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Nutritional Stability of Bambara Groundnut Mutant Lines under Waterlogging Stress: Proximate Composition and Energy Density Responses

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Abstract: Background: Water logging is an increasing challenge in many Nigerian farming systems, particularly in flood-prone and poorly drained areas. In addition to reducing crop yield, waterlogging can affect the nutritional quality of food crops by altering seed composition. Bambara groundnut (*Vigna subterranea*) is an important indigenous legume valued for its balanced nutrient content and potential to improve household nutrition, yet information on its nutritional response to water logging stress is limited. **Objectives:** This study assessed the effects of waterlogging stress on the proximate composition and energy value of colchicine- and phytogenic silver nanoparticle (AgNP)-derived Bambara groundnut mutant lines, with emphasis on nutritional quality. **Materials and Methods:** Water logging stress was imposed at the flowering stage by maintaining 0.5–1.0 cm standing water above the soil surface for 10 days. Seeds harvested at maturity were analyzed for moisture, ash, crude protein, crude fiber, lipid, carbohydrate and calorific value using standard AOAC methods. Data were analyzed using one-way ANOVA ($p < 0.05$) with Tukey's test, and multivariate analyses were used to examine relationships among nutritional traits. **Results:** Water logging significantly influenced seed proximate composition across genotypes and treatments. The combined treatment PTG01 (0.025% colchicine + 20 mg/L AgNP) recorded the highest moisture (≈10.41%), lipid (≈10.38%) and calorific value (≈1575.13 kJ/100 g), indicating better retention of energy-rich nutrients under stress. The highest protein content was observed in MOK01 (0.05% colchicine) (≈24.90%), alongside elevated crude fiber (≈9.10%). In contrast, PTG01 (0.05% colchicine + 40 mg/L AgNP) recorded the lowest protein content (≈18.36%). Multivariate analysis separated the mutant lines into energy-dense and protein–fiber-enriched nutritional groups. **Conclusion:** Overall, water logging-induced nutritional changes were genotype- and treatment-dependent, with distinct protein–fiber–enriched and energy-dense seed profiles emerging among the mutant lines. These outcomes demonstrate the potential of Bambara groundnut to contribute meaningfully to dietary protein adequacy and household energy supply in flood-prone Nigerian agro-ecologies, reinforcing its relevance for food and nutrition security.

Keywords: Bambara groundnut; water logging stress; proximate composition; nutritional quality; induced mutation; food security; Nigeria

1. Introduction

Flood-prone and poorly drained agro-ecologies are expanding under increasingly erratic rainfall patterns associated with climate change, making waterlogging a growing threat to crop productivity, food quality, and food security in vulnerable agricultural systems (Tyagi *et al.,* 2024). Beyond growth suppression, waterlogging induces root-zone hypoxia, disrupting nutrient acquisition, carbon–nitrogen metabolism, and reserve deposition, thereby altering the protein, lipid, carbohydrate, and mineral profiles of harvested grains and seeds (Pan *et al.,* 2021; Manghwar*et al*., 2024). Recent syntheses and meta-analyses show that waterlogging can measurably shift crop quality traits, reinforcing the need for nutrition-focused evaluation of stress responsesnot only agronomic screening (Yang*et al.,* 2024).

In Nigeria, where plant-based diets dominate and protein-energy malnutrition remains a public health concern, underutilized indigenous legumes with high nutrient density are increasingly promoted as affordable nutrition solutions. Bambara groundnut (*Vigna subterranea*) is particularly important in this regard because it provides a balanced macronutrient profile and has strong potential for climate-resilient food systems (FAO *et al.,* 2023). Recent compositional studies continue to confirm the crop's nutritional value across diverse landraces and processing conditions, supporting its relevance for dietary improvement and local value chains (Mabitsela et al., 2025)

However, nutritional resilience to waterlogging is not guaranteed, particularly during reproductive development, when hypoxia disrupts seed-filling processes and biochemical stability, leading to altered reserve deposition and nutritional quality (Pan *et al.,* 2021; Yang *et al.,* 2024). Consequently, breeding and allied innovations that can stabilise seed composition under hypoxia are increasingly attractive. Colchicine-induced polyploidisation is a recognized plant-breeding tool capable of reshaping physiological performance and biochemical traits through genome duplication and metabolic amplification, while green/biogenic silver nanoparticles (AgNPs) have recently been reviewed and demonstrated to modulate plant stress responses via improved redox balance, membrane stability and metabolic regulation, effects that may translate to seed quality outcomes under stress (Mangena & Mushadu, 2023).

Against this background, the present study introduces a nutrition-centred evaluation of colchicine- and phytogenic AgNP-derived Bambara groundnut mutant lines subjected to prolonged waterlogging stress, focusing on proximate composition (moisture, ash, lipid, crude fibre, protein, carbohydrate) and calorific value, alongside integrated trait relationships. This approach aligns with the nutrition scope by linking crop stress adaptation directly to nutritional quality and dietary relevance under Nigerian and comparable sub-Saharan production realities (Manghwar*et al*., 2024).

