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VARIABILITY IN NUTRITIONAL QUALITY AND EVALUATING THE EFFICACY OF SSR MARKERS IN CHARACTERIZATION OF SELECTED BAMBARA GROUNDNUT ACCESSIONS

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ABSTRACT

Variety, soil conditions, climatic change, time, and biodiversity are some of the factors contributing to the inconsistent evaluation of Bambara groundnut accessions over the years. Finding Bambara groundnut accessions with excellent nutritional contents and molecular markers for efficient genetic diversity screening was the aim of this research. Five polymorphic SSR markers and ten Bambara groundnut accessions were used in the experiment. This study aimed to identify Bambara groundnut accessions with high nutritional contents and molecular markers for effective genetic diversity screening. The experiment consisted of Ten Bambara groundnut accessions and five polymorphic SSR markers. Nine proximate and phytochemical parameters were observed among the studied accessions. Wide variability was recorded among the accessions on the total flavonoid content, total phenolic content, crude protein, crude fiber, moisture content, ether extract, nitrogen free extract and total sugar content. The total flavonoid content (TFC) varied among the accessions of Bambara groundnut ranging from 5.58 – 29.19 mgGAE/g with FUKV8 recording the highest. Total phenolic content ranges from 13.48 to 5.85 mgGAE/g, crude protein ranges from 26.74% to 15.14% with FUKV6 recording the highest. The microsatellite markers under analysis had polymorphism information content (PIC) values ranging from 0 to 89%, with an average of 52%. In genetic diversity investigations, CEDG294 and PR-32 had the greatest PIC values, indicating efficiency. It is possible to classify FUKV6, FUKV18, FUKV19, and FUK10 as high-nutrient types, FUKV1 and FUKV2 as low-nutrient types, and the remaining ones as medium-nutrient types.

Keywords: *Vigna subterranea* L. Verdc, Proximate Analysis, Accessions, phytochemical analysis, SSR Markers, Yield

INTRODUCTION

The herbaceous, intermediate, annual Bambara groundnut is thought to primarily self-pollinate (Heller et al., 1997). The crop's morphological structure is similar to that of the groundnut (*Arachis hypogaea*) in that, following fertilization, the pale-yellow flower stalk bends downward, bearing its pods beneath the earth. Depending on the habitat and landraces, it typically has a reproductive cycle of 90 to 150 days and two primary contrasting development habits: the branched form and the bunched habit (Berchie et al., 2010). Bambara groundnut germplasm has been preserved for many centuries as landraces, which are frequently genetically and phenotypically varied (Aliyu et al., 2016). Their cultivation has a positive impact on food security by generating income for subsistence farmers, reducing over-reliance on a limited number of crops for food, fuel and fodder while requiring fewer inputs than conventional agriculture systems (Halimi et al., 2019). Africa produced more than 70% of the 230,619 Mt of dry Bambara groundnut grain seeds produced worldwide on 354,870 hectares, according to FAOSTAT (2020). Despite its many advantages, Bambara Groundnut is still an underutilized species that has long been grown and helped the world's poorest people have access to food and nourishment (Ibrahim et al., 2022). Quest for food surpasses food accessibility due to the steadily increase in human population. If not properly tackled, this will lead to a starvation, particularly in the developing countries. Previous studies have examined various aspects of Bambara groundnut, including genetic diversity, agronomic traits, nutritional composition, and phytochemical properties (Karikari et al., 2017). Selection for genetically diverse accessions in recent weather changes, and improved performance is a better attempt to speed up breeding programs and ensuring food security (Karikari et al., 2017).

Molecular markers such as SSRs and SNPs have been utilized to assess genetic diversity within Bambara groundnut accessions, while phytochemical analyses have focused on flavonoids, phenolic compounds, and other bioactive substances (Esan, et al., 2023). Since molecular markers associated with desirable features have been utilized to speed up plant breeding operations, the availability of trustworthy molecular markers is crucial for plant breeding. DNA-based markers have been established in many agricultural crops (Esan, et al., 2023). Majority of studies on the genetic diversity in Bambara groundnut rely heavenly on agronomic and phenotypic characters (Olukolu et al., 2012) with little results on the use of molecular assets using isozyme markers (Molosiwa et al., 2015), random amplified polymorphic DNA (RAPD) markers (Molosiwa et al., 2013; Uba et al., 2021), amplified fragment length polymorphism (AFLP) markers (Ntundu et al., 2004) and diversity arrays technique (DArT) markers (Osundare et al., 2023). DNA markers are classified based on their detection methods into three categories: hybridization-based, polymerase chain reaction (PCR)-based, and DNA sequence-based markers (Kaur et al., 2018). Microsatellites, also known as simple sequence repeats (SSR), consist of nucleotide sequence motifs flanked by specific sequences and are found throughout the genomes of most eukaryotes (Veldsman et al., 2023). These markers originate from slippage events that occur randomly within

