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Morphological variability of *Euphorbia sepium* N.E. Br. across the Sudanian and Sudano-Guinean zones of Benin Republic (West Africa): Implications for conservation

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ABSTRACT

Euphorbia sepium is a galactogenic plant used by livestock farmers in Benin to improve milk production in cows. Despite its proven medicinal and ethnobotanical importance, little is known about its morphological diversity. To assess the morphological diversity of *E. sepium* in the Sudanian and Sudano-Guinean zones of Benin, fifteen traits including eight quantitative and seven qualitative were studied on 54 individuals of this species. A Principal Component Analysis, a Hierarchical Ascending Classification, an analysis of variance of data and a Pearson linear correlation were performed to describe the intraspecific and inter-region diversity. The results exhibited significant variability in the morphological traits. Also, most of the traits showed a coefficient of variations higher than 30 %, revealing a high level of morphological diversity within the species. Three clusters with distinct phenotypic attributes were identified regardless the climatic zones considered. The major discriminating morphological traits include total plant height, crown height, collar diameter, number of stems, leaf width and length, and petiole length. Individuals in cluster 3 had the highest values of total height (6.94 ± 0.49 m), crown height (5.15 ± 1.42 m), collar diameter (24.45 ± 19.46 cm) and number of stems while those in cluster 1 showed the highest values for leaf length (6.88 ± 0.36 cm), leaf width (3.11 ± 0.46 cm) and petiole length (0.61 ± 0.12 cm). The study also revealed significant and positive correlation between plant height and crown height ($r = 0.86$; $p < 0.001$), plant height and collar diameter ($r = 0.57$; $p < 0.001$), plant height and number of stems ($r = 0.49$; $p < 0.001$). Also, between leaf length and width, leaf width and petiole length, we found a significant and positive correlation. This study provided useful information on the phenotypic variability of *E. sepium* and can be base for future conservation, domestication, and breeding programs.

1. Introduction

Euphorbia sepium N.E. Br. is a species of the Euphorbiaceae family. The species is distributed in sub-Saharan and Sahel regions of northwest Africa (Riina et al., 2021). Previous studies on this species conducted in the Sahel, used the name *Euphorbia balsamifera* Aiton, but this now only applies to populations distributed in the Canary Islands and the Atlantic coast of Morocco and Western Sahara (Riina et al., 2021). The current taxonomy is supported by strong phylogenomic evidence (Villaverde et al., 2018; Riina et al., 2021). Both *E. sepium* and *E. balsamifera* have been used in traditional medicine to treat several diseases, as well as affections caused by pathogenic organisms (Akoègninou, 2006; Arbonnier, 2009; Riina, 2020). In fact, the aqueous extracts preparations of leaves, stem and roots of this plant hold the properties of inhibiting the development of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella* spp, and *Candida albicans* (Kamba and Hassan, 2010; Bashir, 2015). Moreover, it has been reported that aqueous extracts of *E. sepium* (reported as *E. balsamifera* in the study) stem bark have a repellent effect on *Anopheles gambiae* (Idris et al., 2014), which is the most important mosquito vector of malaria in

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Africa (Scholte et al., 2006; Neafsey et al., 2021). The insecticidal effect of this species was also indicated in various countries such as Burkina Faso (Bambara and Tiemtoré, 2008), and Nigeria (Suleiman and Suleiman, 2014; Abubakar et al., 2021), especially when used as leaf powder against *Callosobruchus maculatus* Fab. a dreaded cowpea seed pest and against *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), a grain borer.

In Benin, *E. sepium* is one of the plant species priority medicinal for rural communities (Imorou et al., 2021). This plant would be rarely present in the natural vegetation in Benin according to rural people who use it mainly as a galactogenic plant to solve problems related to milk production in breastfeeding women and to improve milk production in cows (Salifou et al., 2017; Imorou et al., 2021). All the organs (stem, leaves and root) of the species are used by herders in different galactogenic receipts (Agani et al., 2021; Atchouké et al., 2021). In Benin, so far, little is known on the distribution of *E. sepium* in the country. The study of Riina et al. (2021) reported the presence of this species in the Sudanian and Sudano-Guinean zones. Besides, the ethnobotanical study conducted on the biodiversity of galactogenic plants in nine municipalities across the Sudanian and Sudano-Guinean zones of the country confirmed the presence of the species in these two climatic zones (Imorou et al., 2021). Despite the various uses of *E. sepium*, little is known about its morphological variability. Studies in other African Euphorbiaceae species have been conducted revealing diverse sources of variation in the plant traits, leaves, fruits and seeds (Djaha et al., 2017; Salazar-Villa et al., 2020; Adebusuyi et al., 2021; Tiendrébéogo et al., 2021). Many other studies have also pointed out climatic factors as responsible for variation in morphological traits of several tropical individual species (Souza et al., 2018; Houehanou et al., 2019; Lawin et al., 2021; Li et al., 2021). Reliable data on the morphological variability of *E. sepium*, a species identified as highly exploited by herders and agro-pastoralists in the agro-ecological zones of Benin, are required to develop an optimal valorization strategy and its preservation.

The objective of this study was to evaluate the morphological variability of *E. sepium* across the Sudanian and Sudano-Guinean zones in order to identify its local morphotypes in Benin. Specifically, we aimed to (i) determine the intra-specific diversity of *E. sepium* in the study area and (ii) investigate the effect of climatic zone on this variability in the country. The outcomes of this study will serve as a basis for the improvement, domestication, and conservation of the species in Benin.

2. Materials and methods

2.1. Study area

The study was conducted in Sudanian and Sudano-Guinean zones in Benin. These zones are located between 7°30′ - 12°35′ latitude North and 1°55′–3°40′ longitude East. In the Sudano-Guinean zone, rainfall is unimodal, with annual total rainfall varying between 900 mm and 1200 mm. Mean annual temperature is 32 °C, and the relative humidity is 65 % (Ahoyo et al., 2021). In the Sudanian zone, the rainfall is also unimodal but mean annual rainfall is often less than 1000 mm; the relative humidity is less than 65 % while temperature varies between 24 °C and 31 °C (Gandji et al., 2020; Ahoyo et al., 2021). The Table 1 summarizes the ecological characteristics of these zones in Benin Republic.

E. sepium has long, narrow leaves and dense fruits. Botanically, this species seems to be neglected by botanists (Riina, 2020). According to studies by Riina (2020) and Biaou et al. (2022), this species propagates vegetatively from cuttings. Individual plants were selected in the municipalities of Banikoara and Gogounou in the Sudanian zone and in Kalalé and Nikki in the Sudano-Guinean zone (Fig. 1). In fact, the study carried out by Imorou et al. (2021) on galactogenic plants showed that *E. sepium* (reported *as E. balsamifera* in the cited study) is one of the species most used by herders and agro-pastoralists in these municipalities.

Table 1
Climatic characteristics of Sudanian and Sudano-Guinean zones in Benin Republic[a].

Characteristics	Sudanian zone	Sudano-Guinean zone
Latitude	9°45′ - 12°25′N	7°30′ - 9°45′ N
Longitude	2°25′ - 3°40′E	1°55′ - 2°25′ E
Rainfall regime	Unimodal (5–6 months)	Unimodal (5–6 months)
Rainy season	April – October	April – October
Dry season	November – March	November – March
Annual rainfall range (mm)	800–1000	900–1200
Temperature (°C)	24–31	21–40 (mean: 32 °C)
Rel. Humidity (%)	always < 65	65 (mean)
Climate type	Dry tropical	Humid tropical

[a] Information collected from Gandji et al. (2020) and Ahoyo et al. (2021)

2.2. Plant sampling and data collection

Prior to the data collection, plants of *E. sepium* were located in each municipality with the assistance of a local guide and then georeferenced using global positioning system (GPS). In total, 54 individuals were sampled, 25 in the Sudanian zone and 29 in the Sudano-Guinean zone. On each selected individual, parameters such as total plant height, crown height, bole height and collar diameter were measured. Besides, ten (10) leaves were sampled per individual and their length, width and petiole length were measured. Qualitative data included plant architecture, branch shape and color, leaf color, leaf shape and type, and leaf vein color. Descriptors used to characterize this species were inspired by the works on *Jatropa curcas* L. (Tiendrébéogo et al., 2021) and on *Manihot esculenta* Crantz (Djaha et al., 2017; Kosh- Komba et al., 2017) which are species of the same botanical family as *E. sepium.* Overall, fifteen (15) morphological traits including eight quantitative and seven qualitative were selected to assess the diversity of this species (Table 2).