2. **Materials and Methods**

Study site

The experiment was conducted under controlled greenhouse–field conditions at the Monique Garden and Horticultural Arena experimental facility, Ilorin, Nigeria (8.4966^0N/4.5421^0 E). The study area lies within the Southern Guinea Savanna agro-ecological zone, characterized by a tropical climate with distinct wet and dry seasons. The controlled environment allowed uniform imposition of waterlogging stress and minimized confounding environmental variability during the experimental period.

Experimental design and treatments

The experiment was conducted using a completely randomized block design (CRBD) with four replications. The study evaluated M_1 mutant lines of Bambara groundnut previously developed through colchicine treatment and phytogenic silver nanoparticle (AgNP) application, together with their corresponding untreated controls, under waterlogging stress.

Waterlogging stress was imposed uniformly at the flowering stage, and each mutant line and control was represented across replicated experimental units to ensure statistical reliability. The experimental layout minimized positional effects and allowed unbiased comparison of nutritional responses among mutant lines.

At physiological maturity, seeds were harvested only from surviving plants and analyzed for proximate composition. For genotypes in which control plants failed to survive waterlogging stress, nutritional evaluation and statistical comparisons were restricted to the corresponding M_1 mutant lines, in order to avoid invalid control-based inference.

Waterlogging stress simulation

Waterlogging stress was imposed following the procedures established by Olorunwa et al. (2022) to simulate hypoxic soil conditions, with slight modifications to accommodate Bambara groundnut physiology and the controlled greenhouse–field system used in this study. Stress treatment was initiated at the flowering stage (40–50 days after sowing), a phenological phase known to be highly sensitive to excess soil moisture in legumes.

Waterlogging was achieved by flooding each enclosed plot to a uniform depth of approximately 0.5–1.0 cm above the soil surface, ensuring complete rhizosphere saturation while preventing submergence of aerial plant parts. This water level was maintained continuously for ten (10) consecutive days by sealing drainage outlets to avoid water loss and ensure uniform stress exposure across all treatments. Control plots were maintained under normal drainage conditions throughout the experimental period.

At physiological maturity, seeds were harvested from surviving plants, air-dried, and prepared for nutritional analysis.

Proximate analysis of Bambara groundnut seeds

Proximate composition analyses were conducted on post-waterlogging Bambara groundnut seed samples to determine moisture, ash, crude protein, crude fibre, lipid, carbohydrate, and calorific value. All analytical procedures followed standard protocols of the Association of Official Analytical Chemists (AOAC, 2019). Each analysis was performed in triplicate to ensure analytical precision and reproducibility.

Determination of moisture content

Moisture content was determined using the oven-drying method. Approximately 2 g of each finely ground sample was weighed into a pre-weighed moisture dish and dried in a hot-air oven at 105 ± 1 °C for 4 h. The samples were cooled in a desiccator and reweighed. Moisture content was calculated as:

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where:
W_1= weight of sample before drying
W_2= weight of sample after drying

Determination of ash content

Ash content was determined using the dry-ashing method. Exactly 2 g of each sample was weighed into pre-weighed porcelain crucibles and incinerated in a muffle furnace at 550 °C until a light grey ash was obtained. The crucibles were cooled in a desiccator and reweighed. Ash content was expressed as:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Initial weight of sample}} \times 100$$

Determination of crude protein content

Crude protein was quantified using the micro-Kjeldahl method following AOAC (2019), involving digestion, distillation, and titration steps. Exactly 3 g of each finely ground sample was digested with 25 mL concentrated H_2SO_4 in the presence of a catalyst tablet containing $CuSO_4$ (0.15 g), K_2SO_4 (5 g), and TiO_2 (0.1 g) until a clear digest was obtained.

Following digestion, the digest was diluted with 10 mL of 40% NaOH, 100 mL distilled water, and 5 mL $Na_2S_2O_3$ as an anti-bumping agent. Liberated ammonia was distilled into 10 mL boric acid solution and titrated against 0.1 N HCl. A reagent blank was prepared under identical conditions.

Crude protein content was calculated using:

$$\text{Crude Protein (\%)} = \frac{(T_s - T_b) \times 0.1 \times 0.14 \times F}{\text{Weight of sample}} \times 100$$

Where:
T_s= titre value of sample
T_b= titre value of blank
F= nitrogen-to-protein conversion factor

Determination of crude fibre content

Crude fibre content was determined according to AOAC procedures. Five grams (5 g) of each sample was digested with 100 mL trichloroacetic acid (TCA) reagent in a 500 mL Erlenmeyer flask and refluxed for 40 min. After cooling, the mixture was filtered through Whatman No. 1 filter paper and thoroughly washed with hot distilled water.