repetitive sequence stretches (Mayes et al., 2019). Due to their high reproducibility, co-dominance, and informative nature, microsatellites are considered powerful genetic markers and are often the preferred choice in genetic studies.

Currently there is greater decline or complete loss in crop variability, relatively as a result of failure to domesticate wild species and the effect of climate change (Ulian *et al.*, 2020). Regardless of these reliable efforts, issues of narrow genetic variation among cultivated species have increase because breeding approaches have been centered more on the major crop species. Hence, plant breeding techniques have to be stretched to the neglected and underutilized crop species such as Bambara groundnut in order to enhance the already existing major crop species. The main aim of this research is to assess the nutritional and anti-nutritional contents among the selected accessions of Bambara groundnut and identify molecular markers in selected accessions to unearth the potential of this underutilized crop in combatting food security through the application of molecular technologies.

MATERIALS AND METHODS

Experimental Plant Materials

Ten (10) Bambara groundnut accessions were used in this study. These accessions were collected from different Agronomy Laboratory, Department of Agronomy, federal University of Kashere. The accessions (FUKV1, FUKV2, FUKV3, FUKV6, FUKV8, FUKV10, FUKV12, FUKV17, FUKV18 and FUKV19) were selected following Jibrin et al. (2026) recommendations based on their performance and for uniformity, viability and absence of physical damage prior to planting.

Sample Preparation and Analysis

Seed samples were collected in triplicate for each treatment at harvest and stored until further analysis. All samples were rinsed under running water to remove surface dirt. For proximate analysis, the seed samples were oven-dried at 50ºC until a constant weight was achieved. The dry matter content of the seed samples was determined following the method described by Musa et al. (2021). According to this method, samples were dried in a hot-air Memmert oven (MMT-UF110) maintained at 105ºC for 24 hours until a constant weight was reached. The dry matter content was calculated using the following equation:

$$MC = \frac{W1-W2}{W2} \times 100 \quad (1)$$

Where MC = Moisture Content; w_1 = weight of the fresh sample; w_2 = weight of the oven-dried sample

Determination of Dry Ashing

followed the method recommended by Association of Official Agricultural Chemists (Thiex, 2009). Crucibles were cleaned with tap water and dried in a hot-air Memmert oven (MMT-UF110) at 105ºC for 30 minutes. After drying, the crucibles were cooled in desiccators. Then, 2 g of ground seed samples were weighed into each crucible. The crucibles containing the samples were transferred to a heated chamber furnace (CARBOLITE ELF) maintained at 550ºC, where the samples were ashed until they turned light grey in color. The samples were then cooled in a desiccator, and the weight was recorded. The ash content was calculated using the following formula:

$$\%Ash=\frac{(\text{weight of crucible + Ash})-(\text{weight of empty crucible})}{\text{weight of sample}} \times 100 \quad (2)$$

Determination of Crude Protein Content

it was determined from the nitrogen content measured using the Kjeldahl method with a Kjeltec 8400 (FOSS) distillation unit, following the procedure described by Thiex (2009). In this method, 1 g of the sample was placed into a digestion tube (Kjeltec 8400 FOSS), and two catalyst tablets were added. Approximately 12 mL of concentrated (98%) sulfuric acid (H_2SO_4) was then added to the sample. Digestion was carried out at 420ºC for one hour in a fume-hood digester. During distillation, 50 mL of alkali solution (40% NaOH) was used in a 2200 FOSS distillation unit, and the distillate was collected in 4% boric acid. The crude protein content was displayed on the system's screen.

