



Article Variability in Morphological, Biochemical, and Proximate Yield Composition among Predominant Amaranthus hybridus Cultivars in South-West Nigeria

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Abstract: To mitigate food insecurity and the low economic status of amaranth farmers in south-west Nigeria, it is imperative to evaluate the predominant commercial A. hybridus cultivars using key yield and nutrient components to identify promising lines with optimum yield and nutritional quality. The current study evaluated five cultivars of Amaranthus hybridus in south-west Nigeria farms using key morphological, biochemical, and seed-proximate component parameters. The results revealed significant differences in the cultivars' growth and yield indices. The number of leaves ranged from 16.40 to 29.48 (\bar{x} = 22.10) with plant height (\bar{x} = 50.28 cm), while other leaf metrics varied at maturity. Days to 50% flowering ranged from 49 to 90 days. Seed proximate composition also varied significantly. Carbohydrate content ranged from 65.01–76.28%, protein content 6.57–12.42%, ash content 2.73–3.59%, total polyphenols 23.36–29.64 mg/100 g GAE, and total antioxidants 31.6–181.59 mg/100 g AAE. The Pearson correlation matrix and path analysis showed significant relationships among the yield components. The phenotypic variance of the yield components mainly stemmed from environmental factors, indicating their crucial role in the traits' expression. Careful evaluation of the cultivars revealed that AM-1 and AM-3 had the best growth and yield attributes. Additionally, the two cultivars showed a significant degree of relatedness, suggesting a common ancestor. They have the potential to increase productivity, farmers' profitability, and consumers' nutrient gain compared to other cultivars. They are recommended for cultivation and can be further crossed in breeding programs to develop superior lines.

Keywords: amaranth; yield components; morphological characters; seed proximate composition; biochemical analysis

1. Introduction

Africa has a rich variety of plant species that contribute significantly to national and local economies [1]. Leafy vegetables are particularly important among smallholder farmers in Nigeria, and they have a considerable impact on the economies of local communities. They grow naturally in rural environments and are widely cultivated for sale and household consumption [2]. They have been utilized in food and medicine for centuries. Among edible



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). leafy vegetables, Amaranths have gained remarkable recognition in south-west Nigeria, owing to their ease of cultivation and an ever-ready market in the region. Amaranths—especially *Amaranthus hybridus* L.—are preferred by consumers. They are a rich source of essential vitamins, proteins, minerals, and bioactive compounds [3], and the high consumer demand also makes it a choice among local farmers.

Amaranthus hybridus—commonly called green amaranth, slim amaranth, smooth amaranth, or smooth pigweed-belongs to the family Amaranthaceae, the subfamily Amaranthoideae, and the genus Amaranthus which are plants with broad leaves and nutritious grains [4,5]. The genus is often tagged as 'neglected crop' with little scientific attention and often regarded as a 'poor man resource'; however, its enormous nutrient compositions and economic potential deserve attention [6]. A. hybridus (2n = 32) is a dicotyledonous annual herbaceous plant and grows up to 65 cm in height. It has a long taproot (>30 cm) supported with secondary and tertiary roots, making it adaptable to water stress. Additionally, the species is notable for effectively competing with weeds for limited resources, making it suitable for low-input farming. A. hybridus are monoecious and predominantly open-pollinated by natural agents; cross-pollination also occurs among the species or with closely related species, giving rise to sterile F1 generation [7]. Amaranthus hybridus is easy to harvest, edible, and has an excellent nutritional composition. Amaranth grains have significant dietary importance, providing essential nutrients to consumers [5]. The seeds have a good protein content (12–17.6%) [8], higher than major cereals: maize (8.6–12.0%) [9], rice (7–12%) [10,11], wheat (12–15%) [12]. Their gluten-free protein is rich in lysine, providing a viable protein source for gluten-sensitive people [8,13]. Additionally, they possess bioactive components, including antioxidants, which aid in protecting the body against chronic ailments [14].

However, despite the rich diversity of highly nutritious vegetables, food crops, and vast acres of arable farmland, Africa wallows in food insecurity and low living standards [15,16]. The same applies to Nigerian farmers [17], who are often regarded as poor, particularly the rural dwellers who are the major growers of amaranths and other vegetables [18]. Among the many factors contributing to food insecurity, malnutrition, and low socio-economic index among rural farmers are inadequate farm inputs, low irrigation, climate change, land degradation, and obsolete planting methods [19]. Additionally, the cultivation of underperforming cultivars due to the lack of access to superior varieties exacerbates these challenges [20,21]. However, an often underestimated yet significant factor limiting yields on *A. hybridus* farms is the inadvertent mixing of seeds from different varieties, each with different yield capacities. This unintended co-planting of high-performing and low-performing cultivars results in reduced productivity and profitability for farmers compared to what is achievable with elite superior lines. It becomes imperative for breeders and farmers to employ effective strategies to identify and promote the cultivation of best-performing cultivars.

