International Journal of Biochemistry, Biophysics & Molecular Biology
2026, Vol. 11, No. 2, pp. 15–28
https://doi.org/10.11648/j.ijbbmb.20261102.11

Research Article

Molecular and Biochemical Characterization of Black Fonio (*Digitaria iburua*) Accessions Collected in Northern Nigeria

Majiok Koak Nyoach[1,*], Nega Berhane Tessema[2], Happiness Ogba Oselebe[3], Isaac Ishaku Simon[4]

[1]Department of Medical Biotechnology, University of Gondar, Gondar, Ethiopia
[2]Institute of Biotechnology, University of Gondar, Gondar, Ethiopia
[3]Department of Crop Production & Landscape Management, Ebonyi State University, Abakaliki, Nigeria
[4]Department of Crop Production & Landscape Management, Abubakar Tafawa Balewa University, Bauchi, Nigeria

Abstract

Black fonio (Digitaria iburua) is a small annual herbaceous cereal crop, which produces very small grains. Fonio belongs to the family Poaceae, sub-family of Panicoideae, and genus Digitaria. It is believed to have a great potential in solving food crisis and malnutrition, but Limited information is available on various aspects of this crop. This study aimed to characterize nutritional, phytochemical and diversity among the black fonio accessions collected in Northern Nigeria. The study was conducted in Bauchi state Northeast of Nigeria. In this study, 41 black fonio accessions were sowed using alpha lattice design. An analysis on nutritional, phytochemical and diversity among the accessions was carried out. The molecular diversity study of the accessions was carried out using 14 SSR markers. The data obtained in this study were analysed using R-programming for nutritional and phytochemical data and DARWin software for molecular data. The result showed mean proximate contents as follows; moisture (7.97%), Protein (9.343%), lipid (2.96%), fibre (2.16%), Ash (2.11%), Carbohydrate (77.53%), and energy (365.5134 kcal). Microelements detected in D. iburua include Potassium (1.9 mg/l), Zinc (0.55 mg/l), Phosphorous (8.62 mg/l), Sulfur (37.3%), Sodium (1.3 mg/l), Magnesium (0.15 mg/l), Iron (0.15 mg/l), Copper (0.17 mg/l), and Calcium (1.52 mg/l). In addition, phytochemical analysis indicates the present of alkaloid, flavonoid, saponin, phenol and tannin in the selected solvents. Finally, D. iburua displayed three major clusters with genetic similarity at 0.68. In conclusion, the outcome of this study signified that black fonio accessions qualified to be good sources of functional diets for health as well as it could serve as future crops for solving the global food crisis and malnutrition in changing climate.

Keywords

Biochemical, Black Fonio, Genetic, molecular marker, Proximate, Phytochemical

[*]Correspondence: Majiok Koak Nyoach (isaacmajiok84@gmail.com)

Received: 6 July 2026; **Accepted:** 16 July 2026; **Published:** 28 September 2026

1. Introduction

1.1. Background

With an increase of chronic diseases and related pathological complications, health has become the forefront of scientific research for finding novel foods and strategies to combat such public health burdens [5]. The huge rise of consumer interest in whole grains that are healthy and useful for fighting chronic illnesses like diabetes, celiac disease, cancer, cardiovascular disorders, and other chronic illnesses [2, 23]. Infant malnutrition is another major world health problem, especially in developing countries [39]. The World Health Organization (WHO) has estimated that 150 million infants in developing countries are malnourished [39]. This is due to insufficient intake of healthy and nutritious diets [29]. The Solution for such major challenges facing the globe involves boosting the production of healthy and nutritious food for the world's growing population [25, 45]. Whole grains and whole-grain foods are becoming increasingly important and have attracted the attention of many consumers around the world to tackle problems related to chronic diseases and malnutrition [22, 23, 35].

Fonio is one of those cereal grains that have an important role in fighting against chronic diseases and malnutrition due to its high nutritional content and antioxidant activities [36]. It has the potential to be used as a nutraceutical due to its antioxidant, phenolic, and cholesterol-lowering properties [48]. Fonio grains are poor in gluten and are beneficial for people suffering from coeliac disease and overweight people, pregnant and breastfeeding women, as well as people suffering from diabetes [29].

Fonio species belong to the family Poaceae, sub-family Panicoideae, tribe Paniceae, and the genus Digitaria [58]. The genus Digitaria comprises over 300 species [41]. Digitaria species are generally C4 plants with digitated inflorescences but on some occasions, paniculate type is also observed [50]. The species have extraordinary tiny grains about (0.5-1mm in diameter and 0.75-2mm in length) with 1,000 seeds weighing 0.5-0.6g [32]. The caryopsis is tightly enclosed within two brown husks. In *D. iburua*, the husks are intensively dark brown; hence it is commonly named black Fonio in contrast to *D. exilis*, commonly called white Fonio because of its white husks [57]. Fonio millets are tetraploid plants (2n=36 chromosomes) having stable and relatively small genome sizes (1Chromosome = 956Mbp and 904Mbp for *D. exilis* and *D. iburua*, respectively [1].

Fonio plays a critical role in food and nutrition security for several millions of people in many West African Countries including Nigeria [29]. It is commonly used as staple food, especially during shortage periods due to its short life cycle compared to other millet groups like sorghum or rice [1]. Fonio is well appreciated for its tasty and easily digestible grains [7]. The traditional uses of Fonio include stiff porridge, thin porridge, couscous, and alcoholic beverages [58]. Fonio millets can be one of the essential cereal crops for solving the global food crisis in a rapidly growing population and changing climate due to its adaptability to different climatic and marginal environmental conditions and its fast-maturing life cycle [9].

Fonio is not only tolerant to drought but also a very fast maturing crop. It is rich in methionine and Cysteine, the two amino acids vital to human health, and deficient in major cereals such as wheat, rice, and maize [29]. Fonio can well adapt to local pedo-climatic conditions and have the ability to resist different environmental stresses [6, 50]. Fonio millets are among the important traditional cereal crops that significantly contribute to household food security in marginal areas of West Africa [19, 33, 41].