Determination of Crude Fiber

Crude fiber content was determined using a Fibertec™ 2010 (FOSS) hot fiber extraction unit, following the procedure recommended by Thiex (2009). Approximately 2 g of the sample was transferred into clean filter crucibles placed in a crucible stand. The crucible set was loaded with the samples, and the stand was positioned in front of the hot extraction unit. Then, 150 mL of a 0.127 M H_2SO_4 solution was gently added to the unit, and heat was applied for 30 minutes, gradually increasing the temperature from 40ºC to 60ºC. Afterward, the solution was rinsed with boiling water to remove the acid. The residue was then boiled in 150 mL of a 0.313 M NaOH solution and heated again between 40ºC and 60ºC for 30 minutes with a gradual temperature increase. The residue was filtered through a close pad of washed and ignited asbestos in a Gooch crucible. The filtered residue was dried in an electric oven, weighed, then incinerated, cooled, and weighed again. The percentage of crude fiber was calculated using the following formula:

$$CF\% = \frac{d-c}{a} \times 100 \quad (3)$$

Where a is weight of the sample, b is weight of glass crucibles, c is crucible plus sample after drying, d is weight of crucible plus ash

Determination of Total Sugar Content

Sample was grounded and sieved with 2mm sieve and oven dried at 60ºC. 0.5g of dried sample was weighed into a tube and 10mls of 80% ethanol was added and vortex for 2mins and placed in water bath for 30mins at 80ºC to deactivates enzymes and extract sugars after which the sample was centrifuged and the supernatant is obtained. otal sugar content in the samples was measured using the phenol-sulfuric acid method (Thiex, 2009). Approximately 0.1 mL of the sample extract was pipetted, followed by the addition of 1 mL of water. Then, 1 mL of phenol solution and 5 mL of concentrated H_2SO_4 were added to the mixture. The sample was boiled for 20 minutes in a water bath, after which the absorbance was measured at 490 nm using a spectrophotometer.

Nitrogen Free Extract (NFE)

Nitrogen-Free Extract (NFE) is determined by mathematical calculation. It is obtained by subtracting the sum of the percentages of all other measured nutrients from 100%.

$$\%NFE = 100 - (\%moisture + \%CF + \%CP + \%EE + \%Ash) \quad (4)$$

NFE represents soluble carbohydrates and other digestible, easily utilizable non-nitrogenous substances in the feed.

Physicochemical Properties

Total Phenolic Content (TPC)

was determined using a slightly modified Folin–Ciocalteu method as described by Abu Bakar et al. (2009). A 0.15 mL aliquot of the extract solution was mixed with 0.75 mL of Folin–Ciocalteu reagent diluted 1:10 with water. The mixture was allowed to stand for 5 minutes, after which 0.6 mL of

7.5% (w/v) sodium carbonate solution was added. The reaction mixture was then incubated at room temperature for 30 minutes. The total phenolic content was measured using a Thermo Scientific (USA) Spectrophotometer (model 1510) at a wavelength of 765 nm (correlation coefficient $R^2 = 0.9740$). Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh weight (mg GAE/g FW). The total phenolic content was calculated using the following equation:

$$TPC = c\ V/m \quad (5)$$

Where:
TPC = total phenolic content (mg GAE/g fresh extract)
c = concentration of gallic acid obtained from the calibration curve (mg/mL)
V = volume of the extract (mL)
m = mass of the extract (g)

Determination Of Total Flavonoid Content (TFC)

it was determined using a slightly modified colorimetric method by Abu Bakar et al. (2009). A mixture was prepared by combining 1 mL of the extract with 4 mL of sodium nitrite solution (1:5, w/v) and left to stand for 6 minutes. Then, 0.3 mL of aluminum chloride solution (1:10, w/v) was added to the mixture. Afterward, 2.0 mL of 1 M sodium hydroxide solution was introduced, followed by incubation at room temperature for 10 minutes. The total flavonoid content was measured using a Thermo Scientific (USA) Spectrophotometer (model 1510) at a wavelength of 510 nm (correlation coefficient $R^2 = 0.9963$). Results were expressed as milligrams of catechin equivalents (CE) per gram of fresh weight (mg CE/g FW). The total flavonoid content was calculated using the following equation:

$$TFC = c\ V/m \quad (6)$$

Where:
TFC = total flavonoid content (mg CE/g fresh extract)
c = concentration of catechin obtained from the calibration curve (mg/mL)
V = volume of the extract (mL)
m = mass of the extract (g)

Genomic DNA Extraction and PCR Analysis

The fresh and healthy leaves of 3 to 4 weeks old plants was sampled for DNA extraction for molecular studies. The molecular work was conducted at the Centre for Dryland Agriculture, Bayero University, Kano. Genomic DNA was extracted from young leaves of 10 Bambara groundnut accessions using the DNeasy Plant Maxi Kit (QIAGEN), following the manufacturer's instructions as outlined in the DNeasy Plant Handbook. The DNA samples were quantified for concentration and purity using a NanoDrop® ND 1000 Spectrophotometer (Thermo Fisher Scientific). Details of the five polymorphic primers and their sequences are presented in Table 1.