This study aimed to evaluate the key yield components of five major *A. hybridus* cultivars in south-west Nigeria to identify the best-performing yield and desirable nutritional composition. Findings from this study will aid Amaranth growers and breeders in selecting superior lines; it will help to mitigate essential micronutrient deficiency and enhance the socio-economic status of local growers.

2. Materials and Methods

2.1. Plant Materials and Field Evaluation

Commercial cultivars of *Amaranthus hybridus* usually cultivated by local farmers in south-west Nigeria were utilized for this study. The cultivars were collected from the gene bank of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria, and registered as NG/AO/11/08/040, NG/AO/11/08/039, NG/AO/11/042, NHGB/09/09, and NG/TO/AUG/09/007 (all cultivars and information are accessible at NACGRAB in Nigeria). The trial was established at the University of Ilorin Biological Garden in the Guinea savanna zone of Nigeria at Latitude 8°24′ N–8°36′ N and Longitude 4°10′ E–4°36′ E. The annual rainfall is between 990 and 1200 mm, the mean annual tem-

perature is between 33 and 37 °C, and the altitude is 290.9 m above sea level [22]. During the rainy season, the relative humidity is around 75%, while during the dry season, it is around 65%. During the dry season (November to May), the sun shines for 6.5 to 7.5 h per day [22]. A screen house was used for the experiment to protect the plants from pests and human interference.

Seedlings of the amaranth cultivars were grown in the nursery on trays filled with topsoil. Five seedlings of each cultivar were transplanted two weeks after sowing (WAS) into labeled planting pots (20×30 cm) filled with loam–clay soil arranged in Complete Randomized Design in five replicates. The spacing was 1 m between rows and 0.3 m within rows on a plot size of 6.0 m in length and 1.80 m width of 10.8 m² area. Weeding was done manually when required to keep experimental plants free from infestation and competition from weeds. No fertilizer was applied to the plants to simulate a low-input environment; the pots were watered thrice weekly with 75 cL of water to avoid water stress.

2.2. Percentage Germination

The percentage germination of each cultivar was observed and recorded, and twentyfive seeds from each cultivar were evaluated.

2.3. Agronomic Parameters

As amaranths are fast-growing species, the plants were assessed each week till physiological maturity (BBCH stage 71) [23]), and data were collected on both qualitative and quantitative attributes of the five cultivars. Yield components evaluated and also recorded included plant height, the number of leaves, leaf length, leaf width, petiole length, stem girth (BBCH stage 13–71) [23], days to 50% flowering, days to physiological maturity, and a thousand seed weight (TSW). Physiological maturity was determined when the flowers matured; seed head formation was observed, and leaf production began to cease. Qualitative characteristics such as leaf color, seed shape, seed color, and inflorescence color were recorded after nine weeks of growth (BBCH stage 71) [23]; the assessment was based on physical observations. Plant height and leaf dimensions were measured using a meter rule, and stem girth was measured at a height of 10 cm using an electronic vernier caliper (ATD-8656, ATD Tools Inc., Wentzville, MO, USA).

2.4. Proximate Composition Analysis

The proximate composition of the seeds was analyzed according to established procedures described in AOAC [24]. To determine the moisture content, the seeds were subjected to oven drying at 105 °C for 24 h. The seeds were then ground, and the moisture content was determined. Crude lipid extraction was performed using a Soxhlet apparatus with petroleum ether as a solvent. The percentage of crude lipid content was determined according to the protocol established in AOAC [24]. Micro-Kjeldahl's method was used to determine the samples' nitrogen composition using an electrothermal instrument (model MQ3868B/E, Fisher Scientific, Wien, Austria). The total nitrogen content was estimated using the conversion factor N × 5.95, and the resulting values were considered as the percentage of crude protein present in the seeds. Fiber and ash contents were also determined using the protocols described in [24]. The total carbohydrate content of the seeds was determined using the differential method described in [25].

2.5. Phenolic Contents

Total polyphenol content was quantified using Folin–Ciocalteu reagent following protocols described by [26,27]. A mixture of 2.7 mL deionized water, 0.3 mL extracts, 0.3 mL 7% Na₂CO₃, and 0.15 mL Folin–Ciocalteu reagent was vortexed and incubated at 40 °C for 30 min. The absorbance of the solution was measured at 725 nm using gallic acid as a standard.

2.6. Total Antioxidants

To determine the total antioxidant content, 3.8 mL of pre-mixed reagent solution consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was added to 0.2 mL of the extract. The mixture was incubated at 95 °C for one and a half hours and then cooled. The absorbance of the solution was then measured at a wavelength of 695 nm using ascorbic acid as a blank.

