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Conservation Genetics of Baobab (*Adansonia digitata* L.) in the Parklands Agroforestry Systems of Benin (West Africa)

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Abstract

The present study occurred in the three climatic zones of Benin (6°25 - 12° N) and aimed at investigating the level of morphometric and genetic variation and spatial genetic structure within and between threatened baobab populations. A total of 137 individuals from six populations were analysed using morphometric data as well as molecular marker data generated with the AFLP technique. Five primer pairs resulted in a total of 217 scored bands with 78.34% of them being polymorphic. A two-level AMOVA revealed 82.37% of the total variation within populations and 17.63% among populations (P<0.001). Analysis of population structure with allele-frequency based F-statistics revealed a global FST of 0.127±0.072 (P<0.001). The mean gene diversity within populations (Hw) and the average gene diversity among populations (Hb) were estimated at 0.309±0.000 and 0.045±0.072, respectively. Baobabs in the Sudanian and Sudan-Guinean zones of Benin were short and produced the highest yields of pulp, seeds and kernels in contrast to the ones in the Guinean zone. The molecular results indicate some degree of physical isolation of the populations collected in the different climatic zones. We also found morphological differences but further analysis must be done to establish their origin which is certainly an interaction between genotype and environment. Sampling options of the natural populations are suggested for in or ex situ conservation.

Keywords: *Adansonia digitata*, climatic zones, morphometric variation, population structure

Introduction

The multipurpose baobab (*Adansonia digitata* L.) is a key economic species used daily in the diet of rural communities in West Africa (Assogbadjo *et al.*, 2005a,b; Assogbadjo *et al.*, 2008; Codjia *et al.*, 2001, 2003; Sidibe and Williams, 2002). The species contributes to rural incomes (Diop *et al.,* 2005) and has various important medicinal and food uses (Assogbadjo *et al.,* 2005a; Diop *et al.,* 2005; Sena *et al.*, 1998; Sidibé *et al.,* 1996; Sidibé and Williams, 2002; Yazzie *et al.*, 1994).

Within baobab species, there is evidence indicating the existence of a number of local forms differing in habit, vigor, size, quality of the fruits and foliar vitamin content (Assogbadjo *et al.*, 2005a; Gebauer *et al.*, 2002; Sidibé and Williams, 2002). However, information about the ecology, the morphological and genetic variation within and between populations and the productivity of their various organs is lacking (Sidibé and Williams, 2002).

The participatory domestication of indigenous fruits has been proposed as an appropriate means to alleviate poverty (Poulton and Poole, 2001), and could also have positive benefits on the environment since new plantings of baobab would help to restore the declining resources of this important tree.

The main objective of the present study is to define based on molecular analysis a better conservation strategies for the baobab species in Benin. To this aim, Amplified Fragment Length Polymorphism (AFLP) analysis (Vos *et al.*, 1995) was applied to find the intra-specific genetic diversity of those locally recognised morphotypes and on the whole populations in order to assess the genetic diversity and differentiation within and between baobabs in Benin. Because AFLPs are known to map throughout the genome of any particular species analyzed so far, this high-volume DNA fingerprinting techniques gives fast and efficient measurements of genome-wide diversity (Powell *et al.*, 1996). We used this technique to investigate not only if the traditional classifications of *A. digitata* are confirmed by genome-level genetic differentiation but also if there is within the species some genetic variations which should be conserved for the benefit of local people.

Materials and methods

Study areas

The study was conducted in the three climatic zones of Benin (112 622 km^2 and 6.752.569 inhabitants in 2002), located between 6° and 12°50 N and 1° and 3°40 E in West Africa. The zones studied are: the Sudanian zone located between 9°45'-12°25' N, the Sudano-Guinean zone located between 7°30'-9°45' N and the sub-humid Guinean zone (Dahomey Gap) located between 6°25'-7°30' N.

In the Sudanian zone, the annual mean rainfall is often less than 1000 mm and the relative humidity varies from 18% during the harmattan period (December - February)

to 99% in August. The temperature varies from 24°C to 31°C. The Sudanian zone has hydromorphic soils, well-drained soils, and lithosols. The vegetation of this zone is composed of savannas and gallery forests with trees of smaller size.

The mean rainfall in Sudano-Guinean zone is unimodal, from May to October, and lasts for about 113 days with total mean annual varying between 900 mm and 1110 mm. The annual temperature ranges from 25°C to 29°C, and the relative humidity from 31% to 98%. The soils in this zone are infertile mineral soils and ferruginous soils of variable fertility. The vegetation of the Sudano-Guinean transition zone is characterized by a mosaic of woodland, dry dense forests, tree and shrub savannas and forest galleries.

