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Genetic diversity and demographic evolution of baobab (*Adansonia digitata* L., *Bombacoideae, Malvaceae*) populations in Senegalese Sahelian areas

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This study evaluated the spatial genetic structure of baobab (*Adansonia digitata* L.) populations from three agroecological sites located in sahelian zone of Senegal using *ITS1*, *5.8S rDNA* and *ITS2* gene sequences. To determine the extent of isolation, gene sequences were analyzed between and among three sahelian baobab populations. At least 25 haplotypes of baobab (*A. digitata* L.) were revealed in Senegal (6, 9 and 10, respectively in Dakar, Bandia and Widou Thiengoly). Private haplotypes found in each locality show that there is an adaptation of the plant to environmental conditions prevailing in each site. Indeed, nucleotide diversity was more important in Dakar (0.00527); it ranges from 0.00483 to 0.00060 for Bandia and Widou populations, respectively. Curves of mismatch distribution show that the population of Ferlo has undergone a recent demographic expansion. Although Bandia and Dakar populations were polyphyletic; each shows a balanced expansion. Fst values ranging from 0.62946 to 0.90712 correlates a strong genetic differentiation between sites. A correlation between geographic and genetic distances was not highlighted by the Mantel's test but phylogenetic trees of maximum likelihood and Bayesian inference have assigned two clades demonstrating that population of Ferlo (Widou) form a different ecotype from those of Bandia and Dakar.

Key words: *ITS1, 5.8S, ITS2*, *Adansonia digitata,* haplotype, genetic diversity, demographic evolution.

INTRODUCTION

It is important to understand the pattern of variation existing in populations of economically important trees, for use in domestication, conservation, management and tree breeding. Such a distribution should result in formation of distinct geographical races (Zobel and Talbert, 1984) that are adapted to various ecological conditions.

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Abbreviations: AFLP, Amplified fragment length polymorphism; **AIC,** Akaike information criterion; Hd**,** haplotype diversity; **ITS,** internal transcribed spacer; **Pi,** Nucleotide diversity.

This demand is to keep an appropriate level of genetic diversity to guarantee short-term viability and long-term evolutionary potential. In order to manage germplasm resources effectively in fruit tree domestication, one requires knowledge of the amount and distribution of genetic diversity present in natural populations (Mwase et al., 2006).

A. digitata L., the African baobab, is a stem-succulent tree native to the dry regions of tropical Africa (Wickens and Lowe, 2008). *A. digitata* is the oldest known tropical angiosperm species with reliable carbon dating results (Pâtrut, et al., 2007) and the best known of the eight species of *Adansonia*. The genus belongs to *Bombacoideae,* a subfamily of *Malvaceae* (Baum et al., 2004). The species is an autotetraploid species issued from a reduced aneuploid chromosomic type such as 4x = 176 (Baum and Oginuma, 1994). A phylogeographical analysis using polymerase chain reaction-restriction fragment length polymorphism (PCR-RLFP) of DNA chloroplast fragments designed to identify its centre of origin, after many decades of controversy, revealed that *A. digitata* probably originated from West Africa and migrated subsequently throughout the tropical parts of that continent and beyond, by natural and human-mediated terrestrial and overseas dispersal (Pock Tsy et al., 2009). This recent study on chloroplast DNA has shown that there are genetic differences between baobab populations from western and south-eastern Africa. In total, more than 300 uses have been reported for this species, with the most important ones being related to food, medicine and income generation for rural communities (Buchmann et al., 2010; Sarr et al., 2013). According to Sidibe and Williams (2002), anthropogenic pressures on the baobab in its natural habitat justify the absence of natural regeneration throughout its distribution area in Senegal.

Previously, molecular studies have been done to assess genetic diversity in baobabs (Assogbadjo et al., 2009; Kyndt et al., 2009; Pock Tsy et al., 2009; Larsen et al., 2009). Assogbadjo et al. (2009) showed that there was genetic structuring and low to high genetic diversity between baobab populations in different climatic regions of Benin (West Africa). Kyndt et al. (2009) found high levels of genetic structuring present in baobabs at a regional scale (Benin, Ghana, Burkina Faso and Senegal) and within populations, which was unexpected considering its dispersal by bats and human exchanges of seeds. However, Assogbadjo et al. (2009) using amplified fragment length polymorphism (AFLP) markers could not distinguish traditionally classified baobab morphotypes. Pock Tsy et al. (2009) established that the tetraploid *A. digitata*, or its diploid progenitor originated in West Africa and migrated subsequently throughout the continent, and beyond, through natural and human–mediated terrestrial and overseas dispersal. Larsen et al. (2009) developed and tested 18 microsatellite primers (SSR92 primers) for tetraploid *A. digitata* and its relatives showing different alleles per locus and different allele sizes. Most of the published results on baobab are from West Africa. However, there is limited published data from molecular studies in southern Africa. In spite of the paucity of genetic diversity information on baobab, domestication of some priority indigenous fruit species has been advanced in southern Africa (Akinnifesi et al. 2008). According to Larsen et al. (2009), it is pertinent to carry out gene flow studies in baobabs to provide insight into dispersal processes that shape the genetic structure. In addition, they indicated that estimates of seed dispersal and differentiation between populations are vital for monitoring impacts from human influence and for forecasting consequences of climate change.

Over time, baobab demography has been influenced substantially by anthropogenic factors (land-use patterns, trampling and browsing by domesticated livestock, clearing during cultivation), climate (prolonged drought), elephant damage (Wilson, 1988; Edkins et al., 2008), and fire (Chirwa et al., 2006) which have had adverse impact on genetic diversity. It is known that positive correlation exists among the levels of genetic diversity and fitness in plants (A´vila-di´az and Oyama, 2007). For baobab domestic-cation to succeed, it therefore requires understanding of the species' genetic diversity, since it is the fabric of evolution, the base material on which adaptation depends. High levels of genetic diversity confer a greater ability to respond to threats such as diseases, parasites, predators and environmental change (Amos and Harwood, 1998).

The only study on the population genetics of baobab, performed by a research group in Benin (Assogbadjo et al., 2006), indicated some degree of physical isolation of the populations collected in the three climatic zones of Benin, and inferred some impact of the environment and geographic distance on the level of genetic structuring among the analyzed populations. However, the study area was restricted in size. This study aimed at studying the levels of spatial structuring of baobab at different geographic scales. Specifically, we conducted a population genetic study of 11 baobab populations from four West African countries where the species is abundant and widely distributed in parkland agroforestry systems (Benin, Ghana, Burkina Faso, and Senegal). The goal of the research was to build and enhance a database for species conservation and domestication in the West African region (Kyndt et al., 2009).

Prior studies of chloroplast DNA markers (*psbA-trnH*, *trnL-trnF*) and the nuclear internal transcribed spacer (ITS) (*5.8S rRNA, ITS-1 and ITS-2*), combined or not with morphological traits, have been used to assess the genetic diversity and phylogenetic relationships within *Adansonia*. These data identified three lineages: one containing the Malagasy species, one containing the Australian species, and one containing the African species. A recent phylogenetic analysis (Pettigrew et al., 2012) demonstrated that *Adansonia kilima sp.* Nov. is a

Figure 1. Map of Senegal showing the geographical location of the studied baobab (*Adansonia digitata* L.) populations.

new diploid species from Africa, which co-exists with *A. digitata* in Africa. *A. digitata* and *A. kilima* were found to be genetically similar, suggesting that tetraploidy evolved relatively recently.