But despite all these good qualities, Fonio millets remain the least studied among the cereal crops in general and millets in particular. Fonio ranked at the top among the African orphan crops that were not given enough attention [50]. Compared with major cereals, such as wheat and rice, Fonio is a type of underutilized cereal grain with potential for food applications [6, 58]. Due to its high nutritional content, Fonio ranked first among the most nutritious grain crops [1]. Unfortunately, the use and cultivation of Fonio, especially the black fonio, have been ignored in different perspectives. That is why this research aimed to study the nutritional, molecular and phytochemical characteristics of *D. iburua*.

1.2. Statement of the Problem

In many West African Countries including Nigeria, the production of white Fonio is widely practiced, while that of black Fonio is undermined [48]. Black Fonio is poorly known as far as its agronomic performances, nutritional compositions, and genetic and molecular characterizations are concerned. Despite its potential, black Fonio remains a marginal plant and has long been neglected in national and international research programs. Most of the research emphasized the management of Fonio harvest and post-harvest in which most focus is put on the white Fonio. Limited and scattered information is available on varietal breeding, molecular and phytochemicals characterization as well as nutritional contents of this crop especially in the study area and beyond. In addition, there is a variation of data generated by different researchers on the nutritional compositions of this crop which is why this study aimed to characterize genetic diversity, phytochemical and nutritional compositions of *D. iburua* accessions.

1.3. Significance of the Study

This research would greatly serve as a guideline in determining quality varieties based on the molecular characterization in the study area. It would increase the interest and need for black fonio as a major food diet for the people suffering from different chronic diseases and malnutrition due to the outcome generated in this study. This would equally help different sectors like plant breeders and molecular biologist, in identifying and determining useful landraces; food processing industries, in introducing marketable processed black Fonio products, health departments in introducing Fonio as nutraceutical in management of chronic illness, and agricultural companies in maximizing the production of black Fonio millet in large-scale production and harvesting system.

1.4. Objectives of the Study

1.4.1. General Objective

The general objective of this study was to characterize genetic diversity, nutritional and phytochemical compositions of Nigerian black Fonio (*D. iburua*) accessions.

1.4.2. Specific Objectives

The specific objectives of this study were: -

1) To determine proximate (crude protein, crude lipid, crude fibre, moisture, carbohydrate, ash and gross energy) compositions among black Fonio accessions.
2) To determine mineral compositions among black Fonio accessions.
3) To determine the essential amino acids content in black Fonio grains.
4) To analyses the antioxidant assay of black Fonio grains through qualitative and quantitative phytochemical screening.
5) To study genetic diversity among black Fonio accessions using SSR markers.

2. Materials and Methods

2.1. Description of the Study Area

The field and laboratory experiments for nutritional and phytochemical content were conducted in Bauchi State, North-East Nigeria. The site is between latitude 09º 00' 55.9" to 13º 01' 02.4" N and longitude 09º 46' 55.3" to 11º 47' 06.7" E. The mean annual rainfall of the area is 1072mm, with higher total annual potential evapotranspiration of 1200mm. The mean diurnal temperature varied between 13.1ºC and 31.6ºC [37].

2.2. Genetic Materials

The research study was carried out using 41 *Digitaria iburua* accessions which were collected in Northern Nigeria and were kept in the gene bank in the University of Abomney Calavi in the republic of Benin. All the accessions were originally collected from four locations in Northern Nigeria, namely, Kaduna, Nasarawa, Abuja, and Plateau.

2.3. Experimental Design

The field trial for this study employed a completely randomized experimental design with three repetitions at 5% significance level was carried out as described by [17]. The test samples were taken from the dried grain of black fonio accessions for proximate, phytochemical, minerals and essential amino acid contents analysis. For molecular analysis, two weeks young leaves were collected from each accession for DNA extraction.

2.4. Sample Collection for Molecular Analysis and Extraction of Genomic DNA

Young leaves (two weeks old) were collected from the forty-one accessions in the research trials at Abubakar Tafawa Balewa University (ATBU), Bauchi Northern Nigeria. Young leaves that were collected from each genotype were dried at room temperature. The dried leaves samples were packet with presence of silica gel and transported to the Republic of Benin, Laboratory of Genetics, Biotechnology, and Seed Science (GBIOS) in the University of Abomney Calavi for molecular analysis.

Genomic DNA was isolated from each of the forty-one accessions of black Fonio using the cetyl trimethyl ammonium bromide (CTAB) method in which, 1 gram of each leaf sample was ground using liquid nitrogen. From that ground sample, fine powder was taken with 5 ml pre-heated extraction buffer and incubated at 65^{0}C for 1 hour, then kept at 4 ℃ for 15 mins.

Fourteen primers as described by [41, 46, 55] were used. The PCR reactions were performed in 25 µl reaction mixture containing 100 ng of each genomic DNA, 2.5 mM MgCl2, 0.25 mM dNTPs, 400 mM of each primer, 1 µl Taq DNA polymerase.

2.5. Preparation of Sample for Nutritional Analysis

Samples of black Fonio grains were collected from the field trial. The grain samples of black Fonio accessions were washed with distilled water and dried. Each sample was ground separately into a fine powder using mortar and pestle and was stored in an air-tight plastic container for nutritional analysis.

2.6. Proximate and Biochemical Analysis

The experiment for nutritional analysis of black Fonio was conducted in Biochemistry Laboratory at ATBU. The nutritional analysis was done using fine powder of the already ground samples of *D. iburua*. The moisture, ash, fat, fiber, protein, mineral, and essential amino acids content were determined. All the determinations were done in triplicate, and the results were expressed as the average result of the value except for the mineral content.

2.6.1. Reagents Preparation

Reagents of different concentrations were prepared according to the protocol used by [48] for the analysis of protein, carbohydrate, fat or lipid, crude fiber, ash, and moisture content of black Fonio millets. Proximate composition was determined according to the standard method of the Association of Official Analytical Chemists' Approved Methods (AOAC) [27].

2.6.2. Determination of Protein

The protein was determined using Kjeldahl method. Protein-containing sample was digested using concentrated sulfuric acid in the presence of the Kjeldahl catalyst. Standard alkali was added to digest the sample and the ammonia produced is steam distilled into a standard solution of Hydrochloric acid. Titration of acid indicates the ammonia (NH3) distilled, and hence the nitrogen content from which crude protein calculated using AOAC procedures [27]. The procedure was repeated for all the accessions.

$$\text{Protein}\ \ (\%) = \text{Tv} \times 0.041 \times \text{N} \times \text{Va} \times \frac{100}{\text{Ms}} \times \text{va} \tag{1}$$

Where *Tv* = titer volume (ml); *N* = Normality; *Va* = volume of aliquot (ml); *Ms* = sample weight (g) and *6.25* = conversion factor for protein from% nitrogen.

