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Impact of land-use on tree and fruit morphometric variation of the bitter kola (*Garcinia kola* Heckel) in Benin: Insight for domestication and production.

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Garcinia kola **is an IUCN globally vulnerable species native in Benin where it is extinct in the wild. It is also one of the top ten priority non-timber forest products in Benin because of its socio-economic and medicinal values. Still, *G. kola* is neglected and underutilized. The morphological variation of *G. kola* traits was investigated in two land-use types (home gardens versus farmlands) where it is found in Benin with the goal of informing its domestication and production. A total of 79 trees identified in both land use types were characterized based on seventeen tree growth, leaves, fruit and seeds descriptors. Results found no significant difference between land-use types for tree height, first ramification height, crown height, crown diameter growth, blade width, petiole length, petiole diameter and number of seeds. However, stem diameter, blade length, fruit length, fruit width, husk weight, seed length, seed width and seed weight showed significant differences between land-use types. Eight (stem diameter, blade length, fruit length, fruit width, husk weight, seed length, seed width and seed weight) out of the initial descriptors were the most discriminant of trees according to land-use types. Values of most discriminant descriptors were low in home gardens and higher in farmlands. This study shows that land-use management can affect *G. kola* production, highlights the potential of domestication of the species, and suggests the need to fix morphological traits of fruits and nuts which are the most sought *G. kola* products.**

Key words: Benin, Clusiaceae, land-use management, morphological diversity, threatened species.

INTRODUCTION

Garcinia kola Heckel (Clusiaceae) is a medium size tree that grows up to 15-17 m high (Agyili et al., 2006). It is commonly known as "bitter kola" in English, "faux kola" or "petit kola" in French. The tree is endemic to humid lowland rainforest vegetation of central and western Africa (Akoegninou et al., 2006; Agyili et al., 2007;

Kanmegne and Omokolo, 2008). *G. kola* is one of the most valuable trees because of its socio-economic importance and medicinal attributes. The seed, commonly known as bitter kola, is a masticatory and a major kola substitute shared at social ceremonies. Bitter kola is a stimulant that has a bitter astringent and resinous taste when eaten (Yakubu et al., 2014). The seeds are often used as aphrodisiac. They are used in folk medicine and in many herbal preparations for the treatment of ailments such as laryngitis, liver disorders, and bronchitis (Farombi and Owoeye, 2011). Because of its high interest, harvesting of the different organs of *G. kola* has been very heavy (Akoegninou et al., 2006; Agyili et al., 2007; Assogbadjo et al., 2017), making it extinction-threatened in several West and Central African countries (Yakubu et al., 2014).

Garcinia kola is found in Benin and belongs to the top ten priority non-timber forest products in Benin (Assogbadjo et al., 2017). It is only found in the Southern part of the country which corresponds to the sub-humid Guinean zone (Akoegninou et al., 2006) where population density is the highest with the most destructive anthropogenic activities. Bitter kola is an IUCN globally vulnerable species but extinct in the wild in Benin (Neuenschwander et al., 2011). The genetic diversity of the species might therefore be strongly reduced in the future if no appropriate conservation measures are taken. From a phytogenetic viewpoint, characterization and evaluation of morphological and agronomic traits are essential to the identification, conservation and utilization of genetic resources (Gepts, 2006). Studies on morphological variation of *G. kola* are however scarce. Dah- Nouvlessounon et al. (2016) recently explored the intra specific variability in one of the occurring region of the species, namely the Ouémé Region and reported a strong variation. They suggested a strong potential of the species for domestication. However, intra-specific variation may be caused by several factors which if captured could optimize the species domestication.

So far, no quantitative assessment is available on factors that are responsible for the observed variation in trees and fruits in the species. Previous studies demonstrated that land-use types can affect population structure and morphometric traits of multipurpose trees such as *V. paradoxa* (Djossa et al., 2008; Akpona et al., 2015). An exploratory survey in its occurrence areas in Benin shows that *G. kola* is found in home gardens and fields close to houses (Dah-Nouvlessounon et al., 2015). Assuming that land-use could also have an impact on *G. kola*, the current study aims at understanding how in its area of distribution, land-use types affect tree and fruit characteristics of *G. kola*.

