South African Journal of Botany 156 (2023) 286–297

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Conservation status and phenotypic diversity of natural populations of *Vitex doniana* sweet in southern Benin assessed using quantitative morphometric traits

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A R T I C L E I N F O

Article History:
Received 22 May 2022
Revised 9 March 2023
Accepted 13 March 2023
Available online xxx

Edited by Dr. S.J. Siebert.

Keywords:
Vitex doniana
Black plum
in-situ Conservation
Natural populations, Morphological diversity
Quantitative traits
Benin

A B S T R A C T

Vitex doniana is an important but endangered multipurpose wild fruit tree species well known in Sub-Saharan Africa, particularly in Benin. The present study assessed its current conservation status and morphological diversity within its natural populations in Benin. The study was conducted in 28 villages well distributed in 5 districts in southern Benin. The *in-situ* conservation status was assessed through direct field observations and transect walks. A total of 125 randomly selected trees were characterized using 13 quantitative morphological descriptors. Our results showed high morphological variability among trees based on the traits such as trunk circumference, crown attributes and diameter of leaf petiole. Positively significant correlations were found between most dendrometric parameters and leaf and fruit-related traits. The cluster analysis classified natural populations into three phenotypic groups, with the Cluster 2 encompassing the best accessions showing the greatest height, trunk diameter and the largest crown but with leaves moderate in size. However, the transect analysis performed revealed highly fragmented natural populations due to intense human activities. Therefore, awareness needs to be raised to protect this species and there is also an urgent need to develop domestication, breeding schemes and *in-situ* conservation programs dedicated to this species.

1. Introduction

In Sub-Saharan Africa, forests contain many multipurpose tree species that are highly useful for human populations due to their contribution to health, food and energy supply and other aspects of human well-being (Codjia et al., 2003; Natta et al., 2011). Some multipurpose trees have been identified as priority species that need special attention because of their socioeconomic importance, the high anthropic pressure, the extinction risks, and the absence of conservation and research programs (Eyog Matig et al., 2002). In Benin, *Vitex doniana* Sweet, commonly known as black plum, is one of the ten priority but endangered forest tree species and it has been recognized as a potential candidate for domestication (Ahoton et al., 2011).

Black plum belongs to the Verbenaceae family and the genus *Vitex* in which *Vitex doniana* is the most widespread species (Padmalatha et al., 2009). Black plum is a highly valued tree native to tropical Africa where the species occurs from Senegal to Somalia and South Africa. It is also reported in Comoros and Seychelles (Arbonnier, 2000; Ky, 2008). It is a cross-pollinating species mainly carried by insects and birds (Sinébou et al., 2016). *Vitex doniana* is a multipurpose fruit tree highly appreciated by local communities. The immature leaves are the predominant part of the plant which is used and consumed as a leafy vegetable (Sanoussi et al., 2012). The ripe fruit pulp is processed into a refreshing drink. Apart from its leaves, which are in high demand, the black plum tree also has many other domestic uses. For instance, it is reported that the bark and root are used in traditional pharmacopeia (Adetoro et al., 2013). An infusion of its leaves is said to cure arterial hypertension (Bolanle et al., 2014). The domestication of black plum and its integration into diverse agroforestry systems are important components of a strategy for sustainable exploitation of its genetic resources as well the improvement of land use in Africa (Cemansky, 2015; Ofori et al., 2014).

Despite its nutritional, medicinal and economic importance, this multipurpose species is subjected to climate variability and various

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https://doi.org/10.1016/j.sajb.2023.03.023
0254-6299/

anthropic pressures, mainly due to intensive harvest of the young leaves for sauce making and the cutting up of branches for firewood, threatens the survival of the species (N'Danikou et al., 2015). In addition, the seeds of this species have a very low germination ability (Sanoussi et al., 2012).

Recent agroforestry initiatives aimed at integrating tree species with marketable non-timber products into existing farming cropping systems. These species provided farmers with non-timber forest products and timber to improve their livelihood through the diversification of their income sources (Leakey and Tchoundjeu, 2001). In this process, particular importance should be given to species that are traditionally used by people for their food and medical needs. Most of the studies conducted on *V. doniana* in Benin focused on the knowledge and uses of the species by local communities (Dadjo et al., 2012), on the propagation of the species from organs other than seeds (Achigan Dako et al., 2014; Sanoussi et al., 2012) and on the effect of seed pretreatment on the germination and seedling growth (Ahoton et al., 2011).

The lack of scientific data and information related to ecology of the species, its morphological and molecular genetic diversity, its production and management in traditional agroforestry systems was mentioned in a report on food forest species (Eyog Matig et al., 2002). However, studies focusing on the assessment of the variability of natural populations of *V. doniana* addressing morphological variability in Benin are rare, except that recently conducted by Hounkpèvi et al. (2016) on possible induction of morphological variation in the species by climate change. In addition, there are not yet well standardized morphological descriptors to perform adequate morphological characterization of this species. Likewise, Kouyaté and van Damme (2002) argued that the sustainable use and management of trees or shrubs require an analysis of the morphological variability of the species in order to differentiate individuals and target interesting morphotypes to reproduce.

The objectives of the present study are to i) assess the current conservation status of *V. doniana* and ii) quantify the morphological diversity of the species and its distribution within natural populations. These will help in developing efficient strategies for *in-situ* conservation of *Vitex doniana* and a sustainable utilization of its genetic resources through domestication and integration in the agroforestry system in southern Benin.

2. Materials and methods

2.1. Study area

The study was carried out in Zou, Ouémé and Atlantic Departments (southern Benin) where the species is widespread and found in natural populations. This region, situated between latitudes 6°25′N and 7°30′N, belongs to the Guinean climatic zone of Benin characterized by a bimodal rainfall distribution (April-June and September-November) with a mean annual rainfall of 1200 mm (Assogbadjo et al., 2006). The mean daily temperatures range from 25 °C to 29 °C while the relative humidity varies between 69% and 97%. In this zone, the soils are either deep ferrallitic or rich in clay, humus and minerals (Assogbadjo et al., 2009). The native vegetation consists of dense semi-deciduous forests and Guinean savannas. The active vegetation period lasts 7 to 8 months. Data were collected from five districts including Abomey-Calavi in the Atlantic Department, Adjohoun and Bonou in the Ouémé Department, Zangnanado and Ouinhi in the Zou Department (Fig. 1).

2.2. Assessment of the conservation status of V. doniana in southern Benin

The conservation status of natural populations of black plum in the study area was assessed through field survey and transect walks (TW) following the methodology described by World Bank (2005) and well applied by Marchewka et al. (2013) and recently by Atindehou et al. (2022). The TW is a technical methodological approach routinely used in ecological studies to estimate animal/plant biodiversity and abundance (Burnham et al., 1980; Butler et al., 2006). Practically, transect walk is used to identify problems and opportunities regarding resource use and access to resources in the various parts of the visited area (World Bank, 2005). It is a tool for describing and showing the location and distribution of resources, features, landscape, and main land uses along a given transect (Fauna and Flora International, 2013).