This current study was undertaken to assess genetic diversity and differentiation in subpopulations of baobab sampled from three sahelian zones in Senegal in order to choose genetically divergent individuals for *in vitro* cloning and other conservation measures. The specific objectives were to examine the genetic diversity within and among populations, the degree of genetic differentiation among populations and the mode of demographic expansion of different populations in these localities.

MATERIALS AND METHODS

Study site

In Senegal, phytogeographic regions are determined by rainfall. Basically, parallel isohyetes allow one to distinguish three regions from north to south: The sahelian (rainfall: 500 to 700 mm), the soudanian (rainfall: 700 to 900 mm) and the guinnean (rainfall: up to 1000 mm) regions. The three sites sampled are within the sahelian zone where the rainy season last from July to September (Figure 1). The choice of the three study sites was motivated by their contrasting floristic and ecological features although all in the Sahelian zone. Classified forest of Bandia (Latitude 14° 34' 60'' N, Longitude 16° 58' 0" E), 65 km away from Dakar, is located in the region of Thies and is therefore a protected natural site. It also includes the first private reserve of Senegal (1500 ha) and a natural stand of dense and ancient baobabs (Naegele, 1967). Dakar site is located on the peninsula of Cape Verde (Latitude 14° 40' 20 " N, Longitude 17° 25' 54" W), which is the most western point of the Sedimentary Basin of Senegal, as a spur bordered by the Atlantic Ocean. The formerly lush vegetation of this basaltic and rocky promontory, so contrasting with the arid hinterland, explains its name. Dakar is the capital city of Senegal and is increasingly urbanized. Natural wood forest stands have almost disappeared and individuals of emblematic baobabs are scattered throughout the city. Widou Thiengoly (Latitude 15° 20' N, Longitude 15° 26' W) is located in the Ferlo region, which is an agro-sylvopastoral and experimental site. It has been the subject of several reforestation programs and exclosure plots (19400 ha) subjected to controlled management (1975-1981). Ecological monitoring has been ongoing since 1981 (Hiernaux, 2006). Widou Thiengoly is also with the study area of OHM.i Tessekéré (Observatoire Homme Milieux. International), underpinned by an integrative socio-ecological systems in the Sahel. This area is typical of the African Sahel and is a bioclimatic transition zone between the Sahara area to the north and the savannas to the south. It is marked by ecological and human crises due to consecutive droughts (rainfall deficit, anthropic pressure on the environment, and changes in major ecological balances). This area is included in the Pan-African development and reforestation program, called the “Great Green Wall representing a fight against drought combined with promotion of rural development.

Sampling and records of vegetation

A. digitata leaves were collected, after the rainy season, from individuals in three populations located in Bandia forest (Thiès region), Dakar (Dakar region) and Widou Thiengoly (Ferlo, Louga region) sites, respectively. As recognized by Kyndt et al. (2009), a baobab population was defined as a group of baobab trees randomly and naturally distributed in a traditional agroforestry system within a 30 km maximum radius. Two different populations are isolated from each other by a distance of at least 50 km. For each population located in each different site, 15 to 20 individuals were sampled (Bandia: 15; Dakar: 15; Widou Thiengoly: 20) and encoded using the first letter of the locality. The 3 populations of baobab represented 50 individuals in total.

DNA extraction

For each sample, 25 mg of fresh leaves were ground with a 750 µl

of extraction buffer MATAB (Mixed Alkyl Trimethyl Ammonium Bromide) preheated to 65°C as suggested by Gawal and Jarret (1991). The mixture was homogenized by vortexing and the tubes were incubated in a water bath at 65°C for 20 min, with a stir every 5 min to promote cell membrane degradation and the release of the DNA. The samples were then cooled at room temperature for 5 min. Cellular debris and proteins were eliminated by adding 750 µl of chloroform:isoamyl alcohol (CIAA solution; 24:1). The samples were centrifuged for 20 min at 13,000 rpm at 20°C. An aliquot of 600 µl of supernatant was collected for each sample, transferred to a new, sterile Eppendorf tube and an equal volume of isopropanol, cooled to -20°C, was added to precipitate nucleic acids. Microtubes containing DNA pellets were cooled at -20°C for 2 h and centrifuged again as previously. A series of centrifugation and washing of DNA was conducted using 70% ethanol. In order to degrade RNA, 6 µl RNase was added and the mixture was incubated at 37°C for 1 h. The extracted DNA was quantified and stored at -20°C.

Nuclear DNA amplification and sequencing

The nuclear ribosomal DNA region including *5.8S rDNA* and the internal transcribed spacers *ITS-1* and *ITS-2* were amplified using the primers designed by Sun et al. (1994). They were composed respectively, of AB101 (5' ACG AAT TCA TGG TCC CGT GAA GTG TTC G 3') and AB102 (5' TAG AAT TCC CCG GTT CGC TCG CCG TTA C 3'). The amplification was performed in a reaction volume of 25 µl containing 18.3 µl of water, 2.5 µl buffer (10x), 1 µl of additional $MgCl_2$, 0.5 additional of dNTPs, 0.25 µl of each primer, 0.2 µl of Taq polymerase and 2 µl of template DNA. Amplification conditions were done as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min and elongation of the complementary DNA strand at 72°C for 1 min. A final extension at 72°C for 10 min completed the PCR. The visualization of the DNA fragments was done by gel electrophoresis in 1.5% agarose with a 0.5 x TAE buffer and stained with ethidium bromide. The size of the fragments was determined using a molecular weight marker (MW) composed of known size of several DNA fragments. For each individual, two PCR amplifications of 25 µl were carried out for sequencing. DNA sequencing step was performed by Macrogen (South Korea).

Genetic data analysis

The sequences were aligned by BioEdit software and TCS1.21 software (Clement et al., 2000) was used for the determination of haplotypes. This software also helped construct the network of haplotypes for estimating the plausibility of links between haplotypes in the network, using a 95% threshold. Nucleotide diversity (Pi) and haplotype diversity (Hd) were calculated by the DnaSp 5.10.01 software (Rozas et al., 2010). Tajima's D and Fu's F statistics (Tajima, 1989; Fu, 1997) were used to test for deviations from neutrality. Correlation between geographic distance and genetic was assessed with a Mantel Test (Mantel, 1967), implemented in XLSTAT software (Addinsoft, 2012). In all these analyzes, deletions were considered as a fifth state character (Felsenstein, 1985; Sanderson, 1989).

The phylogenetic tree was estimated using maximum likelihood in MEGA 5 software (Tamura et al., 2011). The Akaike information criterion (AIC) was used to estimate the best model of evolution. The GTR model was applied for reconstruction. The robustness of the nodes was assessed for 1000 bootstrap repetitions. The Bayesian approach was implemented by the Mrbayes 3.1.2 software (Huelsenbeck and Ronquist, 2001). The distribution of posterior probabilities in the tree reconstruction using the Bayesian approach was estimated by MC3 in which four chains were used simultaneously (three of which were "heated" gradually). One million (1,000,000) generations were performed for each chain by sampling parameters every 1000 generations. The convergence degree of the chains can be verified by examining the evolution of the likelihood function during the course of the "cold" chain to determine the burn-in period. Generations made during that period are removed from the analysis and the estimates become subsequent. Conservatively, the first 250,000 generations were discarded (25% of trees constructed) and inferences are then made on 750,000 generations, which corresponds to 75% of the trees constructed.