PCR reactions were performed in a total volume of 16 µL, containing 40 to 50 ng of template DNA, 0.8 µM of each primer (forward and reverse), 8 µL of 2× Power Taq PCR MasterMix, 3.2 µL of DNA, and 3.2 µL of nuclease-free water. Amplification was carried out in a thermal cycler (T100™, Bio-Rad, Hercules, CA, USA). The optimized cycling conditions were: initial denaturation at 94 °C for 2 minutes; 35 cycles of 94 °C for 1 minute, annealing at 45–60 °C (depending on the primer) for 1 minute, and extension at 72 °C for 30 seconds; followed by a final extension at 72 °C for 5 minutes and a hold at 10 °C (Usman et al., 2020). PCR products were separated on a 3% MetaPhor™ agarose gel (Lonza) using 1X TBE buffer. The gel was stained with GelView dye and run at a constant voltage of 80 V for 60 minutes. Visualization was done under UV light, and band scoring was performed using the Bio-Imaging system (GelDoc™ XR, Bio-Rad) (Usman et al., 2020).

Molecular Data Analysis

The polymorphism information content (PIC) value for each SSR locus was calculated using the formula $PIC = 1 - \sum pi^2$, where pi represents the frequency of the ith allele among the 20 Bambara groundnut accessions studied (Weir et al., 2006). The POPGENE 1.31 software (Yeh et al., 1999) was used to compute the observed number of alleles, effective number of alleles (Ne), expected heterozygosity (He), Nei's expected heterozygosity (Nei's), and the Shannon's information index (I).

Table 1: Primer Sequence of Five (5) Polymorphic SSR Markers

Primers	Forward Sequence	Reverse Sequence
PR-32	TTCACCTGA ACC CCT TAA CC	AGGCTTCACTCACGGGTATG
PR-48	TACCTGCATTCGGGACAGTT	TTCACTCTTTCTTGATCACATGC
CEDG024	CATCTTCCTCACCTGCATTC	TTTGGTGAAGATGACAGCCC
mBam3Co07	GGGTTAGTGATAATAAATGGGTGTG	GTCATAGGAAAGGACCAGTTTCTC
CEDG294	CACCTTCTTAATCTCTTCACC	GGGTTTCTCTTAATTCATTGAGTC

RESULTS AND DISCUSSION

Nutritional Value

This study reported the variation in the nutritional value among the studied Bambara groundnut accessions in Kashere (Table 2). The nutritional values reported were percentage moisture content (MC), ash content, ether extract (EE), crude protein (CP), crude fibre (CF), nitrogen free extract (NFE), total sugar, total flavonoid content (TFC) and total phenolic contents (TPC). It was observed that FUKV1 recorded the highest percentage of moisture content (10.6%) followed by FUKV10 (8.06%) while FUK17 recorded the least with 5.46%. The moisture content ranges from 10.6 – 5.46 % among the studied accessions (Table 2). The percentage ash content ranges from 3.70 – 3.30 % with FUKV19 recording the highest while FUKV10 gave the lowest value (3.30%). The studied accessions gave variations in their ash content as reported in Table 2. In ether extract, variability was observed in the percentage ether extract among the studied Bambara groundnut accessions (Table 2). FUKV2 gave the highest ether extract with 8.95 % followed by 7.96 and 7.98 % by FUK10 and FUK12 respectively. The least ether extract was recorded by FUK17 with 4.282 %. The crude protein in this study differ substantially in quantity among the studied accessions varying from 26.4% – 15.14 % (Table 2). FUKV6 recorded the highest CP (26.4%) while FUKV12 gave the least CP (15.14%). The percentage crude fiber observed within the studied Bambara groundnut accessions varied with FUKV17 recording the highest (5.42 %). This was followed by FUKV8 and FUKV10 with 4.77 and 4.40 % respectively.

