The relative role of soil, climate, and genotype in the variation of nutritional value of *Annona senegalensis* fruits and leaves

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ABSTRACT

Annona senegalensis Pers is a multipurpose tree species valued for food and medicinal uses in Africa. Although there have been attempts to document the proximate composition of fruits and leaves, little is known about the relative role of soil, climate, and genotype on the nutritional quality. The present study evaluated the variation of the proximate composition of fruits and leaves in populations from Benin and Mozambique. It further assessed the impact of soil, climate and genotype on the proximate composition. Data were collected from four populations genetically different and analyzed using descriptive statistics, analysis of variance (ANOVA), principal component analysis, redundancy analysis (RDA), and variance partitioning. Results revealed significant variation in the proximate composition of fruits and leaves among the studied populations. Ashes and fibers in fruits, and lipids in leaves were 4.8-fold, 2.5- fold, and 1.25-fold higher respectively, in populations from Mozambique. Fruits moisture and lipids content were rather 1.4-fold and 1.10-fold higher in populations from Benin. Moisture and lipids were respectively 6-fold and 1.27-fold higher in fruits than in leaves, while ashes, fibers and proteins were approximately twice higher in the leaves than in the fruits. Genetic groups, climate and soils were found to influence this variation. All three factors explained 74.4% of the variation of nutritional value of fruits and leaves, 31.9% of which was exclusively due to genetic variation, 2.8% to the interaction of climate and soils, 24.1% to the interaction of soil and genetic variation, and 15.5% to the interaction of all three factors. Our study shows that genetic variation and soil properties better than climate, explain the variation of nutritional value of *A. senegalensis* fruits and leaves and further provides essential information that could be harnessed in the domestication and breeding program of the species for its edible parts.

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1. Introduction

Wild edible fruit trees are valuable resources for the livelihoods particularly in African rural communities[1–3]. They provide shade; natural medicine; bed and sleeping mats; building material; or firewood[4,5]. In addition to being a source of income, they are integrated into diets, especially during food shortages, and thereby contribute significantly to food security [6–8]. From a nutritional perspective, wild edible fruit trees were further reported to be a valuable source of proteins, lipids, fibers, ashes, oils, vitamins, and various minerals with benefits for the human diet and health[9,10]. *Annona senegalensis* Pers. (fam. Annonaceae) commonly known as the wild soursop,is one of Africa's most important wild edible tree species. All parts of the plant are used in traditional medicine to heal many diseases including asthma and cough[11], epilepsy and malaria [12,13,], dizziness, diabetes, and several venereal diseases [14, 15]. The fruit is eaten fresh and contributes to human and wild animal diets[16,17,18. The leaf is used as a food seasoning and serves as a vegetable[18–21]. Despite its high use in Africa, studies on the nutritional potential of the edible parts of the plant are limited and most of the existing ones were carried out in Nigeria. The reported values vary greatly among studies. For instance, fibers in fruits were 4.22% in contrast to20.6%[16,22]; ashes in the leaves were 2.0% in contrast to 13.1% [23,24]. Such a variation could be linked to soil properties, climatic conditions, and genetic background. No previous research on *A. senegalensis* has attempted to explicitly investigate the variation of its nutritional value in relation to soil, climate, and genotype. Understanding this is however essential in designing sustainable management strategies and the improvement of nutrition of poor rural people who mostly depend on the species. For example, it can also guide domestication and breeding programs of *A. senegalensis*. It can guide selection criteria in terms of synergies and trade-offs for proximate composition parameters. In fact, some populations might have higher contents in some proximate composition parameters and lowers in others. Therefore, someone selecting those populations for their high content in some parameters should be aware that they are also selecting materials with lower contents in other proximate composition parameters. Our findings in previous studies showed genetic differences within and among populations from Benin and Mozambique [25]. These populations are also subject to different climatic conditions and grow on different soils [25]. This offers an interesting opportunity to test how factors such as genetic variation, climate, and soil properties can solely and interactively affect the nutritional composition of the edible parts.

Previous studies showed the influence of soils on the nutritive value of *Adansonia digitata* L. edible parts collected in genetically different populations[26]; and the influence of a climatic gradient on the nutritional value of *Monotheca buxifolia* (Falc.) A. DC wild edible fruits[27]. Based on these previous insights, we expected that variation in the proximate composition of *A. senegalensis* could be related to genotype, soil, and climate although their relative role could not be predicted. This study was designed to fill the knowledge gap related to factors that influence the nutritive value of *A. senegalensis* and provide information that can guide breeding and domestication programs on the species. The aims of the study were to (i) assess the variation in the proximate composition of *A. senegalensis* fruits and leaves between genetically different populations of *A. senegalensis* from Benin and Mozambique; and (ii) disentangle the relative importance of the influence of the genetic variation, climate, and soil on this variation.

