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Morphological characterization of *Amaranthus cruentus* L. mutant lines derived from local and preferred *Amaranthus* cultivar

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ABSTRACT

     Amaranthus species are of underutilized orphan crops grown in tropical and sub-tropical regions of the world. Benin's most appreciated *Amaranthus cruentus* L. «local» cultivar is susceptible to saline stress. This study aimed to create genotypes agronomically, at least, as good as the «local» cultivar and useful for further saline tolerance breeding using mutation breeding. The morphological diversity among 19 gamma-irradiated *A. cruentus* mutant lines and «local» cultivar (control) were investigated, through a randomized complete block design with three replications, using 18 phenotypical traits (12 quantitative and 6 qualitative). The results show that among the six qualitative traits, only terminal inflorescence shape was discriminant. However, permutation analysis of variance revealed significant variability in quantitative traits: leaf width (CV=19%), Branches length (CV=47%), plant height (CV=25%), stem diameter (CV=29%), number of branches (21%) number of leaves (CV=25%). Multivariate analysis of quantitative traits showed the first two principal components contributing to 78.30% of observed variability. Correlation analysis revealed the significance of number of leaves, number of branches, plant height and leaf width for selecting better genotypes for biomass production. Mutant lines L1, L2, L6, L16, L18 and L23 showed high performances for traits cited above and could, therefore, be a good source of genes.
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Keywords: food production, mutation breeding, phenotypic variation, orphan crops, Republic of Benin

INTRODUCTION

Amaranthus is a genus from Amaranthaceae family, probably originated from America, comprising approximately 70 species cultivated as leafy vegetables, grains and ornamental crops in different parts of the world (Brenner et al., 2000; Janovská et al., 2012; Gerrano et al., 2017). Highly nutritious (17–32.6% of proteins on dry weight basis and well-balanced amino acid profiles), both the grain amaranth and leaves have been reported to be utilized (Kareem et al., 2011) for human as well as for animal food (Slabbert et al., 2004; Mlakar et al., 2009; Gamel et al., 2006; Venskutonis and Kraujalis, 2013; Das, 2016; Thapa and Blair, 2018). Like a large number of traditional leafy vegetables in West Africa (Adjatin et al., 2013), amaranth has been recognized as a cheap source of proteins and therefore could help vulnerable people to diversify their diets particularly in sub-Saharan Africa (Olaposi and Adunni, 2010; Adjatin et al., 2013; Gueco et al., 2016; Akin-Idowu et al., 2016).

In amaranth production system, salinity has been reported as serious problem. This is the case in Benin where Wouyou et al. (2016, 2017a) reported that salinity reduces seeds germination as well as plant root and above ground growth (biomass) of different *A. cruentus* cultivars. The most appreciated «local» cultivar showing high biomass: number of leaves, number of branches and leaf width was found to be particularly susceptible to that stress (Wouyou et al., 2016). Apart from the implantation of halophile species capable of the salt's excess extraction (Amar et al., 2022) which implies a real free space challenge, the solution could be to diminish the salt rate in the soil, through amendment, and in irrigation water or to wash salt through a good drainage, but those practices are reported to be very costly and considered by Dasgan et al. (2002) as temporary solutions. Creating saline tolerant variety, but first, with the same agronomic traits of interest (high biomass) in this context turns out to be the more economic way to reduce the harmful salinity effect on cultures (Poustini and Siosemardeh, 2004).

Genetic variability exploration could help find new genotypes agronomically (biomass), at least, as good as the local cultivar and more tolerant to saline stress. Schreiber et al. (2018) reported that genetic variability in plants is caused by various factors such as: mutation (which can create entirely new alleles in a population), random mating and recombination between homologous chromosomes during meiosis. Mutation breeding in crop plants has become an effective tool in hands of plant breeders and above all in crops having narrow genetic base (Hamzekhanlu et al., 2011). It is, nowadays, reported as an efficient means completing existing germplasm for cultivar improvement. Many mutants have been identified as donors of desirable traits in breeding program such as Amaranth mutant lines "C15/3", "C27/5" and "C82/1" for overall nutrition values (Hamzekhanlu et al., 2011; Acharya et al., 2006; Žiarovská et al., 2013 ; Kečkešová et al., 2012).

In amaranth, mutation breeding has been effectively used for cultivar improvement and generating polygenic variability (Joshi et al., 2018). So far, Joshi et al. (2018) reported the development of four amaranth cultivars for different purposes: 'Centenario' (improved grain yield) in Peru, 'New Asutake' (early maturity) in Japan, 'Sterk' (tolerance to moisture and heat stress) in Russia and 'Pribina' (genetically fixed increased weight of 1000 seeds) in Slovakia (Gomez-Pando et al., 2009; Joshi et al., 2018). More often, these genetic changes are induced by mutagenic agents such as x-rays, gamma rays, beta rays, neutrons, chemicals, high temperature and low pH (Nwankwo et al., 2019) and changes observed in the crop can be different. X-ray particles were reported by Nwankwo et al. (2019) to induce significant differences among inflorescence weight in different *A. hybridus* accessions. Furthermore, Gamma irradiation significantly enhanced 1000 seed weight in *A. cruentus*, and promising mutant lines named C26 and C82 with higher 1000 seed weight have been developed (Hricová et al., 2016). Likewise, Joshi et al. (2018) reported that putative mutant lines of *A. hypochondriacus*

and *A. cruentus* containing 2% more protein than non-treated lines were developed through gamma irradiation (Keckesova et al., 2012).

