Plant Ecology and Evolution 153 (3): 455–465, 2020
https://doi.org/10.5091/plecevo.2020.1576

REGULAR PAPER

Natural intraspecific trait variation patterns of the wild soursop *Annona senegalensis* (Annonaceae) along a climatic gradient in Benin, West Africa

Achille Hounkpèvi[1,*,‡], Valère Kolawolé Salako[1,‡], Janine Conforte Fifonssi Donhouédé[1], Emilienne Houévo Daï[1], Frédéric Tovissodé[1], Romain Glèlè Kakaï[1] & Achille Ephrem Assogbadjo[1,2]

[1]Laboratoire de Biomathématiques et d'Estimations Forestières, Faculty of Agronomic Sciences, University of Abomey-Calavi, 04 BP 1525 Cotonou, Republic of Benin
[2]Laboratory of Applied Ecology, Faculty of Agronomic Sciences, University of Abomey-Calavi, 05 BP 1752 Cotonou, Republic of Benin
*Corresponding author: hounkpeviachille@gmail.com
‡These authors contributed equally to this work

Background and aims – Geographic patterns of phenotypic variability can inform understanding of the resilience potential of plant species to environmental hazards such as climate change. Such understanding provides support for conservation and domestication efforts. Here, we investigated natural morphological variation of the individuals, fruits, seeds, and leaves of the tropical shrub *Annona senegalensis* Pers. along a climatic gradient.

Methods – Morphological data were collected on shrubs, fruits, seeds, and leaves of 150 shrubs from five populations in the three climatic zones of Benin. Linear mixed effects models were used to test the variability of the morphological traits of the species and also to estimate the variance components in order to tease apart the importance of each source of variation. The most important morphological descriptors discriminating climatic zones were identified using a stepwise discriminant analysis. Redundancy analysis was then used to determine the relationships between discriminant morphological traits and bioclimatic variables.

Key results – Morphological traits of *A. senegalensis* varied greatly both within and among climatic zones. A substantial part (42%) of the among-climatic zones phenotypic variability in the species was attributable to climate, mainly rainfall and temperature. Morphological traits such as big shrubs, big fruits, and high number of seeds per fruit were associated with high mean annual rainfall and low mean temperature of the warmest quarter.

Conclusions – The findings suggest an important zonal adaptation of the species to climate variability. The phenotypic diversity pattern that we highlighted can be useful when designing conservation policies for the species. However, quantitative genetics through common garden or reciprocal transplantation experiments related to the species' populations would enable to explore the heritable part of the observed variability to support effective conservation and domestication efforts.

Keywords – *Annona senegalensis*; bioclimatic variables; climate variability; plant morphology; zonal adaptation.

Plant Ecology and Evolution is published by Meise Botanic Garden and Royal Botanical Society of Belgium
ISSN: 2032-3913 (print) – 2032-3921 (online)

INTRODUCTION

Conservation and domestication of indigenous edible plant species for the diversification of subsistence agriculture has emerged as a promising approach to enhance human livelihood (Leakey et al. 2007; Fandohan et al. 2017). The wild soursop (*Annona senegalensis* Pers., Annonaceae) is a tropical fruit shrub under domestication (Pinto et al. 2005). This is due to its high nutritional, medicinal, and economic importance for African rural communities across its geographical distribution range (Orwa et al. 2009; Mustapha 2013; Okhale et al. 2016). Fruits, leaves, and flowers of *A. senegalensis* are used for human and livestock consumption and medical treatments. Several phytochemical constituents including triterpenes, anthocyanins, glucids, coumarins, flavonoids, and alkaloids were identified in leaf, bark, and root of the species and are related to known medicinal properties such as anti-oxidant, analgesic, antiplasmodial, haemostatic, spermatogenic, insecticidal, antimicrobial, anthelmintic, antidiarrheal, anti-inflammatory, antispasmodic, anticonvulsant, antimalarial, antitripasonal, cytotoxic, antivenomous, hypnotic, and antinociceptive (Mustapha 2013; Okhale et al. 2016). This highlights the high cultural importance of the species and attests its potential for local food and pharmacological industries.

Besides its uses, phytocompounds, and biological activities, other interesting aspects of *A. senegalensis,* including distribution and ecology (Pinto et al. 2005) and molecular genetic diversity (Kwapata et al. 2007) have been documented. However, few studies, to our knowledge, have explored the morphological variation within this shrub species (Folorunso & Olorode 2006) despite the importance of a quantitative evaluation of the plant's morphological variability in planning conservation and domestication policies (Pauku et al. 2010; Gouwakinnou et al. 2011; Ewédjè et al. 2012; Padonou et al. 2017). Plant morphology is well known to be under the synergetic control of genotype and environment (Guerin et al. 2012). An important implication is that the pattern of this morphological variability is a useful tool for understanding the resilience potential of plant resources to environmental hazards, such as climate change (Hounkpèvi et al. 2016), in order to secure conservation and domestication efforts.

In Benin (West Africa), *A. senegalensis* is widespread across the three climatic zones (Guinean, Sudano-Guinean, and Sudanian) of the country (Adomou 2005) and thus offers opportunity to investigate how climatic gradient (dry–wet) determines variation of specific morphological traits. This study examined the morphological traits of shrub, leaves, fruits, and seeds of the wild soursop, and assessed their variations across contrasting environmental conditions in Benin. Considering the high genetic diversity observed in natural populations of *A. senegalensis* across a latitudinal gradient in Malawi (Kwapata et al. 2007), we expect a high variability in all morphological traits. In addition, we expect a significant differentiation between *A. senegalensis* populations across climatic zones as an expression of phenotypic plasticity or local adaptation of plants to variation in environmental conditions (Pigliucci 2006). In particular, we predict to detect a significant relationship between bioclimatic parameters and morphological traits that discriminate climatic zones. We expect so because bioclimatic parameters form an integral part of the factors differentiating the three climatic zones of Benin (Adomou et al. 2006).

MATERIAL AND METHODS

Target species

The wild soursop (*Annona senegalensis* Pers., Annonaceae) is a tropical, aromatic shrub with edible fruits that is 2–6 m, or occasionally up to 11 m, in height (Orwa et al. 2009). It occurs from Senegal to Sudan and is often found within woodland savannah understory, in swamp forests, along riverbanks, or in fallows (Arbonnier 2004; Orwa et al. 2009). Areas from 0 to 2400 m in elevation, 17–30°C of mean annual temperature, and 700–2500 mm of mean annual rainfall are suitable for the species (Orwa et al. 2009). The species has alternate, simple, and oblong to ovate leaves. Its flowers are solitary or in groups of 2–4, arising above the leaf axils. The edible fruit is egg-shaped and formed by many fused carpels. The seeds are numerous, cylindrical, oblong, and orange-brown (Orwa et al. 2009).