The residue was dried at 105 °C overnight, cooled in a desiccator, and weighed (W_1). It was subsequently ashed in a muffle furnace at 500 °C for 6 h, cooled, and reweighed (W_2). Crude fibre was calculated as:

$$\text{Crude Fibre (\%)} = \frac{W_1 - W_2}{W_0} \times 100$$

Where:
W_0= initial dry weight of sample
W_1= weight of crucible + fibre + ash
W_2= weight of crucible + ash

Determination of total carbohydrate content

Total carbohydrate content was estimated by difference, following AOAC (2019):

$$\text{Total carbohydrate (\%)} = 100 - (\text{\%Moisture} + \text{\%Ash} + \text{\%Fat} + \text{\%Protein} + \text{\%Fibre})$$

Determination of calorific value

Calorific value (energy content) was calculated from the proximate composition data using standard Atwater conversion factors for protein, carbohydrate, and lipid and expressed as kJ per 100 g dry matter.

Statistical analysis

Proximate composition data were analysed using one-way analysis of variance (ANOVA) within genotype to evaluate treatment effects. All analyses were performed in Python, and results were expressed as mean ± standard deviation (SD). Where significant differences were detected at $p < 0.05$, mean separation was conducted using Tukey's Honest Significant Difference (HSD) test with compact letter display.

Multivariate analyses, including principal component analysis (PCA), standardized heatmaps, and correlation matrices, were used to explore relationships among proximate traits and visualize treatment-specific nutritional patterns (Montgomery, 2020; Jolliffe & Cadima, 2016).

3. Results

Proximate composition of Bambara groundnut seeds under waterlogging stress

Waterlogging stress significantly influenced the proximate composition of Bambara groundnut seeds across colchicine- and phytogenic silver nanoparticle (AgNP)-derived mutant lines ($p < 0.05$). Clear genotype- and treatment-dependent differences were observed for all measured traits, including moisture, ash, protein, crude fibre, lipid, carbohydrate content, and calorific value. Numerical summaries are provided in Supplementary Table 1, while graphical trends are illustrated in Figures 1–4.

Moisture and ash content

Moisture content varied significantly among treatments ($p < 0.05$) (Figure 1A). The highest moisture content was recorded in PTG01 treated with 0.025% colchicine + 20 mg/L AgNPs (≈ 10.41%), representing more than a threefold increase relative to the PTG01 control (≈ 3.39%). In contrast, the lowest moisture content was observed in the PTG01 control and the PTG01 0.025% colchicine treatment (≈ 3.39–3.76%). In MOK02, moisture content remained relatively stable, with the mutant 0.025%–20 mg/L treatment (≈ 4.73%) closely matching the control (≈ 4.65%).

Ash content also differed significantly across treatments ($p < 0.05$) (Figure 1B). The highest ash content was observed in the MOK01 0.05% colchicine mutant (≈ 4.36%), followed closely by PTG01 0.05% colchicine (≈ 4.17%). The lowest ash values were recorded in the MOK02 0.025%–20 mg/L mutant (≈ 3.40%) and its control (≈ 3.45%), both falling within the same statistical group.

Protein and crude fibre composition

Protein content showed marked treatment-dependent variation ($p < 0.05$) (Figure 2A). The highest protein concentration across all genotypes was recorded in the MOK01 0.05% colchicine mutant (≈ 24.90%), exceeding the PTG01 control (≈ 20.47%) by over 4 percentage points. Elevated protein levels were also observed in PTG01 0.025%–20 mg/L (≈ 23.56%) and PTG01 0.05% colchicine (≈ 23.42%). In contrast, the lowest protein content occurred in PTG01 treated with 0.05% colchicine + 40 mg/L AgNPs (≈ 18.36%).

Crude fibre content varied significantly among treatments ($p < 0.05$) (Figure 2B). The highest fibre levels were recorded in MOK01 0.05% colchicine (≈ 9.10%) and the

MOK02 control (≈ 8.54%). Conversely, the lowest fibre content was observed in PTG01 0.025%–20 mg/L (≈ 4.37%) and PTG01 0.05%–40 mg/L (≈ 4.99%), indicating reduced structural carbohydrate accumulation in these lines.

Lipid and carbohydrate content

Lipid content differed significantly among treatments ($p < 0.05$) (Figure 3A). The highest lipid concentration was recorded in PTG01 0.025%–20 mg/L (≈ 10.38%), closely followed by PTG01 0.025% colchicine alone (≈ 9.42%). These values were more than double that of the PTG01 control (≈ 4.60%). The lowest lipid content was observed in the MOK02 control (≈ 3.49%).

Carbohydrate content also varied significantly ($p < 0.05$) (Figure 3B). The highest carbohydrate content was recorded in PTG01 0.05%–40 mg/L (≈ 61.35%) and the PTG01 control (≈ 60.67%). In contrast, the lowest carbohydrate content occurred in MOK01 0.05% colchicine (≈ 43.96%) and PTG01 0.025%–20 mg/L (≈ 47.32%), indicating greater carbohydrate depletion in these treatments under waterlogging stress.

Calorific value

Calorific value differed significantly across treatments ($p < 0.05$) (Figure 4). The highest calorific value was recorded in PTG01 0.025%–20 mg/L (≈ 1575.13 kJ/100 g), followed by PTG01 0.05%–40 mg/L (≈ 1543.33 kJ/100 g). The lowest calorific value occurred in the MOK01 0.05% colchicine mutant (≈ 1465.10 kJ/100 g), which was comparable to the MOK02 control (≈ 1465.33 kJ/100 g).