2.3. Data analysis

Quantitative data were processed using descriptive statistical analyses with calculation of mean, minimum, maximum and coefficient of variation. A Principal Component Analysis (PCA) and Hierarchical Ascending Classification (HAC) with projection on the factorial plane was then performed to identify the different morphotypes within *E. sepium* in the study area. The clusters obtained from HAC were subject to one-way ANOVA followed by the Student Newman Keuls test at 5 % (probability level) to identify the discriminating quantitative traits of each morphotype. ANOVA results are presented using boxplots. Pearson's linear correlation and the performance analytics package of the R software were used to assess the relationship between the measured quantitative traits. The effect of area on the variability of the species was also assessed by performing student's t-test. For this analysis, phytogeographical zones were considered as a factor. All the analyses were performed using R Core Team (2020).

Qualitative data collected were submitted by simple frequencies and then analysed by the chi-square test of independence for their dependence on the two climate zones.

3. Results

3.1. Qualitative traits variability in E. sepium

Two plant architectures were encountered within the species: dendroid shrub (56 %) (Table 3, Fig. 2a) and shrub (Fig. 2b) with many branches (44 %) at the base (Table 3). The branches are straight (71 %), especially in shrubs, or zigzag (29 %) in dendroid shrubs. With regards to other qualitative traits such as color of the stem, leaf color, leaf shape, leaf type, and leaf vein color, no difference was observed.

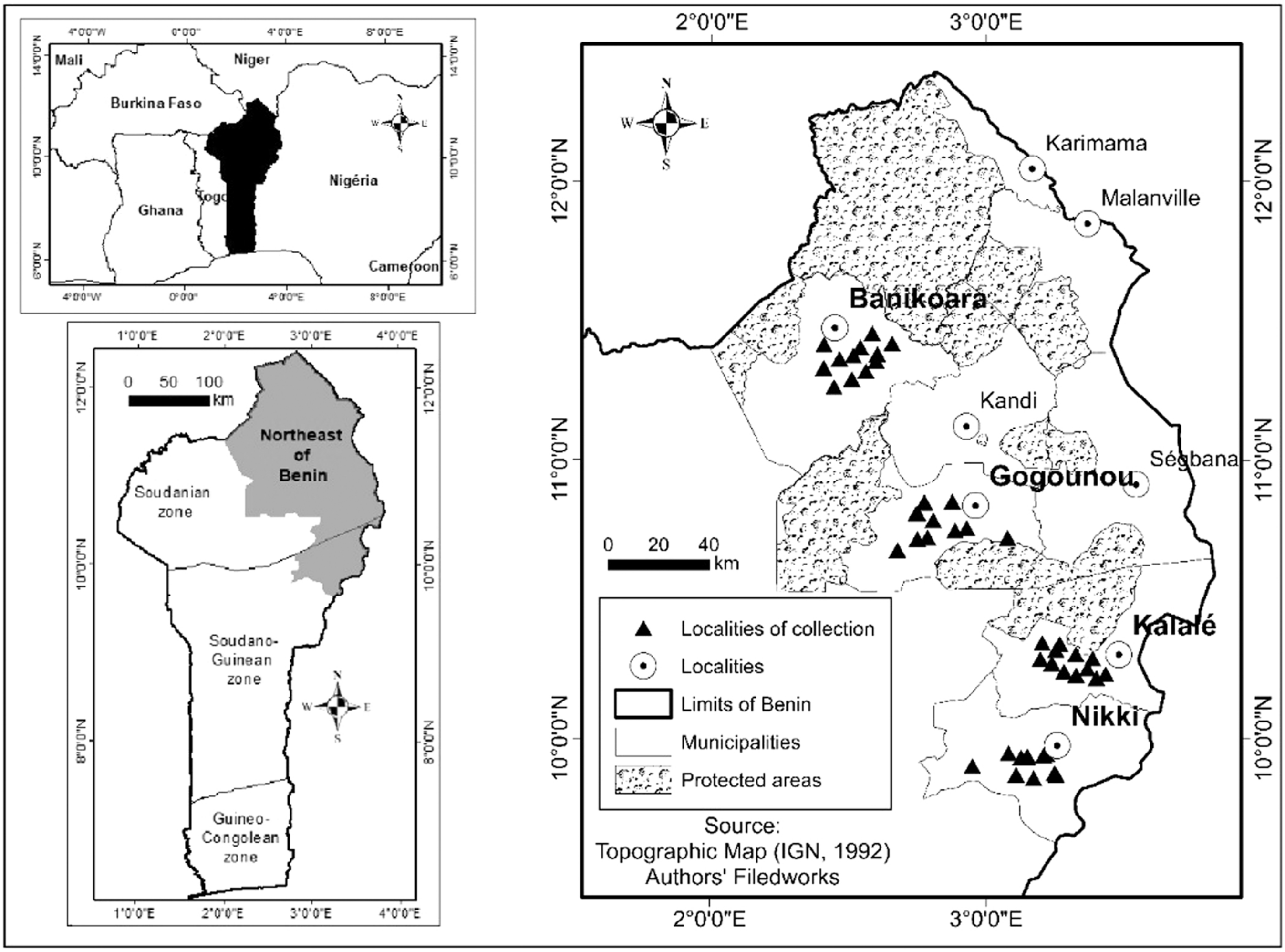


Fig. 1. Map showing data collection sites for E. sepium individuals.

Table 2
Morphological variables used to characterize *E. sepium* individuals.

Type	Variables		Codes	Data collection method	Measuring instrument
Quantitative	Vegetative	Total height (m)	ToH	Distance from the ground surface to the end of the plant top calculated using the equation [ToH = (Distance from observer × tan A) + Base height from ground to position of clinometer]	A handheld clinometer was used to measure plant height (Blozan, 2006)
		Crown height (m)	CrH	Distance from the first branch to the top of the plant (Difference between plant height and bole height: ToH – BoH)	Metric tape was used to measure bole height and collar diameter
		Bole height (m)	BoH	Distance from the ground surface to the first branch of the plant	
		Collar diameter (cm)	CoD	Basal diameter of the plant (Distance between two ends of the stem)	
		Number of main branches	NuB	Simple counting	
	Leaf	Leaf width (cm)	LeW	Lateral distance of the leaf	Double decimeter of 30 cm
		Leaf length (cm)	LeL	Distance between the point of leaf insertion on the stem and the tip of the apex of the terminal leaflet	
		Petiole length (cm)	PeL	Distance between the point of attachment of the petiole on the main vein and the point of attachment on the plant	
Qualitative	Vegetative	Plant architecture	PlA	Assessment by visual observation	
		Shape of the branch	ShB	Assessment by visual observation	
		Color of the branch	CoB	Assessment by visual observation	
	Leaf	Color	LeC	Assessment by visual observation	
		Shape	LeS	Assessment by visual observation	
		Type	LeT	Assessment by visual observation	
		Vein color	LVc	Assessment by visual observation	

Table 3
Diversity of qualitative traits in the 54 accessions of *E. sepium*.