RESULTS

Polymorphism and genetic diversity

The results of the polymorphism analysis and genetic diversity are shown in Table 1. Genes targeted in this study were not amplified in F17 and F19 individuals. Of 715 bp of aligned sequences obtained from the 48 remaining accessions, 668 were conserved sites, 42 sites were variables, 13 sites were singletons and 28 were parsimony informative.

The number of variable sites was highest in the population of Dakar (17), followed by Bandia (14) and finally the Ferlo site in Widou Thiengoly (3). The number of parsimony informative sites is however higher at Bandia (12) than Dakar (11), whereas Ferlo has only one parsimony informative site (Table 2). Despite the relatively low nucleotide diversities in Dakar (0.00527 ± 0.00210) and Bandia (0.00483 ± 0.00210), haplotype diversities were high: 0.648 ± 0.134 and0.638 ± 0.129, respectively. By contrast, Ferlo, had low nucleotide and haplotype diversities (0.00060 ± 0.00024 and 0.363 ± 0.131, respectively).

Distribution and haplotypes network

On the set of sequences, 25 haplotypes were found. The haplotype found most frequently was H16, which was found in nine individuals, mainly from the Ferlo site (Widou Thiengoly). The haplotypes H1, H9 and H10 are common to both Dakar and Bandia. By constrast, Bandia has four private haplotypes (H3, H4, H5, and H6) and Dakar has six (H8, H11, H12, H13, H14 and H15). The site of Widou Thiengoly (Ferlo) presents only private haplotypes, meaning that no haplotypes were shared between Ferlo and the other two sites (Tables 3 and 4).

In the network (Figure 2), each ellipse represents a haplotype, and their size is proportional to the number of individuals corresponding to the haplotype. The lines between haplotypes represent mutational steps. The haplotype network shows three groups. Each group has a main haplotype and derived haplotypes. The first group consists of 13 individuals from Bandia and two individuals from Dakar for a total of 15 individuals. This group has a main haplotype (eight individuals) and five derived haplotypes. Dakar accessions prevail in the second

Table 1. Polymorphism of nuclear DNA (*ITS1*, *5.8S* and *ITS2*) of baobab populations in Senegal.

Parameter	Numbers of site
Total number of sequences	48
Conserved Sites (C)	668
Variable Sites (V)	42
Singleton Sites (S)	13
Informative Sites on parsimony (ISP)	28

Table 2. Polymorphism and genetic diversity in populations.

Parameter	Bandia	Dakar	Widou Thiengoly (Ferlo)
Number of Sequences (N)	15	15	18
Conserved sites (C)	692	689	650
Sites variables (V)	14	17	3
Singleton Sites (S)	2	6	2
Parsimony informative sites (ISP)	12	11	1
Nucleotide Diversity	0.00483 ± 0.00210	0.00527 ± 0.00210	0.00060 ± 0.00024
Haplotype Diversity	0.638 ± 0.129	0.648 ± 0.134	0.363 ± 0.131

Table 3. Distribution of individuals in the identified haplotypes

Haplotype (H)	Number of Individual	Individual	Haplotype (H)	Number of Individual	Individual
H1	8	B2, B5, B8, B9, B10, B13, D9, D15	H14	1	D11
H2	3	B7, B11, B14	H15	1	D5
H3	1	B6	H16	9	F1, F2, F8, F9, F11, F15, F16, F18, F20
H4	1	B15	H17	1	F14
H5	1	B3	H18	1	F3
H6	1	B1	H19	1	F6
H7	5	D1, D3, D4, D7, D12	H20	1	F7
H8	1	D8	H21	1	F10
H9	2	B4, D2	H22	3	F5, F13, F19
H10	2	B12, D10	H23	1	F4
H11	1	D6	H24	1	F17
H12	1	D14	H25	1	F12
H13	1	D13			

B, Bandia; D, Dakar; F, Widou Thiengoly (Ferlo).

Table 4. Haplotypes found in each site.

Site	Haplotypes	Individuals
Bandia	H1, H2, H3, H4, H5, H6, H9, H10	B2, B2, B5, B8, B9, B10, B13, B7 B11, B14, B6, B15, B3, B1, B4, B12
Dakar	H1, H7, H8, H9, H10, H11, H12, H13, H14, H15	D9, D15; D1, D3, D4, D7, D12; D8; D2; D10, D6, D14, D13, D11; D5
Widou Thiengoly (Ferlo)	H16, H17, H18, H19, H20, H21, H22, H23, H24, H25	F1, F2, F8, F9, F11, F15, F16, F18, F20, F14, F3, F6, F7, F10, F5, F13, F19, F4, F17, F12

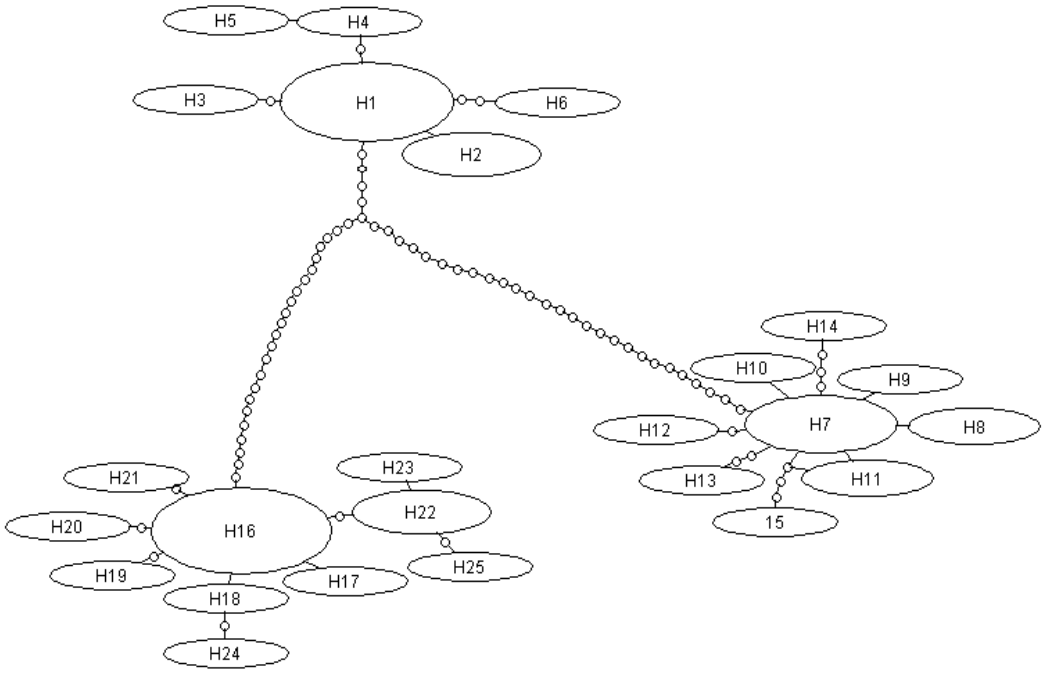

Figure 2. Relationships between haplotypes of baobab populations (*A. digitata* L.) from the three sites.