Multivariate patterns of proximate traits

Standardized multivariate analysis revealed clear, treatment-specific nutritional structuring among the Bambara groundnut mutant lines (Figure 5). The heatmap distinguished energy-dense from protein–fibre-enriched nutritional profiles. In particular, PTG01 mutants subjected to combined colchicine and AgNP treatments (0.025%–20 mg/L) clustered strongly with high lipid content and elevated calorific value, reflecting superior retention of energy-related reserves under waterlogging stress. In contrast, MOK01 colchicine-derived mutants (0.05%) formed a separate cluster characterized by high protein and crude fibre content, indicating a shift toward structural and nitrogen-rich seed composition.

Correlation analysis further resolved the relationships among proximate traits. Carbohydrate, lipid, and calorific value exhibited strong positive correlations, confirming their coordinated contribution to seed energy density under hypoxic conditions. Protein showed a moderate positive association with crude fibre, suggesting linked regulation of nitrogen allocation and structural carbohydrate deposition. Conversely, moisture content displayed weak or negativecorrelations with most macronutrients, indicating dilution effects associated with reduced post-stress seed desiccation.

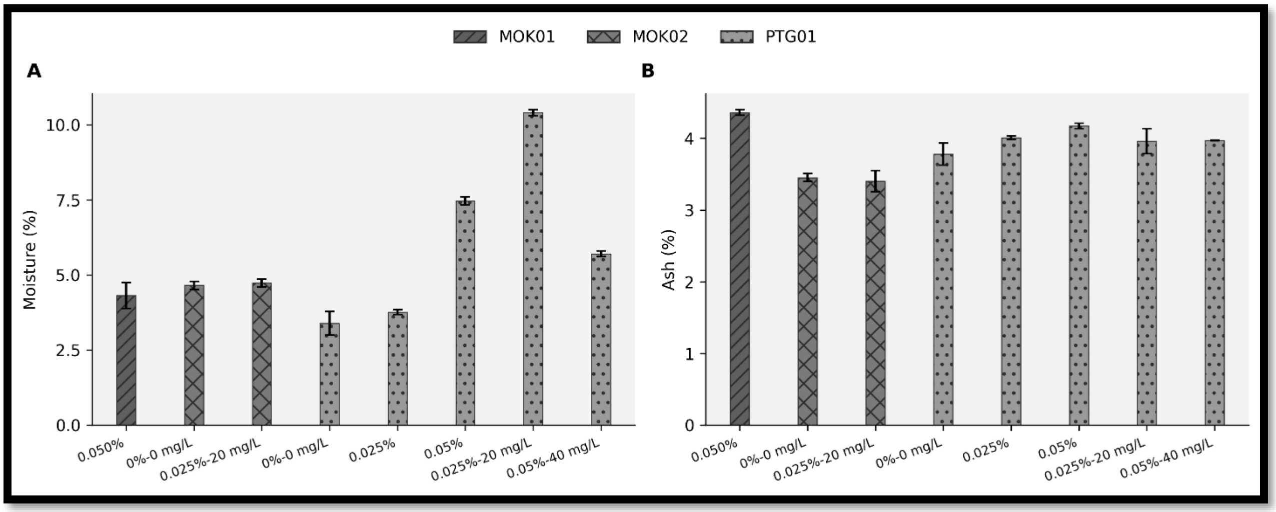

Figure 1. Moisture and ash content of Bambara groundnut seeds after waterlogging stress

(A) Moisture content and (B) ash content of Bambara groundnut seeds harvested from colchicine- and phytogenic silver nanoparticle (AgNP)-derived mutant lines following waterlogging stress. Bars represent mean values ± standard error (SE). Treatments are shown along the x-axis, while genotypes are distinguished by bar patterning. Different treatments exhibited significant variation within traits ($p < 0.05$).