MATERIALS AND METHODS

Studied species

G. kola (Heckel) in the Clusiaceae or Guttiferae family, known as bitter kola, is a perennial tree occurring in West and Central Africa forests. In West Africa, *G. kola* grows in Benin, Côte d'Ivoire, Ghana, Liberia, Nigeria, Togo, Senegal and Sierra Leone. In Central Africa, it is found in Cameroon, Democratic Republic of Congo, Gabon, Central African Republic and Equatorial Guinea. It is a dicotyledonous plant naturally found in the coastal areas and low land plains up to 300 m above sea level with an average of 2000-2500 mm of rainfall. The species occurs where temperatures are uniformly 30 to 32°C and the relative humidity ranges between 76 and 93% (Agyili et al., 2006). It is a medium sized evergreen tree, up to 30 m tall and with a fairly narrow crown (Figure 1) (Agyili et al., 2006; Akoegninou et al., 2006). The leaves are simple, 6-14 cm long and 2-6 cm across, shiny on both surfaces and spotted with resin glands. The small flowers are covered with short, red hairs (Iwu, 1993). The fruit is a drupe of 5-10 cm in diameter and with weights of 30-50 g (Figure 1). It is usually smooth and contains a yellow red pulp. The fruit changes color during maturation from green to orange, and each fruit contains 1-4 white seeds covered by a brown coat (Figure 1) (Agyili et al., 2006).

Study area

In Benin, *G. kola* occurs in the Guinean ecological zone as defined by White (1983). This zone is located in the Southern part of Benin between latitude 6°25 N and 7°30 N. The present study was conducted in the three administrative regions (Atlantique, Ouémé and Plateau) where the species was identified in Southern Benin (Figure 2). Southern Benin has a subequatorial climate with two rainy seasons and two dry seasons of unequal length. The region is characterized by a rain fall gradient from 900 mm in the West to 1300 mm in the East (Adomou et al., 2007). The mean annual rainfall is 1200 mm. The annual average temperature ranges between 25 and 29°C and the relative humidity between 69 and 97%. The soils are either deep ferrallitic or rich in clay, humus and minerals (Adomou, 2005).

Sampling

Trees were only identified in two basic land-use: homegardens and farmlands as already reported by Dah-Nouvlessounon et al. (2015). Morphological data were collected between June and July 2017 during fruit production period of the species. Communes where the species is present were identified by consulting databases then trees were accessed using a snow-balling method. In each commune, fruiting trees were purposively selected by using a minimum distance of 50 m to avoid closely related individuals. A total of 79 tree accessions were sampled of which 60

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Figure 1. *G. kola* a) tree standing in a homegarden; b unripe and ripe fruits; c) seeds

in homegardens, and 19 in farmlands. The nineteen individuals in farmlands are statistical sufficient for comparison of species-specific trait values (Perez-Harguindeguy et al., 2013). On each sampled tree, ten leaves collected from the first lateral branch and five randomly harvested fruits were considered as in Dah-Nouvlessounon et al. (2016).

Data collection and processing

Characterization of *G. kola* was based on 17 morphological variables related to tree trunk and canopy (tree growth), fruits, leaves and seeds characteristics. Tree growth variables are stem diameter at 1.30 m above the ground (dbh), total height, first ramification height, crown height, and crown diameter. Variables related to leaves are leaf blade length, leaf width, petiole length, and petiole diameter. Fruits' variables are fruit length, fruit thickness, fruit weight, fruit husk weight, and number of seeds per fruit while variables measured on seeds was seed length, seed thickness, and seed wet weight. The tree growth characteristics were accessed using the formula in Table 1. The leaf blade length and width, the petiole length were measured using a decimeter while stem diameter were measured using a pentadecameter. The length and the width of the fruits and the nuts were taken using a slide foot. Fruit weight, fruit husk weight and seed wet weight were measured using a balance of precision 0.01 with a maximum range of 500 g.

Statistical analyses

Mean values, and standard error of mean and coefficient of variation of each morphological traits were calculated for all the trees and trees within each land-use type. A two-independent samples t-test was performed to test for differences in morphological traits between land-use types. To identify the most important traits that discriminated trees regarding land-use types, a canonical discriminant analysis was applied to all morphological traits. The most discriminant traits were then used to plot trees according to their land use types in the canonical axes. All analyses were run using R software version 3.4.3 (R Core Team, 2018).

