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Nutritional Variations Among Amaranth Accessions Under Diverse Environmental Conditions in Malawi

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Abstract: This study assessed the chemical composition of amaranth leaves from six different accessions (MN-BH-01, PE-UP-BH-01, PE-LO-BH-01, CK-BH-01, NU-BH-01, and LL-BH-04) grown in various locations in Malawi. Key nutritional traits, including crude protein, calcium, zinc, iron, and potassium content, were analyzed, revealing significant variability influenced by genotype–environment interactions. MN-BH-01 exhibited the highest protein, calcium, zinc, and potassium levels, making it a promising candidate for nutritional enhancement. PE-UP-BH-01 had elevated iron content, while LL-BH-04 showed superior crude protein in certain locations. Nutrient composition varied significantly across different environmental conditions, emphasizing the impact of these interactions on nutrient accumulation. Cluster analysis and AMMI analysis identified consistent accessions (MN-BH-01 and NU-BH-01) valuable for breeding nutrient-rich varieties. Farmers preferred NU-BH-01 for its flavor, yield, and marketability, while MN-BH-01 was less favored due to its bitterness. These findings offer valuable insights for developing climate-resilient and biofortified amaranth varieties, contributing to food security and nutrition in Malawi and similar regions.

Keywords: amaranth accessions; nutrient composition; genotype-by-environment interactions; protein content; cluster analysis

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1. Introduction

Amaranth (*Amaranthus* spp.) is a highly nutritious and drought-tolerant pseudo-cereal known for its rich protein, mineral, and vitamin content [1]. It has garnered attention as a sustainable food source, especially in regions facing food insecurity and climatic challenges. The leaves of amaranth, along with its seeds, are valued for their nutritional density, making them an excellent dietary supplement [2]. Recent studies have highlighted the essential nutrients present in amaranth leaves, such as protein, calcium, iron, zinc, and potassium, which are vital for overall health and well-being, particularly in regions with high malnutrition rates [3–5].

Although extensive research has been conducted on the nutritional profile of amaranth seeds, studies focusing on the nutrient composition of amaranth leaves remain limited [6,7]. Furthermore, nutrient variations across different genotypes and environmental conditions have not been fully explored. Some amaranth accessions have demonstrated enhanced nutrient accumulation under stress conditions, indicating their potential for developing nutrient-rich and climate-resilient varieties [8,9].

The bioavailability of nutrients in amaranth is influenced by both genetic and environmental factors, including soil type, water availability, and climate conditions [10,11]. Studies suggest that soil amendments, temperature fluctuations, and water stress can significantly affect the nutrient composition of amaranth leaves [12,13]. However, limited research has been conducted on how these factors interact in specific agroecological zones, particularly in sub-Saharan Africa.

In Malawi, amaranth is a significant traditional vegetable that plays a crucial role in household nutrition and food security, particularly in rural areas. However, there is a lack of comprehensive studies on the nutritional variations among different amaranth accessions grown under diverse environmental conditions in the country. This study aims to provide the first comprehensive assessment of amaranth nutrient composition in Malawi, offering insights that can guide breeding strategies for nutrient-rich and climate-resilient varieties in similar agroecological regions worldwide. Understanding how genotype–environment interactions influence nutrient composition is crucial for optimizing the production of nutrient-dense varieties suitable for Malawi's agroecological zones.

The study aims to evaluate the chemical composition of amaranth leaves from various accessions grown in different locations across Malawi. Specifically, it aims to identify key nutritional traits, assess the impact of environmental factors on nutrient accumulation, and explore the potential of amaranth as a biofortified crop. The findings from this study will inform breeding strategies for developing nutrient-rich and climate-resilient amaranth varieties that are well-suited for Malawi and similar environments.

2. Materials and Methods

2.1. Plant Material Selection

In this study, we built upon our previous research by incorporating a new amaranth accession, CK-BH-01, which exhibited promising traits in initial assessments [14]. Alongside the five previously chosen accessions, we conducted a nutrition analysis on these six accessions: MN-BH-01, LL-BH-04, NU-BH-01, PE-UP-BH-01, PE-LO-BH-01, and CK-BH-01. These accessions represent a diverse range of genetic backgrounds and phenotypic characteristics, forming a robust basis for evaluating the nutritional potential of amaranth cultivars in different environments.

2.2. Study Sites and Experimental Design

Table 1 presents the agroecological characteristics of the four study locations in Malawi, selected to represent diverse environmental conditions. Thyolo Research and Mzimba-Champhira fall within the humid high-altitude zone, characterized by high rainfall (>1000 mm), moderate temperatures (15–20 °C), and fertile soils. Kasungu-Chulu represents the transitional middle-altitude zone, with moderate rainfall (500–1000 mm), temperatures ranging from 18–26 °C, and well-draining sandy soils. Salima-Chipoka, situated in the semi-arid low-altitude zone, experiences lower rainfall (<500 mm), higher temperatures (27–35 °C), and sandy, low-fertility soils [15]. These sites vary in humidity (40–85%), light intensity, and soil properties, providing a representative environmental framework for assessing the nutrient composition of amaranth accessions across different agroecological zones.

Table 1. Agroecological characteristics of the study locations in Malawi.