2.7. Data Analyses

Morphological, yield, and biochemical composition data were recorded from each replicate. Analysis of variance (ANOVA) followed by multiple comparison procedures was used to compare the datasets. Fisher's protected least significant difference (PLSD) post-hoc test [28] was used for comparison between groups using SPSS statistical package version 20 (IBM Corp., Armonk, NY, USA). The Pearson correlation matrix of the characters was constructed using GraphPad Prism 8.0.2. Canonical variable analysis (CVA) was performed in R using MASS and tidyverse packages to identify multivariate dispersion and visualize the first two canonical variates among *Amaranthus hybridus* cultivars. Genetic variability assessment of the characters evaluated was also carried out in R using variability package. The unweighted pair group method of arithmetic average (UPGMA) dendrogram was conducted using paleontological statistics software (PAST 4.0.3) [29].

3. Results

3.1. Germination Percentage

Germination commenced four days after sowing (DAS). The germination rate was impressive, with all the *Amaranthus* cultivars studied having more than 90% emergence, except AM-5 with a slightly lower germination percentage of 83.33%, while AM-1 and AM-3 had 100% emergence (Figure 1).



Figure 1. Mean germination (%) of five cultivars of *A. hybridus* four days after sowing (bars represent the standard deviation of the mean). Different letters after the bars indicate significant mean differences (p < 0.05), Fisher's PLSD.

3.2. Quantitative and Morphological Characters

Statistical analysis revealed variation among the cultivars for the characters evaluated (Figure 2). Plant height recorded nine weeks after sowing (WAS) varied between 37.70–60.70 cm among the cultivars (Figure 2a). Plant height was highest in AM-1 (60.70 cm), followed by AM-3 (59.30 cm) and AM-4 (51.40 cm). AM-5 produced the shortest plant

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stands with an average height of 37.70 cm. Regarding leaf production, the average number of leaves produced by the cultivars was 5.60, 7.40, 13.80, 18.60, and 22.10 for 1, 3, 5, 7, and 9 WAS, respectively. AM-5 had the highest number of leaves at maturity ($\bar{x} = 29.48$ leaves) (Figure 2b). Another cultivar with above-average leaf production at maturity was AM-3 (23.74 leaves), whereas AM-2 had the lowest number of leaves at maturity and below-average leaf production throughout the weeks of the study. AM-1 had an impressive leaf length with mean values of 9.30, 10.14, 11.92, 13.24, 13.74, and 14 cm at 4, 5, 6, 7, 8, and 9 WAS, respectively, well above the cultivars' averages of 4.40, 5.72, 6.64, 7.96, 10.12, and 10.82 cm within the same period (Figure 2c). Leaf length was significantly shorter in AM-2, AM-4, and AM-5, with mean length below average throughout the study. At maturity, AM-5 had the shortest leaf length (7.88 cm) compared to the cultivars' average of 10.82 cm.



Figure 2. Variations in the morphological parameters of the cultivars over the weeks of evaluation, using the mean values of replicates and standard deviation (bars represent the standard deviation of the mean). (a) shows the plant height of the cultivars through the weeks; (b) shows variations in the number of leaves; variations in leaf length, leaf width, petiole length, and stem girth are depicted in (c-f), respectively. wk—weeks.

The average leaf widths for weeks 6, 7, 8, and 9 were 2.76, 2.99, 3.63, and 5.33 cm, respectively; AM-1 and AM-3 had above-average widths of 4.02, 5.38, 5.96, 6.04 cm; and 2.86, 3.10, 3.36, and 6.12 cm, respectively. AM-1 and AM-3 had the best leaf metrics at maturity, well above the cultivars' average (Figure 2c,d). AM-5 had the lowest leaf metrics at maturity, with a mean length and width of 7.88 cm and 3.68 cm, respectively. Petiole length varied significantly between the cultivars. AM-4 produced leaves with the shortest petiole length (1.60 cm) at maturity. Its leaf length and width were also below average compared to other cultivars, with 9.90 cm and 4.74 cm, respectively. Petiole length generally varied between 1.60 cm and 6.80 cm and was longest in AM-3 (Figure 2e). AM-1 stems had the most considerable girth at maturity with a mean of 1.62, 2.44, 2.68, 2.82, and 2.80 cm at weeks 5–9, respectively, above the cultivars' average of 1.26, 1.60, 1.91, 2.18, and 2.81 cm at the same period, within which AM-3 and AM-5 were below average. AM-2 also had an above-average girth at maturity (3.20 cm), while AM-4 had the lowest stem girth (2.54 cm), as shown in Figure 2f. The mean values of the morphological characters at physiological maturity are presented in Table 1. AM-1, AM-3, and AM-4 had the best vigor.

