thoroughly mixed for 5 minutes, and then centrifuged at 12,000 rpm for 15 minutes. The resulting supernatant was carefully extracted using a pipette and transferred into a fresh 1.5 mL tube.

To precipitate DNA, 400 µL of chilled (20°C) isopropanol was introduced, followed by incubation for 30 minutes. The mixture was then centrifuged at 10,000 revolution per minute for 10 minutes. After discarding the supernatant, the concentrated residue was rinsed with 500 µL of 70% ethanol at 70°C and centrifuged again for 10 minutes at 10,000 revolution per minute. The tubes were left to air-dry on blotting paper for 120 minutes before the precipitate was resuspended in 100 µL of ultrapure water. The concentrations of DNA were then determined using a Nanodrop and stored at -18°C until polymerase chain reaction analysis.

Polymerase Chain Reaction (PCR) analysis

Eleven (11) polymorphic simple sequence repeats markers among the microsatellite markers earlier used by Olodo et al., *[29]* on *Digitaria exilis* were examined on the forty-one (41) *Digitaria iburua* accessions. A 25µL PCR reaction mixture was prepared with 1.25 mM $MgCl_2$, 1 mM dNTPs, 2.5µL each of the primers, 0.15 U Taq DNA polymerase, and 30 ng genomic DNA. The thermal cycle involved initial denaturation at 94°C (60 s), 35 cycles of 94°C (30 s), 50–57°C (30 s), and 72°C (60 s), followed by a final extension at 72°C (5 min). The PCR products were mixed with 1.5 µL of 10x gel loading dye and then loaded onto a 1% agarose gel prepared using 1x TAE buffer. Electrophoresis was conducted at 100V for 45 minutes. The gels were then stained with SYBR Safe and visualized under an ultra-violet transilluminator.

Nutritional and Phytochemical Analysis

The whole and dried grains of black fonio (*Digitaria iburua*) obtained after harvest were evaluated for their

nutritional and phytochemical components. The grains were first separated and sieved, then washed with distilled water. After the washing step, they were transferred to an oven and subjected to drying at 60°C for 48 hours. The dried grains were then ground into flour using a mechanical crusher, and the resulting flour was sieved using a sieve with a diameter of 500 µm. The moisture content was measured using a standard procedure outlined by AOAC [30]. This method relies on measuring the sample's mass reduction when heated to a constant weight at 105 °C.

Similarly, crude protein content was determined by measuring the total nitrogen in the sample using the Kjeldahl method [30]. The procedure entails breaking down the sample using sulfuric acid and a selenium catalyst, facilitating the transformation of organic nitrogen into ammonium sulfate. The nitrogen concentration is subsequently determined and adjusted using a grain-specific conversion factor to estimate the protein content. The conversion factor is 6.25 for fonio [31]. The lipid content was assessed using the Soxhlet extraction method [32]. In this process, oils are extracted from the sample using hexane in a Soxhlet apparatus. After extraction, the solvent is evaporated, and the remaining residue was heated in an oven at 105°C for 30 minutes to achieve drying. The lipid content of the sample was determined by the difference in weight before and after extraction.

To determine the mineral contents in *D. iburua,* calcium, zinc, magnesium, copper, potassium, sodium and phosphorus were measured in the ash using an electronic scanning microscope coupled with energy-dispersive spectroscopy. The fonio samples were first incinerated at a specific temperature to ensure complete combustion of organic material. The resulting ash was then analyzed, with the electronic scanning microscope providing detailed imaging and the energy-dispersive spectroscopy allowing for precise quantification of the mineral elements present.

Data Analysis

Molecular analysis

The DNA bands were analyzed according to the fragment length of each allele, with the size of the fragments determined by comparing their positions to the 50 and 100 bp molecular markers. Alleles were recorded as either present (1) or absent (0). Data analysis was conducted using PowerMarker version 3.25[33], and summary statistics were calculated. The genetic distance [34] for phylogenetic tree construction was also computed utilizing the same software. Allele frequencies were prepared with PowerMarker and used to construct a dendrogram in MEGA-X software employing the UPGMA (unweighted pair group method with arithmetic average) cluster analysis [35]. The values of the PIC were determined based on this formula outlined by Anderson et al., [36]. AMOVA (analysis of molecular variance) was conducted using GenAlEx software to assess the molecular genetic variance within and between populations.

Nutritional analysis

Descriptive statistics was used to assess the nutritional variability of the germplasm. Additionally, correlation analysis, clustering analysis and principal component analysis (PCA), and clustering analysis (CA) were conducted in R software. Multivariate analyses were conducted to assess the relationships between accessions. Similarly, hierarchical clustering on principal components was also conducted to categorize phenotypic groups. Finally, the mean values of all nutritional traits of all identified clusters were compared using boxplots. All these analyses were performed in R software.