2. Materials and methods

2.1. Sampling

Data was collected during the rainy season (from December to February in Mozambique) and (from May to July in Benin), in four populations genetically different and distributed in Benin republic (Western Africa) and Mozambique (Southern Africa). The characteristics of the study area are summarized in Table 1. Nine individuals of *A. senegalensis* were selected per population (36 in total) due to the unavailability of many plants bearing ripened fruits with no fungi attack at the time of data collection. Although higher sample size would have been better, we assumed that it was sufficient to draw acceptable conclusions, following some previous studies. For example [26], selected only 3 individuals in genetically different populations in order to study variation in biochemical composition of baobab's (*Adansonia digitata*) pulp, leaves and seeds in relation to soil types and tree provenances. We collected fruit and leaf samples on each plant. Soil samples were collected from around three individuals per population (12 in total) at a mean depth of 40 cm. A minimum of 1 km was observed between two sampled trees in order to capture variability in soil. Since populations were genetically different, sampling was done randomly regardless of age, size and stand density to select the three individuals[28]. Leaves samples

Table 1

Characteristics of the study area. Population codes - Ben-BGN: North Borgou population from Benin; Ben_MPE: Mekrou Pendjari population from Benin; Moz_MEC: Mecula population from Mozambique; Moz_MAV: Mavago population from Mozambique.

Provenances	Sampled districts	Longitude	Latitude	Altitude (m)	Mean annual rainfall	Mean annual temperature	Major soil type
Benin	Ben_BGN	3,55885	10,93528	308	1000–1200	From 24 to 31 °C.	Ferruginous soils with concretions.
	Ben_MPE	0,85198	10,51104	167	950–1000		Soils on ferruginous crystalline rocks
Mozambique	Moz_MEC	37,10937	−12,07651	281	Mean annual rainfall varies from 800 to 1200 but increase from MEC to MAV	20–26 °C (dry season) and 30 °C (wet season)	Feralsols, luvisols, acrisols
	Moz_MAV	36,01857	−12,08318	1087			

Source: [30,31]; Fieldwork 2019–2021.

were packed in a flat paper container, while fruits samples were inserted into ziplock bags, packed in cooler box containing ice. All samples were immediately and frequently transported from the field to the lab. Sampled leaves had a specific position on the branches: Only leaves located at the 1st, 3rd, 5th, 7th, and 9th position on the third main branch (from the terminal bud) of each plant were harvested as previously described in Ref. [29].

2.2. Proximate composition analysis

Fruits and leaves were washed in the laboratory and air-dried to constant weight. Then, ground separately using a ceramic mortar and pestle and stored in air-tight polyethylene bags in a desiccator. After determination of moisture content, the dried powdered samples were used for all analysis. The proximate composition of fruits and leaves were determined following the official methods of the Association of Official Analytical Chemists[32]. Analysis was first carried out on fruits and then on leaves. The moisture content was determined by drying 2 g of fresh samples in an oven at 105 °C to constant weight for 24 h. The dried sample was then incinerated in a muffle furnace at 550 °C for 6 h, cooled, and weighed to determine ashes content. A Soxhlet apparatus[33] was used to determine crude lipids using 2 g samples and extracting with petroleum ether (40–60 °C). The crude fibers were determined by digesting 2 g of dry sample in 1.25% hydrochloric acid (HCl) and sodium hydroxide (NaOH). The nitrogen content was determined by macro- Kjeldahl method[34] and crude protein was calculated by multiplying the measured nitrogen by a factor of 6.25. All determinations were conducted in triplicate.

2.3. Soils and bioclimatic data

The bioclimatic data were extracted from the CHELSA database (Climatologies at High Resolution for the Earth's Land Surface Areas). For this purpose, the GPS coordinates of each individual were recorded and used in QGIS 3.16.2 [35]. The GPS coordinates were used to extract bioclimatic data considering the last data available over 30 years (1979–2013). A total of 19 bioclimatic variables were downloaded (Table S1, supplementary data). Methods are described in http://chelsa-climate.org/wp-admin/download-page/CHELSA_tech_specification.pdf