In this study, all the *V. doniana* individuals from a territory of a locality were considered as a natural population. In each identified and delimited black plum population, a rapid survey of the covered area (field observation) was first made to get a general view on its conservation status. To better appreciate this status, two to three transects of 40 m by 150 m were made depending on the size of the populations that almost covered a surface ranging from about 0.75 to 3 km^2. This level of appreciation was conducted in order to quantify, at a small scale, the impact of anthropogenic activities on these black plum populations and explore their capacity of regeneration. In general, transects were positioned following different directions to ensure a good coverage and representativeness of the black plum population site (Fig. S1). Also, contiguous transects were not made to avoid double counting of trees. In some populations, transects were divided into smaller transect segments (40 $m \times 50$ m) that were later combined after survey. Four observers (two researchers and two local informants) walked along the length of each transect. During the walk, the survey team members counted, recorded, and discussed any observation which could be of relevance to black plum genetic resources management and utilization. Indeed, in each transect, mutilated trees were identified and counted, and any injuries observed (leaves exploitation, branch and whole tree cut, among others) were carefully categorized and recorded. Furthermore, the investigating team members interviewed any local people met during the walk to get their perceptions on the conservation status of black plum resources in their localities. The abundance of juvenile plants, an indicator of the species regeneration, was also documented. Besides, observers moved slowly in order to minimize disruption during scoring. After the walk, the information collected during the transect walk is used to draw a diagram or map and provides a basis for discussion amongst participants. In general, a couple of hours was devoted to the transect walk in each black plum population.

2.3. Morphological diversity assessment

2.3.1. Tree sampling and characterization

The trees were sampled in the 5 municipalities of the study area from June to August, falling in the rainy season and therefore offers optimal conditions for the development of trees. The localities were selected on the basis of the effective occurrence and accessibility of the plant in natural populations. Thus, per population, 30 individuals spaced at least by 20 m apart were selected so that physically and genetically related individuals were not sampled (Graudal, 1998; Kouyaté, 2005). Exceptionally in Abomey-Calavi, five individuals were sampled due to the limited number of available trees. All the natural populations identified have been geo-referenced using a Global Positioning System (GPS).

The evaluation of the morphological variability between and within *V. doniana* populations was carried out by using a combined system of description. Indeed, combined morphological descriptors of *Detarium microcarpum* (Agbo et al., 2018; Kouyaté, 2005), a species of the same class (*Magnopsideae*) as *V. doniana* and *Anarcadium occidentale* L. (Chipojola et al., 2009) were primarily tested for the morphological characterization of black plum. Besides, descriptors used

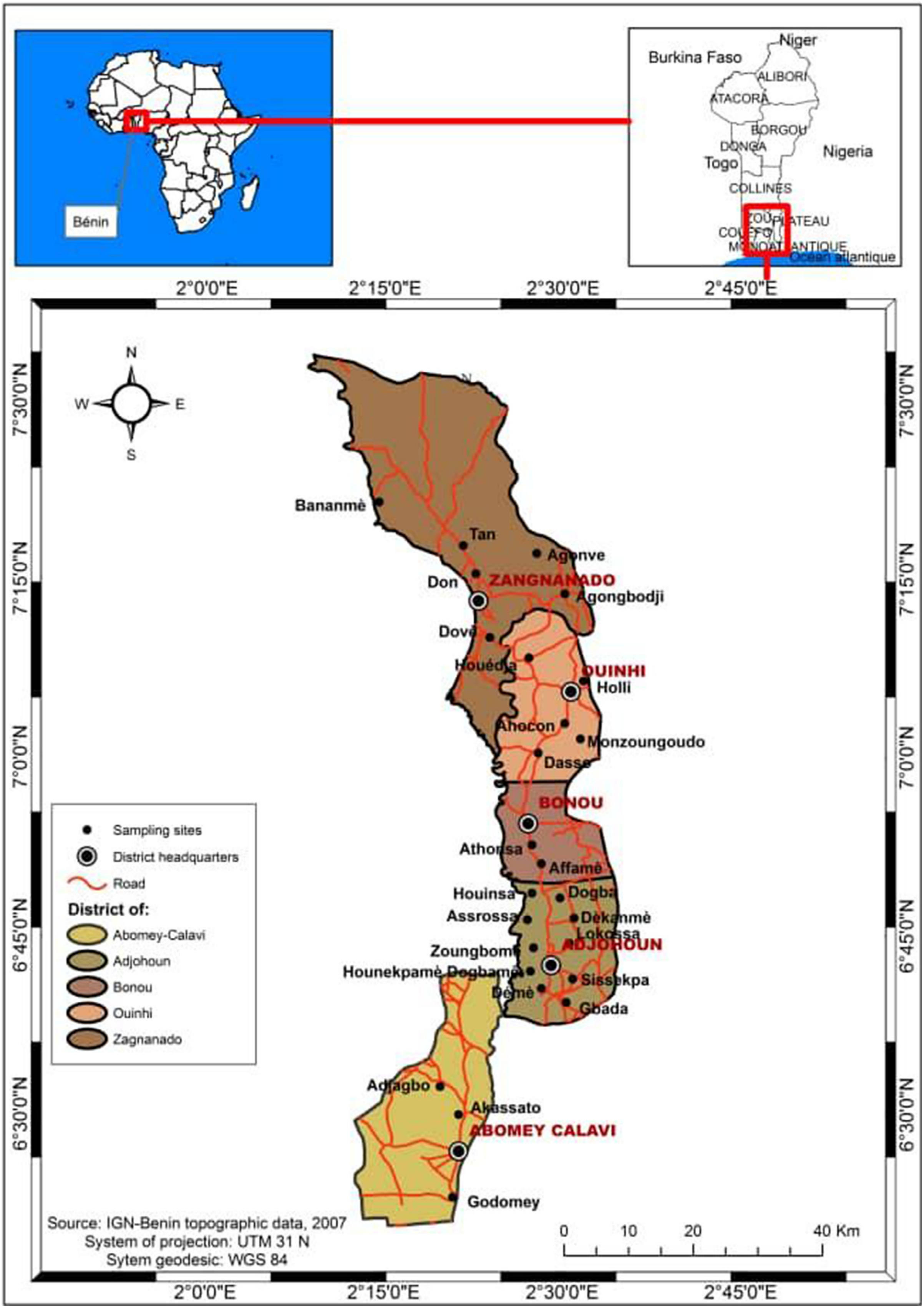

Fig. 1. Geographic localization of study area showing the surveyed villages.

Table 1
Quantitative traits used for morphological description of black plum.

Organs	Abbreviate	Description	Unit
Vegetative	C1.30m	Circumference of the tree	m
	HT	Total height of the tree	m
	SH	Crown surface / area	m^2
	Hr	Height of the first basal branch	m
Leaves	LFe	Leaf length	cm
	Dp	Diameter of the petioles	mm
	Lfo	Length of the largest leaflet	cm
	Lafo	Width of the largest leaflet	cm
	Lp	Length of petiolules	cm
	Nfo	Number of leaflets	–
Fruits	Lf	Fruit length	mm
	Lef	Fruit thickness	mm
	Pf	Fruit weight	g

by Hounkpèvi et al. (2016) were additionally considered. A total of 13 morphological descriptors were definitively retained after the preliminary on-field observations. The measurements were realized on different parts of the tree namely: trunk, crown, leaves and fruits (Table 1). Data were recorded on 125 trees, 375 leaves and 325 fruits.

2.3.2. Measurement of dendrometric parameters

The trunk circumference (C1.30 m) of the trees was measured using a Pi tape at 1.30 m above ground level and is expressed in meter.