Induction of genetic diversity and its use in amaranth breeding programs turns out to be essential for creating performant mutant lines to meet agronomic and nutritional goals. Inducting mutation could then help to create and identify amaranth genotypes agronomically interesting (high biomass) that can thrive despite biotic or non biotic constraints. Checking the morphologic diversity obtained inducing mutation in *A cruentus* «local» cultivar, this research aimed at characterizing nineteen gamma-irradiated *A. cruentus* mutant lines using phenotypic traits.

MATERIALS AND METHODS
Plant material
Mutant lines and stabilization

Seeds of «local» and preferred *Amaranthus* cultivar subjected to 200 grays of a mutagenic agent Gamma at the International Agency of Atomic Energy (AIEA) at Vienna were used to generate mutant lines. Based on traits of interest (high biomass), 19 mutant lines (L1, L2, L3, L6, L8, L9, L10, L11, L12, L13, L14, L15, L16, L17, L18, L19, L20, L21 and L23) were identified and 01 non-gamma-irradiated local cultivar (Lo) used as a control. The obtained mutant lines were self-pollinated until the sixth generation lines (M_6) using « single-seed descent » technique (Janwan et al., 2013) at the experimental site of the International Institute of Tropical Agriculture (IITA / Benin, (latitude: N 6° 25' 260'' and longitude: E 2° 19' 682''; altitude: 15 meter above sea level) in Abomey-Calavi (Benin Republic) from November 2018 to March 2020. The site is located in southern Benin characterized by a sub-equatorial climate with an annual mean temperatures ranging from 26 to 28°C and an annual rainfall varying between 800 to 1400 mm (Yabi and Afouda, 2012). At each self-pollinated generation, seedlings from individual selected plant were grown in 3 rows on a 3 m long and 1.5 m wide plot. Five plots were used per line. Plants were spaced 50 cm apart within rows and 50 cm between rows

with a total of 18 plants per plot. Among those 18 plants grown per plot, the 5 well developed and phenotypically close based on plant height, number of branches and leaves production were identified and their flowers were covered from appearance, against stranger pollen with envelope made of tracing paper. At seeds maturity stage, seeds were harvested per plant per line and dried. Seedlings from one of the five selected plants per plot per line were transplanted onto one plot at the next generation.

Experimental design

Seeds of sixth generation from each 19 mutant lines plus the «local» cultivar (used as control) were manually sown in April 2020 in separated pots filled of sandy loam in nursery. At the 4–5 leaf stage, seedlings were transplanted to the experimental field (latitude: N 6° 29' 3'' and longitude: E 2° 16' 38'' and located at Abomey-Calavi (Benin Republic)) in given plots. Plots were arranged in a randomized complete block design with three repetitions. An experimental unit (plot) consisted of 10 individuals of a mutant line or «local» cultivar planted in two rows on 2 m long and 1 m-wide raised beds (Akin-Idowu et al., 2016). Plants were spaced 50 cm apart within rows and 1 m between rows, with an inter-plot spacing of 1 m as used by Akin-Idowu et al. (2016). The experimental plots were kept weeds-free for the duration of the study and poultry dropping was applied around each seedling one week after transplantation at the dose of 1 t/ha according to Souleymane et al. (2018). Chemicals pesticides such as Acarius (Abamectine 18 g/L) (used at a dose of 300 l/ha) and Mancozeb in wettable powder form (at the dose of 3 kg/ha) were used to control pests and diseases.

Data collection

Twelve (12) quantitative and six (6) qualitative phenotypical traits were assessed on five randomly selected plants within each plot as used by Akin-Idowu et al. (2016). Data on quantitative traits included plant height, number of leaves, number of branches per

plant, branch length, leaf length, leaf width, petiole length, stem diameter, leaf area, inflorescence length (flower length), days from emergence to 50% flowering, and 1000-seed weight. Traits such as plant height, number of leaves, number of branches per plant, branch length, leaf length, leaf width, petiole length, stem diameter and leaf area were recorded when the plants initiated the flowering (Thapa and Blair, 2018). The 8th leaf from the ground was xeroxed on rectangular sheet. The leaf copy was stamped out and weighted. The leaf's copy weight, the weight and the area of the rectangular sheet were proportionally used to estimate the leaf area per plant. *Amaranthus* descriptors, as used by Andini et al. (2013) and Akin-Idowu et al. (2016), were considered to access the morphological qualitative traits (leaf pigmentation, stem pigmentation, petiole pigmentation, inflorescence color, seed color and terminal inflorescence shape). Traits such as leaf pigmentation, stem pigmentation and petiole pigmentation were recorded as the plants grew according to Thapa and Blair (2018). Inflorescence length (flower length), inflorescence color and terminal inflorescence shape were recorded when the plants reached maximum height. Criterions used to ensure that the plants reach maximum height was total blossoming and seed production. Description of all traits and the different codes used are given in Table 1.