RESULTS

Impact of land-use on morphological characteristics of bitter kola

Impact of land-use tree growth characteristics of bitter kola

Tree growth traits were relatively dispersed in both land-use types (CV >25%) except for total height and crown height which were less dispersed. The highest variation was found in first ramification height followed by stem diameter (dbh), and crown diameter (Table 2). There was no significant difference between land-use types for total height, first ramification height, and crown height. However, stem diameter was significantly different between land-use types. The largest trees were encountered in farmlands with a mean dbh of 51.53 cm; whereas the thinnest trees were observed in home gardens with mean dbh of 41 cm (Table 2).

Impact of land-use on leaf size characteristics of bitter kola

Compared to tree growth traits, most of the morphological descriptors of leaves were relatively less dispersed (CV < 25 %) in both land-use types (Table 3). The highest variation was recorded in petiole diameter in both home gardens and farmlands. There was no significant difference between blade leaf width, petiole

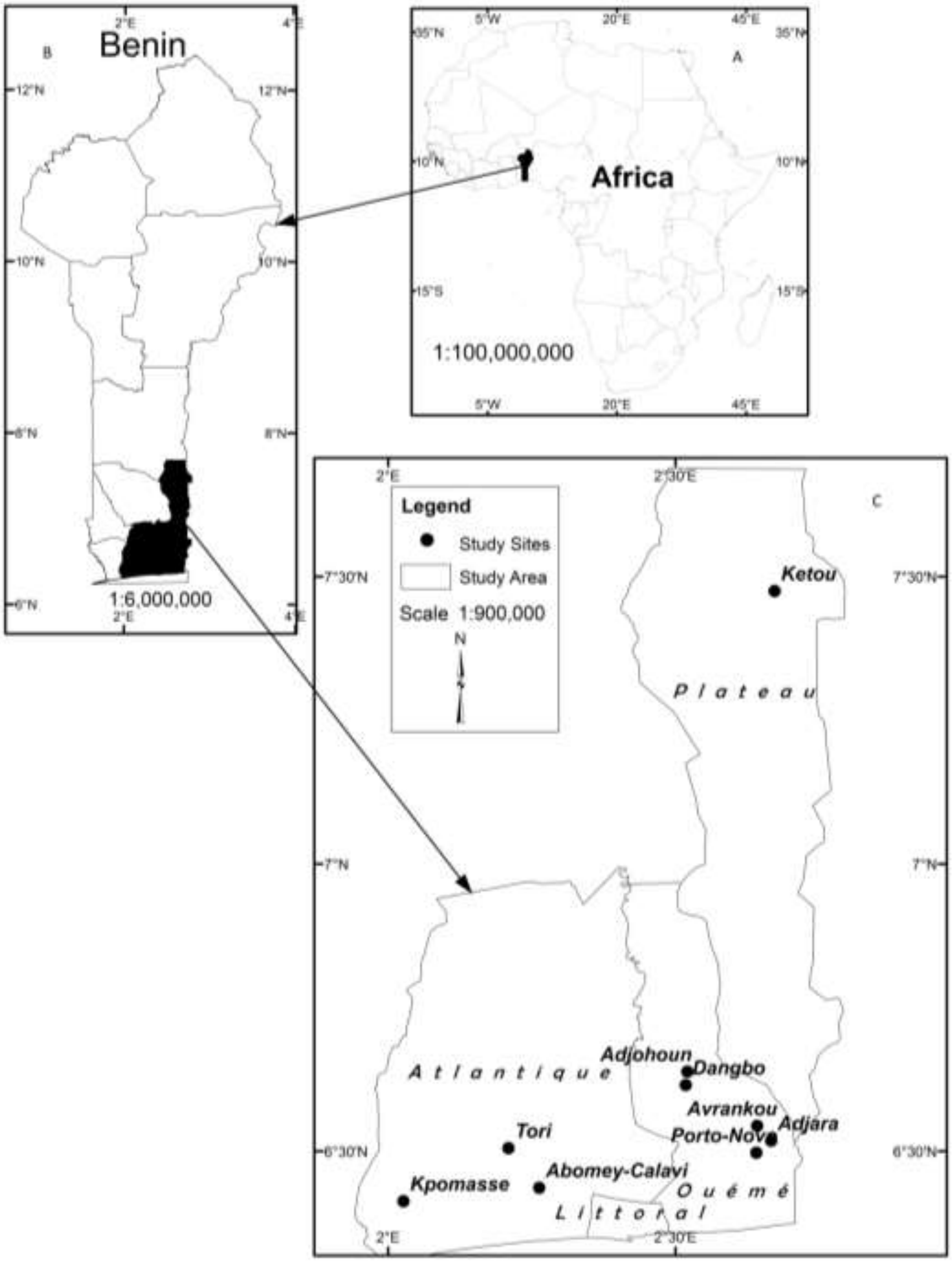

Figure 2. Study area showing (A) the location of Benin in Africa, (B) the position of the three regions studied in Benin, and (C) the Communes where the trees were located.

length and petiole diameter and land-use types. Leaf blade length in home gardens and farms differed significantly (Table 3). The leaf blade was longer in farmlands (129.52 mm) than in home gardens (115.76 mm).

Impact of land-use on fruits and seed's characteristics of bitter kola

In general, most fruit traits had relatively low variation (CV < 25%) except for husk weight and number of

Table 1. Tree growth characteristics.

Growth parameter	Formula	Explanations	References
Stem diameter (Dbh)	$Dbh = \frac{C}{\pi}$	The stem diameter of the tree (dbh) was determined by measuring the circumference (C) at 1.30 m using a pentadecameter.	-
Dbh for multi-stemmed tree	$Dbh = \sqrt{d_1^2 + d_2^2 + \ldots + d_n^2}$	d1…dn is the Dbh of stem 1… stem n	Saıdou et al. (2012)