The total height of the tree was measured using a SUNNTO Finland clinometer. Indeed, two targets, one at the foot of the tree (V1) and the second at the top of the shaft (V2) were necessary for the determination of this parameter. Then, the operator-tree distance (L) was measured using a 50-meter decameter. The formula of Rondeux (1999) and recently used by Saidou et al. (2012) for calculating total tree height, was used:

$$H = \frac{(V2 - V1) \times L}{100}$$

where L is the distance separating the operator from the tree when shooting shots.

The height of the first basal ramification with respect to the ground was measured using a 10 m sliding decameter.

The average crown diameter was determined from the measurement of the North-South diameter (D1) and the East-West diameter (D2) of the crown using a decameter and the formula proposed by Rondeux (1999) and recently used by Saidou et al. (2012).

$$D = \sqrt{\frac{D1^2 + D2^2}{2}}$$

where D is the average crown diameter, D1 the North-South diameter and D2 the East-West diameter.

The crown surface (in m^2) at the ground was calculated after the determination of the average diameter of the crown according to the formula proposed by Rondeux (1999)

$$S = \frac{\pi \times D^2}{4}$$

where D is the average diameter of the crown.

2.3.3. Measurement of leaf parameters

Measurements of leaf parameters (Table 1) were performed on three fresh, healthy and fully opened leaves. The length of the leaves (LFe in cm) was determined using a 50 cm ruler, and the leaf petiole diameter (Dp in mm) using a digital Vernier caliper brand Stainless Hardened with a precision of ±0.02 mm. The width of the leaves (Lafo in mm) and the length of the petiolules (Lp in cm) were measured using a graduated rule and the number of leaflets (Nfo) obtained by simple counting. The length of compound leaves was measured from the base of the petiole to the tip of the terminal leaflet. The leaflets are taken from the point of attachment of the petiolule to the tip of the leaflet. The width of the leaflets was measured from the half length of the leaflets and the petiolule length was measured from the base of the petiolule to the insertion point of the leaflet. Fig. 2 illustrates the measurements of these leaf-related parameters during trait documentation.

2.3.4. Measurement of fruit parameters

Measurements of fruit parameters were performed only on studied populations in three of the five localities surveyed. Indeed, the black plum populations in Ouinhi and Zangnanado did not fruit during the field study period. This could be explained by the strong anthropic pressure exercised on these two populations. In each population with fruiting individuals, data were recorded on twenty-five fruits collected on five individuals except in the locality of Abomey-Calavi where only three of the five individuals identified had fructified during the collection period. The length (Lf) and the thickness (Lef) of the fruits were measured in millimeters using a precision digital caliper ± 0.02 mm. The length was taken from the attachment point of the fruit to the branch extending to the extremity of the fruit while the thickness was measured at the level of the largest diameter of the fruit. Fruit weight (Pf) in grams was determined using a Mettler scale.

2.4. Statistical analysis

Descriptive parameters such as mean, coefficient of variation and range were determined on the morphological parameters to describe their variability within the studied collection. The variation classes were made using a scale proposed and tested by Ouédraogo (1995) and taken up by Kouyaté (2005): (1) weak variation (CV = 0–10%); (2) average variation (CV = 10–15%); (3) fairly large variation (CV = 15–44%); and (4) significant variation (CV> 44%).

Analysis of the variance (ANOVA) was performed to estimate the variability among populations for the parameters measured. Pearson

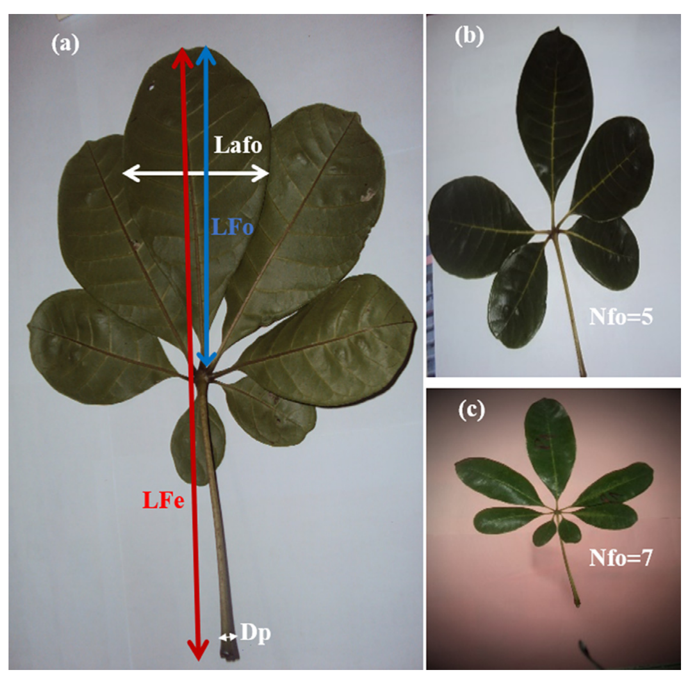

Fig. 2. Illustration of the morphological traits measured on the leaf of *Vitex doniana* (a) whole leaf measurements, b) and c) leaf with 5- and 7 leaflets, respectively. Lafo: width of the largest leaflet; Lfo: length of the largest leaflet; LFe: leaf length; Dp: Diameter of the petioles; Nfo: number of leaflets.

correlations coefficients, used as a measure of similarity, were computed to estimate the relationships among the studied variables; a correlogram was drawn using the *corrplot* package (Wei and Simko, 2017). Hierarchical cluster analysis on principal components was performed using FactoMineR (Sebastien et al., 2008) to determine the clustering structure of the trees and the respective morphological characteristics of inferred phenotypic groups. ANOVA analysis followed by the mean comparison test of Tukey was then performed for comparing the different clusters. All the data analysis was performed using R version 4.1.2 (R Core Team, 2021).

3. Results

3.1. Taxonomy and botanical description

Vitex doniana Sweet. Hort. brit., ed. 1, 2: 323 (1826); FWTA 2: 446; FT 495; FTEA 62.

Syn.: *Vitex cuneata* Thonn. (1827), *Vitex cienkowskii* Kotschy & Peyr. (1867), *Vitex pachyphylla* Baker (1900).

Black plum tree with its different organs are shown on Fig. 3; see also Hutchinson and Dalziel (1963) and Akouegninou et al. (2006). It is a deciduous small to medium-sized tree up to 25 m tall (Fig. 3a); bole branchless for up to 11 m, up to 90 cm in diameter, often slightly fluted at base; bark surface greyish white to pale greyish brown, fissured and scaly, inner bark yellowish white, darkening to brown; crown rounded; young branches shortly hairy and glabrescent.

Leaves are opposite and digitate compounds with mostly 5 and occasionally 6–7 leaflets (Fig. 3b); stipules absent; petiole 5–20 cm long; petiolules up to 2.5 cm long. Leaflets are obovate to elliptical (4–25 cm × 2.5–10.5 cm), notched to rounded or shortly acuminate at apex, entire, and nearly glabrous. The inflorescence is an axillary cyme (Fig. 3c) up to 10 cm long and 16 cm wide, orange-brown hairy; peduncle 2–7.5 cm long; bracts up to 6 mm long. The flowers, bisexual, are zygomorphic, 5-merous; pedicel up to 2 mm long; calyx conical, 3–5 mm long, with short teeth, enlarging in fruit; corolla white to pale purple, tube 6–8 mm long, curved, limb 4-lobed, lobes c. 3 mm long and middle lower lobe up to 4.5 mm long; stamens 4, inserted in the corolla tube, 2 long and 2 short; ovary superior, obovoid, 4-celled, style c. 7 mm long. Flowering and fruiting occur between February and October. The edible fruit is an obovoid to oblong-ellipsoid drupe (Fig. 3e, f), 2–3 cm long, purplish black, fleshy, with woody, 4-celled stone, up to 4-seeded. Seeds are without endosperm.