Statistical analysis

Quantitative data were analyzed using descriptive statistic (mean, minimum, maximum and coefficient of variation). Permutation analysis of variance (Gleason, 2013) under the package RVAide Memoire of R 3.6.0 (R Core Team, 2019) was performed for assessing variation within amaranth mutant lines and the local cultivar. K-means non-hierarchical cluster analysis was performed using R Procedure CLUSTER based on normalized Euclidean distance matrices and dendrogram was constructed by Procedure TREE. The linear relationships among the 12 different quantitative traits were studied using the Pearson-correlation coefficient. Principal component analysis (PCA) was executed from correlation matrices using R Procedure pca in FactoMiner package in order to assess the patterns of phenotypic traits variation considering all 12 qualitative variables simultaneously.

Table 1: List of quantitative and qualitative traits evaluated, their description and abbreviations used, adapted from Andini et al. (2013), (Mbwanbo, 2013) and Akin-Idowu et al. (2016).

Morphological traits	Code	Description
Quantitative variables		
Number of leaves	NL	Number of leaves on the main stem
Number of branches	NB	Number of branches on the main stem
Leaf length	LL	Length of the 8th leaf from the ground (Mbwanbo, 2013)
Leaf width	LW	Width of the 8th leaf from the ground
Branch length	BL	Length of the 8th branch from the ground

Petiole length	PL	Length of the petiole of the 8th leaf from the ground
Plant height	PH	Plant height at flowering
Stem diameter	SD	
Leaf area	LA	Area of the 8th leaf from the ground
Flower length	FlL	Length of inflorescence was measured from the down most branch to the top of inflorescence of the main stem in cm
Days to 50% flowering	D-50-F	days from emergence to 50% flowering
1000-seed weight	1000-SW	Weight of thousand seeds in g

Qualitative variables

Leaf pigmentation	LP	Entire lamina purple/pink (1), Basal area pigmented (2) Central spot (3), Two stripes (V shaped) (4), One stripe (V shaped) (5), Margin and vein pigmented (6), One pale green / chlorotic spot on normal green (7), Normal green (8), and Dark green (9)
Stem pigmentation	SP	Green (1), Pink or purple (2)
Petiole pigmentation	PP	Green (1), Dark green (2), Purple (3), and Dark purple (4)
Inflorescence color	IC	Yellow (1), Green (2), Pink (3), and Red (4)
Seed color	SC	Dark (1), Cream (2), Cream + black (3), Gold (4), and Pink (5)
Terminal inflorescence shape	TIS	Spike panicle (dense) (1), Short (2)

Source: Andini et al. (2013), (Mbwanbo, 2013) and Akin-Idowu et al. (2016).

RESULTS

Qualitative variability

No difference was observed across the 19 mutant lines for leaf pigmentation, stem pigmentation, petiole pigmentation, inflorescence color and seeds color among the mutant lines (Figure 1). Leaves, stem, petiole and inflorescence were all green. Like their relative local cultivar, the seeds of each mutant line were constituted of cream and black grains. However, some difference was observed for terminal inflorescence shape. While the inflorescences of three mutant lines (L8, L12 and L19) were made of panicle with short branches, the remaining (mutant lines) and the relative local cultivar's were spike (dense).

Quantitative variability

High coefficient of variation was observed for leaf area (29%), leaf width (19%), branch length (47%), plant height (25), stem diameter (29%), number of branches (21%) and number of leaves (25%). Day to 50% flowering and thousand seed weight had low values of coefficient of variation (Table 3).