Parameter	Thyolo Research	Mzimba-Champhira	Kasungu-Chulu	Salima-Chipoka
Agroecological Zone	Humid High-Altitude Zone	Humid High-Altitude Zone	Transitional Middle-Altitude Zone	Semi-Arid Low-Altitude Zone
Coordinates	16.0691° S, 35.1420° E	12.3320° S, 33.5964° E	12.8090° S, 33.3110° E	13.9920° S, 34.5096° E
Altitude (m)	1200–1400	1100–1300	800–1000	500–700
Temperature (°C)	15–20	15–20	18–26	27–35
Rainfall (mm/year)	>1000	>1000	500–1000	<500
Humidity (%)	70–85	65–80	50–65	40–55
Light Intensity	Moderate (cloud cover)	Moderate (cloud cover)	High (clear skies)	Very High (strong sunlight)
Soil Type	Clay loam, fertile	Sandy loam, moderate fertility	Sandy, well-draining	Sandy, low fertility

Field experiments were conducted using a randomized complete block design (RCBD), with each block consisting of 20 plants per accession. The plants were spaced 30 cm apart within rows and 60 cm between rows to promote optimal growth and minimize competition, as shown in Figure 1. The trials took place during the 2022/2023 rainfed cropping season across three agroecological zones in Malawi. Agronomic practices, including fertilizer application, cultivation, weeding, and pest and disease control, were carried out according to the methods outlined by Nyasulu et al. [16].

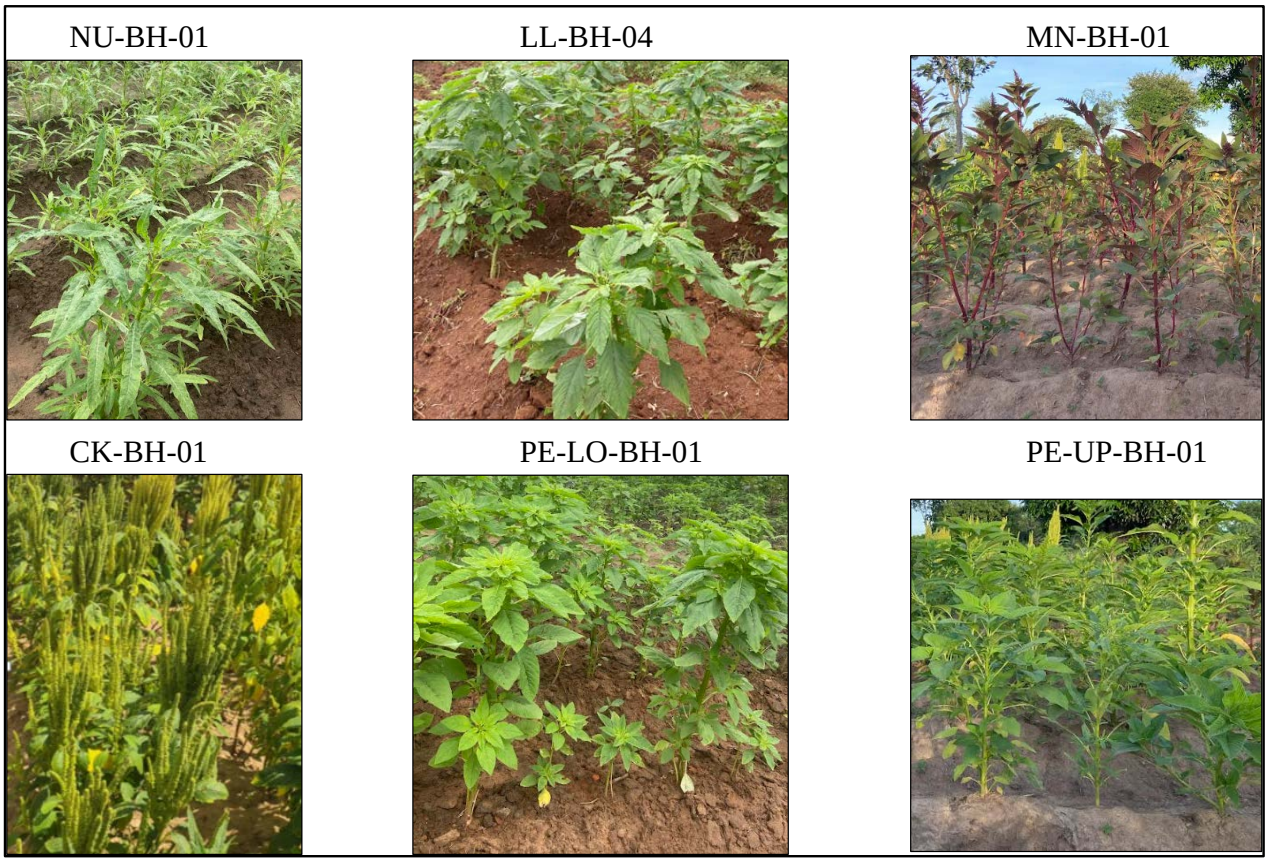

Figure 1. Six amaranth accessions in the field during the experimental work.

2.3. Soil Preparation and Fertilization

Soil samples were collected from each study site before and after fertilizer application to assess variations in soil chemical properties. Samples were analyzed for pH, electrical conductivity (EC), organic matter (OM), nitrogen (N), phosphorus (P), and potassium

(K) using standard soil analysis protocols. Changes in soil properties following fertilizer application were recorded, particularly increases in pH, EC, N, P, and K, with a slight decrease in OM (Table 2).

Table 2. Soil chemical composition before and after fertilizer application in the trial sites.

