Genetic and morphological diversity in populations of *Annona senegalensis* Pers. occurring in Benin (western) and Mozambique (southern) Africa.

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Abstract

Background: Understanding morpho-genetic diversity and differentiation in species with relatively large distribution is crucial for conservation and sustainable management of their genetic resources. The present study focused on *Annona senegalensis*, an important multipurpose wild plant, distributed exclusively in natural ecosystems but facing several threats. The study assessed the genetic and morphological diversity, structure, and differentiation of the species among populations from western Benin and southern Mozambique (Africa). It further evaluated environment (climatic) determinants of the morphological variation of the species.
Methods: 154 individuals from four populations were phenotyped based on 19 plant, fruit, and leaf traits and further genotyped using 10 polymorphic nuclear microsatellite (nSSR) markers.
Results: The results indicated strong differences in plant, fruit, and leaf morphological traits between the western and the southern populations. Furthermore, the studied populations were characterized by a high genetic diversity, with an average genetic diversity index of 1.02. Western populations showed higher heterozygosity values (0.61-0.71) than southern populations (0.41-0.49). These two regions were clearly differentiated into two different genetic groups, with further genetic subdivisions reflecting the four different populations. Genetic variation between regions was higher (69.1%) than among populations (21.3%) and within populations (9.6%). Four distinct morphological clusters were obtained, which were associated with the four genetic groups representing each population. Nevertheless, climate, mainly precipitation and temperature indexes explained relatively higher variation in morphological traits in the western populations

(40.47%) than in the southern (27.98%). Our study suggests that both environment and genetics play important role in the morphological traits variation of *A. senegalensis*.

Introduction

The growing changes in land use and climate are leading to the loss of the natural habitats of many useful wild edible fruit trees (*Anuragi et al., 2016*). As a consequence, some of these species are threatened and have a narrow or fragmented distribution (*Chichorro et al., 2019*; *IUCN, 2022*). Sustainable management and conservation of such useful species require a better understanding of the existing diversity to better capture their potential. However, such information is available for only a limited number of species, and many species are yet to be documented. The need for this information is urgent considering the rapid environmental changes.

Population diversity is any measure that quantifies the magnitude of genetic and morphological variability within a population (*Hughes et al., 2016*). The more diverse a population is, the more it can adapt to a changing environment (*Sheidai et al., 2014*). Morphological traits have been used as a tool to characterize the unexplored potential of germplasm for developing elite genotypes, i.e., resilient, productive, and nutritive ones (*Folorunso and Modupe 2007*). Yet, the morphological variability observed in wild populations is the expression of the signal of genetic diversity which may further be shaped by the environmental conditions. For instance, the morphological variability of *Prunus serotina* Ehrh was reported to be influenced by temperature and precipitation extremes (*Guzman et al., 2018*). *Vitex doniana* Sweet fruit trees were also reported to be influenced by environmental traits, mainly climate factors (*Hounkpevi et al., 2016*). However, the morphological variability found in *Polygonum aviculare* L. species was reported to rather have a strong genetic basis (*Mosaferi et al., 2015*). Therefore, although both genetic diversity and environmental conditions can drive variation in the observed phenotypes, their relative importance varies across taxa.

Species with a wide range of distribution often grow under diverse climatic conditions which gives the opportunity to study how genes are expressed and the probable response of their populations to future climate change. *Annona senegalensis*, also known as the wild custard apple, is an edible fruit plant widely distributed in Africa (*Orwa et al., 2009*). It is native to tropical east and northeast, west and west-central, and southern Africa, as well as southern subtropical Africa (*Orwa et al., 2009*). In mainland Africa, its main southern distribution spans South Africa, Mozambique, and Botswana. The main northern distribution encompasses countries like Benin, Niger, Burkina-Faso, and Mali in Western Africa. *A. senegalensis* is a perennial woody plant, anemophily, predominantly outcrossing. It is a diploid species from the Annonaceae family, one of the largest tropical and subtropical families. It has a high nutritional, medicinal, and economic importance for African rural communities, contributing significantly to household livelihoods and income (*Mapongmetsem et al., 2012*; *Donhouede et al., 2022*). Different parts of this species are also used in traditional medicine to treat diseases such as tuberculosis, gastritis, and snake bites, among others (*Okhale et al., 2016*). As a traditional food plant in Africa, *A. senegalensis*