Morphological traits	Class	Frequency (%)
Plant architecture	Dendroi shrub	56
	Shrub	44
Shape of the branche	Right	71
	Zigzag	29
Color of the branche	Gray/dirty white	100
Leaf color	Green	100
Leaf shape	long-oval	100
Leaf type	Simple	100
Leaf vein color	Light green	100

3.2. Quantitative traits variability in *E. sepium*

Total plant height ranged from 1.15 to 7.25 m with an average of 2.74 ± 1.24 m. The average crown and bole heights of the species were 2.26 ± 1.02 m and 0.84 ± 2.06 m, respectively. The coefficients of variation (CV) showed high values especially for total height (CV = 45 %), crown height (CV = 45 %), bole height (CV = 44 %), collar diameter (CV = 81 %) and number of main branches (CV = 89 %) indicating high variability within the species in the study area (Table 4).

3.3. Intra-specific variability of *E. sepium* in the study area

3.3.1. Morphological characteristics of *E. sepium*

The first two PCA axes explained 58.50 % of the morphological variation between *E. sepium* plants (Table 5). In addition, each of these two axes had an eigenvalue greater than one (1). These two axes can therefore be used to explain the variations in morphological traits between the *E. sepium* individuals studied. The projection of the variables and individuals on the PCA systems showed that individuals BnkE2, KalE14 and NikE13 were the tallest in total height, crown height, the biggest in collar diameter, and had the most main branches (Fig. 3). The results also showed that KalE3, KalE6, KalE7, KalE8 and GogE3 had the highest values of petiole length and leaf width. BnkE2 was among the individuals with the longer and wider leaves (Fig. 3).

3.3.2. Trees' clustering and morphological groups identification

The hierarchical clustering on PCA grouped *E. sepium* individuals into three (3) different clusters or morphological clusters (Fig. 4). Cluster 1 (55.55 % of total individuals) essentially gathered *E. sepium* individuals from Banikoara, Nikki and Kalalé (Table 6). Cluster 2 (37.04 % of total individuals) was composed mainly of individuals from Gogounou. Cluster 3 (7.41 % of total individuals) was composed of four individuals including one from Banikoara, one from Nikki and two from Kalalé.

3.3.3. Discriminant traits of the three morphological groups of *E. sepium*

Significantly differences were observed ($p < 0.05$ to $p < 0.001$) between clusters for most of the quantitative traits (Fig. 5). Considering

Table 4
Descriptive statistic of morphological traits of *E. sepium*.

Morphological traits	Mean + standard deviation	Minimum	Maximum	CV (%)
Plant body				
Total height (m)	2.74 ± 1.24	1.15	7.25	45
Crown height (m)	2.26 ± 1.02	0.58	6.00	45
Bole height (m)	0.84 ± 2.06	0.05	15.00	44
Collar diameter (cm)	12.73 ± 10.30	1.54	50.00	81
Number of branches	4.63 ± 4.12	1.00	30.00	89
Leaf				
Leaf length (cm)	6.42 ± 0.86	3.60	7.57	13
Leaf width (cm)	2.63 ± 0.69	1.33	4.57	26
Petiole length (cm)	0.52 ± 0.16	0.17	0.80	31

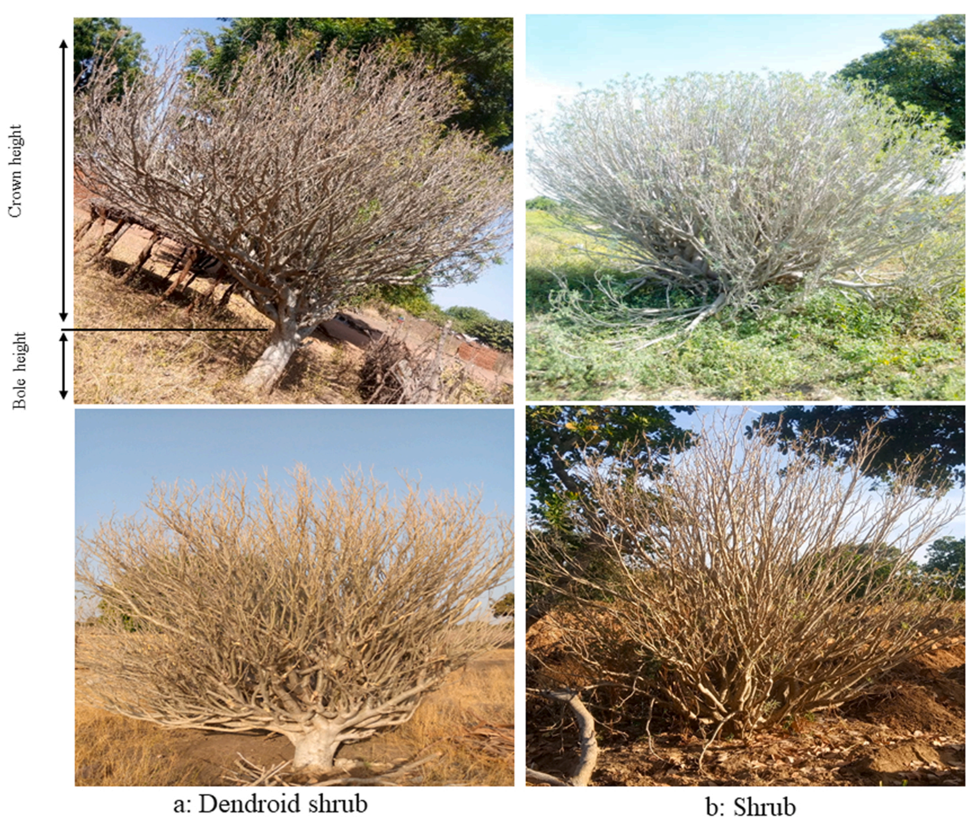


Fig. 2. Diversity of tree architecture in E. sepium.

Table 5
Eigen values and proportion of variation explained by the first three principal component for different morphological traits of *E. sepium*.

Variable	PCA1	PCA2	PCA3
PCA eigenvalues and proportions			
Eigen value	2.71	1.97	1.14
Proportion (%)	33.90	24.60	14.35
Cumulative (%)	33.90	58.50	72.85

total plant height, individuals from cluster 3 showed the highest height of 6.94 ± 0.49 m (Fig. 5A). Individuals from cluster 3 also presented the highest values of crown height (5.15 ± 1.42 m) and collar diameter (24.45 ± 19.46 cm) (Fig. 5B and D). However, individuals from cluster 1 had significantly ($p < 0.05$) longer (6.88 ± 0.36 cm), wider (3.11 ± 0.46 cm) leaves and longer petioles (0.61 ± 0.12 cm) than those from clusters 2 and 3 (Fig. 5F, G and H). In contrast, the bole height was the same across clusters ($p = 0.205$) (Fig. 5C).

3.4. Correlation analysis for quantitative traits of *E. sepium*

The Pearson linear correlation coefficient showed a high correlation between morphological traits (Fig. 6). The plant height was significantly and positively correlated with crown height ($r = 0.86$; $p < 0.001$), collar diameter ($r = 0.57$; $p < 0.001$) and number of branches ($r = 0.49$; $p < 0.001$). The results also showed a positive and significant correlation ($r = 0.35$; $p < 0.01$) between collar diameter and number of branches. In addition, it has been observed a highly significant positive correlation between the three leaf traits (Fig. 6). Thus, leaf length was positively and significantly correlated with leaf width ($r = 0.65$; $p < 0.001$) and petiole length ($r = 0.45$; $p < 0.001$). Leaf width was also positive and significant correlated ($r = 0.52$; $p < 0.001$) with petiole length. However, between the collar diameter, the width of the leaf ($r < -0.28$; $p < 0.05$) and the length of the petiole ($r < -0.34$; $p < 0.05$), a significant and negative correlation was observed. The highest correlation coefficient ($r = 0.86$) was observed between total plant height and crown height.