Site	Time	pH	EC (µS/cm)	OM (%)	N (%)	P (ppm)	K (ppm)
Thyolo-Research	Before	5.71	313.22	2.01	0.11	55.31	41.80
	After	6.01	360.74	1.90	0.13	68.62	127.61
Mzimba-Champhira	Before	6.22	391.15	0.61	0.03	18.73	57.53
	After	6.31	375.42	0.84	0.08	35.68	160.01
Salima-Chipoka	Before	6.32	378.03	0.67	0.04	25.40	65.53
	After	6.63	443.51	0.22	0.06	32.32	159.91
Kasungu-Chulu	Before	6.40	418.52	0.43	0.03	22.50	56.54
	After	6.42	434.31	0.74	0.07	42.50	169.16

2.4. Sample Preparation

Leaf samples for nutrient analysis were collected from the middle section of mature amaranth plants, as this part is considered the most representative of the overall nutrient composition. Sampling from the middle minimizes variability that can arise from leaves at different growth stages or positions (bottom, middle, or top) [17,18]. To ensure uniformity, plants were randomly selected across each planting field in different regions, avoiding bias related to their position within the plot. This random sampling method helped mitigate potential variation due to micro-environmental factors influencing plant growth. Samples were collected at six weeks, when the plants were fully established and deemed optimal for nutrient analysis. A total of 72 samples were collected, labeled, and placed in plastic bags for transportation to the laboratory. Upon arrival, the leaves were washed with tap water to remove any contaminants, then chopped into smaller pieces to facilitate drying. The samples were oven-dried at 60 °C to preserve heat-sensitive compounds and ensure consistent nutrient content. To ensure the accuracy and consistency of the drying process, the oven temperature was regularly monitored, and the drying time was recorded to guarantee uniform moisture removal across all samples. To determine moisture content, a subset of the dried samples was further oven-dried at 105 °C for three hours to achieve complete water removal. Following drying, all leaf samples were finely milled into a powder using a blender to prepare for chemical analysis. Each sample was analyzed in triplicate, and the mean of the three replicates was used for further analysis to ensure consistency and minimize experimental error. This comprehensive process ensures consistent, reproducible sample preparation for accurate nutrient profiling.

2.5. Proximate Analysis

The moisture, ash, fiber, and crude protein content were determined following the recommended methods of the Association of Official Analytical Chemists (AOAC). For moisture content, 5 g of each sample was weighed and dried in an oven at 60 °C for 24 h for fresh samples, then further dried at 105 °C ± 1 °C until a constant weight was achieved. The moisture content was calculated as the percentage of weight loss after drying. Ash content was determined using the dry ashing method, where 5 g of the sample was incinerated in a muffle furnace at 550 °C for 5 h until a constant weight was obtained, and the ash content was expressed as a percentage of the initial sample weight. Crude protein content was analyzed using the Kjeldahl method, involving digesting 0.5 g of the sample with concentrated sulfuric acid (H_2SO_4) and a catalyst (copper sulfate) at 400 °C until a clear

solution was obtained. The digest was neutralized with 40% sodium hydroxide (NaOH), and the liberated ammonia was distilled into 4% boric acid solution, followed by titration with 0.1 N hydrochloric acid (HCl). The total nitrogen content was determined, and the crude protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25 [19]. To ensure analytical accuracy, appropriate blanks and standard reference materials were used.

2.6. Mineral Content

Mineral elements such as potassium (K), calcium (Ca), iron (Fe), and zinc (Zn) were analyzed using the dry ashing method followed by atomic absorption spectrophotometry (AAS) as per AOAC-recommended procedures [20]. In this method, 5 g of the dried and finely ground sample was placed in a clean, pre-weighed crucible and ashed in a muffle furnace at 550 °C for 5 h to eliminate organic matter completely. After cooling, the ash was dissolved in 10 mL of 1 M HCl, filtered through Whatman No. 42 filter paper to obtain a clear solution. The filtrate was then diluted to 25 mL with deionized water before analysis. AAS was conducted using element-specific hollow cathode lamps at their respective wavelengths, with calibration curves generated from certified standard solutions. Each sample was analyzed in triplicate, and appropriate quality control measures, including reagent blanks and standard reference materials, were included to ensure analytical accuracy.

2.7. Statistical Analysis

Data were analyzed using a general linear model (GLM) to assess the effects of different amaranth accessions and environmental conditions (locations) on nutrient composition. The GLM included amaranth accession and environment as fixed factors, with replication as a random effect, allowing for the evaluation of genotype, environment, and their interaction effects. This approach was chosen for its ability to handle multiple variables while accurately partitioning variance across sources. Means for proximate and mineral elements were compared using Tukey's honest significant difference (HSD) test at a significance level of 0.05 to determine significant differences between accessions and locations. Hierarchical cluster analysis using Ward's method with Euclidean distance was conducted to classify accessions based on their nutrient profiles, aiding in the identification of patterns in nutrient composition across different accessions. To further assess genotype-by-environment interactions (G × E) for nutrient traits, the additive main effects and multiplicative interaction (AMMI) model was utilized, as it effectively captures both main effects and interaction patterns in multi-environment trials. Bar graphs were created using GraphPad 10.4.1 to visually depict the differences in nutrient composition across accessions and sites. Statistical analyses were performed using GenStat (21st edition) for GLM modelling and R (version 4.4.2) for advanced statistical processing, ensuring robust and comprehensive data analysis. These methods were selected for their ability to handle complex datasets and provide detailed insights into genotype-by-environment interactions relevant to this study.

3. Results

3.1. The Chemical Composition of Amaranth Leaves

The chemical composition of amaranth leaves varied significantly across accessions (Table 3, $p < 0.001$). Overall, MN-BH-01 consistently demonstrated superior nutritional quality, with the highest concentrations of crude protein (43.18%), calcium (8.00 mg/100 g), zinc (15.12 mg/100 g), and potassium (19.47 mg/g). In contrast, PE-UP-BH-01 exhibited the highest iron content (77.16 mg/100 g) and also had elevated potassium levels. Ash content was highest in NU-BH-01 (15.64%), while the lowest was found in PE-LO-BH-01 (12.93%). Other accessions showed notable variability in key nutrients, such as protein,

calcium, and zinc. For example, crude protein content ranged from 38.91% in NU-BH-01 to 43.18% in MN-BH-01, while iron levels ranged from 50.04 mg/100 g in PE-LO-BH-01 to 77.16 mg/100 g in PE-UP-BH-01. These findings highlight the diverse nutritional profiles across accessions, emphasizing the potential for selecting varieties with enhanced nutrient content for breeding and biofortification efforts.