plays an important role in the context of food security and its domestication has the potential to improve nutrition, foster development, and support sustainable land use. However, A. senegalensis is facing several threats due to its high local exploitation and land use changes that have resulted in severe degradation of its habitat (*Kwapata et al., 2007*, *Ba et al., 2021*). Despite several past studies have highlighted that this species will likely disappear without any conservation efforts (*Campbell and Popenoe 1988*), genetic data that would assist in this procedure is still largely missing for this species. Only one study assessed the genetic diversity of this species and was based on only three microsatellite markers and three populations occurring in Malawi (*Kwapata et al., 2007*). In western Africa, some authors reported high morphological variability in *A. senegalensis* populations and attributed 42% of this variability to climate (*Hounkpevi et al., 2020*). Whether such morphological variation can still occur in a larger geographical range is unclear. Furthermore, whether the observed role of climate at the local scale can expand to a larger geographical range is essential to better understand the species' response to environmental conditions. The use of molecular markers is known as one of the best tools to study genetic material and explore genetic diversity in plants (*Feng et al., 2016*). Simple sequence repeat (SSR) or microsatellite markers are codominant, easily automated, highly polymorphic, highly reproducible, and cost-effective. Therefore, they have been widely used to assess genetic diversity among populations of a given taxon (*Gomes et al., 2020; Rohini et al., 2020; Senkoro et al., 2020; Xue et al., 2021; Eken et al., 2022*). The present study aimed to understand the morpho-genetic diversity, structure, and differentiation in A. senegalensis populations and the role of climate and genetic in phenotypic variability. Specifically, we have assessed, (i) the genetic diversity, population structure, and differentiation; (ii) the morphological diversity, and structure; (iii) the overlapping between genetic and morphological clustering of individuals; and (iv) the relative importance of climate in the morphological variation.

Materials & Methods

Study area

The study was carried out in Niassa Special Reserve – NSR, Mozambique (southern Africa), and in the Sudanian zone, Benin (western Africa), two locations where the species is best known and used. NSR is located in Northern Mozambique approximately between latitudes 12°8′40″ N and 12°22′40″ N; and longitudes 37°21′00″ E and 37°45′00″ E (Fig.1). It covers approximately 42 000 km2 and has been described as the largest protected area of Mozambique and the third largest in Africa (*Ryan et al., 2016; Mbanze et al., 2019*). Seventy-two percent of the total area of NSR is covered by dry Zambezian Miombo woodlands that are dominated by *Brachystegia spiciformis, Brachystegia boehmii* and *Julbernardia globiflora* (*White 1983*). The climate is tropical sub-humid, with a dry and relatively hot period between May and October. The annual rainfall is on average 900 mm per year increasing from the east (800 mm) to the west (1200 mm). Temperature ranges between 20 and 26 °C during the dry season and is on average 30 °C during the wet season (*Ribeiro et al., 2008*). About 60.000 people are living inside the reserve and are concentrated around the two main villages of Mecula (Moz_MEC) in the east and

Mavago (Moz_MAV) in the west and along the main road (*NCP, 2017; SRN, 2008; Mbanze et al., 2021*). Slash-and-burn agriculture is the main livelihood activity of the population (*Cunliffe et al., 2009*).

The Sudanian zone is located in Northern Benin between latitudes 9°45' N and 12°25' N and longitudes 0°45′ E and 3°55′ E (Fig.1) and is characterized by a tropical dry climate with two seasons (rainy and dry). The mean annual rainfall in this zone is often below 1000 mm and the temperature is relatively higher than in Mozambique, varying from 24 to 31 °C (*Adomou et al., 2006*). The vegetation is composed of dry forests, woodlands, savannahs, and riparian forests. Common tree species in the area include *Isoberlinia*, *Combretum* spp., *Acacia* spp., *Hyparrhenia* spp., *Loudetia* spp., and *Andropogon* spp. (*Adomou et al., 2006; Gnanglè et al., 2012*). North Borgou (Ben_BGN) and Mekrou pendjari (Ben_MPE) are the two main phytogeographical districts of the Sudanian zone of Benin. People living in the Sudanian zone of Benin are mainly farmers.