Fig. 3. Morphology of a *Vitex doniana* tree in a fallow land near to Ouinhi locality: a) tree, b) leaves, c) flowered branch, d) infructescence and e) mature fruits (Photos Adoukonou-Sagbadja & Ahoyo 2015, 2016, 2017).

3.2. Vulnerability of natural populations in the study area

Field observations and transect walks in the study area showed a high fragmentation of natural populations of black plum due to intense anthropogenic activities (Fig. 4). These activities were reported to be related to human habitat (35% of respondents) and agricultural land extensions (86% of respondents), leaves (41% of respondents) and wood exploitation of trees (71% of respondents). In the surveyed localities in Atlantic and Ouémé Departments, profound fragmentation of natural populations was clearly observable with only rare isolated adult trees detected, mainly in the relict / sacred forests. In Ouinhi and Zagnanado districts in Zou Department where tree density was found more important, 54 completely or partially defoliated trees, 33 trees with cut branches and 27 cut trees were recorded in the transects analyzed (Fig. S2), showing intense anthropic pressures on these natural populations.

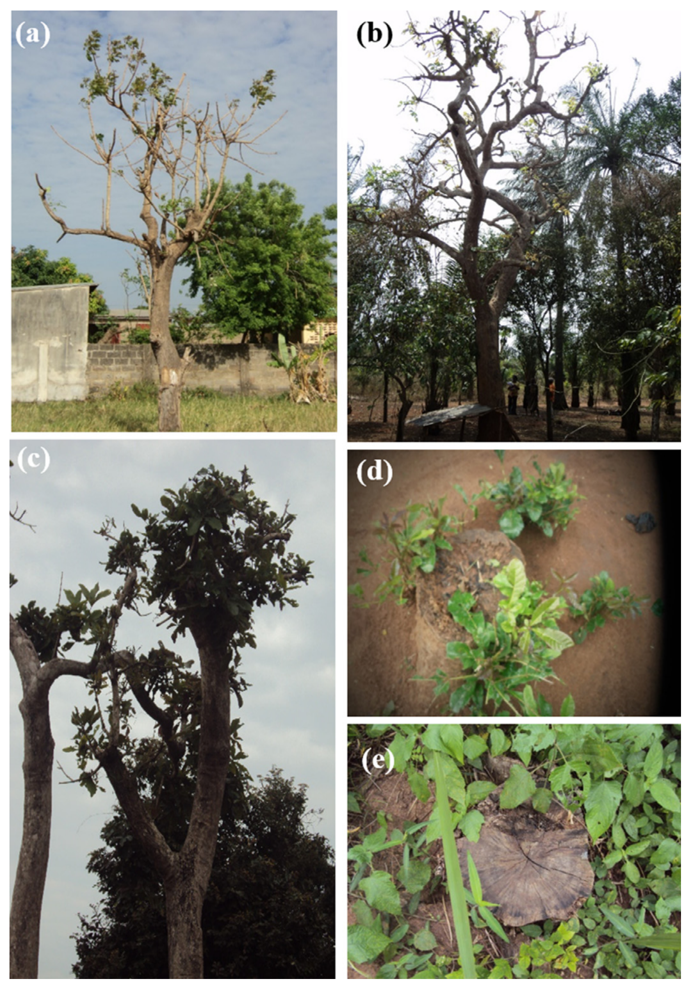

Fig. 4. Mutilated trees showing strong anthropic pressures on natural *Vitex doniana* populations in the study area: (a) & (b) Completely defoliated trees, (c) Trees with all branches cut, (d) & (e) Completely cut trees (Photos Adoukonou-Sagbadja & Ahoyo 2015, 2016, 2017).

In all the studied areas, the mutilated parts of the trees (leaves and branches) were intended mainly for leaves exploitation as traditional leafy vegetables and the branches as fire woods. Most of these trees died after many years of exploitation. Complete trees' cutting for wood exploitation was also observed in the study area and is gaining importance in Ouinhi and Zagnanado districts. Many living trees observed during the surveys of 2015 and 2016 were found cut down in the 2017 expedition. Furthermore, intense seasonal exploitation of juvenile plants in addition to bush fire destruction limits their development into adult plants in most of the study locations (Fig. 5). These observations were confirmed by local populations, indicating that black plum is endangered in the study area with its remaining natural populations highly vulnerable.

3.3. Phenotypic diversity in natural black plum populations in southern Benin

3.3.1. Inter-population morphological variability

The variance analysis on the morphometric traits showed a highly significant difference ($P< 0.001$) between populations for crown surface (SH) and leaf petiole diameter (Dp) (Table 2). The tree circumference (C1.30 m) and tree height (HT) were also significantly different among populations ($P< 0.05$). However, the leaf parameters such as length of leaf (LFe), length of the leaflets (Lfo), length of the petiolules (Lp), number of leaflets (Nfo) and height of the first basal ramification did not significantly vary ($P> 0.05$) among populations.

The descriptive analysis of the parameters measured between populations (Table 3) showed that the coefficient of variation (CV) ranged from 7.66% (number of leaflets) to 75.02% (crown area). For all populations, leaf length (LFe) presented an average variation. Traits such as circumference (C1.30 m), total tree height (HT), height of first basal ramification (Hr), petiole diameter (Dp), leaflet length (Lfo), leaflet width (Lafo) and petiolule length (Lp) showed a fairly large variation (CV = 15−44%). The only variable with a very large variation (CV> 44%) was the crown surface (SH).

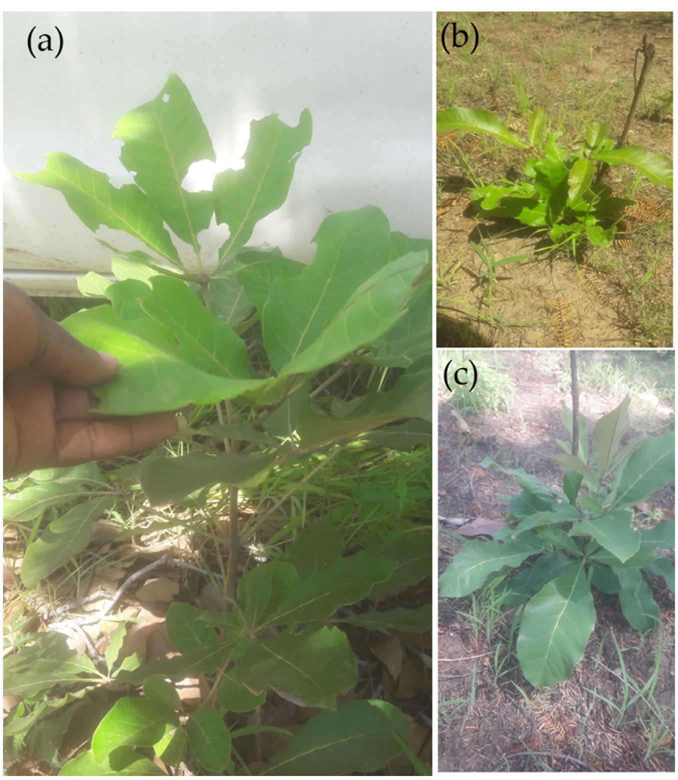

Fig. 5. Juvenile plants of *Vitex doniana*: (a) unexploited plant, (b) & (c) regenerated plants after human exploitation or bush fire destruction (Photos Adoukonou-Sagbadja 2017, 2018).