Soil analysis focused on the determination of parameters such pH (in water), sodium (Na), potassium (K), calcium (Ca), carbon (C), nitrogen (N), ratio carbon to nitrogen (C: N), sand, silt, clay, and organic matter. The pH was measured using a pH meter in a ratio soil/water of 1/2.5. Sodium and potassium were determined by flame emission spectrometry while calcium was determined by atomic absorption spectrophotometry as previously described in Ref. [36]. The Kjeldahl method was used to measure nitrogen[37]. Organic carbon was measured by the oxidation of organic matter using potassium dichromate ($K_2Cr_2O_7$) in the presence of hot concentrated sulfuric acid; the remaining dichromate after oxidation of the organic matter was read in a colorimeter. The obtained value was used in a regression equation to determine the organic carbon. The ratio of carbon to nitrogen (C: N) informs on the degree of evolution of the organic matter and the biological activity[38]. If the C: N ratio is less than 10, the organic matter is not molded; for $10 <$ C: N < 15, the organic matter is well decomposed; for $15 <$ C: N < 25, the organic matter is weakly decomposed while not decomposed for C: N > 25. The sand was determined by sieving while silt and clay were determined using Robinson's pipette method[39].

2.4. Statistical analysis

All the analyses were carried out in R statistical software version 4.1.2 [40]. Descriptive statistics (arithmetic mean, standard error of mean, and coefficient of variation) per population for each proximate composition parameter were calculated. Then, an analysis of variance (ANOVA) was used to test whether the nutritional parameters varied significantly 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 tests, respectively. In the case of severe non-normality (*p-value* < 0.01), the corresponding non-parametric test (i.e., Kruskal-Wallis) was applied. When the ANOVA indicated a significant difference, a Student Newman and Keuls (SNK) test was applied as multiple comparisons tests in the package *agricolae* (de Mendiburu, 2020) to separate means. A principal component analysis (PCA) was first carried out on proximate composition parameters to explore the relationship among the nutritional parameters. This analysis was implemented to understand the patterns of correlations (negative or positive) between the proximate composition parameters. This pattern of relationship among proximate composition parameters can guide selection of germplasm (provenances) in terms of synergies and trade-offs for proximate composition parameters. To evaluate the influence of climate conditions, soil parameters, and genetic variation on the proximate composition of *A. senegalensis* fruits and leaves, we performed a redundancy analysis within the "vegan" package. Due to the large number of soil and climate variables, we run two separate PCA on soil and climate variables to reduce their dimensions. The first axis of the PCA on soil variables saved 82.10% of the total variation in soil properties (see Table S2, supplementary file). For the PCA on climate variables, the first two axes saved together 84.25% of the total variation in climate variables (see Table S3, supplementary file). The loads of each tree on these three axes were extracted from the PCA results to represent variation in soils and climate variables. The proximate composition parameters and the genetic groups were added for each tree to generate a matrix including, its proximate composition, genetic group, soil properties (scores on the first principal component), and climate data (scores on first two principal components). The obtained matrix was submitted to the redundancy analysis with the function *rda*. In this analysis, parameters of proximate composition were considered as response variables, and genetic group, soil properties, and climate data as explanatory variables. To disentangle the influence of the three factors (soil, genetics, and climate) on the variation of proximate composition, we run a variance partitioning (also referred as commonality analysis) with the function *varpart* on the *rda* results in the package "vegan". The variance partitioning analysis breaks down the variance explained by predictor

variables in the redundancy analysis. It quantifies the unique variance explained by individual predictor variables as well as their overlap with other variables in the model.

3. Results

3.1. Proximate composition of A. senegalensis fruits and leaves

There was significant differences in the proximate composition of *A. senegalensis* fruits and leaves among the studied populations (Tables 2 and 3). Moisture content in the fruits ranged from 58.11% to 67.81%; ashes from 0.88% to 4.53%; fibers from 11.92% to 31.17%; lipids from 2.52% to 4.90% and proteins from 4.72% to 4.73% (Table 2). Fruits from Benin populations had 1.41-fold lipids and 1.10-fold moisture content than those from Mozambique, while an opposite trend was found for ashes and fibers which were respectively almost four times and twice higher in fruits from Mozambique. The highest values for lipids (4.90%) and moisture content (67.81%) were found in Benin (Ben_BGN) and the highest values for fibers (26.53%) and ashes (4.47%) were found in Mozambique (Moz_MAV). Protein contents in fruits were similar (4.72% and 4.73%) in the two populations of Mozambique (Table 2).