Sampling and data collection

A total of 154 individuals of *Annona senegalensis* from the two geographical regions (Fig 1; Table 1) were analyzed for genetic diversity and population structure. Due to unavailability of trees bearing mature fruits in some populations, morphological data focused on a total of 147 individuals. In each region, two populations were selected and within each population, samples were collected along a linear transect of 30 km, within a minimum distance of 100 m to 10 km to avoid sampling siblings. Twenty-seven to sixty individuals were sampled within each population and used for genetic and morphological analysis (Table 1).

Samples of leaves and fruits from all the individuals were brought to the laboratory for morphological analysis. For the genetic analysis, fresh leaves were kept in silica gel while in the field and stored at -80 °C once in the laboratory until DNA extraction.

DNA extraction and nSSR amplification

Total genomic DNA was extracted from 50 mg of ground leaves using the InnuSPEED Plant DNA Kit (Analytik Jena Innuscreen GmbH, Germany) according to the manufacturer's protocol. The average yield and purity were assessed using spectrophotometry by OD230, OD260, and OD280 readings (Nanodrop 2000, Thermo Fisher Scientific, and Waltham, MA, USA) and visualized by electrophoresis in 1% agarose gels under UV light. Samples were genotyped based on ten polymorphic nuclear single sequence repeats (nSSRs) previously developed: LMCH4, LMCH6, LMCH11 (*Escribano et al., 2004*), LMCH29, LMCH43, LMCH48, LMCH78, LMCH79, LMCH119, and LMCH122 (*Escribano et al., 2008*). Based on an initial survey, we selected these nSSR markers since they produced robust and highly polymorphic bands among the entire collection of samples. Amplifications were performed in 15 µL reactions containing 1.25U MyTaq DNA polymerase and 1X MyTaq Reaction Buffer (meridian, Bioscience), 0.4 µM each primer (Table 2), and 100 ng of genomic DNA under the following PCR conditions: initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 sec,

annealing at 55 °C for 30 sec (except for LMCH29 where the temperature was 45 °C), followed by 72 °C for 1 min, and a final extension at 72 °C for 5 min. Forward primers were labeled with a fluorescent dye on the 5′-end. PCR products were separated by capillary electrophoresis on a CEQ™ 8000 capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA) and allele sizes were determined using GeneMapper 3.2 (Applied Biosystems; UK).

Data on morphological traits

Six morphological descriptors were measured on plants, namely total height (Tot.hei), bole height (Bol.hei), crown height (Crown.hei), trunk diameter at breast height (Dbh), crown diameter (Crown.diam) and crown shape (Crown.shp). The bole height is the height from the ground to the first big branch and the crown height is the difference between total height and bole height. The crown shape was derived from the ratio of crown height over crown diameter. To determine the crown diameter, four radii were measured from the projection of the crown on the ground (*Glèlè Kakaï et al., 2011; Hounkpèvi et al., 2016*). At least 40 leaves and 40 ripe fruits were collected per individual. Seven morphological fruit descriptors were measured and included fruit length (Fruit.leng), fruit width (Fruit.wid), fruit dry weight (Fruit.wei), number of seeds per fruit (Fruit.nseeds), seeds weight (Seeds.wei), pulp dry mass (Pulp.mass), fruit shape (Fruit.shp, the ratio fruit length to the fruit width) (*Hounkpèvi et al., 2016; Lawin et al., 2021*). Six quantitative descriptors were measured on leaves, including leave length (Leav.len), leave width (Leav.wid), limb length (Limb.len), petiole length (Petiol.len), leave dry weight (Leav.wei) and the ratio of leaves length to petiole length (Leav.len_Petiol.len) (*Sun et al., 2020*; *Mollick et al., 2021*). Fruits and leaves were further oven-dried at 105°C until constant weight for the determinations of fruit dry weight, pulp mass, seeds dry weight, and leaves dry weight. After measuring the fruit dry weight, each fruit was split manually and the seeds were separated from the pulp. The number of seeds per fruit were then counted, and the seeds weight and pulp mass were weighed afterward. Weights were measured using a 0.01 g precision scale while a centimeter rule and a digital caliper with a 0.01 mm level of precision were used for all others measurements (Table 3).