The rainfall regime in the Guinean zone is bimodal from April to June and from September to November, with a mean annual rainfall of 1200 mm. The mean temperature varies between 25°C and 29°C and the relative humidity between 69% and 97%. The soils are either deep ferrallitic, and of low fertility or alluvial and heavy clay soils. The vegetation in this zone has been strongly affected by various agricultural activities and now forms a mosaic of cultivated lands and small relic forest patches. The original vegetation was dense semi-deciduous forests and Guinean savannas. This zone represents about 10% of Benin and supports 60% of the country's inhabitants.

Sampling for DNA fingerprinting

In each climatic zone, 2 populations of baobab were sampled. Tab. 1 summarises the characteristics of the sampled populations, including geographic zone of origin and co-ordinates. Six to 35 individuals were sampled within each population and used for the morphometric, productivity and AFLP analyses. In this study, a baobab population was defined as a group of baobab trees randomly and naturally distributed in a traditionnal agroforestry system that can be assimilated to a circle with a maximum of 50 km radius. Two different populations are isolated from each other by a geographical distance of at least 50 km. Within a population, baobab individuals were randomly selected at a distance of at least 100 m, in order to avoid the sampling of genetically related individuals. In total, six populations of baobab represented by 137 individuals were collected in the aforementioned zones. For each baobab, four or five leaves were harvested and dried in silica gel for DNA extraction and AFLP analysis.

Genetic data analysis

DNA were isolated following the MATAB protocol (Kelly *et al*., 2004) whereas AFLP analysis were performed as described by Vos *et al.* (1995) with minor modifications. For each individual, the DNA fingerprints were scored by visual inspection for presence (1) or absence (0) of specific AFLP-bands (Fig. 1). Only distinct, major bands were scored. For statistical analyses, allele-frequency based analyses of genetic diversity and structure were performed using AFLPsurv version 1.0. (Vekemans, 2002) which is based on the methods described by Lynch and Milligan (1994). Nei's (1973) gene diversity (also known as expected heterozygosity) as well as global and pairwise genetic differentiation (F_{ST}) values were computed. Significance of the genetic differentiation between groups was tested by comparison of the observed F_{ST} with a distribution of F_{ST} under a hypothesis of no genetic structure, obtained by means of 1000 random permutations of individuals among groups. Moreover, a model-based (Bayesian) clustering method was applied on the presence/absence matrix to infer genetic structure in the dataset, using the software Structure version 2.0. (Pritchard *et al*., 2000).

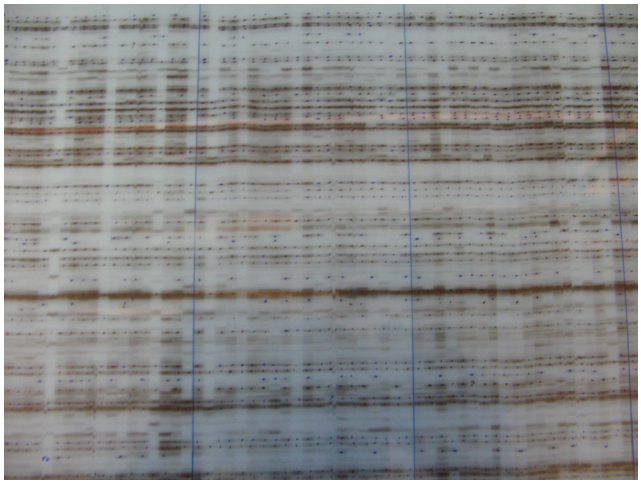

Fig. 1. AFLP electrophoresis showing the scored bands

Assessing and analyzing morphometric data in baobab populations

The morphological characteristics of each baobab were studied (at the abovementioned populations). For each baobab sampled, the trunk diameter was measured at breast height (1.3 m) (DBH). Tree height, crown diameter and the number of branches were also determined. If fruiting, the number of capsules was counted and their shape noted. To estimate the productivity in pulp, seeds and kernels, 600 fruits were sampled in each population. Length and weight of total fruit and its contents (pulp + seeds) was determined. The seeds were then removed by soaking the contents in water. The seeds were counted and then oven-dried at 50 to 60°C for 48 hours. The dry seeds were boiled for 30 min in order to remove the seed coat, which is a traditional technique for extracting the kernel. Kernels were dried at 40 to 50°C for 48 hours and weighed. The weight of the pulp (WP) in each fruit was obtained by the following formula: WP= Wsp - Ws, where Wsp is the weight of the capsule's contents (seed with pulp), and Ws is the weight of the seed without pulp. For each product (pulp, seeds or kernel), the mean productivity was calcu-

lated per tree allowing the calculation of average yield for each population. With SASv8 software, analyses of variance and the Newman and Keuls test were performed on morphological data to describe and compare baobab populations within and between the climatic zones.