2.6.3. Determination of Lipid Content

The principle was because lipophilic (non-polar) constituents of a sample were readily extracted into organic solvent. The lipid extraction was done on each black Fonio accession following the AOAC procedure as described by [48]. Petroleum ether (B.P.40-60 ℃) was used as solvent, and the extraction was carried using Soxhlet extractor.

$$fat\ \ (\%) = WF - \frac{W}{Sw} \times 100 \tag{2}$$

Where *Wf* = weight of the receiver flask and fat deposit, *W* = weight of the empty receiver flask only, and *SW* = weight of the sample used.

2.6.4. Determination of Ash

The principle is based on the loss in weight that occurs after igniting the sample in a muffle furnace at a temperature of about 600 ℃ which causes the organic matter to burn completely without affecting the ash constituents. Then, the sample was removed, cooled in a desiccator at room temperature, and weighted. The procedure for analysis was done following AOAC method [27] and the percentage of ash content in each accession was calculated using the below formula.

$$\text{Ash}\ \ (\%) = (\text{W1} - \frac{\text{W2}}{\text{Sw}}) \times 100 \tag{3}$$

Where W1 = weight of the ash plus crucible after ashing, W2 = weight of the empty crucible, and Sw = weight of the sample taken.

2.6.5. Determination of Moisture Contents

The principle involved a difference in weight before and after drying carried through 100 ℃ constantly in 24 hours by following the procedures provided by AOAC [27]

$$\text{Moisture}\ \ (\%) = \left(\text{W1} - \frac{\text{W2}}{\text{Sw}}\right) \times 100 \tag{4}$$

Where W1 = weight of the cap and fresh sample, W2 = weight of the dry sample and cap, and SW = sample weight.

2.6.6. Determination of Crude Fiber

The sample material for each accession was analyzed for crude fiber content. Firstly, each sample was treated with petroleum ether (B.P 40-60 ℃) for removal of fat. The defatted sample was treated with boiling H2SO4 and later with boiling NaOH, and the residue left after the subtraction of the ash was taken as fiber. The overall determination of ash content in each accession was carried out following AOAC method as described by [27].

$$\text{Crude}\ \ \text{fiber}\ \ (\%) = \left(\text{W1} - \frac{\text{W2}}{\text{Sw}}\right) \times 100 \tag{5}$$

Where *W1* = weight of the porcelain crucible and sample before ashing, *W2* = weight of the porcelain crucible containing ash, and *Sw* = weight of the sample.

2.6.7. Determination of Total Carbohydrate Content

The total carbohydrate was determined by the difference method in which the percentages of crude protein, crude fiber, crude fat, ash content, and moisture content were subtracted from 100% (AOAC) [27].

$$\text{Carbohydrate}\ \ (\%) = [100\% - \%\ \ (\text{Protein} + \text{Fiber} + \text{Lipid} + \text{Moisture})] \tag{6}$$

2.6.8. Determination of Gross Energy

The energy value from each accession was estimated by the following equation:

$$\text{Food}\ \ \text{energy}\ \ (\frac{\text{Kcal}}{\text{g}}) = (\%\text{TC} - \%\text{CF}) \times 4 + (\%\text{TF} \times 9) + (\%\text{CP} \times 4) \tag{7}$$

Where *TC* = total carbohydrate, *CF* = crude fiber, *TF* = total fat, and *CP* = crude protein.

2.7. Determination of Essential Amino Acids Content in Black Fonio grains

UV-Visible spectrophotometer analysis of essential amino acids present in the powdered sample of black Fonio was carried out using the method described by [40] with slight modification. 1gram of black Fonio powdered sample was measured and transferred into 100 ml beaker. 25 ml of ethyl acetate was added to elude the sample. Silica gel was added into the burette tube as the stationary phase and 25 ml of ethanol (100%) was added after as the mobile phase. For extraction of impurities in the sample, Sodium sulfate was added to the burette. After the burette setting was finished, the sample was added to the burette, and the collection bottle was placed below the burette. 10 ml of the eluded sample was collected at the end. The sample of purified amino acids was purred into the cuvette along with a blank sample that contained Ethyl acetate and the two were run in the UV-Visible Spectrophotometer instrument (752N) at different wavelengths for specific determination of essential amino acids.

2.8. Determination of Minerals Composition Among Black Fonio Accessions

The mineral contents of the fine ground grains sample of each black Fonio accession were determined by atomic absorption spectrophotometer (AAS) (AA32ON Wincom Shangia). The analysis was carried out following the AOAC method as described by [35] with slight modification. 1 g of digested sample was obtained from each accession and was dissolved using 37.5 ml of HCL and 12.5 ml of NHO3. The samples were heated on hotplate, and the solutions were diluted to 100 ml with deionized water. Then the filtrate of each sample was used for determination of mineral contents by atomic absorption spectrophotometer using acetylene as source of energy for atomization. The Sulfur and phosphorous content among grains of black Fonio accession were determined through gravimetric and molybdate methods as described by [10, 54] respectively with slight modification. For determination of sulfur content, 2 g of each sample was measured in electric balance and was dissolved in 100 ml of water. Barium chloride was used to test sulfate. The test result showed the present of barium sulfate as indicated by the formation of white precipitate. The amount of sulfur in percent was calculated by considering the weight of filter paper, the weight of BaSO4 in filter paper, the molecular mass of barium sulfate and the molecular mass of SO4 in BaSO4 and the results were reported in percent. The procedure was repeated for each of the samples of black Fonio grain. For determination of Phosphorous, 0.27 g of potassium antimony titrates was added into another bottle. Then, 4 g of Ammonium heptamolybdate was added in 100 ml of the wash bottle. 17.6 g of 0.1 M of ascorbic acid was added into 100 ml of distilled water. The absorbance was measured at 880 nm using UV-Visible spectrophotometer alongside water as blank sample. The procedure was repeated for each of the black Fonio accession grains sample under study and the result was recorded.