Results

Genetic diversity indices

The eleven primers amplified the DNA samples and exhibited polymorphism across all accessions. A summary of the genetic diversity indices obtained from these SSR markers is presented in Table 1. A sum of 50 alleles were recorded, with the number of alleles of each marker varying between 3 and 8, averaging 4.55 alleles per primer. DE-ARC008 marker generated the highest number of alleles per locus (8), seconded by DE-ARC010 with 6 alleles, while DE-ARC028 and DE-ARC029 produced the lowest. They each generated 3 alleles. The PIC scores of the 11 markers spanned from 0.24 to 0.59 (Table 1). Observed heterozygosity varied between 0.20 and 0.95, while the effective number of alleles ranged from 1.25 to 20. Genetic diversity values spanned from 0.26 to 0.65, with a mean value of 0.48.

Genetic diversity, cluster analysis and population structure

The admixture model-based clustering analysis was applied to examine the population structure of the forty-one (41) *Digitaria iburua* accessions. The optimal cluster number (K=4) was determined using the lowest Bayesian Information Criterion (BIC) (Figure 1a, 1b). The sub-populations, highlighting genetic differentiation or clustering among the accessions are shown in Figure 2.

Furthermore, the genetic relationship between *Digitaria iburua* accessions were evaluated using a dissimilarity matrix-based neighbor-joining methodology. The genotypes were clearly categorized into 3 principal groupings using the neighbor-joining tree technique. At a 5% confidence interval, Cluster 1 comprised 27 accessions, Cluster 2 had 5, and Cluster 3 contained 8, regardless of their collection site (Figure 3). Molecular variance analysis indicated that within-group variation contributed 89% of the overall genetic variation, while between-group variation was 11% (Table 2; Figure 4). Additionally, the genetic distance between pairs of sources of collection varied from 0.61 to 0.79 with higher similarity between Nasarawa and Kaduna accession. Abuja accessions are closely related to Nasarawa accession (0.702) when compared to Kaduna and Plateau accessions (Table 3).

Table 1: Descriptive statistics of the genetic markers

Marker	Major Allele Freq.	Allele No	Gene Diversity	H	PIC
DE-ARC011	0.52	5	0.62	0.95	0.56
DE-ARC003	0.67	5	0.5	0.61	0.46
DE-ARC028	0.54	3	0.55	0.93	0.45
DE-ARC020	0.85	3	0.26	0.27	0.24
DE-ARC015	0.57	4	0.54	0.83	0.44
DE-ARC018	0.48	4	0.64	0.71	0.57
DE-ARC029	0.55	3	0.53	0.8	0.43
DE-ARC008	0.79	8	0.36	0.39	0.35
DE-ARC010	0.82	6	0.32	0.2	0.3
DE-ARC019	0.51	4	0.65	0.71	0.59
DE-ARC025	0.83	5	0.3	0.27	0.28
Mean	0.65	4.55	0.48	0.61	0.43

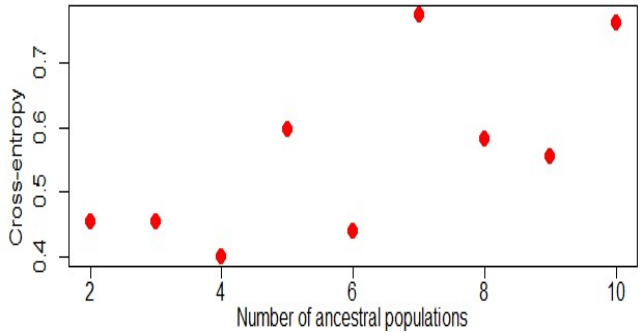

Figure 1a: Admixture model-based clustering

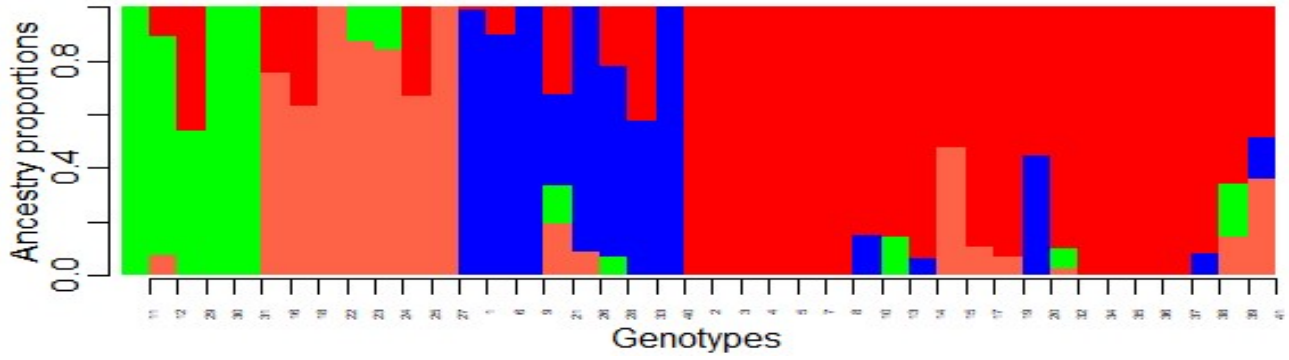

Figure 1b: Bar plot accession showing four sub population (Green=1, red=2, blue=3, gray=4)