Results

Intra specific genetic variation and structuring of baobab species in Benin

When bands from all 137 individuals were considered, levels of polymorphism within populations varied between 89.4% and 98.2%, reflecting a high level of polymorphism and variation within populations. The highest estimate of the likelihood of the data, conditional on a given number of clusters, was obtained when clustering all genotypes into six gene pools. Results indicated that the genetic structuring of the sampled individuals was correlated with their geographic origin. Nei's gene diversity (expected heterozygosity) within populations ranged between 0.26 and 0.37. A three level AMOVA partitioned

Tab. 1. Morphological characteristics and mean production per individual for six baobab populations

Morphological feature	Guinean				Sudano-guinean				Sudanian			
	P1		P2		P3		P4		P5		P6	
	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
DBH (cm)	149.23^{a}	66.89	147.15^{a}	57.29	176.35^{b}	33.64	173.04^{b}	40.32	201.51^{c}	97.88	202.55^{c}	54.90
Hm (m)	21.15^{a}	3.45	18.90^{b}	2.83	13.79^{c}	1.96	13.50^{c}	1.87	15.27^{d}	5.18	18.70^{b}	4.27
Dcrown (m)	14.27^{a}	3.69	14.22^{a}	1.13	16.95^{b}	5.99	16.58^{b}	4.62	16.56^{b}	4.66	16.58^{b}	3.98
NBranches	7^{a}	2.17	7^{a}	2.32	10^{b}	3.02	11^{b}	2.83	7^{a}	2.22	7^{a}	3.98
Ncaps/tree	49^{a}	46.12	67^{b}	36.15	188^{c}	70.77	225^{d}	203.50	137^{e}	92.54	138^{e}	132.51
Wcaps/tree (kg)	20.28^{a}	18.33	25.69^{b}	29.38	32.05^{c}	11.37	34.13^{d}	8.58	28.28^{e}	21.62	34.07^{f}	34.71
Lcaps (cm)	21.71^{a}	4.85	22.71^{a}	4.85	19.89^{b}	3.96	18.89^{b}	3.96	16.89^{c}	5.14	16.59^{c}	5.14
ThicknessCaps	0.45^{a}	0.15	0.45^{a}	0.15	0.43^{b}	0.09	0.43^{b}	0.09	0.43^{b}	0.07	0.43^{b}	0.07
WP/tree (kg)	3.62^{a}	3.19	1.93^{b}	1.55	6.13^{c}	1.98	6.51^{c}	1.46	4.94^{d}	3.75	4.83^{d}	3.83
Nseeds/tree	10969^{a}	9523	9326^{b}	8576	27565^{c}	9168	27635^{c}	8140	21188^{d}	20231	25455^{e}	20876
Wseeds/tree (kg)	4.22^{a}	3.97	4.62^{a}	2.68	9.21^{b}	7.06	11.09^{c}	10.85	16.93^{d}	16.31	17.04^{e}	16.72
Wkernel /tree(Kg)	1.40^{a}	1.32	1.54^{a}	0.89	2.31^{b}	2.10	2.70^{b}	2.57	3.67^{c}	2.35	3.70^{c}	3.62

Tab. 2. Correlation between distance based on individual morphological features and pairwise genetic dissimilarity values

Morphological feature	Correlation with genetic diversity	Probability
DBH (cm)	-0.00726	0.3168
Hm (m)	-0.06655	0.0497*
Dcrown (m)	-0.00568	0.4356
NBranches	0.07911	0.0198*
Ncaps/tree	0.05868	0.1584
Wcaps/tree (kg)	0.04529	0.1980
Lcaps (cm)	-0.00188	0.4851
ThicknessCaps	-0.14078	0.0099*
WP/tree (kg)	0.04825	0.2277
Nseeds/tree	0.04071	0.3069
Wseeds/tree (kg)	0.04441	0.2376
Wkernel /tree(Kg)	0.04441	0.2673

*= Significant (Probability < 0.05)

Legend Tab. 1 and Tab. 2: DBH= diameter at breast height; Hm= Height of tree; Dcrown= diameter of the crown; NBranches= Number of branches; Ncaps= Number of capsules; WCaps= weight of capsules; Lcaps= Length of capsule (cm); Thickness Caps= Thickness of Capsule; WP= Weight of pulp; Nseeds= number of seeds; Wseeds= Weight of seeds; Wkernel= Weight of kernel; σ= standard deviation; P= population

NB: In the same line, figures with the same letters are not significantly different.