2.9. Phytochemical Screening Among the Black Fonio Accessions

Samples of grains from the black Fonio accessions were collected from the experimental field at ATBU. The sample was ground into a fine powder using a clean mortar and pestle. The analysis was carried out using the method described by [20, 51] with slight modification. Three solvents (Chloroform, N-Hexane, and Methanol) were used for the extraction of secondary metabolite present in the black Fonio grains. 100 grams of powdered sample were packed into three separate round bottom flasks for sample extraction using the three solvents. The extraction was conducted with 250 ml of each solvent mixture for 24 hours. At the end of the extraction, the respective solvents were concentrated under reduced pressure and kept in the water bath (at 50 ℃). The extracted experimental solutions were stored in the refrigerator for qualitative and quantitative analysis of secondary metabolites present in the black Fonio grains.

2.9.1. Qualitative Phytochemical Screening

Preliminary phytochemicals screening was performed for the secondary metabolites using the standard protocols described by [26]. Mayer's test, Hager's test, and Dragendorff's test were performed for Alkaloids. Legal test was performed for identifying glycosides; the Ferric chloride test was followed to determine the presence of tannins and polyphenolic compounds. Flavonoids were tested through an alkaline test, and the results were analyzed through the change in color or form.

2.9.2. Quantitative Phytochemical Screening

Quantitative phytochemical screening of black Fonio millet was carried out by adapting the method used by [20, 51] with slight modification. Three secondary metabolites (Alkaloids, Tannin, and Phenolic compounds) were analyzed using a UV-Visible spectrophotometer, while Saponin was determined using double extraction gravimetric method.

2.10. Data Analysis

The proximate, minerals, amino acids and quantitative phytochemical data were subjected to analysis of variance (ANOVA) at 5% probability level, and the results were reported as mean ± standard deviation. Tukey HSD and Pearson correlation test were carried out to determine the relationship between the traits proximate and mineral composition. Nutritional and Physicochemical data were analyzed to determine the effect of each component. However, all data were analyzed using the general model procedures of R programming software version 4.1.1 (2021-08-10). For molecular data analysis, the allelic data for PCR-based SSR markers were converted to a presence (1), absence (0) matrix for analysis. Tree Construction and Principal Coordinate Analysis were calculated for single data of dissimilarity matrices based on the presence/absence of alleles using the Jaccard coefficient as implemented in DARwin 5 software. Cluster analysis was performed using the unweighted neighbour-joining method with 1,000 bootstraps.

3. Result and Discussion

3.1. Results

3.1.1. Proximate Composition of Black Fonio Accessions

Food proximate composition is usually studied to analyse the nutrient profile and its amounts by percentage or weight in the sample. Proximate analysis was undertaken for black Fonio grain samples and the analysis of variance showed highly significant difference among the accessions ($P \leq 0.05$) for crude protein (9.34%), Moisture (7.97%), lipid (2.96%), crude fiber (2.16%), total carbohydrate (77.53%), and energy (365.5Kcal). Table 3 outlined the details of overall means, the maximum, minimum, and coefficient of variation for proximate parameters compositions of black Fonio (*D. iburua*) accessions.

The post hoc test done by Tukey honesty significance different among the accessions (Table 4) outlined the proximate composition in term of mean ± standard deviation for all the forty-one black Fonio accessions. In this study, the maximum crude protein content was recorded from the accession NGNA001 (11.92%), while the minimum crude protein was recorded from accession NGPL003 (6%). Significantly, maximum value of lipid content was recorded from accession NGPL008 (4.91%), while the minimum value was recorded from accession NGNA014 (1.89%). In terms of crude fiber content, maximum value was recorded from accession NGAB008 (3.76%), while the minimum crude fiber content was recorded from accession NGPLOO5 (1.02%). In terms of Ash and Moisture content, maximum values were recorded from accession NGAB004 (3.3%) and accession NGAB003 (9.99%), while the minimum values were recorded from accession NGPL010 (1.47%) and accession NGPL007 (6%) respectively. Significantly, maximum value of total carbohydrate content was recorded from accession NGPL007 (82.64%) while the minimum value was recorded from accession NGNA014 (73.87%). The maximum energy content was recorded from the accession NGPLOO6 (388.81 Kcal), and the minimum value was recorded from accession NGNA014 (349.25 kcal).

3.1.2. Mineral Compositions Among Black Fonio Accessions

In this study, the analysis of variance done on minerals composition of black Fonio accessions showed significant differences ($P \leq 0.05$) for Potassium, Zinc, Sodium, Iron, Calcium, Phosphorous, and Sulfur, whereas the study showed no significant difference ($P \leq 0.05$) among the accession for magnesium and Copper contents. The result (Table 5) showed that the calcium content ranged from (1.2±0.0 mg/l) to (1.9±0.7 mg/l). Copper content among the accessions ranged from (0.04±0 mg/l) to (0.6±0.4 mg/l). The iron content ranged from (0.02±0 mg/l) to (0.3±0.2 mg/l). Potassium content among the accession ranged from (1.4±0.3 mg/l) to (2.6±0.7 mg/l). For Zinc, the value ranged from (0.3±00 mg/l) to (0.8±0.1 mg/l). Phosphorus, the value ranged from (7.23±0.00 mg/l) to (9.56±0.37 mg/l). For Sulphur content among the accessions, the value ranged from (24.44±0.84%) to (53.24±3.74%). For Magnesium the content ranged from (0.02±0 mg/l) to (0.4±0.5 mg/l).

3.1.3. Pearson's Correlation Among Proximate and Mineral Compositions of Black Fonio Accessions

In this study, Pearson's correlation analysis was run to examine the relationship between proximate and mineral compositions among black fonio accessions. The correlation analysis showed significant negative associations between energy and crude Protein, (r= -0.81) energy and crude Fiber (r= -0.80), energy and moisture content (r= -0.78) ($P<0.05$). On the other hand, highly significant positive association was observed between Energy and Lipid (r= 0.93), energy and Carbohydrate (r= 0.90), and slightly significance positive relationship was observed between energy and Ash (r= 0.35) ($P<0.05$). There was also significant positive relationship between Lipids and Carbohydrate (r= 0.83), Lipids and Ash content (r=-0.45), on the contrary, there was significant negative relationship between Lipids and Fiber content (r= -0.66), Lipids and Protein (r= -0.86), and Lipids and Moisture content (r= -0.72). There was a significant positive correlation between Carbohydrate and Ash (r= 0.48), Meanwhile, negative correlation was observed between carbohydrate and crude fiber (r= -0.60) carbohydrate and protein (r= -0.93), carbohydrate and moisture content (r= -0.85). On the other hand, there was no non-significant relationship observed between ash and fiber. Phosphorous and sulfur also showed positive relationship (r= 0.7). Positive relation exists between sulfur and sodium (r= 0.265), phosphorus and sodium (r= 0.298), and sulfur and zinc (r= 0.328). Meanwhile, there was a negative correlation between copper and potassium (r= -0.147), Copper and Sodium (r= -0.185). There was very slightly no correlation between some other mineral and proximate compositions among black Fonio accessions.