Study area

The study was conducted in the Republic of Benin (between 6°25′N and 12°50′N and 0°45′E and 3°50′E) in West Africa. The country has an altitudinal gradient from south to north starting from less than 10 m to more than 650 m. The climatic profile of the country shows three biogeographical zones (fig. 1). The Guinean zone is located between 6°25′N and 7°30′N and is characterised by a subequatorial climate with two rainy and two dry seasons. The rainfall is bimodal with an average rainfall of 1200 mm per year. The temperature varies between 25 and 29°C, and the relative humidity between 69 and 97%. The Sudano-Guinean zone, located between 7°30′N to 9°45′N, is a transitional zone with two rainy seasons but now tending towards one. The annual rainfall fluctuates between 900 and 1110 mm, the temperature ranges from 25 and 29°C, and the relative humidity from 31 to 98%. The Sudanian zone (9°45′N to 12°50′N) located in the northern part of the country has a tropical dry climate with two seasons (one rainy and one dry). The mean annual rainfall in this zone is often below 1000 mm, the relative humidity varies from 18 to 99%, and the temperature from 24 to 31°C (Adomou et al. 2006). Based on the homogeneity of the floristic patterns, ten phytogeographical districts are distinguished, namely Atacora chain, Mékrou-Pendjari, and North-Borgou in the Sudanian zone; South-Borgou, Zou, and Bassila in the Sudano-Guinean zone; and Coastal, Plateau, Pobè, and Valley of Ouémé in the Guinean zone (Adomou 2005).

Data collection

Based on the known distribution of the species in Benin (Akoegninou et al. 2006), two phytodistricts were selected in the Guinean zone (Plateau and Valley of Ouémé), two in the Sudano-Guinean zone (Bassila and South-Borgou), and one in the Sudanian zone (Mékrou-Pendjari) (fig. 1). In each of the selected phytodistricts, two to three sites were considered after a field prospection with local people. A distance of at least 15 km was observed between sites. The selected sites were either in fallows or shrub savannahs. We did not consider sites in farmlands to limit interference of humans and animals as much as possible although the selected sites might have experienced such disturbances (e.g., fire and grazing) in the past. On each site, ten to 15 shrubs were randomly selected, with a distance of at least 100 meters between two consecutive shrubs (Turnbull 1975). This was done in order to avoid genetically close individuals and to capture an important amount of the morphological diversity of the species. The selected shrubs per site were considered as belonging to the same subpopulation, while shrubs from a given phytodistrict were considered as from the same population. The GPS coordinates of each shrub were recorded. The morphological data were collected on a total of 150 shrubs from five populations in the three climatic zones. The data were collected at four levels: entire shrub, fruits, seeds, and leaves. On each shrub, diameter at the basis, total height, and number of branches were recorded. Five to ten ripe fruits were randomly harvested from each shrub following Cornelissen et al. (2003). Total length, width (middle diameter), and the fresh mass of each selected fruit were measured using an electronic digital caliper (0.01 mm resolution) and a scale

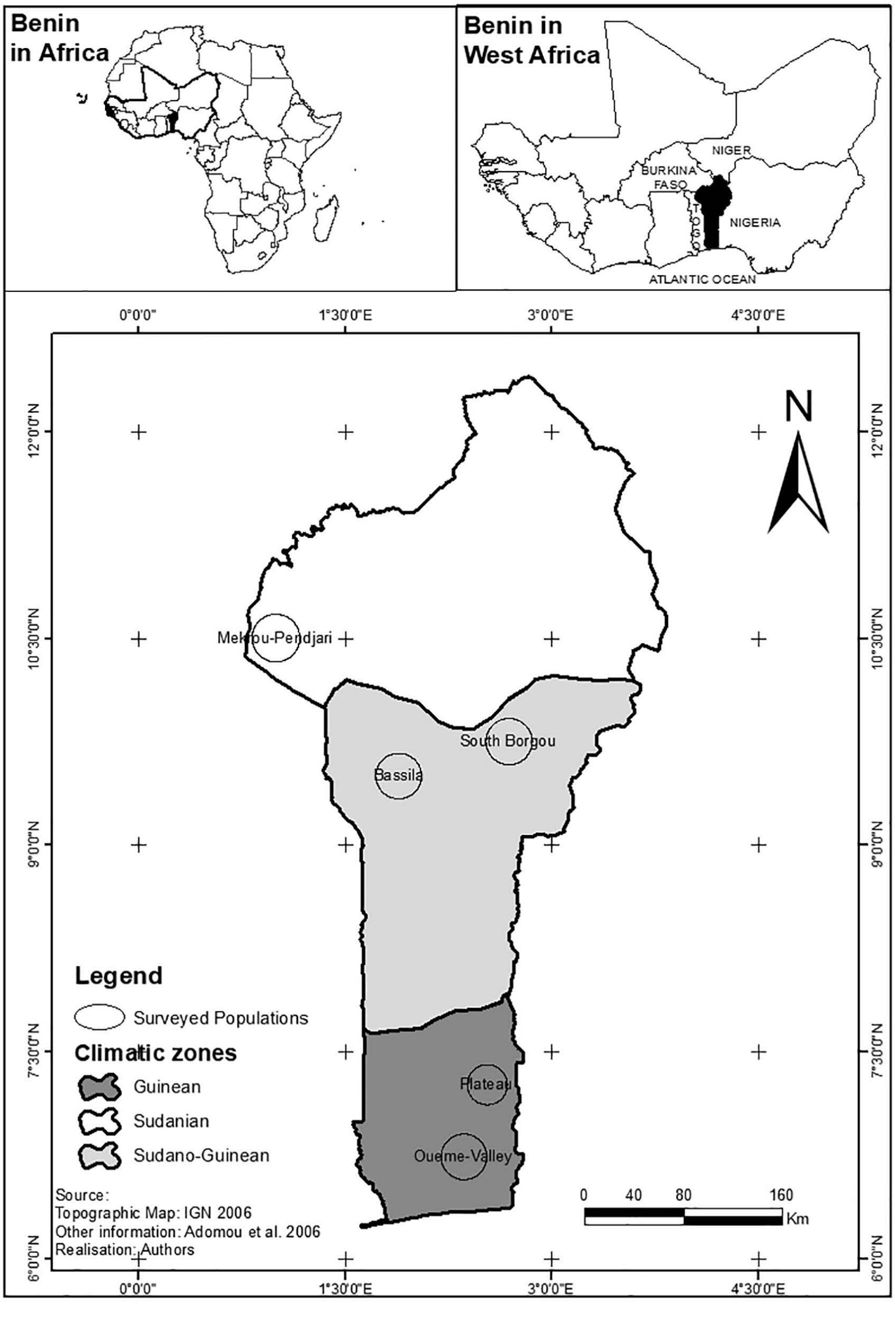

Figure 1 – Climatic zones of Benin and location of the surveyed populations.