Cultivar	PH (cm)	NL	LL (cm)	LW (g)	PL (cm)	SG (cm)
AM-1	$60.70 \text{ a} \pm 21.8$	$19.40\mathrm{bc}\pm3.95$	14.00 a \pm 2.5	$6.04~\mathrm{a}\pm1.8$	$6.22~\mathrm{a}\pm0.68$	$2.80~\mathrm{a}\pm0.18$
AM-2	41.30 ab \pm 19.1	$16.40~\mathrm{c}\pm7.8$	$10.50~b\pm2.5$	$6.06~\mathrm{a}\pm1.5$	$4.38b\pm0.74$	$3.20~\mathrm{a}\pm0.68$
AM-3	59.30 a \pm 5.7	23.74 ab \pm 3.2	11.82 ab \pm 1.3	$6.12~\mathrm{a}\pm0.9$	$6.80~\mathrm{a}\pm0.40$	$2.78~\mathrm{a}\pm0.18$
AM-4	$51.40~\mathrm{ab}\pm12.7$	$21.48~\mathrm{bc}\pm5.4$	$9.90 \text{ bc} \pm 0.8$	$4.74~\mathrm{ab}\pm0.8$	$1.60~\mathrm{c}\pm0.06$	$2.54~\mathrm{a}\pm0.22$
AM-5	$37.70~b\pm5.0$	$29.48~a\pm3.7$	$7.88~\mathrm{c}\pm1.6$	$3.68~b\pm0.4$	$3.60b\pm0.48$	$2.76~a\pm0.28$
Average	50.08	22.10	10.82	5.33	4.52	2.82
LSD	19.40	6.69	2.48	1.43	1.57	1.06
CV %	32.56	29.23	24.86	29.83	48.34	27.30
<i>p</i> value	0.05	0.008	0.001	0.03	0.00	0.78 *

Table 1. Vegetative growth variables of five A. hybridus cultivars at physiological maturity.

Data are means of five replicates \pm SD. Values carrying the same letter(s) along the same column are not significantly different at *p* < 0.05, Fisher's PLSD. PH—plant height; NL—number of leaves; LL—leaf length; LW—leaf weight; PL—petiole length; SG—stem girth; LSD—Least Significant Difference; CV—Coefficient of Variation; *p* values with * indicate not significant.

3.3. Qualitative and Yield-Related Parameters

Qualitative characteristics examined at physiological maturity (9 WAS) showed that leaf color and seed shape were green and oval for all the cultivars (Table 2). The seed color for AM-1 and AM-2 is golden brown. The seed color and shape were similar to the planted seeds; there was no observable difference in the seed morphology of the planted seed and at harvest. The inflorescence color was green for AM-1, pink for both AM-2, AM-3, and AM-5, and white for AM-4. The leaf type was all entire and round for AM-1, AM-2, AM-3, and AM-5, whereas AM-4 is entire and spiral.

The number of days to 50% flowering and maturity of the cultivars is shown in Figure 3. Early flowering was observed in AM-4, which attained 50% flowering at 39 DAS (days after sowing) and reached maturity at 49 DAS; AM-1 at 71 DAS and reached maturity at 84 DAS, followed by AM-3 at 72 DAS and maturity at 84 DAS. Flowering and maturity were delayed in AM-2, which flowered at 73 DAS and reached maturity at 91 DAS. Among all the cultivars evaluated, AM-5 exhibited late maturity, with an average of 80 days to 50% flowering and maturity at 90 DAS. A thousand seeds of each cultivar were weighed; AM-1 had the highest mean weight of 0.926 g, AM-3 (0.4740 g), and AM-2 (0.3740 g). AM-5 had the lowest mean weight of 0.145 g.



Figure 3. Variability in the days to flowering and maturity of five cultivated *A. hybridus*. Different letters above the bars (uppercase for days to 50% flowering; lowercase for days to maturity) indicate statistically significant mean differences (p < 0.05), Fisher's PLSD.

Cultivar Code	Accession Number	ccession Number Leaf Seed Color Shape		Seed Color	Fluorescence Color	Stem Color	or Leaf Type	
AM-1	NG/AO/11/08/040	green	oval	golden brown	green	green	entire and round	
AM-2	NG/AO/11/08/039	green	oval	golden brown	pink	purple	entire and round	
AM-3	NG/AO/11/042	green	oval	deep brown	pink	purple	entire and round	
AM-4	NHGB/09/09	green	oval	black	white	green	entire and spiral	
AM-5	NG/TO/AUG/09/007	green	oval	brown	pink	purple	entire and round	

Table 2. Qualitative characters of the five varieties of A. hybridus.