Table 2
Results from the analysis of variance for the studied traits.

Parameters	RSS	RDF	Error	Frequency
C1.30m	13.4	120	0.11	3.72 *
HT	881.7	120	7.35	3.83 *
SH	163,388.5	120	1361.6	16.36 ***
Hr	37.7	120	0.31	2.16
LFe	1919.8	120	16.00	1.65
Dp	140.5	120	1.17	6.19 **
Lfo	778.1	120	6.48	0.57
Lafo	190.4	120	1.59	0.34
Lp	24.1	120	0.20	2.27
Nfo	18.5	120	0.15	1.40

RSS: residual sum of squares; RDF: residual degrees of freedom; * $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$; C1.30m: circumference of the tree; HT: total height of the tree; SH: crown surface; Hr: height of the first basal branch; LFe: leaf length; Dp: Diameter of the petioles; Lfo: length of the largest leaflet; Lafo: width of the largest leaflet; Lp: length of petiolules; Nfo: number of leaflets.

The trunk circumference of *V. doniana* individuals was on average 1.04 ± 0.34 m. The height of the first basal branching (Hr) of *V. doniana* populations was on average 2.49 ± 0.52 m. On average, the tree crown was 8.27 ± 2.86 m tall. The floor area of the crown (SH) was 60.15 ± 43.13 m^2 on average. Black plum leaves had an average length of 29.55 ± 4.04 cm. The leaflets were on average 16.59 ± 2.53 cm long and generally 8.18 ± 1.25 cm wide. The leaves had petioles averaging 5.83 ± 4.04 mm in diameter and the leaflets had petiolules averaging 1.84 ± 0.46 cm. The number of leaflets per leaf varied from 4 to 7 leaflets.

3.3.2. Intra-population morphological variability in black plum

With regard to the variability within the studied populations, a fairly large variation (CV = 15 - 44%) was noted among the individuals sampled at Abomey-Calavi for most of the traits (Table 4). In this population, very large variation (CV > 44%) was found for trunk circumference and crown surface (SH). In addition, this population was characterized by trees having on average the highest values for the dendrometric parameters. The populations from Adjohoun and Bonou also showed quite a large variation (CV = 15−44%) for all the measured traits. Within these two populations, there was a variation in the crown area that was very important in Adjohoun (CV = 63.36%) and quite significant in Bonou (38.97%). These two populations contained individuals of average size, circumference and crown surface. The Adjohoun population was characterized by individuals with the largest leaflets (Lfo = 8.32 cm) while the Bonou population was

Table 3
Descriptive statistics of quantitative variables for all populations.

Variables	Average	SD	CV (%)	Minimum	Maximum
C1.30m	1.04	0.35	33.58	0.55	2.03
Hr	2.49	0.57	22.90	1.26	4.60
HT	8.53	2.83	33.16	4.73	20,03
SH	60.15	45.13	75.02	6.65	299.82
LFe	29.55	4.04	13.68	20.00	40.56
Dp	5.83	1.16	20.05	3.42	8.64
Lfo	16.59	2.52	15.24	11.40	22.13
Lafo	8.18	1.24	15.24	5.50	10.90
Lp	1.84	0.45	24.80	1.00	3.23
Nfo	5.00	0.39	7.66	4.00	7.00

CV: coefficient of variation; SD: Standard deviation; C1.30m: circumference of the tree; HT: total height of the tree; SH: crown surface; Hr: height of the first basal branch; LFe: leaf length; Dp: diameter of the petioles; Lfo: length of the largest leaflet; Lafo: width of the largest leaflet; Lp: length of petiolules; Nfo: number of leaflets.

Table 4
Morphological characteristics of the five populations of *Vitex doniana* (intra-population variation).

	Abomey-Calavi Mean ±SD	CV (%)	Adjohoun Mean ±SD	CV (%)	Bonou Mean	CV (%)	Zangnanado Mean	CV (%)	Ouinhi Mean	CV (%)
C1.30m	1.3 ± 0.6	44	1.2 ± 0.4	30	1.1 ± 0.4	32	0.9 ± 0.3	32	0.9 ± 0.3	31
HT	10.3 ± 2.5	24	9.9 ± 4.2	43	8.2 ± 1.8	21	8.2 ± 2.4	29	7.5 ± 1.6	22
SH	168.1 ± 107.7	64	74 ± 46.9	63	61.1 ± 23.8	39	56 ± 30.8	55	31.6 ± 17.9	57
Hr	2.7 ± 1	37	2.3 ± 0.5	23	2.7 ± 0.5	18	2.4 ± 0.6	23	2.5 ± 0.6	23
LFe	32.1 ± 4.8	15	29.2 ± 4.4	15	30.1 ± 4.6	15	28.3 ± 3.9	14	30.2 ± 2.7	9
Dp	5.2 ± 0.6	13	5.6 ± 1.2	21	5.9 ± 1.2	20	5.3 ± 0.8	16	6.6 ± 1.2	18
Lfo	17.1 ± 3.4	20	16 ± 2.6	16	17 ± 2.8	16	16.6 ± 2.5	15	16.7 ± 2.1	13
Lafo	8.2 ± 1.4	17	8.3 ± 1.4	16	8.2 ± 1.5	19	8.2 ± 1	12	8 ± 1.1	13
Lp	2 ± 0.7	34	1.9 ± 0.4	21	1.7 ± 0.4	25	2 ± 0.5	25	1.8 ± 0.4	24
Nfo	5 ± 0.8	15	5 ± 0.4	9	5 ± 0.4	8	5 ± 0.2	5	5 ± 0.4	7

CV: coefficient of variation; SD: standard deviation; C1.30m: circumference of the tree; HT: total height of the tree; SH: crown surface; Hr: height of the first basal branch; LFe: leaf length; Dp: diameter of the petioles; Lfo: length of the largest leaflet; Lafo: width of the largest leaflet; Lp: length of petiolules; Nfo: number of leaflets.

characterized by trees with the highest values for the height of the first basal branching (Hr = 2.68 m). The variations observed in the Zangnanado and Ouinhi populations for all the traits measured were quite important, especially for the dendrometric parameters. These populations were characterized by trees having on average the lowest values for the parameters considered. The individuals found on these two sites were small with small circumferences and a very small crown surface (56 m^2).

The length of black plum fruits (Lf) was on average 22.72 ± 1.43 mm (Table 5). The minimum and maximum values were observed in Adjohoun (20.01 mm) and Abomey-Calavi (24.87 mm), respectively. The fruits of *V. doniana* weighed on average 10.03 ± 1.03 g. The fruits weight (Pf) varied from 8.88 g at Adjohoun to 12.86 g in Abomey-Calavi. On average, the fruits had a thickness (Lef) of 31.46 ± 1.91 mm. The minimum and maximum values were 21.24 mm in Adjohoun and 28.45 mm in Abomey-Calavi. Variability among populations for fruit morphological descriptors was low (CV = 6.30−10.24%).