Pearson correlation coefficient

Of the 78 correlation coefficients derived, 47 were positive and highly significant at $p < 0.001$ (Table 4). The highest correlation was between number of branches and number of leaves ($R = 0.96$). The number of leaves was highly correlated with number of branches, plant height, stem diameter, leaf area and days to 50% flowering. The number of branches correlated significantly ($p < 0.001$) with plant height, stem diameter, leaf area, flower length and days to 50% flowering. Leaf length exhibited highly significant ($p < 0.001$) and positive association with branch length, petiole length, stem diameter and leaf area. Petiole length correlated significantly ($p < 0.001$) with plant height, leaf area and days to 50% flowering. Plant height correlated significantly ($p < 0.001$) with stem diameter, flower length and days to 50% flowering. Leaf area exhibited highly significant ($p < 0.001$) and positive association with days to 50% flowering. 1000-seed weight correlated negatively with most of the morphological traits ($R = 0.71$). Number of leaves correlated significantly ($p < 0.01$) Leaf length, leaf width, petiole length and flower length. Number of branches correlated significantly ($p < 0.01$) with leaf length, leaf width and petiole length. Leaf length exhibited significant ($p < 0.01$) correlation with leaf width, plant height and days to 50% flowering. Leaf width correlated significantly ($p < 0.01$) with branch length, plant height, stem diameter, leaf area and days to 50% flowering. Branch length correlated significantly ($p < 0.01$) with petiole length, plant height, stem diameter, leaf area and days to 50% flowering. Petiole length correlated significantly ($p < 0.01$) with stem diameter ($R=0.66$). Plant height correlated significantly ($p < 0.01$) with flower length ($R = 0.59$). Flower length is positively correlated with days to 50% flowering ($R=0.69$).

### *Principal component analysis*

A principal component analysis (PCA) was performed based on the 12 quantitative traits. The first two principal components (PCs) contributed to 78.30% of the variability among the 19 mutant lines and their relative local cultivar for the 12 quantitative traits studied (Figure 2). PC_1, accounting for 68.8% of the variation had number of leaves, number of branches per plant, branches length, leaf length, leaf width, petiole length, plant height, stem diameter, leaf area, inflorescence length and days to 50% flowering as the variables with the largest positive coefficient, while 1000-seed weight had negative coefficient (Figure 3). PC_2, accounting for an additional 9.5% of the total variation had high coefficient of variation observed for 1000-seed weight.

Cluster analysis based on morphological quantitative characters

Based on the cluster analysis carried out using the k-means non-hierarchical clustering three groups were observed (Figure 4). Cluster 1 included the local cultivar and fifteen lines (L1, L11, L15, L3, L16, L23, L6, L17, L18, L21, L9, L14, L10, L20, and L13) having average values for all the traits : number of leaves (39), number of branches (33), leaf length (32.89 cm), leaf width (8.4 cm), branch

length (18.84 cm), petiole length (13.74 cm), plant height (102.84 cm), stem diameter (4.23 mm), leaf area (117.47 cm²), flower length (40.84 cm), Day to 50 % flowering (66 days) and grains weight (0.38 g) (Table 4). Assembled apart in the cluster I, the mutant lines L1, L11, L15, L3, L16, L23, L6, L17 and L18 showed higher performances for ten (10) quantitative morphological traits (number of leaves: 42, number of branches: 35, leaf length: 34.63 cm, leaf width: 8.78 cm, branch length: 21.13 cm, petiole length: 14.38 cm, plant height: 110.71cm, stem diameter: 4.12 mm, leaf area: 129.82 cm², and flower length: 41.62 cm) than the mutant lines L21, L9, L14, L10, L20, L13 and the local cultivar (number of leaves: 35, number of branches: 29, leaf length: 30.67 cm, leaf width: 7.92 cm, branch length: 15.90 cm, petiole length: 12.92 cm, plant height: 92.72 cm, stem diameter: 3.52 mm, leaf area: 101.59 cm², and flower length: 39.84) grouped in the same cluster. The cluster II composed of one mutant line L2 with the highest values for almost all the traits. Remarquably, the mean value for days to 50% flowering was highest (79 days), indicating late maturing. Cluster III comprised of three mutant lines (L8, L12 and L19) having the lowest mean values for all traits compared to the clusters I and II.
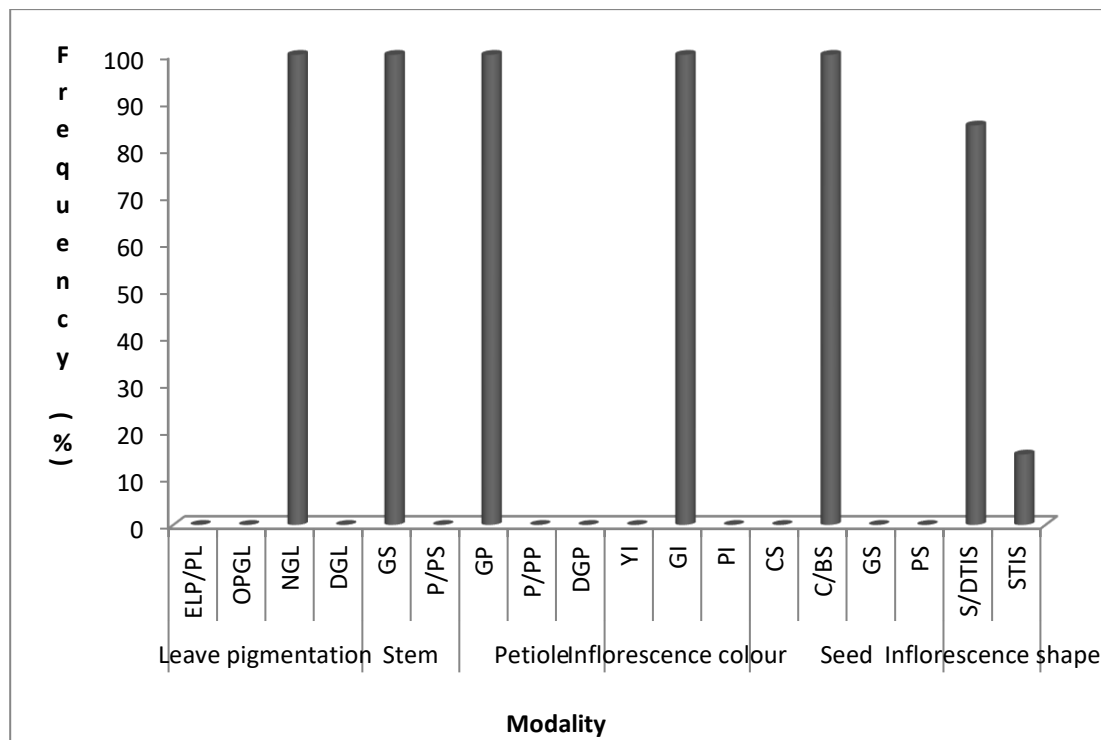
Note: ELP/PL = Entire lamina purple/pink leaves, OPGL = One pale green leaves, NGL = Normal green leaves, DGL = Dark green leaves, GS = Green stem, P/PS = Pink or purple stem, GP = Green petiole, DGP =Dark green petiole, PP = Purple petiole, DPP = Dark purple petiole, YI = Yellow inflorescence, GI = Green inflorescence, PI = Pink inflorescence, CS = Cream seed, C/BS = Cream + black seed, GS = Gold seed, PS = Pink seed, S/DTIS = Spike/Dense panicle (inflorescence shape), STIS = Short panicle (inflorescence shape).