3.4. Seed Proximate Composition

Total carbohydrate content was high in all the cultivars, ranging from 65.01% (AM-3) to 76.28% (AM-4), averaging 70.76%. AM-1 and AM-3 had the highest total protein content (12.42 and 11.35%, respectively), crude fiber content (9.16 and 9.81%, respectively), and ash content (3.59 and 3.06%, respectively) (Table 3). In all the cultivars, total protein content ranged between 6.57 and 12.42%; crude fiber content ranged between 5.56 and 9.81%, with AM-4 having the lowest yield for both parameters. The average percentage of total lipid content in the different cultivars was 5.51%, while the average ash content was 3.01%. Total polyphenols and antioxidants were highest in AM-1 with 29.64 and 181.59 mg/100 g, respectively. The lowest contents of total polyphenols (23.36 mg/100 g) and antioxidants (131.64 mg/100 g) were recorded in AM-5 and AM-4, respectively. The biochemical and proximate seed compositions of AM-1 and AM-3 were not significantly different for the parameters evaluated except for the total lipid and moisture contents. Analysis of variance (ANOVA) revealed significant differences between the cultivars (p < 0.05) based on the proximate parameters assessed. However, total ash and polyphenol contents were not significantly different.

Table 3. Seed proximate compositions, total polyphenols, and antioxidant contents of the *A. hybridus* cultivars at maturity.

Cultivars	TCC (%)	TPC (%)	TLC (%)	CFC (%)	MC (%)	AC (%)	TP (mg/100 g GAE)	TA (mg/100 g AAE)
AM-1 AM-2 AM-3 AM-4 AM-5	$\begin{array}{c} 66.52 \ bc \pm 7.83 \\ 72.33 \ ab \pm 6.25 \\ 65.01 \ c \pm 7.19 \\ 76.28 \ a \pm 4.07 \\ 73.65 \ ab \pm 4.21 \end{array}$	$\begin{array}{c} 12.42 \ a \pm 0.37 \\ 10.02 \ b \pm 1.51 \\ 11.35 \ a \pm 1.01 \\ 6.57 \ c \pm 0.93 \\ 7.29 \ c \pm 0.67 \end{array}$	$\begin{array}{l} 4.98 \ c \ \pm \ 0.13 \\ 5.36 \ bc \ \pm \ 0.69 \\ 6.55 \ a \ \pm \ 0.57 \\ 4.51 \ c \ \pm \ 1.09 \\ 6.13 \ ab \ \pm \ 0.90 \end{array}$	$\begin{array}{c} 9.16 \ a \pm 0.46 \\ 6.25 \ bc \pm 0.45 \\ 9.81 \ a \pm 0.42 \\ 5.56 \ c \pm 1.25 \\ 6.96 \ b \pm 1.09 \end{array}$	$\begin{array}{c} 3.33 \ b \pm 0.16 \\ 3.29 \ b \pm 0.43 \\ 4.22 \ a \pm 0.53 \\ 4.16 \ a \pm 0.62 \\ 3.24 \ b \pm 0.98 \end{array}$	$\begin{array}{c} 3.59 \text{ a} \pm 0.64 \\ 2.75 \text{ a} \pm 0.81 \\ 3.06 \text{ a} \pm 0.77 \\ 2.92 \text{ a} \pm 0.52 \\ 2.73 \text{ a} \pm 1.13 \end{array}$	$\begin{array}{c} 29.64 \ a \pm 1.79 \\ 27.04 \ a \pm 2.74 \\ 28.99 \ a \pm 5.10 \\ 26.65 \ a \pm 3.49 \\ 23.36 \ a \pm 7.40 \end{array}$	$\begin{array}{c} 181.59 \ a \pm 12.06 \\ 163.77 \ b \pm 16.02 \\ 180.53 \ a \pm 10.23 \\ 131.64 \ c \pm 6.86 \\ 140.77 \ c \pm 9.91 \end{array}$
Average LSD CV % p value	70.76 8.06 11.63 0.003	9.53 1.29 26.02 0.00	5.51 1.00 18.51 0.002	7.55 1.08 24.50 0.00	3.65 0.80 19.51 0.03	3.01 1.06 26.25 0.45 *	27.14 6.29 17.43 0.25 *	159.66 15.07 14.59 0.00

Data are means of five replicates \pm SD. Means carrying the same letter(s) along the same column are not significantly different at p < 0.05, Fisher's PLSD. TCC—total carbohydrate content; TPC—total protein content; TLC—total lipid content; CFC—crude fiber content; MC—moisture content; AC—ash content; TP—total polyphenols; TA—total antioxidants; GAE—Gallic Acid Equivalent; AAE—Ascorbic Acid Equivalent; LSD—Least Significant Difference; CV—Coefficient of Variation; p values with * indicate not significant.

3.5. Multivariate Analysis

The Pearson correlation matrix of vegetative and yield characters of the amaranth cultivars showed significant positive relationships between some characters (Figure 4). At p < 0.05, plant height and leaf length show a positive correlation (r = 0.87). Furthermore, plant height, leaf width, and petiole length all correlate with seed weight with r values above 0.64 and below 0.80. Petiole length is associated with plant height, leaf length, and leaf width with r values above 0.50 and less than 0.70. Plant height also correlates with leaf width (r = 0.62). However, a negative correlation exists between seed weight and the number of leaves (r = -0.50). Leaf length, leaf width, and stem girth negatively correlate with the number of leaves with r values between -0.50 and -0.75. The number of leaves and leaf length show no relationship with the days of flowering.