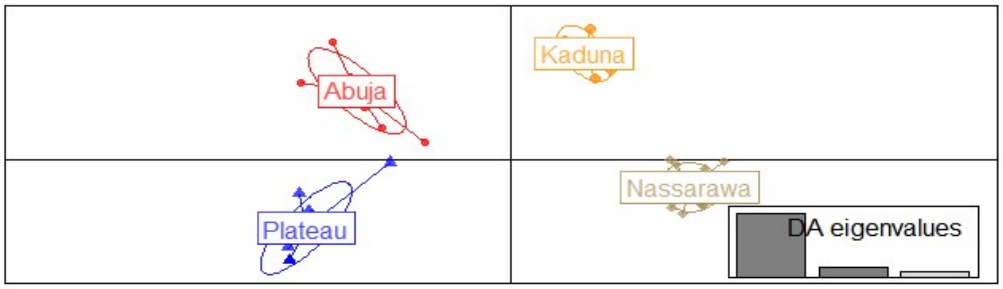

Figure 2: DAPC Scatter Plot, with each colour representing a different Sub-Population.

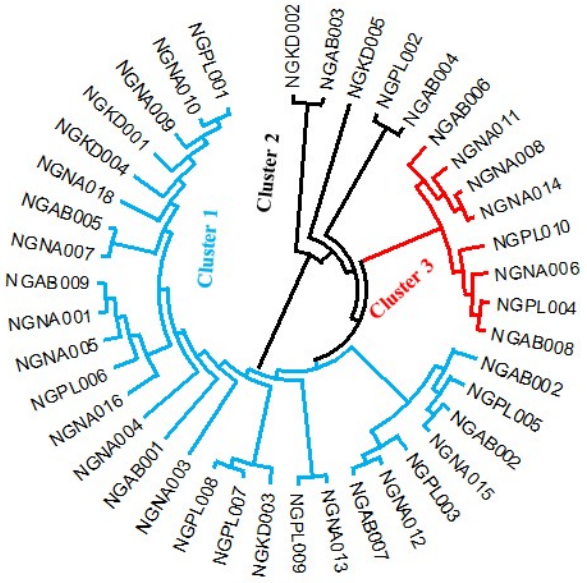

Figure 3: Dendrogram of *D. iburua* Genotypes from Molecular Analysis

Table 2: AMOVA for forty-one (41) Black Fonio Genotypes.

Source	DF	SS	MS	Est. Var.	%Variation	Stat	Value	Prob.
Among Populations	2	5.881	2.941	0.161	11%			
Within Populations	38	51.289	1.350	1.350	89%			
Total	40	57.171		1.511	100%	PhiPT	0.107	0.011

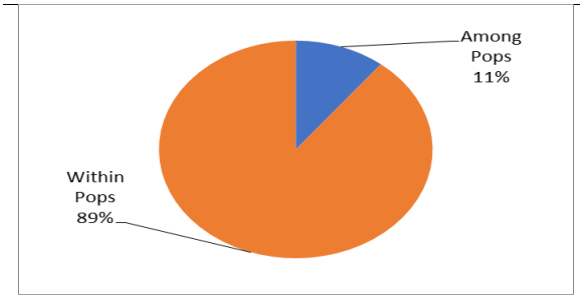

Figure 4: Molecular Variance in *D. iburua* Accessions (Pie Chart)

Table 3: Genetic distance between source of collection. Below diagonal is pairwise genetic distance (D) while above diagonal is pairwise F_{ST} values.

	Abuja	Kaduna	Nasarawa	plateau
Abuja	0	0.018	0.014	0.02
Kaduna	0.713	0	0.004	0.016
Nasarawa	0.702	0.608	0	0.026
Plateau	0.713	0.737	0.786	0

*Nutritional Evaluation of Black Fonio (*Digitaria iburua*)*
Principal component Analysis
The scree plot displays the eigenvalues, highlighting the "elbow" point where additional principal components contribute less to explaining the variance (Figure 5). In this case, the elbow occurs at an eigenvalue of around 2.5. The first and second principal components together account for approximately 34.5% of the variance in the nutritional traits of the *Digitaria iburua* accessions (Figure 6).

Grouping of Accessions and Comprehensive Profiling of Phenotypic Groups
The HCPC analysis utilizing fifteen nutritional traits, classified the accessions into three separate clusters (Figure 7), and this outcome was further supported by the analysis of PCA biplot (Figure 8). The number of landraces varied across these groups, with Cluster 1 containing the highest proportion (48.78% of total accessions), followed by Cluster 3 (31.71%), and Cluster 2 with the fewest (19.51%). Cluster 1 was characterized by high levels of potassium, crude lipid, iron, and carbohydrates. Landraces in Cluster 2 exhibited higher concentrations of iron, zinc, and magnesium. Cluster 3 landraces were distinguished by higher contents of crude fiber, crude protein, copper, phosphorus, and moisture, but had lower levels of sodium and calcium (Figure 8). The variabilities of these nutritional traits across the three clusters are shown in Figure 9.