Table 3. Chemical composition of amaranth leaves on dry matter basis.

Accession	Moisture Content (%)	Ash (%)	Crude Protein (%)	Iron Content (mg/100 g)	Zinc (mg/100 g)	Calcium Content (mg/100 g)	Potassium (mg/g)
CK-BH-01	85.82 ^{c}	15.07 ^{c}	41.03 ^{d}	57.10 ^{b}	14.36 ^{b}	7.92 ^{bc}	18.03 ^{b}
LL-BH-04	85.89 ^{c}	14.49 ^{b}	41.78 ^{e}	57.89 ^{b}	13.13 ^{a}	6.74 ^{a}	17.98 ^{b}
MN-BH-01	85.07 ^{a}	14.56 ^{b}	43.18 ^{f}	68.21 ^{c}	15.12 ^{c}	8.00 ^{c}	19.47 ^{d}
NU-BH-01	85.43 ^{b}	15.64 ^{d}	38.91 ^{b}	75.94 ^{d}	13.12 ^{a}	7.07 ^{a}	17.20 ^{a}
PE-LO-BH-01	85.37 ^{b}	12.93 ^{a}	40.53 ^{c}	50.04 ^{a}	14.45 ^{b}	6.97 ^{a}	18.65 ^{c}
PE-UP-BH-01	85.71 ^{c}	15.36 ^{cd}	40.53 ^{c}	77.16 ^{e}	14.61 ^{b}	7.55 ^{b}	19.73 ^{d}
Grand Mean	85.55	14.67	40.8	64.39	14.13	7.38	18.51
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.172	0.234	0.307	0.794	0.270	0.264	0.284

Note: means within a column followed by different letters are significantly different at $p < 0.05$ according to the least significant difference (LSD) test.

3.2. Cluster Analysis of Nutrient Profiles

Cluster analysis of the rice accessions based on their nutrient profiles revealed four distinct groups (Figure 2). Cluster 1, represented by MN-BH-01, is characterized by elevated levels of protein, iron, and potassium. Cluster 2, consisting of PE-UP-BH-01, is defined by high iron content and moderate potassium levels. Cluster 3, comprising CK-BH-01, LL-BH-04, and PE-LO-BH-01, showcases accessions with balanced nutrient profiles, featuring moderate levels of protein, zinc, and potassium. Lastly, Cluster 4, represented by NU-BH-01, stands out due to its exceptionally high iron content. These clusters underscore the unique nutrient compositions of each accession, providing valuable insights for crop breeding and biofortification strategies.

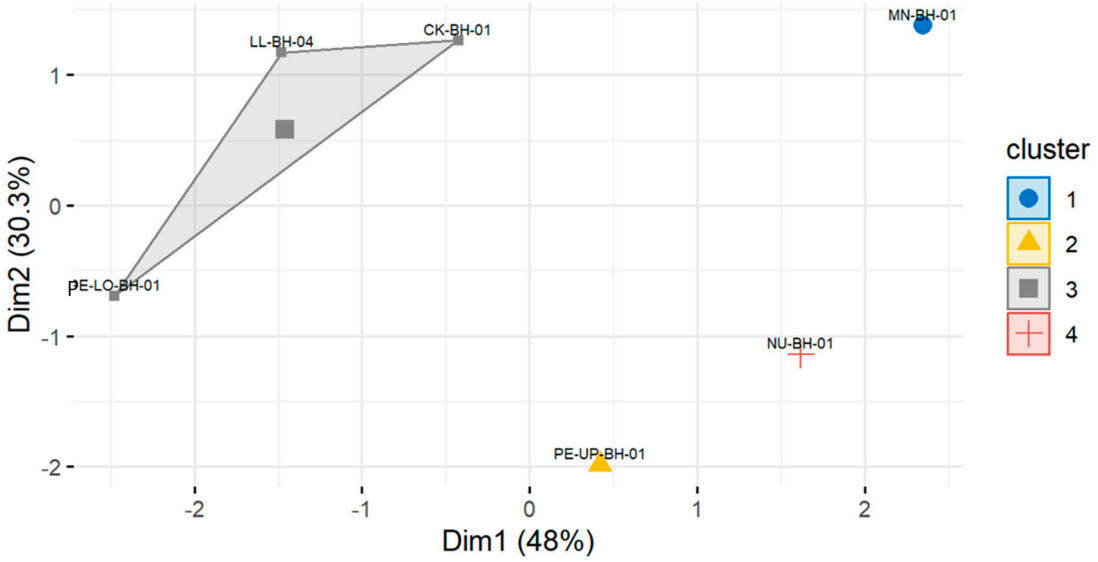

Figure 2. Cluster analysis of nutrient profiles and correlation analysis of the variables.

3.3. Chemical Composition of Amaranth Leaves Across Different Locations and Accessions

The chemical composition of amaranth leaves from different accessions grown across four locations in Malawi—Thyolo Research, Mzimba-Champhira, Kasungu-Chulu, and Salima-Chipoka—revealed significant variations in key nutritional traits, with p-values < 0.01 for all traits (Table 3, Figure 3). Moisture content ranged from 80.50% in Mzimba-Champhira to 90.38% in Kasungu-Chulu, with the highest levels observed in Kasungu-Chulu. Ash content was highest in Salima-Chipoka (16.15%) and lowest in Mzimba-Champhira (13.25%). Crude protein content varied from 31.42% to 56.50%, with Salima-Chipoka showing the highest mean (40.89%). Iron content ranged from 42.29 mg/100 g in Kasungu-Chulu to 88.23 mg/100 g in Salima-Chipoka, indicating regional nutritional differences. Zinc levels ranged from 8.20 mg/100 g in Kasungu-Chulu to 18.95 mg/100 g in Thyolo Research, and calcium content was highest in Thyolo Research (9.12 mg/100 g). Potassium content varied from 14.62 mg/g in Kasungu-Chulu to 21.50 mg/g in Thyolo Research. These findings, highlighted in Table 4 and Figure 3, emphasize the critical role of environmental factors in shaping the nutrient profile of amaranth. This variation supports its potential as a biofortified crop for improved nutrition and provides valuable insights for breeding climate-resilient and nutrient-rich amaranth varieties suited to Malawi and similar environments.