(0.01 g precision), respectively. Afterwards, the fruits were sun-dried for one week in order to facilitate seeds extraction. The extracted seeds were counted and weighted together per fruit. In order to determine the average weight of the seed, seeds extracted per fruit were weighted and the total weight was divided by the number of seeds in the fruit. Concerning leaves' traits, five leaves were harvested on the third main branch (from the terminal bud) of each shrub. Leaves located at the 1st, 3rd, 5th, 7th, and 9th position on the branch were sampled. In order to determine the specific leaf area (SLA) of each leaf, a rectangular section of the leaf considering the greatest length and width of the limb was cut and its length and width were measured to determine the leaf area. The dry weight of the leaf section was determined after oven-drying the sample at 105°C for 72 hours.

The GPS coordinates of each shrub were further used in QGIS software (Qantum GIS Development Team 2016) to extract the bioclimatic data. The bioclimatic information was downloaded from the AfriClim database v.3.0 (Platts et al. 2015) (available at: https://webfiles.york.ac.uk/KITE/AfriClim/ and accessed on 10 Feb. 2017).

Data analysis

The specific leaf area (SLA) was computed using the following equation:

$$SLA_i = \frac{DW_i}{L_i \times W_i}$$

where, SLA_i is the specific leaf area of the i^{th} leaf (g/cm^2), DW_i, L_i, and W_i are dry weight (g), length (cm), and width (cm), respectively, of each leaf rectangular section.

All statistical analyses were performed in R v.3.3.1 (R Core Team 2017). Descriptive statistics parameters such as arithmetic mean, standard error of the mean, coefficient of variation (CV, in %), and coefficient of Skewness were calculated for each morphological trait. The CV was used to describe the morphological trait variability, whereas the coefficient of Skewness was computed in order to evaluate the distribution of the morphological trait per climatic zone. Linear mixed effects models were used to test the variability of each morphological trait of the species across the different sources of variation, namely climatic zone, phytodistrict (i.e., populations), site (i.e., subpopulations), shrubs, and organs (fruits, seeds, leaves). The same models were also used for variance decomposition to estimate the variance components. These variance components were used to assess the relative importance of sources of variation (among-climatic zones, within-climatic zone including among-populations within climatic zone, among-subpopulations within population, among-shrubs within site, and among-fruit/seed/leaf within shrub). For these analyses, climatic zone was considered as fixed factor, while the other factors (population, subpopulation, shrub, and fruit/seed/leaf) were considered as nested random factors. Since sample sizes (number of populations per climatic zone, number of subpopulations per population, number of shrubs considered per subpopulation, number of fruits/leaves per shrub) were unequal, Welch-Satterthwaite approximation was used as a correction method for the degrees of freedom in order to have accurate estimations of P-values (Mason et al. 2003). The models were performed using the *lme4* package (Bates et al. 2015). Tukey's contrasts for multiple comparisons of means from *multcomp* package (Hothorn et al. 2008) was used for means classification among climatic zones (Mangiafico 2015).

The most important morphological descriptors differentiating the species traits among climatic zones were identified using a stepwise discriminant analysis. This analysis was run in the R package *klaR* (Weihs et al. 2005) and based on morphological traits of shrubs, fruits, leaves, and seeds. Since several fruits and leaves were considered per shrub, values of morphological traits related to fruits, seeds, and leaves were considered as adjusted mean values at shrub level. These adjusted means were estimated as the lsmeans in package *emmeans* (Russell 2020) from the former linear mixed models. Finally, we used a redundancy analysis (RDA) to determine the relationships between the discriminant morphological traits and bioclimatic variables. The RDA was run on the standardized mean values of the previously selected discriminant morphological traits and the 21 bioclimatic variables extracted from AfriClim database. The best model was selected using the Akaike Information Criterion (AIC). Non-significant ($\Delta_{AIC} < 2$) bioclimatic variables were sequentially removed with the step function in the *vegan* package (Oksanen et al. 2013) and the final model was considered as the model with the lowest AIC (Cameron & Trivedi 2005).

RESULTS

Morphological variation of *A. senegalensis*

Morphological traits of shrubs – There was a relatively high amplitude of variation of morphological descriptors of shrubs in all climatic zones (CV > 30%). The most important variation was recorded in the Guinean zone (CV > 40%, table 1). The coefficient of Skewness is positive (right asymmetrical distribution) for all the morphological traits except for the height in the Sudanian zone. Diameter and height of the shrubs varied significantly among climatic zones (P-value < 0.05) and the highest values were noted in the Sudano-Guinean zone (13.42 ± 0.55 cm) and in the Guinean zone (3.21 ± 0.18 m) respectively. The number of branches per shrub did not vary significantly (P-value = 0.058) among climatic zones.

The variance decomposition (table 2) revealed that the among-climatic zones part is relatively high for the total height (29.43%), weak for the diameter (14.39%), and very weak for the number of branches per shrub (2.08%). Within climatic zones, the variability due to populations is very weak (< 2%) for all morphological traits of shrubs, while the part due to subpopulations (site) is high especially for diameter and height (more than 45%). The variability due to shrubs within subpopulations is also high for diameter (37.15%) and for height (23.72%) and very high for the number of branches per shrub (88.84%).

Morphological traits of fruits and leaves – The width and length of the fruits were relatively less dispersed around mean values (CV < 25%) than the other morphological traits (table 3). The morphological traits of fruits and leaves varied

Table 1 – **Variation of morphological traits of *A. senegalensis* shrubs along a south–north climatic gradient.**
For the same morphological trait, means followed by different letters (i.e., superscript a and b) are significantly different (P-value < 0.05, Tukey's contrasts test for multiple comparisons of means). n = number of shrubs; SE = standard error of mean; CV = coefficient of variation; Skew. = Skewness.