3.5. Variation of *E. sepium* between climatic zones

3.5.1. Variation of qualitative traits according to climatic zone

On the seven qualitative traits assessed, only two showed two variants. These were plant architecture with the shrub and bushes variants, and the shape of the stem development, straight or zig zag. The Chi2 test of independence showed that the effect of climatic zone was not significant ($p > 0.05$) on these two qualitative traits (plant architecture and stem shape) despite the existence of different variants (Table 7). There was no plant architecture or stem shape that was specific to either of the two climatic zones.

3.5.2. Variation of quantitative traits according to climatic zone

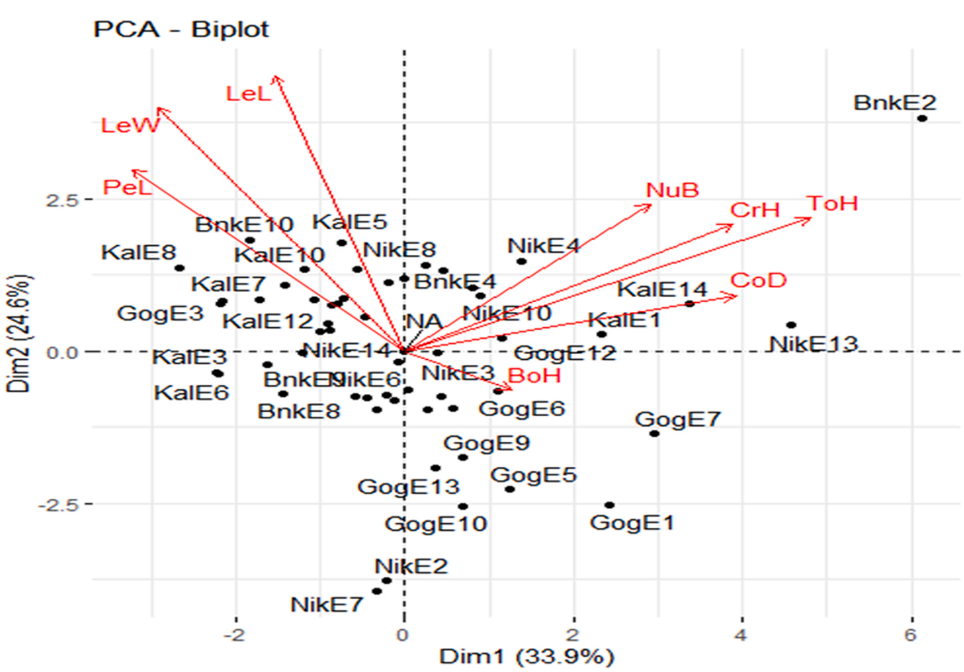
Total plant height varied from 2.54 ± 1.13 m (Sudanian zone) to 2.91 ± 1.32 m (Sudano-Guinean zone) while crown height varied from 2.09 ± 0.68 m (Sudanian zone) to 2.42 ± 1.23 m (Sudano-Guinean zone). In fact, the results showed that, individuals in the Sudanian zone appear to have a greater bole height (1.12 ± 2.98 m) than those in the Sudano-Guinean zone (0.58 ± 0.51 m). Similarly, individuals showed the highest values of collar diameter and number of stems in the Sudanian zone. Regarding leaf parameters, individuals in the Sudano-Guinean zone have wider leaves with a longer petiole.

The climatic zone did not have a significant influence on all the quantitative traits ($p > 0.05$). However, within each zone, a high morphological variability with the species was noted, especially with regard to dendrometry parameters (heights, collar diameter and number of branches) due to the high values of the coefficient of variation (Table 8).

4. Discussion

4.1. Intraspecific variability of *E. sepium*

The present study examined the morphological diversity of *E. sepium* based on 15 morphological traits, eight quantitative and seven qualitative. It also examined the influence of climatic gradient on the morphological diversity of this species. The study showed the level of



ToH: total height, CrH: crown height, BoH: bole height, CoD: collar diameter, LeL: leaf length, LeW: leaf width, PeL: petiole length, NuB: number of branches

Fig. 3. Projection of individuals and traits on axes 1 and 2 of the factorial design.

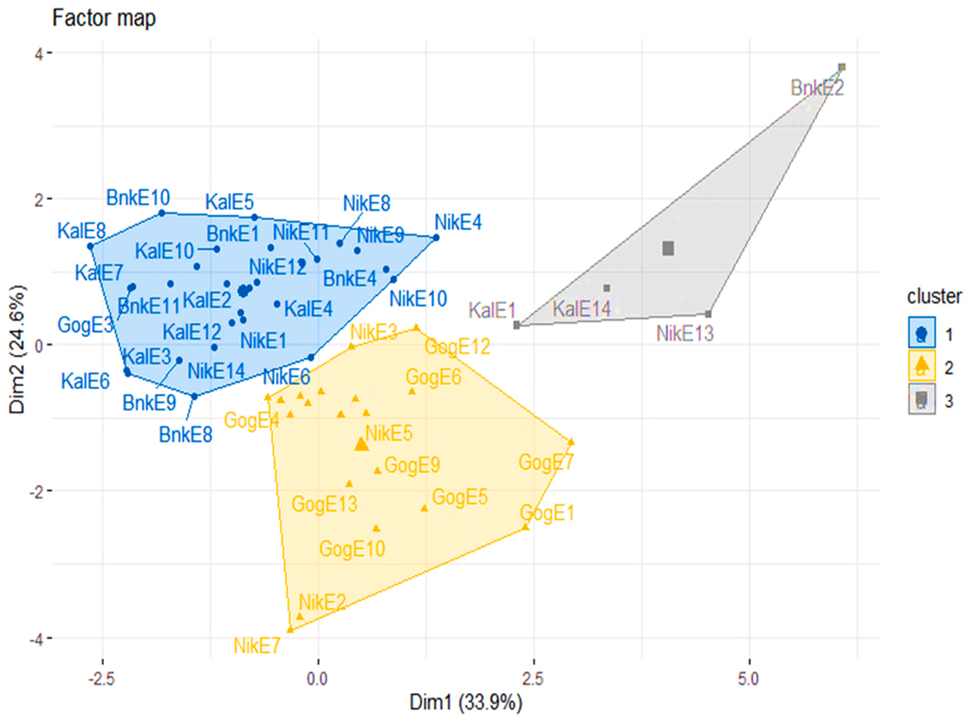


Fig. 4. Hierarchical clustering on principal components analysis (HCPC) showing number of clusters and individuals within each cluster in *E. sepium.*

Table 6
Detailed list of individuals of *E. sepium* in each cluster.

Cluster	Individuals	Number
1	BnkE1, GogE3, NikE1, NikE4, NikE8, NikE9, NikE10, NikE11, NikE12, BnkE3, BnkE4, BnkE5, BnkE6, BnkE8, BnkE9, KalE2, KalE3, KalE4, BnkE10, BnkE11, KalE5, KalE6, KalE7, KalE8, KalE10, KalE11, KalE12, GogE12, GogE14, NikE14	30
2	GogE1, GogE2, NikE2, NikE3, NikE5, NikE6, NikE7, GogE4, GogE5, GogE6, GogE7, GogE8, GogE9, BnkE7, GogE10, GogE11, KalE9, KalE13, GogE13, NikE15	20
3	KalE1, NikE13, BnkE2, KalE14	4

