Annals of Botany **97**: 819–830, 2006
doi:10.1093/aob/mcl043, available online at www.aob.oxfordjournals.org

Patterns of Genetic and Morphometric Diversity in Baobab (*Adansonia digitata*) Populations Across Different Climatic Zones of Benin (West Africa)

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Received: 7 September 2005 Returned for revision: 27 October 2005 Accepted: 19 January 2006 Published electronically: 6 March 2006

• *Background and Aims* Baobab (*Adansonia digitata*) is a multi-purpose tree used daily by rural African communities. The present study aimed at investigating the level of morphometric and genetic variation and spatial genetic structure within and between threatened baobab populations from the three climatic zones of Benin.
• *Methods* A total of 137 individuals from six populations were analysed using morphometric data as well as molecular marker data generated using the AFLP technique.
• *Key Results* Five primer pairs resulted in a total of 217 scored bands with 78·34 % of them being polymorphic. A two-level AMOVA of 137 individuals from six baobab populations revealed 82·37 % of the total variation within populations and 17·63 % among populations ($P < 0{\cdot}001$)· Analysis of population structure with allele-frequency based *F*-statistics revealed a global F_{ST} of $0{\cdot}127 \pm 0{\cdot}072$ ($P < 0{\cdot}001$). The mean gene diversity within populations (H_S) and the average gene diversity between populations (D_{ST}) were estimated at $0{\cdot}309 \pm 0{\cdot}000$ and $0{\cdot}045 \pm 0{\cdot}072$, 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, which were tall and produced only a small number of fruits with a low pulp, seed and kernel productivity. A statistically significant correlation with the observed patterns of genetic diversity was observed for three morphological characteristics: height of the trees, number of branches and thickness of the capsules.
• *Conclusions* The results indicate some degree of physical isolation of the populations collected in the different climatic zones and suggest a substantial amount of genetic structuring between the analysed populations of baobab. Sampling options of the natural populations are suggested for *in* or *ex situ* conservation.

Key words: *Adansonia digitata*, baobab, population structure, morphometric variation, climatic zones.

INTRODUCTION

Baobab (*Adansonia digitata*) is a multi-purpose tree with medicinal properties, numerous food uses of various plant parts, and bark fibres that are used for a variety of applications (Codjia *et al*., 2001, 2003; Sidibé and Williams, 2002).

Baobab and its related species belong to the family Bombacaceae and the genus *Adansonia*. The family includes about 30 genera, six tribes and about 250 species. *Adansonia digitata* is related to seven other species of *Adansonia* that are not well studied except for their descriptions in floras (Baum *et al*., 1998).

The different published chromosome numbers ($2n = 96$, Riley, 1960; $2n = 128$, Schröder and Wickens, 1982; $2n = 144$, Miège, 1974; Baker and Baker, 1968; Miège and Burdet, 1968) suggest *Adansonia digitata* to be cytologically hypervariable and has been interpreted as indicating a polyploid series in the species (Miège, 1974; Morawetz, 1986) based on $x = 8$. Baum and Oginuma (1994) reviewed the chromosome number in Bombacaceae and hypothesized that the African baobab is an autotetraploid originating from aneuploid reduction from $4x = 176$, and they suggested that additional research was needed to clarify the cytology of the Bombacaceae.

Baum *et al*. (1998) argued that the genus *Adansonia* originated in Madagascar and migrated to Africa by long-distance dispersal before the breaking of West Gondwana blocks at the beginning of the Cretaceous. African baobab is associated with the savannah, especially the drier parts, and occurs naturally in traditional agroforestry systems (Wickens, 1982). Some trees have been reported to be over 1000 years old (Wickens, 1982).

The flowering time varies significantly; in general, flowering can occur at any time except during the peak of the dry season, and whether leaves are present or not (Baum *et al*., 1998). Flowers are large, pendulous, solitary or paired in leaf axils, and hermaphrodite (Baum, 1995*a*). The African baobab is known to be bat-pollinated (van der Pijl, 1936; Jaeger, 1945, 1954; Harris and Baker, 1959; Start, 1972;Baum, 1995*a*) like other species/genera of Bombacaceae, such as *Adansonia grandidieri* and *Adansonia suarezensis*, both endemic in Madagascar (Baum, 1995*a*), and *Ceiba pentandra* (Lobo *et al*., 2005). Although its breeding behaviour has not been studied extensively, baobab was classified by Ouedrago (2000) as generally outbreeding.

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Within the species, there is evidence indicating the existence of a number of local types differing in habit, vigour, size, quality of the fruits and vitamin content of the leaves (Gebauer *et al*., 2002; Sidibé and Williams, 2002). For instance, investigations in several bio-climatic zones of Benin showed not only an absence of seedlings and saplings of baobab but also some morphological variability according to the climatic zones (Assogbadjo *et al*., 2005). These observations also apply to Mali where it is frequent to see farmers using their own criteria to distinguish several types of baobab, taking into account bark colour, pulp and leaf taste, or height and width of the tree (Sidibé and Williams, 2002). Until know, quantitative information related to the genetic diversity of baobab is poorly documented (Wickens, 1982; Sidibé and Williams, 2002). Although the biogeography and floral evolution of *Adansonia* species and their systematic relationship with other taxa of Bombacaceae were studied by Baum (1995*b*) and Baum *et al*. (1998), additional studies are needed to consider patterns of genetic diversity in relation to distribution and morphological variability found in the species.