3.4. Correlations between the measured parameters

Pearson correlation coefficients were strong between most of the studied traits (Fig. 6, Table S1). For the vegetative traits, the height of the first basal branching (Hr) was strongly (P<0.0001) correlated with the tree circumference (C1.30 m). This latter was highly correlated with the tree height (HT) and the surface of the crown (SH).

For leaf-related traits, the leaf length (LFe) was highly and positively (P <0.0001) correlated with petiole diameter (Dp), leaflet width (Lafo), length of the leaflets (Lfo) and the length of the petiolules (Lp). Petiole diameter was highly and positively correlated with Lfo and Lafo. Positive and highly significant correlations were also observed between the Lfo and Lafo and on the other hand between Lafo and Lp. In addition, a very significant positive correlation (P <0.001) was observed between Lfo and Lp. Finally, there was a significant association (P <0.05) between Dp and Lp.

Table 5
Biometrical characteristics of *V. doniana* fruits.

Sites	No. Tree	Lf	Lef	Pf
Abomey-Calavi	1	24.87	28.45	12.86
	2	22.37	24.29	9.73
	3	24.63	27.13	11.14
Adjohoun	1	20.01	21.24	9.04
	2	21.00	21.40	9.26
	3	24.94	24.00	10.93
	4	21.56	23.23	9.72
	5	21.77	22.92	8.88
Bonou	1	22.73	23.63	9.51
	2	23.34	24.67	10.01
	3	22.38	24.17	9.56
	4	22.58	23.12	9.75
	5	23.30	24.52	10.10
Average		22.72	31.46	10.03
Standard deviation		1.43	1.91	1.03
CV (%)		6.30	6.07	10.24

Lf: fruit length; Lef: thickness fruit; Pf: fruit weight.

3.5. Structuration of the morpho-dendrometric diversity of V. doniana populations

The principal components analysis showed that twelve components revealed the overall variability within the studied collection (Fig. 7). The first four components, reported in Table 6, were those having eigenvalue >1 and accounted for 72% of the total variability. The first component (Dim.1) revealed 29.5% of variability while the second (Dim.2) accounted for 22.7%. Based on $\cos^2$ values, six out of ten morphological traits significantly ($\cos^2$>0.5) contributed to differentiate the individuals in the population. These discriminant traits were the dendrometric parameters such as HT, C1.30 m and SH, and the parameters related to the leaves such as Lfo, Lfe and Lafo.

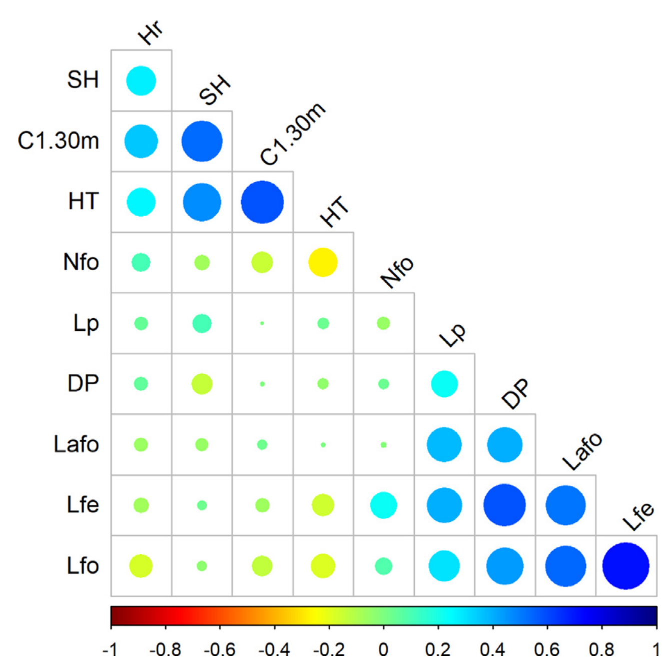

Fig. 6. Pearson standard linear correlation between morphological descriptors of black plum in Benin. C1.30m: circumference of the tree; HT: total height of the tree; SH: crown surface; Hr: height of the first basal branch; LFe: leaf length; Dp: diameter of the petioles; Lfo: length of the largest leaflet; Lafo: width of the largest leaflet; Lp: length of petiolules; Nfo: number of leaflets.

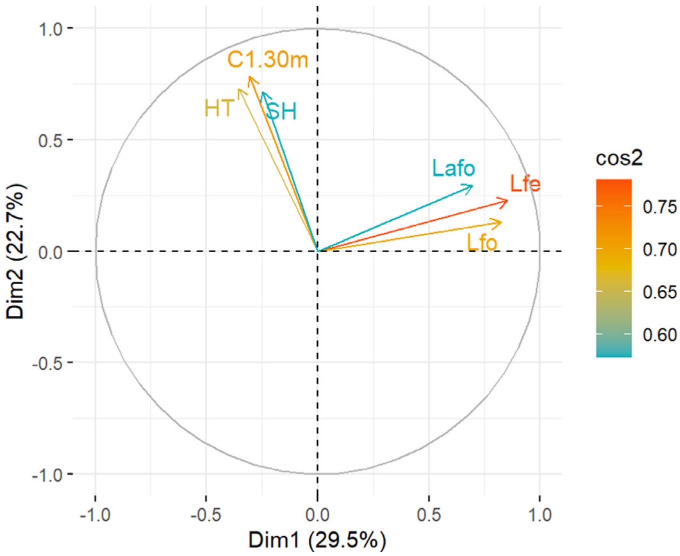

Fig. 7. Principal component analysis showing the relationships between the traits highly discriminating ($cos^2 > 0.5$) the studied population. C1.30m: circumference of the tree; HT: total height of the tree; SH: crown surface; LFe: leaf length; Lfo: length of the largest leaflet; Lafo: width of the largest leaflet.

Three clusters (phenotypic groups) with clearly divergent attributes resulted from Hierarchical Clustering on Principal Components (HCPC) performed based on the quantitative data (Figs. 8 and 9). The cluster 1 (brown1) comprised 67 individuals that were mainly from Ouinhi (30%), Zangnanado (25%), Bonou (24%), Adjohoun (19%) and Abomey-Calavi (1%). The cluster 2 (seegreen3) was composed of 18 trees of which 33% were from Zangnanado, 22% from Adjohoun, 22% from Bonou, 17% from Abomey-Calavi, 6% from Ouinhi. Forty (40) trees composed the cluster 3 (blue) of which 33% were from Adjohoun, 25% from Bonou, 23% from Ouinhi, 18% from Zangnanado and 3% from Abomey-Calavi. The Table 7 illustrates the mean performance of trees within the inferred phenotypic groups. The cluster 2 brought together the best trees. They were big with the greatest height and widest trunk, with the largest crown but got leaves with moderate size. Trees of this cluster were those found in agglomerations benefiting from the protection from their owners and so were under low anthropic pressure. The trees of cluster 3 were slightly less developed than those of cluster 2 but had the largest leaves and leaflets. On the contrary, trees belonging to cluster 1 were smaller and thinner but had leaves and leaflets with moderate size. These trees were found in farmers' fields but did not benefit from any special protection.

Table 6
Inertia percentage and contribution of the variables measured to the different axes of the PCA.