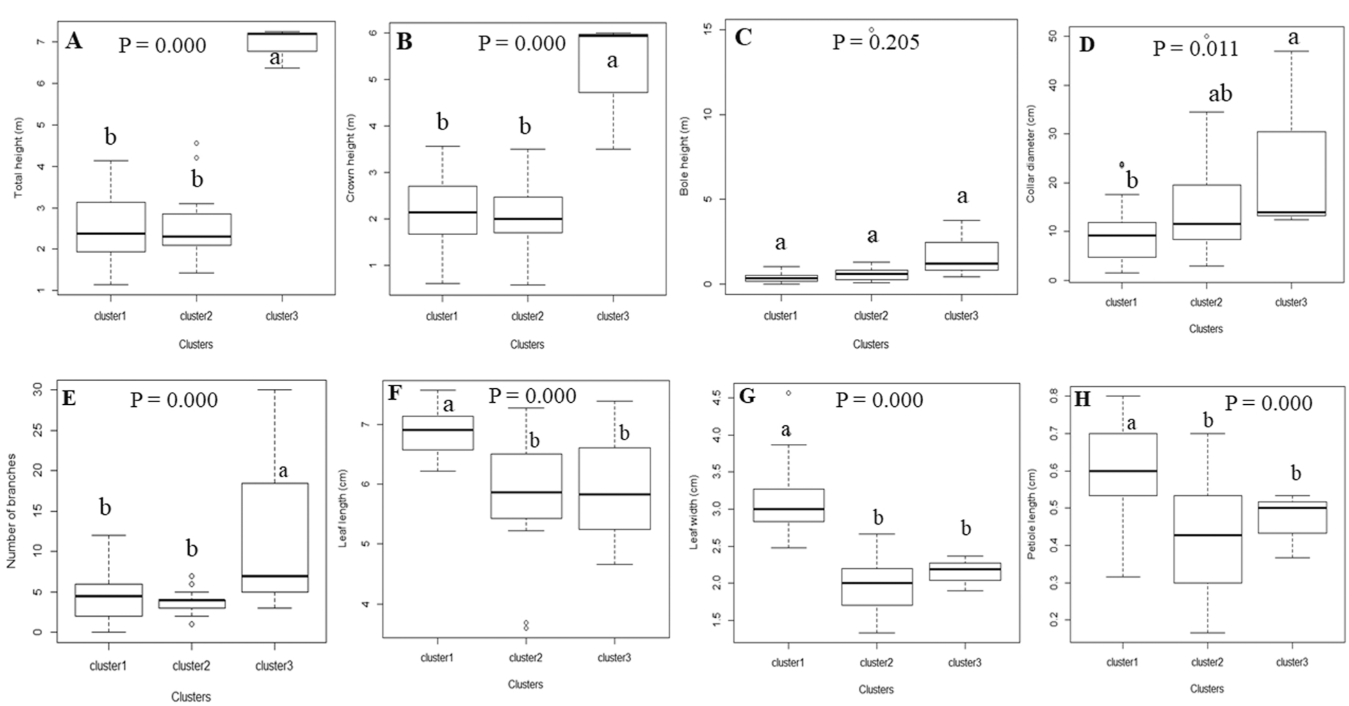


Fig. 5. Variation of mean of the traits discriminating morphological groups in E. sepium: total height (A), crown height (B), bole height (C), collar diameter (D), number of branches (E), leaf length (F), leaf width (G) and petiole length (H). P: probability; Bold values indicate the degree of significance of the probability; Vertical bars represent standard deviation. Bars with the same letter are not significantly different (P > 0.05) according to Student Newman-Keuls test.

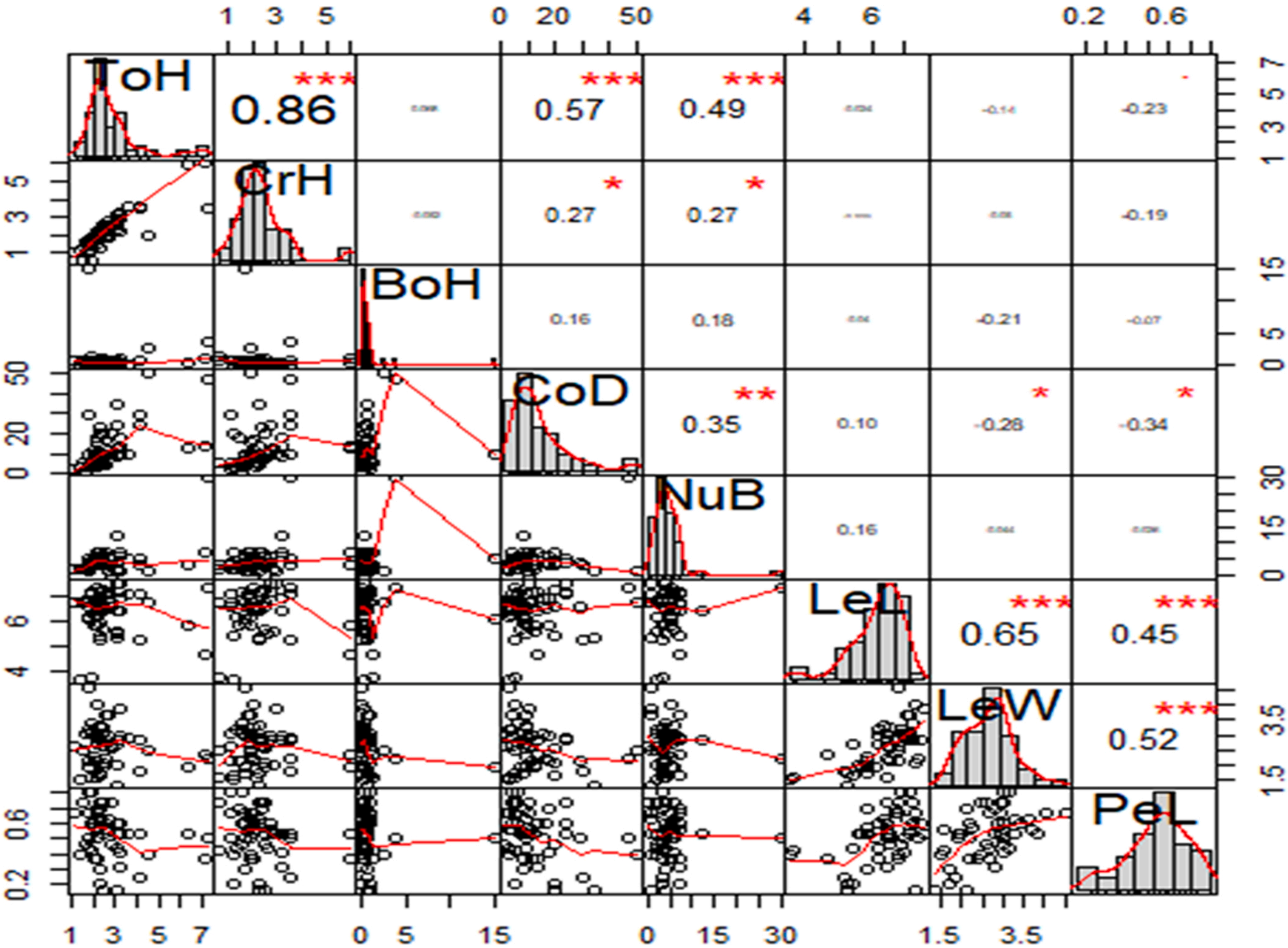


Fig. 6. Linear Pearson correlation coefficients between different morphological variables.

Table 7
Test for the dependence of qualitative traits on zone.

Qualitative trait	χ2	degree of freedom	P
Architecture plants	0.098	1	0.753
Shape of the branche	0.851	1	0.356

morphological diversity of the sampled individuals of this species in the Sudanian and Sudano-Guinean zones of Benin. In fact, as far as plant architecture is concerned, the results revealed two types. The first type was a low dense shrub while the second type was a dendroid shrub. This could be an expression of variability within the species.

The results showed high values of coefficients of variation (CV). The highest CVs were recorded in number of main branches (89 %) and collar diameter (81 %), respectively. In addition, the least CV was observed for the leaf length (13 %). Seven out of the eight measured traits showed CVs more than 20 %, revealing a high level of morphological diversity among the individuals as pointed out by several authors in different plant species (Khadivi et al., 2018; Raji and Siril, 2021; Mohammad et al., 2022). The most significant result was represented by the identification of three separate main clusters of morphotypes within the species in the study area, based on seven of the eight morphological traits quantitative (plant height, crown height, collar diameter, number

Table 8
Descriptive statistics of morphological traits of *E. sepium* within each climatic zone.