Figure 1: Frequency distribution (%) of qualitative morphological traits in 19 *A. cruentus* mutant lines and «local» cultivar.

Table 2: Descriptive statistics of 12 quantitative traits of 19 amaranth lines and their relative «local» amaranth cultivar.

N°	Traits	Mean	Min	Max	Coefficient of Variation (CV %)
1	NL	36.79	15	54	25
2	NB	30.92	15	45	21
3	LL (cm)	32.69	20.5	46.2	15
4	LW (cm)	8.46	6.2	15.2	19
5	BL (cm)	20.09	10	57	47
6	PL (cm)	13.51	7	18	15
7	PH (cm)	97.65	13.5	150	25
8	SD (mm)	3.69	1.7	6.5	29
9	LA (cm^2)	113.22	11.62	219.12	29
10	FlL (cm)	40	23	63	18
11	Day-50-F	64.90	53	79	09
12	1000-SW(g)	0.39	0.31	0.42	07

Notes: NL = Number of leaves; NB = Number of branches; LL = Leaf length; LW = Leaf width; BL = Branches length; PL = Petiole length; PH = Plant height; SD = Stem diameter; LA = Leaf area; FlL= Flower length; Day-50-F = Days to 50% flowering; 1000-SW = 1000-seed weight
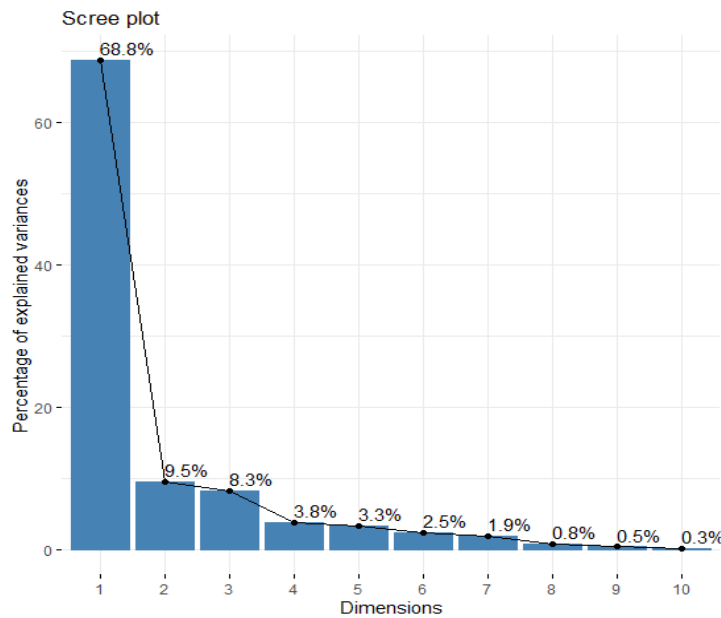

Figure 2: Proportion of variability among *A. cruentus* lines explained by principal components.

Table 3: Pearson-correlation matrix of 12 morphological quantitative traits of 19 Amaranth lines and their relative «local» amaranth cultivar.