Climatic zones	Diameter (cm)			Height (m)			Number of branches		
	Mean ± SE	CV (%)	Skew.	Mean ± SE	CV (%)	Skew.	Mean ± SE	CV (%)	Skew.
Guinean (n = 60)	$9.98^{b} \pm 0.64$	49.86	0.96	$3.21^{a} \pm 0.18$	43.98	0.75	3.88 ± 0.24	48.6	1.5
Sudano-Guinean (n = 60)	$13.42^{a} \pm 0.55$	31.51	1.03	$1.60^{b} \pm 0.07$	32.41	0.72	2.97 ± 0.15	38.74	0.72
Sudanian (n = 30)	$8.63^{b} \pm 0.48$	30.49	0.61	$1.25^{b} \pm 0.08$	34.81	-0.25	3.10 ± 0.24	41.8	1.3

Table 2 – **Variance decomposition (%) for morphological traits of shrubs of *A. senegalensis* among different sources of variation.**

Variance component / sources of variation		Diameter	Height	Number of branches
Among climatic zones		14.39	29.43	2.08
Within climatic zone	**Among populations**	< 0.001	1.12	1.53
	Among subpopulations	48.46	45.73	7.55
	Among shrubs (error)	37.15	23.72	88.84

Table 3 – **Morphological characteristics of fruits, seeds, and leaves of *A. senegalensis* along a south–north climatic gradient.**
For a given characteristic (column), means followed by the same letters (i.e., superscript a, b, or c) are not significantly different (P-value > 0.05, Tukey posthoc test). n = sample size; SE = standard error of mean; CV = coefficient of variation; Skew. = Skewness; SLA = specific leaf area.

Climatic zones	Statistics	Fruit width (mm)	Fruit length (mm)	Fruit length/ width ratio	Fruit weight (g)	Pulp weight (g)	Seeds weight/ Fruit weight ratio	Number of seeds	Seed weight (g) (range)	SLA (g/cm^2) (range)
Guinean (n = 450)	Mean ± SE	$28.40^{a} \pm 0.28$	$28.63^{c} \pm 0.30$	$1.01^{b} \pm 0.01$	$14.61^{c} \pm 0.31$	$13.31^{c} \pm 0.33$	$0.09^{b} \pm 0.01$	$33.06^{b} \pm 0.81$	0.04^{b} (0.001–1.75)	0.008^{b} (0.001–0.026)
	CV (%)	21.22	22.49	13.31	45.55	52.97	183.99	51.86	234.19	36.59
	Skew.	-0.29	-0.55	-0.62	0.31	-1.76	13.75	0.38	16.29	1.52
Sudano-Guinean (n = 600)	Mean ± SE	$28.62^{a} \pm 0.26$	$33.02^{a} \pm 0.29$	$1.19^{a} \pm 0.01$	$18.88^{a} \pm 0.43$	$16.55^{a} \pm 0.40$	$0.15^{a} \pm 0.01$	$39.16^{a} \pm 0.67$	0.06^{a} (0.01–0.25)	0.010^{c} (0.002–0.028)
	CV (%)	21.85	21.28	25.58	55.27	59.86	93.13	41.78	45.61	26.89
	Skew.	-0.35	1.18	2.98	1.29	1.35	4.09	0.49	1.32	2.23
Sudanian (n = 300)	Mean ± SE	$22.39^{b} \pm 0.20$	$24.03^{b} \pm 0.23$	$1.08^{c} \pm 0.01$	$7.22^{b} \pm 0.17$	$6.07^{b} \pm 0.16$	$0.17^{a} \pm 0.01$	$20.40^{c} \pm 0.54$	0.60^{c} (0.01–0.21)	0.011^{a} (0.006–0.021)
	CV (%)	15.21	16.84	13.75	40.24	45.20	67.59	46.08	45.88	18.02
	Skew.	-0.08	-0.16	0.16	0.72	0.71	1.72	0.87	1	1.16

significantly among climatic zones (P-value < 0.05) and the highest values were mostly recorded in the Sudano-Guinean zone. Shrubs from the Sudanian zone produced fewer seeds per fruit (20.40 ± 0.54) but those seeds were heavier (0.60 ± 0.002 g) than elsewhere. Fruits from this climatic zone produced more pulp (16.55 ± 0.40 g) than those of the other regions. Fruits from this region also had an elongated shape (length/with ratio = 1.19 ± 0.01) than fruits from other zones. The specific leaf area (SLA) followed the same trend, with the highest value in the Sudanian zone (0.011 g/cm^2) followed by the Sudano-Guinean zone (0.010 g/cm^2).

The variance decomposition for morphological traits of fruits, leaves, and seeds revealed a relatively high part of variability due to the climatic zone (greater than 20%) except

Table 4 – **Variance decomposition (%) for morphological traits of fruits, seeds, and leaves of *A. senegalensis* among different sources of variation.**
* Depending on the trait.

Variance component / Sources of variation		Fruit width	Fruit length	Fruit length/ width ratio	Fruit weight	Pulp weight	Seeds weight / Fruit weight ratio	Number of seeds	Seed weight	Specific leaf area
Among climatic zones		22.54	25.23	31.03	37.42	29.74	18.22	7.84	5.66	11.88
Within climatic zone	**Among populations**	23.94	14.39	0.48	11.73	14.22	0.00	0.00	1.79	20.74
	Among subpopulations	16.14	8.84	22.06	11.78	10.41	0.64	1.20	0.00	1.42
	Among shrubs	24.24	36.56	23.40	25.89	31.95	46.64	35.19	57.89	52.87
	Among fruits/ leaves/seeds* (error)	13.14	14.98	23.03	13.18	13.68	34.50	55.77	34.66	13.09

for seeds weight/fruit weight ratio (18.22%), specific leaf area (11.88%), number of seeds (7.84%), and seed weight (5.66%). For all traits, the variability due to shrub within subpopulation (site) is high (23–58%), particularly for seed traits and specific leaf area (table 4).

Discriminant morphological descriptors

Four of the twelve morphological descriptors were found to be important to discriminate the shrubs regarding climatic zones. These discriminant morphological descriptors were shrub diameter and height, number of seeds per fruit, and pulp weight. The first two canonical axes were significant (P-value < 0.001) and explained 100% of the variance. The first axis (73.55% of the total variance) discriminated Sudanian shrubs from the Sudano-Guinean's ones based on shrub height, number of seeds per fruit, and pulp weight (fig. 2). The second axis (26.45% of the total variance) performed shrubs discrimination based on shrub diameter. It separated Guinean shrubs from individuals of the other climatic zones (fig. 2). This stepwise discriminant analysis showed an accuracy rate of 87%, revealing then that 87% of the shrubs were perfectly assigned to their respective climatic zone.