Table 5. Neutrality Indices of baobab populations (*A. digitata* L.).

Index	Bandia	Dakar	Widou Thiengoly
Tajima's D	- 0.98282	- 1.31710	- 1.44071
Fu ‘s Fs	1.506	0.704	- 2.135

group (nine haplotypes), with 15 individuals from this population and just two from Bandia. This second group presents a main haplotype (five individuals) and eight derived haplotypes. The third group consists of individuals exclusively originated from the Ferlo (Widou Thiengoly), with a main haplotype of nine individuals and nine derived haplotypes. The first group is separated from the second one by forty (40) mutational steps and from the third group by sixty seven (67) mutational steps. Between the second and the third group, there are ninety one (91) steps of mutation (Figure 2).

Demography of populations

Tajima's D and Fu’s Fs are negative in the Ferlo (Widou Thiengoly). Tajima's D is also negative in the Bandia site and at Dakar unlike Fu’s Fs which is positive in these two localities (Table 5). However, these values are not significant with p-values > 0.10, indicating that neutral evolution cannot be rejected. Mismatch distribution curves for the three populations taken altogether are multimodal (Figure 3a). Considering each site sampled, it appears that only the population of Ferlo presents a unimodal pattern, suggesting a recent expansion. Populations of Bandia and Dakar have multimodal curves revealing that they are in demographic equilibrium (Figure 3b, c and d).

Differentiation and genetic distances

All Fst values between the three populations (Dakar, Bandia and Ferlo) of baobab are high, with probability values highly significant (p less than 0.01). The Fst ranged from 0.62946 (between Dakar and Bandia) to 0.90712 (between Dakar and Ferlo (Widou Thiengoly) showing a partial isolation between these two populations (Table 6). Ferlo population is closed to the exchange of genes, contrary to what is observed between Dakar and Bandia where admixture is noted. The intra-population genetic distances (Kimura 2 parameter Model; Kimura, 1980) are low and vary from 0.001 to 0.006 (Table 7). Between populations, distances vary between 0.015 and

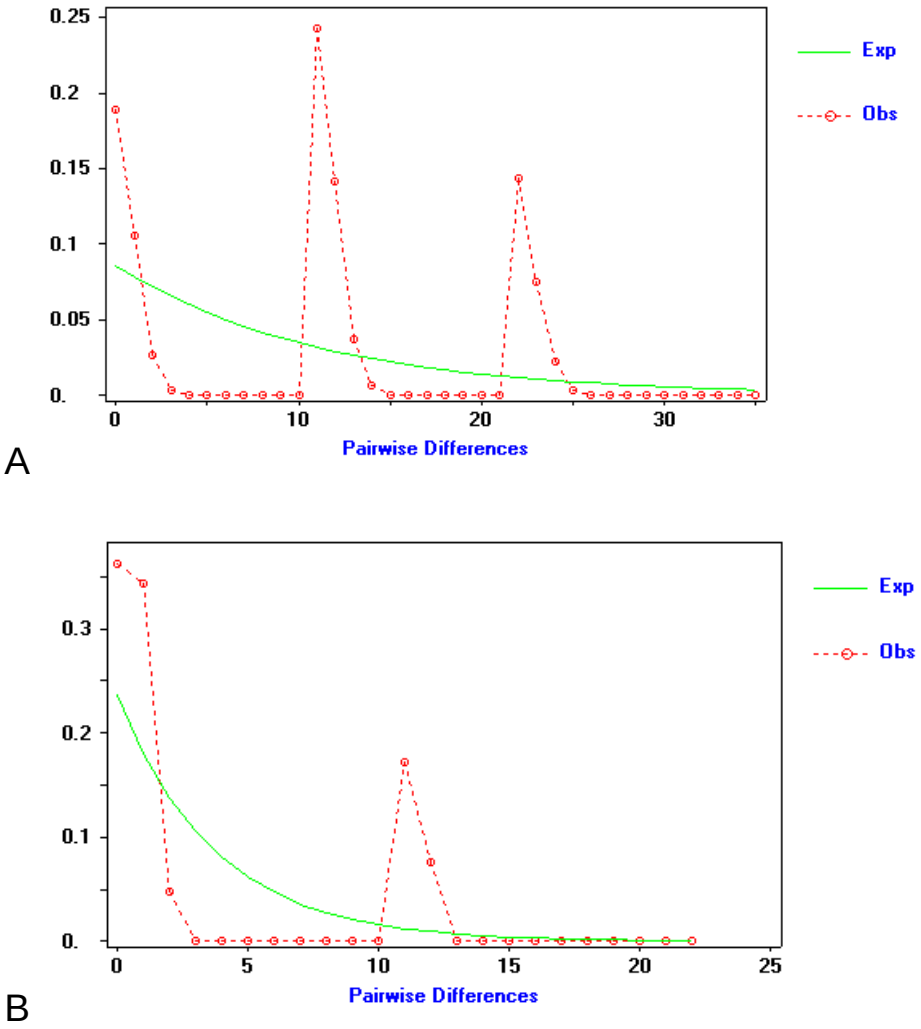

Figure 3. Distribution of the number of differences between haplotypes taken in pairs (mismatch distribution). **A,** All populations; **B,** population of Bandia; **C,** population of Dakar; **D,** population of Ferlo (Widou Thiengoly).

0.035. The highest is found between Dakar and Ferlo (0.035) and the lowest between Dakar and Bandia (0.021). The Mantel's test (Figure 4) revealed no correlation between the matrices of genetic differentiation, Fst and geographic distances (p = 0.500).

Phylogenetic trees

Phylogenetic relationships established with the Bayesian approach revealed the existence of two clades. One clade contained only individuals of the Ferlo (Widou Thiengoly) and is a monophyletic group and the second included those of Dakar and Bandia and is polyphyletic. These two clades were supported by high values of posterior probabilities (Figure 5). The subclades of these clades are not strong because posterior probability values are very low. In the second group, there is no clustering according to the geographic origin of individuals. The same groupings were obtained with the phylogenetic tree by the method of maximum likelihood. Clades are also supported by high values of bootstrap (100%) (Figure 6). This demonstrates that the groups are very strong confirming the very high values of posterior probabilities by the Bayesian approach, and consistent with the TCS analysis.