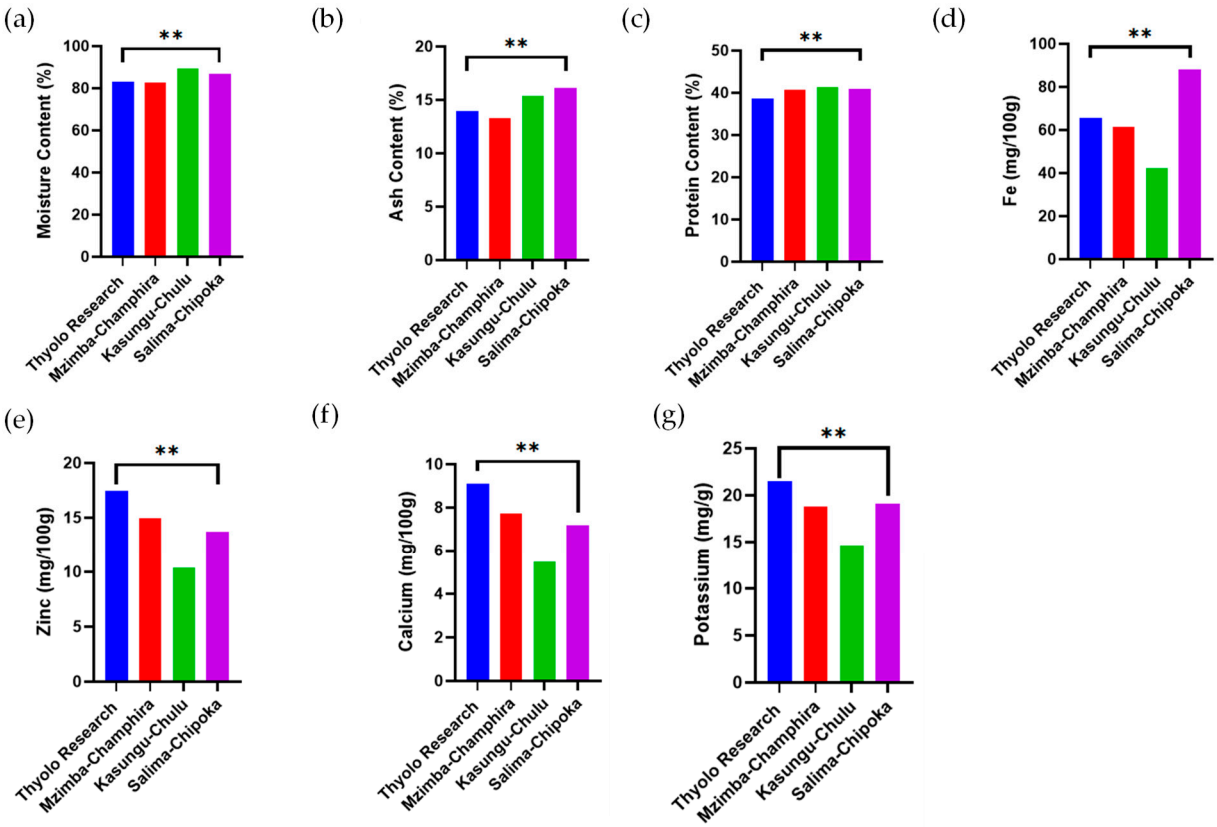

Figure 3. Bar plot illustrating the variation in nutrient composition (% Moisture, % Ash, % Protein, Iron, Zinc, Calcium, and Potassium) across four locations. The bars represent the mean values for each nutrient. Significant differences between locations are indicated by two asterisks (**), corresponding to p-values < 0.01. (**a**) Moisture Content; (**b**) Ash content; (**c**) Protein content; (**d**) Iron content; (**e**) Zinc Content; (**f**) calcium content; (**g**) Potassium content.

Table 4. Chemical composition of amaranth leaves across different locations and accessions.