Traits	NL	NB	LL	LW	BL	PL	PH	SD	LA	FlL	Day-50-F	1000-SW
NL	1.00	-	-	-	-	-	-	-	-	-	-	-
NB	0.96***	1.00	-	-	-	-	-	-	-	-	-	-
LL	0.61**	0.60**	1.00	-	-	-	-	-	-	-	-	-
LW	0.67**	0.65**	0.70**	1.00	-	-	-	-	-	-	-	-
BL	0.53*	0.54*	0.71***	0.71**	1.00	-	-	-	-	-	-	-
PL	0.68**	0.66**	0.78***	0.54*	0.64**	1.00	-	-	-	-	-	-
PH	0.92***	0.92***	0.65**	0.62**	0.64**	0.74***	1.00	-	-	-	-	-
SD	0.81***	0.79***	0.80***	0.70**	0.66**	0.66**	0.83***	1.00	-	-	-	-
LA	0.80***	0.78***	0.74***	0.59**	0.59**	0.73***	0.81***	0.75***	1.00	-	-	-
FlL	0.70**	0.76***	0.52*	0.54*	0.45*	0.48*	0.59**	0.72***	0.50*	1.00	-	-
D-50-F	0.80***	0.75***	0.65**	0.63**	0.57**	0.71***	0.77***	0.81***	0.71***	0.69**	1.00	-
1000-SW	-0.17	-0.09	-0.09	-0.37	-0.04	-0.13	-0.11	-0.03	-0.18	0.14	0.11	1.00

NL = Number of leaves; NB = Number of branches; LL = Leaf length; LW = Leaf width; BL = Branches length; PL = Petiole length; PH = Plant height; SD = Stem diameter; LA = Leaf area; FlL= Flower length; Day-50-F = Days to 50% flowering; 1000-SW = 1000-seed weight.
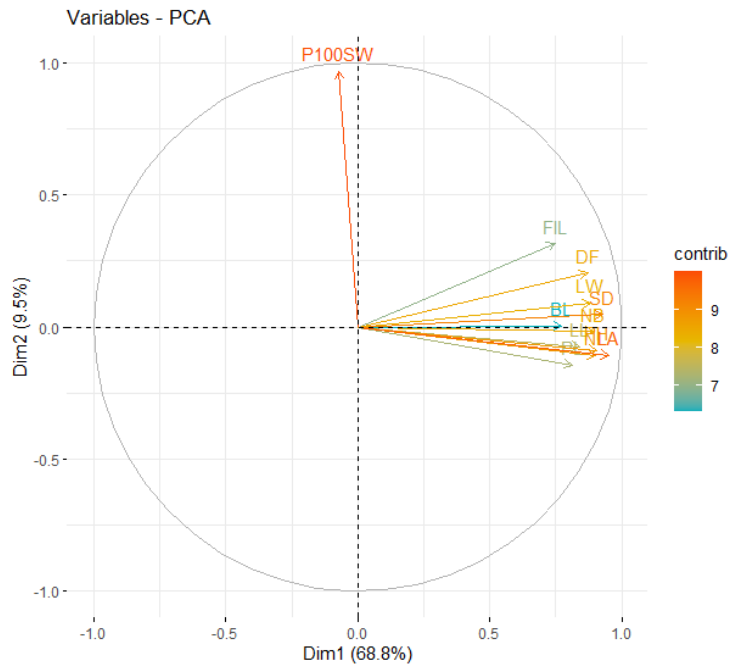
Notes: NL = Number of leaves; NB = Number of branches; LL = Leaf length; LW = Leaf width; BL = Branches length; PL = Petiole length; PH = Plant height; SD = Stem diameter; LA = Leaf area; FlL= Flower length; Day-50-F = Days to 50% flowering; 1000-SW = 1000-seed weight.