Descriptive statistics of nutritional traits in Digitaria iburua
The evaluated *Digitaria iburua* accessions showed considerable variability across all sixteen (16) nutritional traits (Table 4). Moisture content ranged from 6% to 9.99%, with an average of 7.97%. Crude protein averaged 9.34%, varying from 6.7% to 11.92%. Carbohydrate content had a mean of 77.53%, with values ranging between 73.87% and 82.64%. Among all traits analyzed, iron and magnesium had the lowest mean values, measuring 0.09% and 0.13%, respectively. In contrast, carbohydrates recorded the highest mean values of 77.53%.

Relationship among nutritional traits studied
The Pearson correlation coefficients for the nutritional traits of the *Digitaria iburua* germplasm studied are presented in Figure 10. The strongest correlations were observed between crude protein and moisture content (r = 0.80), energy value and crude lipid (r = 0.85), carbohydrate and ash (r = 0.70), carbohydrate and energy value (r = 0.86), and carbohydrate and CL (r = 0.89). Conversely, we observed negative correlations between Ash and MC (r = -0.70), energy value and crude fiber (r = -0.70), energy value and moisture content (r = -0.80), energy value and crude protein (r = -0.75), CL and MC (r = -0.80), CL and CP (r = 0.92), carbohydrate and moisture content (r = 0.90), and carbohydrate and crude protein (r = 0.94).

Composition of nutrient contents of Digitaria iburua
The nutrient composition of the *Digitaria* accessions showed significant variation. Potassium was the most abundant nutrient across all accessions. Phosphorus and sodium had similar levels, with neither exceeding 2.5. Other nutrients, including calcium, zinc, copper, iron, and magnesium, were also present. Similarly, carbohydrates had the highest composition, around 80, followed by crude protein and moisture content. Ash and crude protein showed the lowest composition values in *Digitaria iburua* (Figure 11).

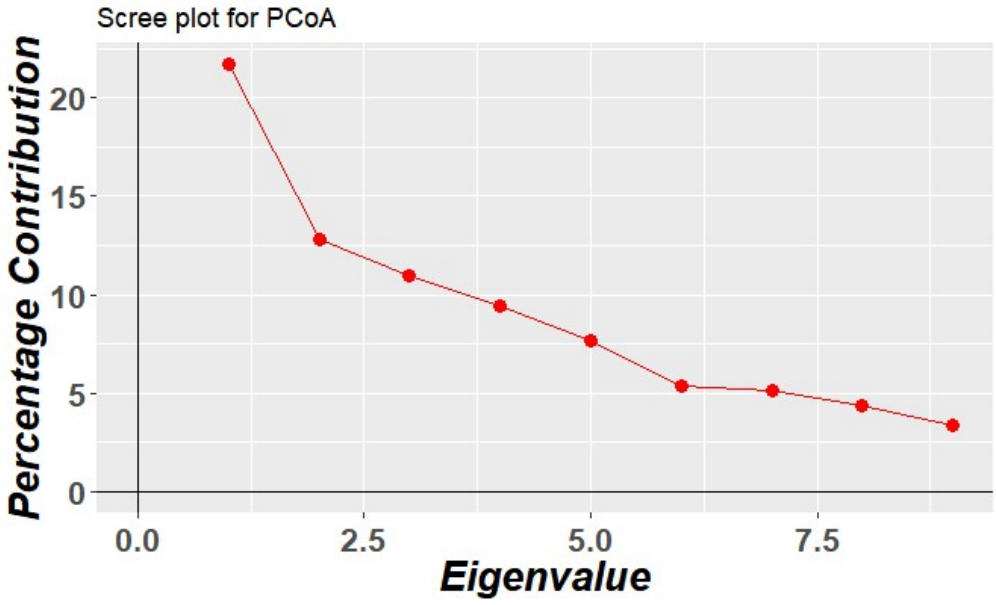

Figure 5: Scree plot showing the eigen values of each principal component.