3.1.4. Essential Amino Acids Profile of Black Fonio Grain

The analysis of variance showed a highly significant difference ($P<0.05$) among the essential amino acids content in black Fonio grains (Table 6). In this study, nine essential and three conditionally essential amino acids were identified in the black Fonio grain sample at wavelength range of 200-400nm at peak of 900-1000. The identified essential amino acids in the black Fonio grain samples were phenylalanine (0.35±0.01 mg/l) at 250 nm, histidine (0.29±0.02 mg/l) at 212.5 nm, isoleucine (0.11±0.01 mg/l) at 201 nm, leucine (0.29±0.02 mg/l) at 280 nm, methionine (0.32± mg/l) at 213.5 nm, lysine 0.29±0.01 mg/l) at 270 nm, (threonine (0.15±0.01 mg/l) at 240 nm, tryptophan (4.33±0.72 mg/l) at 296 nm, and valine (0.32±0.01 mg/l) at 215 nm. In addition, the three conditionally essential amino acids identified in black Fonio were the cysteine (0.38±0.01 mg/l) at 210 nm, proline (0.23±0.02 mg/l) at 215 nm, and arginine (0.21±0.01 mg/l) at 270 nm.

3.1.5. Qualitative Phytochemical Screening

The preliminary qualitative phytochemical screening was done on the black Fonio grain in three different solvents, and the result were summarized in Table 8. The results showed the presence of medicinally active constituent in the three solvents studied. From the test result, Alkaloid, Flavonoids, and Saponin showed positive result in all the extracts of the three solvents, while Tannin only showed positive result in Methanol extract. Glycosides and sterols showed positive result only in N-Hexane extract and Terpenoids showed negative results in all the three solvents.

3.1.6. Quantitative Phytochemical Screening

The quantitative phytochemical screening of black Fonio grain extracts indicated significant present of alkaloid, tannin, flavonoid and phenols ($P\leq0.05$). The analysis showed that the highest concentration of alkaloid in black Fonio was recorded in chloroform extract (208.3±17.97 mg AE/g); while the lowest concentration was recorded in N-Hexane extract (63.51±6.02 mg AE/g). For flavonoid, the highest concentration was recorded in methanol extract (15.46±0.29 mg QE/g), while the lowest concentration was recorded in chloroform extract (1.43±0.21 mg QE/g). Significantly, the highest concentration of phenolic compound in black Fonio grains was recorded in N-Hexane extract (9.29±0.24 mg GAE/g), while the lowest concentration was recorded in Chloroform extract (2.65±0.3 mg GAE/g). The highest concentration of saponin was recorded in methanol extract (47.40±1.50 mg/l) while the lowest concentration was recorded in N-Hexane extract (36.68±3.67). For tannin, the highest and the lowest values were recorded in methanol extract (58.11±8.54 mg GAE/g) and N-Hexane extract (36.68±3.67 mg GAE/g) respectively.

3.1.7. Genetic Diversity and Clustering Pattern of Black Fonio Accessions

Forty-one black Fonio accessions were subjected to SSR marker assay to assess molecular diversity. Fourteenth primers were tested; eleven amplified while three others (DE-ARC016, DE-ARC021, and DE-ARC026) did not amplify. In this study, based on Euclidean distance utilizing the UPGMA, the tested accessions were estimated with SSR data, and distance was achieved as revealed in dendrogram graph (Figure 4). The studied accessions were presented in groups based on the results of the cluster analysis to infer relationships among accessions.

3.1.8. Principal Component Analysis

Principal component analysis (PCA) data for all 41 black Fonio accessions were shown in Fig 5. The analysis classified black Fonio accessions into three major clusters. The Jaccard's similarity coefficients between the accessions were calculated for UPGMA clustering. The forty-one *D. iburua* accessions were divided into three major groups based on their genetic similarity at 0.68 as cluster I, cluster II, and Cluster III. Cluster I was subdivided into two other clusters, such as cluster IA and IB. Cluster IA consisted of twelve accessions which included accessions (I37, I35, I36, I34, I39, I40, I31, I32, I18, I1, I16, and I14). Cluster IB consisted of five accessions which included accession (I4, I3, I6, I5, and I7). Cluster II consisted of nine accessions which included accessions (I33, I9, I41, I23, I38, I2, I25, I7, and I12). Cluster III was subdivided into two sub clusters (IIIA and IIIB). Sub cluster IIIA consisted of twelve accessions which included accession (I24, I21, I19, I22, I15, I13, I28, I27, I29, I30, and I26). Sub cluster IIIB consisted of only three accessions which included accession (I11, I10, and I8). The Jaccard's similarity among the accessions ranged from 0.56 to 0.875 with average edge distance between initial tree and bootstrapped trees of 0.68 and Metric index value and Euclidean index value of 0.98 and 0.55 respectively.

3.2. Discussion

The nutritional analysis was carried out on the grain of black Fonio accessions. The result showed highly significant difference among the forty-one black Fonio accessions in term of moisture content, protein content, crude fiber, crude lipid, ash content, carbohydrate and total energy at ($P<0.05$). Result obtained in this study indicated that the total carbohydrates varied significantly among the accessions and the contents (%) ranged from (74.99±0.30) in accession NGNA018 to (81.44±1.31) in accessions NGPL007 with the average mean value of 77.53%. The highest carbohydrate compositions were recorded from accessions collected in Plateau state and the lowest values were recorded from accessions collected from Nasarawa state. The values obtained in this study are in conformity with values described by [30, 44, 49] on Fonio Carbohydrate content.

