INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
24–0429/2025/33:330310
http://www.fspublishers.org

Full Length Article

Morphological Diversity in Amaranth Lines for Inclusion in the Benin Catalogue of Plant Species and Varieties (CaBEV)

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Received 13 September 2024; Accepted 04 November 2024; Published online 25 December 2024

Editor: Kanval Shaukat

Abstract

Amaranth (*Amaranthus spp*.) is a highly nutritious indigenous vegetable crop in Africa, yet its cultivation remains limited in Benin due to various constraints, including the scarcity of high-quality seeds. This study aimed at to characterize nine new amaranth lines, selected as candidates for inclusion in the Beninese Catalogue of Plant Species and Varieties (CaBEV), using morphological markers. The experiment was conducted under irrigated conditions at the research station of the Vegetable Crops Program of the National Institute of Agricultural Research of Benin, following a Randomized Complete Block Design with three replications. Morphological characterization was performed according to the amaranth descriptor established by the International Union for the Protection of New Varieties of Plants (UPOV). Results indicated significant differences among the lines for several agro-morphological traits ($P \leq 0.05$). In terms of productivity, the AM_NKGN line produced the highest fresh leaf biomass yield (20.92 t/ha), while the Vert_local line had the lowest yield (11.30 t/ha). Additional findings revealed that the ACNL, AVAM_1939, and NGURUMA lines were respectively distinguished by the largest collar diameter (29.87 mm), the highest number of branches (35.60) and the greatest plant height (189.84 cm). Notably, the Vert_local line matured the earliest, reaching physiological maturity 62 days after transplanting (DAT), while the AVAM-1939 line matured the latest (167 DAT). Hierarchical Ascendant Classification (HAC) analysis grouped the lines into three distinct categories. The first group, comprising seven lines, displayed similar yet less productive agro-morphological traits. The second group, represented by the NGURUMA line, was characterized by its large and broad leaves, while the third group, consisting solely of the AVAM-1939 line, showed a high average number of branches. These findings underscore the strong agro-morphological potential of the new candidate lines, supporting their suitability for inclusion in CaBEV. However, multi-location trials are recommended to further assess the genotype-environment interaction for the studied parameters.

Keywords: *Amaranthus* spp.; Agro-morphological potential; Seed; Benin

Introduction

The issue of food and nutrition security has always been a major concern for the ever-growing populations around the world. Despite many efforts to reduce malnutrition, it still affects 767.9 million people worldwide and 57.3 million people in West Africa (FAO 2022). The projected growth of the world's population, accompanied by an increase in food

To cite this paper: Megnonhou S, A Mensah, D Montcho, E Akpo, O Kolade, N Hlanga, C Gandonou, C Agbangla (2025). Morphological diversity in amaranth lines for inclusion in the benin catalogue of plant species and varieties (CaBEV). *Intl J Agric Biol* 33:330310. https://doi.org/10.17957/IJAB/15.2286

demand, particularly in Africa, requires greater attention to underutilized plant species that have significant potential to influence and improve food security for African nations and promote growth and development in Africa (Geisen *et al.* 2021). Benin has a rich diversity of food crops, yet most have received minimal attention in terms of research and the development of policy frameworks to support their efficient commercial and industrial utilization. Amaranth is one of these neglected and underutilized species in Benin and generally in West Africa (Sogbohossou *et al.* 2015). Amaranth is an indigenous African vegetable crop with high nutritional potential that can greatly enhance human health (Zhu 2020). For example, a biochemical analysis by Singhania *et al.* (2023) revealed that amaranth contains substantial levels of starch (29.36%), essential protein (14.25%), fats or lipids (8.18%), and dietary fiber (4.04%). Additionally, from an animal nutrition perspective, Oteri *et al*. (2021) demonstrated that amaranth grains and leaves can be used in many countries as fodder or silage for various animals, including cattle, chickens, pigs, and rabbits. Notably, amaranth is one of the most drought-tolerant crops, with significant commercial and industrial potential that remains largely untapped (Jamalluddin *et al*., 2021).

Amaranth's adaptability allows it to be cultivated successfully in diverse regions and seasons, similar to cereals, due to its resilience in various environmental conditions (Grundy *et al*. 2020). This crop's tolerance to a wide range of climates and its relatively short production cycle make it a valuable vegetable crop with great economic potential (Hoidal *et al.* 2020). Although amaranth is hardy in harsh climates, it thrives best and produces higher yields on fertile, well-drained, loose, and organic-rich soils (Stetter *et al*. 2020).

The development of sustainable value chains for amaranth—from field cultivation to the production of value-added products—could significantly improve the lives of millions of poor rural and urban households in developing countries facing high rates of malnutrition and related health issues. However, amaranth cultivation in Benin encounters challenges, including a lack of improved varieties, weaknesses in the seed system, and limited expertise in cultivation practices (Azandémè-Hounmalon *et al.* 2023). Aglinglo *et al*. (2022) identified four main sources of seed supply for underutilized leafy vegetables in Benin. These include reserves from the previous season, donations from colleagues, the use of wild seedlings, and purchases from informal sources. Currently, none of the amaranth varieties cultivated in Benin are included in the Benin Catalog of Plant Species and Varieties (MAEP 2016). However, according to the Technical Regulations and Annexes (TRA), the production, quality control, and certification of various seed categories apply exclusively to varieties listed in the Benin Catalog of Plant Species and Varieties (CaBEV) or the Regional Catalog of Plant Species and Varieties (COAfEV). This restriction limits the development of local seed programs, including the commercial supply of seeds by small seed companies and community seed systems, both of which are essential for the production and distribution of high-quality seeds.