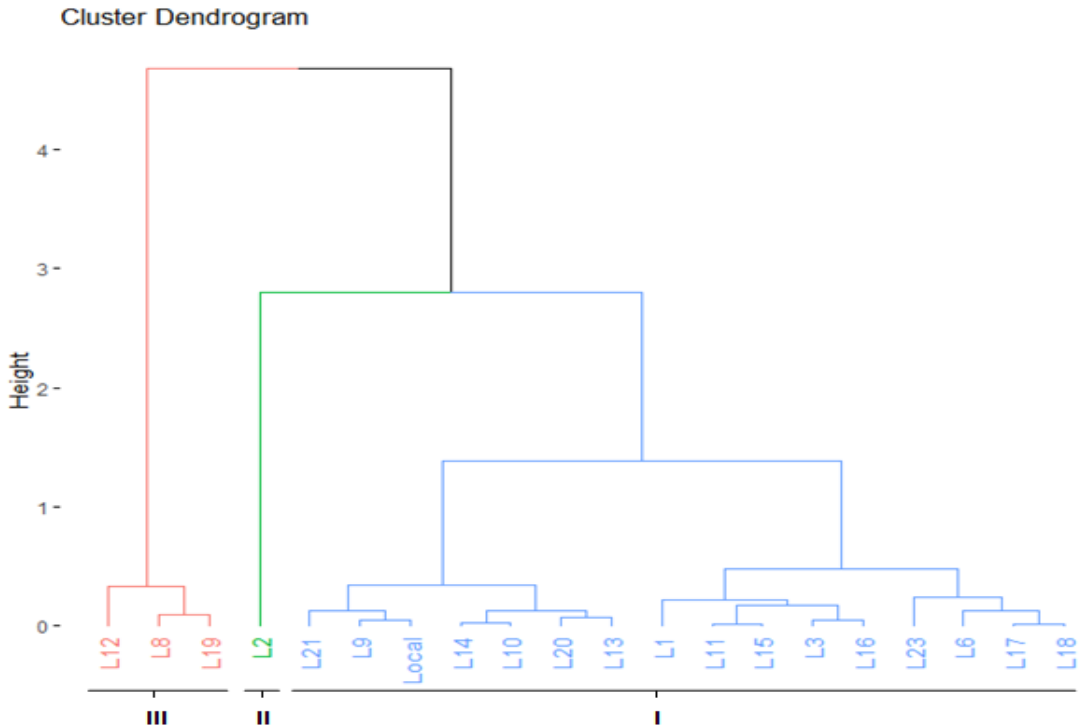**Figure 3**: Phenotypic traits of *A. cruentus* lines contributing to the first two principal components.

Figure 4: Dendrogram of 19 mutant lines of amaranth and the relative «local» cultivar.

Table 4: Cluster analysis for 12 phenotypic quantitative traits in 19 mutant lines of amaranth and the relative «local» cultivar.

Traits	Group (number of lines)		
	Cluster I (16)	Cluster II(1)	Cluster III(3)
NL	39.22	49.67	19.58
NB	32.52	40.8	19.13
LL (cm)	32.89	41.66	28.63
LW (cm)	8.4	14.01	6.95
BL (cm)	18.84	54.99	15.1
PL (cm)	13.74	16.69	11.23
PH (cm)	102.84	135.37	57.37
SD (mm)	4.23	5.84	2.09
LA (cm^2)	117.47	180.97	67.97
FlL (cm)	40.84	49.73	32.08
D-50-F	66.19	79	53.33
1000-SW	0.38	0.39	0.37

Notes: NL = Number of leaves; NB = Number of branches; LL = Leaf length; LW = Leaf width; BL = Branches length; PL = Petiole length; PH = Plant height; SD = Stem diameter; LA = Leaf area; FlL= Flower length; Day-50-F = Days to 50% flowering; 1000-SW = 1000-seed weight.

DISCUSSION

Genetic diversity is evaluated using agro-morphologic traits and molecular markers (Nkhoma et al., 2020; Montcho et al., 2021). Despite the obviousness of the environmental influence on morphological traits, morphological descriptors remain important for assessing genetic diversity, as they are the basis for varietal selection at the farm level (Dagnon et al., 2017; Montcho et al., 2021). The mean morphological quantitative performances indicated the presence of a wide phenotypical variability among the nineteen *A. cruentus* mutant lines and their relative «local» cultivar used in the current work. In a study of 29 accessions from 5 *Amaranthus* species, Akin-Idowu et al. (2016) noticed the agronomic characters such as plant height, stem diameter, number of leaves per plant and leaf area to show wide variability which was useful in accession improvement through selection. Wu et al. (2000) in a field evaluation of an *Amaranthus* genetic resource collection made the same observation for traits such as plant height, seed yield, stem, and leaf color. Similarly in this study, traits such as leaf area, leaf width, branches length, plant height, stem diameter, number of branches and number of leaves had high coefficient of variation (respectively 29%, 19%, 47%, 25%, 29%, 21% and 25%) offering possibilities for selection to improve one or another of these characters in the created mutant lines. Characters such as days to 50% flowering, 1000-seed weight,

petiole length, leaf length and flower length had low coefficient of variation (respectively 7%, 9%, 15%, 15% and 18%). Thus, there are likely fewer chances to get substantial gain through selection among the 19 mutant lines for these traits. The little variability found in this study for seed yield in opposition to the findings by Akin-Idowu et al. (2016) and Wu et al. (2000) finding may be explained by the fact they realized an interspecific assessment whereas our study is about an intraspecific assessment. Also, the mutation might probably not have significant impact on seed yield control genes. This observation is totally opposed to Hricová et al. (2016) finding related to 1000 seed weight improvement in *A. cruentus* through gamma irradiation. The type of cultivar used, other materials and experimental methods used might explain such difference. Jankowicz-Cieslak et al. (2017) affirmed for instance that the effect of different mutagens on the DNA sequence varies with mutagen type and dosage. Moreover, other authors (Mba et al., 2010; Horn and Shimelis, 2013) insisted that the effectiveness of a mutagenic treatment in inducing genetic variations in crop plants depends on many factors among other the genetic constitution of tested varieties and treatment dose. In fact, Hricová et al. (2016) used in their work seeds of *A. cruentus* 'Ficha' previously treated by a dose of 175 Gy (Gray) of γ-radiation. Instead, seeds of a Benin «local» cultivar treated by a dose of 200 Gy were used in the current study. It would also be important to stress that induced mutations are random events so, even a given set of irradiation conditions might not result in the same mutation events for different genotypes (Greene et al., 2003; Horn and Shimelis, 2013; Jankowicz-Cieslak et al., 2017). It nevertheless turns out that gamma irradiation is one of the main physical mutagens used to induce genetic variation (Horn and Shimelis, 2013).