The aim of the present study was to assess the level of genetic variation within and between populations of baobab sampled across different climatic zones in Benin and to investigate their relationships with morphometric variation found in the same populations, in order to draw conclusions to aid in the management and conservation of the species. Amplified fragment length polymorphism (AFLP) analysis (Vos *et al*., 1995) was performed together with an assessment of some morphometric characters in order to find the intraspecific genetic diversity of baobab populations collected in Benin. AFLP has already been applied successfully to many tropical plant species such as rice (Mackill *et al*., 1996), tea (Paul *et al*., 1997), banana (Loh *et al*., 2000) and papaya (Kyndt *et al*., 2005), and has been shown to reveal significant levels of DNA polymorphism in plants (Vos *et al*., 1995). The advantages of this technique include the large number of loci assayed, high levels of polymorphism, high reproducibility, no requirement of prior sequence knowledge, and genome-wide distribution of markers (Powell *et al*., 1996). In the absence of codominant marker methodologies (e.g. SSRs), Krauss (2000) indicated that dominant AFLP markers are a useful alternative for the estimation of genetic diversity and population structure.

MATERIALS AND METHODS

Study areas

The study was performed on baobab (*Adansonia digitata* L.) populations established within three climatic zones of Benin, where this species is considered as threatened. In fact in the South of Benin (Guinean Zone), the species is considered as diabolic and the seedlings and saplings are systematicaly removed from the traditional agroforestry systems by the farmers (Assogbadjo *et al*., 2005). In contrast, in the middle and north of Benin (Sudan-Guinean and Sudanian zones), the species has an economical and cultural value and is over-exploited for food and medicinal purposes by local people. Consequently, the study was undertaken in the different climatic zones of Benin: the Sudanian zone, located between 9 °45′–12 °25′N, the Sudan-Guinean zone, located between 7 °30′–9 °45′N, and the sub-humid Guinean zone, located between 6 °25′–7 °30′N (Fig. 1). In the Sudanian zone, the mean annual rainfall is often less than 1000 mm and the relative humidity varies from 18–99 % (highest in August). The temperature varies from 24–31 °C. The Sudanian zone has hydromorphic soils, well-drained soils, and lithosols. The vegetation of this zone is mainly composed of savannas with trees of smaller size. The rainfall in the Sudan-Guinean zone is unimodal, from May to October, and lasts for about 113 d with an annual total rainfall varying between 900–1110 mm. The annual temperature ranges from 25–29 °C, and the relative humidity from 31–98 %. The soils in this zone are ferruginous with variable fertility. The vegetation of the Sudan-Guinean transition zone is characterized by a mosaic of woodland, dry dense forests, tree and shrub savannas and gallery forests. In the Guinean zone the rainfall is bimodal with a mean annual rainfall of 1200 mm. The mean annual temperature varies between 25–29 °C and the relative humidity between 69–97 %. The soils are either deep ferrallitic or rich in clay, humus and minerals. The primal vegetation consists of dense semi-deciduous forests and Guinean savannas.

Sampling

In each climatic zone, two populations of baobab were sampled. Table 1 summarizes 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 traditional 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.

Assessing genetic data in baobab populations

DNA isolation. DNA was extracted from dried leaves following the Matab method developed for the shea tree (*Vitellaria paradoxa*; Kelly *et al*., 2004). Dried leaf samples (50 mg) were flash-frozen in a tube filled with liquid nitrogen and mixed to a homogenous slurry with 5 mL DNA extraction buffer (100 mM Tris–HCl, pH 8·0, 20 mM EDTA, 1.4 M NaCl, 1 % PEG 6000, 2 % mixed alkyl trimethyl ammonium bromide, 0·5 % sodium sulphite). The tube was then incubated at 74 °C for 25 min. Samples were washed with chloroform (CIAA, 24 : 1) to remove cellular debris and protein. Following centrifugation at 9000 *g* for