To improve access to quality seeds for producers, it is crucial to promote and register new, improved amaranth varieties in Benin. In this context, the present study was aimed at to conduct a genetic diversity analysis based on morphological markers of nine new amaranth lines, which are candidates for inclusion in the Benin Catalog of Plant Species and Varieties (CaBEV).

Materials and Methods

Study area

The experiments in this study were carried out at the vegetable crops program (PCM) research station, located at the Agonkanmey Agricultural Research Center of the Benin National Institute of Agricultural Research, in the commune of Abomey-Calavi. The municipality of Abomey-Calavi is located in the Atlantic department of the Republic of Benin. It lies between latitude 6°22' and latitude 6°30' N and longitude 2°15' and longitude 2°22' E. It is bounded in the north by the commune of Zè, in the south by the Atlantic Ocean, in the east by the commune of Cotonou and So-Ava and in the west by the communes of Ouidah and Tori-Bossito (DGCS-ODD 2019). The experimental soil was a sandy loam with a pH ranging between 6 and 7 and an organic matter content of 0.79%, a carbon content of 0.58%, a nitrogen content of 0.05%, and a cation exchange capacity of 0.18 meq/100 g based on an analysis done earlier in 2017 (Mensah *et al.* 2019a).

Plant material

The plant material used in this study comprised seeds from nine distinct lines of amaranth. Of these, two lines were supplied by the Faculty of Agronomic Sciences at the University of Abomey-Calavi (FAST/UAC), five lines were obtained from the World Vegetable Center and two lines were local accessions provided by the National Institute of Agricultural Research of Benin (INRAB) (Table 1).

Experimental design

The experiment was carried out using a randomized complete block design with three replications. Treatments included the nine distinct lines of amaranth being evaluated as listed in Table 1. Each plot measured 6 m² (5 m x 1.2 m) and contained four rows with 25 plants per row, totaling 100 plants per plot. Transplanting was done at a spacing of 0.20 m x 0.20 m, with one plant per hole, resulting in a planting density of 250,000 plants per hectare.

Test procedures and measured parameters

The experiment was conducted in accordance with the

Table 1: List of the lines studied

N	Lines names	Species	Provenances
1	Vert_local	*Amaranthus cruentus*	INRAB
2	Rouge_local	*Amaranthus cruentus*	INRAB
3	L2	*Amaranthus cruentus*	FAST/UAC
4	L23	*Amaranthus cruentus*	FAST/UAC
5	AM_NKGN	*Amaranthus hybridus*	World Vegetable Center
6	NGURUMA	*Amaranthus dubius*	World Vegetable Center
7	A2004	*Amaranthus cruentus*	World Vegetable Center
8	ACNL	*Amaranthus hypochondriacus*	World Vegetable Center
9	AVAM_1939	*Amaranthus dubius*	World Vegetable Center

recommendations of the INRAB Technical Economic Reference Code for the conduct of amaranth in Benin (Mensah *et al.* 2019b). The seedlings were monitored continuously from sowing until the end of the trial. Within each block, measurements and characterizations were conducted exclusively on the seedlings in the central rows of each plot. Characterization was performed both by individual plant and by line. Twenty plants per line and replicate were randomly selected and marked for observations and measurements. The traits studied, listed in Table 2 followed the amaranth descriptors established by the International Union for the Protection of New Varieties of Plants (UPOV 1999) and the Manual of Procedures for the Approval and Registration of Varieties in the Benin Catalog of Plant Species and Varieties (MAEP 2017).

Statistical analysis of data

The characterization data were analyzed using the R.4.0.2 software. A simple variance analysis was performed to determine whether significant differences exist between the lines for the studied traits. Then, the Newman-Keuls test was used to compare the means at a 5% probability level ($P \le 0.05$). The relationships between variables were studied using the total correlations matrix and a Principal Components Analysis (PCA). The PCA helped visualize how the variables are structured and how the individuals are distributed. A Hierarchical Ascending Classification (HAC) was used to group individuals with similar behaviors.

Results

Qualitative characters

Primary qualitative characteristics related to the leaves, stems and inflorescences of the different lines: Table 3 presents the primary characteristics related to the leaves, stems and inflorescences of the different lines. This table reveals a slight dissimilarity between the different lines with respect to their primary qualitative characteristics. All the lines have leaves at the pointed apex except the NGURUMA line which has leaves at the slightly rounded apex. In addition, the lines have dark green or light-colored limbs with the exception of the Rouge_local line which has purple-colored limbs. Regarding the position of the widest

Fig. 1: The vertex shapes, colors and position of the widest part of the leaves of the lines