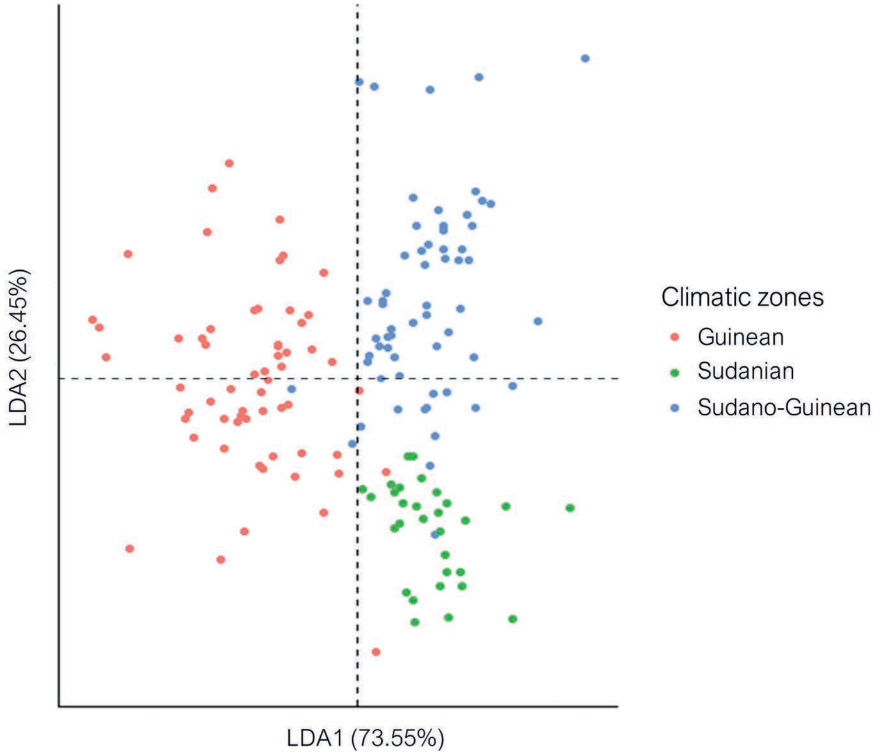

Figure 2 – Projection of *A. senegalensis* individuals regarding morphological descriptors in the system defined by the two discriminant functions.

Table 5 – Significance and scores of bioclimatic variables discriminating morphological traits of shrub, fruit, seed, and leaf of *A. senegalensis*.

bio4 = temperature seasonality; bio5 = maximum temperature of the warmest month; bio6 = minimum temperature of the coolest month; bio10 = mean temperature of the warmest quarter; bio15 = rainfall seasonality; bio16 = rainfall of the wettest quarter; bio17 = rainfall of the driest quarter; mimq = moisture index of the moist quarter; RDA = axes from the redundancy analysis; d.f. = degrees of freedom, F = Fisher statistics related to the test; Pr(>F) = probability of the significance of the bioclimatic variable in discriminating morphological traits.
Bold values indicate attribution of the variables to the RDA axis.

Bioclimatic variables	Permutation ANOVA				Scores on axes	
	d.f.	Variance	F	Pr(>F)	RDA1	RDA2
bio4	1	5.023	5.79	0.014	**-0.732**	-0.099
bio5	1	6.814	7.85	0.001	**-0.324**	-0.214
bio6	1	6.452	7.44	0.002	**-0.295**	0.068
mimq	1	7.407	8.54	0.001	-0.069	**0.156**
bio10	1	8.384	9.66	0.001	**-0.819**	-0.183
bio15	1	3.606	4.16	0.021	**-0.093**	0.057
bio16	1	3.836	4.42	0.017	**-0.141**	0.128
bio17	1	2.069	2.39	0.075	0.024	**0.066**
Residual	141	122.325				

Relationships between discriminant morphological traits and bioclimatic parameters

Eight of the 21 bioclimatic variables were identified as relevant (table 5) to describe the relationships between morphological traits and bioclimatic conditions (AIC = 738; adjusted r-squared = 0.42; d.f. = 8; F-value = 14.288; P-value = 0.001). Two redundancy axes were significant (P-value < 0.05) and then considered because together they explained 96.5% of the total variation in morphological traits due to the bioclimatic variables. The first axis (RDA1) explaining 87.13% was a linear combination of the temperature seasonality (bio4), mean temperature of the warmest quarter (bio10), maximum temperature of the warmest month (bio5), and of the minimum temperature of the coolest month (bio6). On this axis, all the discriminant morphological traits (diameter and height of shrub, number of seeds, and pulp weight) were loaded. These traits were negatively affected by the temperature seasonality (bio4), maximum temperature of the warmest month (bio5), and the minimum temperature of the coolest month (bio6). As far as the second axis (RDA2, 9.37% of total variation) was concerned, the remaining three bioclimatic variables were considered but none of the discriminant morphological traits was loaded on this axis (tables 5 & 6). The projection of the shrubs in the RDA correlation triplot defined by the two first axes (fig. 3) showed that shrubs from the Sudanian zone seemed more influenced by rainfall seasonality (bio15), rainfall of the wettest quarter (bio16), and the moisture index of the moist quarter (mimq). While shrubs from Sudano-Guinean zone and in some extend those from the Guinean zone were mainly under the effects of the maximum temperature of the warmest month (bio5) and of the rainfall of the driest quarter (bio17).

DISCUSSION

This work assessed the phenotypic diversity of *A. senegalensis* and its potential resilience to environmental hazards like climate change/variability. Such information can be useful to strengthen and secure conservation and domestication efforts regarding the species. In particular, the study examined morphological traits of *A. senegalensis* at four levels (shrub, leaf, fruit, and seed) and assessed their variations along a climatic gradient.

We found a relatively important variability in morphological traits of shrubs, leaves, and seeds than of fruits (tables 1, 3). Moreover, a relatively high part of this variability was recorded among climatic zones, especially for the fruit traits (fruit weight, fruit length/width ratio, pulp weight, fruit length, and fruit width) and shrub traits (diameter and height). This finding is further supported by the first two canonical functions from the discriminant analysis that showed a clear separation of shrubs from the climatic zones (fig. 2). The major discriminant traits (shrub height and pulp weight) indicated a differentiation in growth, and the tallest shrubs (also with medium pulp weight) were observed in the Guinean zone. This can be attributed to the greater availability of water in this zone. The greater rainfall might also explain why the biggest and heaviest fruits are found in the Guinean zone (table 3). The number of branches per shrub was relatively constant along the climatic gradient suggesting that this morphological trait cannot be used as a discriminant parameter among climatic zones.

Our findings show a substantial part of the phenotypic variability in *A. senegalensis* due to climate, suggesting that the species has an important phenotypic plasticity, and this plasticity is known to confer a significant adaptation ability against environmental stresses (Pigliucci 2006; Sana et al.

Table 6 – Scores of discriminant morphological descriptors on redundancy axes (RDA).
Bold values indicate attribution of the variables to the RDA axis.