The proximate composition of *A. senegalensis* leaves from the different populations is summarized in Table 3. There were significant variations between the different populations for all parameters except proteins and moisture content. Moisture ranged from 8.44% to 11.97%; ashes from 4.71% to 5.90%, fibers from 29.80% to 45.63%; lipids from 1.95% to 2.87%; and proteins from 1.88% to 2.24%. Ashes and lipids were respectively 1.10 and 1.25 times higher in Moz populations than in Ben populations while fibers were slightly higher in Ben than in Moz. However, the highest value for fibers (45.63%) was found in Moz_MAV and the highest value for ashes (5.90%) and lipids (2.87%) was found in Moz_MEC. Moreover, some values obtained for ashes in the Benin population were similar to those found in Moz_MAV; and in some cases, the lipid contents of Moz samples were similar to those from Ben_BGN (Table 3). Comparing the proximate composition of fruits and leaves (Tables 2 and 3), moisture and lipids were respectively six times and 1.27 times higher in fruits than in leaves while ashes, fibers, and proteins were approximately two times higher in leaves than in fruits.

3.2. Relationships between macronutrients of fruits and leaves of A. senegalensis

Fig. 1 shows the projection of proximate composition parameters in the principal component axes. The first two axes explained 61.70% of the initial information related to proximate composition of *A. senegalensis* fruits and leaves. Parameters such as ashes, fibers, and proteins in fruits; and ashes in leaves were positively correlated with the first axis, while moisture and lipids in the fruits were negatively correlated with this axis. Therefore, fruits with higher ash content also had higher fibers and protein content but lower lipids content and moisture. Only the moisture and protein content in the leaves were loaded on the second axis. Moisture in the leaves showed a positive correlation with the second axis and proteins a negative correlation with it, indicating that leaves with high content of proteins often have low moisture.

Table 2
Proximate composition of *A. senegalensis* fruits.

| Parameters	Statistics	Populations			
		Benin		Mozambique	
		Ben_BGN (n = 9)	Ben_MPE (n = 9)	Moz_MEC (n = 9)	Moz_MAV (n = 9)
Moisture (%)	Mean ± SE	67.81a±22.60	65.23b ± 21.74	58.11d ± 19.37	62.04c±20.68
	CV (%)	5.22	2.71	0.81	1.51
	Min	64.20	63.10	57.29	61.05
	Max	71.70	68.20	58.56	63.64
Ashes (%)	Mean ± SE	0.88b ± 0.29	0.99b ± 0.33	4.40a±1.46	4.53a±1.51
	CV (%)	2.04	22.71	1.88	5.13
	Min	0.86	0.78	4.25	4.17
	Max	0.92	1.34	4.54	4.90
Fibers (%)	Mean ± SE	11.92d ± 3.97	15.67c±5.22	21.90b ± 7.30	31.17a±10.39
	CV (%)	3.57	10.27	8.37	4.92
	Min	11.46	13.50	18.33	18.33
	Max	12.36	17.50	23.71	23.71
Proteins (%)	Mean ± SE	ND	ND	4.73a±1.57	4.72a±1.57
	CV (%)	ND	ND	26.15	11.21
	Min	ND	ND	0.34	0.66
	Max	ND	ND	1.01	0.89
Lipids (%)	Mean ± SE	4.90a±1.63	2.70bc±0.90	2.52c±0.84	2.84b ± 0.94
	CV (%)	2.88	5.49	14.01	2.27
	Min	4.70	2.56	1.98	2.75
	Max	5.10	2.94	2.97	2.94

Population codes are the same as indicated in Table 1. ND: not determined. Means ± standard error of mean.
Means followed by the same letter between populations are not statistically different from each other at *p-value* < 0.01.

Table 3
Proximate composition of *A. senegalensis* leaves.

Parameters	Statistics	Populations			
		Benin		Mozambique	
		Ben_BGN	Ben_MPE	Moz_MEC	Moz_MAV
Moisture (%)	Mean ± SE	11.97a±3.99	11.49a±3.83	8.94a±2.98	8.44a±2.81
	CV (%)	32.54	36.82	5.57	21.33
	Min	5.67	6.54	8.14	6.06
	Max	16.94	16.83	9.54	10.10
Ashes (%)	Mean ± SE	5.12b ± 1.70	4.71b ± 1.57	5.90a±1.96	4.99b ± 1.66
	CV (%)	7.20	12.81	8.26	6.47
	Min	4.61	3.85	5.16	4.52
	Max	5.69	5.59	6.57	5.53
Fibers (%)	Mean ± SE	37.05b ± 12.35	38.95b ± 12.98	29.80c±9.93	45.63a±15.21
	CV (%)	9.46	23.08	6.29	3.74
	Min	33.86	29.79	27.27	43.25
	Max	44.04	54.99	32.62	48.21
Proteins (%)	Mean ± SE	2.18a±0.72	2.24a±0.74	1.88a±0.62	1.91a±0.63
	CV (%)	22.64	27.14	30.95	8.28
	Min	1.43	1.62	1.19	1.76
	Max	3.10	3.62	2.77	2.13
Lipids (%)	Mean ± SE	2.58a±0.86	1.95b ± 0.65	2.87a±0.95	2.80a±0.93
	CV (%)	34.50	33.79	1.91	0.94
	Min	1.66	0.99	2.75	2.76
	Max	4.33	2.82	2.95	2.85