Knowledge of correlations among different characters is essential to design an effective breeding strategy for any crop (Mazid et al., 2013). Phenotypic quantitative traits considered in this study are important because they have direct or indirect effect on leaf yield (number and area). The negative correlation between 1000-seed weight and almost all the above ground biomass production parameters (plant height, number of leaves, number of branches per plant, branch length, leaf length, leaf width, petiole length, stem diameter and leaf area) considered in this study indicates that most of the heavy seed productive mutant lines are fewer biomass productive. So, whenever the purpose is to enhance for instance leaves production, low seed weight productive mutant line should be selected. This finding is helpful for selection since *A. cruentus* is produced in Benin for leaves yield.

Among the 12 quantitative traits, eleven (number of leaves, number of branches per plant, branches length, leaf length, leaf width, petiole length, plant height, stem diameter, leaf area, inflorescence length and days to 50% flowering) were strongly associated with the PC_1. Almost the same characters were also highlighted by Andini et al. (2013) and Tejaswini et al. (2017) as important characters for characterization of *Amaranthus* accessions. If the variable 1000-seed weight had a negative and little representation on PC_1, it was the only one trait that appeared strongly in PC_2. This indicates that 1000-seed weight is negatively correlated with most of the traits studied, hence it could not be possible to select for high vegetative component in the set of mutant lines without negatively affecting seed yield.

Cluster analysis based on principal components subdivided the set of the 19 mutant lines and their relative local cultivar into three major clusters. The cluster I gathered the local cultivar and fifteen mutant lines (L1, L11, L15, L3, L16, L23, L6, L17, L18, L21, L9, L14, L10, L20, and L13) with average values for all the traits but divided in two groups of which the second (L1, L11, L15, L3, L16, L23, L6, L17 and L18) showed higher performances than the first (L21, L9, L14, L10, L20, L13 and the «local» cultivar) for all the traits apart from days to 50% flowering and thousand seed weight. Alone in the cluster II, the mutant line L2 had the highest values for almost all the traits. These results show that the mutant lines

L1, L2, L3, L6, L11, L15, L16, L17, L18, and L23 are more interesting than their relative local cultivar as far as almost all the above ground biomass parameters (number of leaves, number of branches, leaf length, leaf width, branch length, petiole length, plant height, stem diameter, leaf area and flower length) are concerned. Thus, that set of ten mutant lines constitutes good samples for selection for above ground biomass yield improvement. The mutant lines L8, L12 and L19, especially characterized by lowest numbers of days to flowering, clustered together in the cluster III having the lowest value for all the characters considered in this study. They neither are good for above ground biomass improvement nor for grain interest. Thus, higher the numbers of days to flowering, high are the above ground biomass yields. This is in alignment with findings by Janovská et al. (2012) who reported that the length of vegetative phase is very important for amaranth cultivation, because many of the amaranths genotypes are sensitive to day-length; they remain in the vegetative period for a long time and create seeds after day-shortening. As far as qualitative traits are concerned, they were almost not discriminant for the studied populations except the terminal inflorescence shape of Panicle with short branches for the three mutant lines L8, L12 and L19. The mutation might have less effect on qualitative trait control genes.

Conclusion

The twelve morphological quantitative traits used in the current study revealed wide genetic diversity among the nineteen mutant lines and the «local» cultivar. Traits such as leaf area, leaf width, branches length, plant height, stem diameter, number of branches and number of leaves should be considered for *A. cruentus* mutant genotypes selection. Mutant lines L1, L 2, L6, L16, L18 and L23 having high number of leaves, number of branches and leaf width could be a good source of genes for the saline tolerance breeding purpose. The current study makes available useful genotypes for saline tolerance breeding objective. It enhances effective *A. cruentus* resources'

availability for farmers and supports the selection of *Amaranthus* genotypes for traits of interest in further breeding programs.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of this study. Material preparation, plants raising and Data collection were performed by AOEKK, RA and BNG. Supervision of the mutant lines' stabilization and characterization trials were done by AAM, NFH, AW, DM, CBG, LA and CA. AOEKK analyzed the data and wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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