Total height	$H = \frac{V_2 - V_1}{L}$	L = sightings distance of 10 m between the operator and the tree. V1 = first sighting, at the foot of the tree V_2 = second sighting, at the top of the tree	Rondeux (1999)
First ramification height (Hfr)		L = sightings distance of 10 m between the operator and the tree. V1 = first sighting, at the foot of the tree V_3 = second sighting, at the first ramification	
Crown height (Hcr)		H= total height, Hfr= first ramification height	-
Crown diameter (Dcr)		D_1 = crown North–South diameter D_2 = crown East–West diameter	-

Table 2. Tree growth variation according to land-use type.

Parameter	Overall (n=79)		Homegardens (n=60)		Farms (n=19)		P
	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV	
Dbh (cm)	43.79 ± 1.79	36.46	41.00 ± 1.92	36.35	51.53 ± 3.80	32.16	0.011
Total Height (m)	14.83± 0.33	19.86	14.91 ± 0.40	20.74	14.57 ± 0.57	17.05	0.665
First Ramification Height (m)	3.08 ± 0.15	42.75	3.06 ± 0.17	42.42	3.15 ± 0.32	44.81	0.803
Crown Height (m)	11.74 ± 0.31	23.75	11.85 ± 0.38	24.89	11.42 ± 0.52	19.71	0.565
Crown Diameter (m)	9.12 ± 0.28	27.48	9.00 ± 0.32	27.12	9.49 ± 0.63	28.84	0.462

Means followed by the same letter on a row are not statistically different at probability P< 0.05; SE, standard error of mean; CV, coefficient of variation (%).

seeds. However, the fruit traits did not vary in the same way between land-use types. In home gardens, a relatively high variation was observed only in number of seeds while it was observed in husk weight and fruit weight in farmlands (Table 4). The highest overall trait variation as well as the highest variation in home gardens was observed in number of seeds. The highest variation was recorded in husk weight in farmlands. There was a significant difference between land-use types for fruit length, fruit width, fruit weight, and husk weight. No significant difference was observed between land-use types for the number of seeds (Table 4). In farmlands, fruit length (56.06 mm), fruit width (59.53 mm), fruit weight (109.97 mm) and husk weight (103.23 mm) were higher than fruit length (52.02 mm), fruit width (55.55 mm), fruit weight (88.50 mm) and husk weight (85.43 mm) in home gardens. All seed traits had relatively low variation (CV < 25%) in both land-use

Table 3. Leave traits variation according to land-use type.