The moisture content of black Fonio accessions ranged from (6.32±0.49) in accession NGPL002 to (9.58±0.09) in accession NGNA018 with mean value of 7.97%. Similar result that corresponded this study on the moisture content of Fonio have been reported by [48] and [30] indicating (7.00%) and (11.72±0.20%) respectively. In addition, the value reported in this study closely correspond with the value (7.48±0.15%) reported by [12] on moisture content in black Fonio millet. The moisture content obtained in this study among black Fonio accessions indicated that it's ideal for long term storage, suitable extended shell life, and reduction of bacterial or fungal spoilage on the grains. The findings agreed to an extent with the work of [8].

Millets are rich in a unique type of resistant starch that has been recognized as a functional fiber that is vital for body health [34]. Crude fiber is an integral valuable nutrient for healthy probiotics that normalizes bowel movement, lowers blood cholesterol and blood sugar levels and prevents colorectal cancers [2, 9, 22, 58] reported similar findings. Regarding crude fiber content among black Fonio accession evaluated in this study, the crude fiber content ranged from (1.17±0.01) in accession NGPL004 to (3.44±0.32) in accession NGAB005 with the overall mean value of (2.16%). This values nearly correspond to the value (1.03%) reported by [48], but higher than the value (0.857±0.01) reported by [48] in the grain of black Fonio and lower than the value (7.23±0.006) reported by [11, 21]. In general, the high fiber content among black Fonio accessions is an indication that black Fonio is good for maintaining gut and body health and at the same time can be good components in the diets of patients suffering from obesity, constipation, and gallstones. Similar analysis was reported by [34].

The protein content among black Fonio accessions ranged from (6.43±0.44) in accession NGPL003 to (11.48±0.21) in accession NGNA007 with mean value of 9.34%. The highest values were obtained from accessions in Nasarawa followed by the accessions of Abuja, while the lowest protein contents were obtained from the accessions of Plateau and Kaduna. The results obtained in this study correspond with the result (9.53%) protein content reported by [7] and [31], (8.05%) reported by [48] and (8.75%) reported by [49]. In general, the average protein levels of the black Fonio accessions studied were at least similar or higher than those of common cereals like barley, tef, wheat, maize, pearl millet, rye, rice and sorghum [7]. But unlike protein present in other cereals, the protein available in Fonio is considered nutritionally superior because of its high levels of amino acid profile and its gluten free natures which make it a suitable alternative to other cereals in the case of celiac disease and gluten free diet [7, 8]. The high protein content among the accessions signified that black Fonio in this study can have great nutritional importance, especially in developing countries to curb the menace of protein energy malnutrition. Similar output was outlined by [48].

Crude lipid obtained in this study ranged from (2.03±0.1) in accession NGNA014 to (4.6±0.3) in accessions NGPL008 with average mean value of 2.96% among the black Fonio accessions. The highest values of lipid content were obtained among the accessions from Plateau State followed by the accessions from Kaduna state, while the lowest values of lipid content were obtained from the accessions from Nasarawa followed by the accessions from Abuja. The ranges of lipid content in black Fonio obtained in this study exactly fall within the range (1.3-5.2%), reported by Salaudeen and Orhevba, (2021) and (4.0%) reported by [48]. In contrast, [7] reported the lowest lipid content in Fonio (0.98±0.01%) which is lower compared to the ranges of values obtained in the current study. This may be due to variation among the species of Fonio and the varieties involved in the process, or it may be due to environmental or analytical influences.

The ash content among black Fonio accession ranged from (1.6±0.1%) in accession NGNA009 to (3.1±0.1%) in accession NGAB004 with mean value of 2.11%. This corresponds with the range of ash content reported by [49] and the value reported by [48]. The high ash content among the black Fonio accessions showed reflection of mineral content preserved in the Fonio grain. Similar analysis was stated by [13] and [18] demonstrating that the ash content indicates the presence of mineral matter in food and it is a non-organic part of food which aids in the metabolism of other compounds such as protein, fat and carbohydrate.

Minerals if present in diet are responsible for the metabolic activities, rigid bone formation, transmission of nerve impulses, regulation of water and salt balance among others. Minerals like Calcium and phosphorus are essential minerals for a healthy diet. Calcium helps in formation of strong bones, teeth, muscle contraction, while phosphorus is essential for the maintenance of cells, formation and support of healthy bones and teeth [4] and [22]. Just like Ethiopia tef, Fonio has been reported to content sufficient amount of mineral for healthy and nutritional supplementation to the body [9]. The mineral content among black Fonio accession evaluated under this study showed a significant difference among the accessions at ($P \leq 0.05$) for Potassium, Zinc, Sodium, Iron, Calcium, Phosphorous, and Sulfur, whereas the study showed no significant difference ($P \leq 0.05$) among the accession for magnesium and Copper contents. The result as displayed in Table 5 showed that the calcium content among the accessions ranged significantly from (1.2±0.0 mg/l) to (1.9±0.7 mg/l) with the accessions from Nasarawa having recorded the highest values and accessions from Kaduna with least recorded values. The premise of calcium in sufficient quantity is essential for teeth, bones health and further for protection against cancer, diabetes and high blood pressure. Similar results were reported by [48, 53, 58]. Copper content among the accessions ranged from (0.04±0 mg/l) to (0.6±0.4 mg/l) with the accessions from Nasarawa recording the highest values and the accessions from Abuja recording the lowest values. The iron content among the accessions ranged from (0.02±0 mg/l) to (0.3±0.2 mg/l) with the accession NGPL004 recorded for the highest value. Iron is the primary component for making hemoglobin in the body. The iron content reported in this corresponds to the value reported by [3, 38, 39]. Potassium content among the accession ranged from (1.4±0.3 mg/l) to (2.6±0.7 mg/l). For Zinc, the value ranged from (0.3±00 mg/l) to (0.8±0.1 mg/l).

Phosphorus content among the black Fonio accessions ranged from (7.23±0.00 mg/l) to (9.56±0.37 mg/l) with accessions from Abuja recording for the highest values followed by the accessions from Nasarawa. For Sulphur content among the accessions, the value ranged from (24.44±0.84%) to (53.24±3.74%) with accession NGPL007 recording the highest value and NGAB008 recording for the least value. For Magnesium the content ranged from (0.02±0 mg/l) to (0.4±0.5 mg/l. Magnesium is one of the essential minerals for brain, heart and blood health. It helps promote the bone health. Similar results were reported by [34]. But the result reported by [48] is different from the result of the current study, this may be attributed to methodological or instrumental difference, or it may be due to environmental variation.