Location	Accession	Moisture Content (%)	Ash (%)	Crude Protein (%)	Iron Content (mg/100 g)	Zinc (mg/100 g)	Calcium Content (mg/100 g)	Potassium (mg/g)
Thyolo Research	PE-UP-BH-01	82.18 ^{bc}	12.40 ^{b}	40.14 ^{h}	47.63 ^{c}	18.49 ^{i}	9.26 ^{jk}	22.19 ^{mn}
	PE-LO-BH-01	82.45 ^{cd}	11.67 ^{ab}	40.32 ^{h}	51.11 ^{de}	18.95 ^{i}	9.29 ^{jk}	23.37 ^{o}
	CK-BH-01	83.48 ^{e}	17.47 ^{j}	31.42 ^{a}	65.62 ^{gh}	18.26 ^{i}	10.55 ^{l}	22.72 ^{no}
	NU-BH-01	84.57 ^{f}	13.90 ^{cdef}	43.26 ^{ij}	93.29 ^{m}	18.09 ^{i}	9.81 ^{kl}	22.69 ^{no}
	LL-BH-04	83.34 ^{e}	15.64 ^{i}	33.67 ^{bc}	69.61 ^{ij}	12.73 ^{ef}	6.70 ^{def}	16.93 ^{gh}
	MN-BH-01	83.38 ^{e}	12.46 ^{b}	43.24 ^{i}	65.88	18.20 ^{i}	9.12 ^{ijk}	21.14 ^{lm}
Mean		83.23 ^{b}	13.92 ^{b}	38.68 ^{a}	65.52 ^{c}	17.46 ^{d}	9.12 ^{d}	21.50 ^{c}
Mzimba-Champhira	PE-UP-BH-01	83.37 ^{e}	14.59 ^{defg}	36.21 ^{d}	83.05 ^{l}	13.89 ^{g}	7.40 ^{fgh}	19.24 ^{i}
	PE-LO-BH-01	85.13 ^{g}	13.72 ^{cd}	39.39 ^{gh}	76.54 ^{k}	12.55 ^{ef}	6.16 ^{bcde}	16.61 ^{fg}
	CK-BH-01	81.75 ^{b}	11.39 ^{a}	57.57 ^{l}	50.93 ^{d}	16.74 ^{h}	9.20 ^{jk}	17.97 ^{h}
	NU-BH-01	82.91 ^{de}	13.40 ^{c}	40.28 ^{h}	49.02 ^{cd}	12.00 ^{de}	6.18 ^{bcde}	16.28 ^{efg}
	LL-BH-04	82.93 ^{de}	14.83 ^{ghi}	32.50 ^{ab}	68.07 ^{hi}	17.97 i	9.06 ^{ijk}	22.07 ^{mn}
	MN-BH-01	80.50 ^{a}	11.54 ^{ab}	38.35 ^{fg}	41.52 ^{b}	16.39 h	8.41 ^{hij}	20.78 ^{jkl}
Mean		82.77 ^{a}	13.25 ^{a}	40.72 ^{b}	61.52 ^{b}	14.92 ^{c}	7.74 ^{c}	18.83 ^{b}
Kasungu-Chulu	PE-UP-BH-01	89.28 ^{jk}	15.55 ^{hi}	39.20 ^{gh}	42.13 ^{b}	10.00 ^{b}	5.25 ^{b}	14.02 ^{b}
	PE-LO-BH-01	89.59 ^{k}	14.72 ^{fghi}	33.39 ^{bc}	30.66 ^{a}	10.52 ^{bc}	5.35 ^{bc}	14.86 ^{bcd}
	CK-BH-01	90.38 ^{l}	14.69 ^{efgh}	36.57 ^{de}	39.18 ^{b}	11.41 ^{cd}	5.75 ^{bcd}	15.62 ^{cdef}
	NU-BH-01	89.93 ^{kl}	16.86 ^{j}	34.54 ^{c}	56.14 ^{f}	10.91 ^{bc}	6.03 ^{bcd}	15.18 ^{cde}
	LL-BH-04	88.64 ^{ij}	13.78 ^{cde}	56.50 ^{l}	31.39 ^{a}	8.20 ^{a}	4.03 ^{a}	12.02 ^{a}
	MN-BH-01	88.59 ^{i}	16.68 ^{j}	47.57 ^{k}	54.21 ^{ef}	11.38 ^{cd}	6.39 ^{cdef}	16.03 ^{efg}
Mean		89.40 ^{d}	15.38 ^{c}	41.29 ^{c}	42.29 ^{a}	10.40 ^{a}	5.47 ^{a}	14.62 ^{a}
Salima-Chipoka	PE-UP-BH-01	88.00 ^{hi}	18.89 ^{l}	46.58 ^{k}	135.85 ^{p}	16.06 ^{h}	8.29 ^{hij}	23.46^{0}
	PE-LO-BH-01	84.30 ^{f}	11.59 ^{ab}	34.60 ^{c}	41.83 ^{b}	15.76 ^{h}	7.08 ^{efg}	19.74 ^{ij}
	CK-BH-01	87.67 ^{h}	16.72 ^{j}	38.57 ^{fg}	72.66 ^{j}	11.03 ^{bcd}	6.19 ^{bcde}	15.80 ^{def}
	NU-BH-01	84.32 ^{f}	18.41 ^{kl}	37.56 ^{ef}	105.31 ^{n}	11.46 ^{cd}	6.26 ^{bcde}	14.66 ^{bc}
	LL-BH-04	88.66 ^{ij}	13.72 ^{cd}	44.47 ^{j}	62.50 ^{g}	13.62 ^{fg}	7.19 ^{efg}	20.88 ^{kl}
	MN-BH-01	87.80 ^{h}	17.56 ^{jk}	43.58 ^{ij}	111.24 ^{o}	14.48 ^{g}	8.09 ^{ghi}	19.95 ^{ijk}
Mean		86.79 ^{c}	16.15 ^{d}	40.89 ^{b}	88.23 ^{d}	13.73 ^{b}	7.18 ^{b}	19.08 ^{b}
p value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: means within a column followed by different letters are significantly different at $p < 0.05$ according to the least significant difference (LSD) test.

3.4. Additive Main Effects and Multiplicative Interaction (AMMI) Analysis

The AMMI analysis showed significant genotype-by-environment (G × E) interactions in the nutritional composition of amaranth accessions across the four locations (Figure 4). Moisture content was stable in CK-BH-01, MN-BH-01, and NU-BH-01, especially in Kasungu-Chulu, while PE-LO-BH-01 and PE-UP-BH-01 were more affected by the environment (Figure 4a). Ash content was consistent in CK-BH-01 and NU-BH-01 but varied more in Mzimba-Champhira and PE-UP-BH-01 (Figure 4b). Crude protein levels were steady in NU-BH-01, CK-BH-01, and MN-BH-01, while environmental factors had a stronger effect in Mzimba-Champhira and Thyolo Research (Figure 4c). Iron content was stable in LL-BH-04 and NU-BH-01 in Kasungu-Chulu but varied in other locations (Figure 4d). Zinc content was consistent in CK-BH-01, MN-BH-01, and NU-BH-01, with stronger environmental interactions in Salima-Chipoka and Thyolo Research (Figure 4e). Calcium and potassium levels were stable in CK-BH-01, MN-BH-01, and NU-BH-01, but location differences were seen in other accessions (Figure 4f,g).