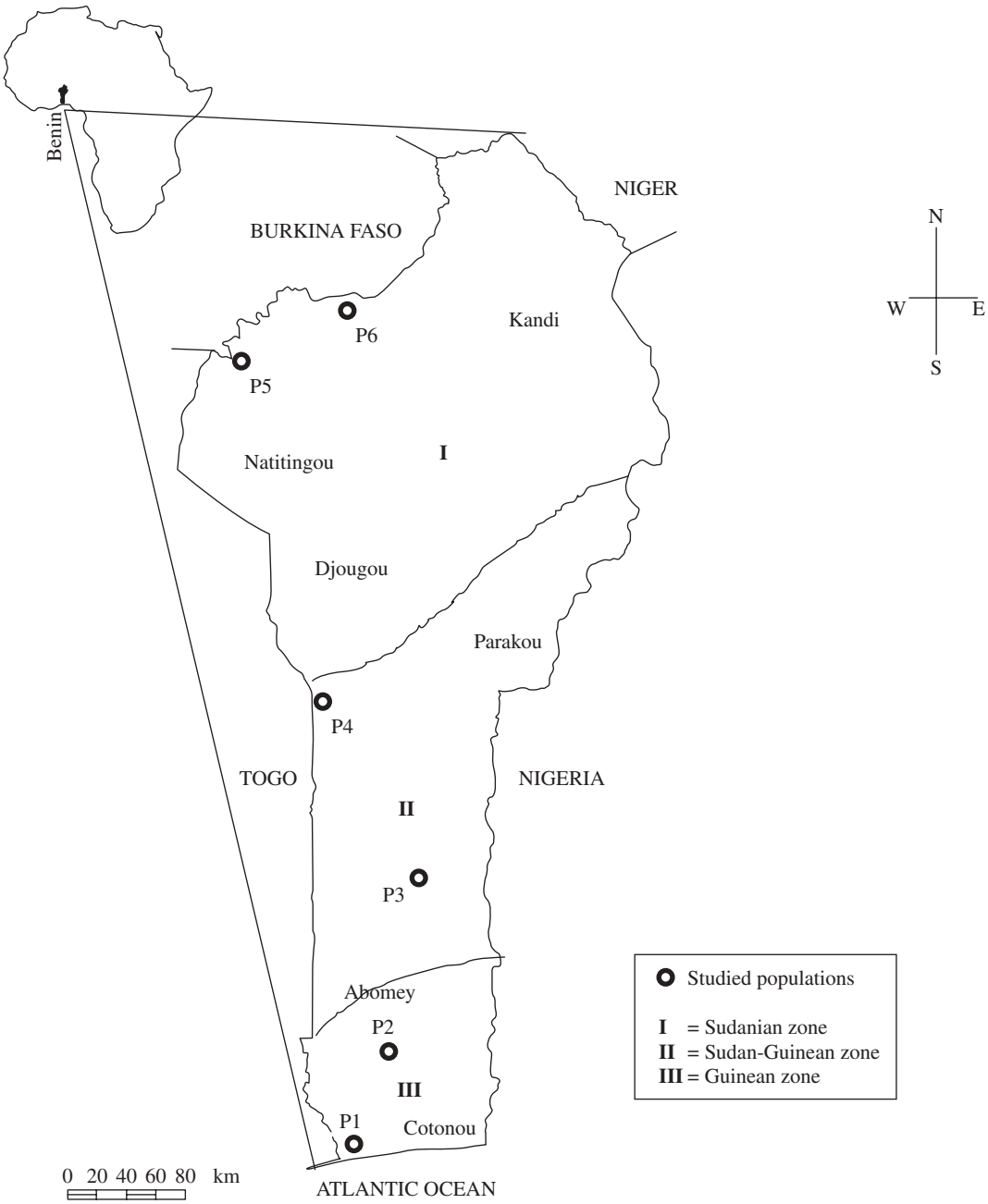

FIG. 1. Map showing the studied populations and the main towns of Benin.

15 min, the liquid phase was transferred into 15-mL tubes, and 5 mL of ice-cold isopropanol was added to precipitate the DNA. The resulting DNA pellets were resuspended in 400 mL sterile water and then stored at 4 °C until further use.

AFLP analysis. The AFLP analysis was carried out essentially as described by Vos *et al.* (1995) with minor modifications. All primers and adaptors were obtained from Genset (Paris, France). AFLP templates were prepared by simultaneous digestion of about 500 ng of DNA with *Eco*RI and *Mse*I. Ligation of the restriction fragments to the adapters was performed in the same step. A 1 : 5 dilution of the restricted and adapter-ligated DNA was used as a template in the pre-amplification reactions. Two different sets of pre-amplification products were generated using an *Eco*RI-primer carrying zero selective nucleotides (E-0) in combination with a *Mse*I-primer with two selective nucleotides (either M-AC or M-GC). For the final selective amplification, the 1 : 10 diluted pre-amplified DNA was amplified using a γ-[^{33}P]-ATP labelled *Eco*RI-primer carrying two selective nucleotides (either E-GT, E-GA, E-TC or E-AT) in combination with an *Mse*I-primer including four selective nucleotides (either M-ACGG, M-ACGC, M-ACGA, M-GCGA or M-GCGG). Pre-selective and selective primer pairs were chosen based not only on the

TABLE 1. *Characteristics of the analysed populations of baobab, collected in three climatic zones in Benin*

Geographic zones	Climatic zones	Population code	Code for individuals	Number of collected samples	Main Geographic co-ordinates
South	Guinean	P1	Numbers between 1 and 30	26	6°24·621′N 001°51·795′E
		P2	Numbers between 31 and 60	30	06°54·249′N 002°15·326′E
Centre	Sudan-Guinean	P3	Numbers between 61 and 70; 77 and 105	35	07°43·214′N 002°11·744′E
		P4	Numbers between 71 and 76	6	08°42·705′N 001°38·694′E
North	Sudanian	P5	Numbers between 106 and 125; 136 and 145	30	10°15·986′N 001°18·601′E
		P6	Numbers between 126 and 135	10	11°00744′N 000°59·570′E

result of an initial screening for polymorphism among a limited number of samples but also on a check for band consistency and repeatability. Following amplification, an equal volume of formamide loading dye was added to the PCR products. After denaturation, the products were separated electrophoretically on 5 % denaturating polyacrylamide gels, and bands were visualized using autoradiography.

Genetic data analysis. For each individual, the DNA fingerprints were scored by visual inspection for presence (1) or absence (0) of specific AFLP-bands. Only distinct, major bands were scored. The resulting data matrices were analysed using the Treecon (Version 1.3b; Van de Peer and De Wachter, 1994) and NTSYS-pc software (Version 2.10L; Rohlf, 2000). Genetic similarities based on Jaccard's coefficient (Jaccard, 1908) were calculated using the SIMQUAL module of NTSYS-pc or the DISTANCE ESTIMATION option of Treecon. A dendrogram was generated from the similarity matrix by the unweighted pair-group method using arithmetic averages (UPGMA; Sokal and Michener, 1958). Reliability of clusters in each dendrogram was tested by bootstrap analysis (Felsenstein, 1985) with 1000 replications using Treecon. Additionally, a principal co-ordinate analysis (PCO; Gower, 1966) based on the genetic similarity matrix was performed using the DCENTER and EIGEN algorithms of the NTSYS-pc software package (Rohlf, 2000).