Figure 4. Correlations matrix of morphological traits at maturity. The matrix of correlations between the vegetative and yield characters of the *A. hybridus* cultivars. Analysis was made from the mean of replicates. Traits with correlations significant at p < 0.05 are enclosed in white boxes. PH—plant height; NL—number of leaves; LL—leaf length; LW—leaf width; PL—petiole length; SG—stem girth; SW—seed weight; DF—days to flowering; GR—germination (%).

Canonical variable analysis (CVA) was conducted based on the data obtained. The resulting CVA plot contained five clusters of samples (Figure 5); each cluster is dominated by samples of the same cultivar, with few outliers suggesting differential trait expression within each cultivar. The overlap between AM-1 and AM-3 clusters on the CVA indicates close similarity in their morphological and yield traits evaluated. AM-4 and AM-5 are further away, showing rapid divergence from the other cultivars.



Figure 5. Canonical variable analysis was conducted on the five cultivars of *A. hybridus*, with individuals plotted on the two canonical variates. The ellipses represent 95% confidence intervals for each variety's morphological distribution.

The unweighted pair group of arithmetic average (UPGMA) dendrogram based on vegetative and yield characters separated the amaranth cultivars into two groups (Figure 6). AM-4 shows a remarkable divergence from other cultivars, standing alone in Group 1, separated from other cultivars by a genetic distance above 40. Group 2, consisting of other cultivars (AM-1, AM-2, AM-3, AM-5), is further separated into clusters 2A and 2B, with a genetic distance of less than 27. Of all the cultivars, AM-1 and AM-3 show the highest degree of relatedness, with a genetic distance of less than 7.



Figure 6. Dendrogram of genetic relationship (using the unweighted pair group of arithmetic average (UPGMA)) among amaranth cultivars.

The CVA of the cultivars' biochemical and seed-proximate composition contributions to the observed variation (Figure 7) further revealed diversity among the cultivars based on the characters evaluated. Each cultivar formed distinct clusters, showing the characters' effectiveness in characterizing the population. The overlap between AM-5 and AM-4 clusters indicates similarities in seed proximate and biochemical contents.



Figure 7. Canonical variable analysis was conducted on the five varieties of *Amaranthus hybridus*, with individuals plotted on the two canonical variates. Ellipses depict the 95% confidence intervals for each variety's distribution of seed proximate components, total polyphenols, and antioxidant contents.

Genetic variability assessment of the morphological and yield-related traits is presented in Table 4. It differentiates the key factors influencing the phenotypic differences observed among the cultivars and their potential for selection.

Table 4. Variability assessment using genetic parameters for the morphological, biochemical, and proximate components of the cultivars.

Traits	σ^2_p	σ^2_{g}	σ_e^2	GCV
Germination (%)	52.4	46.59	5.81	7.21
No of leaves	49.08	18.08	31.01	19.24
Plant height (cm)	244.56	73.49	171.08	17.12
Leaf width (cm)	2.63	0.82	1.81	16.98
Leaf length (cm)	7.62	4.52	3.09	19.65
Petioles length (cm)	5.41	4.05	1.35	44.40
Stem girth(cm)	0.58	-0.08	0.65	9.79
TCC (g/100 g)	43.44	17.66	25.77	5.94
TPC $(g/100 g)$	7.01	6.27	0.74	26.25
TLC (%)	1.16	0.57	0.59	13.75
CFC (%)	3.88	3.30	0.58	24.08
MC (%)	0.53	0.17	0.35	11.46
AC (%)	0.44	0.04	0.39	7.04
TP (mg/100 g GAE)	26.77	0.87	25.90	3.45
TA (mg/100 g AAE)	622.46	492.86	129.60	13.90

 σ_p^2 = Phenotypic Variance, σ_g^2 = Genotypic Variance, σ_e^2 = Environmental Variance, GCV = Genotypic Coefficient of Variance, GAE—Gallic Acid Equivalent; AAE—Ascorbic Acid Equivalent.

Path coefficient analysis reveals other growth components' direct and indirect effects on leaf yield. Leaf yield was the resultant variable, while plant height, leaf width, leaf length, petioles length, stem girth, TCC, TPC, TLC, CFC, MC, AC, TP, and TA were the independent variables. Their direct and indirect effects are presented in Table 5. Results show that plant height has a considerable direct effect on leaf yield (0.8), which is the highest direct positive effect recorded, followed by TCC (0.62), TLC (0.45), and CFC (0.32). TPC, TA, leaf weight, leaf length, MC, and stem girth negatively affect leaf yield (-0.89, -0.83, -0.57, -0.46, -0.35, -0.10, respectively). Petiole length has a positive indirect effect on leaf yield (1.55). A residual effect of -0.2552 was recorded from other possible external factors not captured in this study. The 13 growth and yield parameters surveyed account for 98.91% of the genetic variance of leaf yield in *A. hybridus*.