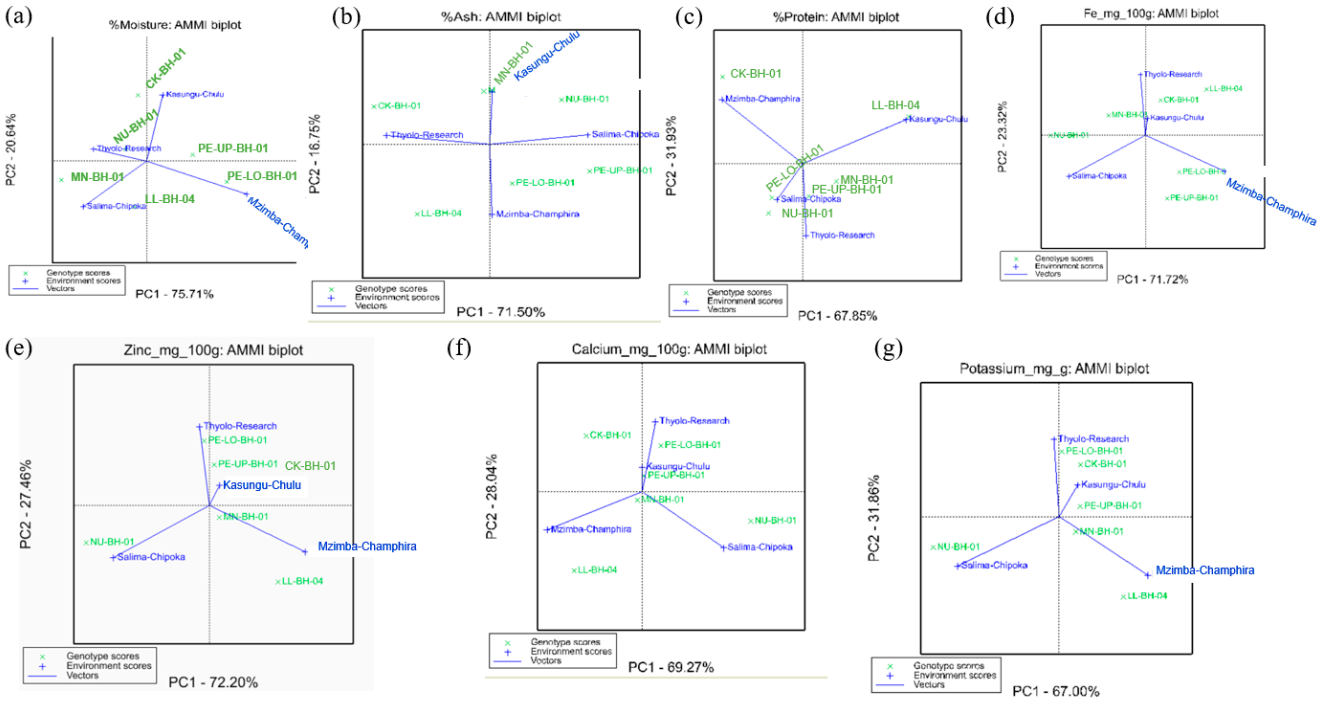

Figure 4. Ammi analysis. (**a**) Moisture content. (**b**) Ash content. (**c**) Crude Protein. (**d**) Iron Content. (**e**) Zinc. (**f**) Calcium content. (**g**) Potassium content.

CK-BH-01, MN-BH-01, and NU-BH-01 were the most consistent across nutritional traits, making them good candidates for breeding. On the other hand, PE-LO-BH-01, PE-UP-BH-01, and LL-BH-04 showed stronger environmental interactions, suggesting their nutrient levels depend more on location. These findings are important for improving amaranth cultivation and breeding in different regions.

3.5. Farmer Preferences and Ranking of Amaranth Accessions

Farmers actively participated in selecting their preferred amaranth accessions based on a range of agronomic and culinary characteristics (Table 5). NU-BH-01 emerged as the top choice among the accessions assessed, thanks to its excellent flavor, high yield, market appeal, and the convenience of cooking without oil. Following closely, CK-BH-01 ranked second, favored for its delicious taste and post-cooking flavor. PE-LO-BH-01 claimed the third spot, valued for its abundant yield, extended harvest window, large leaves, and strong market demand, making it a highly sought-after variety. In fourth place, PE-UP-BH-01 stood out for its numerous branches, large leaf size, and pleasant taste. On the other hand, LL-BH-04 and MN-BH-01 were the least preferred, ranking fifth and sixth, respectively, due to their unappealing flavor profiles. LL-BH-04 received criticism for its poor taste, while MN-BH-01 was noted for its bitterness, rendering it the least favored accession. These findings underscore the significance of both agronomic performance and sensory attributes in shaping farmer preferences for amaranth cultivation and market success.

Table 5. Farmer preferences and ranking of amaranth accessions.