A model-based (Bayesian) clustering method was applied on the presence/absence matrix to infer genetic structure in the six populations using the software Structure version 2.0. (Pritchard *et al*., 2000), applying a 'no admixture' model (250 000 iterations). Without using prior information of the number of populations (USEPOPINFO = 0), different K-values (2–10) were evaluated, in order to estimate the number of genepools present in the dataset. Individuals of the six populations were then assigned probabilistically to the inferred gene pools.

Genetic dissimilarity (DS) was calculated as DS = 1–Jaccard's similarity.

Given that estimation of gene frequencies from dominant markers requires the assumption of Hardy–Weinberg equilibrium, a phenetic method for partitioning of genetic variability among and within populations and regions—analysis of molecular variance (AMOVA)—was executed based on the presence/absence matrix using Arlequin vs. 2.000 (Schneider *et al*., 2000) at three hierarchical levels (among populations within regions, among regions, and overall). This statistical analysis is recognized as an effective tool to define population structure and degree of genetic differentiation, called ϕ_{ST} (Excoffier *et al*., 1992). It has also been shown to be effective in the study of tetraploid species (Jenczewski *et al*., 1999).

Allele frequency-based analyses of genetic diversity and population structure were performed using AFLPsurv version 1.0. (Vekemans, 2002), which is based on the methods described by Lynch and Milligan (1994). Allelic frequencies at AFLP loci were estimated from the binary presence/absence matrix, under the assumption of Hardy–Weinberg equilibrium, from the observed frequencies of fragments using the Bayesian approach proposed by Zhivotovsky (1999). A non-uniform prior distribution of allelic frequencies was assumed with its parameters derived from the observed distribution of fragment frequencies among loci (see note 4 in Zhivotovsky, 1999). Nei's (1973) gene diversity (also know 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 permutation of individuals among groups.

To test for isolation-by-distance, the correlation between genetic dissimilarity (between individuals) or genetic differentiation (F_{ST} between populations) and geographical distance was calculated with the Mantel test (Mantel, 1967) between pairs of populations and between single individuals. Statistical significance was evaluated with 1000 permutations.

Assessing and analysing morphometric data in baobab populations

The morphological characteristics of each baobab were studied in the above-mentioned populations. For each baobab sampled, the trunk diameter was measured at breast height (DBH, 1·3 m). 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) were determined. The seeds were then removed by soaking the contents in water. The seeds were counted and then oven-dried at 50–60 °C for 48 h. 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 40–50 °C for 48 h and weighed. The weight of the pulp (W_p) in each fruit was obtained by the following formula: $W_p = W_{sp} - W_s$, where W_{sp} is the weight of the capsule's contents (seed with pulp), and W_s is the weight of the seed without pulp. For each product (pulp, seeds or kernel), the mean productivity was calculated per tree, thus allowing the calculation of average yield for each population. Using SAS v.8 software, analyses of variance and the Newman and Keuls test were performed on the morphological data to describe and compare baobab populations within and between the climatic zones.

Correlation between morphological/production variables and genetic data

Pairwise morphological diversity values were calculated using the DIST (average taxonomic distance) option in the SIMINT module of NTSYS-pc for each morphological feature individually, as well as for the whole morphological dataset. The correlation between these diversity matrices and Jaccard's genetic dissimilarity coefficients among pairs of individuals was evaluated with a Mantel test (Mantel, 1967) using the MXCOMP module. Statistical significance was evaluated with 1000 permutations.

RESULTS

AFLP patterns

A total of 24 *Eco*RI + 2/*Mse*I + 4 primer combinations were pre-screened for their ability to detect polymorphisms in the species. Five primer pairs were selected (Table 2) based on the number of amplified fragments, their repeatability and the observed polymorphism rate. When bands from all individuals were considered, the five primer combinations resulted in a total of 217 scored bands. Only 47 bands were monomorphic across the complete germplasm set, resulting in 78·34 % of the scored bands being polymorphic. The number of polymorphic bands generated by an individual primer pair ranged from 15 to 51.

TABLE 2. *Primer combination and polymorphism within the species*

Primer pair	Total number of scored bands	Number of polymorphic bands	Polymorphism (%)
E-GT/M-ACGG	62	51	82
E-GT/M-ACGA	34	24	71
E-GA/M-ACGC	50	43	86
E-TC/M-GCGA	48	37	77
E-AT/M-GCGG	23	15	65
Total	217	170	78·34

Cluster analysis

In an UPGMA dendrogram based on Jaccard's similarity coefficient, showing generally low bootstrap support, the studied individuals are largely grouped into two clusters and one mixed group (Fig. 2). Although low bootstrap support was obtained for these clusters, attention is drawn to a notable trend. Cluster 1 (C1) groups the Guinean populations with some baobabs from the Sudan-Guinean populations whilst cluster 2 (C2) is mainly composed of individuals from the Sudanian and Sudan-Guinean populations. The mixed group (Gmix) is composed of a small number of individuals from Guinean and Sudan-Guinean populations.

Principal co-ordinate analysis (PCO) based on the genetic similarity matrix (Fig. 3) showed that the first two eigenvectors explained 70 % of the total variation and were able to separate clusters C1 and C2, as recognized in the UPGMA dendrogram.