DISCUSSION

Studies in genetic diversity within a species are of paramount importance for understanding how a species will respond to environmental changes. Current patterns of genetic diversity can provide important clues to the

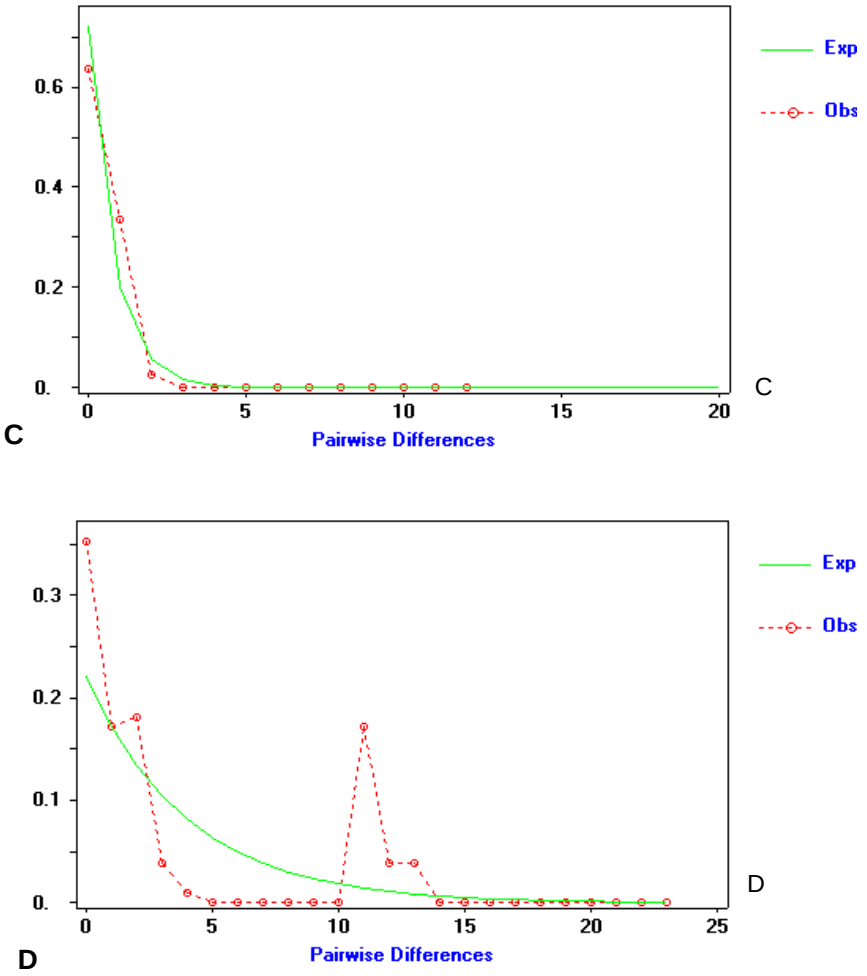

Figure 3. Contd.

history of the species and its current population structure (Heywood and Watson, 1995). In addition, knowledge about population genetics is fundamental for comprehending micro-environmental processes in plant populations that should be utilized in designing management, breeding and conservation strategies (Kyndt et al., 2009). Spatial genetic structuring in tree species is influenced by many biological forces such as gene flow through seed and pollen dispersal, tree density, fragmentation, colonization history, differential mortality, and micro-environmental selection (Kyndt et al., 2009). Genetic variation is the starting point for breeding programs and offers insurances against genetic erosion. Wild trees are genetically structured through natural processes such as mutation, genetic drift, selection, reproductive isolation, and migration (Buiteveld et al., 2007).

Our study reveals that Baobab (*A. digitata* L.) populations are quite diverse in Senegal, mainly in the sahelian zone. Indeed, 25 haplotypes were identified in three locations for 50 individuals sampled. This number of haplotypes is higher than that found by Pock Tsy et al. (2009) who identified five haplotypes in West Africa with a geographical distribution clearly structured. These authors had used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, which is less discriminative than sequencing, and chloroplast DNA as the genetic marker. Each site has a very large number of private haplotypes including the site of Ferlo (Widou Thiengoly), which contains only one haplotype cluster. The haplotype network shows three clusters separated by a large number of mutational steps. Some ellipses include individuals from Dakar and Bandia, which could be explained by the geographical proximity of these two sites that can promote the exchange of seed dispersal (most likely by humans) or pollen flow. More specifically, in agroforestry systems, all these factors may be influenced by human activity leading to many changes in ecosystem processes with various impacts (Allaye Kelly et al., 2004; Sanou et al., 2005). The results obtained by Assogbadjo et al. (2008) indicate a certain degree of physical isolation of popula-

Table 6. Genetic differentiation between populations of baobab (*A. digitata* L.) in Senegal.

Population	Bandia	Dakar	Widou
Bandia	-	-	-
Dakar	0.62946	-	-
Widou	0.85976	0.90712	-

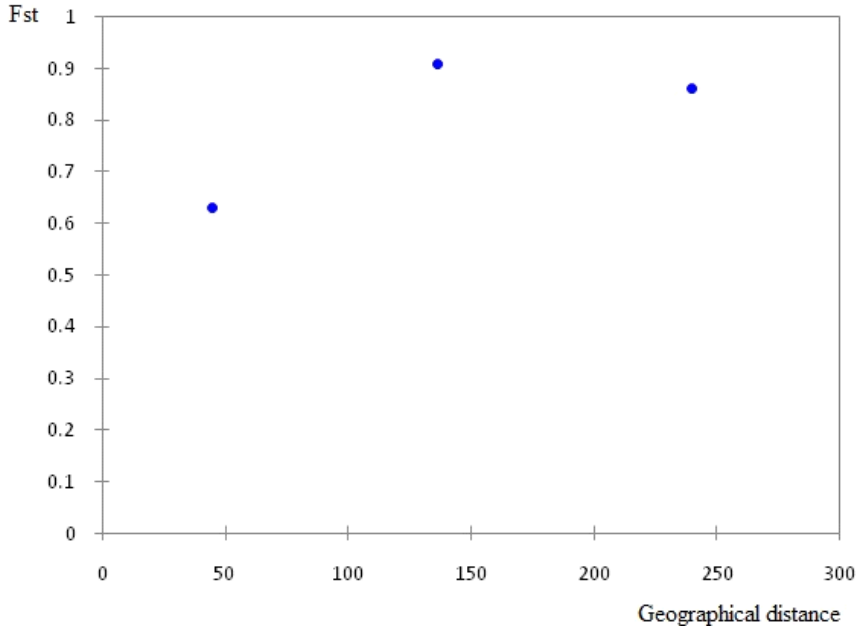

Figure 4. Correlation between geographical distance and genetic differentiation.

tions collected in different climatic zones. Indeed, the Ferlo region is in the forest grazing areas. This region is also the upper limit of baobab expansion in Senegal and has vulnerabilities and ecological characteristics different from those of Dakar, which in turn are also different from those of Bandia. Indeed, the existence of private and individual haplotypes and strong differentia- tion between Widou Thiengoly site and other sites can also be explained by the fact that the stands of baobab (*A. digitata* L.) in this area are considered as vestiges of wetter climatic periods, which cannot regenerate in the current rainfall conditions. Tests of exclosure plots in this area show that the dryer climate that high grazing pressure causes in a major factor preventing regeneration of this species (Miehe, 2002). Private haplotypes found in each locality could, thus, result from an adaptation of baobabs depending on the climate or agro-ecological zone. According to Kyndt et al. (2009), the distribution of seeds and tree improvement should recognize the presence of ecotypes and conservation measures should protect all populations due to the existence of alleles that are important for local adaptation. According to Buiteveld et al. (2007), forest ecosystem will only persist if genetic diversity of forest trees is dynamically maintained in view of environmental changes. The long-term viability of tree species within agroforestry systems depends upon a wide genetic base providing the capacity to adapt to environmental fluctuations or changing farmer requirements, such as changes in species use or planting niche (Lengkeek et al., 2006). Most forest species have evolved into distinct races (ecotypes, provenances), which should be recognized in tree breeding programs, as well as seed distribution for forest planting. Furthermore, it is known that although individuals within a race are similar from past heritage or selection pressures, they may also not necessarily be genetically identical (Zobel and Talbert, 1984).