Means ± standard error of mean; Means followed by the same letter between population are not statistically.
different from each other at *p-value* < 0.01. Population codes are the same as indicated in Table 1.
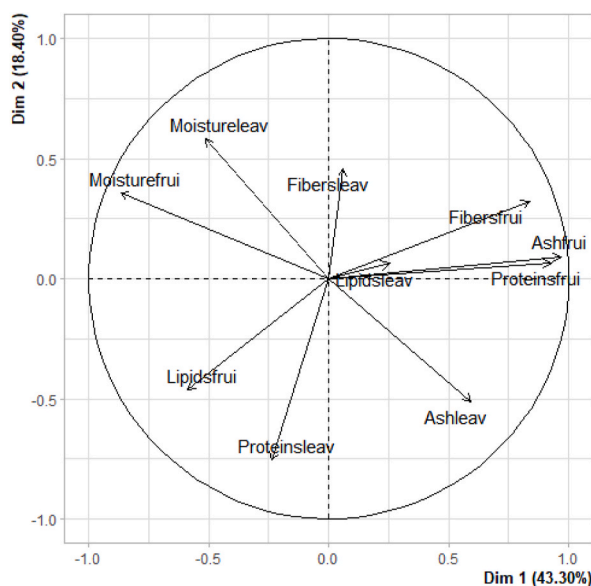
Fig. 1. Relationships between proximate composition of *A. senegalensis* fruits and leaves. Dim 1 and Dim 2 are the first two principal component axes of the PCA.

3.3. Relative importance of the influence of climate, genetic variation, and soil properties on the proximate composition of A. senegalensis fruits and leaves genetic variation

Results of the separate principal component analyses on bioclimatic variables and soil parameters showed that the first axis of bioclimatic variables alone explained 82.10% of the initial information related to climate (Table S2, supplementary data), while the first two axes of soil variables together explained 84.22% of the initial information related to soil (Table S3, supplementary data).. These three axes (with a load of each individual of *A. senegalensis*) in addition to the genetic group of each individual were used in the redundancy analysis (RDA).

The RDA model was overall significant (F = 5.02, *p-value* = 0.003, adjusted R^2 = 74.4%) indicating an overall significant

relationship between soils, climate, genotype on the one hand, and proximate composition of *A. senegalensis* fruits and leaves on the other hand. However, only Soil_PC1, Soil_PC2, and genetic_group were significant (*p-value* < 0.05 , Table 3), indicating that only soil properties and genetic variation had significant influence on the nutritional composition. The first axis (RDA1) explained 58.93% of the total variance and genetic groups (Gen.grp2 – from Mekrou-Pendjari in Benin and Gen.grp4 – from Mavago population in Mozambique) were loaded on this axis. The second axis (RDA2) explained 23.32% of the total variance and soil_PC1, soil_PC2, climate_PC1 and genetic group (Gen.grp3 – from Mecula population in Mozambique) were loaded on this axis (Table 4, Fig. 2). The first PCA axis of soils (Soil_PC1) was a combination of parameters such as sodium (Na), potassium (K), nitrogen (N), and clay with a positive correlation. Parameters such as carbon (C), the ratio carbon to nitrogen (C/N), silt and organic matter had negative correlation. The second axis of soil (Soil_PC2) was a combination of calcium (Ca) and sand. The calcium (Ca) was positively correlated with that axis while the sand content was negatively correlated (Table S3, supplementary data).

Considering the macronutrients on the RDA axes (Table 4), ashes, fibers and proteins in fruits and leaves, as well as lipids and moisture content in leaves were loaded on the first axis, while moisture in fruits and leaves, lipids in fruits, and ashes, fibers, and proteins in leaves were loaded on the second axis. Except for the moisture in leaves, which had a negative correlation with the first axis, all the other macronutrients loaded on the first axis were positively correlated with it. All the variables loaded on the second axis also had a positive correlation with that axis except, ashes, and lipids in leaves.