	Dim.1	Dim.2	Dim.3	Dim.4
Eigenvalue	2.947	2.270	1.162	0.830
Variance (%)	29.466	22.695	11.622	8.304
Cumulative variance (%)	29.466	52.162	63.784	72.088
Correlations between variables and axes				
Lfe	24.765	2.275	1.871	0.771
DP	15.149	2.176	0.249	37.748
Lfo	23.025	0.737	0.178	2.067
Lafo	16.479	3.843	3.065	0.197
Lp	8.038	5.326	1.998	13.246
Nfo	1.411	1.817	62.018	6.352
Hr	1.621	10.615	26.293	13.971
C1.30m	3.158	27.122	0.189	0.594
HT	4.297	23.467	3.212	0.973
SH	2.058	22.622	0.928	24.081

C1.30m: circumference of the tree; HT: total height of the tree; SH: crown surface; Hr: height of the first basal branch; LFe: leaf length; Dp: diameter of the petioles; Lfo: length of the largest leaflet; Lafo: width of the largest leaflet; Lp: length of petiolules; Nfo: number of leaflets.

4. Discussion

The importance of underutilized species for local food security, particularly the wild edible plants in Sub-Saharan Africa, has been largely highlighted by many studies (Adigoun-Akotegnon et al., 2019; Adoukonou-Sagbadja et al., 2006; Dah-Nouvlessounon et al., 2016). To fully harness their potential, knowledge of their genetic diversity is important (Adoukonou-Sagbadja et al., 2007; Aliyu et al., 2016). This study is the first that evaluated the current conservation status and the morphological diversity in natural populations of black plum (*Vitex doniana*) under high anthropic pressures in southern Benin.

4.1. Phenotypic variability in natural populations of black plum in southern Benin

The approach used in the present study was quantitative. High diversity was observed between and within the natural populations of black plum in the study area especially for the dendrometric parameters. This corroborates Hounkpèvi et al. (2016) who reported a strong morphological variability of *V. doniana* populations assessed in three contrasted climatic zones in Benin. These differences can primarily be explained by anthropic pressures on *V. doniana* (pruning, defoliating and debarking of trees) in southern Benin (Bonou et al., 2009). Nonetheless, these differences can also be partly induced by the variations in ecological conditions that predetermine the behavior of the plant in the study area (Assogbadjo et al., 2009, 2006). The different measures taken on the leaves and fruits of *V. doniana* were similar to the observations made by Arbonnier (2002) and Louppe et al. (2008) on the species' biology. The *V. doniana* individuals from southern Benin possessed a height to the first branching relatively high (Hr = 2.49 m) but lesser than that observed by Hounkpèvi et al. (2016) in Guinean and Sudano-Guinean areas in Benin found to be 3.35 and 5.98 m respectively. However, in the Sudanian area, these authors found trees with a height to the first branching close to that obtained in this study. It should be noted that the height to the first ramification is a very interesting descriptor for the identification of individuals with low branches for which fruit and leaf picking will be easier for farmers. This trait will be of high interest for the improvement programs on the species. Similarly, the morphological traits such as tree circumference, total tree height, crown area and petioles diameter of the leaves should be interesting descriptors for the subsequent studies on diversity characterization of the species or for the future improvement programs for the domestication of the species.

4.2. Vulnerability of black plum and strategy for its conservation in southern benin

The present investigation revealed that black plum populations in southern Benin are highly vulnerable, leading to almost disappearance of the species in the southern ecological regions of Benin covering Atlantic and Ouémé departments. This vulnerability was well observable in the Zou department mainly in Ouinhi and Zagnanado districts though natural but highly fragmented populations of black plum were observed in this central ecological areas of Benin during the study. This showed the high human pressure under which *V. doniana* was, due to its intense exploitation as leafy vegetable, fire woods and timbers while the natural regeneration of the tree was found to be very poor in the study area. As it was similarly observed in the north of the country (Oumorou et al., 2010), this situation threatens the survival of the species also in the south. In this area, Akpoyètè et al. (2018) reported the regression of the forest cover

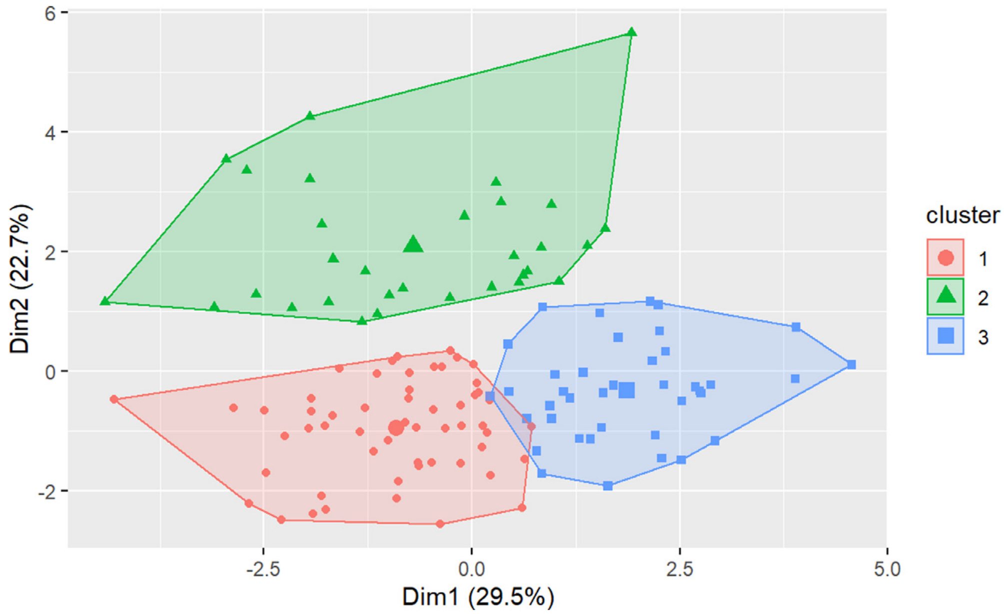

Fig. 8. Plots from hierarchical cluster analysis showing the number of clusters of *V. doniana* accessions based on morphological descriptors.

from 35.5% to 10% between 1986 and 2016. These authors linked the fragmentation of this forest area to human activities particularly agriculture since the cultivated areas increased by 23.2% in 30 years. Apart from the human habitat extension related to the demographic growth which is an important factor influencing forest survival, this area is also subjected to other anthropogenic activities like transhumance, exploitation of timber woods, fire woods and intensive charcoal production. Unfortunately, these activities are conducted in a non-sustainable manner, destroying indifferently juvenile, young and adult trees. Moreover, climate change could also affect to some extent the survival of *V. doniana* tree population but the present study did not particularly address this issue. Hounkpèvi et al. (2016) studying the climate-induced variability of *V. doninia* population in three climatic zones in Benin, showed a relatively low impact of climatic parameters on the distribution of the species. However, with the prediction on climate deterioration in the future, this impact could be highly significant on this species population. Moreover, on other tree species, *Afzelia africana*, Mensah et al. (2014) showed that the structure of its population was strongly influenced by the level of human disturbance and climate variability and this, depending on climatic zone. For instance, in the Guinea climatic area which is wetter compared to Sudano-Guinean and Sudanian areas, the tallest and biggest trees were found where the level of human disturbance was low. Besides, with regard to the high anthropogenic pressure observed, it is urgent to organize the awareness sessions to advise on the rational use of this tree species and define the best conservation strategies in agreement with the local population exploiting these resources.