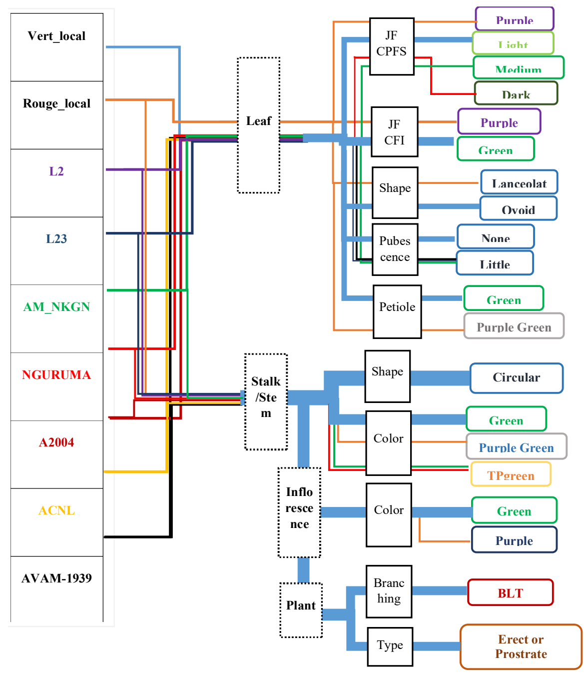

Fig. 2: Main qualitative characters related to leaves, stems and inflorescences of the different lines
Captions: JF CPFS: Younger Leaf Upper Face Color, JF CFI: Younger Leaf Lower Face Color, BLT: Branch Along Stalk

part of the leaves, AM-NKGN, NGURUMA and AVAM_1939 lines have leaves with their widest part being "Moderately towards the base," while all the other lines have leaves with their widest part in the "Middle or towards the base" (Fig. 1). The anthocyanin pigmentation of the cotyledons is only observed in the Rouge local and AM-NKGN lines. Furthermore, among all the lines, only the Rouge local, AM-NKGN and NGURUMA have stems with anthocyanin pigmentation at the base. As regards the form of the inflorescences, they are mostly upright or slightly falling in all lineages.

Main qualitative characteristics related to the leaves, stems and inflorescences of the different lines: Fig. 2 summarizes the main qualitative characteristics of the leaves, stems and inflorescences of the different lines studied. This figure shows that only the Rouge local line has young leaves with purple upper and lower sides. On the other hand, the other lines have young leaves where both sides are green or light green.

Table 2: Details of the measured qualitative and quantitative parameters

Serial Number	Characters	Modalities	Rating	Collection method	Collection period
1	Seed emergence time	fast (< 2 JAS)	1	Observation	At emergence
		slow (2-7 JAS)	2		
		very slow (> 7JAS)	3		
		Irregular (> 10JAS)	4		
2	Cotyledon: Anthocyanin pigmentation	Absent	1	Observation	Seedling observations (3-6 days after emergence at the nursery)
		Present	9		
3	Seedling: pigmentation anthocyanin of hypocotyls	Absent	1	Observation	Seedling observations (3-6 days after emergence at the nursery)
		Present	9		
4	Hypocotyls: color	Green	3	Observation	Seedling observations (3-6 days after emergence at the nursery)
		light purple	5		
		Purple	7		
5	Young leaf: position of the party the widest	In the middle or slightly downward	1	Observation	Seedling observations (3-6 days after emergence at the nursery)
		Moderately down	2		
		Strongly down	3		
6	Young leaf: main color of the face superior	Light green	1	Observation	Seedling observations (3-6 days after emergence at the nursery)
		Medium Green	2		
		Dark green	3		
		red	4		
		purple	5		
7	Young leaf: face color lower	Green	1	Observation	Seedling observations (3-6 days after emergence at the nursery)
		red	2		
		purple	3		
8	Branching index	No branches	1	Observation	Seedling observations (3-6 days after emergence at the nursery)
		Few branches (at the very base of the stem)	2		
		many branches (all at the base of the stem)	3		
		Branches along the stem	4		
9	Leaf pubescence	None	0	Observation	Seedling observations (3-6 days after emergence at the nursery)
		Weak	3		
		Lots	7		
10	The shape of the leaves	Lanceolate	1	Observation	Seedling observations (3-6 days after emergence at the nursery)
		Elliptical	2		
		Cuneiform	3		
		Obovate	4		
		Ovatainate	5		
		Rhombic	6		
		Oval	7		
		Other to specify	8		
11	sheets: top shape	Very sharp	1	Observation	Seedling observations (3-6 days after emergence at the nursery)
		Sharp	3		
		Medium	5		
		Obtuse	7		
		Very obtuse	9		
12	Plant: time of onset of the inflorescence	Early	3	Observation	From seeding to the appearance of the first flower
		Average	5		
		Late	7		
13	Age of 50% Flowering	Early	3	Observation	50% flowering
		Average	5		
		Late	7		
14	Growth habit	Erect	1	Observation	50% flowering
		Prostrate	2		
15	Stem: Color	Green	1	Observation	50% flowering
		Yellow	2		
		pink	3		
		red	4		
		Purple	5		
16	Stem: Strip color	Red	1	Observation	50% flowering
		Violaceous	2		
		Purple	3		
17	Petioles: color	Green	1	Observation	50% flowering
		Light purple	3		
		Purple	5		
		Dark purple	7		
		Dark brown	9		

Table 2: Continued

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18	Inflorescence: Color	Yellow Yellow Green Pink Red Purple	1 2 3 4 5 6	Observation	50% flowering
19	Inflorescence: Type	erect or weak drooping Intermediate strongly dropping Average Long	1 2 3 5 7	Observation	50% flowering
20	Plant: time of Maturity	Early Average Late	3 5 7	Observation	Physiological maturity
21	Stalk: Anthocyanin pigmentation of the base	Absent Present	1 2	Observation	Physiological maturity
22	Stalk: shapes of cross-section	Circular Wavy Discoid	1 2 3	Observation	Physiological maturity
23	Stalk: Color	Brown Black Yellow Pink	1 2 3 4	Observation	Physiological maturity
24	Plant: diameter at the collar	-	-	measured	Flowering: 50% of plants
25	Plant: height	-	-	measured	Flowering: 50% of plants
26	Plant: number of branches	-	-	Counting	Flowering: 50% of plants
27	Plant: weight of fresh leaf biomass	-	-	measured	sixth week after transplanting
28	Fresh leaf yield	-	-	Average fresh leaf biomass weight per plant (PBF/P), then extrapolated to a hectare	sixth week after transplanting
29	Plant: Length of the leaves	-	-	measured	Flowering: 50% of plants
30	Plant: Width of the leaves	-	-	measured	Flowering: 50% of plants