Based on the scores of principal component axes of soils, climate, and genetic groups and on the scores of macronutrients in fruits and leaves in RDA axes (Table 4), moisture and lipids in fruits, and fibers and proteins in leaves were positively associated with soil sodium (Na), potassium (K), nitrogen (N), and clay and negatively associated with soil carbon (C), ratio carbon to nitrogen (C/N), silt, and organic matter. Ashes in leaves were positively associated with sand in soils and negatively associated with the soil calcium (Ca) content. These individuals were mostly from genetic group 3 (Gen.grp3 – from Mecula population in Mozambique). In addition to this, the content of ashes, fibers, and proteins in fruits, and lipids in leaves were positively associated with the genetic group G4 (Gen.grp4 – from Mavago population in Mozambique) and negatively associated with genetic group 2 (Gen.grp2 – from Mekrou-Pendjari in Benin). This suggests that fruits collected from individuals of the genetic group 4 were richer in ashes, fibers, proteins, and their leaves are richer in lipids than those from the genetic group 2. Concerning the genetic group 3, fruits were richer in moisture and fibers, while leaves were richer in proteins. They were mostly collected from soils rich in sodium (Na), potassium (K), nitrogen (N), clay, and poor in calcium (Ca).

Fig. 3 shows the results of the variance partitioning (commonality) analysis. It shows the percentage of the variation in the proximate composition of the fruits and leaves of *A. senegalensis* which is due to the different factors (genetic group, climate, and soil) and their interaction (two-by-two and all three factors). A total of 74.4% of the variation of the nutritional value of fruits and leaves was due to genetic group, climate, and soil. From this, 31.9% was exclusively due to genetic variation, 2.8% to the interaction of climate and soils, 24.1% to the interaction of soil and genetic variation, and 15.5% to the interaction of all three factors (Fig. 3). In total, 71.6% of the variation was explained by genetic variation (exclusively and in common with soil and climate), 43.70% by variation in soil properties (only 1.1% exclusively due to soil, and the remaining 42.6% commonly due to the genetic variation and climate), and 17.27% by climate (in common with soil and genetic group). Finally, 25.6% of the overall variation (residuals) was not due to any of the tree factors, nor to their combined effect (Fig. 3). These results show that genetic variation followed by soil properties are the main drivers of the variation in fruits and leaves proximate composition. The results also showed the important effect of the interaction of soil and genetic variation on proximate composition.

Table 4

Significance of the relationship between proximate composition and soil physico-chemical parameters, bioclimatic variables, and genetic group – summary of the permutation ANOVA test and scores on RDA axes. [ns] : non - significant (*p - value* > 0.05); ** significant at $0.001 < p - value < 0.01$

	Df	Variance	F	Pr (>F)	RDA1 (59.95%)	RDA2 (30.57%)
Factors in the model						
Soil_PC1	1	32.254	8.0844	0.003**	0.39033	**0.7551**
Soil_PC2	1	33.259	8.3362	0.005**	0.44083	**-0.6874**
Climat_PC1	1	3.075	0.7708	0.516 ns	0.34098	**0.7134**
Gen.grp	3	51.692	4.3189	0.008**		
Residual	5	19.948				
Gen.grpG2					**-0.43275**	0.0835
Gen.grpG3					-0.01743	**-0.9456**
Gen.grpG4					**0.94075**	0.3141
Macronutrients of fruits and leaves						
Fruits_Moisture					-0.94562	**1.56740**
Fruits_Ashes					**0.80468**	-0.52269
Fruits_Fibers					**3.97858**	-0.80681
Fruits_Proteins					**0.17345**	-0.10770
Fruits_Lipids					-0.22548	**0.34944**
Leaves_Moisture					**-0.72777**	0.18031
Leaves_Ashes					0.04652	**-0.20369**
Leaves_Fibers					2.27056	**2.35875**
Leaves_Proteins					-0.04872	**0.05154**
Leaves_Lipids					**0.04912**	-0.04687[a]