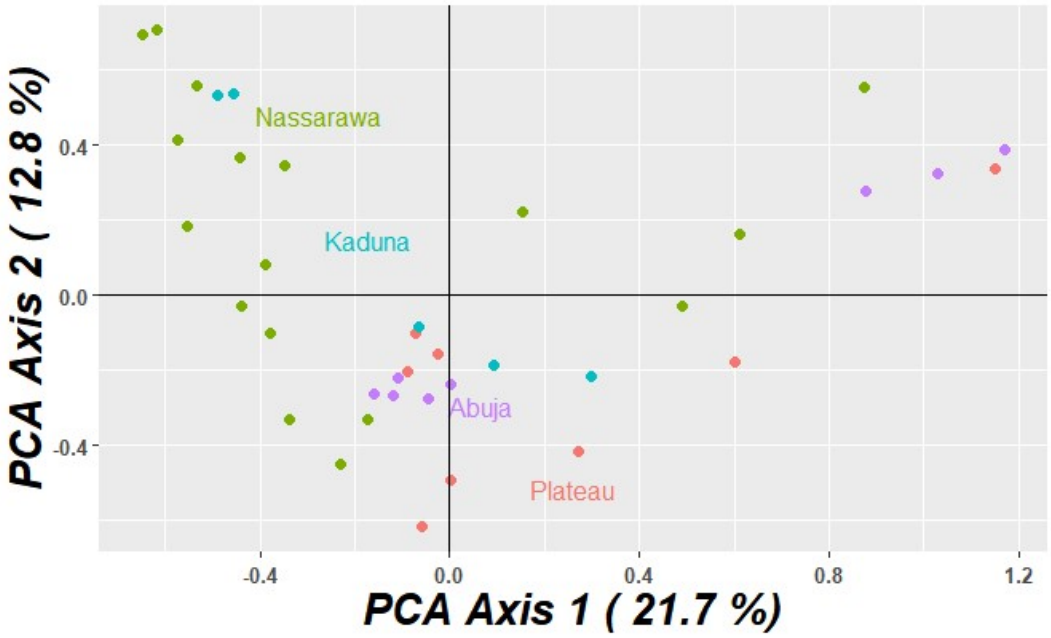

Figure 6: PCA Biplot displaying accessions on Axis 1 and 2.