In general, [48] reported the following proximate composition of black Fonio grain samples; protein (8.75%), lipid (4.0%), fiber (1.03%), moisture (7.0%), carbohydrate (76.95%), and energy (378.64 Kcal). This is like the range of values obtained in the current study. Furthermore, Jideani and Jideani, (2011) also reported the following proximate compositions of flour from Fonio; moisture (6.74±0.06), total ash (0.58±0.01), crude lipid (0.98±0.01), protein content (12.19±0.06), fiber (1.03±0.01), carbohydrate (74.10±0.06), and energy (371.53±0.24). Study shown that, Fonio is a good source of minerals required by both children and pregnant women (Sadiq *et al.*, 2015). These outputs reported by these researchers closely fall within the ranges of value reported in this study.

The result of the present study indicated that black Fonio is an excellent source of amino acids, the essential and some conditionally essential amino acids which were evaluated under this study. The essential amino acids profile of black Fonio grain significantly showed that Tryptophan was recorded at highest concentration (4.33±0.72 mg/l), while Isoleucine was recorded at the lowest concentration (0.11±0.01 mg/l). For Cysteine and Methionine, the vital amino acids that were known only to be found in Fonio grain, the concentration were (0.38±0.01 mg/l) and (0.32±0.00 mg/l) respectively. The other essential amino acids evaluated in this study included; Phenylalanine (0.35±0.0 mg/l), Histidine (0.29±0.02 mg/l), Valine (0.32±0.01 mg/l), Leucine (0.29±0.02 mg/l) Proline, (0.31±0.01 mg/l) Glycine, (0.29±0.01 mg/l), Arginine (0.21±0.01 mg/l), and Threonine (0.15±0.01 mg/l) at wavelength of 200-400nm. The results obtained in this study correspond to the results reported by Sanusi and Ahmad, (2019), But lower than the result reported by [7]. Furthermore, Salaudeen and Orhevba, (2021) reported the following essential amino acids content (%) in Acha grains; Phenylalanine (0.47%), Histidine (0.17%), Isoleucine (0.28%), Leucine (0.91%), lysine (0.19%), Methionine (0.34%), Threonine (0.34%), Tryptophan (0.92%), and Valine (0.52%). The variation among these outputs may be attributed to differences in methods or instruments used or it may be due to varieties or environmental differences.

The proximate, mineral and essential amino acids contents of the black Fonio millet evaluated in this study showed significant variation on most of the parameters studied. This variation could be attributed to environmental influences, variation in geographical location, agronomic or genetic factors or variation in analytical methods involved. Similar analysis was also concluded by [1, 4, 7, 16, 49]. The World health organization (WHO) stated that cereal grains including Fonio supplies important minerals, vitamins, protein, and micronutrients essential for optimal health, growth, and development of the human body [17]. Minerals are among the most important constituents of diet for good health and proper growth. Some of the minerals are integral parts of blood, teeth, muscles, and bones. Hence, their specific amount is required for proper functioning of body systems [47]. The minerals present in Acha are reported to help build tissues, regulate body fluids or assist in various body functions [14].

The correlation analysis was done to evaluate the relationships that exist among proximate and mineral compositions of black Fonio millet. Most of the traits exhibited significant positive correlation ($P \leq 0.05$) with each other. However, few traits exhibited insignificant ($P > 0.05$) correlation and others were negatively correlated. Lipid, energy and ash showed positive relationships while carbohydrate, protein and moisture showed negative correlation.

Just like other cereals, Fonio and indeed many other underutilized species, represent unique sources of a wide variety of bioactive substances including antioxidants which are essential for preventing free-radical induced degenerative disorders like cancers, heart diseases and diabetes [15]. Many plant parts contain bioactive substances that are of pharmacological importance. These bioactive components include phenols, flavonoids and alkaloids, many of which have antioxidant and redox properties [42]. The study on black Fonio grains sample showed significance content of alkaloid, flavonoid, tannin, phenolic compounds and saponin in methanol, chloroform and N-Hexane solvents. It was observed that the chloroform extract had the highest alkaloid content (208.3±17.97 mg GAE/g) compared to methanol (67.56±1.92 mg GAE/g) and N-Hexane extracts (63.51±6.02 mg GAE/g). For flavonoid, the highest content was obtained from methanol extract (15.46±0.29 mg QE/g) followed by N-Hexane extract (2.68±0.18 mg QE/g) and chloroform extract (1.43±0.21 mg QE/g). Flavonoids are group of polyphenols with low molecular weight and their present in the diet is very useful for attenuating hyperglycemia including; preventing long term diabetic complications (Rolnikan and Olas, 2021). Phenolic compound content in black Fonio grains displayed significant difference among the solvents. The highest phenolic content was recorded in N-Hexane extract (9.29±0.24 mg GAE/g) followed by N-Hexane extract (9.29±0.24 mg GAE/g) and the chloroform extract which recorded for the minimum value (2.65±0.3 mg GAE/g). For Saponin the highest value was recorded from Methanol extract (47.40±1.50) while the lowest value was recorded from N-Hexane extract (36.68±3.67). The tannin the highest value was recorded in Methanol extract (58.11±8.54), while the lowest value was recorded from N-Hexane (36.68±3.67). The outcome of this study closely corresponded to the results reported by [42] on phytochemical content of Fonio millets in powder form, aqueous extract and methanol extract. In general, the present of phytochemical compounds in sufficient quantity as indicated in this study, signified that black Fonio can an essential diet with antioxidant activities and treatment for chronic diseases such as cancer and cardiovascular diseases. Similar analysis was stated by [3, 9, 13, 36, 43, 58] indicating that millets especially, Fonio are rich in hidden treasure of highly potent health-promoting phytochemicals.

The presence of proximate constituents, macro and micronutrients and phytochemicals as revealed in this study, implied that the nutritional and antioxidant properties of black Fonio investigated if consumed in sufficient amounts and doses, would contribute greatly towards meeting human nutritional and health requirement for normal growth and adequate protection against malnutrition and some chronic diseases such as cancer, cardiovascular diseases and diabetes. Similar reports on health and nutritional contribution of Fonio were stated by [30, 49, 52, 53].