Table 3: Primary qualitative characteristics of the different lines

Lines Characters	Vert_local	Rouge_local	L2	L23	AM-NKGN	NGURUMA	A2004	ACNL	AVAM_1939
Leaves									
Cotyledon pigmentation	Absent	Present	Absent	Absent	Present	Absent	Absent	Absent	Absent
Position of the widest part	Middle or Bottom	Middle or Bottom	Middle or Bottom	Middle or Bottom	Moderately down	Moderately down	Middle or Bottom	Middle or Bottom	Moderately down
Vertex Shape	Sharp	Sharp	Sharp	Sharp	Sharp	Medium	Sharp	Sharp	Sharp
Main color of the leaf blade	Light green	Purple	Light green	Light green	Medium Green	Dark green	Light green	Medium Green	Light green
Pigmentation of petioles	Absent	Present	Absent	Absent	Absent	Present	Absent	Absent	Absent
Stems									
Hypocotyls: color	Green	Purple	Green	Green	Purple	Light purple	Green	Green	Green
Anthocyanin coloration of hypocotyls	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Absent
Stripe Color	Green	Purple	Green	Green	Purple Green	Purple Green	Green	Green	Green
Base anthocyanin coloration	Absent	Present	Absent	Absent	Present	Present	Absent	Absent	Absent
Inflorescences									
Type	Erected or shallow	Erected or shallow	Erected or shallow	Erected or shallow	Erected or shallow	Erected or shallow	Erected or shallow	Erected or shallow	Erected or shallow

The leaves of the NGURUMA line are ovoid in shape, whereas those of the other lines are lanceolate. Additionally, the figure indicates low pubescence on the leaves of lines AVAM_1939, AM-NKGN, L23 and A2004. However, this pubescence is absent in the leaves of the other lines. This figure also shows that the lines are highly similar in terms of cross-sectional shape and color of the stems. Most lines have stems with a circular cross-section and green coloration, except for the Rouge local line, which has purple stems. The same applies to the inflorescences, which are green in all lines except for Rouge local, where they are purple.

Table 4: Flowering and maturity periods of the different line

Lines	Days until 1st Flowering	Days to 50 % Flowering	Days to Flower Maturity
AVAM_1939	60.66 a	98.00 a	167 a
Rouge_local	47.66 b	56.66 c	76 c
AM_NKGN	46.00 b	67.66 b	83 b
A2004	44.66 b	56.00 c	76 c
ACNL	44.33 b	54.33 c	76 c
L23	37.66 bc	41.00 e	76 c
NGURUMA	37.66 bc	52.66 c	76 c
L2	35.00 bc	46.33 d	76 c
Vert_local	31.00 c	39.00 e	63 d

Key: Means with the same letter within each column do not differ statistically (Tukey *P ≤ 0.05*)

Table 5: Analysis of variance for agro-morphological data

Parameter	Df	Sum Sq	Mean Sq	F value	*P* (>F)
Collar diameter (mm)	8	177.21	22.151	2.0529	0.09777
Leaf width (cm)	8	42.022	5.2528	7.4447	0.0002133 ***
Leaf length (cm)	8	106.37	13.296	1.2109	0.347
Number of branches	8	344.03	43.004	11.773	9.718e-06 ***
Weight of fresh leaf biomass per plant (g)	8	3394.0	424.26	1.9283	0.1179
Plants height (cm)	8	9091.7	1136.47	5.8523	0.0009149 ***
Fresh leaf yield (t/ha)	8	212.13	26.516	1.9283	0.1179

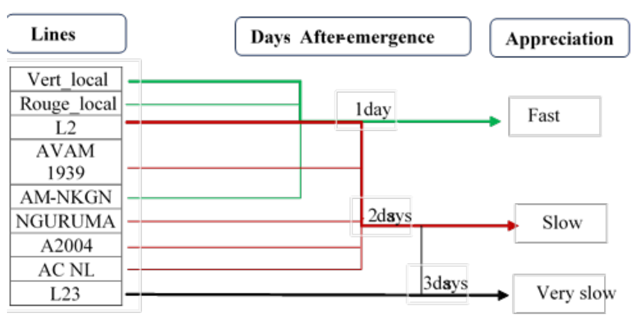


Fig. 3: Seed emergence time of different lines

Quantitative characters

Seed emergence time of different lineages: Fig. 3 illustrates the number of days before the emergence of the different amaranth lines after sowing. The analysis shows variations in the seed emergence ability among the different lines. Some lines, such as Vert_local and Rouge_local, are earlier with rapid emergence (about 1 day after sowing), whereast, the L23 line takes more time to emerge (up to 3 days), indicating slower germination according to the descriptor.