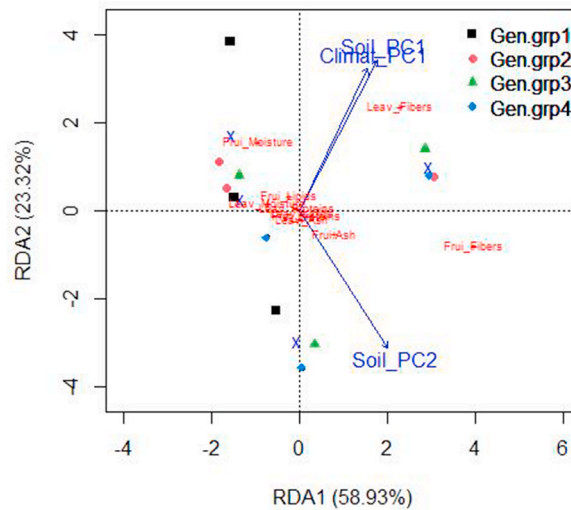[a] Gen.grp stands for genetic group (see Table 1 for detailed description)

Fig. 2. Projection of proximate composition variables, climate and soil axes from the different populations in the RDA correlation plots.
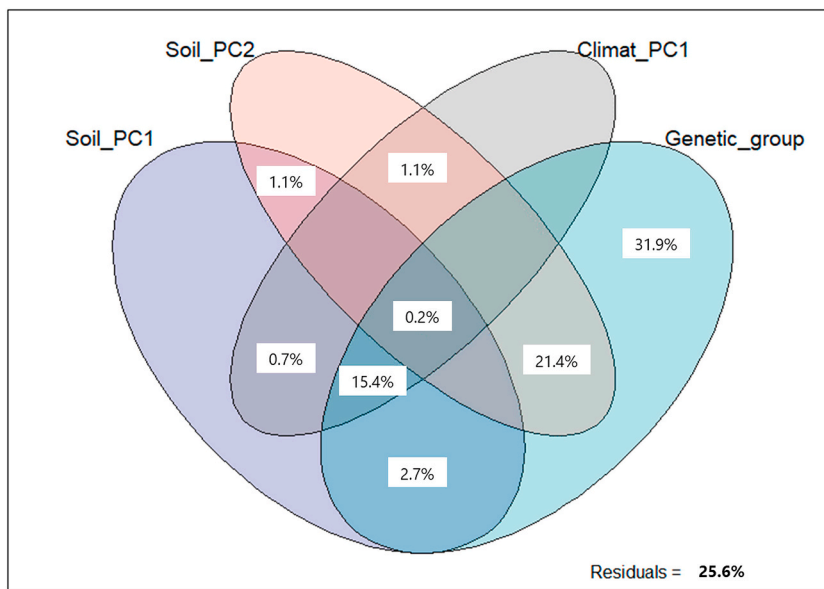

Fig. 3. Venn diagram illustrating the shared and unique variance explained in nutritional value by genetic group, soil, and climate variables.

4. Discussion

In this study, we assessed the variation of the proximate composition of fruits and leaves from *A. senegalensis* between genetically different populations from western (Benin) and Southern (Mozambique) Africa, in relation to climate and soils, and further disentangled the relative contribution of genetic variation, climate, and soils.

As expected, we found significant variation among populations for all parameters, except for proteins in fruits and leaves, and moisture content in leaves (Tables 2 and 3). Moreover, some values obtained for ashes in populations from Benin were similar to those found in Mozambique, particularly in Mavago, and some values found for lipids in populations from Mozambique were similar to those found in Benin, particularly in North Borgou. Since the studied populations are genetically different [25], it appears that the observed variation in the proximate composition of *A. senegalensis* fruits and leaves is not only due to genetic differences among populations, but that other factors are also driving these variations. For instance, the redundancy analysis showed some influence of soil parameters. Moisture and lipids in fruits, as well as fibers and proteins in leaves were positively associated with soil sodium (Na), potassium (K), nitrogen (N), and clay, and negatively associated with soil carbon (C), the ratio carbon to nitrogen (C/N), silt and organic matter. Ashes in leaves were positively associated with soil sand content and negatively associated with soil calcium (Ca). Influence of climate was