Parameter	Overall (n=79)		Homegardens (n=60)		Farms (n=19)		P
	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV	
Blade Length (mm)	119.06 ± 2.01	15.04	115.76 ± 2.25	15.03	129.52 ± 3.59	12.10	0.007
Blade Width (mm)	49.66 ± 0.65	11.65	49.44± 0.78	12.20	50.34 ± 1.15	9.96	0.556
Petiole Length (mm)	12.12 ± 0.21	15.13	11.93 ± 0.23	14.75	12.71 ± 0.46	15.60	0.110
Petiole Diameter (mm)	1.80 ±0.03	16.52	1.80 ± 0.04	16.69	1.80 ± 0.07	16.42	0.948

*Means followed by the same letter on a row are not statistically different at probability P< 0.05. SE, standard error of mean; CV, coefficient of variation (%).

Table 4. Fruit traits variation according to land-use type.

Parameter	Overall (n=79)		Homegardens (n=60)		Farms (n=19)		P
	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV	
Fruit Length (mm)	52.99 ±0.61	10.22	52.02 ± 0.54	8.07	56.06 ± 1.72	13.37	0.004
Fruit Width (mm)	56.51 ± 0.52	8.20	55.55 ± 0.50	6.91	59.53 ± 1.30	9.52	0.001
Fruit Weight (g)	93.66 ± 2.63	24.97	88.50 ± 2.13	18.63	109.97 ± 7.64	30.27	0.000
Husk Weight (g)	87.35 ± 2.58	26.24	85.43 ± 2.55	23.16	103.23 ± 7.45	31.44	0.000
Number of Seeds	2.34 ± 0.08	28.82	2.34 ± 0.09a	30.28	2.36 ± 0.13	24.42	0.921

*Means followed by the same letter on a row are not statistically different at probability P< 0.05. SE, standard error of mean; CV, coefficient of variation (%).

Table 5. Seed traits variation according to land-use type.

Parameter	Overall (n=79)		Homegardens (n=60)		Farms (n=19)		P
	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV	
Seed Length (mm)	28.87 ± 0.79	24.24	27.59 ± 0.89	24.85	32.92 ± 1.36	18.07	0.003
Seed Width (mm)	17.20 ± 0.28	14.67	16.76 ± 0.34	15.72	18.58± 0.34	7.9	0.004
Seed Weight (g)	6.31 ± 0.17	23.66	6.04 ± 0.19	23.98	7.15 ± 0.31	18.76	0.000

*Means followed by the same letter on a row are not statistically different at probability P< 0.05. SE, standard error of mean; CV, coefficient of variation (%).

types. The highest overall trait variation as well as the highest variation in home gardens was observed in seed length followed by seed weight. The highest variation in farmlands was observed in seed weight followed by seed length (Table 5). Seed length (32.92 mm), seed width (18.58 mm) (7.15 mm) and seed weight were significantly higher in farmlands than seed length (27.59 mm),seed width (16.76 mm) and seed weight (6.04 mm) in home gardens (Table 5).

Discriminant morphological descriptors of G. kola according to land-use types

The canonical discriminant analysis indicated that some morphological descriptors separated *G. kola* trees according to land-use types (P < 0.001). The first axis saved 100% of the information. Only 8 out of the 17 initial descriptors were identified as the most discriminant of trees according to land-use types. There were stem dbh, blade length, fruit length, fruit width, husk weight, seed length, seed width and seed weight (Figure 3). Trees in farmlands are bigger, with longer blades, longer and bigger fruits, and longer, bigger and heavier seeds.