Flowering and maturity periods of the different lines: Table 4 shows the different flowering and maturity periods of the various lines studied. This reveals a significant diversity (*P ≤ 0.05*) in the flowering and maturity periods of the different lines. Thus, AVAM_1939 is the slowest in terms of flowering, with its first flowers appearing at 60.66 days after transplanting (DAT) and reaching full maturity at 167 DAT. In contrast, the Vert_local line is the earliest, flowering in just 31 DAT and maturing in 63 DAT. Lines such as Rouge_local, AM_NKGN, A2004 and NGURUMA exhibit intermediate flowering cycles, blooming around 40 to 50 DAT and achieving full maturity at approximately 76 DAT.

Analysis of variance for the measured agro-morphological parameters

The ANOVA (Table 5) reveals significant differences among the lines studied for several agro-morphological parameters. Thus, leaf width, number of branches, and plant height show highly significant differences, with p-values well below 0.05. In contrast, parameters such as collar diameter, leaf length, fresh biomass weight per plant and fresh leaf yield do not show statistically significant variations.

Agro-morphological characteristics of the studied lines

Table 6 presents the results of the Newman-Keuls test on the means of the different agro-morphological parameters measured for the studied lines. From this table, a significant difference is noted between the different lines regarding the evaluated agro-morphological parameters. For instance, the ACNL line exhibits the largest collar diameter (29.87 mm) and is statistically different from the other lines. However, the L23 line shows the smallest diameter compared to the lines Vert_local, Rouge_local, L2 and NGURUMA, which display similar diameters ranging between 23.02 mm and 26.64 mm.

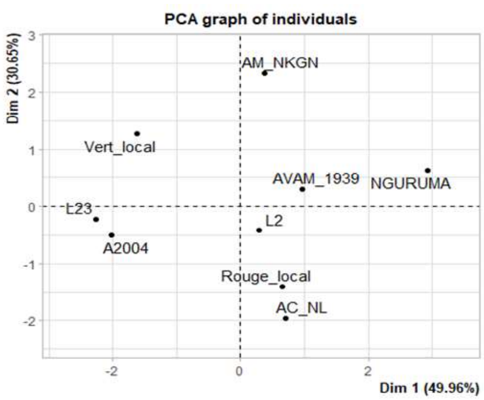
Regarding leaf width, NGURUMA and AM_NKGN have the widest leaves, measuring 10.45 cm and 9.20 cm, respectively, while the other lines exhibit leaf widths ranging from 6.39 cm to 7.73 cm, with no significant statistical difference. For the number of branches, the AVAM_1939 line stands out with 35.60 branches, the highest number among the lines, while Vert_local, NGURUMA and L23 have the lowest values, around 23 to 24 branches. The other lines fall between these extremes, with values ranging from 25.71 to 30.17 branches.

Regarding the fresh leaf biomass weight per plant, the values are similar across all lines, ranging from 45.21 g to 83.68 g, without any significant statistical difference. The same observation was made for the average fresh biomass yield, which statistically remained similar across all lines. However, arithmetic differences were noted, identifying the AM_NKGN line as the most productive in terms of fresh biomass (20.92 t/ha) compared to the Vert_local line, which gave the lowest fresh biomass yield (11.30 t/ha). However, for plant height, a significant disparity is observed among the lines. The NGURUMA line stands out with a height of 189.84 cm, making it to be significantly taller than the other lines, while AVAM_1939 has the shortest height at 124.95 cm.

Table 6: Newman-Keuls test on the means of the different agro-morphological parameters studied for the various lines

Lines	Collar diameter (mm)	Leaf width (cm)	Leaf length (cm)	Number of branches	Weight of fresh leaf biomass per plant (g)	Plants height (cm)	Fresh leaf yield (t/ha)
AVAM_1939	25.55 ab	6.39 b	22.53 a	35.60 a	63.88 a	124.95 c	15.97 a
Rouge_local	23.23 ab	7.18 b	22.66 a	25.71 bc	51.60 a	164.58 b	12.90 a
AM_NKGN	24.51 ab	9.20 a	25.11 a	27.56 bc	83.68 a	136.41 bc	20.92 a
A2004	26.64 ab	7.73 b	27.64 a	30.17 b	64.56 a	152.33 bc	16.14 a
ACNL	29.87 a	7.40 b	22.78 a	29.50 b	63.02 a	150.29 bc	15.75 a
L23	20.23 b	6.87 b	22.40 a	24.72 c	57.90 a	141.90 bc	14.47 a
NGURUMA	25.26 ab	10.45 a	25.70 a	23.92 c	73.21 a	189.84 a	18.30 a
L2	23.02 ab	7.04 b	22.73 a	26.70 bc	51.08 a	156.14 bc	12.77 a
Vert_local	23.09 ab	6.70 b	20.85 a	23.82 c	45.21 a	132.40 bc	11.30 a

Key: Means with the same letter within each column do not differ statistically (Tukey $P \leq 0.05$)



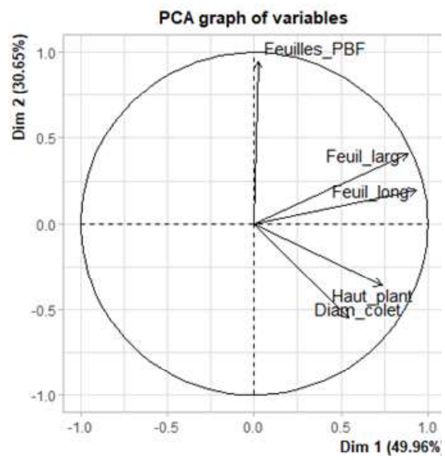


Fig. 4: Distribution of lines according to the Principal Component Analysis

Distribution of lines identified through Principal Component Analysis

The principal components analysis carried out on the characteristics of the different lines studied shows that the first two principal components accounted for 80.61% of all the information collected (Fig. 4). This part of the information is sufficient to guarantee the reliability of the results or the interpretation of the results as it is more than 50%. The projection of the different characteristics in axes 1 and 2 makes it possible to note that the NGURUMA line presented the largest plants with long and large leaves. Meanwhile, the highest fresh biomass weight per plant was recorded in the AM-NKGN line.