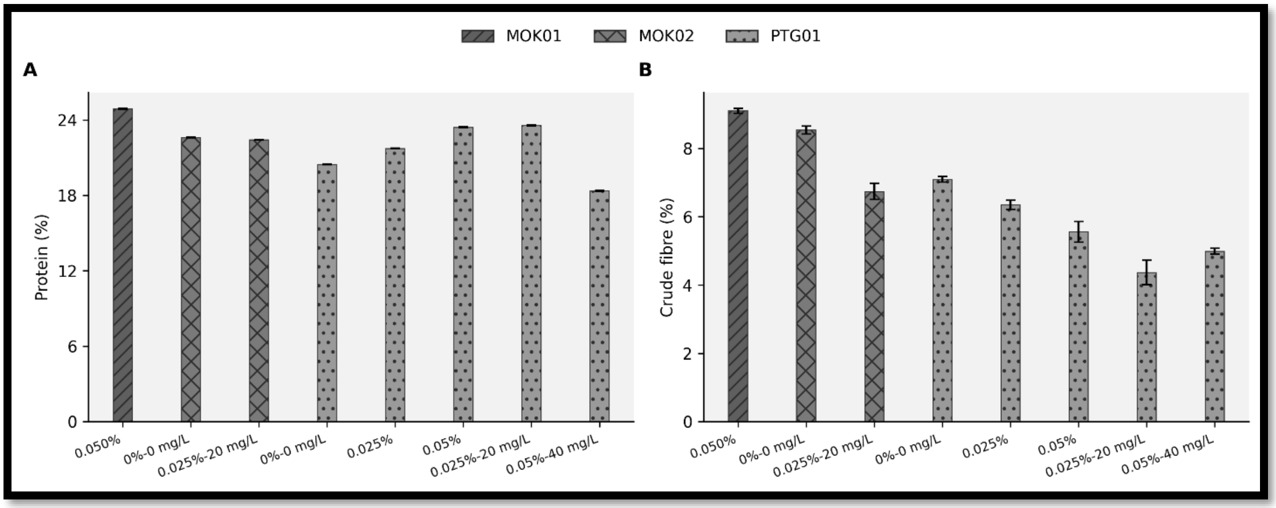

Figure 2. Protein and crude fibre composition of Bambara groundnut seeds under waterlogging stress

(A) Crude protein content and (B) crude fibre content of Bambara groundnut seeds across mutant lines following waterlogging stress. Values are presented as mean ± SE. Genotypes are differentiated by bar patterning, while treatments are indicated along the x-axis. Significant treatment-dependent variation was observed within each trait ($p < 0.05$).

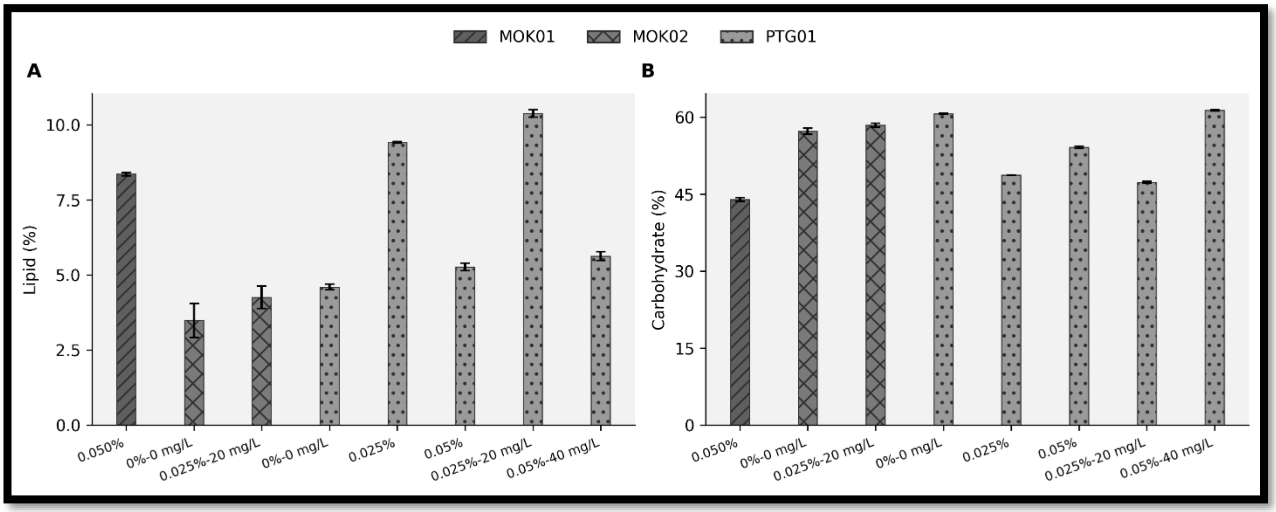

Figure 3. Lipid and carbohydrate content of Bambara groundnut seeds under waterlogging stress

(A) Lipid content and (B) carbohydrate content of Bambara groundnut seeds derived from colchicine- and AgNP-treated lines following waterlogging stress. Bars represent mean ± SE. Variation among treatments reflects differential retention of energy-related reserves under hypoxic conditions ($p < 0.05$).