DISCUSSION

The relatively important variation of morphological descriptors around mean values especially for tree

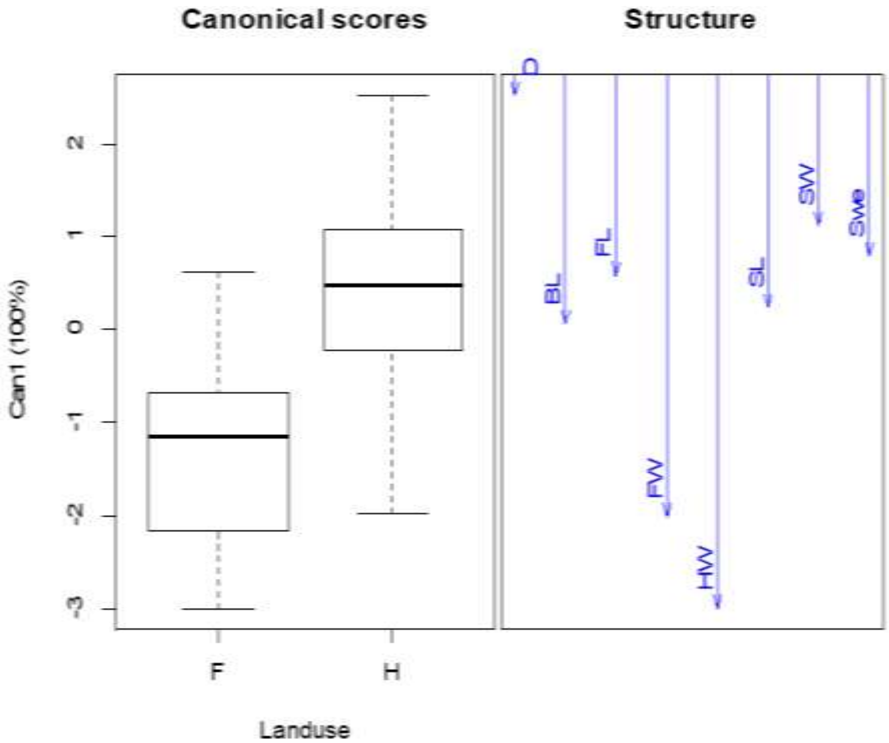

Figure 3. Morphological descriptors discriminating land-use: canonical scores and structures on the first canonical axis. D- diameter, FL= fruit length, FW- fruit width, HW- husk weight, SL- seed length, SW- seed width, SWe- seed weight.

growth and seed traits is an indicator of the potential of the species for domestication through selection of elite trees. Our results reveal that land-use type has an impact on variation of tree diameter, leaf blade length, and fruit and seed size of bitter kola. .The difference between home gardens and farmlands for those traits is possibly linked to different management practices occurring in the land-uses. Although they are both under human management, different practices are done in farmlands and home gardens. The largest *G. kola* trees were found in farmlands. Considering that farmlands are less disturbed than homesteads (Schumann et al., 2010), trees in farmlands might have less pressure such as pruning and debarking than in homesteads and heavy pruning has been shown to negatively affect tree diameter (Kumar et al., 2010; Erkan et al., 2016). Contrary to our findings, Akpona et al. (2015) found no significant difference in trees diameter of shea butter from three land-use types especially parklands, fallows and villages, possibly because trees are not subject to particular management. In comparing fruits and seeds descriptors between the land-use types, fruits and seeds from farmlands were found to be bigger and heavier than those from home gardens. This result may be explained by the fact that farmlands are more fertile than home garden soils. The soil where the trees grow is an important factor that may have affected the morphological traits of the seed and fruit (Assogbadjo et al., 2006). Farmlands are areas where annual crops are actively cultivated. Soil fertilizers originally provided to annual crops also benefit associated trees in farmlands (Aleza et al., 2018). In addition, the findings indicate that dbh, blade length, fruit length, fruit width, husk weight, seed length, seed width and seed weight are the most appropriate traits to discriminate *G. kola* trees among land-use types. Given the degree of variation reported in this study, land-use management may be an important determinant in morphological variability of the species. Selection for improvement of fruit and seed traits would be more effective among trees in farms which recorded the highest values.

Conclusion

This study assessed the variability of tree and fruit morphometry of *G. kola* and tested their differences between home garden and farmland. The observed variability in morphological traits highlights the potential for selection within the species. We also found significant difference between both land uses, with stem diameter, fruits and seeds of the trees being bigger in farmlands possibly due to difference in management practices. Experimental studies are needed to provide better understanding of the species response to different management practices. This morphological study should be completed with amolecular analysis of the intraspecific genetic variation. This would provide insights to define an effective conservation strategy for *G. kola* and would be very useful for the development of breeding programs in order to increase *G. kola* production in Benin.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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