Morphological traits	RDA1	RDA2
Shrub diameter	**1.87**	-0.77
Shrub height	**0.17**	0.14
Number of seeds per fruit	**6.85**	1.62
Pulp weight	**4.57**	-2.11

2018; Wang et al. 2020). The observed multilevel variability may nevertheless also express intraspecific genetic diversity as observed for the species in Malawi (Kwapata et al. 2007). Therefore, quantitative genetic investigations about the studied populations are necessary to examine the heritable part of the observed variability. For example, traits related to seeds (weight and number per fruit) showed the highest overall intraspecific variability (table 3) but a low variability between climatic zones (5.66% and 7.84% respectively). Yet, this important variability of morphological traits (shrubs, leaves, fruits, and seeds) of the species can be considered as an asset for the selection of 'plus trees' in domestication process because different 'ideotypes' can be developed to meet various utilization needs (Akinnifesi et al. 2008; Abasse et al. 2011; Padonou et al. 2017). However, this cultivar selection should consider several shrubs due to the high variability among shrubs within subpopulations (sites). In addition to the low variability observed in seeds traits regarding climatic zone, the species have been reported to display low phenotypic variability at seedlings life stage (Pinto & Sylva 1996).

The redundancy analysis revealed significant relationships between discriminant morphological descriptors of *A. senegalensis* and bioclimatic parameters which explained an important part of the variation (42%) in morphological traits. This supports the strong control of bioclimatic parameters on the among climatic zones variability of discriminant morphological descriptors. However, since topography and soil properties (type, moisture, nutrient content) varied across climatic zones of Benin (Adomou et al. 2006) and are also potential sources of phenotypic diversity (Hounkpèvi et al. 2016; Wang et al. 2020), their impact on *A. senegalensis* shrubs might also be important. Further investigations are required in order to disentangle the role of climate and soil in the morphological diversity of the species. Only 42% of the morphological variability in the species is potentially explained by the considered bioclimatic parameters. In addition to soil, and topography, other factors, not considered in the current study, may contribute to the differentiation of the morphological traits of the species (Wang et al. 2020). For example, disturbances by human (e.g., fire and pruning) and chronic grazing by herbivores (e.g., cattle) or defoliation by insects, might also contribute to the variation in the

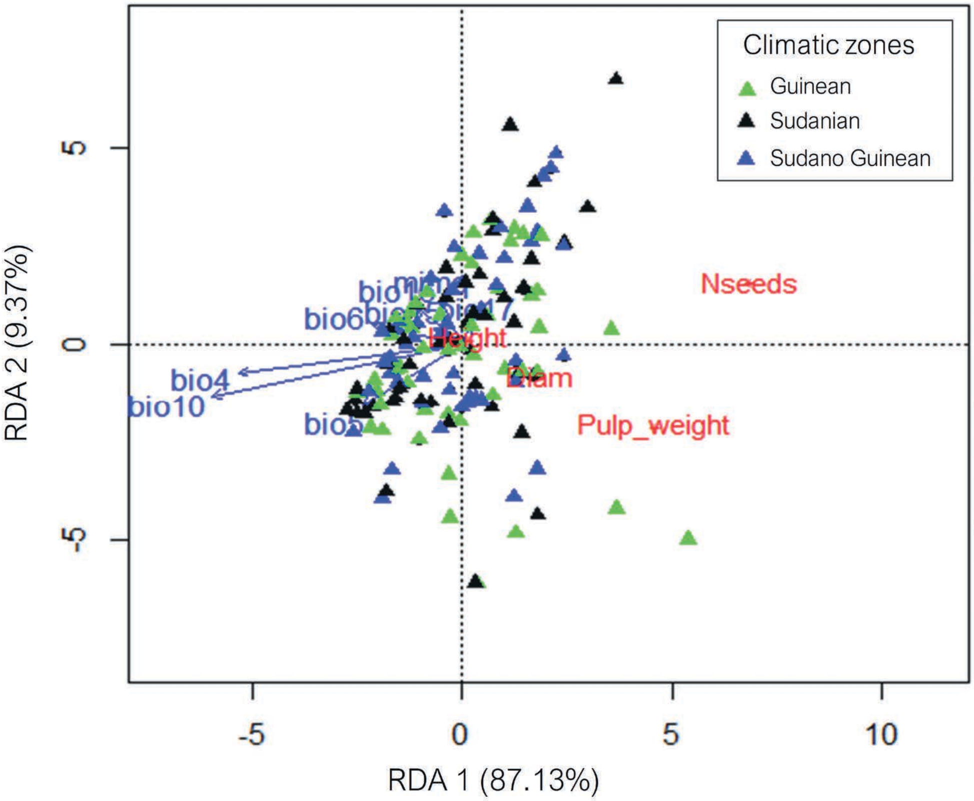

Figure 3 – Projection of *A. senegalensis* shrubs in the RDA correlation triplot defined by the two first axes. bio4 = temperature seasonality; bio5 = maximum temperature of the warmest month; bio6 = minimum temperature of the coolest month; bio10 = mean temperature of the warmest quarter; bio15 = rainfall seasonality; bio16 = rainfall of the wettest quarter; bio17 = rainfall of the driest quarter; mimq = moisture index of the moist quarter; RDA = axes from the redundancy analysis.

species traits, especially because the studied populations are not within protected areas. Some studies (e.g., Semmartin & Ghersa 2006; Venter & Witkowski 2013; Ooi et al. 2014) have shown that in addition to climatic variables, grazing and fire may affect plant morphology and functional traits. Our analyses show that the climatic drivers of the species' phenotypic diversity are mostly related to rainfall (rainfall seasonality, rainfall of the wettest quarter) for which high values in the Guinean and Sudano-guinean zones were associated to good performances regarding fruits characteristics such as big fruits, high number of seeds per fruit, and high pulp content. The phenotypic diversity was also greatly determined by the maximum temperature of the warmest month for which high values in the Sudanian zone was associated to good performance in terms of seeds weight/fruit weight ratio. The positive relationships between rainfall and morphological traits in *A. senegalensis* were also reported for other species including *Vitex doniana* Sweet (Hounkpèvi et al. 2016) and *Adansonia digitata* L. (Assogbadjo et al. 2005) in the same study area, and by Maranz & Wiesman (2003) on *Vitellaria paradoxa* C.F.Gaertn. in Mali and Burkina-Faso. On the contrary, other studies, (e.g., Abasse et al. 2011) found a negative relationship between rainfall and fruit size for *Balanites aegytiaca* Delile in Niger where larger fruit size was found in drier conditions. These contradicting results suggest that rainfall impact on fruit size are species-specific and might be linked to species-specific fitness strategies to environmental conditions. Bioclimatic parameters related to extreme temperatures (minimum temperature of the coolest month, maximum temperature of the warmest month, mean temperature of the warmest quarter) and extreme rainfalls (rainfall of the wettest quarter, rainfall of the driest quarter) were also determinant of the height of *A. senegalensis*. Such relationship of extreme temperature and rainfall with morphological variability have also been found for *V. doniana* in Benin (Hounkpèvi et al. 2016). Several previous studies have reported strong impacts of climatic conditions on morphological traits of several other tree species including *Afzelia africana* Sm. (Houehanou et al. 2019), *A. digitata* (Assogbadjo et al. 2011), *Detarium microcarpum* Guill. & Perr. (Kouyaté & Van Damme 2002), and *V. paradoxa* (Ugese et al. 2010).