Although weakly supported statistically, some general correlation can be observed between clustering and geographic origin, indicating some degree of genetic structuring in these populations. Apparently there is some genetic isolation between Guinean and Sudanian populations, whereas there remains an extensive overlap between Sudanian and Sudan-Guinean populations on the one hand, and the Guinean and Sudan-guinean populations on the other hand. Transition between Guinean and Sudanian populations is probably ensured by their respective links with Sudan-Guinean baobab individuals.

Genetic structuring

The model-based clustering method of Pritchard *et al*. (2000) using a no admixture model with correlated allele frequencies was applied to the data set without using prior information of the number of 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 (which is, by coincidence, equal to the number of populations). The assignment of individuals from the different samples to these six gene pools is shown in Table 3 (also available as a coloured figure online at http://www.MolecularBiotechnology.ugent.be/publications/kyndt2005B). Generally, samples collected in the same climatic zones belong to the same gene pools. Gene pool 1 contains the majority of individuals from the Sudanian zone: all of P6, and 70·5 % of the individuals from P5. Almost all remaining individuals from P5 are assigned to gene pool 4. P3 and P4, both originating from the Sudan-Guinean zone, are very diverse, as their individuals are attributed to different genepools, mainly gene pools 3, 4 and 5. Gene pool 6 contains most samples of P2 and about one third of the individuals from P1, which were both collected in the Guinean zone. Roughly two thirds of the individuals of P1 belong to gene pool 2. These results indicate again that the genetic structuring of the sampled individuals is correlated with their geographic origin.

Genetic diversity

Estimates of within-population genetic diversity were calculated using AFLPsurv and the results are summarized

FIG. 2. UPGMA dendrogram computed using Jaccard's similarity coefficient of 137 individuals sampled across six populations of baobab. Bootstrap values (%) above 20 % are indicated on the branches.

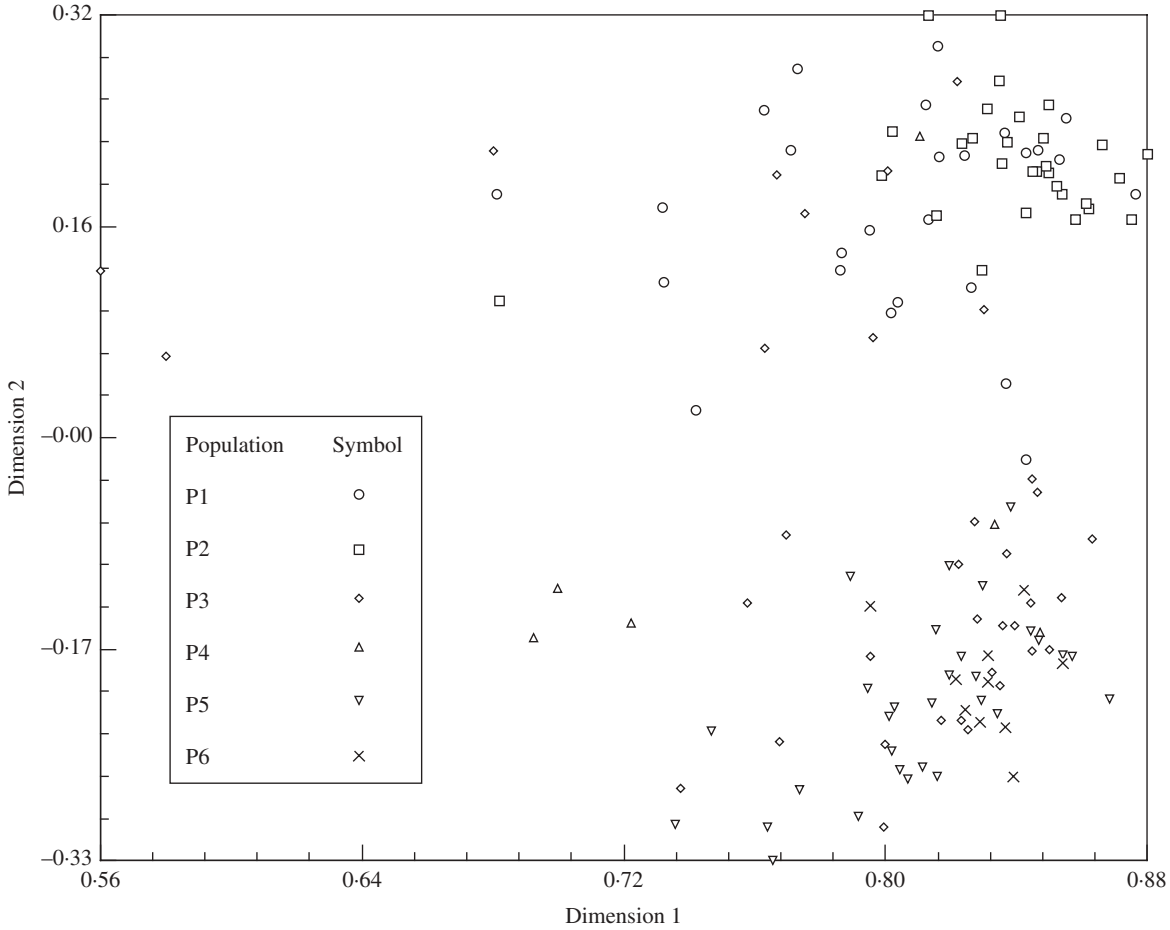

FIG. 3. Principal co-ordinate plot based on Jaccard's coefficient of genetic similarity between 137 individuals sampled across six populations of baobab.