Table 5. Genotypic path analysis of direct and indirect effects of evaluated morphological and yield-related characters on leaf yield in the *A. hybridus* cultivars.

Traits	Plant Height	Leaf Width	Leaf Length	Petioles Length	Stem Girth	TCC	TPC	TLC	CFC	МС	AC	ТР	TA	GCLY
Plant height	0.80	-0.32	-0.45	0.95	-0.10	-0.56	-0.70	-0.04	0.28	-0.23	0.34	0.09	-0.68	-0.64
Leaf width	0.45	-0.57	-0.44	1.20	0.00	-0.58	-0.91	-0.01	0.21	-0.07	0.19	0.08	-0.88	-1.34
Leaf length	0.78	-0.55	-0.46	1.20	-0.02	-0.57	-0.83	-0.12	0.23	-0.05	0.29	0.09	-0.78	-0.80
Petioles length	0.49	-0.44	-0.36	1.55	0.02	-0.68	-0.86	0.29	0.32	0.05	0.18	0.06	-0.83	-0.21
Stem girth	-0.77	0.01	0.09	0.35	-0.10	-0.11	-0.38	0.17	-0.01	0.22	0.01	-0.02	-0.37	0.26
ŤCC	-0.73	0.53	0.43	-1.72	-0.02	0.62	0.92	-0.25	-0.36	0.06	-0.26	-0.07	0.93	0.08
TPC	0.63	-0.59	-0.43	1.51	0.04	-0.64	-0.89	0.11	0.27	0.05	0.21	0.08	-0.86	-0.49
TLC	-0.07	0.02	0.12	1.02	0.04	-0.35	-0.21	0.45	0.18	-0.06	-0.07	-0.03	-0.31	0.73
CFC	0.69	-0.37	-0.33	1.52	0.00	-0.69	-0.75	0.25	0.32	-0.06	0.21	0.06	-0.74	0.11
MC	0.53	-0.12	-0.07	-0.21	-0.07	-0.11	0.12	0.08	0.05	-0.35	-0.04	0.07	0.10	-0.03
AC	1.54	-0.63	-0.76	1.61	0.01	-0.94	-1.07	-0.17	0.39	0.08	0.17	0.13	-0.90	-0.55
TP	2.02	-1.25	-1.16	2.68	-0.06	-1.20	-1.95	-0.40	0.56	-0.64	0.62	0.04	-1.67	-2.41
TA	0.65	-0.61	-0.43	1.56	0.05	-0.69	-0.92	0.17	0.29	0.04	0.19	0.07	-0.83	-0.46

TCC—total carbohydrate content; TPC—total protein content; TLC—total lipid content; CFC—crude fiber content; MC—moisture content; AC—ash content; TP—total polyphenols; TA—total antioxidant; GCNL = genotypic correlation with leaf yield. Note: direct effects are in bold letters.

4. Discussion

Significant differences in plant height, foliage parameters, yield-related traits, and nutrient composition are required to assess plant diversity [25,30–34]. *Amaranthus* exhibits significant heterogeneity with variation in plant habit (erect to prostrate), plant height, number of inflorescences (single to multiple), seed coloration, nutrient content, and yield components [8,33,35]. Thus, there is a vast potential for genetically improving vegetable amaranths, harnessing their heterogeneity. Studies demonstrated substantial variability among *A. hybridus* and its sister species, reflected in both agronomic and qualitative traits [36–41]. All the traits examined in this study showed a remarkable variation in all the cultivars and significantly influenced leaf and seed yield. These findings support the previous reports of high heterogeneity among Nigerian *A. hybridus* accessions [8,36].

Significant associations were observed among the traits evaluated in the current study. AM-5 was the shortest but had the highest foliage yield at maturity; however, it had tiny leaves compared to other cultivars. Subsequently, AM-1, which had the tallest stands, produced fewer long and broad leaves. The negative correlation between plant height and foliage yield agrees with earlier reports [38,42]. Taller plants have fewer leaves compared to shorter stands; this relationship could be due to the genetic system of the species. Amaranths are often found growing in clusters in the field and the wild; this could be an adaptation mechanism developed to help shorter stands rapidly harvest and maximize the little light they receive in the shade of taller amaranth stands. AM-1 should be considered when selecting leaf metrics suitable for consumer demands. Assad et al. [8] and Showemimo et al. [43] also found a positive association between plant height and leaf metrics (length and width) in amaranths; there could be an additive gene action in determining both traits. The correlation between the days to flowering and leaf width was insignificant, in contrast to [44].