Significant morphometric variation was observed between *V. doniana* populations despites their high fragmentation. In addition, important intra-population variability was also observed for instance for dendrometric parameters within populations implying that conservation strategies may also consider the specific variation between individuals within populations. Thus, strategies to conserve the maximum diversity of *V. doniana* should be included in conservation programs. Effective management of forest resources requires the

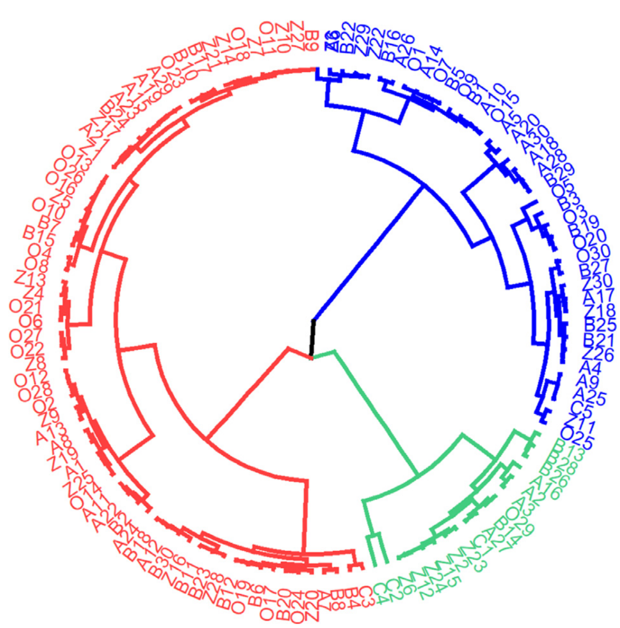

Fig. 9. Dendrogram obtained from hierarchical clustering on principal components based on morphological traits collected on 125 individuals of *V. doniana* from southern Benin. Brown1, seagreen3 and blue colors represent Clusters 1, 2 and 3, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 7
Description of the clusters from hierarchical clustering on principal components. Data are mean followed by standard error. Between the clusters, the traits mean with different letters are statistically different ($P<0.05$).

	Cluster 1	Cluster 2	Cluster 3	$Pr > F$
C1.30m	0.9 ± 0.03c	1.51±0.07a	1.06±0.04b	< 0.0001
HT	7.39±0.28c	12.37±0.54a	8.75±0.36b	< 0.0001
SH	43.8 ± 4.54b	120.71±8.76a	60.29±5.88b	< 0.0001
Hr	2.35±0.07b	2.98±0.13a	2.51±0.08b	0.000
Lfe	28.21±0.41b	27.2 ± 0.79b	32.85±0.53a	< 0.0001
DP	5.45±0.12b	5.17±0.23b	6.76±0.16a	< 0.0001
Lfo	15.86±0.26b	14.98±0.5b	18.55±0.34a	< 0.0001
Lafo	7.76±0.12b	7.4 ± 0.24b	9.24±0.16a	< 0.0001
Lp	1.65±0.05b	1.84±0.09b	2.16±0.06a	< 0.0001
Nfo	5.22±0.05a	5.02±0.09a	5.12±0.06a	0.125

C1.30m: circumference of the tree; HT: total height of the tree; SH: crown surface; Hr: height of the first basal branch; LFe: leaf length; Dp: diameter of the petioles; Lfo: length of the largest leaflet; Lafo: width of the largest leaflet; Lp: length of petiolules; Nfo: number of leaflets.

Fig. 10. Black plum tree conserved through traditional voodoo belief.

identification of priority areas where conservation efforts can be focused (Assogbadjo et al., 2006). For this purpose, a representative sample of remnant natural populations of *V. doniana* can be used to develop *in-situ* and *ex situ* conservation strategies for the species. *In-situ* conservation will consist of defining a locality where the species will be conserved and protected in collaboration with the local population. For instance, localities of Ouinhi and Zagnanado seem to be more appropriate to host an *in-situ* conservation project seeing the enthusiasm of local populations to take care of the species. Participatory domestication of the species should reduce the pressure of the local population on the natural environment thereby contributing to the maintenance of a salutary biodiversity in the area (Mapongmetsem et al., 2012). Successful stem/root cuttings and marcots propagation already reported in black plum (Achigan Dako et al., 2014; Sanoussi et al., 2012) as well as in its sister species *V. madiensis* Oliv. in Central Africa (Mapongmetsem et al., 2012) constitute a good asset for *V. doniana* domestication. The development of *in-situ* conservation strategies could help to better understand the factors and processes that influence the dynamism of natural populations of this species. For this reason, local conservation strategies, such as *on-farm* conservation, home gardens, field conservation should be promoted. Domestication and production through agroforestry systems may help to limit the high anthropic pressure of intense leaf exploitation of the black plum. The introduction of the species into reserves and protected areas should also be another conservation strategy of *V. doniana* in southeastern Benin. During our field data collection, we found some *V. doniana* trees used to house the spirit of traditional *voodoo* (Fig. 10). This traditional practice contributes to the *in-situ* conservation of the species as previously reported in other studies addressing the role of the sacred forests on the conservation of the biodiversity in Africa (Kossi et al., 2021; Oba and Mgumia, 2003; Onyekwelu and Olusola, 2014; Toko Imorou et al., 2018). However, considering the increasing conversion of *voodoo* followers to Christianity, this conservation strategy is threatened. For any *in-situ*/*on-farm* conservation, special attention for the protection of seedlings of the species in the fields and vicinity of dwellings is required. As for the *ex-situ* conservation of the species, collection and maintenance of individuals from different sites in the study area is recommended. Also, gene bank conservation of seeds or *in vitro* plant organs can be made for *ex-situ* conservation of this species.

5. Conclusion

The present study revealed the current conservation status and genetic diversity of the black plum natural populations in Southern Benin. The transect walks analysis performed revealed highly fragmented natural populations due to overexploitation of the trees for human multiple uses. However, the study revealed a great diversity within and between the studied populations through 13 morphological descriptors. The variation was more related to dendrometric parameters such as tree trunk circumference, crown area and height of the first branch. This study identified morphological descriptors that better discriminated individuals of *V. doniana*, which should be taken into account for a subsequent characterization of the species. This study shed light on information that could be used for the conservation, domestication and genetic improvement of the species. Future investigations could address the genetic variability existing within these relic populations using molecular markers.

Authors' contributions

Hubert Adoukonou-Sagbadja (HA-S) designed this study, supervised the field survey as well wrote the first draft of the manuscript. Daryl-Biopaix Ahoyo conducted the field survey, recorded data and participated to the first drafting. Thierry Klanvi Tovignan (TKT) and Fiacre Zavinon analyzed the data. TKT contributed to the final drafting of the manuscript. HA-S, TKT and Léonard Ahoton contributed to the critical revision of the manuscript. All authors read, improved and approved the final manuscript.

Funding

This study was fully funded by LaREGAME of the University of Abomey-Calavi (UAC), Benin Republic through its project on Conservation and Valorization of Endangered Wild Edible Plants in West-Africa.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgement

The authors are grateful to local informants involved in field walks for their significant contribution during the surveys. We also acknowledge Dr. Mathieu Ayénan (World Vegetable Center, Cotonou, Benin) and Dr. Isaac Kodzo Amegbor (Savanna Agricultural Research Institute, Tamale, Ghana) for their critical comments and proofreading of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2023.03.023.

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