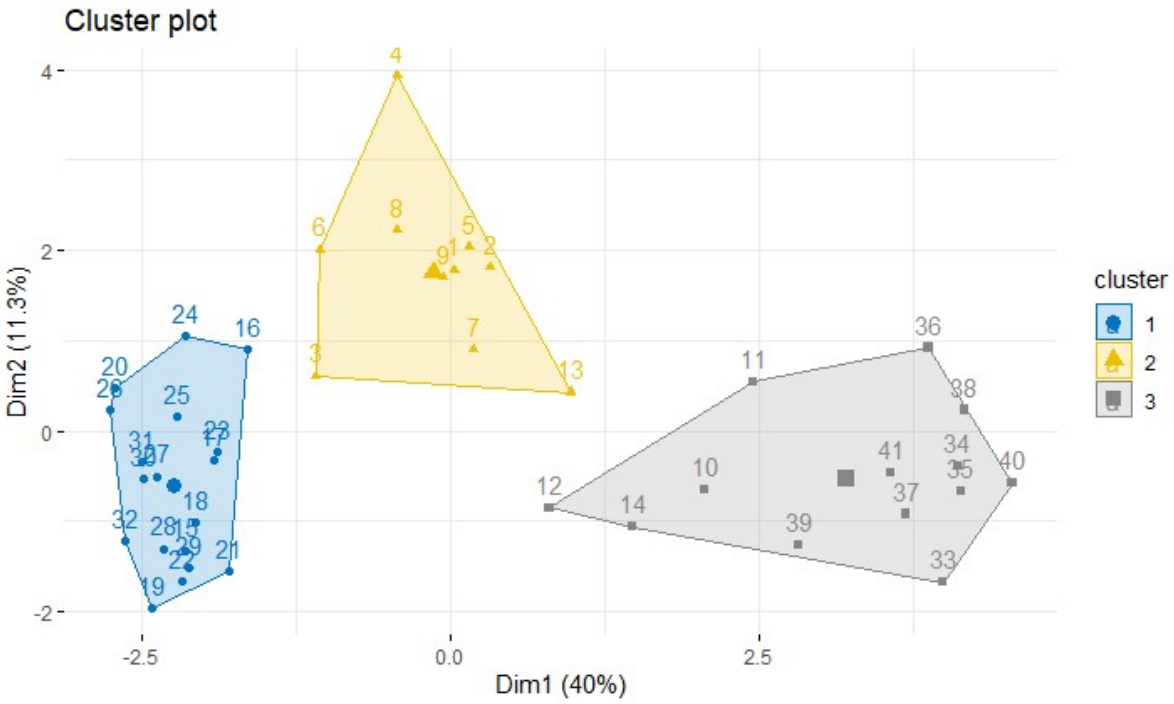

Figure 7: PCA-Based Hierarchical Clustering Revealing Phenotypic Groups

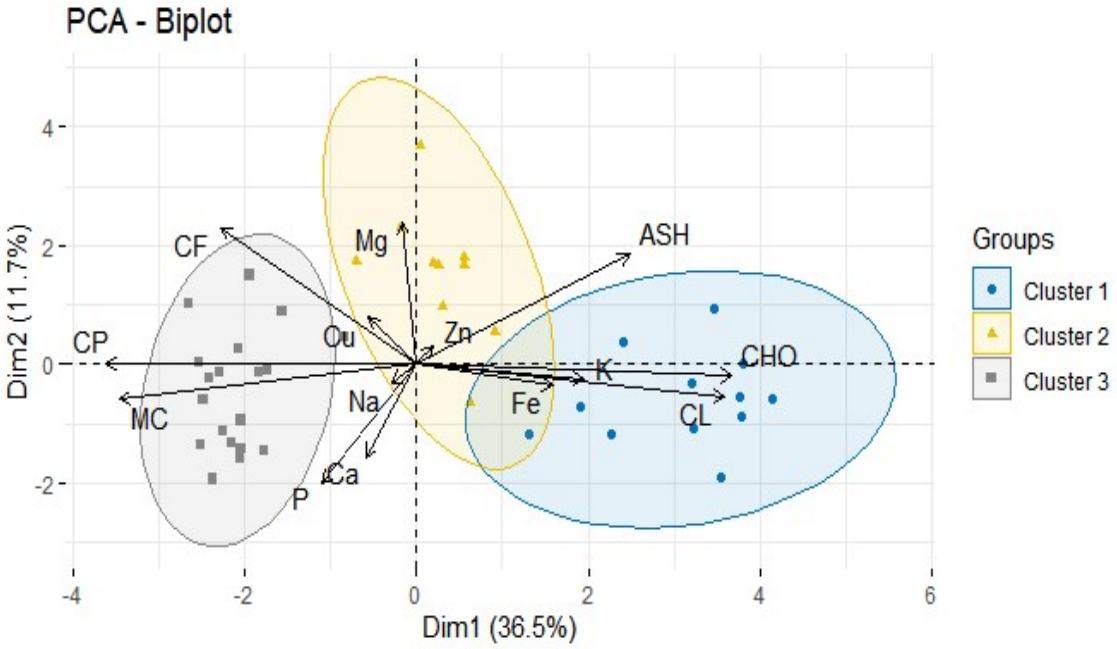

Figure 8: PCA biplot analysis showing traits, their dimensions and dominant traits in each Cluster.

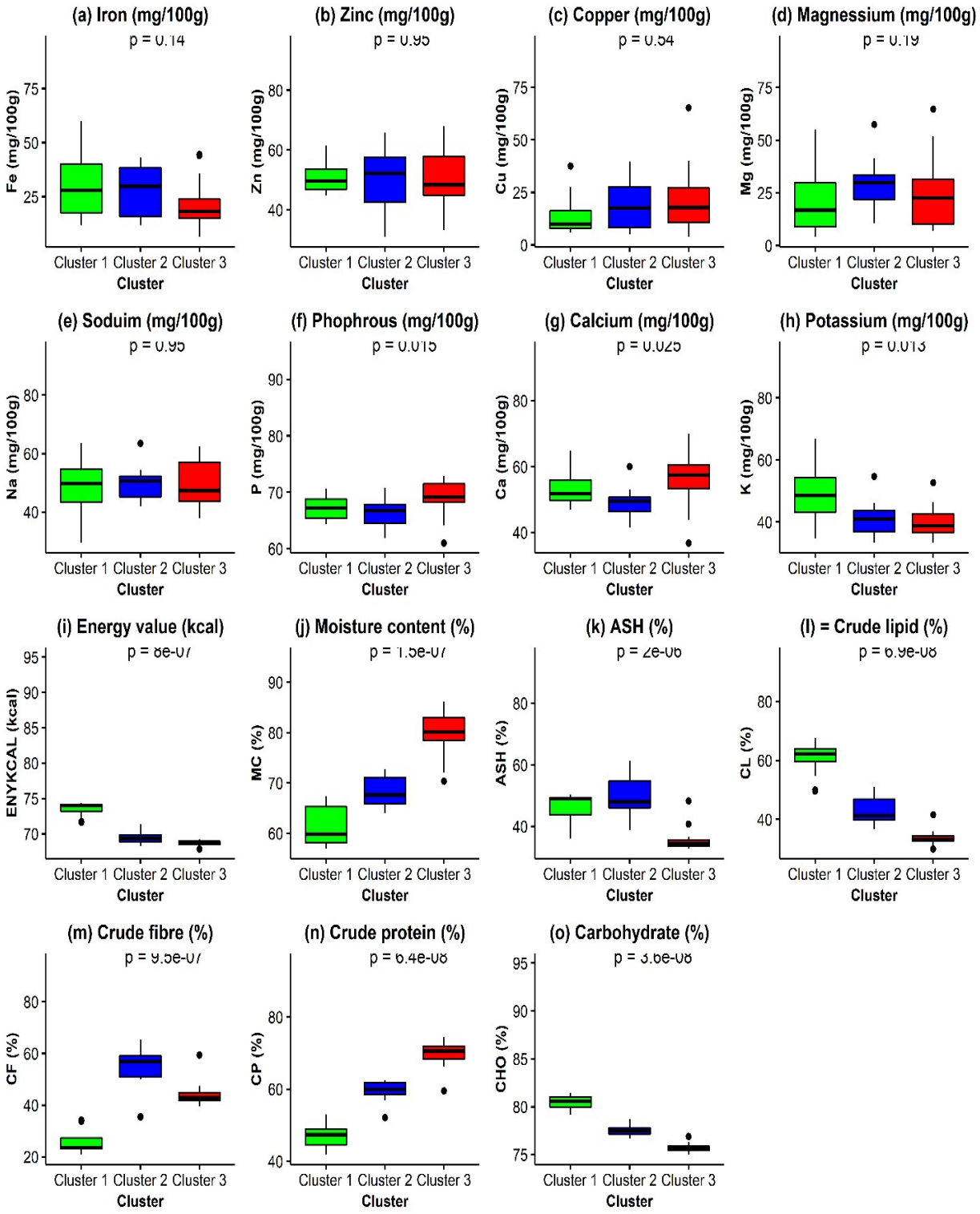

Figure 9: Boxplot showing the differences in *Digitaria iburua* performance based on the identified three clusters.

Table 4. Descriptive statistics of the nutritional traits studied.