Bioclimatic data

Using the GPS coordinates of each individual in QGIS 3.16.2 (Quantum Development Team 2021), bioclimatic data for each individual was extracted from the CHELSA database (Climatologies at High resolution for the Earth's Land Surface Areas). Bioclimatic data considered the last data available over 30 years (1979-2013).

Data analysis

Genetic diversity, population structure, and differentiation

For each geographical area and population, genetic diversity was assessed by calculating the total number of alleles (Ta), mean number of alleles per locus (Na), Shannon's information index (H), mean expected heterozygosity (He), mean observed heterozygosity (Ho), inbreeding coefficient

(FIS), and % of polymorphic loci (PIC) using GenAlEx 6.51 (*Peakall and Smouse, 2012*). An analysis of variance was used to detect significant differences between populations for the measured genetic values using the same software.

The Bayesian program STRUCTURE v.2.3.4 (*Pritchard et al., 2020*) was used to test whether any discrete genetic structure existed among samples. The analysis was performed assuming clusters from K = 1 to K = 10, with 10 repetitions per K. Models were run assuming ancestral admixture and correlated allele frequencies using run lengths of 300,000 interactions for each K after 50,000 burn-in steps. The optimum K was determined using STRUCTURE HARVESTER (Earl and von Holdt 2012), which identifies the optimal K based on both the posterior probability of the data for a given K and the ΔK (*Evanno et al., 2005*). The results of the replicates at the best-fit K identified by STRUCTURE were then post-processed using CLUMPP 1.1.2 (Jakobsson et al., 2007). A principal component analysis (PCoA) was also constructed in GenAlEx 6.51 (*Peakall and Smouse, 2012*) to detect the genetic relatedness among individuals based on Nei's genetic distance. We used an analysis of molecular variance (AMOVA) to quantify the partitioning of genetic variance between the geographical regions, between and within all populations that showed genetic differentiation in STRUCTURE and PCoA analyses. Each AMOVA was run with 10000 permutations at 0.95 significance levels in Arlequin 3.11 (*Excoffier et al., 2005*). The relationships between population pairwise Nei's Da genetic distances and linear geographical distances (isolation by distance) were examined with a Mantel test (Mantel 1967) implemented in Arlequin 3.11 (*Excoffier et al., 2005*) using the same permutation and significance levels.

Morphological diversity and structuring

The mean, standard error, and coefficient of variation of each morphological trait were calculated by country and population. The coefficient of variation (cv) was used to assess the variability of each morphological trait, considering a cv < 25% an indicator of weak variability (*Reza et al., 2017*). A student t-test was first used to compare the traits between the northern and the southern populations. Similarly, an analysis of variance was used to compare the traits among the four populations. The assumptions of normality and Homoscedasticity required to run these tests were checked before, using the Shapiro-Wilks test and the Levene test respectively. When the violation of the assumption of normality is severe ($p < 0.01$), the corresponding non-parametric test (Mann-Whitney or Kruskal-Wallis) was applied. When the ANOVA indicated a significant difference, an SNK-test was applied as a multiple comparison test in the package agricolae (*de Mendiburu, 2020*) to separate means.

To assess the relationship between the morphological descriptors and the bioclimatic variables, a redundancy analysis (RDA) within the "vegan" package was carried out on the least square mean values of the morphological descriptors and bioclimatic variables (Table S, supplementary data). The RDA was first carried out separately for the western populations, and the southern populations. A third RDA analysis was implemented on all the studied populations together. These RDA analyses were intended to assess whether the relative importance of the relationships

between bioclimatic variables and morphological variation was similar for the two regions. All analyses were implemented in R statistical software (version 4.1.2 R Core Team 2021).

Results

Genetic diversity, structure, and differentiation

A total of 156 alleles were found among the 154 A. senegalensis samples. The number of alleles varied from 27 in the southern (Moz_MAV) to 55 in the western (Ben_BGN) (Table 4). The total number of alleles was significantly higher in the populations sampled in western regions than in the southern region ($F = 3.23$, $p=0.023$). This pattern was also recorded in the average number of alleles ($F= 2.05$, $p=0.001$), the Shannon Diversity Index ($F = 1.04$, $p=0.021$), and the observed ($F = 4.24$, $p=0.019$) and expected heterozygosity ($F = 4.47$, $p=0.024$) where these genetic values were higher in western than in southern populations (Table 4). The percentage of polymorphic loci was overall very high and showed the same pattern ($F = 3.39$, $p=0.025$) i.e., higher in the western than in the southern (Table 4). The coefficient of inbreeding (FIS) showed negative values in all sampled populations (Table 4) suggesting a heterozygosity higher than expected under the Hardy-Weinberg assumption. FIS values were lower in the western than in the southern populations ($F= 1.29$, $p=0.012$; Table 4).