Morphological trait	Sudanian zone				Soudano-Guinean zone				Student's t-test	
	Mean	Minimum	Maximum	CV (%)	Mean	Minimum	Maximum	CV (%)	t-value	P
Plant body										
Total height (m)	**2.54 ± 1.13**	1.68	7.25	44	**2.91 ± 1.32**	1.15	7.20	45	-1.106	**0.274**
Crown height (m)	**2.09 ± 0.68**	1.12	3.50	33	**2.42 ± 1.23**	0.58	6.00	51	-1.243	**0.220**
Bole height (m)	**1.12 ± 2.98**	0.05	15.00	61	**0.58 ± 0.51**	0.05	2.60	86	0.917	**0.368**
Collar diameter (cm)	**14.03 ± 11.18**	1.54	46.90	79	**11.60 ± 9.53**	2.47	50.00	82	0.850	**0.399**
Number of branches	**5.68 ± 5.55**	1.00	30.00	90	**3.69 ± 2.04**	1.00	7.00	53	1.672	**0.105**
Leaf										
Leaf length (cm)	**6.42 ± 0.68**	5.23	7.39	11	**6.41 ± 1.00**	3.60	7.57	15	0.016	**0.987**
Leaf width (cm)	**2.52 ± 0.66**	1.33	4.02	26	**2.71 ± 0.71**	1.50	4.57	26	-1.024	**0.310**
Petiole length (cm)	**0.48 ± 0.19**	0.17	0.80	41	**0.56 ± 0.11**	0.37	0.80	20	-1.693	**0.099**

P: probability; t: Student's t-test value; CV: Coefficient of variation of branches, leaf width, leaf length and petiole length). Individuals were mostly distributed in three different clusters with cluster 1 having the greatest number of the individuals (55.55 %) followed by cluster 2 which contained twenty plants. On the other hand, clusters 3 contained only four individuals. Similar results were found by many authors with various african plant species of the same or different botanical families. In fact, in Nigera, the work by Adebusuyi et al. (2021) indicated three distinct morphotypes of *J. curcas*, discriminated by the diameter of the collar, the number of leaves and the height of the plants. In Benin, Gandji et al. (2020) found four morphotypes of *Moringa oleifera* Lam. (Moringaceae) and these were identified with five morphological characters including number of tertiary branches, maximum leaf width and leaf length. Saini et al. (2021) also recorded morphological variability of *Balanites aegyptiaca* (L.) Del. based on morphological descriptors including plant height and girth, leaf length and width. The morphological diversity of *E. sepium* plants thus highlighted through this study would provide an opportunity for breeders to develop novel morphotypes with desirable characteristics and for botanists to make provisions for better conservation of the species. Adoukonou-Sagbadja et al. (2007) and recently Bhandari et al. (2017) indicated that genetic diversity is the basis for plant survival in nature and this genetic diversity was revealed through morphological traits, biochemical characteristics as well as DNA attributes in plant genetic resources (Sharma et al., 2021). In addition, morphological data were collected on individuals of different ages. Therefore, the variability observed within this species could also be related to the age of the shrubs on which data were collected. A positive correlation was recorded between some quantitative morphological traits of *E. sepium* individuals, especially between plant height, crown height and collar diameter, between plant height, crown height and number of branches. The same trend was observed between leaf parameters (length, width, and petiole length). However, a significantly negative correlation was observed between plant collar diameter and leaf petiole length. The knowledge of these different relationships between the characteristic traits of a species is of a great importance as it guides breeders in their studies (Okello et al., 2018; Xue et al., 2020; Mousavi and Nagy, 2021).

4.2. Effect of climatic zones on variability of *E. sepium*

The climatic gradient did not affect ($p > 0.05$) the variability of *E. sepium* as shown by absence of significant morphological variation between individuals in the Sudano-Guinean and Sudanian zones ($p > 0.05$). In fact, the Sudanian zone is characterized by a single dry season (April – October), unimodal rainfall regime, dry season from (November - March) and a temperature ranging from 24° to 31 °C (Gandji et al., 2020; Ahoyo et al., 2021). These climatic characteristics are similar to those of the Sudano-Guinean zone. This could explain the absence of morphological diversity between *E. sepium* individuals from the two climatic zones. Opposite results were reported in *Anogeissus leiocarpa* (Ouédraogo et al., 2013), *Vitex doniana* Sw. (Hounkpèvi et al., 2016), *Tamarindus indica* LINN. (Okello et al., 2018), *Afzelia africana* Sm. (Houehanou et al., 2019), *Moringa oleifera* Lam. (Gandji et al., 2020; Drisya Ravi et al., 2021) and *Cola millenii* K. Schum. (Lawin et al., 2021) from various countries. Results from these authors showed a variation in the morphological traits of species studied according to phytodistricts, agroecological zones or climatic zones. This could be a particularity of *E. sepium* and would justify its geographical distribution (Riina et al., 2021).

The intraspecific variation thus observed within *E. sepium* would be of genetic origin. In fact, various studies had established the link between morphological variation and genetic diversity in plants including those of Kumar et al. (2022) on *Mangifera indica* L. in India, Mahmodi et al. (2022) on *Capparis spinosa* L. in Iran, and Rajarajan et al. (2022) on *Leucaena leucocephala* (Lam.) in India which revealed a high degree of genetic variability described by morphological and molecular markers in both species.

However, the various treatments (branch cutting, leaf removal and tree pruning) that trees undergo may well affect their morphology. In fact, Kelly et al. (2021) reported in their study on *Parkia biglobosa* that the way farmers manage home garden and production systems is one of the important factors affecting the phenotypic traits of trees. This could be the case in the evaluated *E. sepium* individuals that are planted and undergo almost the same treatments such as branch and leaf collection for medicinal purposes and maintenance pruning by the owners.

4.3. Implications of the study for the valorization and conservation of *E. sepium*

Euphorbia sepium is a species widely used by ruminant breeders to improve the dairy performance of cows in Benin but has not been extensively studied for its morphological diversity. This has led to a complete lack of information on the intraspecific diversity of this species. Knowledge of morphological variability is an important step in breeding, genetic improvement, gene bank conservation programs and conservation of plant species in future (Devi et al., 2022). The present study, which highlights three morphotypes within the *E. sepium* species, provides a basic knowledge on its morphological richness in the Sudanian and Sudano-Guinean zones of Benin. This information is essential for the preservation of plant species diversity and increasing their use and valorization (Dansi et al., 2010). Certainly, molecular characterization studies are needed to confirm this morphological variability. Many morphotypes within certain important plant species have disappeared due to agricultural practices, uncontrolled cutting, the absence of conservation measures both *in situ* and *ex situ*, and a lack of knowledge especially due to the absence of documentation (Kimaro and Lulandala, 2013; Yang et al., 2020). Encouraging the conservation of the intraspecific diversity of *E. sepium* is a way of safeguarding the biodiversity of Benin's flora and guaranteeing food security by increasing milk production. This can be done *ex situ* in botanical gardens or *in situ* in fields using the species as a living hedge plant to avoid extinction (Mounce et al., 2017; Griffith et al., 2020).

5. Conclusion

This morphological characterization study of *E. sepium* provided new knowledge on the phenotypic diversity of this species in the Sudanian and Sudano-Guinean zones of Benin. It allowed the identification of three morphotypes within this species that could offer a potential for improvement. This high morphological diversity could be applied in various breeding and conservation programmes of this high value galactogenic plant species. However, molecular characterization studies are needed to confirm the morphological diversity here reported. As the species is a galactogenic plant, phytochemical characterization studies of the three morphotypes identified would make it possible to detect any difference among these morphotypes, if it exists, in the stimulation of milk production in cows.