Values of Tajima's D and Fu's Fs are negative in the Ferlo (Widou Thiengoly) but are not significant. According to Excoffier et al. (2005), a negative Tajima's D could correspond to a demographic expansion. Negative and non-significant values in the Ferlo site suggest a moderate demographic expansion. This last hypothesis is confirmed by the mismatch distribution curves that are unimodal in this locality. This contrasts those of Bandia

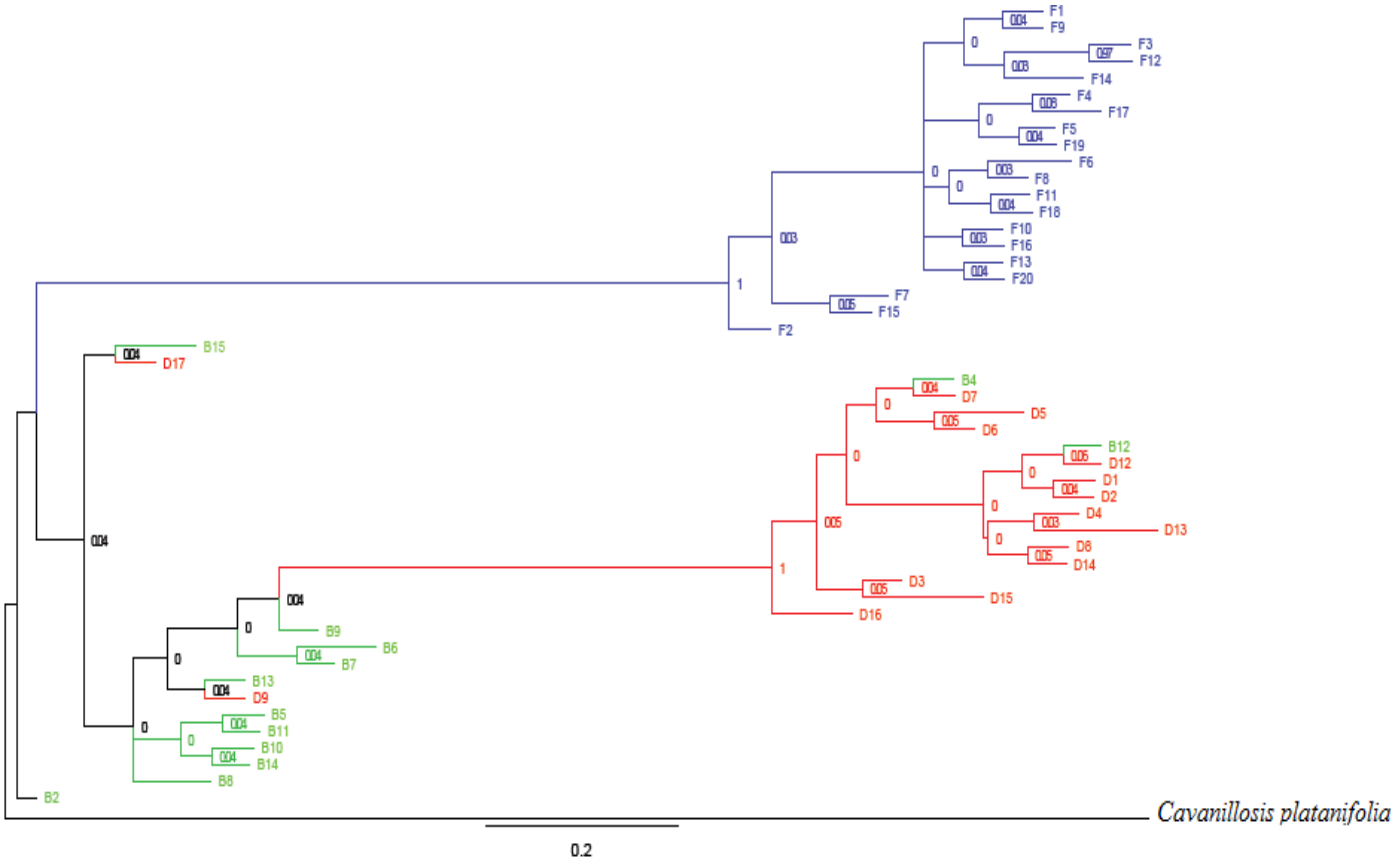

Figure 5. Phylogenetic relationships of baobab (A. *digitata* L.) populations with the method of Bayesian inference.

and Dakar, whose curves are multimodal and reveal that the populations of these two sites are in equilibrium. Fst values ranged from 0.62946 to 0.90712 and increased with the distance. These values were higher than those found by Kyndt et al. (2009) ranging from 0.02 to 0.28. Strong genetic differentiation is observed between Dakar and Bandia despite the potential for dispersal of seeds and pollen (by bats) within populations.

Genetic distances within populations are much lower than between populations. This indicates significant genetic differentiation between populations of different sites. The intra-population genetic distances (Kimura 2 parameter Model; Kimura, 1980) are low and vary from 0.001 to 0.006 (Table 7). Between populations, distances vary between 0.015 and 0.035. The highest is found between Dakar and Ferlo (0.035) and the lowest between Dakar and Bandia (0.021). The Mantel's test gives a value of P-value equal to 0.5 and does not reveal any correlation between geographic distance and genetic differentiation despite higher Fst values due to the distance. Distribution of seeds by humans could prevent isolation by distance. The active exchange of seeds in local markets allows improve gene flow between populations, the maintenance of genetic variation within populations and reduce genetic differentiation between populations (Chung et al., 2000). On the other hand, a weak positive and significant correlation (Z = 0.12, p = 0.64) between genetic distance and real geographic distance on the spot was reported by Munthali et al. (2013). According to these authors, gene flow is not directly influenced by the distance isolation. The organi-zation of genetic diversity appears to result essentially from spatially restricted gene flow with some influen-ces of seed exchange between humans (Kyndt et al., 2009).