The Bayesian clustering program STRUCTURE found the highest LnP(D) and ΔK values for $K = 2$ differentiating the samples collected in Benin from the ones collected in Mozambique (Fig. 2). Nevertheless, STRUCTURE further revealed a secondary high LnP(D) and ΔK values at K=4 differentiating the four populations, Ben_BGN, Ben_MPE, Ben_MEC, and Ben_MAV into different genetic clusters (Fig. 2). Despite an overall high genetic integrity found in most samples, the results showed some signs of admixture between the genetic groups found in Benin, and the ones sampled in Mozambique, although this admixture is negligible (Fig. 2). The same geographical pattern was retrieved by principal coordinate analysis (PCoA) (Fig. 3). The first two coordinates of PCoA explained 35.9% of the total variation. Samples were spatially separated considering the two main geographic areas (Benin and Mozambique), but also by populations following the K=4 (Fig. 2), clustering result found in STRUCTURE. The degree of spatial separation was lower for the two Mozambican districts than for the ones from Benin (Fig. 3).

AMOVA revealed that a high proportion of genetic variation was attributable to significant differences between the two regions (69.1%) supported by high levels of genetic differentiation ($FST = 0.305$, $p < 0.001$). In addition, 21.3% of variation occurred among populations while the remaining was found within populations. In addition, the Mantel's test confirmed the existence of a significant positive correlation between Nei's genetic distance and geographic distance for all pairwise populations ($r = 0.212$, $p < 0.001$).

Morphological diversity and structure

The morphological traits of *A. senegalensis* plants varied significantly between northern and southern populations. Plants of the southern population were significantly bigger (15.89 ± 2.10, cm) and taller (5.72 ± 0.75, m) than those from the northern population (5.89 ± 0.62, cm; 2.56 ± 0.27, m respectively) (Table 5). Irrespective of the populations, the values of the cv were high (cv > 25%) for all traits. However, the northern population had the highest values of cv irrespective of the traits, except for the crown shape. A significant difference was also noted among sub-populations. However, the sub-population Ben_BGN (from the northern population) had a similar DBH to that of Moz_MEC (from the southern population). Within the southern population; the sub-populations Moz_MEC and Moz_MAV had similar values for bole height. The variability decreased from populations (65.55% - 31.04%) to sub-populations (65.00% - 15.49%) but was still relatively high for the northern and southern populations, respectively. The DBH, bole height, crown height, crown diameter, and crown shape were more dispersed in Moz_MAV sub-population while total height was more dispersed in Ben_MPE (Table 5).

The morphological parameters of fruits and leaves varied significantly among populations (Table 6). Mozambique showed the highest values for fruit traits, and Benin the highest values for leaf traits (Table 6). Considering the four sub-populations, results showed that Moz_MAV had the highest value for fruit length, fruit shape, fruit dry weight, number of seeds, seeds weight, pulp dry mass, limb length, leaf length and leaves width. Ben_BGN had the highest value for fruit width, while the highest value for petiole length and leaves weight was recorded in Ben_MPE. Fruits from Mozambique were found to be more elongated (higher fruit shape) than the ones from Benin. However, some traits showed similar values between populations (Table 6). It is for example the case of the ratio of leaf length to petiole length. Furthermore, Ben_BGN and Moz_MEC presented similar values for fruit length and the number of seeds per fruit. Both regions and their respective populations showed high cv regarding all traits, except fruit length, width, and fruit shape (Table 6). For fruit length, cv values vary from 15.53% to 15.96% in western populations and from 14.53% to 18.76% in southern. For fruit width, cv values vary from 11.68% to 13.43% in western populations and from 12.46% to 14.58% in southern populations and for fruit shape, cv values vary from 7.41% to 10.03% in western populations and from 8.96% to 12.45% in southern populations. The hierarchical clustering of the individuals based on their morphological traits resulted in four clusters (Fig. 4).