14.70% among the three regions of Benin and 5% of genetic variation among populations within regions. Analysis of population structure with allele-frequency based *F*-statistics revealed a global F_{ST} of 0.127±0.072 (P= 0.001). The total gene diversity (Ht) was estimated to be 0.355±0.02 while the mean gene diversity within populations (Hw) and the average gene diversity among populations (Hb) were estimated at 0.309 and 0.045±0.072, respectively. Pairwise genetic distances between populations (F_{ST}), calculated using AFLPsurv 1.0, were statistically significant (P<0.001). Within the same climatic region, the genetic distance is generally lower than 0.05, whilst genetic distance between populations located in the different climatic zones were larger than 0.05. Mantel tests comparing genetic differentiation and geographic distance per population showed a significant correlation of 0.758 (P<0.001), indicating isolation by distance.

Morphological data, productivity in analyzed populations

Morphological data and productivity of the analyzed baobab individuals varied significantly (P<0.05) among populations and climatic zones. In the Sudanian zone, the baobabs have large girths and crowns, and numerous fruits with a high pulp, seed, and kernel production (Tab. 1).

Baobabs from the Sudano-Guinean zone are short, their diameter at breast height is intermediate between DBH values measured in the Guinean and Sudanian region. Populations in this zone produce the highest yield of pulp, seeds and kernels. In the Guinean zone, the individuals were tall but of a small diameter at breast height. These baobabs have capsules with high length and thickness but produce only a small number of fruits with a low pulp, seed and kernel productivity (Tab. 1).

Discussion and Conclusions

Relationship between morphometric data and genetic variation

Morphometric data (Tab. 1) show significant difference within and among baobab populations across the climatic zones. Environmental effects on the biotic variables have also been observed in other edible trees in Africa. Maranz and Wiesman (2003) showed for the shea tree (*Vitellatria paradoxa*) a significant relationship between trait values (fruit size and shape, pulp sweetness, and kernel content of the species) and abiotic variables (temperature and rainfall) in sub-Saharian Africa north of the equator. Also, Soloviev *et al.* (2004) showed for *Balanites aegyptiaca* and *Tamarindus indica* (savanna trees) the significant influence of different climatic zones of Senegal on fruit pulp production. Moreover, Silva-Montellano and Eguiarte (2003) were able to detect genetic differentiation in populations of *Agave lechuguilla* along a latitudinal transect in the Chihuahuan desert. The pattern of population differentiation along this transect was congruent with patterns of morphological and reproductive differentiation found (Silva-Montellano and Eguiarte, 2003).

In this study, we observed some parallel patterns of morphological and genetic diversity in baobab. Although it may well be that the variation observed in morphology and other morphometric characters studied in baobab were significantly correlated with abiotic factors of environment (Assogbadjo *et al*., 2005), there is also no doubt that part of this variation within baobab populations could be explained by genetic differentiation. Indeed, the molecular results indicate some degree of physical isolation of the populations collected in the different climatic zones. We also found morphological differences but further experiments (e.g. mapping studies) are needed to identify specific genes or genome regions that might have a direct influence on the observed morphometric variation.

Conservation of baobab genetic diversity in Benin

As baobab seeds exhibited orthodox behaviour (Razanameharizaka *et al*., 2006), they can be conserved *ex situ* in seed banks and also *in situ* or *in circa* as living trees. Strategies for prioritizing the conservation of genetic diversity need to consider the level of diversity in an area, and condition of, a particular region. The best option should be to conserve seeds from non desirable baobab (Assogbadjo *et al.*, 2008) in *ex situ* in gene banks and the living desired trees *in situ* as seeds and service suppliers. The high levels of genetic variation present within populations suggested that large numbers of samples from a few populations would capture a sufficient amount of the species' genetic variability. However, such a practice would increase the chance of missing rare alleles, particularly in disjoint populations, which also expresses extreme phenotypes for phenological traits related to climatic adaptation. We suggested in these cases to sample for *ex situ* gene conservation, populations from different geographic areas and individuals from all morphotypes to maximize genetic diversity for *ex situ* collections thereby increasing probability of conserving rare alleles. We have also recommended to sample seeds deployed for *ex situ* conservation in gene banks from a high number of individuals and within all climatic zones and morphotypes, thereby avoiding low genetic diversity within seedlots and consequently low risk of inbreeding depression and high adaptive capacity to environmental variation in trees to be planted within the parklands agroforestry systems. This could be done using the core collection concept and is important since it can allow the sampling of different classes of alleles (widespread, localized and rare).

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