TABLE 3. *Summary of the results of a model-based (Bayesian) clustering method using the software Structure v. 2.0 (Pritchard* et al.*, 2000) with the 'no admixture' model (250 000 iterations). Individuals are assigned probabilistically to gene pools, based on the AFLP data. Figures are the proportion of estimated membership to each of six inferred gene pools for genotypes of a given population (P1–P6)*

	Inferred gene pool					
Population	1	2	3	4	5	6
P1	0·000	0·623	0·000	0·000	0·038	0·338
P2	0·000	0·069	0·000	0·000	0·033	0·898
P3	0·001	0·147	0·284	0·401	0·120	0·047
P4	0·000	0·038	0·333	0·000	0·500	0·129
P5	0·705	0·000	0·001	0·294	0·000	0·000
P6	1·000	0·000	0·000	0·000	0·000	0·000

in Table 4. Nei's (1973) gene diversity (expected heterozygosity) within populations ranged between 0·26 (P5) and 0·37 (P4). Levels of polymorphism within populations varied between 89·4 % (P4) and 98·2 % (P1; Table 4), reflecting a high level of polymorphism and variation within populations.

TABLE 4. *Genetic diversity within six baobab populations*

Population	*H*	s.d.	*P* (%)
P1	0·35	0·01	98·2
P2	0·28	0·01	91·2
P3	0·32	0·01	94·9
P4	0·37	0·01	89·4
P5	0·26	0·01	90·8
P6	0·28	0·01	93·1

H = Nei's gene diversity (expected heterozygosity); s.d. = standard deviation of *H*; *P* (%) = level of polymorphism within population.

The assessment of genetic variability distribution among and within populations and geographical regions was estimated by an analysis of molecular variance (AMOVA). A two-level AMOVA (Table 5) of 137 individuals from six baobab populations revealed 82·37 % of the total variation within (intra) populations and 17·63 % among (inter) populations (ϕ_{ST}= 0·176). A three-level AMOVA (Table 5) partitioned 14·70 % among the three regions and 5 % of genetic variation among populations within regions. All values were statistically significant ($P < 0·001$).

Analysis of population structure with allele frequency-based *F*-statistics revealed a global F_{ST} of 0·127 ± 0·072

TABLE 5. *AMOVA for 137 individuals of baobab from six populations using 217 AFLP markers. The* P*-value is the probability of obtaining an equal or more extreme value by chance alone, estimated from 1023 permutations*

Source of variation	d.f.	SSD	Variance component	% of total	*P*-value
Two-level					
Among populations (ϕ_{ST})	5	642·21	4·85	17·63	<0·001
Within populations	131	2970·32	22·67	82·37	
Three-level					
Among regions	2	498·94	4·15	14·70	<0·001
Among populations/ within regions	3	143·28	1·42	5·02	<0·001
Within populations	131	2970·32	22·67	80·28	

TABLE 6. *Pairwise genetic differentiation (*F_{ST}*, below diagonal) between populations, as calculated with the Bayesian method using AFLPsurv 1·0, and mean distance (km) between pair of populations (above diagonal)*

	P1	P2	P3	P4	P5	P6
P1	–	72·5	97·5	212·5	410	435
P2	0·05	–	156·5	258·5	456	496
P3	0·09	0·15	–	147·5	323	333
P4	0·10	0·18	0·02	–	201	210
P5	0·18	0·23	0·08	0·15	–	71
P6	0·17	0·21	0·10	0·16	0·02	–

($P = 0{\cdot}001$). The total gene diversity (H_T) was estimated to be $0{\cdot}355 \pm 0{\cdot}02$, while the mean gene diversity within populations (H_S) and the average gene diversity among populations (D_{ST}) were estimated at 0·309 and $0{\cdot}045 \pm 0{\cdot}072$, respectively. Pairwise genetic distances between populations (F_{ST}), calculated using AFLPsurv 1.0, were statistically significant ($P < 0{\cdot}001$; Table 6). Within the same climatic region, the genetic distances were generally lower than 0·05, whilst genetic distances between populations located in the different climatic zones were larger than 0.05 (Table 6). Maximum and minimum F_{ST} were 0·23 between P2–P5, and 0·02 between P3–P4 and P5–P6 respectively.

Spatial autocorrelation

Mantel tests comparing genetic differentiation and geographic distance per population showed a significant correlation of 0·758 ($P < 0{\cdot}001$), indicating isolation by distance (Wright, 1943, 1946). The genetic dissimilarity (1 – Jaccard's similarity coefficient) between pairs of baobab individuals increased significantly with geographical distance, and a statistically significant correlation of 0·276 was found using the Mantel test.

Morphological data, productivity in analysed populations

Morphological data and productivity of the analysed baobab individuals varied significantly ($P < 0{\cdot}05$) among populations and climatic zones. In the Sudanian zone (zone I, see Fig. 1), the baobabs have large girths and crowns, and numerous fruit with a high pulp, seed and kernel production (Table 7). Baobabs from the Sudano-Guinean zone (zone II) are short, and the 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 (zone III), 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 low pulp, seed and kernel productivity (Table 7).

Correlation between morphological/production variables and AFLP data

The correlation between the diversity matrix based on all morphological features and the Jaccard's genetic dissimilarity coefficients was not significant ($r = 0{\cdot}036$, $P = 0{\cdot}327$). However, when analysing each morphological characteristic individually (Table 8), statistically significant correlations ($P < 0{\cdot}05$) were detected between the observed patterns of genetic variation and three morphological features: (1) height of the trees, (2) number of branches, and (3) thickness of the capsules.