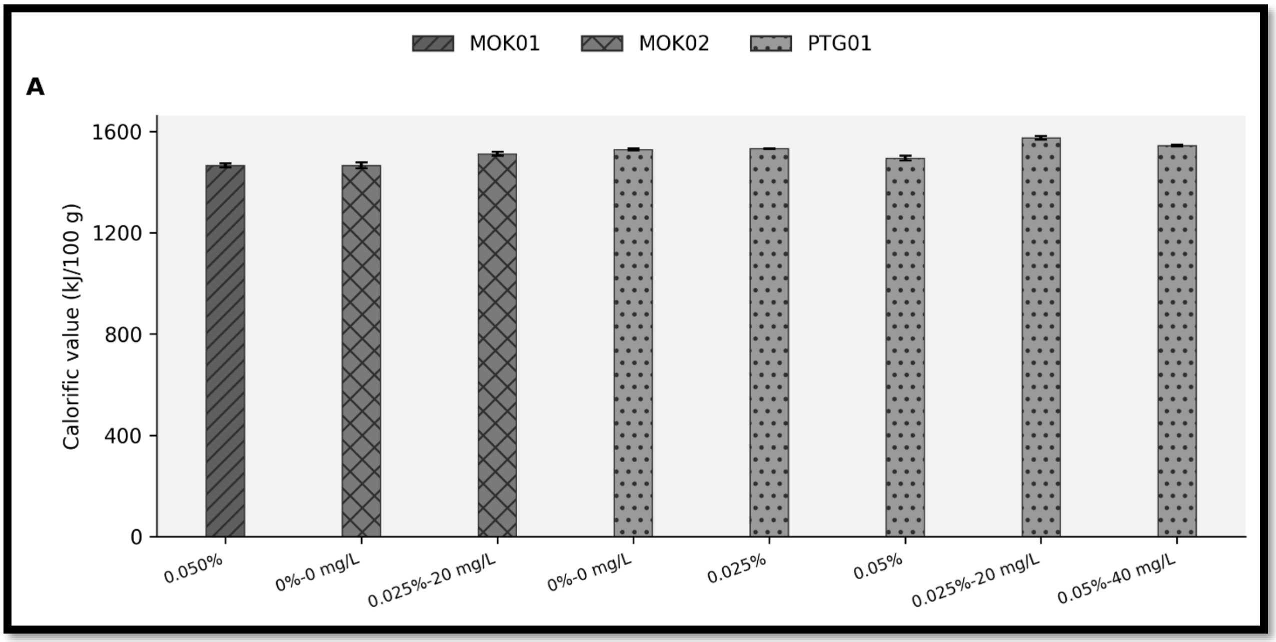

Figure 4. Calorific value of Bambara groundnut seeds after waterlogging stress

Calorific value (kJ/100 g) of Bambara groundnut seeds harvested from mutant lines subjected to waterlogging stress. Values are means ± SE. Differences among treatments indicate variable preservation of energy density under prolonged soil saturation ($p < 0.05$).

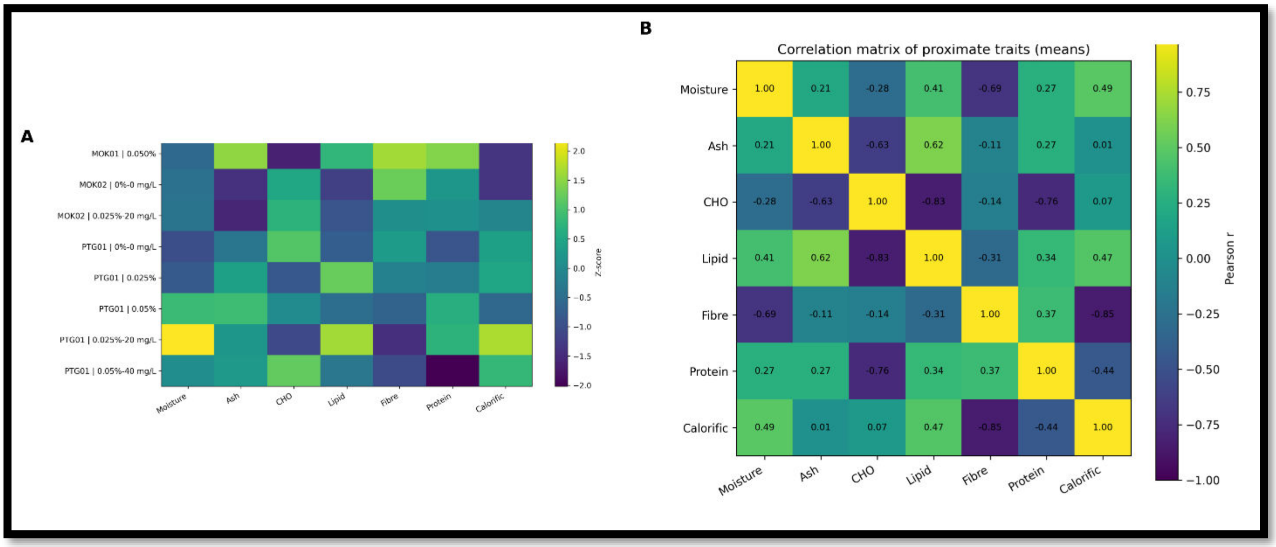

Figure 5.Multivariate relationships among proximate traits of Bambara groundnut under waterlogging stress.

(A) Standardized heatmap (z-scores) showing genotype- and treatment-specific nutritional profiles across colchicine- and phytogenic silver nanoparticle (AgNP)-derived mutant lines following waterlogging stress. Warmer colours indicate relatively higher trait values, while cooler colours indicate lower values. (B) Correlation matrix illustrating pairwise relationships among proximate traits based on treatment means. Strong positive correlations were observed among lipid, carbohydrate, and calorific value, whereas fibre showed negative associations with energy-related traits. Moisture exhibited weak or negative relationships with most macronutrients.

4. Discussion

This study demonstrates that prolonged waterlogging stress significantly alters the proximate composition of Bambara groundnut seeds, and that the magnitude and direction of these nutritional changes depend strongly on genotype and treatment. The observed variation in moisture, mineral content, protein, fibre, lipid, carbohydrate, and calorific value confirms that waterlogging affects not only yield-related traits but also nutritional quality, an aspect increasingly emphasized in food and nutrition research (Manghwar*et al.,* 2024).