Overlap between genetic and morphological clusters

The Chi-Square test performed to test the association between morphological clusters and genetic clusters (Pearson Chi-Square = 209.771, DF = 9, p = 0.000; Likelihood Ratio Chi-Square = 195.358, DF = 9, p = 0.000) suggested significant association. For instance, 86.67% of individuals in morphological cluster 1 correspond to genetic cluster 1; 70% of individuals in the morphological cluster 3 corresponds to the genetic cluster 3 (Table 7).

Influence of bioclimatic variables on the morphological variation

The redundancy analysis showed that there is a significant correlation between morphological traits and bioclimatic variables. Furthermore, this relationship varies in diverse ways according to the two regions. In all cases, only the first two axes were significant (p = 0.001) and explained the extent to which variation in morphological traits is related to bioclimatic variables. In the western populations, the model considered 9 out of the 19 bioclimatic variables (F = 7.7245, p = 0.001, adjusted R2 = 0.404). The first axis (RDA1) explained 80.77% of the total variance and was a combination of mean diurnal air temperature range (chelsa_b_1), temperature seasonality (chelsa_b_3), mean daily maximum air temperature of the warmest month (chelsa_b_4), annual range of air temperature (chelsa_b_6), mean daily mean air temperatures of the wettest quarter (chelsa_b_7), mean daily mean air temperatures of the warmest quarter (chelsa_b_9), mean monthly precipitation amount of the coldest quarter (chelsa_b_18). The second axis (RDA2) explained 8.56% of total variation and combined mean annual air temperature (chelsa_bio) and annual precipitation amount (chelsa_b_11).

In the southern populations, the model considered only 5 out of the 19 bioclimatic variables (F = 5.3517, p = 0.001, adjusted R2 = 0.2798). The first axis (RDA1) explained 67.17% of the total variance and was a combination of mean annual air temperature (chelsa_bio), mean diurnal air temperature range (chelsa_b_1), temperature seasonality (chelsa_b_3) and mean daily maximum air temperature of the warmest month (chelsa_b_4). The second axis (RDA2) explained 21.74% of total variation and only considered annual range of air temperature (chelsa_b_6).

While considering together the western and the southern regions, all populations merged, the model considered 11 out of the 19 bioclimatic variables (F = 12.489, p = 0.001, adjusted R2 = 0.46398). The first axis (RDA1) explained 83.44% of the total variance and was the combination of isothermally (chelsa_b_2); temperature seasonality (chelsa_b_3); mean daily minimum air temperature of the coldest month (chelsa_b_5); mean daily mean air temperatures of the wettest quarter (chelsa_b_7); mean daily mean air temperatures of the driest quarter (chelsa_b_8); annual precipitation amount (chelsa_b_11); precipitation amount of the wettest month (chelsa_b_12); precipitation amount of the driest month (chelsa_b_13); precipitation seasonality (chelsa_b_14) and mean monthly precipitation amount of the wettest quarter (chelsa_b_15). The second axis (RDA2) explained 6.19% of the variation and only considered mean daily mean air temperatures of the warmest quarter (chelsa_b_9). However some of these bioclimatic variables such as mean annual air temperature, temperature seasonality, mean monthly precipitation amount of the coldest quarter, annual range of air temperature and the mean monthly precipitation amount of the wettest quarter were not statistically significant in these different models (Table 8).

In western populations, trunk diameter at breast (Dbh), total height (Tot_hei), bole height (Bol. hei), crown height (Crown. hei), crown diameter (Crown. diam), crown shape (Crown.shp), fruit length (Fruit. leng), fruit width (Fruit. wid), fruit shape (Fruit.shp), fruit dry weight (Fruit. wei), number of seeds per fruit (Fruit. nseeds), seeds weight (Seeds. wei), pulp dry mass (Pulp. mass), leave width (Leav. wid), petiole length (Petiol. len), the ratio leaves length/petiole length