DISCUSSION

Partitioning of genetic variation

To our knowledge, this is the first study using molecular markers to investigate the genetic diversity and spatial genetic structure in baobab. Because the species is threatened in the traditional agroforestry system of Benin, where Assogbadjo *et al*. (2005) reported an absence of seedling and saplings, it was highly important to undertake this study in the country in order to propose better management and adequate conservation strategies.

Baobab may be an aneuploid/polyploid species (Baum and Oginuma, 1994) comprising populations that vary in chromosome numbers and have duplicated portions of the genome. As polyploids may retain more than two allele copies at one locus, these organisms are less sensitive to population bottlenecks. Accordingly, genetic analyses executed during this study can only be used as descriptive tools; their results may not be directly comparable to those of other studies.

The genetic variability within the six analysed baobab populations, measured as Nei's gene diversity, ranged from 0·17 to 0·28. This is slightly higher than values observed in, for example, *Primula farinosa* (from 0·12 to 0·18; Reisch *et al*. 2005) and comparable to the average value of 0·22 observed in other long-lived perennials using RAPD markers (Nybom and Bartish, 2000). In tropical forest trees (Hall *et al*., 1994), as well as in temperate evergreen woody plants (Soo Oh *et al*., 1996), within-population genetic diversity parameters did not vary greatly between populations, and this was also observed for *Adansonia digitata*. The lowest values of gene diversity within and differentiation between populations are observed in the Sudanian region, while the

TABLE 7. *Morphological characteristics and mean production per individual for six baobab populations*

	Guinean				Sudano-guinean				Sudanian			
	P1		P2		P3		P4		P5		P6	
Morphological feature	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
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
Height of tree (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
Diameter of the crown (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
Number of branches	7[a]	2·17	7[a]	2·32	10[b]	3·02	11[b]	2·83	7[a]	2·22	7[a]	3·98
Number of capsules per 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
Weight of capsules per 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
Length of capsule (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
Thickness of capsule (cm)	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
Weight of pulp per 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
Number of seeds per tree	10969[a]	9523	9326[b]	8576	27565[c]	9168	27635[c]	8140	21188[d]	20231	25455[e]	20876
Weight of seeds per 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
Weight of kernels per 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

* DBH = diameter at breast height.
Numbers with the same letters within a row are not significantly different.

TABLE 8. *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
Height of tree (m)	−0·06655	0·0497*
Diameter of the crown (m)	−0·00568	0·4356
Number of branches	0·07911	0·0198*
Number of capsules per tree	0·05868	0·1584
Weight of capsules per tree (kg)	0·04529	0·1980
Length of capsule (cm)	−0·00188	0·4851
Thickness of capsule (cm)	−0·14078	0·0099*
Weight of pulp per tree (kg)	0·04825	0·2277
Number of seeds per tree	0·04071	0·3069
Weight of seeds per tree (kg)	0·04441	0·2376
Weight of kernels per tree (kg)	0·04441	0·2673

* = Significant (probability < 0.05).
[†] DBH = diameter at breast height.

Guinean and Sudan-Guinean populations have slightly higher values of gene diversity and between-population differentiation. This can be explained by the fact that Sudanian populations may have been established by a small number of settlers. Apparently, the threatening of baobab in the Guinean zone does not result in a lower diversity within populations from this region.

Analysis of molecular variance within and between the six analysed populations of baobab suggests that these populations retain relatively high levels of genetic variation within populations and relatively little variation among populations, as confirmed by two independent estimates of genetic differentiation, Bayesian (F_{ST} = 0·127), and AMOVA-based (ϕ_{ST} = 0·176).

The level of differentiation between these six baobab populations is therefore slightly higher than values observed for outcrossing species such as *Alchornea latifolia*, *Dendropanax arboreus*, *Inga thibaudiana* and *Protium glabrum* (less than 4 %; Schierenbeck *et al*., 1997), *Digitalis obscura* (15·2 %; Nebauer *et al*., 1999), *Leucopogon obtectus* (13·3 %; Zawko *et al*., 2001), *Aster tripolium* (17·5 %; Krüger *et al*., 2002), or *Vitellaria paradoxa* (14·8 %; Bouvet *et al*., 2004), but substantially lower than reported for selfing plant species such as *Cerastium fischerianum* (39·1 %; Maki and Horie, 1999), *Senecio vulgaris* (38·9 %; Müller-Schärer and Fischer, 2001) or *Antirrhinum subaeticum* (82·3 %; Jimenez *et al*., 2002). The relatively low level of differentiation between baobab populations corresponds to general observations for outcrossing plants and long-lived perennials (Nybom and Bartish, 2000). AMOVA results for RAPD studies on outbreeding vs. selfing plants have been summarized by Bussell (1999). He classified plants with low ϕ_{ST} (less than 0·413) as outbreeders, while values for inbreeders were reported to be generally higher than 0·45. The estimated ϕ_{ST} value of 0·176, observed for baobab in this study, confirms the species to be outbreeding, as suggested before by Ouedroaogo (2000). However, we have to keep in mind that we used AFLP, and not RAPD (as Bussell, 1999 and Nybom and Bartish, 2000), and that the marker system utilized can influence the estimates of variability to a certain degree. Several studies confirmed that the African baobab is bat-pollinated (van der Pijl, 1936; Jaeger, 1945, 1954; Harris and Baker, 1959; Start, 1972). Possible wind (Wickens, 1982) or ant pollination (Humphries, 1982) was discounted by Baum (1995*a*), although bush babies (*Otolemur crussicaudatus* and *Galago senegalensis*), known to feed on the flowers, might play a pollinating role (Coe and Isaac, 1965).