FUKV3 and FUKV6 gave the least percentage of crude fiber with 3.17 and 3.27 respectively (Table 2).

The nitrogen free extract (NFE) was also found to differ among the studied Bambara groundnut accessions (Table 2). FUKV12 was observed to have the highest NFE (62.48 %) followed by FUKV8 with NFE of 62.19 %. The least NFE was recorded by FUKV10 with 52.19 % during the period of the experiment (Table 2). Total sugar content of the ten Bambara groundnut accessions was not found to be same among when estimated. FUKV8 gave the highest total sugar content with 61.54 % while FUKV2 recorded the least total sugar content with 50.65 % (Table 2). The total flavonoid content (TFC) varied among the accessions of Bambara groundnut (Table 2) ranging from 5.58 – 29.19 mgGAE/g. The least TFC was observed in FUK17 and the highest was recorded by FUKV8 indicating a wide variability among the accessions. FUKV1, FUKV2, FUKV3, FUKV6, FUKV10 and FUKV8 showed an estimated higher TFC content compared to FUKV17, FUKV12, FUKV18 and FUKV19 that recorded low quantity. The total phenolic content (TPC) was observed in the studied Bambara groundnut accessions (Table 2) with variability in the estimated quantity. FUKV17 gave the highest TPC (13.48 mgGAE/g) followed by FUKV3 that recorded 10.65 mgGAE/g TPC. The least TPC was recorded by FUKV1 with an estimated 5.85 mgGAE/g. This showed a wide gap between the estimated TPC in the studied Bambara groundnut accessions (Table 2). This study showed wide variability among the evaluated 10 Bambara groundnut accessions in Kashere. These findings are supported by the reports from Atoyebi et al. (2017) that showed significant difference among the 20 Bambara groundnut evaluated. The findings of this study is similar with the work of Akpalu, et al. (2010).

Nutrient parameters are essential not only for breeding programs aimed at genetic improvement and developing improved crop varieties but also for selecting specific germplasm lines or accessions to address particular needs, especially related to health challenges (Gonné et al., 2013). Many developing countries, particularly in Africa, face widespread malnutrition, leading to significant loss of life, especially among infants (Sanilkumar and Muthu, 2013). While these conditions often result from micronutrient deficiencies, Bambara groundnut has been identified as a crop with the potential to help alleviate such nutritional challenges (Ijarotimi, 2013).

Some African accessions of Bambara groundnut, known for their potential to alleviate many nutritional challenges, were randomly selected for evaluation in this study following initial screening. The goal was to identify those best suited to address specific nutrition and health-related issues. These accessions could be utilized in complementary foods or as ready-to-use foods (RTUF) (Bamshaiye et al., 2011). Among the 10 African Bambara groundnut accessions evaluated, FUKV6, FUKV18, and FUKV19 are recommended for addressing protein deficiency due to their high energy content.

Table 2: Summary of the Mean Values for the Nine Proximate and Phytochemical Properties Evaluated in Bambara Groundnut Accessions

Bambara Accessions	Moisture Content (%)	Ash Content (%)	Ether Extract (%)	Crude Protein (%)	Crude Fiber (%)	NFE (%)	Total Sugar	Flavonoids (mgGAE/g)	Phenol (mgGAE/ g)
FUKV1	10.6	3.60	7.59	16.96	3.75	57.51	55.67	15.65	5.85
FUKV2	6.34	3.70	8.95	15.20	4.16	53.89	52.65	19.58	10.41
FUKV3	6.88	3.46	6.92	19.80	3.17	59.77	57.88	20.39	10.65
FUKV6	6.24	3.56	5.26	26.74	3.27	54.93	53.88	25.03	9.98
FUKV8	7.32	3.52	6.67	15.52	4.77	62.19	61.54	29.19	9.39
FUKV10	8.06	3.30	7.96	24.09	4.40	52.17	50.65	19.29	9.23
FUKV17	5.46	3.66	4.28	21.57	5.43	59.60	58.06	5.58	13.48
FUKV12	6.58	3.64	7.98	15.14	4.18	62.48	60.85	5.88	10.01
FUKV18	6.26	3.34	7.36	25.61	4.05	53.38	52.86	6.43	8.92
FUKV19	6.52	3.70	4.59	25.73	3.98	55.48	53.50	8.57	9.31

NFE = Nitrogen Free Extract

Consequently, these accessions could help mitigate protein deficiency-related diseases such as marasmus, kwashiorkor, and cachexia, thereby contributing to the prevention of protein–energy undernutrition. Additionally, the results show that accessions FUKV8 and FUKV12 contain the highest carbohydrate content among the evaluated samples. This makes them a reliable source of glucose for the body, offering protection against ketone formation and ketoacidosis. Therefore, consuming Bambara groundnut, particularly accessions FUKV8 and FUKV12, can provide an important dietary nutrient and key source of caloric energy.