Despite the narrow geographical coverage of our data, this study provides evidence in support of great variability in the morphological traits of the species and the important role of climatic conditions in shaping this variability. However, data from a larger geographical range are needed to better understand the role of climate but also other sources of variation on the morphology of the species. Indeed, considering the large geographical distribution of the species, data collected only in Benin (a small part in terms of longitudinal expansion), may not provide enough information on the role of climate in determining the morphological characteristics of the species. Moreover, future studies of this kind should include as much as possible other environmental parameters such as topography and soil features so that the role of each component of the environment is clarified.

CONCLUSION

The present study investigated phenotypic diversity in *A. senegalensis* (shrubs, leaves, fruits, and seeds) and its relation to climate in order to support the conservation and the domestication of the species. Based on data collected in Benin, findings revealed significant morphological variability in the species among climatic zones as well as within climatic zones. These findings suggest an important phenotypic plasticity of the species towards environmental hazards like climate change and or variability. Quantitative genetic studies will be useful in order to explore the heritable part of the observed variability. Yet, conservationists can already find relevant information from the documented phenotypic diversity pattern while designing conservation policies for the species.

ACKNOWLEDGEMENTS

The authors are grateful to Athanase Kintonou and Simon Awokou for their significant help during field work. The authors are also thankful to the field guides for their determination during data collection.

REFERENCES

Abasse T., Weber J.C., Katkore B., Boureima M., Larwanou M., Kalinganire A. (2011) Morphological variation in Balanites aegyptiaca fruits and seeds within and among parkland agroforests in eastern Niger. *Agroforestry Systems* 81: 57–66. https://doi.org/10.1007/s10457-010-9323-x

Adomou A.C. (2005) Vegetation patterns and environmental gradients in Benin: implications for biogeography and conservation. PhD thesis. Wageningen University, Wageningen, the Netherlands.

Adomou A.C., Sinsin B., Van der Maesen L.J.G. (2006) Phytosociological and chorological approaches to phytogeography: a meso-scale study in Benin. *Systematics and Geography of Plants* 76(2): 155–178. https://www.jstor.org/stable/20649708

Akinnifesi F.K., Sileshi G., Ajayi O.C., Chirwa P.W., Kwesiga F.R., Harawa R. (2008) Contributions of agroforestry research and development to livelihood of smallholder farmers in Southern Africa: 2. Fruit, medicinal, fuel wood and fodder tree systems. *Agricultural Journal* 3(1): 76–88.

Akoegninou A., van der Burg W.J., van der Maesen L.J.G. (2006) Flore analytique du Bénin. Cotonou & Wageningen, Backhuys Publishers.

Arbonnier M. (2004) Trees, shrubs and lianas of West African dry zones. France, Editions Quae.

Assogbadjo A.E., Glèlè Kakaï R., Edon S., Kyndt T., Sinsin B. (2011) Natural variation in fruit characteristics, seed germination and seedling growth of *Adansonia digitata* L. in Benin. *New Forests* 41: 113–125. https://doi.org/10.1007/s11056-010-9214-z

Assogbadjo A.E., Sinsin B., Van Damme P. (2005) Caractères morphologiques et production des capsules de baobab (*Adansonia digitata* L.) au Bénin. *Fruits* 60(5): 327–340. https://doi.org/10.1051/fruits:2005039

Bates D., Maechler M., Bolker B., Walker S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48. https://doi.org/10.18637/jss.v067.i01

Cameron C., Trivedi P. (2005) Microeconometrics: methods and applications. , Cambridge, UK, Cambridge University Press.

Cornelissen J.H.C., Lavorel S., Garnier E., Diaz S., Buchmann N., Gurvich D.E., Reich P.B., ter Steege H., Morgan H.D., van der Heijden M.G.A., Pausas J.G., Poorter H. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* 51(4): 335–380. https://doi.org/10.1071/BT02124

Ewédjè E.-E.B.K., Parmentier I., Natta A., Ahanchédé A., Hardy O.J. (2012) Morphological variability of the tallow tree, *Pentadesma butyracea* Sabine (Clusiaceae), in Benin. *Genetic Resources and Crop Evolution* 59: 625–633. https://doi.org/10.1007/s10722-012-9802-1

Fandohan A.B., Chadaré L.J., Gouwakinnou G.N., Tovissodé C.F., Bonou A., Djonlonkou S., Houndélo L., Sinsin C., Assogbadjo A.E. (2017) Usages traditionnels et valeur économique de *Synsepalum dulcificum* au Sud-Bénin. *Bois & Forêts des Tropiques* 332: 17–30. https://doi.org/10.19182/bft2017.332.a31330

Folorunso A.E., Olorode O. (2006) Biosystematic studies in Annonaceae I. Vegetative and Floral Morphological Studies of some species of *Annona* in Nigeria. *Research Journal of Botany* 1(3): 118–124. https://doi.org/10.3923/rjb.2006.118.124

Gouwakinnou G.N., Assogbadjo A.E., Lykke A.M., Sinsin B. (2011) Phenotypic variations in fruits and selection potential in *Sclerocarya birrea* subsp. *birrea*. *Scientia Horticulturae* 129(4): 777–783. https://doi.org/10.1016/j.scienta.2011.05.041

Guerin G.R., Wen H., Lowe A.J. (2012) Leaf morphology shift linked to climate change. *Biology Letters* 8(5): 882–886. https://doi.org/10.1098/rsbl.2012.0458

Hothorn T., Bretz F., Westfall P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal* 50: 346–363. https://doi.org/10.1002/bimj.200810425

Houehanou T.D., Prinz K., Hellwig F., Assogbadjo A.E., Gebauer J., Glèlè 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. https://doi.org/10.1007/s10722-019-00773-x

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. https://doi.org/10.1007/s10722-016-0409-9

Kouyaté A.M., Van Damme P. (2002) Caractères morphologiques de *Detarium microcarpum* Guill. et Perr. au sud du Mali. *Fruits* 57(4): 231–238. https://doi.org/10.1051/fruits:2002020

Kwapata K., Mwase W.F., Bokosi J.M., Kwapata M.B., Munyenyembe P. (2007) Genetic diversity of *Annona senegalensis* Pers. populations as revealed by simple sequence repeats (SSRs). *African Journal of Biotechnology* 6(10): 1239–1247.