Moisture and mineral responses under waterlogging stress

The elevated seed moisture content observed in PTG01 mutants subjected to combined colchicine–AgNP treatment is consistent with reports that waterlogging delays post-maturation desiccation due to hypoxia-induced impairment of carbohydrate metabolism and reduced solute-driven water loss (Pan *et al.,* 2021; Olorunwa*et al.,* 2022). Similar increases in seed or grain moisture under flooding have been reported in soybean and cowpea, where oxygen deprivation interferes with normal seed drying processes (Olorunwa*et al.,* 2023).

In contrast, the relatively stable and lower moisture content recorded in MOK02 mutants and controls suggests better recovery of membrane integrity and desiccation capacity following stress. From a nutritional standpoint, this is advantageous because lower seed moisture improves storability and reduces post-harvest deterioration, a key concern for the utilization of legumes in tropical environments.

Ash content, reflecting total mineral accumulation, increased notably in colchicine-derived MOK01 and PTG01 mutants. This aligns with evidence that induced polyploidy can enhance mineral uptake and retention by altering root physiology and improving ion transport efficiency (Tossi*et al.,* 2022). Similar increases in ash and mineral content have been reported in colchicine-induced polyploids of fenugreek and other legumes, where genome duplication enhanced nutrient deposition in edible tissues (Mangena &Mushadu, 2023). Enhanced mineral density under stress is nutritionally relevant, given widespread micronutrient deficiencies in plant-based diets.

Protein and Fibre Accumulation: Nutritional Trade-offs

Protein enrichment was most pronounced in the MOK01 0.05% colchicine mutant, exceeding values reported for the corresponding controls. This observation agrees with studies showing that polyploidisation can increase protein concentration by enhancing nitrogen assimilation, amino acid biosynthesis, and translational capacity in seeds (Yadav *et al.,* 2022; Han *et al.,* 2024). Similar protein increases have been documented in colchicine-derived mutants of legumes and oilseeds under abiotic stress, suggesting that genome duplication can buffer nitrogen metabolism against hypoxic disruption (Mangena &Mushadu, 2023).

Crude fibre content followed a contrasting pattern, with higher fiber levels in MOK01 mutants and lower values in PTG01 combined treatments. Waterlogging has been shown to induce structural carbohydrate deposition in some genotypes as part of stress adaptation, while in others it suppresses cellulose synthesis due to limited carbon availability (Pan *et al.,* 2021; Yang *et al.,* 2024). Nutritionally, higher fiber content enhances gut health but may reduce energy density and nutrient bioavailability, highlighting a trade-off between protein–fiber enrichment and energy concentration among the mutant lines.

Energy-related traits and calorific value under hypoxia

The substantial lipid accumulation and elevated calorific value observed in PTG01 mutants receiving combined colchicine–AgNP treatment indicate superior preservation of energy-dense reserves under waterlogging stress. Lipid biosynthesis is typically constrained under hypoxia because fatty acid synthesis is oxygen-dependent; therefore, enhanced lipid retention suggests improved metabolic stability (Manghwar*et al*., 2024).

Comparable increases in seed oil content under stress mitigation treatments have been reported in soybean and groundnut using nanomaterials, where improved redox balance and membrane stability supported lipid metabolism (Tokarz *et al.,* 2024). Additionally, induced genetic modification and chromosomal alteration can influence seed oil biosynthesis and biochemical profiles in plants. For example, polyploidy has been shown to affect natural metabolite production and biochemical traits (Madani *et al.,* 2021), and induced mutations have been associated with changes in fatty acid and oil content in crop species (Rauf *et al.,* 2023).

reductions in carbohydrate content observed in some mutant lines are consistent with established evidence that waterlogging-induced hypoxia promotes the diversion of soluble sugars toward anaerobic respiration and survival pathways, thereby limiting carbohydrate accumulation in developing seeds (Pan*et al.,* 2021; Manghwar*et al*., 2024). However, the maintenance of high calorific value in PTG01 combined treatments suggests that enhanced lipid accumulation effectively compensated for carbohydrate depletion, preserving overall dietary energy density under stress.

Multivariate nutritional strategies revealed by integrated analysis

Multivariate analysis clearly separated mutant lines into distinct nutritional strategy groups. Energy-dense PTG01 mutants clustered with high lipid and calorific value, whereas MOK01 colchicine-derived mutants clustered with elevated protein and fibre. Similar multivariate separations between energy-focused and structural or protein-focused nutritional profiles have been reported in legumes subjected to flooding and drought stress (Manghwar*et al.,* 2024).

Correlation analysis further supported these patterns, with strong positive associations among lipid, carbohydrate, and calorific value, consistent with coordinated regulation of energy-related traits reported in grain legumes (Pan*et al.,* 2021). The strong negative relationship between fibre and calorific value mirrors findings in soybean and cowpea, where increased structural carbohydrate deposition under stress reduced energy density (Olorunwa*et al.,* 2022). Moisture content showed weak or negative associations with most macronutrients, reinforcing its role as a dilution factor rather than a contributor to nutritional quality.