also observed although non-significant. Soil, climate and genotype are among the most reported factors that influence the nutritive value in plant foods[41,42]. In this study, genetic variation, and soil better than climate stood out as the most influencing factors of the proximate composition of *A. senegalensis* fruits and leaves. Our findings show that all the three factors explained 74.4% of the variation in nutritional value, of which 31.9% were exclusively due to genetic variation. This indicates that an important part of the variation of the proximate composition in *A. senegalensis* fruits and leaves is exclusively due to allelic variation. However, gene expression seems to be dependent of specific climate and soil conditions as indicated by their interactions: 2.8% for climate and soils, 24.1% for soil and genetic variation, and 15.5% for all three factors. Soil effect on the nutritive value of plant foods was also observed in many others fruits trees. For example, Kowalczyk and colleagues observed strong effects of soil nitrogen on nutritional status of apple fruits and leaves[43]. Highly basic soils, rich in carbon, clay, fine silt, and organic matter were also reported to positively affect the concentration of iron, potassium, vitamin C, carbohydrates, zinc, proteins, and lipids in baobab fruits, leaves and seeds [26]. In the present study, no exclusive effect of climate was noted, but the combined effect of climate and soil, climate and genetic variation, and climate, soil, and genetic variation were high. Therefore, in the case of *A. senegalensis,* a breeding program targeting the proximate composition, should mainly focus on genotypes and soils, and to a lower extent, on climate. It was also observed that 25.6% of the variation was neither due to genetic variation, soil, or climate, nor to their interaction. This could be explained by the effect of other biotic (e.g., competition, age and size of trees, leaves and fruits), and abiotic (e.g., other soil properties and climate variables, exposure to sunlight) factors not considered in this study, which might also interfere and play an important role in the observed variation. On the other hand, the range of moisture content in the leaves, was similar to values (7.7%; 11.78%) reported in previous studies [23,24]. However, fruits had low moisture levels (58.11%–67.81%) when compared to the reported values in other Annona species such as *Annona cherimoya* (79.26%), *Annona reticulata* (73.00%), *Annona muricata* (81.23%), *Annona atemoya* (76.63%) and *Annona squamosa* (81.66%–83.40%), respectively [44,45]. Foods with a moisture content of 50–95% are referred to as high-moisture foods[46]. The high moisture content in *A. senegalensis* fruits shows that they can easily be exposed to microbial infections after short-term storage. Fibers in fruits and leaves were higher than most values (4.22%; 15.87%; 20.6% and 20.15%; 33%) reported in the literature for *A. senegalensis* fruits[16,22,47] and leaves [23,24]. However, values observed for fibers in fruits from the two populations from Benin and Mecula (Mozambique) were similar to those reported by [16,22]. Crude fibers are considered as an indicator of non-digestible carbohydrates and lignin in food [48]. The consumption of fruits rich in fibers play an important role in the prevention of various diseases such as cardiovascular diseases[49], constipation [50], pancreatic and colorectal cancer; diabetes among others [51–53]. Our findings agreZakarie with previous studies that reported that *A. senegalensis* is a veritable source of dietary fibers[24]. Ashes in leaves were slightly higher than the value reported by[24]. Considering the fruit, ashes content was low when compared to 10.83% and 13.1% found in previous studies [22,47], but the values obtained in the two populations from Mozambique were similar to those reported by [16]. Low ash contents are an indication of low mineral content. Protein and lipid values in fruits were lower than the previously reported: 13.7%; 10.16%; 8.78% and 8.3% respectively for protein and lipids in *A. senegalensis* fruits [16,22,47]. However, values reported for proteins and lipids in this study were higher when compared to 1.97%; 2.13% and 0.96%; 1.55% reported for proteins and lipids respectively in fruits of two cultivars of *A. squamosa* [45]. Concerning the leaves, most values reported for lipids in this study were within previously reported ranges of 2% and 2.94%[23,24]. However, protein values were higher than the 0.85% reported by Ref. [24], but similar to the 2.71% reported by Ref. [23]. These values of proteins in *A. senegalensis* could significantly contribute to the daily protein requirement of the human body and help prevent nutrition-related diseases like kwashiorkor and marasmus[54].

5. Conclusion

The current study is the first attempt to link variation in the proximate composition of *A. senegalensis* with genetics, climate, and soils. We reported a high variation of the proximate composition in fruits and leaves. Genotype, followed by soils contributed the most to the observed variation. As such, a breeding program targeting a specific parameter of the proximate composition of *A. senegalensis* should consider genotype and soils. However, some factors not considered here might also influence the nutritional composition observed and constitute limitations for the present study (25.6 % of variation not explained). These factors include the age and size of trees, exposure to sunlight, time of the day at which samples were collected, and transport of samples from field to the lab. The proximate composition reported in this study showed that the species is rich in nutrients, but the high moisture content observed in fruits can make storage difficult. Therefore, further research focusing on the post-harvest storage and processing would be beneficial for a better use of the species and the preservation of its nutritional and health benefits.

Declarations

Author contribution statement

Janine Conforte Fifonssi Donhouedé, Kolawolé Valère Salako, Ana IF. Ribeiro-Barros, Achille Ephrem Assogbadjo, and Natasha Ribeiro: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

5.1. Data availability statement

Data will be made available on request.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19012.

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