Hierarchical ascending classification of the different lines

The Hierarchical Ascending Classification (HAC) made it possible to group the lines having similar agro-morphological parameters. This classification presented on Fig. 5 reveals a grouping of the nine lines into three main categories. The first category consists of 7 lines with similar but less efficient agro-morphological parameters. In contrast, the second category consists of a single lineage (NGURUMA) which stands out from the other groups by its long and wide leaves. The last category was also a single line (AVAM_1939), which is distinguished by a high performance, in terms of the average number of branches.

Correlation between the different measured parameters

Fig. 6 shows the correlation between the different parameters of the characterized lines. This figure shows that there is a perfect positive correlation between leaf length, collar diameter, weight of fresh biomass, leaf width and branch number. On the other hand, a negative correlation is reported between the height of the plants, the date of start of flowering, the date to 50% flowering and the date of maturation.

Discussion

This study investigated the morphological diversity within nine new amaranth lines to document important agronomic traits and thus facilitate their inclusion in the Benin Catalog of Plant Species and Varieties (CaBEV). This characterization, which was based on twenty-eight morphological markers, revealed significant morphological variability within the studied amaranth lines.

However, the qualitative parameters considered in this study did not reveal a high phenotypic diversity within the

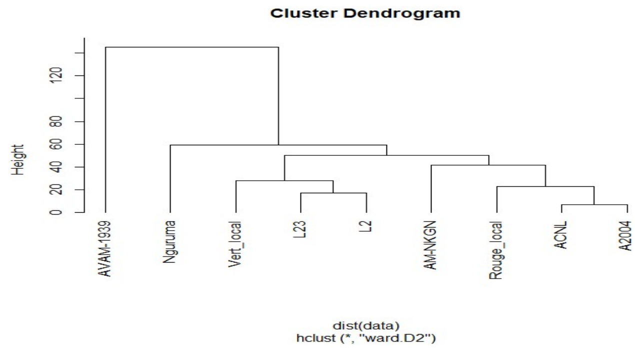


Fig. 5: The hierarchical ascending classification of the different lines

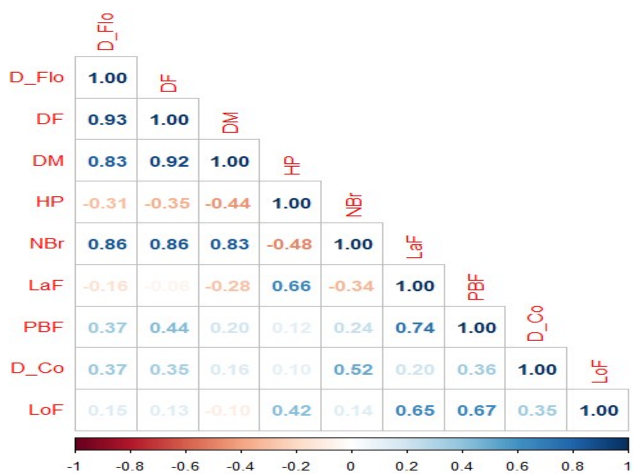


Fig. 6: Correlation between various parameters of the lines (D_flo = days to start of flowering, DF = days to 50% flowering, DM = days to maturity, HP = plant height, NBr = number of branches, LaF = Leaf width, PBF = Weight of fresh leaf biomass per plant, DCo = collar diameter, LoF = Leaf length)

characterized lines. Indeed, the qualitative traits related to leaves, stems, and inflorescences were almost similar for most of the lines. These observed morphological similarities could be due to very close phylogeny of some of the studied lines. In fact, the lines from the Faculty of Science and Technology of the University of Abomey-Calavi (L2 and L23) characterized in this study originated from the Vert_local line of the National Institute of Agricultural Research of Benin (INRAB). This line underwent induced mutation (irradiation by ultraviolet rays) to produce a population in which the L2 and L23 lines were identified for their salt resistance (Wouyou *et al*. 2017). Thus, the L2 and L23 lines come from the same parent, the Vert_local. This could explain the morphological similarity observed between the L2, L23 and Vert_local lines. Moreover, the low polymorphic nature of the morphological markers used could also justify the absence of significant differences between the lines regarding the qualitative parameters. This agrees with a similar study by Thapa and Blair (2018), who had difficulty distinguishing South American species (*A. caudatus, A. quitensis*) from Central American species (*A. cruentus, A. hypochondriacus*) using morphological markers, but other authors such as Bojórquez-Velázquez *et al*. (2021); Rathod *et al.* (2021); Jamalluddin *et al.* (2022) and Vats *et al*. (2023) were able to do so using molecular methods. Furthermore, next-generation Genotyping by Sequencing (GBS) by Wu and Blair (2019) and Jamalluddin *et al*. (2022) differentiated cultivars from wild accessions and various species in an amaranth collection. Essentially, the use of molecular markers can complement the botanical or morphological descriptors of amaranth species to make their diversity studies more efficient.