Phylogenetic trees reveal the existence of two clades supported by very high bootstrap values (maximum likelihood tree) and posterior probability (Bayesian approach). One of the clades contained individuals from Dakar and Bandia and the other exclusively those of the Ferlo (Widou Thiengoly). It shows a structuring between populations of Ferlo which form a different ecotype from that one met in Bandia and Dakar. In addition to the distance between these regions, exchange of seeds between Ferlo and Bandia on the one hand, and between Dakar and Ferlo on the other hand would be low. Indeed, localities near Dakar as Bandia forest contain baobabs (*A. digitata* L.) and the exchange of seeds per trade is easier between these localities. It is also rare to have the bat-pollination between baobab populations separated by such distances. Knowledge about population genetics is,

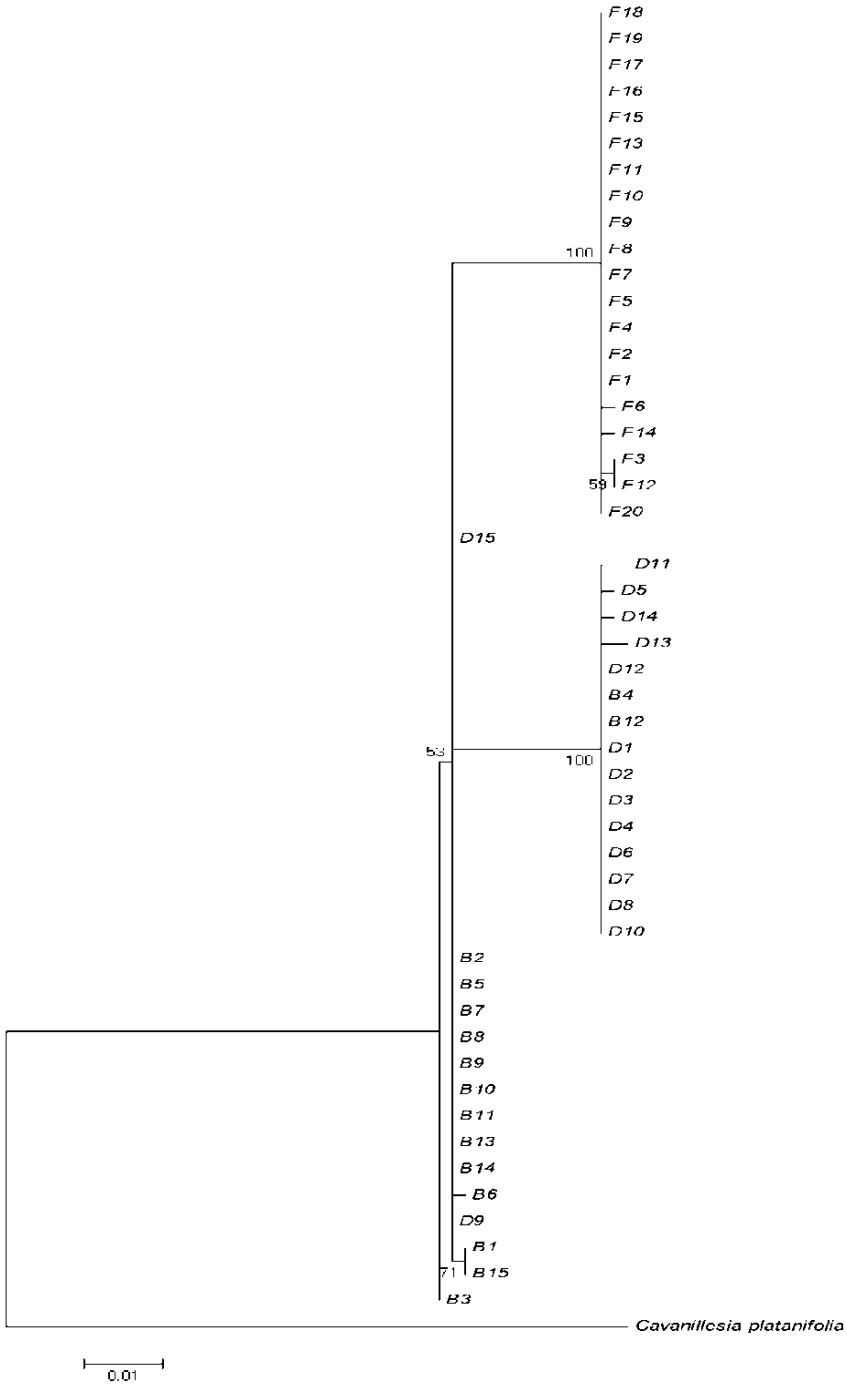

Figure 6. Phylogenetic relationships of baobab (*A. digitata* L.) populations with the method of maximum likelihood.

Table 7. Genetic distances of baobab (*A. digitata* L.) populations in Senegal.

Population	Within population	Population	Between population		
			Bandia	Dakar	Widou T.
Bandia	0.005	Bandia	-	-	-
Dakar	0.006	Dakar	0.015	-	-
Ferlo (Widou T.)	0.001	Ferlo (Widou T.)	0.021	0.035	-

however, of key importance for understanding micro-evolutionary processes in plant populations and supporting or developing appropriate use and conservation strategies (Lengkeek et al., 2006). Human trade promotes gene flow between remote populations of baobab (*A. digitata* L.) and pollination by bats between populations less distant from each other.

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REFERENCES

A´vila-di´az I, Oyama K (2007). Conservation Genetics of an endemic and endangered epiphytic *Laelia speciosa* (*Orchidaceae*). Am. J. Bot. 94 (2):184-193.

Addinsoft (2012). XLSTAT software, Paris, France.

Akinnifesi FK, Chirwa PW, Ajayi OC, Sileshi G, Matakala P, Kwesiga FR, Harawa H, Makumba W (2008). Contributions to agroforestry research to livelihood of smallholder farmers in southern Africa:1. Taking stock of the adaptation, adoption and impact of fertilizer tree options. Agric. J. 3:58-75.

Allaye Kelly B, Hardy O J, Bouvet J-M. (2004). Temporal and spatial genetic structure in Vitellaria paradoxa (shea tree) in an agro-forestry system in Southern Mali. Mol. Ecol. 13:1231-1240.

Amos WW, Harwood J (1998). Factors affecting levels of genetic diversity in natural populations. Phil. Trans. R. Soc. B 353:177-186.

Assogbadjo AE, Glèlè Kakaï R, Chadare F, Thomson L, Kyndt T, Sinsin B, Van Damme P (2009). Genetic fingerprint-ing using AFLP cannot distinguish traditionally classified baobab morphotypes. Agroforest. Syst.75:157-165.

Assogbadjo AE, Glèlè Kakaï R, Chadare FJ, Thomson L,Kyndt T, Sinsin, Van Damme P (2008). Folk classification, perception and preferences of baobab products in West Africa: consequences for species conservation and improvement. Eco. Bot. 62 (1):74-84.

Assogbadjo AE, Kyndt T, Sinsin B, Gheysen G, Van Damme P (2006). Patterns of Genetic and Morphometric Diversity in Baobab (*Adansonia digitata*) Populations across Different Climatic Zones of Benin (West Africa). Ann. Bot. 97:819-830.

Baum DA, DeWitt Smith S, Yen A, Alverson WS, Nyffeler R, Whitlock BA, Oldham RL (2004). Phylogenetic relationships of *Malvatheca* (*Bombacoideae* and *Malvoideae*; *Malvaceae s.l.*) as inferred from plastid and nuclear DNA sequences and their bearing on the mallow radiation. Am. J. Bot. 91:1862-1870.

Baum DA, Oginuma K (1994). A review of chromosome numbers in *Bombacacae* with new counts for *Adansonia*. Taxon. 43:11-20.

Buchmann C, Prehsler S, Hartl A, Vogl CR (2010). The Importance of Baobab (*Adansonia digitata* L.) in Rural West African Subsistence-Suggestion of a Cautionary Approach to International Market Export of Baobab Fruits. Ecol. Food Nutri. 49:145-172.