Cultivars with the best foliage attributes (AM-1, AM-2, and AM-3) also had the highest seed weight, consistent with earlier reports [8]. This could result from the increased leaf surface area and enhanced photosynthesis [45], enhancing grain yield. These cultivars have long petioles, which help amaranths compete for sunlight and escape shade effect, increasing light penetration to boost photosynthetic rate and grain yield [44]. Assad et al. [8] and Baturaygil and Karl [46] reported variation in the number of days to flower in *Amaranthus* spp., as also observed in this study. However, the authors noted that this variation was solely due to genotypic factors. Late flowering is advantageous to farmers; it gives them more time to harvest leaves. Early flowering cultivars like AM-4 are unsuitable for commercial purposes; delayed flowering cultivars are more profitable.

The canonical variable analysis based on growth and yield traits shows an intrinsic and rapid divergence among the cultivars. AM-4 and AM-5 cultivars formed distinct clusters, with no conjunction with other samples from sister cultivars, indicating an intrinsic intraspecific divergence among the species. The dendrogram of genetic relatedness also shows a high degree of similarity between each cluster. AM-1 and AM-3, which are the most related, have a negligible genetic distance, as also revealed in the canonical variable analysis; this indicates a common origin.

The quest to meet the food demands of the increasing population in Africa has inadvertently shifted the attention of growers and breeders to selecting crops for higher productivity over nutrient value. The unavoidable consequence is the present-day reality of a nutrient-deficient population [47,48]. The nutrient values of the accessions were also examined for consumer suitability. Analysis of the proximate composition of the seed showed that the grains are rich in protein, lipids, and fiber. The protein and lipids contents peaked at 12.42 and 6.55%, respectively. This is a deviation from previous reports [49,50]. They documented protein contents of 14.7% and 16.1% and lipid contents of 5.8% and 8.3% in selected Indian and Polish cultivars, respectively. The differences can be attributed to their genetic systems, different evolutionary paths within the genus, and the influence of regional climate conditions and cultivation methods. The average ash content in the studied cultivars (3.01%) corresponds well with earlier reports [49,50]. The cultivars were found to be relatively high in crude fiber content. Fibers and non-digestible carbohydrates help to lower low-density lipoprotein and overall blood cholesterol levels. Additionally, research has shown that fiber-rich foods may lower blood pressure and inflammation, among other heart-healthy advantages [51]. The total antioxidants of the five cultivars studied ranged from 131.6 to 181.59 (mg/100 g AAE), which is similar to a previous report (140.22 to 199.93 mg/100 g AAE) [27]. The antioxidant activities of amaranths are significantly higher than those of common cereals such as rice (85.49 mg AAE/100 g), barley (3.46 mg AAE/100 g), maize (26.94 mg AAE/100 g), and wheat (14.17 mg AAE/100 g) [27,52]. The total polyphenol content obtained ranged from 23.36 to 29.64 mg/100 g GAE, which is comparable to 30.79 mg/100 g GAE earlier reported for *A. hybridus* and 27.52 to 30.8 mg/100 g GAE reported among other Nigerian amaranth species [27]. Among the cultivars, AM-1 and AM-3 have higher protein, fiber, polyphenols, and antioxidants, thus promising health benefits.

Since agronomic traits hinge on the contributory effects of genotype and environmental factors [33]. It is necessary to examine these influences to determine their additive share of variability. In the current study, the wide difference between the phenotypic and genotypic variance and their corresponding coefficient of variance shows a significant environmental influence on the morphological traits. Genotypic Coefficient of Variance (GCV) values enable breeders to prioritize traits with higher genetic variability for selection and breeding programs. Additionally, plant heights and leaf metrics in this study, having high GCV values may be more resilient to environmental stresses and exhibit greater adaptability. Path coefficient analysis helps estimate the contributing factors' direct and indirect effects on a trait. It helps to identify associated traits and assess their influence on targeted traits [33,53]. The path coefficient analysis revealed that the traits considered in this study are key predictors of leaf yield in *A. hybridus*, with only 1.09% residual effects. Cultivars that do not maintain a good index in these indices (evaluated traits) may have a low leaf yield and would not be profitable to farmers in the region.

Evaluating cultivars to identify those with desirable yield and nutrient composition is pivotal to overcoming malnutrition and improving the socio-economic status of local farmers. Among the cultivars of *A. hybridus* evaluated in this study, AM-1 and AM-3 showed the best performance and maintained desirable productivity and nutrient value compared to other cultivars under similar growth conditions. Genetic analysis showed a significant degree of relatedness between both cultivars, suggesting a common ancestor that is most likely a superior pure-line *A. hybridus* variety.

5. Conclusions

This study assessed commercially cultivated *A. hybridus* cultivars in south-west Nigeria using key morpho-agronomic and yield-related traits to identify the most suitable for increased productivity and profitability. Altogether, AM-1 and AM-3 showed the best growth and yield indices compared to other cultivars under similar growth conditions and maintained desirable productivity and nutritional value. These cultivars can increase farmers' productivity and offer more nutritional benefits for consumers. They are recommended for cultivation and can be further explored in breeding programs to develop superior lines.

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