(Leav.len_Petiol.len), leave dry weight (Leav. wei) and limb length (Limb. len) were all loaded on RDA1 while only leave length (Leav. len) were loaded in RDA2 (Table 9). Based on the scores of morphological traits and bioclimatic variables on RDA axes (Tables 8, 9; Fig 5), Leav. len was positively influenced by annual precipitation (chelsa_b_11). Dbh, Tot_hei, Bol. hei, Crown. hei, Crown. diam, Fruit. leng, Fruit. wid, Fruit. wei, Fruit. nseeds, Seeds. wei, and Pulp. mass were negatively influenced by mean diurnal air temperature range (chelsa_b_1), mean daily maximum air temperature of the warmest month (chelsa_b_4), annual range of air temperature (chelsa_b_6) and the mean daily mean air temperatures of the wettest quarter (chelsa_b_7).
In the southern populations, all morphological parameters were loaded on RDA1 except Bol. hei, Crown. diam, Crown.shp, and the ratio Leav.len/Petiol.len which were loaded in RDA2. Crown. diam and the ratio Leav.len/Petiol.len were negatively influenced by the mean annual air temperature (chelsa_bio), mean diurnal air temperature range (chelsa_b_1), temperature seasonality (chelsa_b_3) and the mean daily maximum air temperature of the warmest month (chelsa_b_4) (Fig 5.).

When we considered the morphological traits in western and southern regions, all populations merged, morphological parameters were all loaded in RDA 1 except Limb. len, Leav. wid, the ratio Leav.len_Petiol.len, and Leav. wei. Dbh, Tot_hei, Bol. hei, Crown. hei, Crown. diam, Crown.shp, Fruit.leng, Fruit. wid, Fruit.shp, Fruit. wei, Fruit. nseeds, Seeds. wei, Pulp.mass, and Leav. len were positively influenced by the mean daily minimum air temperature of the coldest month (chelsa_b_5) and mean daily mean air temperatures of the driest quarter (chelsa_b_8) (Fig 5). The Petiol. len and the ratio Leav.len/Petiol.len were negatively influenced by the isothermality (chelsa_b_2); temperature seasonality (chelsa_b_3), mean daily mean air temperatures of the wettest quarter (chelsa_b_7); annual precipitation amount (chelsa_b_11), precipitation amount of the wettest month (chelsa_b_12); precipitation amount of the driest month (chelsa_b_13); precipitation seasonality (chelsa_b_14), mean monthly precipitation amount of the wettest quarter (chelsa_b_15) and the mean daily mean air temperatures of the warmest quarter (chelsa_b_9), respectively (Fig 5).

Discussion

Our results revealed a high genetic diversity in the studied populations. A high number of alleles (156) were recorded among 154 *A. senegalensis* samples. Some authors similarly reported a high level of genetic diversity in A. senegalensis (*Kwapata et al., 2007*), in *Annona cherimola* (*Escribano et al., 2007*), and ~~in~~ many other *Annona* genotypes such as *Annona reticulata*, *Annona muricata, Annona atemoya, Annona squamosa* (*Anuragi et al., 2016*). The high genetic diversity among *Annona* population could be related to protogynous basis cross-pollination of *Annona* species (*Anuragi et al., 2016*). The diversity observed in *A. senegalensis* in the present study could also be attributed to the large geographical distance among the studied populations. In addition to this, the outcrossing nature of *A. senegalensis* could favor a stock of genetic diversity in its populations (*Kwapata et al., 2007*).

Our results further showed higher diversity in the western populations than in the southern populations. The percentage of polymorphic loci was also higher in the western populations. The center of origin of *A. senegalensis* could explain the higher level of diversity detected in the western populations. In fact, Most *Annona* species are originated from South America and the Antilles. However, *A. senegalensis*, called African species is thought to have originated in Africa (*Pinto et al., 2005*). The specific name senegalensis is derived from Senegal (Western Africa) which is where the type specimen was collected (*Lizana and Reginato, 1990*). This might also be explained by population size which was bigger in the western than in the southern. However, a small size population can lead to too few heterozygosity which could imply inbreeding as reported by several authors (*Angeloni et al., 2011; Ellegren et al., 2016; Rosenberger et al., 2021*). For instance, the inbreeding coefficient FIS values were lower in western than in southern populations. However, FIS showed negative values in all sampled populations suggesting a number of heterozygotes higher than expected for all populations according to the Hardy-Weinberg Principle. This indicated that there were some gene flows between non-related individuals.