Buiteveld J, Vendramin GG, Leonardi S, Kamer K, GeburekT (2007). Genetic diversity and differentiation in European beech (*Fagus sylvatica* L.) stands varying in management history. Forest Ecol. Manage. 247:98-106.

Chirwa M, Chithila V, Kayambazinthu D, Dohse C (2006). Distribution and Population Structures of *Adansonia digitata* in Malawi. FRIM, Zomba, Malawi. 28 p.

Chung MG, Chung MY, Oh GS, Epperson BK (2000). Spatial genetic structure in a *Neolitsea sericea* population (*Lauraceae*). Heredity 85:490-497.

Clement M, Posadu D, Crandall K (2000). TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9:1657-1660.

De Smedt S, Cuní Sanchez A, Van den Bilcke N, Simbo D, Potters G, Samson R (2011). Baobab (*Adansonia digitata* L.) seedlings under drought: differences between genetic clades. Conference on International Research on Food Security, Natural Resource Management and Rural Development. University of Bonn, October 5-7.

Edkins MT, Kruger LM, Harris K, Midgley JJ (2008). Baobabs and elephants in Kruger National Park: nowhere to hide.. Afr. J. Ecol. 46 (2):119-125. Doi:10.1111/j.1365-2028.2007.00798.x
Evolut. Bioinfo. Online 1:47-50.

Excoffier L, Laval G, Schneider S (2005). Arlequin ver. 3.1:An integrated software package for population genetics data analysis.

Felsenstein J (1985). Confidences limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Fu YX (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915-925.

Gawel NJ, Jarret RL (1991). A modified CTAB DNA extraction procedure for*Musa* and *Ipomoea.* Plant Mol. Biol. Reporter (3):262-266.

Heywood VH, Watson RT (1995). Global Biodiversity Assessment. Cambridge University Press, Cambridge. New York, NY, USA, 1140 p.

Hiernaux P (2006). Le suivi écologique de Widou Thiengoly : Un patrimoine scientifique à développer et valoriser. Rapport de mission. Ministère Environnement et Protection de la Nature. D.E.F.C.C.S. Projet Autopromotion Pastorale dans le Ferlo (PAPF). Centre d'Etudes Spatiales de la Biosphère (CESBIO). 45p.

Huelsenbeck JP, Bollback JP (2001). Application of the likelihood function in phylogenetic analysis. In: Balding DJ, Bishop M. & C. Cannings (eds.). Handbook of Statistical Genetics. pp. 415-439.

Kimura M (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111-120.

Kyndt T, Assogbadjo AE, Hardy OJ, Kakaï RG, Sinsin B, Damme PV, Gheysen G. (2009). Spatial genetic structuring of baobab **(***Adansonia digitata* L.**,** *Malvaceae*) in the traditional agroforestry systems of West Africa. Am. J. Bot. 96 (5):950-957.

Larsen AS, Vaillant A, Verhaegen D, Kjaer ED (2009). Eighteen SSR-primers for tetraploid *Adansonia digitata* and its relatives. Conser. Genet Resour. 1:325-328. DOI 10.1007/s12686-009 9075-y.

Lengkeek AG, Mwangi AM, Agufa CAC, Ahenda JO, Dawson IK (2006). Comparing genetic diversity in agro-forestry systems with natural forest:A case study of the important timber tree *Vitex fischeri* in central Kenya. Agroforest. Syst 6:293-300.

Mantel NA (1967). The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209-220.

Miehe S (2002). Inventaire et Suivi de la Végétation dans le Périmètre Expérimental à Widou Thiengoly dans le cadre du Projet Sénégalo-Allemand Autopromotion Pastorale dans le Ferlo (PAPF). Rapport final 2002 (N°99.2003.4-001.00). Saint Louis, 56p + annexes

Munthali CRY, Chirwa PW, Changadeya WJ, Akinnifesi FK (2013). Genetic differentiation and diversity of *Adansonia digitata* L (Baobab) in Malawi using microsatellite markers. Agroforest. Syst. 87(1):117-130.

Mwase WF, Bjørnstad Å, Stedje B, Bokosi JM, Kwapata MB (2006). Genetic diversity of *Uapaca kirkiana* Muel. Årg. populations as revealed by amplified fragment length polymorphisms (AFLPs). Afr. J. Biotech. 5 (13):1205-1213. DOI:10.5897/AJB06.209

Naegelé AFG (1967). Excursion botanique du 11 décembre 1966 dans la forêt classée de Bandia ». Bulletin de l'Association pour l'avancement des Sciences naturelles au Sénégal, n° 17, janvier-février 1967, 2-7.

Pâtrut A, von Reden KF, Lowry DA, Alberts AH, Pohlman JW, Wittmann R, Gerlach D, Xu DL, Mitchell CS (2007). Radiocarbon dating of a very large African baobab. Tree Physiol. 27(11):1569-1574.

Pettigrew JD, Bell KL, Bhagwandin A, Grinan E, Jillani N, Meyer J, Wabuyele E, Vickers CE (2012). Morphology, ploidy and molecular phylogenetics reveal a new diploid species from Africa in the baobab genus *Adansonia* (*Malvaceae:Bombacoideae*). Taxon 61 (6):1240-1250.

Pock Tsy JML, Lumaret R, Mayne D, Vall AOM, Abutaba YIM, Sagna

M, Raoseta SOR, Danthu P (2009). Chloroplast DNA phylogeography suggests a West African centre of origin for the baobab, *Adansonia digitata* L. (*Bombacoideae, Malvaceae*). Mol. Ecol. 18:1707-1715.
Rozas J, Librado P, Sánchez-Del Barrio JC, Messeguer X, Rozas R (2010). DnaSP Version 5 Help Contents [Help File]. Available with the program at http://www.ub.edu/dnasp/
Sanderson MJ (1989). Confidence limits on phylogenies: the bootstrap revisited. Cladistics 5 (2):113-129.
Sanou H, Lovett PN, Bouvet JM (2005). Comparison of quantitative and molecular variation in agroforestry populations of the shea tree (*Vitellaria paradoxa* CF Gaertn) in Mali. Mol. Ecol. 14:2601-2610.
Sarr O, Diatta S, Gueye M, Ndiaye PM, Guisse A, Akpo LE (2013). Importance des ligneux fourragers dans un système agropastoral au Sénégal (Afrique de l'Ouest). Revue Méd. Vét. 164 (1):2-8.
Sidibe M, Williams JT (2002). Baobab *Adansonia digitata* L. A. Hughes, N. Haq and R. W. Smith (eds.) University of Southampton, Southampton, UK: International Centre for Underutilised Crops. 105p. ISBN 0854327649.
Sun Y, Skinner DZ, Liang GH, Hulbert SH (1994). Phylogenetic analysis of sorghum and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theor. Appl.Genet. 89:26-32.
Tajima F (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595.
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). "MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods". Mol. Biol. Evol. 28 (10):2731-2739.
Wickens GE, Lowe P (2008). The Baobabs:Pachycauls of Africa, Madagascar and Australia. Dordrecht:Springer. 498 p.
Wilson RT (1988). Vital Statistics of the baobab (*Adansonia digitata*). Afr. J Ecol. 26:197-206.
Zobel B, Talbert J (1984). Applied Forest Tree Improvement. John Wiley & Sons. New York, NY, USA. 505 p.