On the other hand, the few phenotypic differences observed within the different lines regarding the qualitative characteristics could be explained by the specificity of the genetic heritage of each species. Gueco *et al*. (2016) reported the diversity of 18 collections of amaranth germplasm in the Philippines and found significant genetic variability among them for qualitative traits. Similarly, Gerrano *et al*. (2017) reported significant differences between the morphological traits of 32 different amaranth accessions through their frequency of distribution and the extent of variation in the gene pool.

Regarding the quantitative parameters considered in this study, the lines showed significant statistical variabilities. For example, the leaf length and width of plants from the different lines varied from 20 to 25 cm and from 8 to10 cm, respectively. These values are slightly higher than those found by Okeke *et al*. (2020) concerning the length and width of leaves of *A. spinosus*. These reported differences could be due to the fact that the lines belong to different species categories. Moreover, these arithmetic differences observed in the means of the different quantitative parameters of the different lines which agrees with previous studies by Okeke *et al*. (2020) and Gueco *et al*. (2016), could be justified not only by the divergence of the cultural techniques applied in each study, but also by the divergence of the ecosystems or environment of the different experiments. However, the plant height, collar diameter, and number of days before flowering found for the lines characterized in this study corroborate the results obtained by Gueco *et al.* (2016) in their study of amaranth species diversity in the Philippines. The seed characterization at the individual lineage level was used to assess the speed or energy of seed germination. Thus, it varied from early to slow for the Vert_local and L23 lines, respectively. This physiological difference in the seeds is probably due to the variability in their size. This agrees with the results obtained by Xavier *et al*. (2019), who studied the germination rate of seeds from different amaranth species in a characterization test and found that commercial seeds ranging in size from 600 to 850 μm had the highest germination rate.

Conclusion

The present study was of crucial importance because it made it possible to make a comparative agro-morphological characterization of nine lines of amaranth, which are candidates for inclusion in the Benin catalog of plant species and varieties. Thus, the different lines characterized had interesting agro-morphological traits that could contribute to improving the productivity of the amaranth-based farming systems in Benin. Analysis of quantitative data revealed statistically significant differences between the study lines. The data available from this study can already be used to register these nine lines in CaBEV and later in the West African Catalog of Plant Species and Varieties (COAfEV). However, the lines showed a low morphological diversity in terms of qualitative traits. As a result, the leaf, stem, and inflorescence quality characteristics were almost identical for most of the lines studied and may be explained by the lack of polymorphic morphological markers in diversity studies. We therefore suggest that the study be supplemented by the use of molecular markers in order to strengthen the results of the characterization work on these various lines of amaranth that are candidates for inclusion in the Benin Catalog of Plant Species and Varieties.

Acknowledgments

We appreciate the National Institute of Agricultural Research of Benin (INRAB) for funding this work.

Author Contributions

SM: Conceptualization; methodology; investigation; data curation; Writing – Original Draft; Writing – Review & Editing. AM: Conceptualization; validation; methodology; resources; writing- reviewing and editing; supervision. DM: Conceptualization; validation; methodology; writing-reviewing and editing; supervision. EA: Conceptualization; validation; methodology; writing- reviewing and editing. OK, NH, CG and CA: Validation; writing, reviewing and editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All the data used to support the findings of the study are available within the research article.

Ethics Approval

All the authors have read, validate and approved the research draft for publication.

References

Aglinglo AL, M Ayenan, J Azagba, D Sognizoun, DA Tchokponhoue (2022). *Feuille de route pour le développement du sous-secteur des semences maraîchères au Bénin. Document de travail*, p:55. World Vegetable Center, Institut National des Recherche Agricole du Benin, Direction de la Production Végétale, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi. Abomey-Calavi, Benin

Azandémè-Hounmalon GY, J Logbo, A Gbèblonoudo Dassou, L Lokossi, E Akpla, KKM Fiaboe, M Tamò (2023). Investigation of amaranth production constraints and pest infestation reduction by basil intercropping. *J Agric Food Res* 12:100627

Bojórquez-Velázquez E, FA Talamantes-Herrera, S Valles, IT Cerritos-Castro, APBDL Rosa (2021). Molecular characterisation of Seed Storage Proteins (SSPs) in grain amaranth. In: *The Amaranth Genome. Compendium of Plant Genomes*, pp:55–79. Adhikary D, MK Deyholos, JP Délano-Frier, (Eds.). Springer, Cham, Switzerland

DGCS-ODD (2019) *Monographie des communes des départements de l'Atlantique et du Littoral: Spatialisation des cibles prioritaires des ODD au Bénin*, p:135, Benin

FAO (2022). Voices of the Hungry. Rome. Available at: www.fao.org/in-action/voices-of-the. (Accessed: November 27, 2022)

Geisen S, K Krishnaswamy, R Myers (2021). Physical and structural characterization of underutilized climate-resilient seed grains: Millets, sorghum and amaranth. *Front Sustain Food Syst* 5:599656

Gerrano AS, WSJV Rensburg, S Mavengahama, M Bairu, S Venter, PO Adebola (2017). Qualitative morphological diversity of *Amaranthus* species. *J Trop Agric* 55:12–20

Grundy MML, KD Momanyi, C Holland, F Kawaka, S Tan, M Salim, BJ Boyd, B Bajka, AI Mulet-Cabero, J Bishop, WO Owino (2020). Effects of grain source and processing methods on the nutritional profile and digestibility of grain amaranth. *Functional Foods* 72:10406