Leakey R.R.B., Tchoundjeu Z., Schreckenberg K., Simons A.J., Shackleton S., Mander M., Wynberg R., Shackleton C., Sullivan C. (2007) Trees and markets for agroforestry tree products: targeting poverty reduction and enhanced livelihoods. In: Garrity D., Okono A.M., Parrott S. (eds.) World agroforestry into the future: 11–22. World Agroforestry Centre, Nairobi, Kenya.

Mangiafico S.S. (2015) An R Companion for the Handbook of Biological Statistics, version 1.09i. New Brunswick, USA, Rutgers Cooperative Extension.

Maranz S., Wiesman Z. (2003) Evidence for indigenous selection and distribution of the shea tree, *Vitellaria paradoxa,* and its potential significance to prevailing parkland savanna tree patterns in sub-Saharan Africa north of the equator. *Journal of Biogeography* 30(10): 1505–1516. https://doi.org/10.1046/j.1365-2699.2003.00892.x

Mason R.L., Gunst R.F., Hess J.L. (2003) Statistical design and analysis of experiments: With applications to engineering and science. 2nd Edition. Hoboken, John Wiley & Sons.

Mustapha A. (2013) *Annona senegalensis* Pers: a multipurpose shrub, its phytotherapic, phytopharmacological and phytomedicinal uses. *International Journal of Science and Technology* 2(12): 862–865.

Okhale S.E., Akpan E., T. F.O., Esievo K.B., Kunle F.O. (2016) *Annona senegalensis* Persoon (Annonaceae): A review of its ethnomedicinal uses, biological activities and phytocompounds. *Journal of Pharmacognosy and Phytochemistry* 5(2): 211–219.

Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H., Wagner H. (2013) Vegan: Community Ecology Package. R package version 2.0-10. Available at https://cran.r-project.org/web/packages/vegan/index.html [accessed 9 Sep. 2020].

Ooi M.K., Denham A.J., Santana V.M., Auld T.D. (2014) Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecology and Evolution* 4(5): 656–671. https://doi.org/10.1002/ece3.973

Orwa C., Mutua A., Kindt R., Simons A., Jamnadass R.H. (2009) Agroforestree database: a tree reference and selection guide. Version 4.0. Nairobi, World Agroforestry Centre (ICRAF). CD-ROM.

Padonou E.A., Tovissodé F.C., Idohou R., Salako V.K., Fantondji L., Vihotogbé R., Fandohan B., Assogbadjo A.E. (2017) Pilot assessment of locally acknowledged morphotypes of *Irvingia gabonensis* (Aubry-Lecomte) Baill. in southwestern Benin (West Africa). *Fruits* 72(5): 306–316. https://doi.org/10.17660/th2017/72.5.6

Pauku R.L., Lowe A.J., Leakey R.R. (2010) Domestication of indigenous fruit and nut trees for agroforestry in the Solomon Islands. *Forest, Trees and Livelihood* 19: 269–287. https://doi.org/10.1080/14728028.2010.9752671

Pigliucci M. (2006) Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology* 209: 2362–2367. https://doi.org/10.1242/jeb.02070

Pinto A.C.D.Q., Sylva E.M. (1996) Graviola para Exportaçao, aspectos Técnicios da Produçao. Brasilia, Embrapa/SPI.

Pinto A.C.D.Q., Cordeiro M.C.R., De Andrale S.R.M., Ferreira F.R., De C. Figueras H.A., Alves R.E., Kinpara D.I. (2005) *Annona species*. Southampton, UK, International Centre for Underutilized Crops, University of Southampton.

Platts P.J., Omeny P.A., Marchant R. (2015) AFRICLIM: high-resolution climate projections for ecological applications in Africa. *African Journal of Ecology* 53(1): 103–108. https://doi.org/10.1111/aje.12180

Quantum GIS Development Team (2016) Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. Available at https://www.qgis.org [accessed 9 Sep. 2020].

R Core Team (2017) R: a language and environment for statistical computing. Vienna, R Foundation for Statistical Computing. Available at https://www.r-project.org/ [accessed 9 Sep. 2020].

Russell L. (2020) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.4. Available at https://cran.r-project.org/web/packages/emmeans/index.html [accessed 9 Sep. 2020].

Sana F., Mansoor H., Farooq A., Muhammad A., Rashid A. (2018) Structural and functional modifications in a typical arid zone species *Aristida adscensionis* L. along altitudinal gradient. *Flora* 249: 172–182. https://doi.org/10.1016/j.flora.2018.11.003

Semmartin M., Ghersa C.M. (2006) Intraspecific changes in plant morphology, associated with grazing, and effects on litter quality, carbon and nutrient dynamics during decomposition. *Austral Ecology* 31(1): 99–105. https://doi.org/10.1111/j.1442-9993.2006.01556.x

Turnbull J.W. (1975) Seed collection sampling consideration and collection techniques. Rome, FAO.

Ugese F.D., Baiyeri P.K., Mbah B.N. (2010) Agroecological variation in the fruits and nuts of shea butter tree (*Vitellaria paradoxa* C.F. Gaertn.) in Nigeria. *Agroforestry Systems* 79: 201–211. https://doi.org/10.1007/s10457-009-9261-7

Venter S.M., Witkowski E.T.F. (2013) Where are the young baobabs? Factors affecting regeneration of *Adansonia digitata* L. in a communally managed region of southern Africa. *Journal of Arid Environments* 92: 1–13. https://doi.org/10.1016/j.jaridenv.2012.12.010

Wang M., Zhang J., Guo Z., Guan Y., Qu G., Liu J., Guo Y., Yan X. (2020) Morphological variation in *Cynodon dactylon* (L.) Pers., and its relationship with the environment along a longitudinal gradient. *Hereditas* 157: 1–11. https://doi.org/10.1186/s41065-020-00117-1

Weihs C., Ligges U., Luebke K., Raabe N. (2005) klaR analyzing German business cycles. In: Baier D., Decker R., Schmidt-Thieme L. (eds) Data analysis and decision support: 335–343. Berlin, Springer-Verlag. https://doi.org/10.1007/3-540-28397-8_36

Communicating Editor: Isabel Larridon.

Submission date: 26 Jan. 2019

Acceptance date: 1 Sep. 2020

Publication date: 23 Nov. 2020