Genetic Diversity Analysis

Ten Bambara Groundnut accessions were screened with 5 genetic diversity SSR primers. All primers amplified with all accessions indicating diversity among them. A total of 5 SSR primers were tested and were found to be polymorphic among the studied (Table 3). The allele numbers and allele sizes of the primers are presented in Table 3. The number of alleles detected by the primers ranged from 1 to 2 among the Bambara groundnut accessions. The least polymorphic microsatellite marker was CEDG024, which exhibited only 1 allele, while all other markers showed 2 alleles (Table 3). A total of 24 polymorphic alleles were obtained from screening 10 Bambara groundnut accessions using the 5 SSR markers. Marker CEDG294 had the highest polymorphism information content (PIC) value of 0.89. Conversely, CEDG024 had the lowest number of alleles per locus (1) and a PIC value of 0.0. Loci polymorphism levels were classified as high (PIC > 0.5), medium (0.25 < PIC ≤ 0.5), or low (PIC ≤ 0.25). This genetic diversity information, along with the identification of specific alleles, genes, or loci and the assessment of genetic relationships among accessions, provides valuable guidelines for selecting parents and designing new breeding strategies to improve Bambara groundnut (Osundare et al., 2023). Lombardi et al. (2014) emphasized that the selection of divergent parental genotypes for breeding should be based on a systematic assessment of genetic distance between genotypes, rather than relying solely on geographical distance.

The polymorphism information content (PIC) values of the analyzed microsatellite markers ranged from 0 to 0.89, with an average of 0.52. Among the five SSR markers used in this study, the CEDG294 primer exhibited the highest PIC value, followed by primer PR-32 with a PIC of 0.73, and mBam3Co07 and PR-48 SSR primers with PIC values of 0.56 and 0.42, respectively. The lowest PIC value was observed for the PR-48 primer at 0.42. PIC values greater than 0.5 are considered informative, indicating a high level of gene variation within the Bambara groundnut accessions. A PIC value of 0.5 or higher denotes high diversity at the locus, while a PIC value around 0.25 indicates low diversity (Esan et al., 2023). Therefore, all primers used in this study were found to be highly informative. These results suggest that the SSR markers used here have strong potential as molecular markers for assisting in the background selection in molecular plant breeding. Furthermore, these findings align with previous reports by Esan et al. (2023), who identified SSR markers associated with genetic diversity in wheat genotypes.

Table 3: Major Allele Frequency, Number of Alleles, Genetic Diversity, and Polymorphism Information Content (PIC) of Functional DNA Primers in 10 Bambara Groundnut Accessions

Primers	Chromosome Position	Major Allele Frequency	No. Observation	No. Alleles	Genetic Diversity	PIC
PR-32	1B	0.958	24	2	0.0799	0.727
PR-48	2D	0.917	24	2	0.1528	0.421
CEDG024	3BL	1.000	24	1	0.0000	0.000
mBam3Co07	4B	0.917	24	2	0.1528	0.561
CEDG294	4D	0.958	24	2	0.0799	0.897
Total				9		
Mean		0.950	24	1.8	0.0931	0.521
SD (±)				0.4	0.0568	0.305

PIC: Polymorphic Information Content

CONCLUSION

In conclusion, among the African Bambara groundnut accessions evaluated for nutritional parameters, FUKV6, FUKV18, FUKV19, and FUK10 can be classified as high-nutrient types. Accessions FUKV1 and FUKV2 fall into the low-nutrient category, while the remaining accessions are considered medium-nutrient types. These findings suggest that certain accessions could be strategically utilized to help eradicate endemic diseases and improve food security. Additionally, the SSR markers CEDG294 and PR-32 are effective tools for studying genetic diversity among Bambara groundnut accessions, as they provide substantial diversity information.

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