Gueco LS, T Borromeo, CD Guzman (2016). Diversity in the morphology of Amaranth (*Amaranthus* sp.) germplasm collection in the Phillipines. *Asian J Agric Food Sci* 4:73–79

Hoidal N, S Jacobsen, A Odone, G Alandia (2020). Defoliation timing for optimal leaf nutrition in dual-use amaranth production systems. *J Sci Food Agric* 13:4745–4755

Jamalluddin N, FJ Massawe, S Mayes, WK Ho, RC Symonds (2022). Genetic diversity analysis and marker-trait associations in *Amaranthus* species. *PLoS One* 5:e0267752

Jamalluddin N, FJ Massawe, S Mayes, WK Ho, A Singh, RC Symonds (2021). Physiological screening for drought tolerance traits in vegetable amaranth (*Amaranthus tricolor*) germplasm. *Agriculture* 10:994

MAEP (2017). *Manuel des procédures d'homologation et d'inscription des variétés au Catalogue Béninois des Espèces et Variétés végétales, INRAB/DPV/PPAAO/ProCAD/MAEP & CORAF/WAAPP*, p:260. Dépôt légal N° 9431 du 12 juin 2017, Bibliothèque Nationale du Bénin, 2ème trimestre

MAEP (2016). *Catalogue Béninois des Espèces et Variétés végétales (CaBEV), INRAB/DPVPPAAO/ProCAD/MAEP & CORAF/WAAPP*, p:339. Dépôt légal N° 8982 du 21 octobre 2016, Bibliothèque Nationale (BN) du Bénin, 4ème trimestre

Mensah ACG, KF Assogba, DAJ Azagba, ORO Ogoutolou, LG Amadji (2019a). Effet du fractionnement d'engrais organique, d'Urée et du Sulfate de Potassium sur la productivité et la conservation des fruits de tomate au Sud du Bénin. *J Appl Biosci* 138:14050

Mensah ACG, R Sikirou, F Assogba Komlan, BB Yarou, GSK Midingoyi, J Honfoga, ME Dossoumou, GN Kpéra, AK Djinadou (2019b). *Guide pratique pour la culture de l'amarante (Amaranthus cruentus) au Bénin. Référentiel Technico-Economique (RTE). MAEP/INRAB/FIDA/ProCar/PADMAR/World Vegetable Center/Bénin*, p:52. Dépôt légal N° 11557, du 26/08/2019, Bibliothèque Nationale (BN) du Bénin, 3ème trimestre

Okeke NF, CV Ilodibia, BE Okoli (2022). A Comparative Morphological Study on *Amaranthus spinosus* L., *Celosia argentea* L. and *Gom phrena celosioides* Mart (Amaranthaceae). *Univ J Plant Sci* 8:1–10

Oteri M, F Gresta, A Costale, VL Presti, G Meineri, B Chiofalo (2021). *Amaranthus hypochondriacus* L. as a sustainable source of nutrients and bioactive compounds for animal feeding. *Antioxidants* 10:876

Rathod KJ, BA Jadeja, RN Nakar (2021). Morphological, biochemical and molecular characterization of *Amaranthus* varieties in India. In: *Proc Natl Acad Sci, India Sec B: Biol Sci* 91:681–694

Singhania N, R Kumar, SP Bishnoi, AB Ray, A Diwan (2023). Bioactive properties and health benefits of *Amaranthus*. In: *Harvesting Food from Weeds*, pp:351-383. Gupta P, N Chhikara, A Panghal (Eds.). Wiley, New York, USA

Sogbohossou OED, EG Achigan-Dako, FA Komlan, A Adam (2015). Diversité et utilisation différentielle des espèces d' *Amaranthus* le long du continuum urbain-rural du sud du Bénin. *Econ Bot* 69:9–25

Stetter MG, M Vidal-Villarejo, KL Schmid (2020). Parallel seed color adaptation during multiple domestication attempts of an ancient new world grain. *Mol Biol Evol* 37:1407–1419

Thapa R, MW Blair (2018). Morphological assessment of cultivated and wild amaranth species diversity. *Agronomy* 11:272

UPOV (1999). *Guidelines for the conduct of tests for distinctness, uniformity and stability for amaranth*, p:35. Geneva, Switzerland

Vats G, D Das, R Gupta, A Singh, A Maurya, S Rajkumar, AK Singh, R Bharadwaj, S Kumar, SK Kaushik, V Gupta, K Singh, R Singh (2023). Validation of genome-wide ssr markers developed for genetic diversity and population structure study in grain Amaranth (*Amaranthus hypochondriacus*). *Agriculture* 13:431

Wouyou A, CB Gandonou, F Assogba-Komlan, D Montcho, AS Zanklan, S Lutts, SL Gnancadja (2017). Salinity resistance of five amaranths (*Amaranthus cruentus*) cultivars at young plant stage. *Intl J Plant Soil Sci* 14:1–11

Wu X, MW Blair (2019). Genotyping by Sequencing (GBS) Polymorphism Diversity in grain amaranths and relatives. *Front Plant Sci* 8:1960

Xavier JB, BDD Andrade, DCD Souza, GC Guimarães, LV Resende, RM Guimarães (2019). Morphological, chemical and physiological characterization of *Amaranthus* spp. seeds. *J Seed Sci* 41:478–487

Zhu F (2020). Dietary fiber polysaccharides of amaranth, buckwheat and quinoa grains: A review of chemical structure, biological functions and food uses. *Polymer Carbohydr* 248:116–819