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CRediT authorship contribution statement

Lucien Imorou conceived the work with advice from Nicodème V. Fassinou Hotegni, Hospel G. Goudou and Léonard E. Ahoton. Lucien Imorou and Hospel G. Goudou collected the data. Lucien Imorou processed the data and performed the statistical analyses. Lucien Imorou drafted the manuscript with significant contribution of Nicodème V.

Fassinou Hotegni, Euloge C. Togbé , Daouda O. Bello, Hubert Adoukonou-Sagbadja and Léonard E. Ahoton. Léonard E. Ahoton and Hubert Adoukonou-Sagbadja supervised the work. All authors read and approved the final manuscript.

Declaration of Competing 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.

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References

Abubakar, R., Suleiman, M., Wagini, N.H., 2021. Phytochemical and insecticidal properties of some botanical extracts against the lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae). Journal of Entomology and Zoology Studies 9 (2), 06–13.

Adebusuyi, G.A., Oyedeji, O.F., Alaje, V.I., Sowunmi, I.L., Dunmade, Y.A., 2021. Morphological diversity in growth characteristics of *Jatropha curcas* L. accessions from South-West Nigeria. Nigerian Journal of Biotechnology 38, 109–119.

Adoukonou-Sagbadja, H., Wagner, C., Dansi, A., Ahlemeyer, J., Daïnou, O., Akpagana, K., Ordon, F., Friedt, W., 2007. Genetic diversity and population differentiation of traditional fonio millet (*Digitaria* spp.) landraces from different agro-ecological zones of West Africa. Theoretical and Applied Genetics 115, 917–931. https://doi.org/10.1007/s00122-007-0618-x.

Agani, Z., Cyrille, B.K., Guénole, A.C., Habirou, S.I., Bello, O., Daouda, H.M., Dossou, J., Babatounde, S., 2021. Préparations galactogènes utilisées par les agroéleveurs au Bénin: espèces végétales, proportions d'organes impliqués et production laitière chez les vaches Borgou. Journal of Applied Biosciences 157, 16161–16171.

Ahoyo, C.C., Houéhanou, T.D., Yaoïtcha, A.S., Prinz, K., Kakai, R.G., Sinsin, B.A., Houinato, M.R., 2021. Traditional medicinal knowledge of woody species across climatic zones in Benin (West Africa). Journal of Ethnopharmacology 265, 113417.

Akoègninou, A., Van der Burg, W.J., Van der Maesen, L.J.G., 2006. Flore analytique du Bénin. (Backhuys Publishers),, Netherlands, p. 1033.

Arbonnier, M., 2009. Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. Troisème Edition, MNHN & Quae,, Paris, p. 573.

Atchouké, G.D.L., Dabadé, D.S., Adéoti, K., Ouikounn, C.G., Bello, O.D., Dossou, J., 2021. Connaissances et pratiques de recettes endogènes pour l'amélioration de la production laitière des vaches locales au Bénin. Journal of Applied Biosciences 160. https://doi.org/10.35759/JABs.161.3 (16576 – 1658).

Bambara, D., Tiemtoré, J., 2008. Efficacité biopesticide de Hyptis spicigera Lam., *Azadirachta indica* A. Juss. et *Euphorbia balsamifera* Ait. sur le niébé *Vigna unguculata* L. Walp. Tropicultura 26, 53–55.

Bashir, L.B., 2015. Phtochimical screening and antibacterial studies of Euphorbia balsamifera leaves (Aguwa). Usmanu Danfodiyo Unniversity, Sokoto (Posgraduate School), p. 88.

Biaou, O.D.B., Balogoun, I., Bello, O.D., Chabi, F., Saïdou, A., Ahoton, E.L., 2022. Propagation by stem cutting of *Euphorbia balsamifera* (Aiton), a galactogenic plant in Benin. Journal of Applied Biosciences 172, 17894–17904.

Blozan, W., 2006. Tree measuring guidelines of the eastern native tree society. Bulletin of the Eastern Native Tree Society 1, 3–10.

R. Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Dansi, A., Adoukonou-Sagbadja, H., Vodouhe, R., 2010. Diversity, conservation and related wild species of Fonio millet (*Digitaria* spp.) in the northwest of Benin. Genetic Resources and Crop Evolution 57, 827–839.

Devi, A., Iqbal, T., Wani, I.A., Sharma, G., Verma, S., Noureldeen, A., Darwish, H., 2022. Assessment of variability among morphological and molecular characters in wild populations of mint [*Mentha longifolia* (L.) L.] germplasm. Saudi Journal of Biological Sciences 29, 3528–3538.

Djaha, K.E., Abo, K., Bonny, B.S., Kone, T., Amouakon, W.J.L., Kone, D., Kone, M., 2017. Caractérisation agromorphologique de 44 accessions de manioc (*Manihot esculenta* Crantz) cultivés en Côte d'Ivoire. International Journal of Biological and Chemical Sciences 11, 174–184.

Drisya Ravi, R.S., Nair, B.R., Siril, E.A., 2021. Morphological diversity, phenotypic and genotypic variance and heritability estimates in *Moringa oleifera* Lam.: a less used vegetable with substantial nutritional value. Genetic Resources and Crop Evolution 68, 3241–3256.

Gandji, K., Tovissodé, F.C., Azihou, A.F., Akpona, J.D.T., Assogbadjo, A.E., Kakaï, R.L.G., 2020. Morphological diversity of the agroforestry species *Moringa oleifera* Lam. as related to ecological conditions and farmers' management practices in Benin (West Africa). South African Journal of Botany 129, 412–422.

Griffith, M.P., Clase, T., Toribio, P., Piñeyro, Y.E., Jimenez, F., Gratacos, X., Sanchez, V., Meerow, A., Meyer, A., Kramer, A., 2020. Can a botanic garden metacollection better conserve wild plant diversity? A case study comparing pooled collections with an ideal sampling model. International Journal of Plant Sciences 181, 485–496.

Houehanou, T.D., Prinz, K., Hellwig, F., Assogbadjo, A.E., Gebauer, J., Glele Kakaï, R.L., Sinsin, B., 2019. Morphological trait variation and relationships of *Afzelia africana* Sm. caused by climatic conditions and anthropogenic disturbance in Benin (West Africa). Genetic Resources and Crop Evolution 66, 1091–1105.

Hounkpèvi, A., Azihou, A.F., Kouassi, É.K., Porembski, S., Glèlè Kakaï, R., 2016. Climate-induced morphological variation of black plum (*Vitex doniana* Sw.) in Benin, West Africa. Genetic Resources and Crop Evolution 63, 1073–1084.

Idris, M.M., Mudi, S.Y., Datti, Y., 2014. Phytochemical screening and mosquito repellent activity of the stem bark extracts of *Euphorbia balsamifera* (Ait). ChemSearch Journal 5, 46–51.

Imorou, L., Togbé, E.C., Fassinou Hotegni, N.V., Bello, D.O., Biaou, B.O., Nuer, A.T., Adoukonou-Sagbadja, H., Ahoton, L.E., 2021. Galactogenic plant diversity, phenology and local in situ conservation practices in agro-ecological zones of Benin Republic. Genetic Resources and Crop Evolution 68, 979–998.

Kamba, A., Hassan, L.G., 2010. Phytochemical screening and antimicrobial activities of *Euphorbia balsamifera* leaves, stems and root against some pathogenic microorganisms. African Journal of Pharmacy and Pharmacology 4 (9), 645–652.

Kelly, B.A., MalÃ, A., DembÃ, S.G., 2021. Variation of *Parkia biglobosa* morphological traits according to land use and agro-climatic zones in Southern Mali. African Journal of Plant Science 15, 20–27.

Khadivi, A., Anjam, R., Anjam, K., 2018. Morphological and pomological characterization of edible fig (*Ficus carica* L.) to select the superior trees. Scientia Horticulturae 238, 66–74.