Nutritional contents	Maximum	Minimum	Mean ± SEM
Moisture content	9.99	6	7.97± 0.42
Crude protein	11.92	6	9.34±0.42
Carbohydrate	82.64	73.87	77.53± 0.66
Crude lipid	4.91	1.89	2.96±0.13
Crude fibre	3.76	1.02	2.16±0.13
Copper	0.98	0.01	0.14±0.09
Calcium	0.87	0.28	0.62±0.11
Sodium	3.9	1.03	1.77±0.35
Zinc	0.87	0.28	0.62±0.10
Magnesium	0.97	0.01	0.13±0.09
Ash	3.3	1.47	2.11±0.16
Potassium	3.9	1.03	1.78±0.34
Iron	0.46	0.01	0.09±0.05
Phosphorus	9.57	7.07	8.46±0.43
Energy value	388.81	349.25	365.51±2.03
Sulphur	34.1	23.25	26.28±1.48

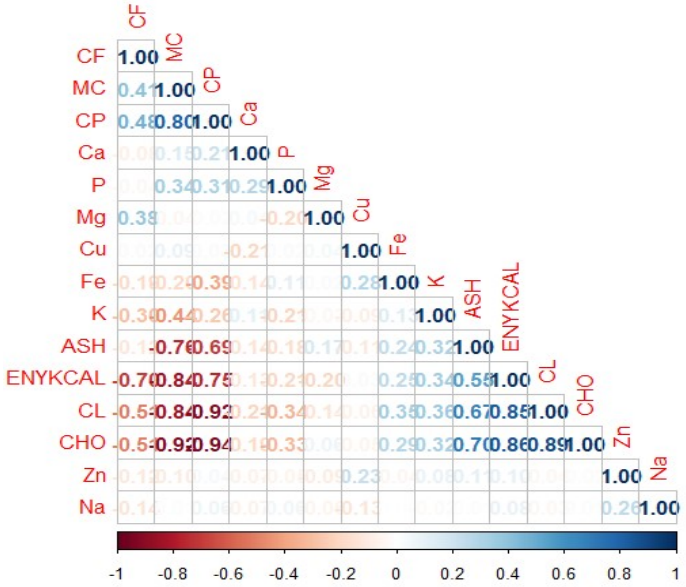

Figure 10: Correlation coefficients among nutritional traits of *Digitaria iburua* accessions

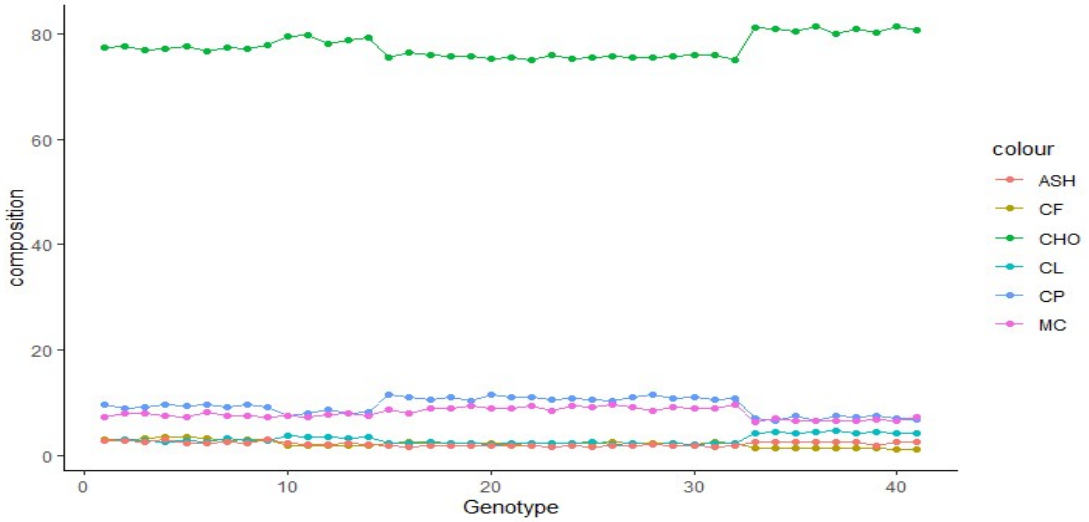

Figure 11: Line graph showing the proximate composition of *Digitaria iburua* accessions. studied.

Discussion

The genetic diversity estimations conducted in this study demonstrated that the eleven simple sequence repeat makers utilized provide highly informative insights into the variation among the *D. iburua* accessions, and the eleven sequence markers could be used for future crop research. The expected heterozygosity (gene diversity) levels (0.26 - 0.62, mean = 0.48) obtained in the present study were greater than those observed in a study of 140 fonio landraces using 12 microsatellite (SSR) markers, where heterozygosity levels were within the range of 0.15 to 0.89, with an average of 0.37. [37]. The high heterozygosity levels could be as a result of the location where the genotypes evaluated in this study were collected, and the primer markers utilized for the analysis. Fonio, being a cross-pollinated crop, exhibits high heterozygosity at multiple loci, indicating active gene transfer within the population and a high degree of variability among the studied genotypes. A greater number of alleles with balanced frequencies significantly enhances the expected average heterozygosity. The average number of alleles per locus in this study (4.55) is higher than the 2.50 reported by Akpan [38] in an earlier study. Furthermore, Bernard et al. [39] recorded an average of 4.71 alleles per locus in *black fonio* genotypes, which exceeds the average allele count observed in this investigation. This difference may be attributed to variations in the molecular markers utilized in each analysis.