The differential nutritional responses observed among mutant lines highlight the potential to tailor Bambara groundnut nutritional profiles through induced mutation strategies under adverse environmental conditions. Protein- and fibre-enriched MOK01 mutants may be valuable for diets requiring higher protein intake, while energy-dense PTG01 combined-treatment mutants are particularly relevant for populations facing caloric insufficiency.These findings support recent calls to integrate nutritional quality metrics into stress-tolerance breeding programs (Manghwar*et al.,* 2024) and reinforce the role of Bambara groundnut as a climate-resilient legume capable of contributing meaningfully to food and nutrition security in flood-prone regions of Nigeria and sub-Saharan Africa.

Conclusion

Waterlogging stress significantly altered the proximate composition of Bambara groundnut seeds, with nutritional responses strongly influenced by genotype and treatment. Colchicine-derived MOK01 mutants exhibited higher protein and crude fibre content, whereas PTG01 mutants subjected to combined colchicine–phytogenic silver nanoparticle treatments maintained higher lipid levels and a higher calorific value under hypoxic conditions. Multivariate analyses revealed distinct nutritional strategies, separating protein–fibre–enriched from energy-dense seed profiles. These findings indicate that induced mutation can be strategically used to improve the nutritional stability of Bambara groundnut under waterlogging stress and highlight the crop's potential role in addressing protein and energy deficiencies in flood-prone agro-ecological zones of Nigeria.

Statements and Declarations

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval

This research involved cultivated plant materials only and did not involve human participants or vertebrate animals. All procedures complied with institutional, national, and international guidelines and legislation.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. Restrictions apply to the availability of these data due to the strict management of various data and technical resources within the research teams.

Authors' contributions (Credit taxonomy)

MJ: Conceptualization, Methodology, Formal analysis, Investigation, Writing—original draft.

FOA: Supervision, Methodology, Validation, Writing—review & editing.
DOAY: Investigation, Data curation, Visualization, Writing—review & editing.
DMC: Investigation, Resources, Project administration, Writing—review & editing.
All authors read and approved the final manuscript.

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Table S1. Proximate composition of Bambara groundnut seeds following Waterlogging Stress Across Colchicine- and AgNP-Derived Mutant Lines

Treatment	Moisture (%)	Ash (%)	CHO (%)	Calorific (kJ/100 g)	Lipid (%)	Crude Fibre (%)	Protein (%)
MOK 01							
0.050%	4.319 ± 0.429^{e}	4.361 ± 0.042^{a}	43.957 ± 0.372^{f}	1465.102 ± 7.705^{d}	8.360 ± 0.052^{c}	9.102 ± 0.075^{a}	24.902 ± 0.028^{a}
MOK 02							
0%-0 mg/L	4.652 ± 0.137^{d}	3.451 ± 0.055^{c}	57.258 ±0.617^{c}	1465.330 ± 10.720^{d}	3.488 ± 0.569^{f}	8.539 ± 0.118^{a}	22.612 ± 0.026^{e}
0.025%-20mg/L	4.729 ± 0.132^{d}	3.402 ± 0.148^{c}	58.459 ±0.388^{b}	1511.079 ± 7.178^{c}	4.259 ± 0.373^{e}	6.741 ± 0.239^{d}	22.409 ± 0.025^{e}
PTG 01							
0%-0 mg/L	3.388 ± 0.398^{f}	3.779 ± 0.155^{c}	60.670 ±0.045^{a}	1528.430 ± 4.693b	4.600 ± 0.094^{e}	7.094 ± 0.081^{b}	20.470 ± 0.023^{f}
0.025%	3.763 ± 0.088^{f}	4.010 ± 0.027^{b}	48.714 ±0.030^{e}	1531.806 ± 0.770^{b}	9.421 ± 0.023^{b}	6.349 ± 0.132^{c}	21.742 ± 0.025^{d}
0.05%	7.463 ± 0.131^{b}	4.171 ± 0.038^{a}	54.118 ±0.240^{d}	1493.521 ± 9.442^{c}	5.270 ± 0.132^{d}	5.560 ± 0.306^{e}	23.419 ± 0.027^{c}
0.025%-20 mg/L	10.408 ± 0.101^{a}	3.959 ± 0.176^{b}	47.318 ±0.181^{e}	1575.129 ± 7.512^{a}	10.384 ± 0.131^{a}	4.371 ± 0.360g	23.559 ± 0.027^{b}
0.05%-40 mg/L	5.703 ± 0.090^{c}	3.970 ± 0.004^{b}	61.349 ±0.160^{a}	1543.334 ± 3.314^{b}	5.628 ± 0.149^{d}	4.989 ± 0.084^{f}	18.360 ± 0.021^{g}

Values represent mean ± standard error (n = 3). Means within each column followed by different superscript letters (a–g) differ significantly at $p < 0.05$ according to Tukey's HSD test. MOK01 had no surviving control under waterlogging stress; therefore, treatment effects for this genotype are interpreted only among its mutant lines. MOK02 and PTG01 controls were included for comparison. CHO = carbohydrate