Kimaro, J., Lulandala, L., 2013. Human influences on tree diversity and composition of a coastal forest ecosystem: The case of Ngumburuni forest reserve, Rufiji, Tanzania. International Journal of Forestry Research, 305874. https://doi.org/10.1155/2013/305874.

Kosh-Komba, E., Toumnou, L.A., Touckia, G.I., Innocent, Z., De Mon-Zoni, L.G., Semballa, S., Yandia, P., Batawila, K., Akpagana, K., 2017. Comparative performance of traditional farming practices and improved practice on the development of cassava in the field in Pissa (Central African Republic). Journal of Experimental Agriculture International 19 (6), 1–10.

Kumar, S., Kaushik, R.A., Jain, D., Saini, V.P., Babu, S.R., Choudhary, R., Ercisli, S., 2022. Genetic diversity among local mango (*Mangifera indica* L.) germplasm using morphological, biochemical and chloroplast DNA barcodes analyses. Molecular Biology Reports 1–11.

Lawin, I.F., Fandohan, A.B., Salako, K.V., Assogbadjo, A.E., Ouinsavi, C.A.I.N., 2021. Morphological variability of fruits of *Cola millenii* K. Schum. from seven phytogeographical districts in Benin: opportunity for domestication. Genetic Resources and Crop Evolution 68, 1225–1242.

Li, Y., Li, S., Lu, X., Wang, Q., Han, H., Zhang, X., Ma, Y., Gan, X., 2021. Leaf phenotypic variation of endangered plant *Tetracentron sinense* Oliv. and influence of geographical and climatic factors. Journal of Forestry Research 32, 623–636.

Mahmodi, N., Sharifi-Sirchi, G.-R., Cheghamirza, K., 2022. Evaluation of molecular and morphological diversity of caper (*Capparis spinosa* L.). Genetic Resources and Crop Evolution 1–26.

Mohammad, N., Dahayat, A., Pardhi, Y., Rajkumar, M., 2022. Morpho-molecular diversity assessment of Indian kino (*Pterocarpus marsupium* Roxb.). Journal of Applied Research on Medicinal and Aromatic Plants, 100373.

Mounce, R., Smith, P., Brockington, S., 2017. Ex situ conservation of plant diversity in the world's botanic gardens. Nature Plants 3, 795–802.

Mousavi, S.M.N., Nagy, J., 2021. Evaluation of plant characteristics related to grain yield of FAO410 and FAO340 hybrids using regression models. Cereal Research Communications 49, 161–169. https://doi.org/10.1007/s42976-020-00076-3.

Neafsey, D.E., Taylor, A.R., MacInnis, B.L., 2021. Advances and opportunities in malaria population genomics. Nature Reviews Genetics 22, 502–517.

Okello, J., Okullo, J.B.L., Eilu, G., Nyeko, P., Obua, J., 2018. Morphological variations in Tamarindus indica LINN. Fruits and seed traits in the different agroecological zones of Uganda. International Journal of Ecology, 8469156. https://doi.org/10.1155/2018/8469156.

Ouédraogo, A., Kakaï, R.G., Thiombiano, A., 2013. Population structure of the widespread species, *Anogeissus leiocarpa* (DC.) Guill. & Perr. across the climatic gradient in West Africa semi-arid area. South African Journal of Botany 88, 286–295.

Rajarajan, K., Uthappa, A.R., Handa, A.K., Chavan, S.B., Vishnu, R., Shrivastava, A., Handa, A., Rana, M., Sahu, S., Kumar, N., 2022. Genetic diversity and population structure of *Leucaena leucocephala* (Lam.) de Wit genotypes using molecular and morphological attributes. Genetic Resources and Crop Evolution 69, 71–83.

Raji, R., Siril, E.A., 2021. Genetic diversity analysis of promising Ceylon olive (*Elaeocarpus serratus* L.) genotypes using morphological traits and ISSR markers. Current Plant Biology 26, 100201.

Riina, R., 2020. Three sweet tabaibas instead of one: splitting former *Euphorbia balsamifera* s.l. and resurrecting the forgotten *Euphorbia sepium*. Euphorbia World 16 (2), 41–45.

Riina, R., Villaverde, T., Rincón-Barrado, M., Molero, J., Sanmartín, I., 2021. More than one sweet tabaiba: Disentangling the systematics of the succulent dendroid shrub *Euphorbia balsamifera*. Journal of Systematics and Evolution 59, 490–503. https://doi.org/10.1111/jse.12656.

Saini, M.K., Prasad, J., Raju, P.V.S., Kothari, S.L., Shukla, J.K., Gour, V.S., 2021. Morphological descriptors and heritability as markers for oil yield in Balanites aegyptiaca (L.) Del.: a potential biodiesel xerophyte. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 91, 695–706.

Salazar-Villa, E., Alcaraz-Meléndez, L., León-Félix, J., Heredia, J.B., Soto-Landeros, F., Angulo-Escalante, M.A., 2020. Morphological variability and oil content of Jatropha platyphylla Müll. Arg. germplasm as determined using multivariate analysis. Scientia Horticulturae 261, 108968. https://doi.org/10.1016/j.scienta.2019.108968.

Salifou, C.F.A., Kassa, K.S., Ahounou, S.G., Moussa, H., Dotché, I.O., Agbozo, J.M., Issifou, M.T., Youssao, I.A.K., 2017. Plantes lactogènes des bovins et leurs modes de préparation dans les élevages traditionnels au Bénin. Livestock Research for Rural Development 29, 29.

Scholte, E.-J., Knols, B.G., Takken, W., 2006. Infection of the malaria mosquito Anopheles gambiae with the entomopathogenic fungus *Metarhizium anisopliae* reduces blood feeding and fecundity. Journal of Invertebrate Pathology 91, 43–49.

Sharma, S., Kaur, R., Kumar, K., Kumar, D., Solanke, A.K.U., 2021. Genetic variability in *Rubus ellipticus* collections assessed by morphological traits and EST-SSR markers. Journal of Plant Biochemistry and Biotechnology 30, 37–55.

Souza, M.L., Duarte, A.A., Lovato, M.B., Fagundes, M., Valladares, F., Lemos-Filho, J.P., 2018. Climatic factors shaping intraspecific leaf trait variation of a neotropical tree along a rainfall gradient. PLOS One 13, e0208512. https://doi.org/10.1371/journal.pone.0208512.

Suleiman, M., Suleiman, H.Y., 2014. Control of *Callosobruchus maculatus* (F.) [Coleoptera: Bruchidae] using leaf powders of *Euphorbia balsamifera* L. and *Lawsonia inermis* L. International Journal of Science Environment and Technology 3, 100–109.

Tiendrébéogo, K.F., Sawadogo, N., Gapili, N., Ouédraogo, M.H., Ouédraogo, R.F., Nanema, K.R., Ouoba, A., Sawadogo, M., 2021. Variability and relationships between characters of physic nut (*Jatropha curcas* L.) in Burkina Faso. Highlights in BioScience 4, 1–11.

Villaverde, T., Pokorny, L., Olsson, S., Rincón-Barrado, M., Johnson, M.G., Gardner, E. M., Wickett, N.J., Molero, J., Riina, R., Sanmartín, I., 2018. Bridging the micro- and macroevolutionary levels in phylogenomics: Hyb-Seq solves relationships from populations to species and above. New Phytologist 220, 636–650. https://doi.org/10.1111/nph.15312.

Xue, J., Gao, S., Fan, Y., Li, L., Ming, B., Wang, K., Xie, R., Hou, P., Li, S., 2020. Traits of plant morphology, stalk mechanical strength, and biomass accumulation in the selection of lodging-resistant maize cultivars. European Journal of Agronomy 117, 126073. https://doi.org/10.1016/j.eja.2020.126073.

Yang, T., Siddique, K.H., Liu, K., 2020. Cropping systems in agriculture and their impact on soil health – a review. Global Ecology and Conservation 23, e01118.