The polymorphic information content (PIC) serves as a measure of a marker's ability to differentiate genetic variations and shows a positive relationship with the allele count per locus [40]. It is an essential method for measuring genetic diversity. A PIC value of 0.5 or higher implies that such markers are suitable for genetic diversity analysis [41]. In this analysis, the DE-ARC019 marker displayed the highest PIC value (0.59), with DE-ARC018 following at 0.57, whereas DE-ARC020 recorded the lowest PIC at 0.24. The mean PIC score of 0.43 recorded in this study aligns with findings from previous research, including a reported value of 0.43 in *D. exilis [*39*]*; 0.58 and 0.38 in Foxtail millet [41]. The outcomes of this investigation suggest that the microsatellite markers used were highly informative and could serve as valuable resources prospective genetic research on the taxonomy of fonio and the management of its genetic resources, especially the DE-ARC019 and DE-ARC018 markers.

The molecular variance evaluation indicated that genetic variability was more in within populations than between populations, conforming to findings from previous studies by various researchers[42;5]. The lower genetic diversity observed between populations as opposed to within populations might be a consequence of farmers selecting for similar traits across different collection sites. Nevertheless, farmers often maintain several genotypes within a single germplasm collection. Molecular screening techniques, such as SSR markers and associated DNA profiling techniques, offer an effective approach to assessing genetic variation independent of environmental influences.

The broad range of values for the nutritional attributes found in the accessions for the majority of the traits further support the significant genotypic variability found in the germplasm under study. The presence of genetic variety in crops such as cowpea [43], common bean [44], and sorghum [45] has been verified by the application of descriptive statistics. There is potential for selection for nutritional traits based on the high variation in proximate composition traits among the 41 black fonio millet accessions, or there may be a chance to create lines with improved proximate composition traits from this gene pool for a future breeding program. According to Lahoz et al. [46], genetic heterogeneity within a collection of genetic resources is a key measure of their significance and forms the basis for developing improved varieties.

The crude protein content in this study varied between 6.0 and 11.92, with an average of 9.3, aligning with previous findings on black fonio [47;48]. Gomez-Becerra et al. [49] state that protein deficiency remains a critical nutritional challenge in Africa. Grain protein level in black fonio millet is therefore a crucial quality measure that can be exploited to design cultivars with high nutritional content. It is noteworthy that a significant amount of crude protein was present in some accessions, including the Nasarawa accessions. This might be expanded into a variation to satisfy Africans' daily fonio millet-based diet and their immediate need for crude protein. Additionally, these

Nasarawa accessions represent prospective genetic resources that may be utilized as parental materials in hybridization to raise the concentration of crude protein in the grain of cultivated black fonio millet to notably higher levels.

The result of the mineral analysis revealed that *D. iburua* also contains high levels of sulfur, phosphorus, and potassium. Ballogou et al. [50] reached a similar conclusion, noting that the primary minerals in the grains of *Digitaria* species were potassium, phosphorus and magnesium. The carbohydrate content observed in this study was high, and is similar to the findings obtained by Idris et al., [47], who conducted nutritional analysis on black and white fonio accessions in Nigeria. The carbohydrates in fonio grains have diverse industrial uses and may act as a partial substitute for barley malt due to their low starch breakdown temperature and high beta-amylase property in both black and white fonio [47].

This study reveals that black fonio accessions in Nigeria have a considerable amount of diversity that could be exploited for future breeding research. This research found four major populations or sub-clusters from the analyzed germplasm using the population structure admixture model and discriminant analysis of principal components. However, Molecular variance evaluation demonstrated that the variation within the population exceeded the variability between populations, which could be as a result of farmers selecting for comparable traits. Furthermore, The Nasarawa accessions recorded the highest crude protein content compared to the other accessions, whereas Plateau accessions had high carbohydrate and crude fat content. Hence, this study offers important knowledge regarding the genetic diversity of *D. iburua* and highlight the potential for genetic improvement to boost its nutritional value.

Authors' contribution(s): Nwogiji Cletus Olando and Uba Charles Uwazuruike developed and authored the research proposal. Nwogiji Cletus Olando performed the experiment, collected and analysed the data, and drafted the manuscript. Majiok Koak Nyoac reviewed the proposal and assisted in study design. Nkpuma Kenneth Chukwuka collected the accessions and supported data collection. Nnamani Catherine Veronica edited the manuscript. Afiukwa Celestine Azubuike interpreted and helped analyse the data. Abdul, S. D., Uyoh E. A., and Opaluwa, H. I. assisted with accession collection, field setup, and field experiments. Enoch G. Achigan-Dako and Oselebe Happiness Ogba supervised the project and secured the research funding.

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