Furthermore, our results showed a high level of genetic differentiation in the studied populations. The Bayesian clustering program STRUCTURE presented the highest LnP(D) and ΔK values for K = 2 differentiating the samples collected in the western from those collected in southern. STRUCTURE also revealed secondary high LnP(D) and ΔK values at K=4 differentiating the four populations Ben_BGN, Ben_MPE (from Western Region), Moz_MEC, and Moz_MAV (from southern region) into four different genetic clusters. These findings were supported by the principal coordinate analysis which showed spatial separation between the western and the southern populations and also between populations within each geographical region. This genetic structure could be explained by the wide geographical accession collection which doesn't make possible any gene flow between the two geographical regions (*Yang et al., 2019*). However, some signs of admixture were observed within the genetic groups found in the western populations, and within the genetic groups found in the southern populations. In addition to this, the degree of spatial separation was lower for the two populations from the southern region than for the western populations. This is probably due to a higher level of admixture in the southern populations. Being from a protected area, and therefore from a relatively close area, gene flow might be more facilitated in our studied populations in southern region.

Analysis of Molecular Variance showed high genetic variation between the two regions (69.1%). The variation was greater among populations (21.3%) than within populations (9.6%). This means that enough populations with few individuals within the population should be considered while designing selection and plant breeding programs on the species to capture large variability. This result doesn't corroborate with the one of Kwapata et al., 2007 who rather reported a higher genetic diversity within *A. senegalensis* populations than among populations in Malawi. This difference in results might be due the largest geographical distribution covered by the present study which could induce a break in genetic traits between populations. Furthermore, Mantel's test confirmed the existence of a significant positive correlation between Nei's genetic distance

and geographic distance for all pairwise populations suggesting that the geographical distribution of the populations contributed significantly to the observed genetic diversity in our study.

Likewise, high morphological traits variation was observed between populations. Individuals from the two populations of the western regions and the two from the southern regions were grouped into four different clusters. The Chi-Square test performed on morphological and genetic data confirmed a significant association between the two, showing that the studied populations are morphologically and genetically connected. The strong association with genetics and morphological data also implies a high local adaptation of the species.

Morphological traits were also found to be highly influenced by the environment, mainly by temperature and precipitation indexes (Fig. 5, 6, 7). In the western region, the Dbh, total height, bole height, crown height, crown diameter, fruit length, fruit width, fruit dry weight, number of seeds per fruit, seeds weight, and pulp dry mass were negatively influenced by air temperature index, suggesting that increases in air temperature over a period can lead to a reduction in those growth parameters of the plants. In the southern region, similar trends were observed in temperature index which negatively influenced other growth parameters such as the crown diameter and the ratio leaves length/petiole length. In the western region, it was also found that leaves are longer when the annual precipitation amount increases. However, when combining both western and southern regions, it was noted that the petiole length is negatively influenced by some bioclimatic variables including annual precipitation amount. This showed that bioclimatic variables can have a contrasting effect on the morphological traits of the plants depending on the environment where the plants are established. The results suggest important phenotypic plasticity of *A. senegalensis* plants in different environments and confirm the results of some authors who state that climate contributes largely to morphological variation in plants (*Guerin et al., 2012*). However, the percentage attributed to climate could not be clearly defined in the present study, since soil and other environmental variables like topography can also induce variability in morphological traits (*Ouédraogo et al., 2019*). Therefore, studies considering data from additional environmental sources are required to better differentiate the extent of the contribution of the genetic background vs. the environment to the morphological traits variation of A. senegalensis. Yet the availability of high genetic diversity in the studied populations is a sign of biological efficiency that can enable *A. senegalensis* to respond in various ways to changes in the environment.

Conclusions

The present study reported the morpho-genetic diversity in populations of *A. senegalensis* from Benin (Western) and Mozambique (Southern). Strong differences were observed in the plant, fruit, and leaf morphological traits between the western and the southern populations. Moreover, the studied populations were characterized by high genetic diversity. Clusters of morphological traits corroborated with genetic differentiation structures showing that the studied populations were both morphologically and genetically connected. Precipitation and temperature extremes were also found to influence the morphological traits of *A. senegalensis*. Our study provides

information that is crucial for sustainable management and the prevention of the future extirpation of the species.

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