Diversity analysis based on morphological traits may be inflated and unrealistic due to environmental influence [45]. This is because Fonio exhibits some level of isomorphism, and it is difficult to distinguish morphologically during the vegetative growth stage. This was also supported by [1, 5, 24, 28, 38, 45, 56]. The diversity study of Fonio at the varieties level was given little to no attention. Most of the studies done on Fonio species focus on morphological differences, while ignoring molecular characterization. The current study on genetic relationships among black Fonio accessions are presented in SSR based UPGMA tree. On the UPGMA tree, *D. iburua* accessions were clustered into three major clusters with Cluster I and III composing of the highest number of accessions and cluster II composing of minimum number of accessions. The SSR markers amplified a total of 68 alleles in black Fonio accessions. The number of alleles per locus ranged from 1 to 6 alleles. The observed heterozygosity among the accessions was 1 for all and the expected heterozygosity ranged from (0) in accession I6 to (0.49) in accession I4. The principal coordinate analysis classified the accessions into five PCs with the first axis contributing the highest percentage of inertia (25.28%) followed by axis two and three and axis five consisting of the less inertia (7.3%). In the study, fragment amplification by the ISSR primers revealed inter- and intra-genetic diversity among the *D. iburua* accessions grown in Northern Nigeria. This indicated that the markers used in this study were effective for the accessions partitioning and identification. This is in agreement with study done by [5, 6], which demonstrated that microsatellite markers are effective in genotyping and fingerprinting of plant accessions. Based on these findings, it could be concluded that there was a significant amount of genetic heterogeneity among the evaluated accessions and that the SSR markers used in the study were informative in discriminating the Fonio accessions. The outcomes of this study are therefore important for the genetic improvement of this indigenous African cereal crop. This is in line with the study done by [17, 55, 59].

4. Conclusion and Recommendations

4.1. Conclusion

Due to an increase in awareness of nutrition and health, attention has turned to improving functional food products containing essential nutrients and bioactive components. This is because functional foods such as whole grain food are equipped with essential nutrients and health providing antioxidant compounds. The current research was undertaken to analyze the nutrient composition and bioactive components profile of Nigerian black Fonio accessions to determine their special qualities. According to this study, black Fonio accessions have shown significance differences for the proximate, minerals, essential amino acids and bioactive compositions investigated. These differences were attributed to the genetic differences among the black Fonio accessions and environmental conditions. Black Fonio accessions have been found to have essential nutritional quality. The nutrients found in these amazing cereal crops include protein, carbohydrate, fiber, essential amino acids and minerals plus many other nutrients and phytochemicals. The present of essential amino acids and antioxidant activities as reported in this study through phytochemical screening and nutritional analysis of grain samples of black Fonio millet indicate that the crop can act not only as nutritional rich diet but also as medicine for maintaining body health. In addition, this study and other studies as well, found that Fonio grains are rich in methionine and cysteine which are vital amino acids needed by body and which cannot be found in other major cereal crops like wheat, maize and sorghum. Furthermore, black Fonio accessions showed significance quantity and quality of phytochemicals which can be essential for the treatment of degenerative diseases, cancer, and hypertension as well as for infectious diseases. In similar extent, the minerals like calcium, magnesium, zinc, sodium and others found in black Fonio grains indicated that the crop can be good for skin, bones and brain health. The results obtained from this study also showed that black Fonio accessions can be potential nutraceutical which could make substantial health contribution through the intakes of essential nutrients, phytochemical and antioxidant compounds. Finally, the variation among the accessions for proximate and mineral compositions was also pictured on the diversity analysis among the black Fonio accessions using SSR markers. This showed that the accessions in this study could be good sources in future breeding and nutritional quality in varying environments. Finally, molecular profiling has been the preferred choice of breeding as these are more reliable and authentic and less influenced by environmental fluctuations. Therefore, this study proved that black Fonio accessions evaluated could be of utmost contribution to the future of human health, nutrition and breeding program and for solving global food crisis and malnutrition.

4.2. Recommendations

The results obtained in this study signify black Fonio accessions as promising nutraceutical grains to be applied for food consumption, nutritional supplementation, antioxidant activities, and breeding program for better quality varieties. But the current study alone is not enough, further research should be done ahead to fully characterize and understand the health and nutritional benefits of Fonio millet. Hence the following areas are highly recommended to be studied further on these amazing cereal crops:

1) Similar research with large sample size.
2) In-vitro antibacterial activities on the different parts of black Fonio millet.
3) In –vitro and in-vivo antioxidant and antidiabetic activities on the grains.
4) Comparative study on amino acids profile, proximate, vitamins and minerals compositions on both white and black Fonio grains.
5) Food processing industries need to include Fonio in food formulation in making cookies, cakes, biscuits, flour etc. for people suffering from celiac diseases and diabetes, and as porridge for pregnant and breast-feeding women, as well as for children.
6) Diversity study using single nucleotide polymorphism (SNP) markers and sequencing among the black Fonio accessions is another area that requires further research.
7) Adoption, usage and evaluation of Fonio millet especially in our Country Ethiopia are highly recommended.

Abbreviations

AAS	Atomic Absorption Spectrophotometer
AE	Atropine Equivalent
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ATBU	Abubakar Tafawa Balewa University
CTAB	Cetyltrimethylammonium Bromide
DARwin	Dissimilarity Analysis and Representation for Window
EDTA	Ethylenediaminetetraacetic Acid
FAO	Food and Agricultural Organization
GAE	Gallic acid Equivalent
GFF3	General Feature Format
GBIOS	Genetics, Biotechnology, and Seed Science
HSD	Honestly Significant Difference
Kcal	Kilocalorie
Mbp	Mega Base Pair
mpa	Mega Pascal
ND	Nanodrop
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
QE	Quercetin Equivalent
rpm	revolution Per Minute
RSP	Repetitive Spark Pair
SNP	Single Nucleotide Polymorphism
SSR	Single Sequence Repeat
UN	United Nation
UPGMA	Unweighted Pair-group Method Using Arithmetic Averages
USA	United States of AMERICA
UV	Ultraviolet
WHO	World Health Organization

Author Contributions

Majiok Koak Nyoach: Conceptualization, Data curation, Funding acquisition, Methodology, Resources

Nega Berhane Tessema: Formal Analysis, Investigation

Happiness Ogba Oselebe: Funding acquisition, Investigation, Project administration

Isaac Ishaku Simon: Conceptualization, Data curation

Conflicts of Interest

The authors declare no conflicts of interest.

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