As also reported for another typical savanna tree, *Vitellaria paradoxa* (Hall *et al*., 1996; Lovett and Haq, 2000), the low level of differentiation and corresponding high amount of gene flow between baobab populations may also partly be explained by the status of baobab as a semi-domesticated species. Since the beginning of (semi-) domestication, rural populations, farmers and traders have played a role in gene flow by facilitating village-to-village transport of fruit. As a result, undomesticated populations may have relatively larger levels of

intrapopulational diversity than domesticated populations (Ledig, 1992).

Genetic differentiation across climatic zones

Among populations in the Sudanian zone, F_{ST} was very low (0·02), precluding the possibility of fixation. This high level of gene flow between the Sudanian populations as expressed by their low genetic differentiation could be explained by the abundance of baobab tree pollinators in the region or a high fruit and seed exchange rate between farmers. Genetic differentiation between populations of the different climatic zones was substantial, indicating a degree of genetic isolation and suggesting some risk of genetic erosion among the baobab populations in Benin.

A strong correlation between genetic and geographical distances between individuals and populations indicates that long-distance dispersal has been rare. The latitudinal isolation among baobab populations could be partially explained by the age and history of populations. From a biogeographic point of view, *A. digitata* is normally found in the Sahelian, Sudano-Sahelian and Sudanian zones, where the average annual rainfall is 300, 500 and 800 mm, respectively (FAO, 1981, 1988; Wickens, 1982; Sidibé and Williams, 2002). However, Wickens (1982) argued that there are extensions of the distribution into forest areas, probably associated with human habitation. Baobab is not likely to be found growing in the Guinean zone of Benin because of the high annual rainfall (>1200 mm) but the occurrence of the species within this zone may be explained by the phenomenon of the Dahomey-Gap, a dry corridor from the plain of Accra in Ghana to Benin in the Guinean Zone of Africa. In the Dahomey-Gap the savannas interrupt the forest block and reach the sea coast. Baobab probably appeared in this zone in the late Holocene about 3700 y BP, and must be associated with the reappearance of the 'little dry season' inland and with the upwelling of cold water in the Benin Gulf (Maley, 1991). These assumptions suggest that the Guinean and Sudanian populations may be of different age and origin, possibly leading to the genetic differentiation found among populations of these climatic zones.

In addition, phenology might play a role in the spatial differentiation between the studied baobab populations. In fact, the flowering time varies significantly. In Benin, the baobab's flowering period coincides with the rainy season (Assogbadjo *et al*., 2005), which varies between the Sudanian and Guinean zones. Due to different flowering times, pollen flow between the Sudanian and Guinean populations could be reduced, resulting in the observed pattern of variation.

Until now, nothing has been known about the genetic diversity of baobab in other areas of distribution. Placed in a broader context for the species, we could assume that the results of this study could be generalized in all West-African regions and some parts of East African regions, where the three climatic zones under investigation are also present (White, 1983). These regions generally comprise areas with very similar bio-climatological characteristics, as illustrated by clima-diagrams from various sites within the Sudanian regional centre of endemism, spanning from Senegal to Ethiopia (White, 1983). However, it would be very interesting to undertake a similar study in all areas where the species is distributed, in order to establish relevant management strategies for each region.

Relationship between morphometric data and genetic variation

Morphometric data (Table 7) show significant differences within and among baobab populations across the climatic zones. Environmental effects on 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) a 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 the 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 were significantly correlated with abiotic factors of the environment (Assogbadjo *et al*., 2005), there is also no doubt that part of this variation within baobab populations could be explained by genetic differentiation. In fact there are some statistically significant correlations between the observed patterns of genetic diversity and three morphological features, i.e. height of the tree, number of branches and thickness of the capsules. These correlations point towards some degree of genetic determinism for these morphological features. However, additional 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.

Consequences for conservation and domestication

Conservation and breeding strategies involving genetic studies are still limited for tropical trees, and exploration of the genetic diversity of African trees is required. Intraspecific diversity has become a fundamental parameter for the management of species with the aim of maintaining their evolutionary potential (Rajagodal *et al*., 2000). In the current study, gene diversity and morphometric variation found between baobab populations across climatic zones of Benin were shown to be significant. The results showed a genetic differentiation among baobab populations collected in different climatic zones. Efficient genetic resource management requires the identification of priority zones where the efforts of conservation can be best focused. Conservation

strategies should involve the morphological qualities that are essential for rural people who use the species on a daily basis. For instance, the baobabs in the Sudanian and Sudan-Guinean zones produce higher yields of pulp, seeds and kernels in comparison with the ones in the Guinean zone. The conservation strategies based on intraspecific variation found between those populations should involve these morphological features. Based on this principle, the strategy for conserving the maximum diversity of *A. digitata* would be to maximize the genetic distance between populations included in a conservation program. A representative sample of the natural populations of baobab could then be used to develop an *in situ* or *ex situ* conservation strategy for the species. *In situ* conservation could consist of defining in each climatic zone a representative locality or unit of conservation. For *ex situ* conservation of the species, provenance trials could be carried out using a provenance from each climatic zone. To start an improvement program, the breeding population should consist of many individual trees selected within a few populations to capture a large proportion of the variation.

ACKNOWLEDGEMENTS

This work was supported by grants from the International Foundation for Sciences (IFS). We thank this institution and its donors. We also thank Dr Els Coart (CLO-DvP, Belgium) and Dr Glele-Kakaï Romain (INRAB, Benin) for their help with the statistical analyses.

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