Accession	Rank	Reasons
PE-UP-BH-01	4	➢ Too many branches ➢ Big leaf size ➢ Nice flavor
PE-LO-BH-01	3	➢ High yielding ➢ High value crop ➢ Big leaves ➢ Can be harvested for a long period of time ➢ Highly marketable
CK-BH-01	2	➢ Delicious ➢ Good flavor after cooking
NU-BH-01	1	➢ Good flavor ➢ High yield ➢ Good for business ➢ Marketable ➢ Can be cooked without cooking oil ➢ Delicious
LL-BH-04	5	➢ Bad flavor
MN-BH-01	6	➢ Bitterness

4. Discussion

Our study highlights significant variations in the nutritional composition of amaranth accessions across the distinct agroecological zones of Thyolo Research, Mzimba-Champhira, Kasungu-Chulu, and Salima-Chipoka, demonstrating differential responses to environmental conditions. These findings align with previous studies emphasizing the pivotal role of local climate in shaping crop performance [21]. The high-altitude zones, such as Thyolo Research, characterized by over 1000 mm of annual rainfall and temperatures between 15 °C and 20 °C, provided optimal conditions for enhanced nutritional performance, consistent with observations that moderate temperatures, adequate precipitation, and moderate light intensity promote both growth and nutritional value. In contrast, the low-altitude Salima-Chipoka region, with less than 500 mm of rainfall, temperatures exceeding 27 °C, and very high light intensity, presented harsher conditions, leading to the expression of stress tolerance traits in certain accessions. These traits helped maintain nutritional consistency, supporting the findings of Reyes-Rosales et al. [22], who identified heat-resistant genotypes in amaranth. Notably, some accessions exhibited consistent nutritional quality across different locations, underscoring the importance of selecting genotypes with broad environmental adaptability for sustainable, nutritionally robust amaranth production [14,23–25]. These results are further validated by the agroecological data from the study locations, which reveal significant contrasts in temperature, rainfall, light intensity, and soil characteristics, reinforcing the need for tailored cultivation strategies to maximize amaranth's nutritional potential under varying environmental conditions.

The influence of environmental factors on amaranth's nutritional composition aligns with findings from previous studies [21], where local climate, particularly temperature and rainfall, was identified as a key determinant. This study not only confirms those findings but also expands the understanding by focusing on the nutritional composition of amaranth leaves, an under-explored aspect in the literature [26]. Unlike most previous studies that concentrated on amaranth grain, our work highlights the nutritional diversity found in amaranth leaves across different agroecological zones, reinforcing the crop's

potential in combating malnutrition in regions that primarily consume amaranth leaves as a vegetable [27].

Additionally, our findings illustrate that soil composition and microclimatic conditions significantly influence nutrient content. In Thyolo Research, higher soil nitrogen levels (0.08% to 0.11%) contributed to enhanced biomass accumulation, corroborating previous studies that underscore nitrogen's role in promoting amaranth growth [28]. Similarly, phosphorus (P) and potassium (K) availability influenced nutritional performance, particularly in Kasungu-Chulu, where increases in P and K availability were associated with improved nutritional outcomes [29]. This highlights the importance of optimizing soil fertility management in different agroecological zones to enhance amaranth's nutritional quality.

Importantly, the study also emphasizes the importance of genotype-by-environment (G × E) interactions in determining amaranth's nutritional composition. We identified several accessions that demonstrated consistent nutritional performance across multiple locations. Among these, CK-BH-01, MN-BH-01, and NU-BH-01 exhibited high consistency in nutritional traits, making them strong candidates for breeding programs focused on enhancing nutritional resilience. In contrast, accessions like PE-LO-BH-01, PE-UP-BH-01, and LL-BH-04 showed strong environmental interactions, indicating their suitability for local adaptation programs. These findings support earlier research suggesting that breeding programs should prioritize genotypes that exhibit stability across various environmental conditions, especially in the face of increasing climate variability.

A critical aspect of successful breeding strategies is integrating farmer feedback to ensure that the selected varieties align with both nutritional and sensory preferences. While MN-BH-01 is nutritionally superior, its bitterness detracts from its appeal to farmers. As farmer preferences heavily influence the adoption of new varieties, breeding programs should prioritize sensory traits, such as flavor, texture, and bitterness, in parallel with nutritional qualities. Future breeding strategies should place a strong emphasis on sensory preferences to optimize both the nutritional benefits and marketability of amaranth varieties. Participating farmers can be actively engaged in the breeding process through participatory breeding programs to gather feedback on sensory traits and overall crop performance.

The identification of accessions that maintain high nutritional consistency across diverse environments is crucial for breeding programs. The cluster analysis revealed that MN-BH-01, while nutritionally superior, faces challenges in adoption due to undesirable sensory traits like bitterness. This highlights the need for breeding programs to balance both nutritional and sensory attributes to improve consumer acceptance [23]. CK-BH-01, with a balanced nutrient profile, and NU-BH-01, which stands out for its high iron content, represent promising breeding targets.

In addition to breeding applications, the identification of nutritionally consistent accessions opens avenues for biofortification initiatives. Amaranth's rich micronutrient content positions it as a promising candidate for biofortification strategies aimed at improving the nutritional quality of staple foods. Future research should explore amaranth's potential in enhancing food products, focusing on developing biofortified or functional foods that address nutrient deficiencies in vulnerable populations. Moreover, molecular studies aimed at identifying the genetic mechanisms underpinning nutrient consistency in amaranth will be crucial for developing targeted breeding strategies that optimize both yield and nutritional content.

5. Conclusions

Our study provides critical insights into significant variations among amaranth accessions across diverse agroecological zones, demonstrating how environmental factors such as soil composition and climate impact the crop's nutritional profile. These findings under-

score the importance of selecting adaptable accessions with consistent nutritional quality, a crucial consideration for breeding programs focused on enhancing climate resilience and food security. Amaranth's potential as a nutrient-rich, climate-resilient crop offers an opportunity to combat malnutrition and food insecurity, particularly in resource-constrained regions. Notably, accessions like CK-BH-01, MN-BH-01, and NU-BH-01 present promising prospects for targeted breeding and biofortification initiatives. To advance breeding strategies, future research should concentrate on elucidating the genetic mechanisms governing nutrient consistency and investigating the interaction between environmental factors and sensory traits. This will facilitate the optimization of breeding approaches for increased yield and nutritional value, ensuring consumer acceptance. The broader implications of this study extend to its potential to guide global food security strategies, advocating for the integration of amaranth into sustainable agricultural systems to enhance nutrition in vulnerable populations worldwide.

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